HIGH THROUGHPUT QUANTIFICATION OF THE FUNCTIONAL GENES ASSOCIATED WITH RDX DEGRADATION USING THE SMARTCHIP PLATFORM

By

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ABSTRACT

HIGH THROUGHPUT QUANTIFICATION OF THE FUNCTIONAL GENES ASSOCIATED WITH RDX DEGRADATION USING THE SMARTCHIP PLATFORM

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a contaminant of concern at many military sites in the US. One approach that may be used to clean up these sites is bioremediation, and six functional genes, *diaA*, *nfsI*, *pnrB*, *xenA*, *xenB*, and *xplA*, have been linked to RDX degradation to date. Quantitative polymerase chain reaction (qPCR) is typically used to detect these genes in environmental samples, but the primer sets previously used do not have good theoretical coverage of the known gene sequences, so they may yield false negative results. To address this, new primer sets were designed with the EcoFunPrimer tool based on sequences collected by the Functional Gene Pipeline and Repository and verified based on residues and motifs. These primer sets were then used to quantify the RDX functional genes in RDX-contaminated groundwater before and after biostimulation, RDX-contaminated sediment and uncontaminated samples (Red Cedar River water and agricultural soils) using the SmartChip Real-Time PCR System. The newly designed primer sets improved upon the theoretical coverage of the previously published ones, and this corresponded to more detections in the environmental samples. All genes except *diaA*, were detected in the environmental samples, with *xenA* and *xenB* being the most predominant. In the sediment samples, *nfsI* was the only gene detected. The primer sets designed in this study may be used for more reliable detection of the RDX functional genes at contaminated sites. However, additional work with high throughput sequencing is required to confirm the specificity of these assays.

Dedicated to my family and friends

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

aa	amino acid
BLAST	Basic Local Alignment Search Tool
BLASTP	protein-protein BLAST
bp	base pair(s)
DNX	1,3-dinitroso-5-nitro-1,3,5-hexahydrotriazine
EPA	Environmental Protection Agency
FunGene	Functional Gene Pipeline and Repository
HMM	HMMER Hidden Markov Model
HPCC	High Performance Computing Center
KBS	Kellogg Biological Station
LRE	Linear Regression of Efficiency
MNX	1-nitroso-3,5-dinitro-1,3,5-hexahydrotriazine
NCBI	National Center for Biotechnology Information
NDAB	4-nitro-2,4-diazabutanal
nt	nucleotide(s)
PS	primer set
PDB	Protein Data Bank
qPCR	quantitative polymerase chain reaction
RDX	royal demolition explosive, hexahydro-1,3,5-trinitro-1,3,5-triazine
rxn	reaction
TNX	1,3,5-trinitroso-1,3,5-hexahydrotriazine

1.0 INTRODUCTION

1.1 General

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a xenobiotic compound that enters the environment through the production, use and disposal of explosives (1). It has contaminated many military sites across the US (2) and is a cause for concern because RDX has a low soil sorption coefficient, which means it is mobile through soil and may contaminate groundwater (3). Through a literature review, the US Environmental Protection Agency (EPA) has found RDX to have toxic effects and identified it as a possible human carcinogen based on evidence of adverse nervous system effects in humans and liver and lung tumors observed in animal experiments (4). While RDX does not have a federal drinking water standard currently, the US EPA set a lifetime health advisory guidance level at 2 µg/L for drinking water (5).

1.2 Biodegradation of RDX

Between the 1970s and 1990s, it was discovered that RDX was susceptible to biodegradation, both aerobically and anaerobically. In 1981, McCormick, Cornell and Kaplan investigated the biological degradation of RDX and the products generated under both aerobic and anaerobic conditions, using activated and anaerobic sewage sludge, respectively, as the inoculum. While the authors did not observe any degradation for the aerobic case, they did find rapid degradation under anaerobic conditions with the production of formaldehyde and nitroso derivatives (6). About a decade later, Binks, Nicklin and Bruce cultured *Stenotrophomonas maltophilia* PB1 from soil samples from an RDX contaminated site. This microorganism utilized RDX as its sole nitrogen source under aerobic and nitrogen-limiting conditions (1). Later work identified additional conditions that RDX may be degraded under, including nitrate-reducing, acetogenic and methanogenic, and bacteria capable of the degradation (7, 8). These studies confirmed the potential for using biodegradation to remediate RDX contaminated sites.

Along with the discovery of bacteria capable of degrading RDX, the genes responsible for this degradation were investigated. To date, six functional genes have been linked to RDX degradation: *diaA*, *nfsI*, *pnrB*, *xenA*, *xenB*, and *xplA* (9, 10). These genes may be used as biomarkers to detect the presence and monitor the level of bacteria capable of degrading RDX at a contaminated site. Also, the genes present at a site will indicate the end products likely to be produced from biodegradation because the enzymes encoded by these functional genes attack the RDX compound in different ways. While Crocker et al. (7) identified seven possible degradation pathways, Fuller et al. (11) noted that there are three main ones: 1) anaerobic nitro-reduction, 2) anaerobic ring cleavage, and 3) aerobic denitration (Figure 1). The known RDX functional genes have all been connected to one of these three pathways.

The most well researched of the RDX functional genes is *xplA*. This gene encodes a cytochrome P450-like enzyme that also contains a flavodoxin domain on the n-terminus (12). It has been identified in multiple aerobic bacteria genera, including *Gordonia*, *Williamsia* and *Rhodococcus*, and it works with the *xplB* gene, a flavodoxin reductase (10). Fournier et al. (13) investigated the metabolites of aerobic RDX degradation using *Rhodococcus* sp. strain DN22 and hypothesized a degradation pathway based on these. During RDX degradation, these authors measured the production of nitrite (NO_2^-), nitrous oxide (N_2O), formaldehyde (HCHO), carbon dioxide (CO_2), ammonia (NH_3), and 4-nitro-2,4-diazabutanal (NDAB, $C_2H_5N_3O_3$); both nitrite and formaldehyde were found to be transient intermediates. The authors hypothesized that the early

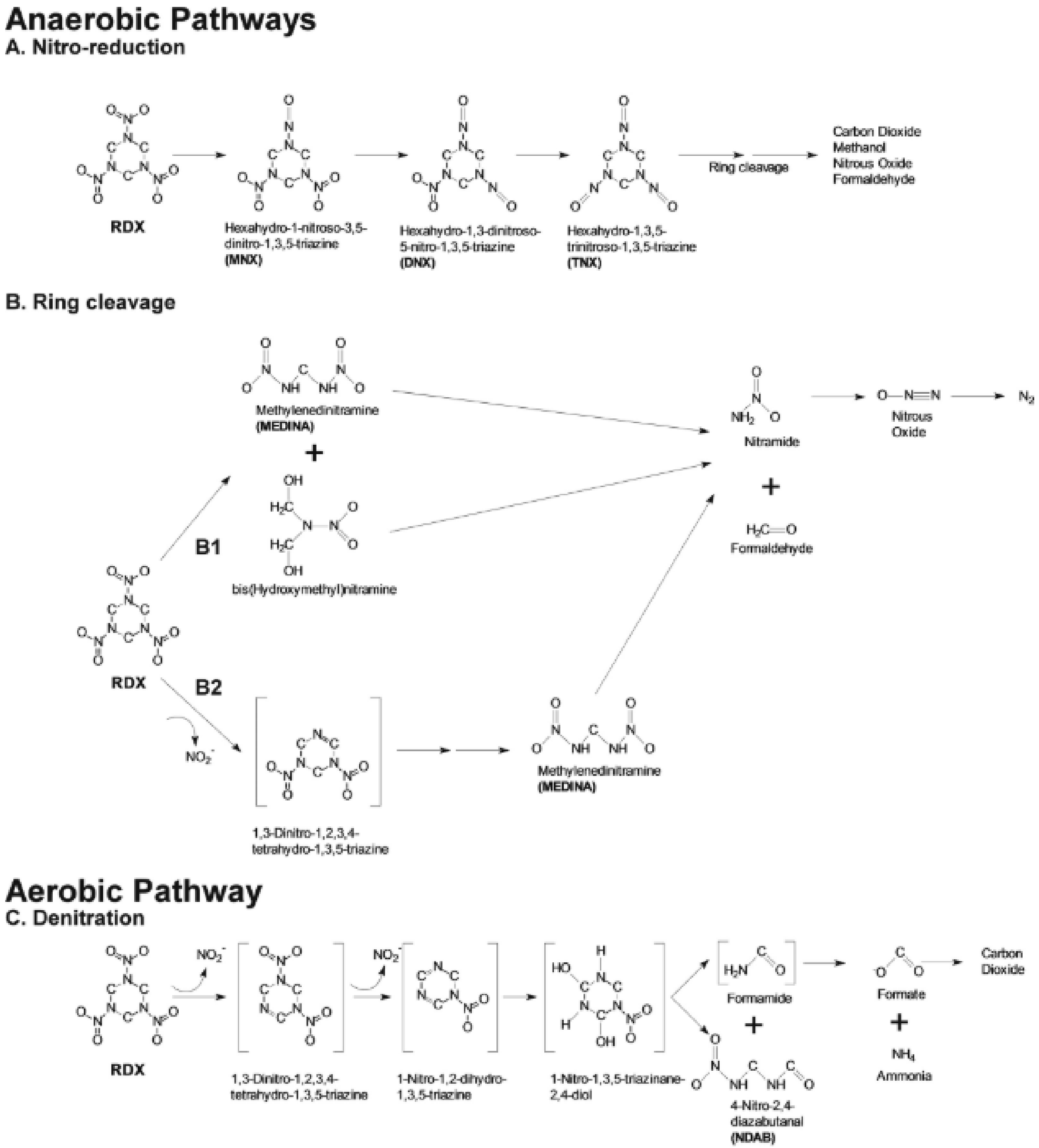

Figure 1. Pathways of RDX metabolism under anaerobic and aerobic conditions (reprinted from Fuller et al. (11) with permission from American Society for Microbiology)

production of nitrite indicated that denitration was the initial mechanism used to breakdown RDX, and this denitration would be catalyzed by the *xplA* enzyme. Seth-Smith et al. (12) supported these findings when they investigated the RDX degradation pathway of *Rhodococcus* *rhodochrous* strain 11Y under aerobic conditions. These authors also observed early production of nitrite, and noted that the other products formed, formate and formaldehyde in this case, would also be explained by a denitration mechanism since denitration would destabilize the molecule for spontaneous ring cleavage to occur. Fuller, Perreault and Hawari (14) determined that *xplA* may also operate under microaerophilic conditions, although the degradation rates are much slower than for aerobic conditions, and NDAB was measured as a breakdown product, which is expected for the aerobic denitration pathway. Therefore, the current evidence indicates that the *xplA* enzyme catalyzes aerobic denitration of RDX (Pathway C in Figure 1).

Another two genes found in aerobic bacteria are *xenA* and *xenB*. Both genes are xenobiotic reductases that have been identified in the *Pseudomonas* genus to date, and the enzymes contain a flavin mononucleotide-containing oxidoreductase domain (10). Even though the bacteria are aerobic, Fuller et al. (15) found RDX degradation to occur under anoxic and anaerobic conditions, and it was severely inhibited under aerobic conditions for *Pseudomonas putida* II-B (*xenA*) and *Pseudomonas fluorescens* I-C (*xenB*). The authors hypothesized that at least for *xenB*, the inhibition may be due to oxygen competing with RDX for the active site. Regarding the breakdown products, formaldehyde and small levels of methylenedinitramine (MEDINA), nitrous oxide, nitrite, and ammonia were measured, although it was inconclusive if the ammonia might have been nitramide or some other product. Based on this, the authors hypothesized that the degradation pathway was the anaerobic ring cleavage route. A later study by Fuller et al. (11) used the same bacteria strains and supported this hypothesis as they did not measure any 1-nitroso-3,5-dinitro-1,3,5-hexahydrotriazine (MNX), 1,3-dinitroso-5-nitro-1,3,5-hexahydrotriazine (DNX) or 1,3,5-trinitroso-1,3,5-hexahydrotriazine (TNX) intermediates during

RDX degradation. The presence of these intermediates would have indicated that these enzymes catalyzed the anaerobic nitro-reduction pathway. While the mechanism of attack is currently not known for *xenA* and *xenB*, these authors hypothesized that it may involve an initial denitration reaction based on the production of nitrite during RDX degradation. This corresponds to Pathway B2 (Figure 1), which includes a denitration step prior to ring cleavage.

Contrary to the previous genes, *diaA* has been isolated from an anaerobic bacterium. It is a diaphorase found among the *Clostridium* genus, and it contains a flavin reductase-like domain with a rubredoxin-like domain on the n-terminus (16). To the author's knowledge, no investigations into the degradation mechanism of *diaA* have been done with whole cell assays. Bhushan et al. (17) investigated the degradation route for RDX using the purified diaphorase enzyme from *Clostridium kluyveri*. They found that *diaA* functioned under anaerobic conditions and was inhibited by oxygen, and they measured the production of nitrite, ammonium, nitrous oxide, formaldehyde, and MEDINA, with MEDINA being a transient intermediate, during RDX degradation. From this, they concluded the degradation pathway involved an initial denitration step followed by ring cleavage. The *diaA* enzyme is expected to catalyze the transformation of RDX to $\text{RDX}^{\cdot -}$ (species I) through a transfer of one redox equivalent (Figure 2). Spontaneous denitration, ring cleavage and decomposition in water completes the degradation pathway. This proposed pathway agrees with the more general anaerobic ring cleavage pathway with an initial denitration step (Pathway B2 in Figure 1).

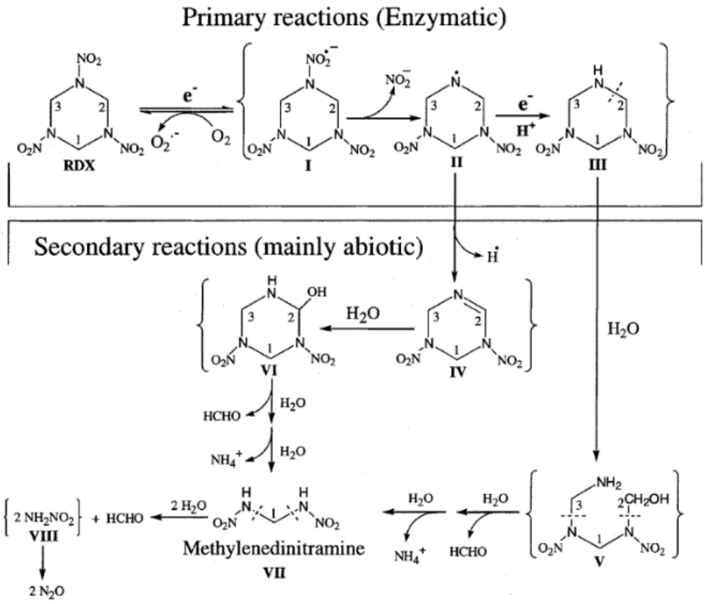

Figure 2. Proposed pathway of diaphorase catalyzed RDX transformation based on stoichiometry of metabolites recovered and NADH consumed (reprinted from Bhushan et al. (17) with permission from Elsevier). Primary reactions involve RDX reduction to RDX anion radical I that undergoes denitrohydrogenation to produce III. Secondary reactions involve ring cleavage and spontaneous decomposition in water. Intermediates shown inside brackets were not detected.

The remaining two genes, *nfsI* and *pnrB*, are both type I oxygen-insensitive nitroreductases. *NfsI* has been isolated from the *Enterobacter* and *Morganella* genera, which are both facultative anaerobes, and *pnrB* from the *Pseudomonas* and *Stenotrophomonas* genera, which are both aerobic. A study on the *nfsI* gene by Kitts, Cunningham and Unkefer (18) investigated the degradation pathway and measured MNX, DNX and TNX as intermediate products produced by *M. morganii* strain B2 under O_2-depleted conditions. These intermediates suggest that *nfsI* functions according to the anaerobic nitro-reduction pathway and may catalyze the reduction of the nitro groups on RDX, which would eventually lead to spontaneous ring cleavage. These authors also measured the production of CO_2, which supported the thought that the ring in the RDX structure had been broken. It is assumed that *pnrB* follows the same pathway as *nfsI,* but not much work has been done to elucidate the products generated by this enzyme. Lee et al. (19)

investigated the degradation of RDX under aerobic conditions with *Pseudomonas* sp. HK-6 and a later study used *Stenotrophomonas maltophilia* OK-5 (20), but both only noted that metabolic intermediates were not detected. While these enzymes are not sensitive to oxygen, Kitts et al. (21) discovered that only the purified *nfsI* enzyme will degrade RDX under aerobic conditions, while whole cell assays with *Enterobacter cloacae* and *Morganella morganii* strain B2 only degraded RDX under anaerobic conditions. For *pnrB*, it is unknown if a similar situation exists because work has only been done with this gene under aerobic conditions.

1.3 Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) has been used extensively to detect and quantify genes of interest in environmental samples. The conventional approach uses a thermocycler that can run either 96 or 384 reactions simultaneously. Reactions include a DNA-binding dye, deoxyribonucleotides (dNTPs), DNA polymerase, $MgCl_2$, stabilizers, primers, and DNA template. Primers include a forward and reverse primer designed to specifically target the gene of interest, and the DNA template is DNA extracted from the environmental samples to be assayed. Many studies exist that used this method to detect and quantify RDX functional genes (22-24).

Conventional qPCR, however, is limited by the number of reactions that can be run, the sample volume and time required and the coverage of the target gene’s diversity by the primer set used. When there are multiple genes of interest for a set of samples, it will quickly become necessary to perform multiple runs with the thermal cycler. The number of runs that may be performed, however, is constrained by the available sample volume. While it may be feasible to test around

20 genes, depending on the original sample volume, DNA concentration and technical replicates desired, this method is impossible for studies investigating hundreds of genes. Lastly, the primer sets designed to be used with conventional qPCR are historically designed based on only one gene sequence, or possibly a consensus sequence. In the case where there is high genetic variability known to exist among a gene, this method may not be capable of capturing the genetic diversity as multiple primer sets would be required. Therefore, it is necessary to consider high throughput options.

High throughput qPCR uses the same methodology as conventional qPCR, but it runs significantly more reactions with smaller volumes in parallel. The SmartChip Real-Time PCR System (SmartChip) is one platform that is capable of high throughput qPCR. With this technology, 5,184 reactions may be run simultaneously with a reaction volume of only 100 nL. This allows multiple genes and/or multiple primer sets for a single gene to be tested against multiple samples using a much smaller sample volume as compared to conventional qPCR. This platform has been used for a wide range of applications including detecting disease biomarkers and antibiotic resistance genes (25, 26). To the author's knowledge, there are no studies to date detecting or quantifying RDX genes using a high throughput qPCR platform.

1.4 qPCR Analysis

Both absolute and relative quantification techniques may be used with high throughput qPCR. The common absolute quantification method requires running reactions with known quantities of a standard simultaneously with the samples. The C_t values from the standard reactions are plotted against the logarithm of the known starting quantity to generate a standard curve, which can be described by the equation of a line: $C_t = m\log\left(\frac{gene\ copies}{rxn}\right) + b$. The gene copies in the sample reactions may then be calculated using this equation. An alternative absolute quantification approach is the linear regression of efficiency (LRE) method, which bases quantification off of fluorescence readings and an optical calibration factor (27). This optical calibration factor is determined from a few reactions with a known standard, and Rutledge and Stewart (27) recommend using lambda gDNA as the universal standard. For relative quantification, the $2^{-\Delta\Delta C_T}$ method is typically used for determining fold changes in the gene levels of environmental samples. In this method, $\Delta\Delta C_T$ is the difference in ΔC_T values between the target sample and reference sample, where ΔC_T is the difference in C_t values between the gene of interest and a reference gene (28). Another relative quantification method uses the standard curve equation for absolute quantification to estimate gene copy numbers and then normalizes the result for the target gene to the 16S rRNA gene. However, a standard curve is not actually generated in this case, instead the amplification efficiency is assumed to be 100% and the y-intercept is set at the threshold cycle (29). While relative quantification does not require sample space to be dedicated to standard curve reactions and therefore permits the maximum number of genes and samples to be tested at one time, it is desirable to be able to perform absolute quantification if needed.

1.5 Study objectives

1. To design a new suite of primer sets for each RDX degrading functional gene, to ensure a high coverage of all gene sequences currently available.
2. To ensure the primer sets work efficiently with the SmartChip Real-Time PCR System, which is a massive-parallel singleplex PCR platform.

3. To quantify RDX functional genes 1) in groundwater at a contaminated site before and after biostimulation, 2) in contaminated sediment and 3) in uncontaminated water and soils.

2.0 HIGH THROUGHPUT QUANTIFICATION OF RDX FUNCTIONAL GENES USING THE SMARTCHIP PLATFORM

2.1 Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) has contaminated many military sites across the US (2). It is released to the environment through its production, use and disposal (1), and typically ends up in groundwater due to its high mobility through soil, as determined by its low soil sorption coefficient (3). RDX is not regulated in drinking water, but the US Environmental Protection Agency (EPA) has set a lifetime health advisory guidance level of 2 µg/L since the compound has been identified as a possible human carcinogen (5).

Bioremediation is one feasible treatment approach for RDX-contaminated sites. Bacteria from many different genera, including *Clostridium*, *Enterobacter*, *Gordonia*, *Pseudomonas*, and *Rhodococcus*, have been found capable of degrading RDX under a wide range of conditions, such as aerobic, anaerobic, nitrate-reducing, and methanogenic (7, 8). Six of the functional genes responsible for this degradation have also been identified: *diaA*, *nfsI*, *pnrB*, *xenA*, *xenB*, and *xplA* (9, 10). These functional genes may be used as biomarkers for detecting the presence of bacteria capable of RDX degradation in environmental samples.

Besides detecting RDX-degrading bacteria, the identification of the functional genes present at a contaminated site can also indicate the products expected to be formed during RDX biodegradation. The three primary degradation pathways identified for RDX are aerobic denitration, anaerobic ring cleavage and anaerobic nitro-reduction (11). Three of the RDX genes, *diaA*, *xenA* and *xenB*, are expected to degrade RDX through the anaerobic ring cleavage route, which produces methylenedinitramine (MEDINA), formaldehyde and nitrous oxide (15, 17). *NfsI* and *pnrB*, on the other hand, are thought to follow the anaerobic nitro-reduction pathway and produce 1-nitroso-3,5-dinitro-1,3,5-hexahydrotriazine (MNX), 1,3-dinitroso-5-nitro-1,3,5-hexahydrotriazine (DNX) or 1,3,5-trinitroso-1,3,5-hexahydrotriazine (TNX) as intermediates before ring cleavage yields carbon dioxide, methanol, nitrous oxide, and formaldehyde (18, 19). Lastly, the *xplA* gene is expected to catalyze the aerobic denitration pathway with carbon dioxide, ammonia, and 4-nitro-2,4-diazabutanal (NDAB) being produced (13). Knowing the expected intermediates and end products provides a second line of evidence that may be used to confirm biodegradation is occurring.

Quantitative PCR (qPCR) has been widely used for detecting and quantifying RDX functional genes in environmental samples. Andeer et al. (22) investigated RDX degradation in contaminated soil and detected the presence of *xplA* in 11 out of 16 soil samples tested. Michalsen et al. (24) monitored *xplA* gene copy levels while testing the feasibility of biostimulation and bioaugmentation for a contaminated aquifer, and Wilson and Cupples (30) quantified *xenA*, *xenB* and *xplA* in contaminated groundwater and sediment samples. However, Fuller et al. (23) tested groundwater from two RDX-contaminated sites and did not detect *xenA*, *xenB* nor *xplA*. They identified multiple potential reasons for this, with one being that the primers used could not amplify the gene variants present. This highlights a concern with conventional qPCR, which is that one primer set may not be capable of amplifying all of the gene variants.

To the author's knowledge, only conventional qPCR with a single primer set for each gene has been used to detect and quantify RDX functional genes to date. Conventional qPCR utilizes a thermal cycler that can only run either 96 or 384 reactions simultaneously, which limits the number of samples and assays included in each run. This can make the methodology expensive and time consuming when there are a lot of samples and assays to test, and sample volume may also become a limiting factor in this situation. The use of a single primer set for each gene also potentially restricts the ability of the gene to be detected, especially for genes that have high sequence diversity that cannot be captured by a single primer set. This could result in false negative results and indicate the need for bioaugmentation when it is not actually necessary. These limitations of conventional qPCR are addressed with high throughput qPCR platforms.

One high throughput qPCR platform is the SmartChip Real-Time PCR System (SmartChip) which can run 5,184 reactions simultaneously with only 100 nL reaction volumes. This system provides multiple configurations for testing a large number of assays and samples, and it has been used for many applications, including detecting antibiotic resistance genes in environmental samples (26). The purpose of this study was to design a suite of assays for each gene to use with the SmartChip platform for detecting RDX functional genes in environmental samples. These suites of assays were designed with the goal of maximizing the theoretical coverage of known gene sequences. This will provide a more efficient method for quantifying all six RDX functional genes and a higher probability that the genes will be detected if they are present at RDX-contaminated sites.

2.2 Methods

2.2.1 FunGene and Primer Design Sequence Sets

A preliminary set of sequences for each of the six RDX genes was collected from the National Center for Biotechnology Information (NCBI), Protein Data Bank (PDB), and UniProt databases. These sequences were subjected to strict criteria concerning the length, identity and residues and motifs to ensure they were relevant to the gene. Those that passed were identified as the FunGene sequence set, which was used to develop the HMMER Hidden Markov Model (HMM) search program for the Michigan State University RDP Functional Gene Pipeline and Repository (FunGene) (31). With this HMM model, FunGene was capable of performing a more thorough search of the NCBI non-redundant protein database. The sequences gathered by FunGene were subjected to the same criteria before being included in the final primer design sequence set. Additional detail regarding the identification of these sequence sets is provided in Appendix A.

2.2.2 Primer Design

New primer sets were designed using the EcoFunPrimer tool (32) available from RDP's website (https://github.com/rdpstaff). This tool designed complementary primer sets for each gene to maximize the overall theoretical coverage of the input primer design sequence set. Input parameters were set for the tool to limit degeneracy among each primer and mismatches allowed between a primer and target sequence to two, melting temperature to between 59°C and 61°C, and primer length to between 18 and 21 nt. However, the primer length for *diaA* was expanded to between 15 and 25 nt in order to meet the T_m requirement. Regions for the forward and reverse primers were also specified to ensure all primer sets for a single gene were amplifying the same region, that the region amplified contained some of the identified residues and motifs, and to control the length of the amplicon generated. Multiple runs were performed with different primer regions and subsets of the initial sequence set to identify the optimum suite of primers. The final suite of assays for each gene were run through RDP's SeqFilters and Probematch (32) tools to determine the theoretical coverage.

Primer sets were also validated on a C1000 Touch thermal cycler with CFX96 real-time platform (Bio-Rad, Hercules, CA) before proceeding with running them on the SmartChip. Additional detail regarding this validation is provided in Appendix B. This test was to confirm the primer sets would amplify efficiently under the SmartChip's cycling parameters. Primer sets were removed if the linear dynamic range did not include at least 4 orders of magnitude, if multiple dilutions in this range had scattered replicates, if the range did not extend to 10^3 copies per reaction or fewer, and if the amplification efficiency was less than 70% or greater than 120%. In the case that removing assays would greatly reduce the theoretical coverage of the assay suite, new primers were designed with NCBI's Primer-BLAST tool to replace them.

2.2.3 Published Primer Sets

Literature was searched to generate a list of currently published quantitative PCR (qPCR) primer sets for each gene. These primer sets were then run against the appropriate primer design sequence set using RDP's SeqFilters and ProbeMatch tools to determine their theoretical coverage and enable a comparison between these and the designed assays. They were also validated like the designed assays on a C1000 Touch thermal cycler with CFX96 real-time platform before running them on the SmartChip (Appendix B).

2.2.4 Environmental Sample Collection and DNA Extraction

Contaminated and uncontaminated water and soil samples were collected for analysis. From an RDX-contaminated naval site, groundwater samples were collected before and after biostimulation with fructose and sediment samples before biostimulation. The groundwater included two wells from a shallow zone aquifer and five from a perched zone aquifer. For the shallow zone aquifer, samples were collected in November 2017 and June 2018 and for the perched zone aquifer, samples were collected in January 2018 and April 2018. Biostimulation was performed in March 2018 using injection wells and an injection trench for the shallow and perched zone aquifers, respectively. The water was collected in 1 L amber glass bottles, shipped overnight on ice, and stored in the dark at 4°C prior to filtration. The sediment sample was collected in a Ziplock bag, shipped overnight and stored at 4°C prior to DNA extraction. For the uncontaminated samples, water was collected from the Red Cedar River in Michigan and agricultural soils were taken from MSU and MSU’s Kellogg Biological Station (KBS). The river water was collected in 1 L plastic bottles and frozen at -20°C until filtration. The agricultural soil samples were collected in Ziplock bags and immediately stored at 4°C until DNA extraction.

For the groundwater and river water samples, DNA was extracted with the DNeasy PowerWater kit (Qiagen, Germany) following manufacturer’s protocols. Approximately 950 mL of groundwater or 250 mL of river water was filtered on a 0.47 µm diameter 0.22 µm filter (GSWG047S6, Millipore) using a vacuum pump and then the filter was stored in the PowerBead tube at -20°C until proceeding with the rest of the extraction protocol. Triplicate extractions were done for the groundwater from each well before biostimulation and from the shallow zone aquifer wells after biostimulation. Duplicate extractions were done for the groundwater from the perched zone aquifer wells after biostimulation and from the Red Cedar River water. DNA was eluted in 50 µL and stored at -20°C.

For the sediment and soil samples, DNA was extracted with the DNeasy PowerSoil kit (Qiagen) following manufacturer's protocols. Approximately 1 g of sediment or soil was used for each extraction and extractions were done in triplicate for each sample. With the contaminated sediment sample, 40 mg of Carnation instant nonfat dry milk (Nestlé, Rosslyn, VA) per g of sediment was added at the beginning of the extraction to improve the DNA yield (33). DNA was eluted in 50 µL and stored at -20°C.

The A260/A280 value was measured for each sample with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE) and the DNA concentration was determined using the appropriate Qubit dsDNA BR or HS assay kits with a Qubit fluorometer (Invitrogen, Eugene, Oregon) following manufacturer's protocols.

2.2.5 Quantitative PCR

RDX genes present in environmental samples were quantified on the SmartChip using the primer sets that passed all of the above tests. These runs included serial plasmid dilutions to generate standard curves and DNA from the environmental samples.

All primers were manufactured by Integrated DNA Technologies (IDT, Coralville, IA) and tested against plasmids containing a partial gene sequence (GenScript, Piscataway, NJ). Gene copy number for the plasmids was calculated as described by Ritalahti et al. (34). Primer and plasmid sequence information is provided in Appendix C.

Samples and assays were dispensed into the 72x72 nanowell chip with the Multisample Nanodispenser. Individual reactions on the chip consisted of a total volume of 100 nL with 1X LightCycler 480 SYBR Green I Master (Roche Applied Sciences, Indianapolis, IN), 0.5 µM each of the forward and reverse primers (2.5 µM each in the case of two forward and/or reverse primers), DNA, and balance PCR grade water. Extracted DNA from the environmental samples was diluted to 20 ng/µL for a total of 0.2 ng in the reaction or run at concentration if the sample concentration was below 20 ng/µL. Cycling was performed following the SmartChip protocol specified by Wang et al. (35).

The 24 assays by 216 samples configuration was used for each SmartChip run. Each chip included the designed and published primer sets for two genes along with the universal bacteria primer set, which targets the 16S rRNA gene (36). Triplicate 10-fold serial plasmid dilutions (10^1-10^7 copies/reaction) were included for each plasmid required to generate the standard curves for the primer sets being used. For the 16S rRNA primer set, the plasmid dilution series also included reactions with 10^8 copies. All environmental samples were run on each chip as biological duplicates or triplicates. A reaction blank and triplicate negative controls using a plasmid with an *aceA* gene insert (10^3 copies/reaction) were also included.

2.2.6 qPCR Analysis

Reactions that did not amplify or were identified as false positives were considered as missing data for all analyses. False positives were reactions that amplified later than the threshold cycle, which was set at 28 as recommended for the SmartChip (37), reactions with C_t values outside of the standard curve range, or reactions that were the only replicate to amplify.

The copy number of each gene per reaction was calculated based on the standard curve for the corresponding primer set and plasmid. This was then analyzed in two ways: 1) copy number per reaction was converted to per mL or per g of starting material, as appropriate, and 2) copy number per reaction was normalized to the 16S rRNA gene copy number per reaction as described previously (29). Heatmaps were generated for the copy numbers per mL or per g of starting material using the gplots v3.0.1 package in R v3.5.1 (38, 39), and bar charts were created in Excel to display the groundwater pre- and post-biostimulation results for each well. Additionally, boxplots were generated from the results of both analysis approaches using the ggplot2 v3.0.0 package (40). For the groundwater results, only assays that had detections in both the pre- and post-biostimulation samples were plotted, and for the sediment and soil results, the assays that had detections in at least one of the samples were plotted.

Statistical analysis of the difference between the pre- and post-biostimulation samples for the gene copy number per mL of groundwater values was performed in R using the car v3.0-2 and matrixTests v0.1.0 packages (41, 42). The Student's independent two-sample t-test, Welch's t-test or Mann-Whitney U test was used for each primer set as appropriate. A detailed explanation of the statistical analysis is provided in Appendix F.

2.3 Results and Discussion

2.3.1 FunGene and Primer Design Sequence Sets

Table 1 summarizes the number of sequences at the beginning and final stages of identifying the FunGene and primer design sequence sets. The elimination criteria specified in Appendix A greatly reduced the number of sequences included in these sequence sets. However, the use of FunGene did increase the number of sequences used for primer design. The accession numbers for the nucleotide and protein sequences included in the final FunGene and primer design sequence sets are reported in Tables A.3-A.8 (Appendix A).

Table 1. Sequence set size for identifying FunGene and primer design sequence sets. "Initial Sequences" are those collected from the NCBI, UniProt and PDB databases and "FunGene Sequences" are those that were gathered by the HMM model.

Functional Gene	Initial Sequences	FunGene Sequence Set	FunGene Sequences	Primer Design Sequence Set
diaA	1,051	96	123,691	16[1]
nfsI	2,926	23	58,706	723
pnrB	1,178	32	61,601	56
xenA	1,852	14	199,903	309
xenB	4,435	58	110,413	425
xplA	7	7	189,222	49[2]

[1] Initial criteria yielded 123 sequences, but these were further filtered to only include *Clostridium*.

[2] Partial sequences were allowed for this gene – 27 sequences included the flavodoxin domain and 30 included the cytochrome P450 domain.

2.3.2 Primer Design

A suite of assays was successfully designed for each gene to have at least 80% coverage of the primer design sequence set according to the EcoFunPrimer Tool, except for *diaA* (Table 2). For *diaA*, the EcoFunPrimer tool was unable to find many regions in the sequences to design primers that met the T_m requirements even with a larger allowable primer length and more variability permitted in the amplicon lengths. This relaxation of the design parameters did yield assays that covered a majority of the *diaA* sequences though. All assays amplify at least one residue and

Table 2. Designed Suite of Assays.

Functional Gene	**Number of Assays**	**Total Coverage (%)**	**Amplicon Length (bp)**	**Residues Amplified**	**Motifs Amplified**
diaA	3	62.4	84-136	2	1
nfsI	8	92.5	181-200	4	1
pnrB	5	83.9	156-189	10	1
xenA	17	81.6	190-200	4	1
xenB	19	90.4	129-134	1	1
xplA	3	96.3[1]	157-165	1	2

[1] The coverage for *xplA* is only based on the 27 flavodoxin sequences

motif, and these may be used in future work to confirm the amplicons generated in qPCR are from the expected gene. All primer sequences are reported in Appendix C.

Results from the qPCR runs on the Bio-Rad thermal cycler to validate the assays are provided in Appendix B. One, five and two assays were removed from the *xenA*, *xenB* and *xplA* gene suites, respectively, due to poor performance on the Bio-Rad thermal cycler. The two assays for *xplA* were redesigned with NCBI’s Primer-BLAST tool, and the new primer sets had similar coverages to the original ones and performed better on the Bio-Rad thermal cycler (Appendix C). For *xenA* and *xenB*, however, these primer sets were not redesigned because their removal did not change the theoretical coverage of the full assay suites.

A breakdown of the coverage for each assay as determined by the EcoFunPrimer tool is presented in Figure 3. The first assay has the highest coverage for each gene and the subsequent assays generally decrease in coverage. Therefore, only the first, or a select few of the assays, could be used for evaluating environmental samples and still maintain a good coverage of the gene of interest.

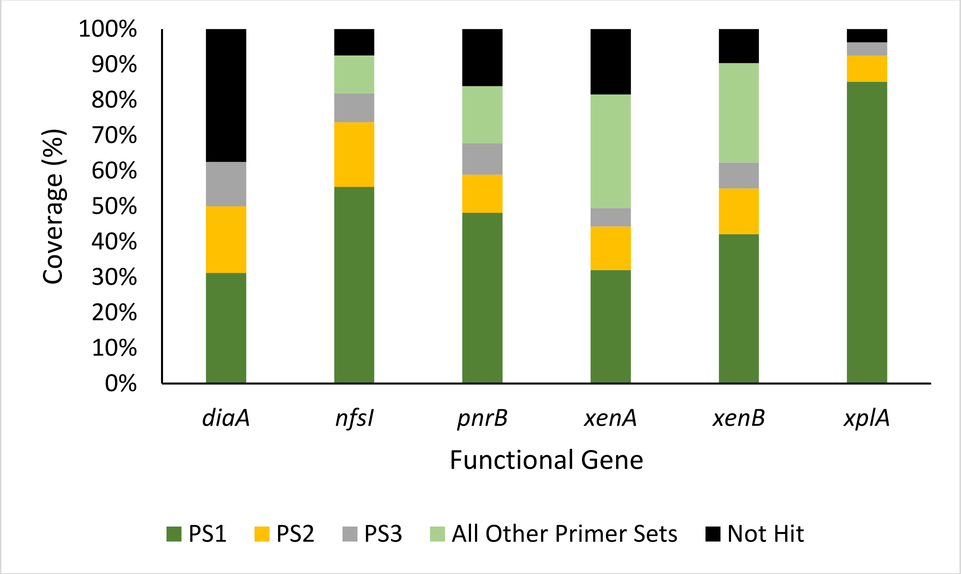

Figure 3. Breakdown of assay coverage. The coverage for *xplA* is only based on the 27 flavodoxin sequences.

The theoretical coverage determined with the SeqFilters and ProbeMatch tools was higher than that from the EcoFunPrimer tool (Tables C.1-C.6 in Appendix C). This occurred because these tools allowed certain mismatches that the EcoFunPrimer tool does not. Along with increasing the coverage of the assays, this analysis also revealed that a single sequence may be amplified by more than one assay. Therefore, the qPCR results from a single gene's suite of assays are not additive, and the full suite should only be used to ensure a high probability that the gene will be detected if it is present in the sample. The copy numbers present may then be estimated based on the assay with the highest initial copy number.

2.3.3 Published Primer Sets

Primer sets already published in literature and used for qPCR were identified for all genes and are listed in Table C.7 (Appendix C). Only *diaA* was found to have no previously published qPCR primer sets. The theoretical coverage of the complete suite of designed assays is consistently higher than the published assays, which supports that the designed assays improve upon those currently used (Figure 4). Only *xplA* had a small amount of improvement with the designed assays, and this was due to a high similarity among most of the *xplA* sequences so one primer set was able to capture most of the sequences.

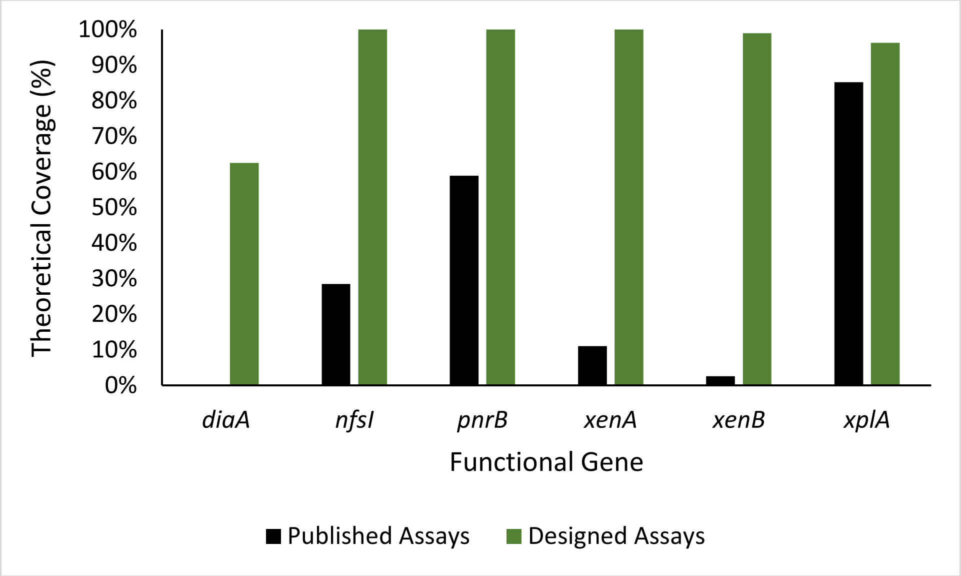

Figure 4. Theoretical Coverage of Published and Designed Assays. The theoretical coverage shown is for the assay with the highest coverage for each gene. For *xplA*, this coverage is only based on the 27 sequences that include the flavodoxin domain as this is the portion of the gene that the assay targeted.

2.3.4 DNA Extraction

The purity and concentration values for each DNA extraction are reported in Tables 3 and 4 for the RDX-contaminated and uncontaminated samples, respectively. The purity values were generally around 1.80 as desired for DNA, except for cases where DNA yield was very low, or less than 10 ng/µL. In most cases for the groundwater samples, the ones from after biostimulation yielded more DNA than those before. The RDX-contaminated sediment sample, MW71, had very low DNA yields even with the addition of dry milk during the extraction process. This is assumed to be partially due to the fact that the sediment appeared very clay-like, and clay would strongly bind DNA (33).

Table 3. Purity and concentration of replicate DNA extracts from groundwater and sediment samples from an RDX-contaminated site

Source	Sample ID	Biostimulation Status	A260/A280			Concentration (ng/µL)		
			1	2	3	1	2	3
Shallow Aquifer	MW32	Pre	1.74	1.94	2.14	11.8	9.78	15.2
		Post	1.86	1.93	1.86	43.7	109	142
	MW62	Pre	1.91	1.96	1.93	101	90	133
		Post	1.70	1.89	1.84	19.4	119	68.0
Perched Aquifer	MW48	Pre	1.48	1.74	1.52	0.079	6.06	13.9
		Post	1.85	1.85	N/A	405	465	N/A
	MW60R	Pre	2.48	1.76	1.29	3.42	0.129	0.098
		Post	1.98	1.74	N/A	48.9	38.2	N/A
	MW64	Pre	2.02	-0.80	1.23	4.11	1.14	2.70
		Post	1.86	1.89	N/A	249	352	N/A
	MW66	Pre	2.15	1.94	1.70	19.8	18.1	12.4
		Post	2.07	1.7	N/A	8.07	43.5	N/A
	MW67	Pre	2.53	6.91	1.83	4.10	4.92	5.75
		Post	2.16	1.64	N/A	0.086	0.102	N/A
Sediment	MW71	Pre	1.04	1.00	1.05	2.31	2.75	2.29

Table 4. Purity and concentration of replicate DNA extracts from uncontaminated soil and water samples

Source	Sample ID	A260/A280			Concentration (ng/µL)		
		1	2	3	1	2	3
KBS Agricultural Soil	T1	1.89	1.69	1.85	31.0	27.9	47.5
	T2	1.65	1.96	1.71	24.4	35.2	32.0
	T3	1.76	1.92	1.90	24.7	27.0	41.7
	T4	1.71	1.89	1.87	37.5	43.8	46.5
MSU Agricultural Soil	Soil E	1.88	1.67	1.87	31.7	17.3	16.3
	Soil F	1.83	1.81	1.57	27.8	14.4	13.8
Red Cedar River	RC_B	1.86	1.95	N/A	26.3	28.1	N/A

2.3.5 Quantitative PCR

The SmartChip C_t values, standard curve characteristics, gene copies per mL or g, and gene copies per 16S rRNA gene for each assay are reported in Appendix D. All samples amplified consistently with the 16S rRNA assay, except one replicate of MW66_Pre on the first chip, confirming that DNA extraction was successful and that qPCR was not inhibited (Figure 5 and Figure 6). The heatmaps also show that the designed assays resulted in more detections of each gene than the published ones, which suggests that the greater theoretical coverage of these assays did translate into better detection. In most cases, the standard curves were also better in terms of amplification efficiency and R^2 values. However, *pnrB* and *xplA* did have a few designed assays with low amplification efficiencies (Table 5). For *pnrB*, this was only PS2 that had a low amplification efficiency, and the other designed assays were comparable to the published ones with regard to standard curve characteristics. For *xplA*, on the other hand, Pub1, Pub2 and Pub4 performed much better than the designed assays, and this suggests that the results for these assays may be more reliable.

RDX functional genes were detected in most of the samples, and almost every gene was detected. The sediment samples (MW71) were the only ones to yield detection of the RDX functional genes by only one primer set, but this may have been due to the low DNA concentration of these samples. This is supported by the lack of gene detection in MW48_Pre.1, which also had very low DNA yield. Regarding the genes detected, *xenA* and *xenB* were the most commonly detected. *NfsI*, *pnrB* and *xplA* were also detected in at least a few of the samples, and *diaA* was the only gene not detected in any of the samples.

The perched zone aquifer had positive detection of every gene except *diaA*, while the shallow zone aquifer only had detections of *pnrB*, *xenA*, and *xenB*. The assays that had detections at both time points yielded gene copies in the range of 10^2 to 10^5 per mL of groundwater, and this value was generally consistent at both time points for each assay, whether looking at the wells individually (Appendix E) or combined for each aquifer (Figure 7a-b). This agrees with the results of Michalsen et al. (24) who found *xplA* gene copies to be present at 10^3 to 10^5 copies per mL in an RDX contaminated aquifer before biostimulation and experience no change after biostimulation. There were a few assays, *xenB* PS1, PS5 and PS7 for the shallow zone aquifer and *pnrB* PS2 and *xenB* PS1 for the perched zone aquifer, that did have a statistically significant difference ($p<0.05$) between the pre- and post-biostimulation levels (Appendix F). For the shallow zone aquifer, this was a decrease in the post-biostimulation level compared to pre-biostimulation, while for the perched zone aquifer, it was an increase (Figure 7a-b). Further sampling would be required to confirm either of these trends. Considering the 16S rRNA normalized results for the groundwater samples, there is a clear decrease in the relative abundance of the genes detected in the post-biostimulation samples (Figure 7c-d). This implies that biostimulation may have initially promoted the growth of a wide range of bacteria, and a later time point may show the RDX degraders gaining dominance. Interestingly, when the genes were also detected in the Red Cedar River samples, they were present at similar levels and relative abundance to those in the contaminated groundwater (Figure 7).

For the sediment and soil samples, most of the detections were with the agricultural soils. All of the genes were detected in the KBS soils, except for *diaA*, and all of the genes, except *diaA* and *pnrB*, were detected in the MSU soils at levels ranging from 10^4 to 10^8 copies per g of soil (Figure 8a). Only one assay, PS7 from *nfsI*, yielded positive detection in the contaminated sediment samples. With this assay, *nfsI* was detected at levels around 10^5 copies per g of contaminated sediment (Figure 8a). This is similar to the findings of Wilson and Cupples (30) who quantified *xenA*, *xenB* and *xplA* in RDX-contaminated sediment at levels around 10^5 copies per g; they did not test for *nfsI*. The uncontaminated KBS soils had a higher copy number, approximately 10^6 copies per g, and the MSU soils had no detection (Figure 8a). Despite the KBS soils having a higher copy number, the contaminated sediment had a higher relative abundance (Figure 8b). This is as expected because the microorganisms capable of RDX degradation should be able to compete better in the RDX contaminated sediment than uncontaminated soils and therefore be more dominant.

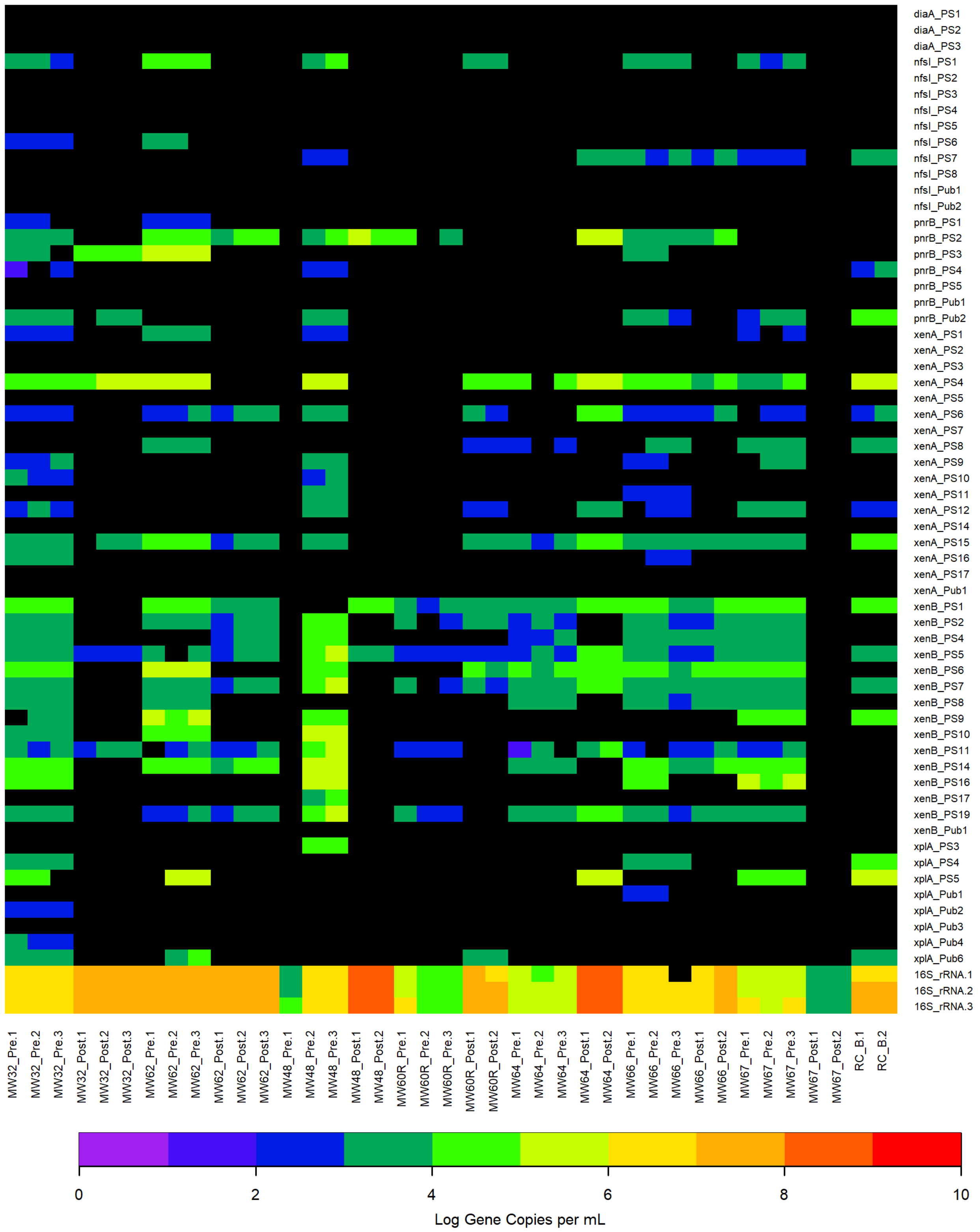

Figure 5. Heatmap of groundwater (MW) and Red Cedar River (RC) log gene copies per mL of groundwater or river water, respectively. Black cells indicate either no amplification or false positive amplification. The 16S_rRNA results correspond to the 3 chip runs performed: 1) *xenB* and *pnrB*, 2) *xplA* and *nfsI*, and 3) *xenA* and *diaA*. The terms "Pre" and "Post" with the samples indicate whether they are pre- or post-biostimulation, respectively.

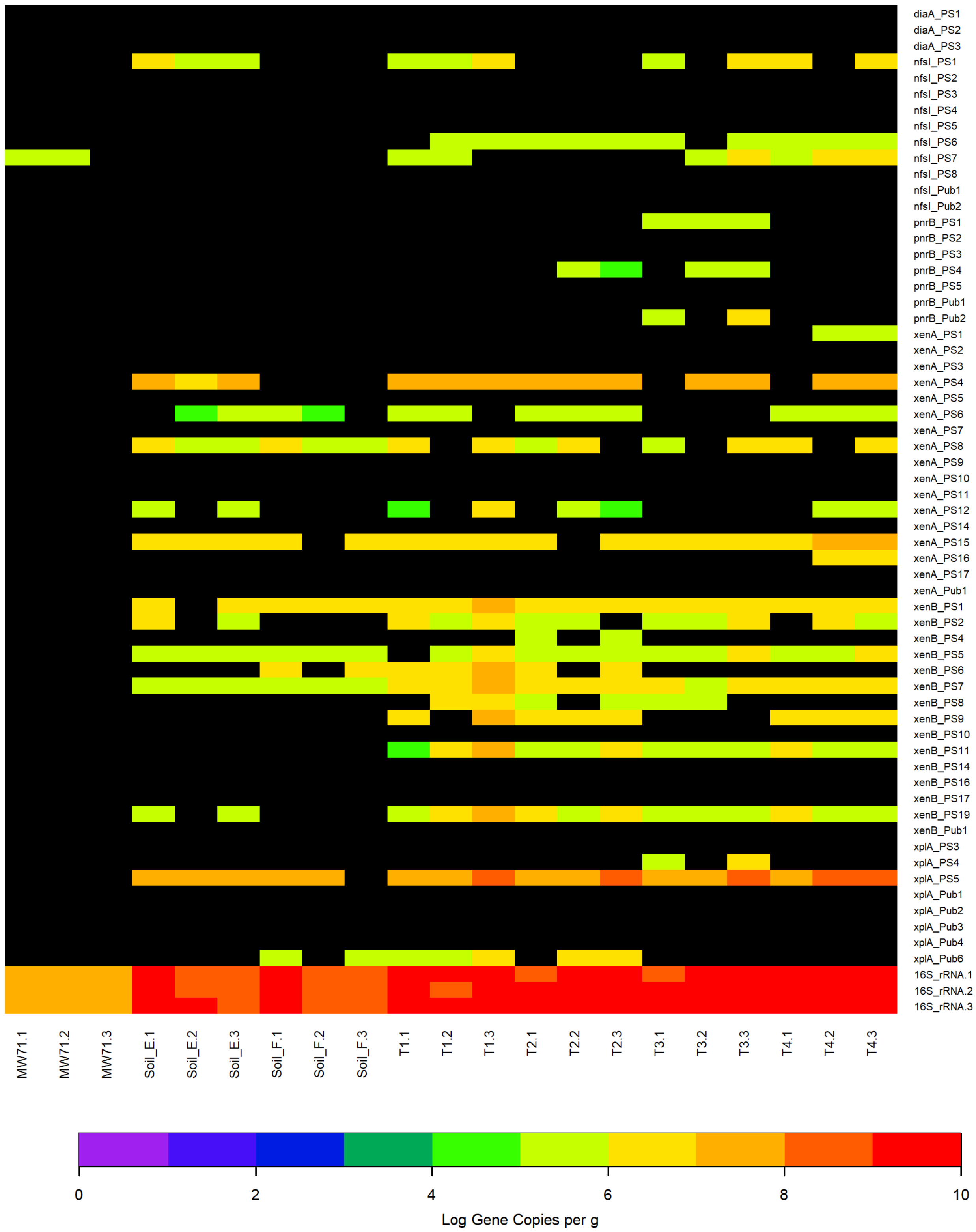

Figure 6. Heatmap of sediment (MW) and soil log gene copies per g of sediment or soil, respectively. Black cells indicate either no amplification or false positive amplification. The 16S_rRNA results correspond to the 3 chip runs performed: 1) *xenB* and *pnrB*, 2) *xplA* and *nfsI*, and 3) *xenA* and *diaA*.

Table 5. Summary of SmartChip Standard Curve Characteristics

Functional Gene	**Assays**	**Log(Linear Range)**		**Efficiency (%)**		**R^2**	
		Min	**Max**	**Min**	**Max**	**Min**	**Max**
diaA	Designed	2 to 7	1 to 7	78.3	102.6	0.986	0.998
nfsI	Designed	2 to 7	1 to 7	76.1	87	0.983	0.995
	Published	2 to 7	2 to 7	76.2	91.9	0.918	0.935
pnrB	Designed	3 to 7	1 to 7	60.1	91.5	0.987	0.997
	Published	2 to 7	2 to 7	89.9	91	0.968	0.980
xenA	Designed	4 to 7	1 to 7	81.5	97.3	0.989	0.998
	Published	3 to 6	3 to 6	74.8	74.8	0.989	0.989
xenB	Designed	4 to 7	1 to 7	68.1	93	0.986	0.996
	Published	3 to 7	3 to 7	68.5	68.5	0.990	0.990
xplA	Designed	4 to 7	2 to 7	40.2	75.4	0.930	0.993
	Published	3 to 7	1 to 7	61.8	97.3	0.984	0.995
16S rRNA	Published	2 to 8	1 to 8	93	107	0.994	0.998

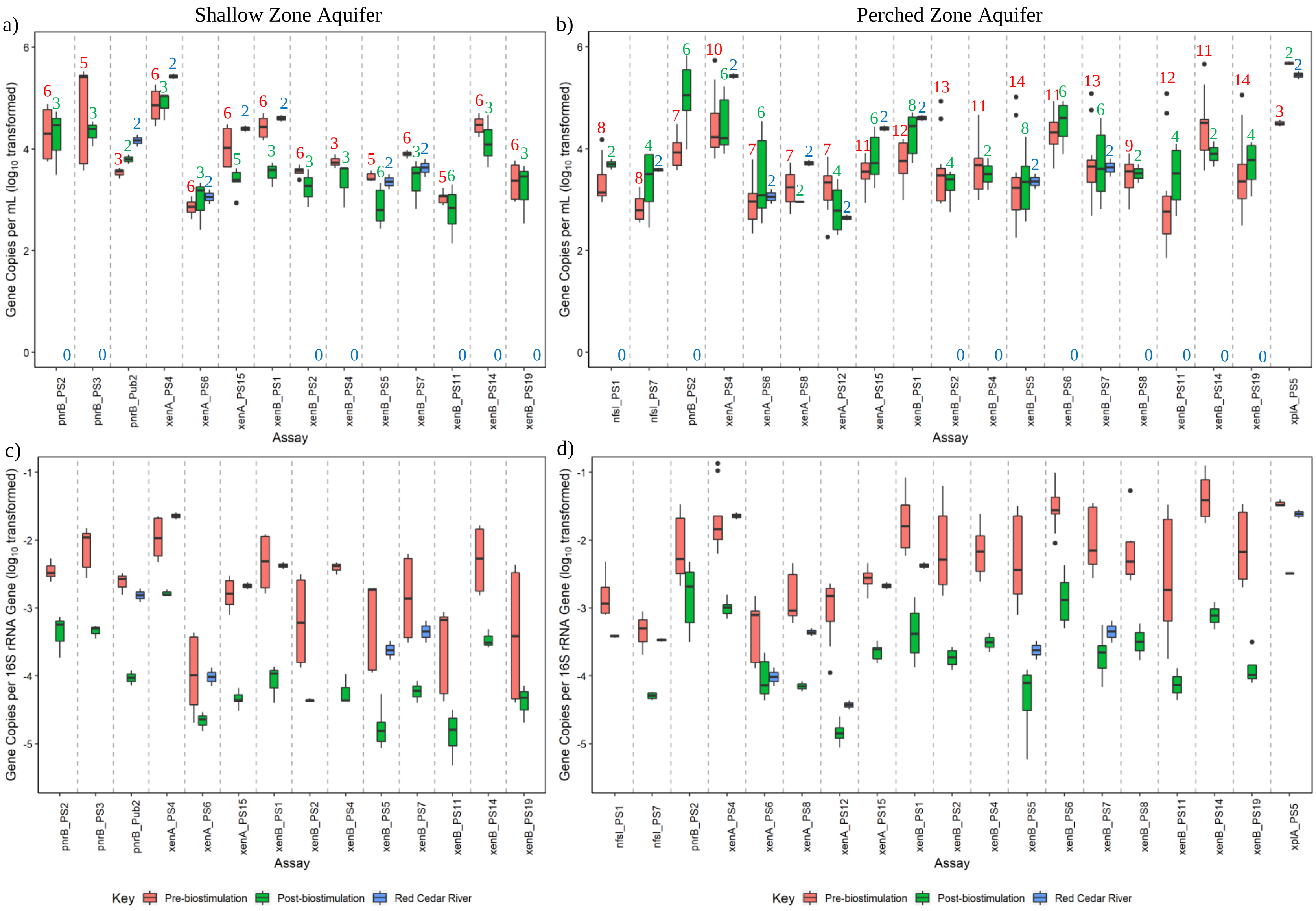

Figure 7. Boxplots of RDX genes in contaminated groundwater and uncontaminated river water. Plots (a) and (c) for the shallow zone aquifer (n≤6) and (b) and (d) for the perched zone aquifer (n≤14) display the results per mL of groundwater and normalized to the 16S rRNA gene, respectively. Results for the Red Cedar River (n=2) are provided for comparison to uncontaminated water. The number of detections for each assay are noted on plots (a) and (b).

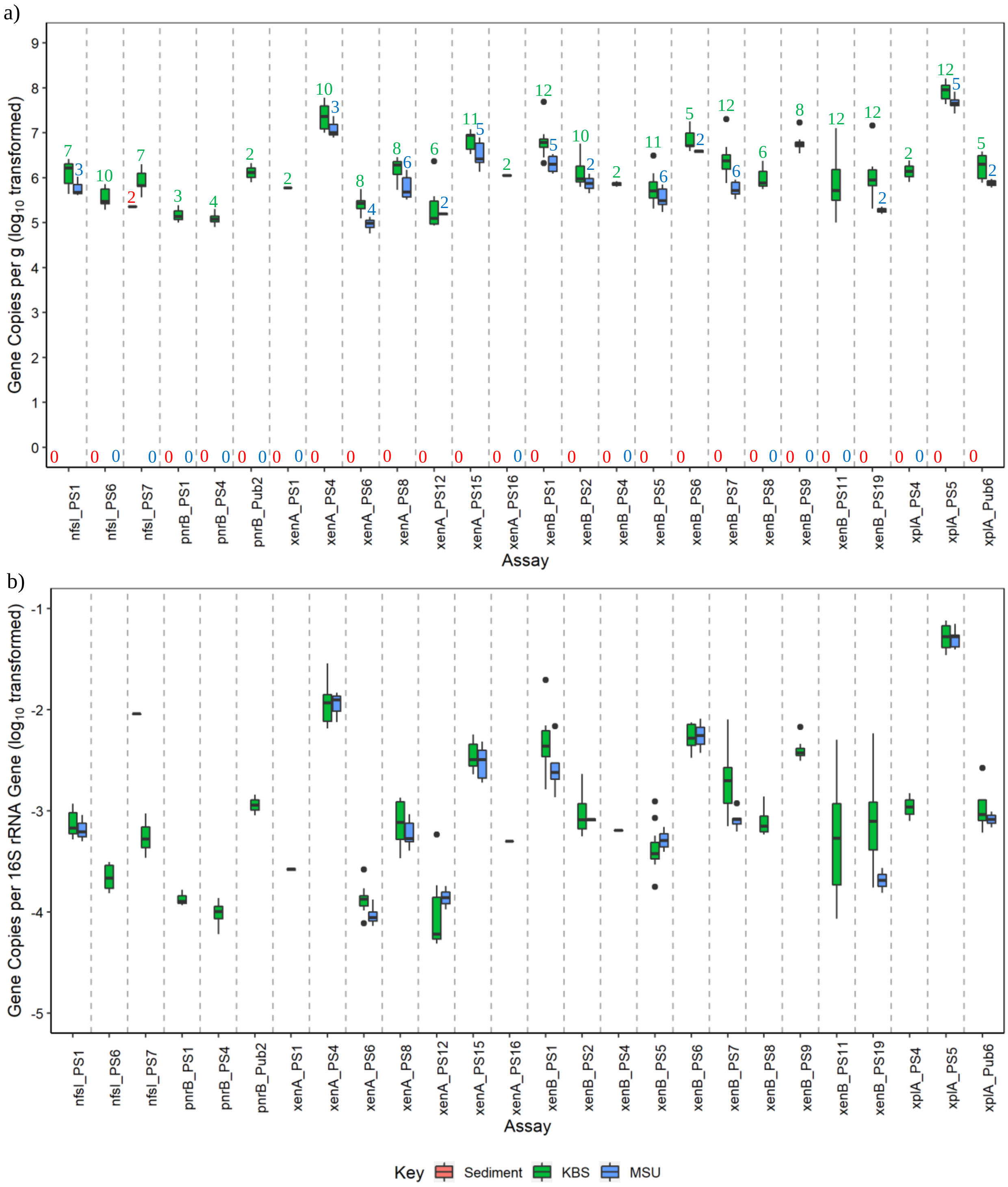

Figure 8. Boxplots of RDX genes in contaminated sediment (n=2) and uncontaminated soil samples (n≤12 for KBS; n≤6 for MSU). Plots (a) and (b) display the results per g and normalized to the 16S rRNA gene, respectively. The number of detections for each assay are noted on plot (a).

3.0 RECOMMENDATIONS AND FUTURE WORK

3.1 Absolute Quantification

3.1.1 Standard Curves

To test all of the designed and published assays (as described in Chapter 2), it is recommended to run 3 chips, with 2 RDX functional genes each, in the 24 assays by 216 samples format. Pairing *diaA* with *xenA*, *nfsI* with *xplA* and *pnrB* with *xenB* maximizes the use of each chip. These arrangements permit 64 samples to be tested with each chip, assuming the functional gene plasmids include 7 10-fold dilutions in triplicate, the 16S rRNA plasmid include 8 10-fold dilutions in triplicate and there is one no template control and one negative control. This permits 32 samples to be tested in duplicate or 21 samples to be tested in triplicate. However, if funds are limited, and only chip can be analyzed, a subset of these assays can be used (Table 6). These were selected based on the assays' combined theoretical coverage, their performance on the SmartChip and the need for only one plasmid per gene. In this case, the 12 assays by 384 samples layout would be used and this would enable 232 samples to be tested, which equates to 116 samples in duplicate or 77 samples in triplicate. The proposed one chip format does increase the potential for false negatives because the total theoretical coverage is less than the three chips approach. Additional assay and sample configurations could be designed depending on the requirements of the project.

Table 6. Recommended assays and plasmids for a single SmartChip run.

Functional Gene	**Assays**	**Plasmids**	**Total Theoretical Coverage (%)**
diaA	PS1	CP012395	62.5
nfsI	PS1	CP001918	99.9
pnrB	PS1 and PS5	CP010979	98.2
xenA	PS1, PS4, PS12, and PS16	CP004045	81.9
xenB	PS2 and PS19	CP014205	86.6
xplA	Pub1	AF449421	85.2
16S rRNA	16S_rRNA	KX858536	N/A

3.1.2 Linear Regression of Efficiency Method

An alternative absolute quantification method that could be used with these assays is the linear regression of efficiency (LRE) method. This method was developed by Rutledge and Stewart (27), and it uses fluorescence readings with an optical calibration factor to calculate the number of target molecules present in the reaction. For the optical calibration factor, a few reactions with lambda gDNA and the CAL1 primer set (43) would need to be included on the chip, but substantial space would be saved for running samples since the plasmid standards would not be required. Rutledge and Stewart (27) found absolute accuracies of ±25% using this method, and Boyle, Dallaire and MacKay (44) determined that the number of target molecules calculated from LRE and average standard curves were highly correlated. This method does require high optical precision to be accurate, however (27). Therefore, it will be necessary to test the precision achieved with the SmartChip platform and ensure the results are reliable before solely relying on this approach.

3.2 Relative Quantification

Other approaches that can maximize the number of samples run on each chip are relative quantification methods. These include the $2^{-\Delta\Delta C_T}$ (28) method and the relative gene copies calculation described by Looft et al. (29). The $2^{-\Delta\Delta C_T}$ method normalizes the target gene to a reference gene and calibrator, and it assumes that the amplification efficiencies of the target and reference genes are the same (28). These two normalizations yield the fold change of the target gene relative to the calibrator. This calibrator could be the sample before biostimulation, and in this case the method would yield the resulting fold change in the target gene from biostimulation. The relative gene copies calculation described by Looft et al. (29) uses the same formula as the standard curve absolute quantification method, but assumes an efficiency of one and sets the y-intercept to the threshold cycle. The result is normalized to the 16S rRNA gene for a measure of the relative abundance. These methods are appropriate in situations where only the fold changes in gene levels or relative abundances are required, such as for bioremediation projects that want to check if biostimulation or bioaugmentation was successful at increasing the gene copies present. Care is recommended in the interpretation of such results, however, as these methods could be useful for comparing results from a specific primer set among all the samples tested, but comparisons between primer sets would not be recommended due to the inherent efficiency assumptions.

3.3 Future Work

3.3.1 High throughput sequencing

Additional work is needed to confirm the specificity of the assays designed in the current research. High throughput sequencing of the gDNA used in qPCR as well as of the amplicons generated through qPCR will aid in this. Sequencing of the gDNA can be used to confirm that the genera expected based on the genes detected are actually present. However, all of the genera that contain these functional genes are not known. This means that cases where the expected genera are not detected will remain inconclusive regarding the accuracy of the qPCR results. Sequencing the qPCR amplicons will help resolve this because the amplicon sequences can be compared against the sequence sets identified here to determine if they are of the expected length and contain the identified residues and motifs. These two efforts together have the potential to both confirm the specificity of the assays and identify new genera containing these RDX functional genes.

3.3.2 Proteomics

The results from high throughput qPCR may also be supported by proteomics. In proteomics, the enzymes associated with functional genes are used as the quantitative biomarker. This enables both the detection of RDX-degraders and confirmation of enzymatic activity. This technique has already been used to detect reductive dehalogenase enzymes from organohalide-respiring bacteria (45). However, it should be noted that no detection through proteomics may only mean degradation is not actively occurring, and not that the bacteria capable of degradation are not present.

APPENDICES

APPENDIX A

Sequence Sets

NCBI, PDB, and UniProt databases were all searched for relevant protein sequences using the search terms in Table A.1. From the initial search results, the expected protein length was determined, and a minimum length criterion of 90% was set to avoid the inclusion of partial sequences. Sequences retrieved from the UniProt database were also used to run individual Basic Local Alignment Search Tool (BLAST) searches within this database. The automatic BLAST search settings were used for this with the exception that the number of hits was increased to 1,000. A subset of the sequences obtained from UniProt were also used to perform a single protein-protein BLAST (BLASTP) search in the NCBI database. Results from these searches were subject to the criteria of ≥90% coverage and ≥40% identity.

Table A.1. Search terms used to gather protein sequences

Functional Gene	Search terms
diaA	"*diaA*"; "*diaA* NOT DnaA"; "diaphorase"; "high molecular weight rubredoxin"
nfsI	"*nfsI*"
pnrB	"*pnrB*"
xenA	"*xenA*"
xenB	"*xenB*"; "xenobiotic reductase B"
xplA	"*xplA*"

After sequences had been gathered from every database, they were dereplicated using the RDP's Clustering tool (32) and aligned using MAFFT v7.215 (46) through the Michigan State University High Performance Computing Center (HPCC). After dereplication, confirmation of these sequences was done based on common protein motifs and residues identified in literature (Table A.2). Jalview (47) was used to view the aligned sequences, mark the residues and motifs as sequence features, and remove sequences that did not match the identified features. At this stage for *diaA*, it was observed that most sequences had lengths less than 270 aa, another set of sequences had lengths greater than 400 aa and a few sequences had gaps between the two motifs. Therefore, two additional criteria were set for the *diaA* FunGene sequence set: 1) maximum length of 270 aa and 2) no gap between the motifs when aligned. The remaining sequences were used as the seed set, or training sequences, for the FunGene HMM model.

In FunGene, the HMM model pulled sequences from the NCBI non-redundant protein database. These sequences were first filtered by setting the minimum score to 90% of the max. The results were reviewed to ensure all of the seed set sequences were present and that the minimum length was only 90% of the max. The exception to this was *xplA*, which was determined to require the inclusion of partial sequences due to many reported sequences only covering one of the two domains present in this gene, so the minimum score was set at 200.

In most cases, a few of the seed set sequences were not present in the list from FunGene because they were from a different database. These sequences were manually added to the others, and all sequences were then vetted based on the previously identified residues and motifs. For *diaA*, the sequences remaining had a high diversity in both the organisms included and the aligned sequences. Therefore, this sequence set was restricted to only the *Clostridium* genus, because it is the only genus that has been linked to *diaA* to date. The final primer design sequence lists are reported in Tables A.3 - A.8.

Table A.2. Identified residues and motifs of functional genes. The positions refer to the reference noted in the "Gene" column.

Gene (reference)	Residue/Motif	Position	Reference	Residue/Motif	Position	Reference
diaA (16)	Y/F/W	180	(48)	W	213	(48)
	C-x-y-C-G-Y[1]	182-187	(48)	C-P-y-C-G-z[2]	215-220	(48)
	C	182	(16)	C	215	(16)
	C	185	(16)	C	218	(16)
	Y	189	(48)	K	222	(48)
	F	206	(48)	F	225	(48)
nfsI (49, 50)	R	10	(49)	F	124	(51, 52)
	K	14	(51-53)	Y	144	(51)
	S[PA]SSXNSQPW[HK]FIV	37-46	(49, 50)	L	145	(51)
	SPSS	37-40	(53)	GXGXXG[3]	153-158	(54)
	S	39	(49, 51, 53)	PIEGF	163-167	(49, 53)
	S	40	(51, 52)	P	163	(51, 53)
	T	41	(51)	I	164	(51)
	Y	68	(51)	E	165	(51)
	F	70	(51, 52)	G	166	(49, 51)
	N	71	(51)	G	192	(49)
	K	74	(51, 52)	HH[SP]XED[FY]NA[TGS]LPKSR	193-207	(49, 50)
	Y	123	(51)			
pnrB (55)	M	1	(55)	Q	117	(55)
	L	6	(55)	R	121	(55)
	R	10	(55)	R	122	(55)
	T	12	(55)	Y	124	(55)
	K	14	(55)	V	125	(55)
	A	15	(55)	H	128	(55)
	P	23	(55)	D	134	(55)
	Q	24	(55)	W	138	(55)
	S[4]	25	(55)	M	139	(55)
	L	30	(55)	Q	142	(55)
	L	31	(55)	YLALG	144-148	(55)
	L	34	(55)	L	151	(55)
	SPSSVNSQPWHF	37-48	(55)	GAA	153-155	(55)
	V	50	(55)	G	158	(55)
	A	51	(55)	DATP	160-163	(55)
pnrB (55)	E	55	(55)	EGFD	165-168	(55)
	A	58	(55)	D	173	(55)
	A	61	(55)	A	174	(55)
	R[4]	67	(55)	LGLRERG	176-182	(55)
	N	71	(55)	T	184	(55)

Table A.2. (cont'd)

Gene (reference)	**Residue/Motif**	**Position**	**Reference**	**Residue/Motif**	**Position**	**Reference**
pnrB (55)	ASHVI	78-82	(55)	S	185	(55)
	R	87	(55)	V	187	(55)
	M	90	(55)	SLG	190-192	(55)
	H	94	(55)	A[4]	195	(55)
	L	95	(55)	T[4]	197	(55)
	L	99	(55)	DFNA	198-201	(55)
	E	102	(55)	L	203	(55)
	DGRF	105-108	(55)	KSRL	205-208	(55)
	A	115	(55)	FTFL	214-217	(55)
	V[4]	116	(55)			
xenA (56)	PMC[MQ]Y[5]	23-27	(57)	R	231	(56, 58)
	M	24	(57, 58)	W	302	(58, 59)
	C	25	(57-62)	F	304	(56)
	Y	27	(57-59)	S	323	(56)
	A	57	(56)	G	325	(56)
	GRI[TS]X(4)GIW[5]	64-74	(57)	R	326	(56)
	Q	99	(56, 58)	A	330	(58)
	H	178	(56, 58, 60)	P	354	(58)
	H	181	(56, 58, 60)	H	357	(58)
	Y	183	(58, 59)	W	358	(58, 59, 61)
	[TS]NXRTDX YGGSX[DE]NR [SA]R[FL]LLE[5]	193-213	(56)			
xenB (56)	T	25	(56)	[TS]NXRTDX YGGSX[DE]NR [SA]R[FL]LLE[6]	188-208	(56)
	A	56	(56)	YGGS	195-198	(63)
	Q	98	(56)	H	225	(56)
	HGA	173-175	(63)	F	288	(56)
	H	173	(56)	A	306	(56)
	N	176	(56)	G	308	(56)
xplA[7] (64)	T	10	(64)	Y	97	(64)
	E	11	(64)	**P**	132	(64)
	T	12	(64)	**P**	136	(64)
	N	14	(64)	**V**	391	(64)
	STYGEG**E**LPA	57-66	(64)	**M**	394	(64, 65)
	Y	59	(64)	**A**	395	(64, 65)
	F	90	(64)	**Q**	438	(65)
	DS**T**Y**DT**YNN	94-102	(64)			

[1] Sieker et al. (48) reports the last amino acid as being maintained as Y by the rubredoxin sequences analyzed. However, the sequence reported by Chakraborty et al. (16) has F in this position so this residue was not used to eliminate sequences.

[2] Sieker et al. (48) reports the second amino acid as being an invariant P by the rubredoxin sequences analyzed. The sequence reported by Chakraborty et al. (16) agrees with this so this residue was used to eliminate sequences.

[3] Bryant et al. (54) reports that *nfsI* conserves this motif which is found among flavoproteins. However, Zenno et al. (50) notes that this motif is not conserved among other members of this protein family so this was not used to eliminate sequences.

[4] Amino acids identified by Kahng et al. (55) as unique to *pnrB*. These were not used to eliminate sequences from the FunGene or primer design sequence sets.

[5] Sequences for the FunGene sequence set were not eliminated based on positions in this motif that could be more than one amino acid, but these positions were used as inclusion criteria for the primer design sequence set.

[6] For determining the FunGene sequence set, this motif was kept strictly to TNQRTDNYGGSLENRARLLLE, which is the specific sequence reported for *xenB* rather than the general motif observed for the OYE family.

[7] For *xplA*, not all of the residues and motifs were used eliminate sequences. The ones indicated in bold were permitted to have some variability, otherwise sequences that had been identified in their respective databases as cytochrome p450 would have been removed.

Table A.3. FunGene and primer design sequences for *diaA*

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
ABC76945	CP000252	Syntrophus aciditrophicus SB	ferric-chelate reductase / Rubredoxin	236	X^2	Q2LS83 (UniProt)
ACV63492	CP001720	Desulfotomaculum acetoxidans DSM 771	flavin reductase domain protein FMN-binding	227	X^2	ACV63492 (NCBI)
ADG82833	CP002028	Thermincola potens JR	flavin reductase domain protein FMN-binding protein	234	X^2	ADG82833 (NCBI)
ADK15010	CP001666	Clostridium ljungdahlii DSM 13528	putative flavin reductase-like protein with rubredoxin domain	229		
AEA33387	CP002606	Hippea maritima DSM 10411	flavin reductase domain protein FMN-binding protein	234	X^2	F2LTY8 (UniProt)
AEG16589	CP002770	Desulfotomaculum kuznetsovii DSM 6115	flavin reductase domain protein FMN-binding protein	228	X^2	F6CLH1 (UniProt)
AGL02744	CP003273	Desulfotomaculum gibsoniae DSM 7213	conserved protein of DIM6/NTAB family	228	X^2	R4KJE5 (UniProt)
AGY74262	CP006763	Clostridium autoethanogenum DSM 10061	Rubredoxin domain containing protein	229		
AHF96662	CP007051	Desulfurella acetivorans A63	High molecular weight rubredoxin	223	X^2	WP_025391426 (NCBI)
AKI97034	CP011232	Kosmotoga pacifica	High molecular weight rubredoxin	229	X^2	WP_047754169 (NCBI)
AKN31180	CP011803	Clostridium carboxidivorans P7	High molecular weight rubredoxin	232	X	AKN31180 (NCBI)
ALU34453	CP012395	Clostridium autoethanogenum DSM 10061	Rubredoxin domain-containing protein	229		
APM40069	CP018335	Clostridium kluyveri	High molecular weight rubredoxin	229	X	WP_073539679 (NCBI)
BAH07534	AP009049	Clostridium kluyveri NBRC 12016	hypothetical protein	229		
CBX27933	FR695868	uncultured Desulfobacterium sp.	High molecular weight rubredoxin	230	X^2	CBX27933 (NCBI)
CEO88390	CDRZ01000090	Syntrophaceticus schinkii	High molecular weight rubredoxin	227	X^2	WP_044664557 (NCBI)

Table A.3. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
EDK34804	CP000673	Clostridium kluyveri DSM 555	Hrb	229	X	EDK34804 (NCBI)
EET85839	ACVI01000074	Clostridium carboxidivorans P7	flavin reductase domain protein FMN-binding	232		
GAG55455	BART01002025	marine sediment metagenome	Marine sediment metagenome DNA, contig: S01H4_C02025, whole genome shotgun sequence	213	X^2	X0YHV6 (UniProt)
GAG71827	BART01000399	marine sediment metagenome	Marine sediment metagenome DNA, contig: S01H4_C00399, whole genome shotgun sequence	233	X^2	X1AR62 (UniProt)
GAH02136	BART01022901	marine sediment metagenome	Marine sediment metagenome DNA, contig: S01H4_S07771, whole genome shotgun sequence	227	X^2	X1C206 (UniProt)
GAH71630	BARU01029844	marine sediment metagenome	Marine sediment metagenome DNA, contig: S03H2_S13408, whole genome shotgun sequence	223	X^2	X1IQV1 (UniProt)
GAI21343	BARV01015951	marine sediment metagenome	Marine sediment metagenome DNA, contig: S06H3_S01427, whole genome shotgun sequence	227	X^2	X1LQG4 (UniProt)
GAI31013	BARV01019462	marine sediment metagenome	Marine sediment metagenome DNA, contig: S06H3_S04938, whole genome shotgun sequence	218	X^2	X1MIC9 (UniProt)
KFI37218	JPEC01000073	Peptococcaceae bacterium SCADC1_2_3	High molecular weight rubredoxin	232	X^2	KFI37218 (NCBI)
KFO68279	JQDQ01000086	Smithella sp. SCADC	High molecular weight rubredoxin	229	X^2	KFO68279 (NCBI)
KJS03435	LADN01000009	Peptococcaceae bacterium BRH_c4a	High molecular weight rubredoxin	227	X^2	KJS03435 (NCBI)
KJS11230	LADP01000035	Peptococcaceae bacterium BRH_c8a	High molecular weight rubredoxin	224	X^2	KJS11230 (NCBI)
KJS76399	JUED01000041	Desulfotomaculum sp. BICA1-6	High molecular weight rubredoxin	224	X^2	KJS76399 (NCBI)
KNZ69687	LGTE01000010	Thermincola ferriacetica	flavin reductase domain-containing FMN-binding protein	239	X^2	KNZ69687 (NCBI)

Table A.3. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
KPJ49086	LIZT01000076	candidate division TA06 bacterium DG_26	High molecular weight rubredoxin	235	X^2	KPJ49086 (NCBI)
KPJ60922	LJNC01000006	Latescibacteria bacterium DG_63	High molecular weight rubredoxin	236	X^2	KPJ60922 (NCBI)
KPJ66142	LIZW01000040	Coxiella sp. DG_40	High molecular weight rubredoxin	236	X^2	KPJ66142 (NCBI)
KPL01198	LJUX01000004	candidate division Zixibacteria bacterium SM23_73_3	High molecular weight rubredoxin	231	X^2	KPL01198 (NCBI)
KPL09964	LJVA01000046	candidate division TA06 bacterium SM1_40	High molecular weight rubredoxin	242	X^2	KPL09964 (NCBI)
KPL14439	LJVE01000050	candidate division WOR_3 bacterium SM1_77	High molecular weight rubredoxin	232	X^2	KPL14439 (NCBI)
KPL19139	LJUY01000010	candidate division Zixibacteria bacterium SM23_81	High molecular weight rubredoxin	253	X^2	KPL19139 (NCBI)
KPL20732	LJUZ01000178	Anaerolineae bacterium SM23_84	High molecular weight rubredoxin	236	X^2	KPL20732 (NCBI)
KPU45454	LKET01000021	Oxobacter pfennigii	high molecular weight rubredoxin	230	X^2	WP_054873804 (NCBI)
KQC07238	LKUC01000307	Smithella sp. SDB	High molecular weight rubredoxin	229	X^2	KQC07238 (NCBI)
KUG22969	LNQE01000929	hydrocarbon metagenome	rubredoxin	229	X^2	A0A0W8FQ88 (UniProt)
KUK51921	LGFZ01000128	Desulfotomaculum sp. 46_296	Rubredoxin	229	X^2	KUK51921 (NCBI)
KUK87408	LGGX01000005	candidate division TA06 bacterium 34_109	flavin reductase-like, FMN-binding	233	X^2	A0A101I288 (UniProt)
KUO39544	LQMQ01000061	Hadesarchaea archaeon YNP_45	High molecular weight rubredoxin	228	X^2	A0A147JSV1 (UniProt)
KYK20996	LSSE01000053	Thermoplasmatales archaeon SG8-52-2	High molecular weight rubredoxin	230	X^2	KYK20996 (NCBI)
KYK23145	LSSI01000040	Thermoplasmatales archaeon SM1-50	High molecular weight rubredoxin	233	X^2	A0A151E3C7 (UniProt)

Table A.3. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
KYK24869	LSSF01000008	Thermoplasmatales archaeon SG8-52-4	High molecular weight rubredoxin	231	X^2	A0A151E8W7 (UniProt)
KYK27312	LSSD01000056	Thermoplasmatales archaeon SG8-52-1	High molecular weight rubredoxin	231	X^2	A0A151EFD3 (UniProt)
KYK33978	LSSG01000019	Thermoplasmatales archaeon SG8-52-3	High molecular weight rubredoxin	230	X^2	KYK33978 (NCBI)
KZL91355	LWAE01000003	Clostridium magnum DSM 2767	high molecular weight rubredoxin	246	X	KZL91355 (NCBI)
OAA21971	JGCK01000033	Kosmotoga sp. DU53	High molecular weight rubredoxin	229	X^2	WP_063727853 (NCBI)
OAA31561	JFHK01000003	Kosmotoga arenicorallina S304	high molecular weight rubredoxin	228	X^2	WP_068345860 (NCBI)
OAA87671	LITS01000008	Clostridium ljungdahlii DSM 13528	High molecular weight rubredoxin	229	X	OAA87671 (NCBI)
OAA90537	LITT01000011	Clostridium ljungdahlii	High molecular weight rubredoxin	229	X	WP_063554973 (NCBI)
OAA95145	LITQ01000001	Clostridium coskatii	High molecular weight rubredoxin	229		
OBR89701	LROS01000079	Clostridium ragsdalei P11	high molecular weight rubredoxin	229	X	WP_065079974 (NCBI)
OBR97507	LROR01000022	Clostridium coskatii	high molecular weight rubredoxin	229	X	OBR97507 (NCBI)
OFY91363	MEPH01000049	Bacteroidetes bacterium RIFOXYA2_FULL_33_7	High molecular weight rubredoxin	233	X^2	OFY91363 (NCBI)
OGC05759	METP01000034	candidate division WOR-1 bacterium RIFCSPLOWO2_02_FULL_46_20	High molecular weight rubredoxin	224	X^2	OGC05759 (NCBI)
OGD15969	MEYH01000043	Candidatus Atribacteria bacterium RBG_19FT_COMBO_35_14	High molecular weight rubredoxin	238	X^2	OGD15969 (NCBI)
OGN89297	MGML01000095	Chloroflexi bacterium RBG_13_46_14	High molecular weight rubredoxin	227	X^2	OGN89297 (NCBI)
OGN89489	MGMM01000043	Chloroflexi bacterium RBG_13_46_9	High molecular weight rubredoxin	228	X^2	OGN89489 (NCBI)

Table A.3. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
OGN93299	MGMP01000067	Chloroflexi bacterium RBG_13_50_10	High molecular weight rubredoxin	229	X^2	OGN93299 (NCBI)
OGN97618	MGMS01000010	Chloroflexi bacterium RBG_13_51_36	High molecular weight rubredoxin	230	X^2	OGN97618 (NCBI)
OGO02426	MGMW01000067	Chloroflexi bacterium RBG_13_53_26	High molecular weight rubredoxin	237	X^2	OGO02426 (NCBI)
OGO02536	MGMX01000156	Chloroflexi bacterium RBG_13_54_8	High molecular weight rubredoxin	229	X^2	OGO02536 (NCBI)
OGO60596	MGOH01000046	Chloroflexi bacterium RBG_19FT_COMBO_48_23	High molecular weight rubredoxin	229	X^2	OGO60596 (NCBI)
OGP54962	MGQF01000035	Deltaproteobacteria bacterium RBG_13_52_11	High molecular weight rubredoxin	230	X^2	OGP54962 (NCBI)
OGP85639	MGQS01000023	Deltaproteobacteria bacterium RBG_16_54_11	High molecular weight rubredoxin	231	X^2	OGP85639 (NCBI)
OGP89539	MGQZ01000066	Deltaproteobacteria bacterium RBG_19FT_COMBO_43_11	High molecular weight rubredoxin	231	X^2	OGP89539 (NCBI)
OGP91237	MGQZ01000015	Deltaproteobacteria bacterium RBG_19FT_COMBO_43_11	High molecular weight rubredoxin	231	X^2	OGP91237 (NCBI)
OGP94917	MGQT01000009	Deltaproteobacteria bacterium RBG_16_54_18	High molecular weight rubredoxin	234	X^2	OGP94917 (NCBI)
OGS20528	MGVC01000068	Elusimicrobia bacterium RIFOXYA2_FULL_39_19	High molecular weight rubredoxin	234	X^2	OGS20528 (NCBI)
OGS21815	MGVD01000012	Elusimicrobia bacterium RIFOXYA2_FULL_40_6	High molecular weight rubredoxin	236	X^2	OGS21815 (NCBI)
OGS24501	MGVO01000041	Elusimicrobia bacterium RIFOXYB2_FULL_50_12	High molecular weight rubredoxin	234	X^2	OGS24501 (NCBI)
OGS31001	MGVL01000010	Elusimicrobia bacterium RIFOXYB2_FULL_46_23	High molecular weight rubredoxin	228	X^2	OGS31001 (NCBI)
OGS34140	MGVN01000219	Elusimicrobia bacterium RIFOXYB2_FULL_49_7	High molecular weight rubredoxin	238	X^2	OGS34140 (NCBI)

Table A.3. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
OGS36449	MGVN01000083	Elusimicrobia bacterium RIFOXYB2_FULL_49_7	High molecular weight rubredoxin	238	X^2	OGS36449 (NCBI)
OGS40611	MGVV01000075	Euryarchaeota archaeon RBG_13_31_8	High molecular weight rubredoxin	234	X^2	A0A1F9YLW4 (UniProt)
OHB81972	MHYO01000240	Planctomycetes bacterium RBG_19FT_COMBO_48_8	High molecular weight rubredoxin	237	X^2	OHB81972 (NCBI)
OHD44682	MIAW01000022	Spirochaetes bacterium GWD1_27_9	High molecular weight rubredoxin	234	X^2	OHD44682 (NCBI)
OHE16697	MICJ01000099	Syntrophobacterales bacterium GWC2_56_13	High molecular weight rubredoxin	233	X^2	OHE16697 (NCBI)
OHE20549	MICK01000095	Syntrophobacterales bacterium GWF2_56_9	High molecular weight rubredoxin	233	X^2	OHE20549 (NCBI)
OIO01048	MNUP01000068	Candidatus Desantisbacteria bacterium CG1_02_49_89	High molecular weight rubredoxin	232	X^2	OIO01048 (NCBI)
OIO87023	MNXI01000071	Actinobacteria bacterium CG2_30_50_142	High molecular weight rubredoxin	233	X^2	OIO87023 (NCBI)
OIP25752	MNYE01000136	Dehalococcoidia bacterium CG2_30_46_9	High molecular weight rubredoxin	228	X^2	OIP25752 (NCBI)
OIP68335	MNYY01000124	Candidatus Atribacteria bacterium CG2_30_33_13	High molecular weight rubredoxin	236	X^2	OIP68335 (NCBI)
OIP93149	MNZQ01000033	Syntrophaceae bacterium CG2_30_58_14	High molecular weight rubredoxin	233	X^2	OIP93149 (NCBI)
OPL11334	LQBH01000070	delta proteobacterium MLS_D	High molecular weight rubredoxin	234	X^2	OPL11334 (NCBI)
OPL19420	LQBI01000006	Candidatus Aegiribacteria bacterium MLS_C	High molecular weight rubredoxin	235	X^2	OPL19420 (NCBI)
OPX18296	MUKB01000025	Candidatus Omnitrophica bacterium 4484_100	High molecular weight rubredoxin	233	X^2	OPX18296 (NCBI)
OPX28692	MVCX01000136	Gemmatimonadaceae bacterium 4484_173	High molecular weight rubredoxin	236	X^2	OPX28692 (NCBI)
SEM25682	FOBS01000008	Syntrophus gentianae	NADH-FMN oxidoreductase RutF, flavin reductase (DIM6/NTAB) family	239	X^2	SEM25682 (NCBI)

Table A.3. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size	FunGene Sequence Set	FunGene Sequence ID (Database)
SFH18630	FOOX01000019	Desulfotomaculum arcticum	NADH-FMN oxidoreductase RutF, flavin reductase (DIM6/NTAB) family	227	X[2]	SFH18630 (NCBI)
SHH39297	FQXL01000005	Clostridium magnum DSM 2767	NADH-FMN oxidoreductase RutF, flavin reductase (DIM6/NTAB) family	246		
WP_027718493[1]	NZ_AUBR01000039.1: c22180-21491	Desulfovirgula thermocuniculi	high molecular weight rubredoxin	229	X[2]	WP_027718493 (NCBI)
WP_028894048[1]	NZ_KI867150.1: 928490-929185	Syntrophorhabdus aromaticivorans	high molecular weight rubredoxin	231	X[2]	WP_028894048 (NCBI)
WP_031515655[1]	NZ_JONT01000011.1: c2640-1936	Desulfotomaculum alkaliphilum	high molecular weight rubredoxin	234	X[2]	WP_031515655 (NCBI)
WP_035588975[1]	NZ_JQLX01000014.1: 152368-153078	Hippea jasoniae	high molecular weight rubredoxin	236	X[2]	WP_035588975 (NCBI)
WP_041584747[1]	NC_007759.1: c1091004-1090300	Syntrophus aciditrophicus	high molecular weight rubredoxin	234	X[2]	WP_041584747 (NCBI)
WP_051965572[1]	NZ_JQKL01000008.1: 84143-84793	Clostridiales bacterium DRI-13	high molecular weight rubredoxin	216	X[2]	WP_051965572 (NCBI)
WP_052217953[1]	NZ_LGTE01000010.1: 87345-88067	Thermincola ferriacetica	high molecular weight rubredoxin	240	X[2]	WP_052217953 (NCBI)
WP_066625043[1]	NZ_FQXL01000005.1: c30610-29912	Clostridium magnum	high molecular weight rubredoxin	232	X	WP_066625043 (NCBI)

[1] These sequences were not on FunGene so were added back in to the primer design sequence set manually.

[2] These sequences were not included in the final primer design sequence set because they did not meet the stricter criteria used in the final review.

Table A.4. FunGene and primer design sequences for *nfsI*

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
AAA62801	M63808	Enterobacter cloacae	oxygen-insensitive NAD(P)H nitroreductase	217	X	Q01234 (UniProt)
ADF62693	CP001918	Enterobacter cloacae subsp. cloacae ATCC 13047	retro-nitroreductase	217		
AEN63793	CP003026	Enterobacter asburiae LF7a	nitroreductase	217	X	G2SB42 (UniProt)
AFM58872	CP003678	Enterobacter cloacae subsp. dissolvens SDM	dihydropteridine reductase	217	X	A0A0M1R1B2 (UniProt)
AFP69024	CP003737	Enterobacter cloacae subsp. cloacae ENHKU01	dihydropteridine reductase	217		
AHW95293	CP007546	Enterobacter asburiae L1	dihydropteridine reductase	217		
AIN21788	CP008897	Enterobacter cloacae ECNIH3	dihydropteridine reductase	217		
AIN27131	CP008905	Enterobacter cloacae ECR091	dihydropteridine reductase	217		
AIV28758	CP009756	Enterobacter cloacae	dihydropteridine reductase	217		
AIX55855	CP009850	Enterobacter cloacae	dihydropteridine reductase	217		
AIX58255	CP009854	Enterobacter cloacae	dihydropteridine reductase	217		
AJB61696	CP010376	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
AJB70122	CP010377	Enterobacter hormaechei subsp. hormaechei	dihydropteridine reductase	217		
AJB80828	CP010384	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
AKL01059	CP011591	Enterobacter asburiae	dihydropteridine reductase	217		
AKZ83319	CP012167	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
ALA01283	CP012165	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
ALL16580	CP012999	Enterobacter sp. E20	dihydropteridine reductase	217		
AMA02343	CP011863	Enterobacter asburiae	dihydropteridine reductase	217		
AMJ71713	CP014280	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217	X	A0A126PS74 (UniProt)
AMX06383	CP014993	Enterobacter asburiae	oxygen-insensitive NAD(P)H nitroreductase	217		
AMY66853	CP010512	Enterobacter cloacae	dihydropteridine reductase	217		
AMZ77222	CP015227	Enterobacter sp. ODB01	NAD(P)H nitroreductase	217		
AOE94628	CP016906	Enterobacter cloacae	NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AOL14985	CP017087	Enterobacter sp. HK169	Oxygen-insensitive NAD(P)H nitroreductase	217		
AOP77117	CP017179	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
AOP81553	CP017180	Enterobacter hormaechei subsp. oharae	NAD(P)H nitroreductase	217		
AOP85852	CP017181	Enterobacter kobei	NAD(P)H nitroreductase	217		
AOP90393	CP017183	Enterobacter xiangfangensis	NAD(P)H nitroreductase	217		
AOP94610	CP017184	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
AOP99046	CP017186	Enterobacter cloacae complex 'Hoffmann cluster III'	NAD(P)H nitroreductase	217		
APR42534	CP018814	Enterobacter cloacae	NAD(P)H nitroreductase	217		
AQT90310	CP019839	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
ARA26060	CP020053	Enterobacter cloacae	NAD(P)H nitroreductase	217		
ARZ77846	CP021749	Enterobacter cloacae	NAD(P)H nitroreductase	217		
ASA06611	CP021776	Enterobacter cloacae	NAD(P)H nitroreductase	217		
ASB74171	CP021896	Enterobacter cloacae	NAD(P)H nitroreductase	217		
ASD58149	CP017990	Enterobacter cloacae complex sp. ECNIH7	NAD(P)H nitroreductase	217		
ASG39592	CP022148	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
ASO98863	CP022532	Enterobacter cloacae	retro-nitroreductase	217		
ASQ16953	CP017475	Enterobacter cloacae	Oxygen-insensitive NAD(P)H nitroreductase	217		
ASQ75974	CP019889	Enterobacter cloacae	NAD(P)H nitroreductase	217		
ATW94206	CP024812	Enterobacter sp. CRENT-193	oxygen-insensitive NAD(P)H nitroreductase	217		
AUJ82597	CP025225	Enterobacter cancerogenus	oxygen-insensitive NAD(P)H nitroreductase	217		
AUM02628	CP020817	Enterobacter sp. Crenshaw	NAD(P)H-dependent oxidoreductase	217		
CBK85782	FP929040	Enterobacter cloacae subsp. cloacae NCTC 9394	dihydropteridine reductase	217		
CQR78470	CTRQ01000008	Enterobacter cloacae	Oxygen-insensitive NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
CUJ19923	CYTA01000035	Achromobacter sp. ATCC13047	Oxygen-insensitive NAD(P)H nitroreductase	217		
CZU19544	FJXF01000001	Enterobacter cloacae	nitroreductase	217		
CZU20024	FJXK01000001	Enterobacter cloacae	nitroreductase	217		
CZU23511	FJXT01000001	Enterobacter cloacae	nitroreductase	217		
CZU24251	FJWG01000001	Enterobacter cloacae	nitroreductase	217		
CZU26231	FJXH01000001	Enterobacter cloacae	nitroreductase	217		
CZU28052	FJWM01000001	Enterobacter cloacae	nitroreductase	217		
CZU33565	FJWK01000001	Enterobacter cloacae	nitroreductase	217		
CZU35296	FJXQ01000001	Enterobacter cloacae	nitroreductase	217		
CZU37660	FJXE01000001	Enterobacter cloacae	nitroreductase	217		
CZU38649	FJWX01000001	Enterobacter cloacae	nitroreductase	217		
CZU41411	FJXL01000001	Enterobacter cloacae	nitroreductase	217		
CZU48432	FJWW01000001	Enterobacter cloacae	nitroreductase	217		
CZU50099	FJXV01000001	Enterobacter cloacae	nitroreductase	217		
CZU50140	FJXN01000002	Enterobacter cloacae	nitroreductase	217		
CZU50741	FJWV01000001	Enterobacter cloacae	nitroreductase	217		
CZU54269	FJWN01000005	Enterobacter cloacae	nitroreductase	217		
CZU57345	FJXP01000001	Enterobacter cloacae	nitroreductase	217		
CZU60949	FJWQ01000004	Enterobacter cloacae	nitroreductase	217		
CZU61229	FJXC01000002	Enterobacter cloacae	nitroreductase	217		
CZU65748	FJWJ01000001	Enterobacter cloacae	nitroreductase	217		
CZU66880	FJWT01000003	Enterobacter cloacae	nitroreductase	217		
CZU68610	FJWY01000004	Enterobacter cloacae	nitroreductase	217		
CZU74858	FJWR01000006	Enterobacter cloacae	nitroreductase	217		
CZU76805	FJWS01000002	Enterobacter cloacae	nitroreductase	217		
CZU83876	FJWO01000002	Enterobacter cloacae	nitroreductase	217		
CZU84775	FJWC01000005	Enterobacter cloacae	nitroreductase	217		
CZU86851	FJWP01000002	Enterobacter cloacae	nitroreductase	217		
CZU87995	FJXA01000007	Enterobacter cloacae	nitroreductase	217		
CZU94381	FJWI01000007	Enterobacter cloacae	nitroreductase	217		
CZV12637	FJWF01000004	Enterobacter cloacae	nitroreductase	217		
CZV18957	FJXB01000006	Enterobacter cloacae	nitroreductase	217		
CZV35823	FJXJ01000020	Enterobacter cloacae	nitroreductase	217		
CZV39330	FJXO01000009	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
CZV40446	FJXU01000001	Enterobacter cloacae	nitroreductase	217		
CZV58614	FJXM01000015	Enterobacter cloacae	nitroreductase	217		
CZV80438	FJWL01000019	Enterobacter cloacae	nitroreductase	217		
CZV87735	FJXD01000009	Enterobacter cloacae	nitroreductase	217		
CZV88602	FJXI01000007	Enterobacter cloacae	nitroreductase	217		
CZV93255	FJXY01000002	Enterobacter cloacae	nitroreductase	217		
CZV99032	FJYF01000001	Enterobacter cloacae	nitroreductase	217		
CZW04811	FJXR01000027	Enterobacter cloacae	nitroreductase	217	X	A0A157GLI8 (UniProt)
CZW07158	FJYC01000004	Enterobacter cloacae	nitroreductase	217		
CZW17832	FJYB01000001	Enterobacter cloacae	nitroreductase	217	X	A0A0P8J201 (UniProt)
CZW26425	FJYM01000001	Enterobacter cloacae	nitroreductase	217		
CZW28558	FJYJ01000002	Enterobacter cloacae	nitroreductase	217		
CZW29068	FJYT01000001	Enterobacter cloacae	nitroreductase	217		
CZW29237	FJYH01000002	Enterobacter cloacae	nitroreductase	217		
CZW32992	FJYN01000001	Enterobacter cloacae	nitroreductase	217		
CZW36114	FJXS01000032	Enterobacter cloacae	nitroreductase	217		
CZW50797	FJYE01000005	Enterobacter cloacae	nitroreductase	217		
CZW62010	FJXZ01000014	Enterobacter cloacae	nitroreductase	217		
CZW65348	FJYY01000001	Enterobacter cloacae	nitroreductase	217		
CZW72685	FJYK01000005	Enterobacter cloacae	nitroreductase	217		
CZW74276	FJYU01000001	Enterobacter cloacae	nitroreductase	217		
CZW74715	FJYA01000007	Enterobacter cloacae	nitroreductase	217		
CZW78701	FJZB01000001	Enterobacter cloacae	nitroreductase	217		
CZW80294	FJZC01000001	Enterobacter cloacae	nitroreductase	217		
CZW80321	FJYQ01000004	Enterobacter cloacae	nitroreductase	217		
CZW83490	FJYS01000002	Enterobacter cloacae	nitroreductase	217		
CZW86349	FJYV01000002	Enterobacter cloacae	nitroreductase	217		
CZW89600	FJYR01000001	Enterobacter cloacae	nitroreductase	217		
CZW91698	FJYW01000002	Enterobacter cloacae	nitroreductase	217		
CZW95394	FJYG01000005	Enterobacter cloacae	nitroreductase	217		
CZW96222	FJZH01000001	Enterobacter cloacae	nitroreductase	217		
CZX00489	FJZD01000001	Enterobacter cloacae	nitroreductase	217		
CZX05303	FJXX01000014	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont’d)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
CZX11286	FJZG01000001	Enterobacter cloacae	nitroreductase	217		
CZX33936	FJZI01000002	Enterobacter cloacae	nitroreductase	217		
CZX34071	FJZK01000002	Enterobacter cloacae	nitroreductase	217		
CZX39460	FJYL01000007	Enterobacter cloacae	nitroreductase	217		
CZX48956	FJZJ01000002	Enterobacter cloacae	nitroreductase	217		
CZX51289	FJZF01000006	Enterobacter cloacae	nitroreductase	217		
CZX54413	FJZM01000004	Enterobacter cloacae	nitroreductase	217		
CZX68213	FJYX01000022	Enterobacter cloacae	nitroreductase	217		
CZX70772	FJZP01000006	Enterobacter cloacae	nitroreductase	217		
CZX71404	FJZE01000006	Enterobacter cloacae	nitroreductase	217		
CZX71419	FJYZ01000030	Enterobacter cloacae	nitroreductase	217		
CZX82876	FJZR01000007	Enterobacter cloacae	nitroreductase	217		
CZX90662	FJZT01000002	Enterobacter cloacae	nitroreductase	217		
CZY00734	FKAA01000001	Enterobacter cloacae	nitroreductase	217		
CZY03920	FJZZ01000001	Enterobacter cloacae	nitroreductase	217		
CZY15676	FJZQ01000005	Enterobacter cloacae	nitroreductase	217		
CZY20210	FJZX01000002	Enterobacter cloacae	nitroreductase	217		
CZY26570	FJZN01000007	Enterobacter cloacae	nitroreductase	217		
CZY37120	FJZO01000010	Enterobacter cloacae	nitroreductase	217		
CZY38711	FKAG01000001	Enterobacter cloacae	nitroreductase	217		
CZY39159	FJZY01000002	Enterobacter cloacae	nitroreductase	217		
CZY40875	FKAB01000002	Enterobacter cloacae	nitroreductase	217		
CZY56648	FKAC01000001	Enterobacter cloacae	nitroreductase	217		
CZY58696	FKAD01000002	Enterobacter cloacae	nitroreductase	217		
CZY59519	FKAM01000001	Enterobacter cloacae	nitroreductase	217		
CZY61426	FKAE01000003	Enterobacter cloacae	nitroreductase	217		
CZY66124	FJZL01000009	Enterobacter cloacae	nitroreductase	217		
CZY72955	FJZS01000005	Enterobacter cloacae	nitroreductase	217		
CZY74135	FKAR01000001	Enterobacter cloacae	nitroreductase	217		
CZY78281	FKAU01000001	Enterobacter cloacae	nitroreductase	217		
CZY85360	FKAP01000002	Enterobacter cloacae	nitroreductase	217		
CZY85613	FKAW01000001	Enterobacter cloacae	nitroreductase	217		
CZY94616	FKAZ01000001	Enterobacter cloacae	nitroreductase	217		
CZZ00442	FKAF01000009	Enterobacter cloacae	nitroreductase	217		
CZZ04384	FKAN01000002	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
CZZ05290	FKBC01000001	Enterobacter cloacae	nitroreductase	217		
CZZ10435	FKBA01000001	Enterobacter cloacae	nitroreductase	217		
CZZ10793	FKAV01000002	Enterobacter cloacae	nitroreductase	217		
CZZ15592	FKBD01000001	Enterobacter cloacae	nitroreductase	217		
CZZ17546	FKBE01000001	Enterobacter cloacae	nitroreductase	217		
CZZ19399	FKAT01000002	Enterobacter cloacae	nitroreductase	217		
CZZ35379	FKBH01000001	Enterobacter cloacae	nitroreductase	217		
CZZ47430	FKAX01000011	Enterobacter cloacae	nitroreductase	217		
CZZ49686	FKBF01000003	Enterobacter cloacae	nitroreductase	217		
CZZ67135	FKAJ01000005	Enterobacter cloacae	nitroreductase	217		
CZZ75660	FKBL01000001	Enterobacter cloacae	nitroreductase	217		
CZZ80303	FKAQ01000006	Enterobacter cloacae	nitroreductase	217		
CZZ87099	FKAS01000007	Enterobacter cloacae	nitroreductase	217		
CZZ90710	FKAH01000010	[Enterobacter] aerogenes	nitroreductase	217		
EFC55379	ABWM02000020	Enterobacter cancerogenus ATCC 35316	oxygen-insensitive NAD(P)H nitroreductase	217	X	D2ZGD5 (UniProt)
EGK62528	AFHR01000020	Enterobacter hormaechei ATCC 49162	oxygen-insensitive NAD(P)H nitroreductase	217	X	F5RUG0 (UniProt)
EIM37332	AJXP01000003	Enterobacter cloacae subsp. cloacae GS1	dihydropteridine reductase	217		
EJO48778	ALNS01000001	Enterobacter sp. SST3	Oxygen-insensitive NAD(P)H nitroreductase	217		
EPY96152	ATHX01000006	Enterobacter cloacae EC_38VIM1	dihydropteridine reductase	217		
ERP02085	AXLJ01000001	Enterobacter sp. MGH 8	oxygen-insensitive NAD(P)H nitroreductase	217		
ESL78320	AYIE01000007	Enterobacter cloacae UCICRE 11	oxygen-insensitive NAD(P)H nitroreductase	217		
ESL83262	AYID01000001	Enterobacter cloacae UCICRE 12	oxygen-insensitive NAD(P)H nitroreductase	217	X	V3E2X6 (UniProt)
ESL91992	AYIG01000007	Enterobacter cloacae UCICRE 9	oxygen-insensitive NAD(P)H nitroreductase	217		
ESM19499	AYIK01000004	Enterobacter cloacae UCICRE 5	oxygen-insensitive NAD(P)H nitroreductase	217		
ESM22487	AYIM01000001	Enterobacter cloacae UCICRE 3	oxygen-insensitive NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
ESM32927	AYIP01000009	Enterobacter cloacae BWH 31	oxygen-insensitive NAD(P)H nitroreductase	217		
ESM44307	AYIR01000011	Enterobacter cloacae BWH 29	oxygen-insensitive NAD(P)H nitroreductase	217		
ESM79363	AYIY01000008	Enterobacter sp. MGH 38	oxygen-insensitive NAD(P)H nitroreductase	217		
ESM83780	AYJA01000005	Enterobacter cloacae 'Hoffmann cluster IV' MGH 34	oxygen-insensitive NAD(P)H nitroreductase	217		
ESN11078	AYJE01000005	Enterobacter sp. MGH 26	oxygen-insensitive NAD(P)H nitroreductase	217		
ESN17841	AYJF01000018	Enterobacter sp. MGH 25	oxygen-insensitive NAD(P)H nitroreductase	217		
ESN26004	AYJH01000005	Enterobacter sp. MGH 23	oxygen-insensitive NAD(P)H nitroreductase	217		
ESN28155	AYJI01000001	Enterobacter sp. MGH 22	oxygen-insensitive NAD(P)H nitroreductase	217		
ESN53371	AYJO01000001	Enterobacter sp. MGH 16	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL38804	JCKK01000002	Enterobacter cloacae UCI 50	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL64446	JCKQ01000001	Enterobacter cloacae UCI 39	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL67820	JCKR01000005	Enterobacter cloacae UCI 36	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL73477	JCKS01000001	Enterobacter cloacae UCI 35	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL78189	JCKT01000002	Enterobacter cloacae UCI 30	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL88446	JCKW01000004	Enterobacter cloacae UCI 24	oxygen-insensitive NAD(P)H nitroreductase	217		
EUL91820	JCKX01000009	Enterobacter cloacae UCI 23	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM08906	JCLB01000007	Enterobacter sp. BIDMC 29	oxygen-insensitive NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
EUM15526	JCLC01000009	Enterobacter sp. BIDMC 28	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM21943	JCLD01000002	Enterobacter sp. BIDMC 27	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM34474	JCLG01000014	Enterobacter sp. BWH 39	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM49863	JCLI01000001	Enterobacter sp. MGH 37	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM53867	JCLJ01000002	Enterobacter sp. MGH 33	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM57100	JCLK01000001	Enterobacter sp. MGH 15	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM63475	JCLM01000004	Enterobacter sp. MGH 12	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM65749	JCLN01000008	Enterobacter sp. MGH 11	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM75320	JCLP01000007	Enterobacter sp. MGH 9	oxygen-insensitive NAD(P)H nitroreductase	217		
EUM82347	JCLQ01000006	Enterobacter sp. MGH 7	oxygen-insensitive NAD(P)H nitroreductase	216		
EUM94544	JCLS01000005	Enterobacter sp. MGH 5	oxygen-insensitive NAD(P)H nitroreductase	217		
EUN03671	JCLV01000014	Enterobacter sp. MGH 2	oxygen-insensitive NAD(P)H nitroreductase	217		
EWG65056	AZUA01000013	Enterobacter sp. DC1	Oxygen-insensitive NAD(P)H nitroreductase	217		
EWG72594	AZUB01000007	Enterobacter sp. DC4	Oxygen-insensitive NAD(P)H nitroreductase	217	X	W7P1G3 (UniProt)
EWG78545	AZXZ01000004	Enterobacter sp. DC3	Oxygen-insensitive NAD(P)H nitroreductase	217		
EZR15844	JJNL01000005	Enterobacter sp. BWH 27	oxygen-insensitive NAD(P)H nitroreductase	217		
KDF43196	JMUQ01000011	Enterobacter cloacae BIDMC 67	oxygen-insensitive NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KDF50040	JMUR01000002	Enterobacter cloacae BWH 43	oxygen-insensitive NAD(P)H nitroreductase	217		
KDF51086	JMUS01000021	Enterobacter cloacae CHS 79	oxygen-insensitive NAD(P)H nitroreductase	217		
KDF59885	JMUT01000007	Enterobacter cloacae MGH 53	oxygen-insensitive NAD(P)H nitroreductase	217		
KDF60961	JMUU01000004	Enterobacter cloacae MGH 54	oxygen-insensitive NAD(P)H nitroreductase	217		
KDF77739	JMUV01000001	Enterobacter cloacae UCI 29	oxygen-insensitive NAD(P)H nitroreductase	217		
KDM55519	JMZV01000009	Lelliottia amnigena CHS 78	oxygen-insensitive NAD(P)H nitroreductase	217		
KGB03739	JPPR01000041	Enterobacter cloacae	oxygen-insensitive NAD(P)H nitroreductase	217		
KGI65104	JRJC01000008	Enterobacter sp. UCD-UG_FMILLET	dihydropteridine reductase	217		
KGY63834	JTBF01000001	Enterobacter cloacae	dihydropteridine reductase	217		
KGZ08118	JTBO01000001	Enterobacter cloacae	dihydropteridine reductase	217		
KHG49224	JTEP01000015	Enterobacter hormaechei subsp. oharae	oxygen-insensitive NAD(P)H nitroreductase	217		
KHK40536	JRUR01000037	Enterobacter sp. E20	dihydropteridine reductase	217		
KHM15979	JUHY01000016	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KHO34010	JSZC01000013	Enterobacter sp. FB	dihydropteridine reductase	217		
KHQ43105	JWRQ01000001	Enterobacter cloacae	dihydropteridine reductase	217		
KHQ47705	JWRR01000001	Enterobacter cloacae	dihydropteridine reductase	217		
KIF96196	JXAE01000012	Enterobacter cloacae	dihydropteridine reductase	217		
KJC02823	JYGA02000001	Enterobacter cloacae	dihydropteridine reductase	217	X	A0A0J0GYZ6 (UniProt)
KJF33504	JZKC01000002	Enterobacter cloacae BIDMC 33A	oxygen-insensitive NAD(P)H nitroreductase	217		
KJI52102	JYME01000018	Enterobacter cloacae	dihydropteridine reductase	217		
KJI63937	JYMG01000012	Enterobacter cloacae	dihydropteridine reductase	217		
KJI67088	JYMF01000003	Enterobacter cloacae	dihydropteridine reductase	217		
KJI77864	JYMJ01000026	Enterobacter cloacae	dihydropteridine reductase	217		
KJI80093	JYMM01000070	Enterobacter cloacae	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KJL53990	JZXS01000010	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJL58524	JZXR01000012	Enterobacter kobei	dihydropteridine reductase	217		
KJL62788	JZXQ01000004	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJL65872	JZXV01000014	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJL71098	JZXT01000012	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJL82019	JZXW01000022	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJL91614	JZXX01000006	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJL96458	JZYB01000016	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJM25698	JZYE01000004	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJM31332	JZYF01000019	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJM39857	JZYG01000008	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217	X	A0A0F0TWZ9 (UniProt)
KJM40163	JZYH01000001	Enterobacter kobei	dihydropteridine reductase	217		
KJM53393	JZYK01000004	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJM60000	JZYM01000008	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJM69539	JZYN01000005	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	215		
KJM73897	JZYP01000010	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJM83489	JZYQ01000002	Enterobacter hormaechei	dihydropteridine reductase	217		
KJM84692	JZYS01000051	Enterobacter kobei	dihydropteridine reductase	217		
KJM96715	JZYT01000006	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN06334	JZYW01000015	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN09901	JZYU01000001	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJN15129	JZYY01000015	Enterobacter hormaechei	dihydropteridine reductase	217		
KJN22542	JZYZ01000004	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN24874	JZYX01000036	Enterobacter cloacae complex sp. 35699	dihydropteridine reductase	217	X	A0A0F1AUY8 (UniProt)
KJN28736	JZZA01000011	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KJN33593	JZZB01000014	Enterobacter cloacae complex sp. 35669	dihydropteridine reductase	217		
KJN40016	JZZC01000012	Enterobacter hormaechei subsp. hormaechei	dihydropteridine reductase	217		
KJN48312	JZZD01000001	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN63831	JZZJ01000015	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN64553	JZZH01000008	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJN78680	JZZK01000012	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	215		
KJN82211	JZZL01000003	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJN90460	JZZM01000002	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJN94148	JZZO01000007	Enterobacter hormaechei	dihydropteridine reductase	217		
KJN96050	JZZN01000005	Enterobacter hormaechei	dihydropteridine reductase	217		
KJO04448	JZZP01000007	Enterobacter hormaechei subsp. hormaechei	dihydropteridine reductase	217		
KJO07497	JZZQ01000006	Enterobacter hormaechei	dihydropteridine reductase	217		
KJO18773	JZZT01000007	Enterobacter hormaechei	dihydropteridine reductase	217		
KJO22169	JZZU01000006	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJO22423	JZZS01000004	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO28651	JZZW01000013	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJO56047	LAAE01000079	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJO61267	LAAD01000004	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJO70875	LAAH01000091	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO72059	LAAG01000015	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO74471	LAAF01000011	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO82549	LAAJ01000019	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO95282	LAAK01000013	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO97513	LAAL01000028	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJO99505	LAAN01000046	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KJP17220	LAAP01000023	Enterobacter asburiae	dihydropteridine reductase	217		
KJP20970	LAAQ01000012	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP25065	LAAS01000087	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP28956	LAAR01000009	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP37881	LAAT01000003	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP47639	LAAW01000021	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP50767	LAAV01000011	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP54695	LAAY01000039	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJP68624	LAAZ01000006	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP71642	LABB01000027	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJP72531	LABA01000005	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP80366	LABC01000008	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJP95304	JZCX01000045	Enterobacter cloacae	dihydropteridine reductase	217		
KJQ14588	JZDA01000017	Enterobacter cloacae	dihydropteridine reductase	217		
KJQ40098	JZDF01000006	Enterobacter cloacae	dihydropteridine reductase	217		
KJW76773	JZKP01000096	Enterobacter asburiae	dihydropteridine reductase	217		
KJW92826	JZKN01000015	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJX00074	JZKO01000105	Enterobacter asburiae	dihydropteridine reductase	217	X	A0A0F3WPM3 (UniProt)
KJX08594	JZKU01000031	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KJX15256	JZLE01000091	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJX20650	JZKM01000005	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KJX34743	JZKY01000001	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KJX47824	JZLB01000004	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KKA58171	JYMH01000005	Enterobacter cloacae	dihydropteridine reductase	217		
KKJ29292	LBLX01000021	Enterobacter hormaechei	dihydropteridine reductase	217		
KKY76395	JSWY01000043	Enterobacter cloacae	dihydropteridine reductase	217		
KLF79275	LDCC01000090	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLF80514	LDCD01000036	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KLF88527	LDCE01000036	Enterobacter asburiae	dihydropteridine reductase	217		
KLF93227	LDCB01000006	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLF95206	LDCF01000038	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLG05662	LDCG01000016	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KLG07420	LDCI01000028	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KLG12786	LDCH01000009	Enterobacter asburiae	dihydropteridine reductase	217		
KLG16574	LDCL01000065	Enterobacter kobei	dihydropteridine reductase	217		
KLG23926	LDCJ01000025	Enterobacter kobei	dihydropteridine reductase	217		
KLP27586	LEEQ01000011	Enterobacter kobei	dihydropteridine reductase	217		
KLP43733	LECX01000031	Enterobacter asburiae	dihydropteridine reductase	217		
KLP47377	LECY01000030	Enterobacter kobei	dihydropteridine reductase	217		
KLP59854	LEDA01000066	Enterobacter asburiae	dihydropteridine reductase	217		
KLP62170	LEDB01000033	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLP75032	LEDE01000031	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLP75103	LEDC01000017	Enterobacter kobei	dihydropteridine reductase	217		
KLP81665	LEDD01000008	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLP83346	LEDG01000028	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLP86873	LEDH01000068	Enterobacter asburiae	dihydropteridine reductase	217		
KLP92362	LEDF01000028	Enterobacter hormaechei	dihydropteridine reductase	217		
KLP99732	LEDK01000036	Enterobacter hormaechei subsp. oharae	dihydropteridine reductase	217		
KLQ16646	LEDL01000009	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KLQ26057	LEDP01000064	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ27743	LEDO01000027	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ30737	LEDQ01000030	Enterobacter cloacae complex sp. GN02548	dihydropteridine reductase	217		
KLQ38423	LEDR01000024	Enterobacter cloacae subsp. dissolvens	dihydropteridine reductase	217		
KLQ46072	LEDS01000040	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KLQ48645	LEDU01000057	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ67113	LEDY01000059	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ72829	LEDZ01000057	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ76728	LEEA01000031	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ85237	LEEB01000032	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ90636	LEEC01000026	Enterobacter kobei	dihydropteridine reductase	217		
KLQ96663	LEEF01000057	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLQ97280	LEEE01000022	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLR02651	LEEG01000062	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLR07252	LEEH01000061	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLR11692	LEEI01000036	Enterobacter hormaechei subsp. hormaechei	dihydropteridine reductase	217		
KLR21134	LEEK01000108	Enterobacter kobei	dihydropteridine reductase	217		
KLR25454	LEED01000008	Enterobacter hormaechei	dihydropteridine reductase	217		
KLR26621	LEEJ01000006	Enterobacter cloacae complex sp. GN02283	dihydropteridine reductase	217		
KLR28966	LEEM01000053	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLR30325	LEEL01000045	Enterobacter kobei	dihydropteridine reductase	217		
KLR40091	LEEN01000024	Enterobacter hormaechei subsp. steigerwaltii	dihydropteridine reductase	217		
KLW07972	LESZ01000004	Enterobacter cloacae	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW13711	LETA01000002	Enterobacter cloacae	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW18492	LETB01000003	Enterobacter sp. BWH52	oxygen-insensitive NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KLW26047	LETD01000004	Enterobacter sp. BWH64	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW32531	LETE01000008	Enterobacter sp. MGH85	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW40786	LETG01000003	Enterobacter sp. MGH119	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW41347	LETH01000009	Enterobacter sp. MGH120	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW50123	LETF01000005	Enterobacter sp. MGH86	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW54414	LETI01000007	Enterobacter sp. MGH127	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW57527	LETJ01000002	Enterobacter sp. MGH128	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW59202	LETL01000008	Enterobacter sp. BIDMC93	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW73177	LETN01000002	Enterobacter sp. BIDMC99	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW81937	LETO01000020	Enterobacter sp. BIDMC100	oxygen-insensitive NAD(P)H nitroreductase	217		
KLW90890	LETR01000001	Enterobacter sp. BIDMC92	oxygen-insensitive NAD(P)H nitroreductase	217	X	A0A0J1VWE2 (UniProt)
KML25527	LDWM01000001	Leclercia adecarboxylata	dihydropteridine reductase	217		
KMN67227	LDUO01000002	Leclercia sp. LK8	dihydropteridine reductase	217		
KOQ80652	LDVU01000068	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KOQ87409	LDVV01000054	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KOQ93714	LDVX01000013	Enterobacter asburiae	dihydropteridine reductase	217		
KPR21094	LJDV01000008	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KPU04692	LJEZ01000041	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KRS24540	LLXN01000111	Enterobacter cloacae	dihydropteridine reductase	217		
KSX59031	LNHM01000049	Enterobacter sp. 50588862	NAD(P)H-dependent oxidoreductase	217	X	A0A0V9DV66 (UniProt)
KSZ07484	LNIO01000058	Enterobacter sp. 50858885	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KTG80664	LPOA01000048	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTG84608	LPOC01000044	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTG86594	LPOB01000070	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTG91605	LPOD01000058	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTG99646	LPOE01000034	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH08142	LPOF01000034	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH09403	LPOG01000025	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH14315	LPOH01000027	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KTH18700	LPOK01000079	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTH20759	LPOI01000057	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH27214	LPOJ01000059	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTH43167	LPON01000023	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH48191	LPOP01000040	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTH52258	LPOO01000023	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH57455	LPOQ01000078	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTH74797	LPOT01000013	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTH87422	LPOV01000007	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KTH90299	LPOW01000033	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTH92960	LPOY01000068	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTI10466	LPPA01000028	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTI39611	LPPI01000062	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTI42379	LPPH01000030	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTI47583	LPPJ01000034	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTI52549	LPPK01000082	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTI60960	LPPL01000071	Enterobacter kobei	NAD(P)H-dependent oxidoreductase	217		
KTI63472	LPPN01000079	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTI63977	LPPM01000046	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTI95150	LPPS01000046	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTI96293	LPPR01000005	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KTJ14364	LPPX01000022	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTJ20738	LPPY01000061	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTJ24913	LPQA01000034	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTJ42059	LPQC01000035	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTJ70678	LPQK01000055	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KTJ82381	LPQI01000024	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KTJ85353	LPQL01000045	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTJ86452	LPQM01000034	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTJ86847	LPQN01000085	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTJ96976	LPQO01000045	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KTK06233	LPQQ01000045	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTK10694	LPQS01000045	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KTK11953	LPQR01000034	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KTK24322	LPQU01000045	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217	X	A0A0W1YUK5 (UniProt)
KTK29608	LPQW01000086	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KTK35666	LPQV01000028	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KTQ45547	LDQG01000023	Enterobacter cancerogenus	dihydropteridine reductase	217		
KTQ52338	LDQD01000040	Enterobacter asburiae	dihydropteridine reductase	217		
KTQ53424	LDQH01000007	Enterobacter cancerogenus	dihydropteridine reductase	217		
KTQ63824	LDQM01000003	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTQ66510	LDQE01000004	Enterobacter asburiae	dihydropteridine reductase	217		
KTQ71393	LDQK01000005	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTQ74078	LDQI01000007	Enterobacter cancerogenus	dihydropteridine reductase	217		
KTQ79589	LDQF01000011	Enterobacter asburiae	dihydropteridine reductase	217		
KTQ83662	LDQJ01000006	Enterobacter cancerogenus	dihydropteridine reductase	217		
KTQ93775	LDQO01000001	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTQ94732	LDQQ01000001	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR00900	LDQS01000004	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR13789	LDQP01000003	Enterobacter xiangfangensis	dihydropteridine reductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KTR16152	LDQT01000012	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR23491	LDQR01000005	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR29804	LDQL01000036	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR33302	LDQN01000023	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KTR45762	LDQU01000004	Enterobacter xiangfangensis	dihydropteridine reductase	217		
KUH52752	LKUI01000012	Enterobacter cloacae subsp. cloacae	dihydropteridine reductase	217		
KUQ00846	LRBY01000036	Enterobacter kobei	NAD(P)H-dependent oxidoreductase	217		
KUQ05118	LRBZ01000098	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUQ21814	LRCD01000196	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KUQ25915	LRCE01000130	Enterobacter asburiae	NAD(P)H-dependent oxidoreductase	217		
KUQ34672	LRCF01000046	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUQ35170	LRCH01000146	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUQ49934	LRCJ01000039	Enterobacter asburiae	NAD(P)H-dependent oxidoreductase	217		
KUQ63953	LRCM01000021	Enterobacter kobei	NAD(P)H-dependent oxidoreductase	217		
KUQ66043	LRCK01000024	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUQ77117	LRCO01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KUQ77282	LRCP01000012	Enterobacter kobei	NAD(P)H-dependent oxidoreductase	217		
KUQ82043	LRCN01000031	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KUQ87332	LRCQ01000042	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KUQ91727	LRCT01000115	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KUQ96880	LRCS01000043	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUR08289	LRCU01000001	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KUR30674	LRCZ01000028	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVI51697	LRIO01000127	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KVI62307	LRIR01000050	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVI67583	LRIS01000034	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVI94875	LRIY01000034	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KVJ04049	LRIZ01000023	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ05337	LRJA01000101	Enterobacter asburiae	NAD(P)H-dependent oxidoreductase	217		
KVJ12397	LRJB01000012	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ18342	LRJC01000012	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ24582	LRJD01000012	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ30752	LRJE01000012	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ36558	LRJH01000034	Enterobacter cloacae subsp. cloacae	NAD(P)H-dependent oxidoreductase	217		
KVJ37673	LRJF01000001	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ52360	LRJI01000001	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ68336	LRJM01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KVJ71757	LRJL01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVJ72350	LRJP01000086	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVJ75509	LRJN01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVJ78395	LRJO01000006	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVJ82232	LRJQ01000052	Enterobacter asburiae	NAD(P)H-dependent oxidoreductase	217		
KVJ94409	LRJT01000045	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK03536	LRJS01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK05669	LRJU01000028	Enterobacter hormaechei subsp. oharae	NAD(P)H-dependent oxidoreductase	217		
KVK07627	LRJV01000034	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK13877	LRJY01000075	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK18908	LRJX01000048	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK25305	LRIN01000103	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		
KVK30750	LREQ01000063	Enterobacter hormaechei	NAD(P)H-dependent oxidoreductase	217		
KYH19405	LUGN01000001	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
KYJ78922	LOBN01000007	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
KYO08671	LEDX02000020	Enterobacter ludwigii	NAD(P)H-dependent oxidoreductase	217		
KYO15497	LEDV02000008	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H-dependent oxidoreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KYQ76748	LOMM01000013	Enterobacter sp. SENG-6	NAD(P)H-dependent oxidoreductase	217		
KZP49864	LVTX01000044	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZP50052	LVTR01000036	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZP59420	LVTS01000042	Enterobacter cloacae complex sp. GN04787	NAD(P)H nitroreductase	217		
KZP70673	LVTZ01000030	Enterobacter cloacae subsp. dissolvens	NAD(P)H nitroreductase	217		
KZP71339	LVTV01000012	Enterobacter hormaechei subsp. oharae	NAD(P)H nitroreductase	217		
KZP74228	LVTP01000097	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
KZP84796	LVUH01000015	Enterobacter hormaechei subsp. oharae	NAD(P)H nitroreductase	217		
KZP87247	LVUA01000010	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZP87438	LVTT01000222	Enterobacter cloacae complex sp. GN04826	NAD(P)H nitroreductase	217	X	A0A0X6Z623 (UniProt)
KZP91282	LVUG01000049	Enterobacter asburiae	NAD(P)H nitroreductase	217		
KZQ01202	LVTU01000021	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ10184	LVUM01000022	Enterobacter kobei	NAD(P)H nitroreductase	217		
KZQ11844	LVTQ01000236	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
KZQ19715	LVUI01000022	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ24451	LVTY01000016	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ26350	LVUF01000120	Enterobacter cloacae complex sp. GN05526	NAD(P)H nitroreductase	217		
KZQ41025	LVUS01000006	Enterobacter cloacae subsp. dissolvens	NAD(P)H nitroreductase	217		
KZQ42475	LVUD01000011	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ56649	LVUJ01000020	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ65574	LVUX01000034	Enterobacter kobei	NAD(P)H nitroreductase	217		
KZQ74737	LVUK01000184	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KZQ88537	LVUZ01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ90092	LVUQ01000023	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ99425	LVVC01000001	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZQ99976	LVUU01000057	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZR24251	LVVD01000021	Enterobacter hormaechei subsp. steigerwaltii	NAD(P)H nitroreductase	217		
KZR30349	LVVA01000021	Enterobacter cloacae complex sp. GN06232	NAD(P)H nitroreductase	217	X	A0A162E6U0 (UniProt)
KZR40420	LVVI01000034	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
KZR42159	LVVE01000067	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OAE36549	LXPT01000036	Enterobacter cloacae	NAD(P)H nitroreductase	217		
OAE65687	LXPZ01000039	Enterobacter cloacae	NAD(P)H nitroreductase	217		
OAH34057	LSTS01000023	Enterobacter xiangfangensis	NAD(P)H-dependent oxidoreductase	217		
OAR71532	LSUR01000039	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
OAR85305	LSUS01000023	Enterobacter cloacae	NAD(P)H-dependent oxidoreductase	217		
OAT39275	LXES01000032	Enterobacter soli ATCC BAA-2102	oxygen-insensitive NAD(P)H nitroreductase/dihydropteridine reductase	217		
OAY17414	LZCS01000019	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OAZ41327	LZEN01000029	Enterobacter cloacae	NAD(P)H nitroreductase	217		
OAZ94693	LYYC01000010	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OBS89924	LSQV01000012	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OEG80433	LJDP02000122	Enterobacter cloacae complex 'Hoffmann cluster III'	NAD(P)H nitroreductase	217		
OEG91103	LJDJ02000001	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OEG95508	LJED02000157	Enterobacter kobei	NAD(P)H nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
OEH05625	LJEO02000112	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
OEH07809	LJER02000001	Enterobacter asburiae	NAD(P)H nitroreductase	217	X	A0A1D3B951 (UniProt)
OEH12797	LJEW02000099	Enterobacter kobei	NAD(P)H nitroreductase	217		
OEH18070	LJEY02000079	Enterobacter asburiae	NAD(P)H nitroreductase	217		
OEH22099	LJDO02000071	Enterobacter sp. ST121:950178628	NAD(P)H nitroreductase	217		
OEI71308	MDVW01000019	Enterobacter sp. ku-bf2	NAD(P)H nitroreductase	217		
OFU74459	LTZU01000008	Enterobacter sp. HMSC16D10	NAD(P)H-dependent oxidoreductase	217		
OHY48340	MBTX01000065	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
OHY64544	MBMT01000089	Enterobacter cloacae complex 'Hoffmann cluster IV'	NAD(P)H nitroreductase	217		
OIR48669	MKER01000005	Lelliottia nimipressuralis	NAD(P)H nitroreductase	217		
OIR53486	MKEQ01000001	Enterobacter hormaechei ATCC 49162	NAD(P)H nitroreductase	217		
OJH28178	JSVH01000104	Enterobacter kobei	dihydropteridine reductase	217		
OOC92560	MTFV01000010	Enterobacter cloacae	NAD(P)H nitroreductase	217		
OOK78025	MPZO01000003	Pedobacter himalayensis	NAD(P)H nitroreductase	217		
OOV73515	MWLI01000072	Enterobacter cloacae	NAD(P)H nitroreductase	217		
OQD51350	MTKD01000001	Enterobacter cancerogenus	NAD(P)H nitroreductase	217		
ORC20908	MWMD01000002	Enterobacter cloacae subsp. cloacae	NAD(P)H nitroreductase	217		
ORC31004	MWME01000004	Enterobacter cloacae subsp. cloacae	NAD(P)H nitroreductase	217		
ORD23492	NCRW01000002	Enterobacter cloacae	NAD(P)H nitroreductase	217		
SAA02736	FKDK01000001	Enterobacter cloacae	nitroreductase	217		
SAA04403	FKDF01000001	Enterobacter cloacae	nitroreductase	217		
SAA10055	FKBM01000001	Enterobacter cloacae	nitroreductase	217		
SAA11604	FKBN01000003	Enterobacter cloacae	nitroreductase	217		
SAA12898	FKBB01000015	Enterobacter cloacae	nitroreductase	217		
SAA13613	FKCA01000002	Enterobacter cloacae	nitroreductase	217		
SAA21926	FKAO01000008	Enterobacter cloacae	nitroreductase	217		
SAA27147	FKBJ01000006	Enterobacter cloacae	nitroreductase	217		
SAA27968	FKBO01000005	Enterobacter cloacae	nitroreductase	217		
SAA42770	FKCR01000005	Enterobacter cloacae	nitroreductase	217		
SAA52862	FKBG01000006	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
SAA55253	FKBI01000010	Enterobacter cloacae	nitroreductase	217		
SAA65037	FKCT01000006	Enterobacter cloacae	nitroreductase	217		
SAA91716	FKAY01000012	Enterobacter cloacae	nitroreductase	217		
SAB04137	FKCI01000001	[Enterobacter] aerogenes	nitroreductase	217		
SAB05543	FKBZ01000001	Enterobacter cloacae	nitroreductase	217		
SAB09583	FKCV01000001	Enterobacter cloacae	nitroreductase	217		
SAB12814	FKCO01000001	Enterobacter cloacae	nitroreductase	217		
SAB16149	FKBP01000031	Enterobacter cloacae	nitroreductase	217		
SAB23482	FKCD01000022	Enterobacter cloacae	nitroreductase	217		
SAB26478	FKBK01000023	Enterobacter cloacae	nitroreductase	217		
SAB29498	FKDC01000006	Enterobacter cloacae	nitroreductase	217		
SAB39454	FKCW01000003	Enterobacter cloacae	nitroreductase	217		
SAB49507	FKDI01000024	Enterobacter cloacae	nitroreductase	217		
SAB49971	FKCM01000004	[Enterobacter] aerogenes	nitroreductase	217		
SAB53712	FKCQ01000022	Enterobacter cloacae	nitroreductase	217		
SAB54213	FKCU01000005	Enterobacter cloacae	nitroreductase	217		
SAB57257	FKBY01000002	Enterobacter cloacae	nitroreductase	217		
SAB60405	FKCJ01000002	Enterobacter cloacae	nitroreductase	217		
SAB60523	FKCK01000001	Enterobacter cloacae	nitroreductase	217		
SAB61877	FKBW01000001	Enterobacter cloacae	nitroreductase	217		
SAB64872	FKCF01000003	Enterobacter cloacae	nitroreductase	217		
SAB66050	FKCE01000001	Enterobacter cloacae	nitroreductase	217		
SAB69185	FKBU01000001	Enterobacter cloacae	nitroreductase	217		
SAB71343	FKBX01000001	Enterobacter cloacae	nitroreductase	217		
SAB72692	FKDA01000001	Enterobacter cloacae	nitroreductase	217		
SAB73941	FKDB01000003	Enterobacter cloacae	nitroreductase	217		
SAB76967	FKCB01000005	Enterobacter cloacae	nitroreductase	217		
SAB78266	FKCN01000002	Enterobacter cloacae	nitroreductase	217		
SAB81491	FKDD01000004	Enterobacter cloacae	nitroreductase	217		
SAB83561	FKDJ01000001	Enterobacter cloacae	nitroreductase	217		
SAB91962	FKCL01000001	Enterobacter cloacae	nitroreductase	217		
SAB92460	FKDL01000005	Enterobacter cloacae	nitroreductase	217		
SAC00944	FKDH01000009	Enterobacter cloacae	nitroreductase	217		
SAC19627	FKCH01000002	Enterobacter cloacae	nitroreductase	217		
SAC22435	FKCC01000021	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SAC23165	FKDE01000027	Enterobacter cloacae	nitroreductase	217		
SAC40547	FKDP01000002	Enterobacter cloacae	nitroreductase	217		
SAC52878	FKDQ01000001	Enterobacter cloacae	nitroreductase	215		
SAC58935	FKDS01000001	Enterobacter cloacae	nitroreductase	215		
SAC59184	FKDM01000001	Enterobacter cloacae	nitroreductase	217		
SAC61352	FKDR01000002	Enterobacter cloacae	nitroreductase	217		
SAC64471	FKDV01000001	Enterobacter cloacae	nitroreductase	217		
SAC64485	FKDN01000004	Enterobacter cloacae	nitroreductase	217		
SAC66563	FKDT01000001	Enterobacter cloacae	nitroreductase	217		
SAC72423	FKCY01000007	Enterobacter cloacae	nitroreductase	217		
SAC91605	FKCZ01000009	Enterobacter cloacae	nitroreductase	217		
SAC93538	FKDG01000029	Enterobacter cloacae	nitroreductase	217		
SAD00330	FKEA01000003	Enterobacter cloacae	nitroreductase	217		
SAD06557	FKDW01000002	Enterobacter cloacae	nitroreductase	217		
SAD20032	FKEM01000002	Enterobacter cloacae	nitroreductase	217	X	A0A155XML1 (UniProt)
SAD20572	FKEG01000001	Enterobacter cloacae	nitroreductase	217	X	A0A0X4EH75 (UniProt)
SAD25447	FKEJ01000001	Enterobacter cloacae	nitroreductase	217		
SAD28852	FKEF01000001	Enterobacter cloacae	nitroreductase	217		
SAD30055	FKDZ01000005	Enterobacter cloacae	nitroreductase	217		
SAD30063	FKEN01000001	Enterobacter cloacae	nitroreductase	217		
SAD38094	FKEB01000002	Enterobacter cloacae	nitroreductase	217		
SAD43294	FKER01000001	Enterobacter cloacae	nitroreductase	217		
SAD44220	FKEL01000005	Enterobacter cloacae	nitroreductase	217		
SAD47002	FKET01000001	Enterobacter cloacae	nitroreductase	217		
SAD52735	FKEC01000005	Enterobacter cloacae	nitroreductase	217		
SAD53317	FKEO01000008	Enterobacter cloacae	nitroreductase	217		
SAD55041	FKEV01000001	Enterobacter cloacae	nitroreductase	217		
SAD58068	FKEU01000002	Enterobacter cloacae	nitroreductase	217		
SAD61074	FKEW01000001	Enterobacter cloacae	nitroreductase	217		
SAD61815	FKEE01000002	Enterobacter cloacae	nitroreductase	217		
SAD65095	FKEI01000005	Enterobacter cloacae	nitroreductase	217		
SAD70767	FKES01000002	Enterobacter cloacae	nitroreductase	217		
SAD84247	FKDU01000008	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SAD91670	FKEH01000007	Enterobacter cloacae	nitroreductase	217		
SAD95622	FKEZ01000001	Enterobacter cloacae	nitroreductase	217		
SAD95647	FKDO01000014	Enterobacter cloacae	nitroreductase	217		
SAE00086	FKEK01000010	Enterobacter cloacae	nitroreductase	217		
SAE13724	FKDY01000016	Enterobacter cloacae	nitroreductase	217		
SAE13834	FKEX01000002	Enterobacter cloacae	nitroreductase	217		
SAE24346	FKFJ01000001	Enterobacter cloacae	nitroreductase	217		
SAE29034	FKFG01000002	Enterobacter cloacae	nitroreductase	217		
SAE36397	FKFK01000001	Enterobacter cloacae	nitroreductase	217		
SAE39710	FKFF01000002	Enterobacter cloacae	nitroreductase	217		
SAE47717	FKFH01000002	Enterobacter cloacae	nitroreductase	217		
SAE49498	FKFI01000001	Enterobacter cloacae	nitroreductase	217		
SAE49828	FKFB01000003	Enterobacter cloacae	nitroreductase	217		
SAE51062	FKEP01000008	Enterobacter cloacae	nitroreductase	217		
SAE57309	FKFE01000009	Enterobacter cloacae	nitroreductase	217		
SAE67731	FKFC01000002	Enterobacter cloacae	nitroreductase	217		
SAE71105	FKFQ01000001	Enterobacter cloacae	nitroreductase	217		
SAE74949	FKEQ01000016	Enterobacter cloacae	nitroreductase	217		
SAE76723	FKFA01000011	Enterobacter cloacae	nitroreductase	217		
SAE80945	FKFM01000002	Enterobacter cloacae	nitroreductase	217		
SAE93277	FKFO01000001	Enterobacter cloacae	nitroreductase	217		
SAE94808	FKFR01000001	Enterobacter cloacae	nitroreductase	217		
SAE96551	FKFP01000002	Enterobacter cloacae	nitroreductase	217		
SAF01451	FKFD01000010	Enterobacter cloacae	nitroreductase	217		
SAF11915	FKFS01000007	Enterobacter cloacae	nitroreductase	217		
SAF35596	FKGF01000001	Enterobacter cloacae	nitroreductase	217		
SAF37355	FKFU01000002	Enterobacter cloacae	nitroreductase	217		
SAF37740	FKGG01000001	Enterobacter cloacae	nitroreductase	217		
SAF42163	FKGA01000001	Enterobacter cloacae	nitroreductase	217		
SAF45631	FKFW01000003	Enterobacter cloacae	nitroreductase	217		
SAF46279	FKFN01000008	Enterobacter cloacae	nitroreductase	217		
SAF50080	FKGK01000001	Enterobacter cloacae	nitroreductase	217		
SAF56133	FKFT01000005	Enterobacter cloacae	nitroreductase	217		
SAF67525	FKGL01000001	Enterobacter cloacae	nitroreductase	217		
SAF71051	FKGC01000008	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
SAF74441	FKGH01000002	Enterobacter cloacae	nitroreductase	217		
SAF75369	FKGT01000001	Enterobacter cloacae	nitroreductase	217		
SAF83751	FKFZ01000006	Enterobacter cloacae	nitroreductase	217		
SAF87875	FKGI01000002	Enterobacter cloacae	nitroreductase	217		
SAF90882	FKFV01000007	Enterobacter cloacae	nitroreductase	217		
SAF91144	FKGB01000003	Enterobacter cloacae	nitroreductase	217		
SAF96241	FKGP01000002	Enterobacter cloacae	nitroreductase	217		
SAF96936	FKFY01000005	Enterobacter cloacae	nitroreductase	217		
SAG02935	FKGO01000004	Enterobacter cloacae	nitroreductase	217		
SAG10926	FKGM01000002	Enterobacter cloacae	nitroreductase	217		
SAG12298	FKHA01000001	Enterobacter cloacae	nitroreductase	217		
SAG12543	FKGX01000001	Enterobacter cloacae	nitroreductase	217		
SAG29285	FKGS01000003	Enterobacter cloacae	nitroreductase	217		
SAG29371	FKHD01000001	Enterobacter cloacae	nitroreductase	217		
SAG36680	FKFX01000025	Enterobacter cloacae	nitroreductase	217		
SAG37487	FKGU01000005	Enterobacter cloacae	nitroreductase	217		
SAG41943	FKGZ01000002	Enterobacter cloacae	nitroreductase	217		
SAG44047	FKHC01000002	Enterobacter cloacae	nitroreductase	217		
SAG44883	FKGR01000002	Enterobacter cloacae	nitroreductase	217		
SAG54405	FKHF01000001	Enterobacter cloacae	nitroreductase	215		
SAG91102	FKHO01000001	Enterobacter cloacae	nitroreductase	217		
SAG94334	FKHN01000001	Enterobacter cloacae	nitroreductase	217		
SAH11013	FKHB01000008	Enterobacter cloacae	nitroreductase	217		
SAH11674	FKHI01000003	Enterobacter cloacae	nitroreductase	217		
SAH16545	FKGV01000011	Enterobacter cloacae	nitroreductase	217		
SAH16889	FKHP01000001	Enterobacter cloacae	nitroreductase	217		
SAH23588	FKGJ01000023	Enterobacter cloacae	nitroreductase	217		
SAH24378	FKHT01000001	Enterobacter cloacae	nitroreductase	217		
SAH36146	FKGE01000027	Enterobacter cloacae	nitroreductase	217		
SAH47684	FKHX01000001	Enterobacter cloacae	nitroreductase	217		
SAH59601	FKHE01000009	Enterobacter cloacae	nitroreductase	217		
SAH60362	FKHU01000003	Enterobacter cloacae	nitroreductase	217		
SAH64810	FKHY01000005	Enterobacter cloacae	nitroreductase	217		
SAH65951	FKHQ01000007	Enterobacter cloacae	nitroreductase	217		
SAH66437	FKID01000001	Enterobacter cloacae	nitroreductase	217		

Table A.4. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SAH79547	FKHL01000008	Enterobacter cloacae	nitroreductase	217		
SAH81040	FKHK01000005	Enterobacter cloacae	nitroreductase	217		
SAH93782	FKIA01000005	Enterobacter cloacae	nitroreductase	217		
SAH93923	FKHV01000002	Enterobacter cloacae	nitroreductase	217		
SAI02736	FKIC01000004	Enterobacter cloacae	nitroreductase	217		
SAI09028	FKHR01000003	Enterobacter cloacae	nitroreductase	217		
SAI14426	FKHW01000005	Enterobacter cloacae	nitroreductase	217		
SAI14706	FKIB01000002	Enterobacter cloacae	nitroreductase	217	X	A0A145CU16 (UniProt)
SAI49348	FKHS01000030	Enterobacter cloacae	nitroreductase	217		
SAI56111	FKHZ01000005	Enterobacter cloacae	nitroreductase	217		
SAJ03134	FKIW01000007	Enterobacter cloacae	nitroreductase	217		
SAJ04635	FKIS01000007	Enterobacter cloacae	nitroreductase	217		
SAJ07412	FKIX01000006	Enterobacter cloacae	nitroreductase	215		
SAJ14952	FKIU01000007	Enterobacter cloacae	nitroreductase	217		
SAJ19176	FKIQ01000008	Enterobacter cloacae	nitroreductase	217		
SAJ28726	FKIR01000014	Enterobacter cloacae	nitroreductase	217		
SAZ11274	FKLS01000001	Enterobacter kobei	nitroreductase	217		
SBL27928	FKZC01000001	Klebsiella oxytoca	Oxygen-insensitive NAD(P)H nitroreductase	217		
SCZ31110	FMVY01000002	Enterobacter sp. NFIX45	nitroreductase / dihydropteridine reductase	217		
SEO76491	FODW01000001	Enterobacter sp. NFIX58	nitroreductase / dihydropteridine reductase	217		
SFI03138	FOQN01000001	Enterobacter sp. NFIX59	nitroreductase / dihydropteridine reductase	217		
SHG59995	FQWJ01000002	Pantoea sesami	nitroreductase / dihydropteridine reductase	217		
SMF89767	LT840187	Enterobacter cloacae	nitroreductase / dihydropteridine reductase	217		

Table A.5. FunGene and primer design sequences for *pnrB*

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AAM95987	AF532912	Pseudomonas putida	oxygen-insensitive NADPH nitroreductase	217	X	AAM95987.1 (NCBI)
AAN68044	AE015451	Pseudomonas putida KT2440	6,7-dihydropteridine reductase	217		
ABQ79389	CP000712	Pseudomonas putida F1	dihydropteridine reductase	217		
ABY97978	CP000926	Pseudomonas putida GB-1	6,7-dihydropteridine reductase	217		
ADR60849	CP002290	Pseudomonas putida BIRD-1	PnrB	217	X	ADR60849.1 (NCBI)
AFK67306	CP003588	Pseudomonas putida ND6	dihydropteridine reductase	217		
AFO51068	CP003734	Pseudomonas putida DOT-T1E	dihydropteridine reductase	217		
AGA74160	CP003738	Pseudomonas putida HB3267	dihydropteridine reductase	217		
AGN78716	CP005976	Pseudomonas putida H8234	dihydropteridine reductase	217		
AJA15421	CP009974	Pseudomonas putida S12	dihydropteridine reductase	217		
AJQ47913	CP010979	Pseudomonas putida S13.1.2	dihydropteridine reductase	217		
ANC03605	CP011789	Pseudomonas putida	dihydropteridine reductase	216		
ANC82375	CP015202	Pseudomonas putida B6-2	NAD(P)H nitroreductase	217		
ANI04291	CP015876	Pseudomonas putida SJTE-1	NAD(P)H nitroreductase	217		
ANI33970	CP011525	Pseudomonas sp. JY-Q	dihydropteridine reductase	217		
AOX09888	CP016212	Pseudomonas putida JB	NAD(P)H nitroreductase	217		
APE98345	CP017073	Pseudomonas putida	NAD(P)H nitroreductase	217		
APO83023	CP018743	Pseudomonas sp. DRA525	NAD(P)H nitroreductase	217		
AUZ60049	CP026332	Pseudomonas sp. XWY-1	Oxygen-insensitive NAD(P)H nitroreductase/ Dihydropteridine reductase	217		
BAN55223	AP013070	Pseudomonas putida NBRC 14164	nitroreductase	217		
CAK15120	CT573326	Pseudomonas entomophila L48	oxygen-insensitive NADPH nitroreductase	217		
EJT82690	ALXA01000164	Pseudomonas putida S11	dihydropteridine reductase	217		
EMR47500	ALPV02000011	Pseudomonas putida LS46	dihydropteridine reductase	217		
ENY79724	APBQ01000004	Pseudomonas putida TRO1	dihydropteridine reductase	217		
ERL01707	AOUR02000030	Pseudomonas putida LF54	dihydropteridine reductase	217		
ERT16443	AXDX02000175	Pseudomonas putida SJ3	dihydropteridine reductase	217		
ESP86245	AYKV01000005	Pseudomonas putida S12	dihydropteridine reductase	217		
KIC80666	JWLN01000026	Pseudomonas sp. C5pp	dihydropteridine reductase	217		
KKO15840	AYRY01000007	Pseudomonas putida KG-4	dihydropteridine reductase	217		

Table A.5. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KMU95024	LFYQ01000023	Pseudomonas putida	dihydropteridine reductase	217		
KMY34721	LDJF01000018	Pseudomonas putida	dihydropteridine reductase	217		
KPM64697	LKKT01000041	Pseudomonas putida	dihydropteridine reductase	217		
KPM68139	LKKS01000025	Pseudomonas putida	dihydropteridine reductase	217		
KWW19341	LNNI01000002	Stenotrophomonas rhizophila	NAD(P)H-dependent oxidoreductase	217		
KZO52039	LWDW01000078	Pseudomonas putida	NAD(P)H nitroreductase	217		
OAH42514	LSTU01000078	Pseudomonas monteilii	NAD(P)H-dependent oxidoreductase	217		
OAS06505	LSUZ01000180	Pseudomonas putida	NAD(P)H-dependent oxidoreductase	217		
OCT29702	MCBG01000022	Pseudomonas putida	NAD(P)H nitroreductase	216		
OCT31398	MCBH01000011	Pseudomonas putida	NAD(P)H nitroreductase	216		
OCT33640	MCBI01000011	Pseudomonas putida	NAD(P)H nitroreductase	216		
OCT40086	MCBJ01000011	Pseudomonas putida	NAD(P)H nitroreductase	216		
ORL51207	NBWB01000012	Pseudomonas putida	oxygen-insensitive NAD(P)H-dependent nitroreductase NfsB	217		
ORL69362	NBWA01000016	Pseudomonas putida	oxygen-insensitive NAD(P)H-dependent nitroreductase NfsB	217		
SIS12998	LT707061	Pseudomonas putida	dihydropteridine reductase	217		
SKB88244	LT799039	Pseudomonas putida	dihydropteridine reductase	217		
SMC56309	FWXQ01000002	Pseudomonas sp. URIL14HWK12:I5	dihydropteridine reductase	217		
WP_043200951.1[1]	NZ_JENB01000025.1: c56599-55946	Pseudomonas putida	oxygen-insensitive NAD(P)H-dependent nitroreductase NfsB	217	X	WP_043200951.1 (NCBI)
WP_060497226.1[1]	NZ_BCBJ01000003.1: c3014-2361	Pseudomonas sp. NBRC 111133	NAD(P)H nitroreductase	217	X	WP_060497226.1 (NCBI)
WP_060515567.1[1]	NZ_BCBI01000057.1: c38546-37893	Pseudomonas sp. NBRC 111132	NAD(P)H nitroreductase	217	X	WP_060515567.1 (NCBI)
WP_063915060.1[1]	NZ_LWBV01000011.1: c46838-46185	Pseudomonas sp. p21	NAD(P)H nitroreductase	217	X	WP_063915060.1 (NCBI)
WP_085599282.1[1]	NZ_NEJU01000002.1: c242079-241426	Pseudomonas sp. B10(2017)	NAD(P)H nitroreductase	217	X	WP_085599282.1 (NCBI)
WP_085615138.1[1]	NZ_NEJL01000004.1: 218166-218819	Pseudomonas	MULTISPECIES: NAD(P)H nitroreductase	217	X	WP_085615138.1 (NCBI)

Table A.5. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
WP_085624491.1[1]	NZ_NEIN01000005.1: 167504-168157	Pseudomonas	MULTISPECIES: NAD(P)H nitroreductase	217	X	WP_085624491.1 (NCBI)
WP_085665187.1[1]	NZ_NEIR01000013.1: c164785-164132	Pseudomonas sp. B5(2017)	NAD(P)H nitroreductase	217	X	WP_085665187.1 (NCBI)
WP_085706301.1[1]	NZ_NEIO01000023.1: c44082-43429	Pseudomonas sp. B8(2017)	NAD(P)H nitroreductase	217	X	WP_085706301.1 (NCBI)
WP_085720572.1[1]	NZ_NEJN01000002.1: 424849-425502	Pseudomonas sp. B22(2017)	NAD(P)H nitroreductase	217	X	WP_085720572.1 (NCBI)

[1] These sequences were not on FunGene so were added back in to the primer design sequence set manually.

Table A.6. FunGene and primer design sequences for *xenA*

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AAF02538	AF154061	Pseudomonas putida	xenobiotic reductase A	363	X	5CPL (PDB)
AAN66878	AE015451	Pseudomonas putida KT2440	XenA	363		
AAO55915	AE016853	Pseudomonas syringae pv. tomato str. DC3000	xenobiotic reductase A	363		
AAY37233	CP000075	Pseudomonas syringae pv. syringae B728a	NADH:flavin oxidoreductase/NADH oxidase	363		
AAY40303	AY957609	Pseudomonas putida DOT-T1E	xenobiotic reductase	363		
ABQ77442	CP000712	Pseudomonas putida F1	NADH:flavin oxidoreductase/NADH oxidase	363		
ABZ00028	CP000926	Pseudomonas putida GB-1	NADH:flavin oxidoreductase/NADH oxidase	363		
ACA74393	CP000949	Pseudomonas putida W619	NADH:flavin oxidoreductase/NADH oxidase	363		
ADR58970	CP002290	Pseudomonas putida BIRD-1	XenA	363		
AEJ14509	CP002870	Pseudomonas putida S16	xenobiotic reductase A	360		
AFJ57172	CP003041	Pseudomonas fluorescens A506	xenobiotic reductase A	363		
AFK70926	CP003588	Pseudomonas putida ND6	NADH:flavin oxidoreductase/NADH oxidase	363		
AFO48989	CP003734	Pseudomonas putida DOT-T1E	NADH:flavin oxidoreductase/NADH oxidase	363		
AGA74837	CP003738	Pseudomonas putida HB3267	XenA	363		
AGE26148	CP004045	Pseudomonas poae RE*1-1-14	xenobiotic reductase A	363		
AGN80144	CP005976	Pseudomonas putida H8234	NADH:flavin oxidoreductase	363		
AGZ36485	CP003961	Pseudomonas sp. VLB120	NADH:flavin oxidoreductase/NADH oxidase	363		
AHC84020	CP006978	Pseudomonas monteilii SB3078	NADH:flavin oxidoreductase	363		
AHC89391	CP006979	Pseudomonas monteilii SB3101	NADH:flavin oxidoreductase	363		
AHD15905	CP007012	Pseudomonas sp. FGI182	NADH:flavin oxidoreductase	363		
AHG40605	CP007014	Pseudomonas syringae CC1557	NADH:flavin oxidoreductase	363		
AHH54488	KF827430	Pseudomonas putida	xenobiotic reductase	363		
AHZ78908	CP007620	Pseudomonas putida	xenobiotic reductase A	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
AIB36087	CP007637	Pseudomonas simiae	NADH:flavin oxidoreductase	363		
AIB41642	CP007638	Pseudomonas sp. WCS374	NADH:flavin oxidoreductase	363		
AJA12330	CP009974	Pseudomonas putida S12	NADH:flavin oxidoreductase	363		
AJG12026	CP010359	Pseudomonas plecoglossicida	NADH:flavin oxidoreductase	363		
AJP51761	CP010896	Pseudomonas fluorescens	xenobiotic reductase A	363		
AJQ50999	CP010979	Pseudomonas putida S13.1.2	NADH:flavin oxidoreductase	363		
AJZ96515	CP005975	Pseudomonas fluorescens PICF7	NADH:flavin oxidoreductase	363		
AKA86083	CP011117	Pseudomonas fluorescens	xenobiotic reductase A	363		
AKF45677	CP005969	Pseudomonas syringae pv. syringae B301D	NADH:flavin oxidoreductase, Old Yellow Enzyme family	363		
AKF51813	CP006256	Pseudomonas syringae pv. syringae HS191	NADH:flavin oxidoreductase, Old Yellow Enzymefamily	349		
AKT31150	CP011972	Pseudomonas syringae pv. actinidiae ICMP 18884	NADH:flavin oxidoreductase	363		
ALD96243	CP005970	Pseudomonas syringae UMAF0158	oxidoreductase	363		
ALU60317	CP013183	Pseudomonas syringae pv. lapsa	NADH:flavin oxidoreductase	363		
AMA45169	CP013997	Pseudomonas monteilii	NADH:flavin oxidoreductase	363		
AMS21491	CP014868	Pseudomonas fluorescens	NADH:flavin oxidoreductase	363		
ANC81490	CP015202	Pseudomonas putida B6-2	NADH:flavin oxidoreductase	363		
ANI02283	CP015876	Pseudomonas putida SJTE-1	NADH:flavin oxidoreductase	363		
ANI35820	CP011525	Pseudomonas sp. JY-Q	NADH:flavin oxidoreductase	363		
AOE57546	CP012179	Pseudomonas syringae pv. actinidiae ICMP 18708	NADH:flavin oxidoreductase	363		
AOS73499	CP017296	Pseudomonas fluorescens	NADH:flavin oxidoreductase	363		
BAN53045	AP013070	Pseudomonas putida NBRC 14164	xenobiotic reductase A	369	X	T2H290 (UniProt)
BAP43811	AP014637	Pseudomonas sp. StFLB209	xenobiotic reductase A	363		
CAK16750	CT573326	Pseudomonas entomophila L48	xenobiotic reductase A	363		
CRM05256	CVTN01000034	Pseudomonas sp. 31 R 17	NADPH dehydrogenase	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
CRM18142	CVTU01000039	Pseudomonas sp. 44 R 15	NADPH dehydrogenase	363		
CRM29370	CVTJ01000044	Pseudomonas sp. 25 E 4	NADPH dehydrogenase	363		
CRM64863	CVTK01000058	Pseudomonas sp. 28 E 9	NADPH dehydrogenase	363		
CRM71811	CVTV01000057	Pseudomonas sp. 37 R 15	NADPH dehydrogenase	363		
CRM85450	CVTO01000162	Pseudomonas sp. 24 E 13	NADPH dehydrogenase	363		
CRN00641	CVTX01000123	Pseudomonas sp. 34 E 7	NADPH dehydrogenase	363		
CRN04937	LN865164	Pseudomonas sp. URMO17WK12:I11	NADPH dehydrogenase	363		
EEB57589	ABSM01000035	Pseudomonas syringae pv. tomato T1	xenobiotic reductase A	363		
EFQ64130	AEAZ01000013	Pseudomonas fluorescens WH6	xenobiotic reductase A	363		
EGC00475	AEWE02000142	Pseudomonas sp. TJI-51	NADH:flavin oxidoreductase	363		
EGH10960	AEAE01000300	Pseudomonas syringae pv. morsprunorum str. M302280	xenobiotic reductase A	363		
EGH21037	AEAG01000233	Pseudomonas syringae pv. mori str. 301020	xenobiotic reductase A	363		
EGH29481	AEAH01000519	Pseudomonas syringae pv. japonica str. M301072	NADH:flavin oxidoreductase/NADH oxidase	363		
EGH52142	AEAJ01000375	Pseudomonas syringae Cit 7	NADH:flavin oxidoreductase/NADH oxidase	363		
EGH59050	AEAK01000183	Pseudomonas syringae pv. maculicola str. ES4326	xenobiotic reductase A	363		
EGH63875	AEAL01000063	Pseudomonas syringae pv. actinidiae str. M302091	xenobiotic reductase A	363		
EGH74239	AEAO01000647	Pseudomonas syringae pv. aceris str. M302273	NADH:flavin oxidoreductase/NADH oxidase	363		
EGH79809	AEAN01001032	Pseudomonas syringae pv. aptata str. DSM 50252	NADH:flavin oxidoreductase/NADH oxidase	363		
EGH87466	AEAF01000431	Pseudomonas syringae pv. lachrymans str. M301315	xenobiotic reductase A	363		
EGH90517	AEAP01000239	Pseudomonas syringae pv. tabaci str. ATCC 11528	xenobiotic reductase A	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
EGH99022	AEAM01000441	Pseudomonas syringae pv. lachrymans str. M302278	xenobiotic reductase A	363		
EGI03899	ABZR01000560	Pseudomonas syringae pv. oryzae str. 1_6	xenobiotic reductase A	363		
EIK59498	AHPN01000001	Pseudomonas fluorescens SS101	xenobiotic reductase A	363		
EIK69323	AHPP01000002	Pseudomonas synxantha BG33R	xenobiotic reductase A	363		
EIK94421	AJWX01000024	Pseudomonas sp. M47T1	xenobiotic reductase A	363		
EKG41581	AKCJ01000141	Pseudomonas syringae pv. avellanae str. ISPaVe013	xenobiotic reductase A	363		
EKN47358	AMQP01000035	Pseudomonas viridiflava UASWS0038	XenA	363		
EKX82219	AMWJ01000173	Pseudomonas putida CSV86	NADH:flavin oxidoreductase/NADH oxidase	363		
ELP99815	AMXK01000040	Pseudomonas syringae BRIP34876	NADH:flavin oxidoreductase	363		
ELQ06431	AMXL01000008	Pseudomonas syringae BRIP34881	NADH:flavin oxidoreductase	363		
ELQ11581	AMZX01000026	Pseudomonas syringae BRIP39023	NADH:flavin oxidoreductase	363		
EMR45280	ALPV02000017	Pseudomonas putida LS46	XenA	363		
ENY77786	APBQ01000060	Pseudomonas putida TRO1	NADH:flavin oxidoreductase/NADH oxidase	363		
EPJ87167	ATLM01000017	Pseudomonas sp. CFT9	putative xenobiotic reductase	363		
EPJ95771	ATLR01000010	Pseudomonas sp. CF149	xenobiotic reductase A	363		
EPL10706	ATLQ01000053	Pseudomonas sp. CF150	putative xenobiotic reductase	363		
EPM44393	AOKE01000198	Pseudomonas syringae pv. actinidiae ICMP 19098	xenobiotic reductase A	363		
EPM69780	AOJT01001410	Pseudomonas syringae pv. actinidiae ICMP 18886	xenobiotic reductase A	363		
EPM72486	AOJV01000035	Pseudomonas syringae pv. theae ICMP 3923	xenobiotic reductase A	363		
EPM89582	AOJX01000059	Pseudomonas syringae pv. actinidiae ICMP 19068	xenobiotic reductase A	363		
EPM98481	AOJZ01000055	Pseudomonas syringae pv. actinidiae ICMP 19104	xenobiotic reductase A	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
EPN12185	AOKB01000050	Pseudomonas syringae pv. actinidiae ICMP 9855	xenobiotic reductase A	363		
EPN15537	AOKC01000356	Pseudomonas syringae pv. actinidiae ICMP 19100	xenobiotic reductase A	363		
EPN23908	AOKD01000233	Pseudomonas syringae pv. actinidiae ICMP 19099	xenobiotic reductase A	363		
EPN31631	AOKH01000282	Pseudomonas syringae pv. actinidiae ICMP 18883	xenobiotic reductase A	363		
EPN40178	AOKI01000532	Pseudomonas syringae pv. actinidiae ICMP 19095	xenobiotic reductase A	363		
EPN53783	AOKJ01000053	Pseudomonas syringae pv. actinidiae ICMP 19094	xenobiotic reductase A	363		
EPN67578	AOKM01001490	Pseudomonas syringae pv. actinidiae ICMP 19101	xenobiotic reductase A	363		
EPN69472	AOKL01000421	Pseudomonas syringae pv. actinidiae ICMP 19079	xenobiotic reductase A	363		
EPN76784	AOKQ01000469	Pseudomonas syringae pv. actinidiae ICMP 18801	xenobiotic reductase A	363		
EPN80270	AOKN01000101	Pseudomonas syringae pv. actinidiae ICMP 19097	xenobiotic reductase A	363		
ERH47346	AVQG01000058	Pseudomonas fluorescens EGD-AQ6	NADH:flavin oxidoreductase	363		
ERL01089	AOUR02000063	Pseudomonas putida LF54	NADH:flavin oxidoreductase	363		
EST13798	AYJQ01000069	Pseudomonas putida S610	flavin oxidoreductase / NADH oxidase family protein	363		
ETK39784	AOJA01000060	Pseudomonas fluorescens FH5	NADH:flavin oxidoreductase	363		
EXL33003	JALJ01000005	Pseudomonas syringae pv. syringae str. B301D-R	NADH:flavin oxidoreductase, Old Yellow Enzyme family	363		
EZI24854	AHIP01000041	Pseudomonas extremaustralis 14-3 substr. 14-3b	NADH:flavin oxidoreductase	363		
EZP67781	JFYX01000007	Pseudomonas sp. RIT357	putative xenobiotic reductase	363		
GAO92257	BBWG01000029	Pseudomonas syringae pv. actinidiae	xenobiotic reductase A	363		
KAK39242	AOKO01000022	Pseudomonas syringae pv. actinidiae ICMP 18884	xenobiotic reductase A	363		

Table A.6. (cont’d)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KCU97759	AOKP01000131	Pseudomonas syringae pv. actinidiae ICMP 9617	xenobiotic reductase A	363		
KEY89873	JMIT01000001	Pseudomonas sp. WCS358	NADH:flavin oxidoreductase	363		
KEZ27011	AJXI01000065	Pseudomonas syringae pv. tabaci str. 6605	NADH:flavin oxidoreductase	363		
KEZ65469	ACHU02000949	Pseudomonas syringae pv. tabaci str. ATCC 11528	NADH:flavin oxidoreductase	363		
KFE45277	JPQV01000033	Pseudomonas syringae	NADH:flavin oxidoreductase	363		
KFF42841	JPRX01000001	Pseudomonas sp. BRG-100	NADH:flavin oxidoreductase	363		
KFF83486	JPNN01000077	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KGF65078	JRMB01000001	Pseudomonas lutea	NADH:flavin oxidoreductase	363		
KGI92449	JRPO01000027	Pseudomonas sp. H2	NADH:flavin oxidoreductase	363		
KGK26984	JOJY01000001	Pseudomonas plecoglossicida	NADH:flavin oxidoreductase	363		
KGK92869	JRRA01000032	Pseudomonas syringae pv. tomato	NADH:flavin oxidoreductase	363		
KHL75754	JTCJ01000042	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KIC81698	JWLN01000016	Pseudomonas sp. C5pp	NADH:flavin oxidoreductase	363		
KIQ06542	JXQW01000001	Pseudomonas fulva	NADH:flavin oxidoreductase	363		
KIQ11179	JXQT01000019	Pseudomonas simiae	NADH:flavin oxidoreductase	363		
KIQ36105	JXQO01000012	Pseudomonas viridiflava	NADH:flavin oxidoreductase	363		
KIR19740	JXCQ01000069	Pseudomonas fluorescens	NADPH dehydrogenase	363		
KIU52287	JXOG01000163	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KIY17823	JWJF01000103	Pseudomonas amygdali pv. tabaci	NADH:flavin oxidoreductase	363		
KKI23303	LAZV01000188	Pseudomonas syringae pv. persicae	NADH:flavin oxidoreductase	363		
KKO13881	AYRY01000025	Pseudomonas putida KG-4	NADH:flavin oxidoreductase	363		
KKY54047	LCWS01000002	Pseudomonas amygdali pv. tabaci str. ATCC 11528	NADH:flavin oxidoreductase	363		
KKY58242	LCWT01000004	Pseudomonas amygdali pv. lachrymans	NADH:flavin oxidoreductase	363		
KMM79283	JYKX01000004	Pseudomonas deceptionensis	NADH:flavin oxidoreductase	363		

Table A.6. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KMM85239	JYLA01000003	Pseudomonas taetrolens	NADH:flavin oxidoreductase	363		
KMM98537	JYKZ01000007	Pseudomonas psychrophila	NADH:flavin oxidoreductase	363		
KMU95130	LFYQ01000021	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KMY03695	AYTM02000002	Pseudomonas syringae KCTC 12500	NADH:flavin oxidoreductase	363		
KMY36699	LDJF01000007	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KOG06597	LGAR01000010	Pseudomonas syringae pv. aceris	NADHflavin oxidoreductase/NADH oxidase	363		
KOP54490	JTHM01000050	Pseudomonas coronafaciens pv. porri	NADH:flavin oxidoreductase	363		
KOP61167	JUEU01000015	Pseudomonas coronafaciens pv. porri	NADH:flavin oxidoreductase	363		
KOX64919	LBHT01000065	Pseudomonas psychrophila	NADH:flavin oxidoreductase	363		
KPA91268	JSYZ01000007	Pseudomonas fuscovaginae	NADH:flavin oxidoreductase	363		
KPA99436	JTBY01000019	Pseudomonas fuscovaginae	NADH:flavin oxidoreductase	363		
KPB14520	LGKR01000102	Pseudomonas savastanoi	xenobiotic reductase A	363		
KPB17767	LGKT01000094	Pseudomonas syringae pv. syringae	NADHflavin oxidoreductase	363		
KPB26448	LGKU01000017	Pseudomonas syringae pv. syringae	NADHflavin oxidoreductase	363		
KPB51484	LGKZ01000009	Pseudomonas coronafaciens pv. oryzae	Xenobiotic reductase A	363		
KPB65778	LGLA01000016	Pseudomonas amygdali pv. myricae	Xenobiotic reductase A	363		
KPB72305	LGLD01000041	Pseudomonas syringae pv. maculicola	Xenobiotic reductase A	363		
KPB76072	LGLE01000038	Pseudomonas syringae pv. maculicola	Xenobiotic reductase A	363		
KPB90331	LGLH01000075	Pseudomonas syringae pv. maculicola	Xenobiotic reductase A	363		
KPB97366	LGLI01000078	Pseudomonas amygdali pv. lachrymans	Xenobiotic reductase A	363		
KPC09767	LGLJ01000060	Pseudomonas amygdali pv. lachrymans	Xenobiotic reductase A	363		
KPC20153	LGLK01000033	Pseudomonas amygdali pv. lachrymans	Xenobiotic reductase A	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KPC27726	LGLN01000067	Pseudomonas syringae pv. cilantro	Xenobiotic reductase A	363		
KPC57944	LGLQ01000039	Pseudomonas amygdali pv. morsprunorum	Xenobiotic reductase A	363		
KPG76757	LHOY01000006	Pseudomonas libanensis	NADH:flavin oxidoreductase	363		
KPL65379	JRXH01000017	Pseudomonas viridiflava	NADH:flavin oxidoreductase	363		
KPM58581	LKKS01000137	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KPM60167	LKKT01000088	Pseudomonas putida	NADH:flavin oxidoreductase	363		
KPW12300	LJPO01000074	Pseudomonas syringae pv. atrofaciens	NADH:flavin oxidoreductase/NADH oxidase	363		
KPW32857	LJPS01000116	Pseudomonas coronafaciens pv. atropurpurea	Xenobiotic reductase A	370	X	A0A0P9I8N7 (UniProt)
KPW36430	LJPR01000084	Pseudomonas syringae pv. apii	Xenobiotic reductase A	363		
KPW36510	LJPQ01000094	Pseudomonas amygdali	Xenobiotic reductase A	363		
KPW43710	LJPT01000173	Pseudomonas syringae pv. antirrhini	Xenobiotic reductase A	370	X	A0A0N8QM38 (UniProt)
KPW59084	LJPU01000046	Pseudomonas syringae pv. berberidis	Xenobiotic reductase A	363		
KPW59535	LJPW01000078	Pseudomonas caricapapayae	Xenobiotic reductase A	363		
KPW75204	LJPY01000106	Pseudomonas amygdali pv. ciccaronei	Xenobiotic reductase A	363		
KPW80628	LJPZ01000081	Pseudomonas syringae pv. coriandricola	Xenobiotic reductase A	363		
KPW86204	LJQB01000027	Pseudomonas congelans	NADH:flavin oxidoreductase/NADH oxidase	374	X	A0A0P9MDZ2 (UniProt)
KPX08211	LJQE01000123	Pseudomonas syringae pv. cunninghamiae	Xenobiotic reductase A	374	X	A0A0P9R9Q3 (UniProt)
KPX11492	LJQC01000036	Pseudomonas syringae pv. coryli	Xenobiotic reductase A	363		
KPX22183	LJQG01000076	Pseudomonas amygdali pv. dendropanacis	Xenobiotic reductase A	363		
KPX27260	LJQJ01000579	Pseudomonas ficuserectae	xenobiotic reductase A	363		
KPX34269	LJQK01000021	Pseudomonas coronafaciens pv. garcae	Xenobiotic reductase A	372	X	A0A0P9QQ78 (UniProt)

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KPX49079	LJQM01000029	Pseudomonas syringae pv. helianthi	Xenobiotic reductase A	370	X	A0A0N8RPQ9 (UniProt)
KPX65160	LJQQ01000034	Pseudomonas syringae pv. lapsa	NADH:flavin oxidoreductase/NADH oxidase	363		
KPX69394	LJQP01000214	Pseudomonas amygdali pv. lachrymans	Xenobiotic reductase A	374		
KPX70385	LJQR01000162	Pseudomonas syringae pv. maculicola	Xenobiotic reductase A	363		
KPX70929	LJQO01000285	Pseudomonas amygdali pv. photiniae	Xenobiotic reductase A	363		
KPX86489	LJQS01000035	Pseudomonas amygdali pv. mellea	Xenobiotic reductase A	363		
KPX89519	LJQU01000429	Pseudomonas amygdali pv. mori	Xenobiotic reductase A	363		
KPX92876	LJQV01000249	Pseudomonas amygdali pv. myricae	Xenobiotic reductase A	363		
KPX96612	LJQW01000339	Pseudomonas savastanoi pv. nerii	Xenobiotic reductase A	363		
KPY06286	LJQX01000038	Pseudomonas coronafaciens pv. oryzae	Xenobiotic reductase A	363		
KPY11776	LJQY01000398	Pseudomonas syringae pv. philadelphi	Xenobiotic reductase A	370		
KPY23643	LJRA01000112	Pseudomonas coronafaciens pv. porri	Xenobiotic reductase A	363		
KPY25300	LJRB01000250	Pseudomonas syringae pv. papulans	NADH:flavin oxidoreductase	374	X	A0A0Q0CDH2 (UniProt)
KPY31821	LJRC01000246	Pseudomonas syringae pv. primulae	XenA	370	X	A0A0P9YD88 (UniProt)
KPY42460	LJRF01000212	Pseudomonas syringae pv. ribicola	XenA	383	X	A0A0P9YU58 (UniProt)
KPY51316	LJRE01000090	Pseudomonas syringae pv. rhaphiolepidis	Xenobiotic reductase A	363		
KPY61375	LJRH01000089	Pseudomonas syringae pv. solidagae	Xenobiotic reductase A	374	X[1]	A0A0P9ZH38 (UniProt)
KPY70038	LJRJ01000190	Pseudomonas savastanoi pv. savastanoi	Xenobiotic reductase A	363		
KPY74635	LJRK01000019	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase/NADH oxidase	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KPY84223	LJRL01000017	Pseudomonas amygdali pv. tabaci	Xenobiotic reductase A	374	X	A0A0Q0AXV3 (UniProt)
KPY86895	LJRM01000070	Pseudomonas syringae pv. tagetis	Xenobiotic reductase A	363		
KPY92773	LJRO01000438	Pseudomonas tremae	Xenobiotic reductase A	363		
KPY97352	LJRN01000003	Pseudomonas syringae pv. tomato	Xenobiotic reductase A	363		
KPZ03551	LJRP01000027	Pseudomonas syringae pv. aptata	NADH:flavin oxidoreductase/NADH oxidase	363		
KPZ13212	LJRR01000300	Pseudomonas syringae pv. viburni	Xenobiotic reductase A	363		
KPZ23504	LJRT01000106	Pseudomonas coronafaciens pv. zizaniae	Xenobiotic reductase A	363		
KPZ26452	LJRS01000052	Pseudomonas viridiflava	XenA	363		
KPZ30393	LJRU01000298	Pseudomonas syringae pv. theae	hypothetical protein	363		
KQN59517	LMLL01000014	Pseudomonas sp. Leaf58	NADH:flavin oxidoreductase	363		
KQQ66475	LMOA01000002	Pseudomonas sp. Leaf127	NADH:flavin oxidoreductase	363		
KRA21433	LMGQ01000006	Pseudomonas sp. Root569	NADH:flavin oxidoreductase	363		
KRD00894	LMIY01000006	Pseudomonas sp. Root9	NADH:flavin oxidoreductase	363		
KRP45927	JYLH01000006	Pseudomonas libanensis	NADH:flavin oxidoreductase	363		
KRP46498	JYLJ01000023	Pseudomonas synxantha	NADH:flavin oxidoreductase	363		
KRP49716	JYLI01000011	Pseudomonas poae	NADH:flavin oxidoreductase	363		
KRP61178	JYLK01000004	Pseudomonas trivialis	NADH:flavin oxidoreductase	363		
KRP65217	JYLL01000044	Pseudomonas veronii	NADH:flavin oxidoreductase	363		
KRP65781	JYLM01000003	Pseudomonas orientalis	NADH:flavin oxidoreductase	363		
KRP66441	JYLM01000002	Pseudomonas orientalis	NADH:flavin oxidoreductase	363		
KRP69737	JYLN01000010	Pseudomonas sp. DSM 29164	NADH:flavin oxidoreductase	363		
KRP82190	JYLO01000004	Pseudomonas sp. DSM 29167	NADH:flavin oxidoreductase	363		
KRP90091	JYLP01000014	Pseudomonas sp. DSM 28142	NADH:flavin oxidoreductase	363		
KTB59618	LKEJ01000147	Pseudomonas viridiflava ICMP 13104	NADH:flavin oxidoreductase	363		
KTB74906	LKEK01000013	Pseudomonas sp. ICMP 3272	NADH:flavin oxidoreductase	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KTB76290	LKEO01000137	Pseudomonas syringae ICMP 13102	NADH:flavin oxidoreductase	363		
KTB82988	LKEL01000109	Pseudomonas syringae pv. syringae PD2774	NADH:flavin oxidoreductase	363		
KTB87381	LKEM01000009	Pseudomonas syringae pv. syringae PD2766	NADH:flavin oxidoreductase	363		
KTB93776	LKEP01000009	Pseudomonas syringae ICMP 11293	NADH:flavin oxidoreductase	363		
KTB99733	LKGU01000023	Pseudomonas syringae ICMP 11292	NADH:flavin oxidoreductase	363		
KTC05025	LKGW01000110	Pseudomonas sp. ICMP 10191	NADH:flavin oxidoreductase	363		
KTC07361	LKGV01000012	Pseudomonas syringae ICMP 11168	NADH:flavin oxidoreductase	363		
KTC10692	LKGX01000091	Pseudomonas marginalis ICMP 11289	NADH:flavin oxidoreductase	363		
KTC28179	LKBK01000086	Pseudomonas sp. ICMP 19500	NADH:flavin oxidoreductase	363		
KTC43005	LKBL01000055	Pseudomonas sp. ABAC21	NADH:flavin oxidoreductase	363		
KTC46527	LKBQ01000178	Pseudomonas syringae pv. actinidiae ICMP 19497	NADH:flavin oxidoreductase	363		
KTC55621	LKCH01000071	Pseudomonas syringae ICMP 19498	NADH:flavin oxidoreductase	363		
KTC61416	LKCI01000009	Pseudomonas syringae ICMP 19499	NADH:flavin oxidoreductase	363		
KTS93911	LDSM01000036	Pseudomonas parafulva	NADH:flavin oxidoreductase	363		
KUR49064	LNKZ01000080	Pseudomonas syringae pv. tomato	NADPH dehydrogenase	363		
KUR49358	LNKY01000017	Pseudomonas syringae pv. tomato	NADPH dehydrogenase	363		
KWS08243	LIHR01000120	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KWS14735	LIHS01000025	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KWS21119	LIHT01000075	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KWS28871	LIHU01000017	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KWS32435	LIHW01000155	Pseudomonas syringae pv. papulans	NADH:flavin oxidoreductase	363		
KWS37873	LIHV01000184	Pseudomonas syringae pv. rhaphiolepidis	NADH:flavin oxidoreductase	363		
KWS44549	LIHY01000180	Pseudomonas amygdali pv. myricae	NADH:flavin oxidoreductase	363		
KWS47726	LIHX01000084	Pseudomonas savastanoi pv. nerii	NADH:flavin oxidoreductase	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KWS54718	LIHZ01000086	Pseudomonas amygdali pv. morsprunorum	NADH:flavin oxidoreductase	363		
KWS65852	LIIA01000109	Pseudomonas amygdali pv. morsprunorum	NADH:flavin oxidoreductase	363		
KWS68338	LIIB01000018	Pseudomonas amygdali pv. morsprunorum	NADH:flavin oxidoreductase	363		
KWS80777	LIID01000003	Pseudomonas amygdali pv. eriobotryae	NADH:flavin oxidoreductase	363		
KWS85568	LIIE01000028	Pseudomonas amygdali pv. dendropanacis	NADH:flavin oxidoreductase	363		
KWT12181	LIIJ01000048	Pseudomonas syringae pv. avii	NADH:flavin oxidoreductase	363		
KWV68804	LCYB01000047	Pseudomonas fluorescens	NADPH dehydrogenase	363		
KWV71504	LCYC01000059	Pseudomonas fluorescens	NADPH dehydrogenase	363		
KWV78740	LCYD01000008	Pseudomonas fluorescens	NADPH dehydrogenase	371	X	A0A109L0F8 (UniProt)
KWV89736	LCYA01000018	Pseudomonas fluorescens	NADPH dehydrogenase	363		
KWW14945	LNNI01000023	Stenotrophomonas rhizophila	NADH:flavin oxidoreductase	363		
KXK68425	JFBC01000827	Pseudomonas monteilii	NADH:flavin oxidoreductase	358		
KYC14061	LQXW01000089	Pseudomonas sp. ABFPK	NADH:flavin oxidoreductase	363		
KZL39595	LAGV01000018	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
KZO51922	LWDW01000089	Pseudomonas putida	NADH:flavin oxidoreductase	363		
OAE17352	LVWZ01000003	Pseudomonas brenneri	NADH:flavin oxidoreductase	363		
OAG90515	LKEH01000025	Pseudomonas viridiflava	NADH:flavin oxidoreductase	363		
OAH45484	LSTU01000060	Pseudomonas monteilii	NADH:flavin oxidoreductase	363		
OAI88493	LUCV01000029	Pseudomonas putida	NADH:flavin oxidoreductase	363		
OAK57773	LVHH01000060	Pseudomonas putida	NADH:flavin oxidoreductase	363		
OAS18859	LSUZ01000066	Pseudomonas putida	NADH:flavin oxidoreductase	363		
OBS40910	LYUP01000003	Pseudomonas syringae pv. syringae	NADH:flavin oxidoreductase	363		
OCW24493	MAUE01000026	Pseudomonas sp. S1E40	NADH:flavin oxidoreductase	363		
OCX20361	MDEN01000062	Pseudomonas graminis	NADH:flavin oxidoreductase	363		

Table A.6. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
ODJ90924	MBPF01000034	Pseudomonas viridiflava	NADH:flavin oxidoreductase	363		
OEC35283	LYSO01000199	Pseudomonas sp. AP42	NADH:flavin oxidoreductase	363		
OHC34750	MHZO01000006	Pseudomonadales bacterium RIFCSPLOWO2_12_60_38	NADH:flavin oxidoreductase	363		
OHC39719	MHZP01000088	Pseudomonadales bacterium RIFCSPLOWO2_12_FULL_59_450	NADH:flavin oxidoreductase	363		
OHW41413	MIFU01000003	Pseudomonas sp. 06C 126	NADH:flavin oxidoreductase	363		
OJT21760	MPOR01000060	Pseudomonas sp. FSL W5-0203	NADH:flavin oxidoreductase	363		
OKO48625	MPBM01000021	Pseudomonas sp. BTN1	NADH:flavin oxidoreductase	363		
OKP71313	MRXZ01000008	Pseudomonas fluorescens	NADH:flavin oxidoreductase	363		
OKS49835	MOMJ01000096	Pseudomonas syringae pv. actinidiae	NADH:flavin oxidoreductase	363		
OKS50752	MOMK01000092	Pseudomonas syringae pv. actinidiae	NADH:flavin oxidoreductase	363		
OKS53916	MOML01000088	Pseudomonas syringae pv. actinidiae	NADH:flavin oxidoreductase	363		
OKS69485	MOMM01000098	Pseudomonas syringae pv. actinidiae	NADH:flavin oxidoreductase	363		
OKS71121	MOMN01000097	Pseudomonas syringae pv. actinidiae	NADH:flavin oxidoreductase	363		
OLS64627	MKZO01000004	Pseudomonas sp. GM4FR	NADPH dehydrogenase	363		
OLY75578	LPNO01000009	Pseudomonas sp. ATCC PTA-122608	NADH:flavin oxidoreductase	363		
ONH40299	MNPU01000025	Pseudomonas gessardii	NADH:flavin oxidoreductase	363		
ONH50659	MNPW01000014	Pseudomonas cedrina subsp. cedrina	NADH:flavin oxidoreductase	363		
SCX36276	FMUC01000016	Pseudomonas sp. NFACC25	2,4-dienoyl-CoA reductase	363		
SDE90858	FNBM01000001	Pseudomonas seleniipraecipitans	2,4-dienoyl-CoA reductase	363		
SDG60929	FNCO01000002	Pseudomonas abietaniphila	2,4-dienoyl-CoA reductase	363		
SDH68626	FNCX01000005	Pseudomonas sp. BS3767	2,4-dienoyl-CoA reductase	363		
SDN38921	FNHT01000005	Pseudomonas sp. BS3759	2,4-dienoyl-CoA reductase	363		
SDN46203	FNHM01000008	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SDO43711	FNJH01000001	Pseudomonas congelans	2,4-dienoyl-CoA reductase	363		
SDQ45065	FNKR01000003	Pseudomonas gessardii	2,4-dienoyl-CoA reductase	363		

Table A.6. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
SDR29758	FNKM01000002	Pseudomonas grimontii	2,4-dienoyl-CoA reductase	363		
SDW49665	FNNY01000004	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SDZ12916	FNPP01000010	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SEC23539	FNSP01000004	Pseudomonas panacis	2,4-dienoyl-CoA reductase	363		
SEC41669	FNRS01000001	Pseudomonas taetrolens	2,4-dienoyl-CoA reductase	363		
SEE40898	FNTR01000004	Pseudomonas proteolytica	2,4-dienoyl-CoA reductase	363		
SEF02887	FNUD01000002	Pseudomonas deceptionensis	2,4-dienoyl-CoA reductase	363		
SEI50856	FNYJ01000001	Pseudomonas sp. NFR16	2,4-dienoyl-CoA reductase	363		
SEQ15057	FOEO01000004	Pseudomonas sp. NFACC02	2,4-dienoyl-CoA reductase	363		
SES71110	FOHW01000001	Pseudomonas graminis	hypothetical protein	363		
SFB33384	FOKB01000004	Pseudomonas simiae	2,4-dienoyl-CoA reductase	363		
SFH24896	FOPR01000003	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SFI81213	FOQB01000011	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SFL75637	FOTU01000004	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SFN84275	FOVV01000004	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SFO25780	FOWH01000003	Pseudomonas syringae	2,4-dienoyl-CoA reductase	363		
SFW23264	FPJB01000001	Pseudomonas sp. NFACC10-1	2,4-dienoyl-CoA reductase	363		
SFY24249	FPJI01000017	Pseudomonas sp. NFPP02	2,4-dienoyl-CoA reductase	363		
SFY40182	FPJJ01000010	Pseudomonas sp. NFR02	2,4-dienoyl-CoA reductase	363		

[1] This sequence was not included in the final primer design sequence set because it did not meet the stricter criteria used in the final review.

Table A.7. FunGene and primer design sequences for *xenB*

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AAF02539	AF154062	Pseudomonas fluorescens	xenobiotic reductase B	349	X	Q9RPM1 (UniProt)
AAN66545	AE015451	Pseudomonas putida KT2440	Xenobiotic reductase B	349		
AAY90620	CP000076	Pseudomonas protegens Pf-5	xenobiotic reductase B	349		
ABA73025	CP000094	Pseudomonas fluorescens Pf0-1	putative reductase	349	X	Q3KGT1 (UniProt)
ABP83887	CP000680	Pseudomonas mendocina ymp	NADH:flavin oxidoreductase/NADH oxidase	349		
ABQ77120	CP000712	Pseudomonas putida F1	NADH:flavin oxidoreductase/NADH oxidase	349		
ABZ00357	CP000926	Pseudomonas putida GB-1	NADH:flavin oxidoreductase/NADH oxidase	349		
ACA71491	CP000949	Pseudomonas putida W619	NADH:flavin oxidoreductase/NADH oxidase	349		
ADR58656	CP002290	Pseudomonas putida BIRD-1	XenB	349		
AEA67461	CP002585	Pseudomonas brassicacearum subsp. brassicacearum NFM421	putative reductase	349		
AEB57203	CP002620	Pseudomonas mendocina NK-01	NADH:flavin oxidoreductase/NADH oxidase	349		
AEJ14844	CP002870	Pseudomonas putida S16	NADH:flavin oxidoreductase/NADH oxidase	349		
AEV61380	CP003150	Pseudomonas fluorescens F113	NADH:flavin oxidoreductase, Old Yellow Enzyme family	349		
AFJ54804	CP003041	Pseudomonas fluorescens A506	xenobiotic reductase B	351		
AFK70508	CP003588	Pseudomonas putida ND6	NADH:flavin oxidoreductase/NADH oxidase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AGA75223	CP003738	Pseudomonas putida HB3267	NADH:flavin oxidoreductase	349		
AGD93208	JX406851	uncultured bacterium	xenobiotic reductase B	349	X	L7Y4X7 (UniProt)
AGE24813	CP004045	Pseudomonas poae RE*1-1-14	xenobiotic reductase B	351		
AGL83161	CP003190	Pseudomonas protegens CHA0	N-ethylmaleimide reductase NemA	349		
AGN80394	CP005976	Pseudomonas putida H8234	NADH:flavin oxidoreductase	349		
AGS77941	KF055345	Pseudomonas putida	xenobiotic reductase B	349		
AGZ33755	CP003961	Pseudomonas sp. VLB120	NADH:flavin oxidoreductase	349		
AHC34158	CP006852	Pseudomonas sp. TKP	NADH:flavin oxidoreductase	351		
AHC84358	CP006978	Pseudomonas monteilii SB3078	NADH:flavin oxidoreductase	349		
AHC89729	CP006979	Pseudomonas monteilii SB3101	NADH:flavin oxidoreductase	349		
AHD16245	CP007012	Pseudomonas sp. FGI182	NADH:flavin oxidoreductase	349		
AHL32686	CP007410	Pseudomonas brassicacearum	NADH:flavin oxidoreductase	349		
AHZ69210	CP005960	Pseudomonas mandelii JR-1	xenobiotic reductase B	349	X	A0A024E9G9 (UniProt)
AHZ79197	CP007620	Pseudomonas putida	NADH:flavin oxidoreductase	349		
AIB35284	CP007637	Pseudomonas simiae	NADH:flavin oxidoreductase	351		
AIB40980	CP007638	Pseudomonas sp. WCS374	NADH:flavin oxidoreductase	351		
AIC18479	CP008696	Pseudomonas chlororaphis	NADH:flavin oxidoreductase	349		
AIG05209	CP008896	Pseudomonas fluorescens	NADH:flavin oxidoreductase	351		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
AIN56779	CP009365	Pseudomonas mosselii SJ10	NADH:flavin oxidoreductase	349		
AIS14518	CP009290	Pseudomonas chlororaphis subsp. aurantiaca	NADH:flavin oxidoreductase	349		
AJA12635	CP009974	Pseudomonas putida S12	NADH:flavin oxidoreductase	349		
AJG11724	CP010359	Pseudomonas plecoglossicida	NADH:flavin oxidoreductase	349		
AJO77016	CP010892	Pseudomonas sp. MRSN12121	NADH:flavin oxidoreductase	349		
AJP51036	CP010896	Pseudomonas fluorescens	xenobiotic reductase B	351		
AJQ50713	CP010979	Pseudomonas putida S13.1.2	NADH:flavin oxidoreductase	349		
AJZ92320	CP005975	Pseudomonas fluorescens PICF7	NADH:flavin oxidoreductase	351		
AKA26193	CP011110	Pseudomonas chlororaphis	NADH:flavin oxidoreductase	349		
AKA85083	CP011117	Pseudomonas fluorescens	NADH:flavin oxidoreductase, Old Yellow Enzyme family	351		
AKK00454	CP011020	Pseudomonas chlororaphis	NADH:flavin oxidoreductase	349		
AKS05896	CP011507	Pseudomonas trivialis	NADH:flavin oxidoreductase	351		
AKV07122	CP010945	Pseudomonas fluorescens NCIMB 11764	NADH:flavin oxidoreductase	349	X	A0A0K1QN81 (UniProt)
ALE89407	CP012676	Pseudomonas sp. L10.10	NADH:flavin oxidoreductase	349		
ALI02756	CP012830	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349	X	A0A0N7H0C5 (UniProt)
ALI06144	CP012831	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
ALN20415	CP013124	Pseudomonas mendocina S5.2	alkene reductase	349		
ALQ02030	CP012680	Pseudomonas brassicacearum	NADH:flavin oxidoreductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AMB80784	CP013861	Pseudomonas fragi	alkene reductase	349		
AMB85847	CP014135	Pseudomonas agarici	alkene reductase	349		
AMK29616	CP014343	Pseudomonas putida	NADH:flavin oxidoreductase	349		
AMN79393	CP014546	Pseudomonas azotoformans	alkene reductase	351		
AMO78612	CP014158	Pseudomonas citronellolis	N-ethylmaleimide reductase	349		
AMQ86030	CP014205	Pseudomonas sp. MS586	alkene reductase	349		
AMS13897	CP014867	Pseudomonas chlororaphis	alkene reductase	349		
AMS20787	CP014868	Pseudomonas fluorescens	alkene reductase	351		
AMT87710	CP014947	Pseudomonas koreensis	alkene reductase	349		
AMW85394	CP012400	Pseudomonas fluorescens	NADH:flavin oxidoreductase, Old Yellow Enzyme family	351	X	A0A143GMM1 (UniProt)
AMZ72190	CP015225	Pseudomonas fluorescens	alkene reductase	349		
ANC84610	CP015202	Pseudomonas putida B6-2	alkene reductase	349		
ANF84946	CP015600	Pseudomonas antarctica	NADH:flavin oxidoreductase	351		
ANH97038	CP015852	Pseudomonas koreensis	alkene reductase	349	X	A0A1A9JD21 (UniProt)
ANI01920	CP015876	Pseudomonas putida SJTE-1	alkene reductase	349		
ANI17272	CP015878	Pseudomonas citronellolis	alkene reductase	349		
ANI36191	CP011525	Pseudomonas sp. JY-Q	NADH:flavin oxidoreductase	349		
ANI53064	CP011566	Pseudomonas sp. DR 5-09	NADH:flavin oxidoreductase	349		
ANI62042	CP011567	Pseudomonas sp. GR 6-02	NADH:flavin oxidoreductase	349	X	A0A173JP35 (UniProt)
ANJ57850	CP014870	Pseudomonas sp. A3(2016)	alkene reductase	349		
ANY88072	CP016634	Pseudomonas putida	N-ethylmaleimide reductase	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
AOE64385	CP014262	Pseudomonas corrugata	alkene reductase	349	X	A0A1B3CH67 (UniProt)
AOE66706	CP015637	Pseudomonas fluorescens	alkene reductase	351		
AOE72527	CP015638	Pseudomonas fluorescens	alkene reductase	351		
AOE78173	CP015639	Pseudomonas fluorescens	alkene reductase	351		
AOS39011	CP014693	Pseudomonas brassicacearum	alkene reductase	349		
AOS72848	CP017296	Pseudomonas fluorescens	alkene reductase	351		
APC15562[1]	CP017886 (1521004-1522053)	Pseudomonas frederiksbergensis	Alkene reductase	349	X	A0A1J0EHH6 (UniProt)
BAN52746	AP013070	Pseudomonas putida NBRC 14164	xenobiotic reductase B	349		
BAO60736	AP014522	Pseudomonas protegens Cab57	xenobiotic reductase B	349		
BAQ73051	AP014627	Pseudomonas sp. Os17	xenobiotic reductase B	349		
BAQ79279	AP014628	Pseudomonas sp. St29	xenobiotic reductase B	349		
BAV29269	AP017423	Pseudomonas sp. LAB-08	NADH:flavin oxidoreductase	349		
BAV73391	AP014623	Pseudomonas chlororaphis subsp. aurantiaca	xenobiotic reductase B	349		
CAY47641	AM181176	Pseudomonas fluorescens SBW25	putative reductase	351		
CCH36864	CAIG01000006	Pseudomonas pseudoalcaligenes CECT 5344	NADH:flavin oxidoreductase/NADH oxidase	349		
CDF93320	CBLV010000095	Pseudomonas sp. SHC52	NADH:flavin oxidoreductases, Old Yellow Enzyme family	349	X	A0A0A1HSW6 (UniProt)
CDM41878	HG916826	Pseudomonas pseudoalcaligenes CECT 5344	NADH:flavin oxidoreductase/NADH oxidase	349		
CDR92503	LK391695	Pseudomonas pseudoalcaligenes	NADH:flavin oxidoreductase/NADH oxidase	349		
CEL27966	CDMF01000001	Pseudomonas fluorescens	N-ethylmaleimide reductase	349	X	A0A0B7D4B5 (UniProt)

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
CRI58840	LN847264	Pseudomonas sp. CCOS 191	N-ethylmaleimide reductase	349		
CRL48432	LN854573	Pseudomonas sp. URMO17WK12:I11	N-ethylmaleimide reductase	349	X	A0A0S4HRC9 (UniProt)
CRM01181	CVTI01000009	Pseudomonas sp. 24 R 17	N-ethylmaleimide reductase	351		
CRM02824	CVTK01000026	Pseudomonas sp. 28 E 9	N-ethylmaleimide reductase	351		
CRM03580	CVTJ01000021	Pseudomonas sp. 25 E 4	N-ethylmaleimide reductase	351		
CRM17195	CVTM01000031	Pseudomonas sp. 31 E 5	N-ethylmaleimide reductase	349		
CRM27102	CVTL01000022	Pseudomonas sp. 31 E 6	N-ethylmaleimide reductase	349		
CRM28733	CVTT01000038	Pseudomonas sp. 58 R 3	N-ethylmaleimide reductase	351	X	A0A1B5D928 (UniProt)
CRM30878	CVTG01000018	Pseudomonas sp. 24 E 1	N-ethylmaleimide reductase	351		
CRM45217	CVTU01000090	Pseudomonas sp. 44 R 15	N-ethylmaleimide reductase	351		
CRM47242	CVTP01000021	Pseudomonas sp. 58 R 12	N-ethylmaleimide reductase	351		
CRM51727	CVTN01000065	Pseudomonas sp. 31 R 17	N-ethylmaleimide reductase	351		
CRM55034	CVTS01000040	Pseudomonas sp. 25 R 14	N-ethylmaleimide reductase	351		
CRM56446	CVTO01000117	Pseudomonas sp. 24 E 13	N-ethylmaleimide reductase	351		
CRM60541	CVTV01000050	Pseudomonas sp. 37 R 15	N-ethylmaleimide reductase	351		
CRM85006	CVTH01000092	Pseudomonas sp. 8 R 14	N-ethylmaleimide reductase	351		
CRM94062	CVTW01001110	Pseudomonas sp. 22 E 5	N-ethylmaleimide reductase	351		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
CRM95738	CVTW01001144	Pseudomonas sp. 22 E 5	N-ethylmaleimide reductase	349	X	A0A1B5FAM7 (UniProt)
CRN01384	CVTX01000141	Pseudomonas sp. 34 E 7	N-ethylmaleimide reductase	351		
EFQ64781	AEAZ01000007	Pseudomonas fluorescens WH6	NADH:flavin oxidoreductase/NADH oxidase	349	X	E2XN25 (UniProt)
EGB95234	AEWE02000091	Pseudomonas sp. TJI-51	NADH:flavin oxidoreductase	349		
EIK63596	AHPN01000001	Pseudomonas fluorescens SS101	xenobiotic reductase B	351		
EIK68118	AHPP01000002	Pseudomonas synxantha BG33R	xenobiotic reductase B	351		
EIK70206	AHPO01000001	Pseudomonas fluorescens Q8r1-96	xenobiotic reductase B	349		
EIK98083	AJWX01000002	Pseudomonas sp. M47T1	xenobiotic reductase B	349		
EIM14524	AHOT01000027	Pseudomonas chlororaphis O6	xenobiotic reductase B	349		
EJF73535	AKVH01000058	Pseudomonas sp. Ag1	xenobiotic reductase B	351	X	J1IR65 (UniProt)
EJL01909	AGBM01000001	Pseudomonas fluorescens Q2-87	xenobiotic reductase B	349	X	J2Y524 (UniProt)
EJL08155	AHHJ01000004	Pseudomonas chlororaphis subsp. aureofaciens 30-84	xenobiotic reductase B	349		
EJL97622	AKJB01000085	Pseudomonas sp. GM102	NADH:flavin oxidoreductase	349	X	J2V2F2 (UniProt)
EJL98803	AKJV01000061	Pseudomonas sp. GM16	NADH:flavin oxidoreductase	349	X	J2MLJ3 (UniProt)
EJM14726	AKJS01000132	Pseudomonas sp. GM21	NADH:flavin oxidoreductase	349		
EJM19669	AKJT01000028	Pseudomonas sp. GM18	NADH:flavin oxidoreductase	349	X	J2P8U7 (UniProt)
EJM24862	AKJQ01000056	Pseudomonas sp. GM25	NADH:flavin oxidoreductase	349		
EJM45429	AKJR01000019	Pseudomonas sp. GM24	NADH:flavin oxidoreductase	349		
EJM64761	AKJK01000070	Pseudomonas sp. GM50	NADH:flavin oxidoreductase	349	X	J2SCW5 (UniProt)

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
EJM86044	AKJI01000027	Pseudomonas sp. GM60	NADH:flavin oxidoreductase	349		
EJM89053	AKJH01000036	Pseudomonas sp. GM67	NADH:flavin oxidoreductase	349		
EJN19686	AKJE01000087	Pseudomonas sp. GM79	NADH:flavin oxidoreductase	349	X	J3I9U5 (UniProt)
EJN30950	AKJF01000061	Pseudomonas sp. GM78	NADH:flavin oxidoreductase	349		
EJN32926	AKJD01000056	Pseudomonas sp. GM80	NADH:flavin oxidoreductase	349	X	J3IPY2 (UniProt)
EJN39968	AKJC01000028	Pseudomonas sp. GM84	NADH:flavin oxidoreductase	349		
EJO92734	ALKM01000018	Pseudomonas mendocina DLHK	NADH:flavin oxidoreductase	349		
EJZ56958[1]	CM001561 (1441846-1440797)	Pseudomonas fluorescens R124	NADH:flavin oxidoreductase	349	X	K0WBQ2 (UniProt)
EKN47447	AMQP01000035	Pseudomonas viridiflava UASWS0038	NADH:flavin oxidoreductase	349		
ELQ17266	AMZW01000070	Pseudomonas fluorescens BRIP34879	xenobiotic reductase B	351		
EMI07731	AOGS01000008	Pseudomonas sp. Lz4W	NADH:flavin oxidoreductase/NADH oxidase	349		
EMR45607	ALPV02000017	Pseudomonas putida LS46	NADH:flavin oxidoreductase	349		
ENY79318	APBQ01000013	Pseudomonas putida TRO1	NADH:flavin oxidoreductase/NADH oxidase	349		
EPA95962	APIO01000051	Pseudomonas sp. G5(2012)	NADH:flavin oxidoreductase	349		
EPB93281	ASJX01000087	Pseudomonas plecoglossicida NB2011	NADH:flavin oxidoreductase	349		
EPJ83344	ATLM01000040	Pseudomonas sp. CFT9	putative reductase	351		
EPJ87362	ATLN01000082	Pseudomonas sp. CFII68	xenobiotic reductase B	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
EPJ96296	ATLR01000008	Pseudomonas sp. CF149	xenobiotic reductase B	349		
EPL13953	ATLQ01000011	Pseudomonas sp. CF150	putative reductase	351		
ERH53411	AVQF01000001	Pseudomonas mendocina EGD-AQ5	NADH:flavin oxidoreductase	349		
ERH56204	AVQG01000023	Pseudomonas fluorescens EGD-AQ6	NADH:flavin oxidoreductase	351		
ERI51154	AVOF01000414	Pseudomonas sp. EGD-AK9	NADH:flavin oxidoreductase	349		
ERL02865	AOUR02000011	Pseudomonas putida LF54	NADH:flavin oxidoreductase	349		
ERT19414	AXDX02000028	Pseudomonas putida SJ3	NADH:flavin oxidoreductase	349		
ESP81685	AYKV01000339	Pseudomonas putida S12	NADH:flavin oxidoreductase	349		
ESU56671	AXUO01000023	Pseudomonas mosselii SJ10	NADH:flavin oxidoreductase	349		
ESW55952	AKXH02000078	Pseudomonas fluorescens BBc6R8	NADH:flavin oxidoreductase, Old Yellow Enzyme family	351	X	V7E6V6 (UniProt)
ETD36775	AYUD01000017	Pseudomonas chlororaphis subsp. aurantiaca PB-St2	NADH:flavin oxidoreductase	349		
ETF08564	AYMZ01000003	Pseudomonas moraviensis R28-S	NADH:flavin oxidoreductase	349		
ETK14997	AOHM01000129	Pseudomonas sp. FH1	putative reductase	351		
ETK20007	AOHN01000007	Pseudomonas sp. FH4	xenobiotic reductase B	351		
ETK40031	AOJA01000053	Pseudomonas fluorescens FH5	NADH:flavin oxidoreductase	351		
ETM67429	ANIR02000002	Pseudomonas sp. M1	NADH:flavin oxidoreductase	349		
EUB72080	AKJN02000008	Pseudomonas sp. GM41(2012)	12-oxophytodienoate reductase	349		
EUB88069	AKJP02000001	Pseudomonas sp. GM30	12-oxophytodienoate reductase	349	X	W6VUU6 (UniProt)

Table A.7. (cont’d)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
EXF91143	AFOY02000028	Pseudomonas fluorescens HK44	NADH:flavin oxidoreductase	349	X	A0A010SIM9 (UniProt)
EZI29412	AHIP01000004	Pseudomonas extremaustralis 14-3 substr. 14-3b	NADH:flavin oxidoreductase	351		
EZP27080	JFYN01000041	Pseudomonas sp. RIT288	putative reductase	349	X	A0A031FTW4 (UniProt)
EZP65263	JFYX01000027	Pseudomonas sp. RIT357	putative reductase	351	X	A0A031IVN5 (UniProt)
KDD66191	AZQQ01000100	Pseudomonas mandelii PD30	NADH:flavin oxidoreductase	349	X	A0A059KW36 (UniProt)
KER98673	JOVT01000001	Pseudomonas mendocina	NADH:flavin oxidoreductase	349		
KES20626	JNCW01000027	Pseudomonas sp. AAC	NADH:flavin oxidoreductase	349		
KEX92696	JOJW01000600	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KEY85454	JMIT01000004	Pseudomonas sp. WCS358	NADH:flavin oxidoreductase	349		
KFB13754	AMCD02000001	Pseudomonas mendocina S5.2	NADH:flavin oxidoreductase	349		
KFF43528	JPRX01000001	Pseudomonas sp. BRG-100	NADH:flavin oxidoreductase	351		
KFX68328	AWSQ01000006	Pseudomonas taeanensis MS-3	NADH:flavin oxidoreductase	349		
KGE65450	ASGY01000188	Pseudomonas fluorescens LMG 5329	NADH:flavin oxidoreductase	351		
KGF63359	JRMB01000002	Pseudomonas lutea	NADH:flavin oxidoreductase	349		
KGI93390	JRPO01000017	Pseudomonas sp. H2	NADH:flavin oxidoreductase	349		
KGK27307	JOJY01000001	Pseudomonas plecoglossicida	NADH:flavin oxidoreductase	349		
KGU84577	AUPB01000037	Pseudomonas mediterranea CFBP 5447	NADH:flavin oxidoreductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KHA75274	JSFK01000001	Pseudomonas chlororaphis	NADH:flavin oxidoreductase	349	X	A0A0A6DL87 (UniProt)
KHK62616	JQGJ02000014	Pseudomonas frederiksbergensis	NADH:flavin oxidoreductase	349	X	A0A0B1Z038 (UniProt)
KHL72640	JTCJ01000582	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KIC82144	JWLN01000013	Pseudomonas sp. C5pp	NADH:flavin oxidoreductase	349		
KIF57554	JTGH01000018	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KIF57970	JTGG01000017	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KIH84784	JXDG01000014	Pseudomonas batumici	NADH:flavin oxidoreductase, old yellow enzyme family	349	X	A0A0C2EFI2 (UniProt)
KII35693	JSAL01000011	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KII36337	JSAK01000003	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KIK86261	JTKF01000026	Pseudomonas sp. W15Feb9B	NADH:flavin oxidoreductase	349	X	A0A0C2R9U2 (UniProt)
KIP94325	JXQY01000013	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KIQ06552	JXQW01000001	Pseudomonas fulva	NADH:flavin oxidoreductase	349	X	A0A0D0L692 (UniProt)
KIQ15503	JXQT01000001	Pseudomonas simiae	NADH:flavin oxidoreductase	351		
KIQ34136	JXQO01000022	Pseudomonas viridiflava	NADH:flavin oxidoreductase	349		
KIQ58286	JXNZ01000156	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KIR14312	JXOE01000052	Pseudomonas fluorescens	N-ethylmaleimide reductase	349		
KIR20920	JXCQ01000036	Pseudomonas fluorescens	N-ethylmaleimide reductase	351		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KIY40023	JYKS01000023	Pseudomonas sp. 10-1B	NADH:flavin oxidoreductase	349		
KJH75266	JZRI01000119	Pseudomonas sp. ES3-33	NADH:flavin oxidoreductase	349		
KJH87387	JYHW01000021	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KJU76655	JTFL01000133	Pseudomonas pseudoalcaligenes	NADH:flavin oxidoreductase	349		
KJZ38146	LACE01000008	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KJZ45649	LACD01000008	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349		
KJZ51510	LACF01000033	Pseudomonas fluorescens	NADH:flavin oxidoreductase	351		
KJZ60693	LACG01000012	Pseudomonas fluorescens	NADH:flavin oxidoreductase	351		
KJZ63606	LACH01000053	Pseudomonas fluorescens	NADH:flavin oxidoreductase	349	X	A0A0F4V499 (UniProt)
KKA05202	JZXC01000030	Pseudomonas kilonensis	NADH:flavin oxidoreductase	349		
KKO13364	AYRY01000036	Pseudomonas putida KG-4	NADH:flavin oxidoreductase	349		
KKX64351	JTEN01000036	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KMM80195	JYKX01000002	Pseudomonas deceptionensis	NADH:flavin oxidoreductase	349		
KMM90164	JYKY01000010	Pseudomonas lundensis	NADH:flavin oxidoreductase	349		
KMM94028	JYLB01000002	Pseudomonas lini	NADH:flavin oxidoreductase	349		
KMN01515	JYKZ01000002	Pseudomonas psychrophila	NADH:flavin oxidoreductase	349		
KMN14846	JYLF01000002	Pseudomonas sp. DSM 29166	NADH:flavin oxidoreductase	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KMN19599	JYLG01000002	Pseudomonas sp. DSM 28140	NADH:flavin oxidoreductase	349		
KMT57316	LFMW01000001	Pseudomonas sp. KG01	NADH:flavin oxidoreductase	351		
KMU97538	LFYQ01000002	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KMY37166	LDJF01000005	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KNH21892	LFQK01000047	Pseudomonas syringae	NADH:flavin oxidoreductase	349	X	A0A0L1M0A7 (UniProt)
KNH46712	LFQO01000022	Pseudomonas lini	NADH:flavin oxidoreductase	349		
KNX79790	JHEE01000030	Pseudomonas sp. 250J	NADH:flavin oxidoreductase	349		
KOX63247	LBHT01000123	Pseudomonas psychrophila	NADH:flavin oxidoreductase	349		
KOY02903	LIUV01000004	Pseudomonas sp. 655	NADH:flavin oxidoreductase	349	X	A0A0M9B180 (UniProt)
KPG71943	LHOY01000037	Pseudomonas libanensis	NADH:flavin oxidoreductase	351		
KPG81095	LHPA01000026	Pseudomonas sp. RIT-PI-o	NADH:flavin oxidoreductase	349		
KPG89508	LHPC01000077	Pseudomonas sp. RIT-PI-q	NADH:flavin oxidoreductase	349		
KPG90359	LIGE01000047	Pseudomonas sp. RIT-PI-r	NADH:flavin oxidoreductase	349	X	A0A0P6RJL3 (UniProt)
KPL63051	JRXH01000090	Pseudomonas viridiflava	NADH:flavin oxidoreductase	349		
KPM61121	LKKS01000116	Pseudomonas putida	NADH:flavin oxidoreductase	353		
KPM63840	LKKT01000050	Pseudomonas putida	NADH:flavin oxidoreductase	349		
KPN92360	LIRD01000002	Pseudomonas sp. In5	NADH:flavin oxidoreductase	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KPW58318	LJPW01000107	Pseudomonas caricapapayae	NADH:flavin oxidoreductase	349		
KPX45515	LJQM01000119	Pseudomonas syringae pv. helianthi	NADH:flavin oxidoreductase	349		
KPY47232	LJRF01000107	Pseudomonas syringae pv. ribicola	NADH:flavin oxidoreductase	349		
KPY82151	LJRM01000168	Pseudomonas syringae pv. tagetis	NADH:flavin oxidoreductase	349		
KPZ26549	LJRS01000052	Pseudomonas viridiflava	NADH:flavin oxidoreductase	349		
KQB53953	LLWH01000124	Pseudomonas endophytica	NADH:flavin oxidoreductase	349		
KQM55866	LMKI01000001	Pseudomonas sp. Leaf15	alkene reductase	351		
KQN52963	LMLH01000007	Pseudomonas sp. Leaf48	alkene reductase	349	X	A0A0S8ZHZ0 (UniProt)
KQO43820	LMMB01000001	Pseudomonas sp. Leaf83	alkene reductase	349		
KQT66828	LMQZ01000001	Pseudomonas sp. Leaf434	alkene reductase	349		
KQV22463	LMCV01000002	Pseudomonas sp. Root329	alkene reductase	349		
KQW33059	LMDO01000014	Pseudomonas sp. Root401	alkene reductase	349		
KQZ80538	LMGK01000024	Pseudomonas sp. Root562	alkene reductase	349		
KRA08390	LMGQ01000025	Pseudomonas sp. Root569	alkene reductase	351		
KRC91733	LMIY01000033	Pseudomonas sp. Root9	alkene reductase	351		
KRP46219	JYLH01000005	Pseudomonas libanensis	NADH:flavin oxidoreductase	351		
KRP52035	JYLI01000005	Pseudomonas poae	NADH:flavin oxidoreductase	351		
KRP56740	JYLJ01000002	Pseudomonas synxantha	NADH:flavin oxidoreductase	351		
KRP60927	JYLK01000005	Pseudomonas trivialis	NADH:flavin oxidoreductase	351		
KRP66147	JYLM01000003	Pseudomonas orientalis	NADH:flavin oxidoreductase	351		

Table A.7. (cont’d)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
KRP72691	JYLO01000021	Pseudomonas sp. DSM 29167	NADH:flavin oxidoreductase	351		
KRP73729	JYLN01000002	Pseudomonas sp. DSM 29164	NADH:flavin oxidoreductase	351	X	A0A0R3AUE0 (UniProt)
KRP78024	JYLL01000009	Pseudomonas veronii	NADH:flavin oxidoreductase	351		
KRP95344	JYLP01000006	Pseudomonas sp. DSM 28142	NADH:flavin oxidoreductase	351		
KRV76043	LKJO01000074	Pseudomonas citronellolis	NADH:flavin oxidoreductase	349		
KRW79973	LKKN01000043	Pseudomonas citronellolis	NADH:flavin oxidoreductase	349		
KSW24251	LKAX01000007	Pseudomonas sp. ADP	NADH:flavin oxidoreductase	349		
KTB60191	LKEF01000041	Pseudomonas fluorescens ICMP 11288	NADH:flavin oxidoreductase	351	X	A0A0W0HHA2 (UniProt)
KTB63178	LKEI01000025	Pseudomonas fluorescens	NADH:flavin oxidoreductase	351		
KTC10619	LKGX01000091	Pseudomonas marginalis ICMP 11289	NADH:flavin oxidoreductase	349		
KTC17938	LKGY01000077	Pseudomonas marginalis ICMP 9505	NADH:flavin oxidoreductase	351		
KTC30003	LKBK01000063	Pseudomonas sp. ICMP 19500	NADH:flavin oxidoreductase	351		
KTC30506	LKBM01003010	Pseudomonas sp. ABAC61	NADH:flavin oxidoreductase	349		
KTC64218	LKBO01000144	Pseudomonas fluorescens ABAC62	NADH:flavin oxidoreductase	351		
KUJ91836	LGEP01000163	Pseudomonas sp. 63_8	NADH:flavin oxidoreductase/NADH oxidase	349		
KVV02262	LLWI01000080	Pseudomonas sp. TAD18	N-ethylmaleimide reductase	349		
KVV03976	LLWJ01000070	Pseudomonas sp. TAA207	N-ethylmaleimide reductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
KWR82998	JWMC01000019	Pseudomonas sp. PI1	alkene reductase	349		
KWU51469	LRMR01000007	Pseudomonas fluorescens	alkene reductase	351	X	A0A0X7K752 (UniProt)
KWV70004	LCYB01000031	Pseudomonas fluorescens	N-ethylmaleimide reductase	351		
KWV73294	LCYC01000048	Pseudomonas fluorescens	N-ethylmaleimide reductase	351		
KWV82444	LCYD01000004	Pseudomonas fluorescens	N-ethylmaleimide reductase	351		
KWW17213	LNNI01000011	Stenotrophomonas rhizophila	alkene reductase	349		
KXK70423	JFBC01000463	Pseudomonas monteilii	NADH:flavin oxidoreductase	349		
KYC15044	LQXW01000067	Pseudomonas sp. ABFPK	alkene reductase	349		
KZN18510	LUKJ01000003	Pseudomonas fluorescens	alkene reductase	349	X	A0A166P6Y4 (UniProt)
KZO57322	LWDW01000001	Pseudomonas putida	alkene reductase	349		
OAB51206	LRSO01000018	Pseudomonas thivervalensis	alkene reductase	349		
OAE13969	LUXZ01000001	Pseudomonas simiae	alkene reductase	351		
OAG90436	LKEH01000025	Pseudomonas viridiflava	NADH:flavin oxidoreductase	349		
OAH44117	LSTU01000067	Pseudomonas monteilii	alkene reductase	349		
OAJ46344	LKEG01000053	Pseudomonas marginalis	NADH:flavin oxidoreductase	351		
OAK58391	LVHH01000054	Pseudomonas putida	alkene reductase	349		
OAS06649	LSUZ01000179	Pseudomonas putida	alkene reductase	349		
OBP13010	LZYC01000004	Pseudomonas sp. EGD-AKN5	alkene reductase	349		
OBZ25625	MAUK01000008	Pseudomonas protegens	alkene reductase	349		
OBZ31261	MAUL01000003	Pseudomonas protegens	alkene reductase	349		
OCT21678	MCBI01000076	Pseudomonas putida	alkene reductase	349		
OCT22860	MCBH01000057	Pseudomonas putida	alkene reductase	349		
OCT23449	MCBG01000061	Pseudomonas putida	alkene reductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
OCT36411	MCBJ01000053	Pseudomonas putida	alkene reductase	349		
OCW26635	MBDT01000005	Pseudomonas sp. S3E12	alkene reductase	351		
OCW30538	MAUE01000001	Pseudomonas sp. S1E40	alkene reductase	351		
OCX11190	MDEN01000069	Pseudomonas graminis	alkene reductase	349		
ODJ90848	MBPF01000034	Pseudomonas viridiflava	alkene reductase	349		
OEC50081	LYSO01000044	Pseudomonas sp. AP42	alkene reductase	351		
OEC69559	LXJP01000033	Pseudomonas sp. AP19	alkene reductase	351		
OFJ41211	MKQJ01000035	Pseudomonas koreensis	alkene reductase	349		
OFS70239	LWMZ01000089	Pseudomonas sp. HMSC08G10	alkene reductase	349		
OHC26220	MHZK01000211	Pseudomonadales bacterium RIFCSPHIGHO2_02_FULL_60_43	alkene reductase	349		
OHC32763	MHZO01000025	Pseudomonadales bacterium RIFCSPLOWO2_12_60_38	alkene reductase	351		
OHC41724	MHZP01000028	Pseudomonadales bacterium RIFCSPLOWO2_12_FULL_59_450	alkene reductase	351		
OHR79983	LTNB01000814	Pseudomonas sp. HMSC75E02	alkene reductase	349		
OHW38538	MIFU01000097	Pseudomonas sp. 06C 126	alkene reductase	351		
OIN11636	MDGK01000015	Pseudomonas extremorientalis	alkene reductase	351		
OIN44232	MDDQ01000035	Pseudomonas azotoformans	alkene reductase	351		
OIN52527	MDDR01000026	Pseudomonas costantinii	alkene reductase	351		
OJT28512	MPOR01000026	Pseudomonas sp. FSL W5-0203	alkene reductase	351		
OJT48555	MQUK01000011	Pseudomonas moraviensis	alkene reductase	349		
OKA19855	MPJC01000009	Pseudomonas sp. A4R1.5	alkene reductase	349		
OKA23865	MPJD01000018	Pseudomonas sp. A4R1.12	alkene reductase	349		
OKK42947	MRUA01000010	Pseudomonas protegens	alkene reductase	349		
OKK46238	MRTZ01000005	Pseudomonas protegens	alkene reductase	349		
OKK61234	MRUB01000004	Pseudomonas protegens	alkene reductase	349		
OKK65769	MRUC01000003	Pseudomonas protegens	alkene reductase	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
OKO46042	MPBM01000048	Pseudomonas sp. BTN1	alkene reductase	351		
OKP74931	MRXZ01000001	Pseudomonas fluorescens	alkene reductase	351		
OLU02012	MSTQ01000009	Pseudomonas reinekei	alkene reductase	349		
OLY74077	LPNO01000020	Pseudomonas sp. ATCC PTA-122608	alkene reductase	351		
OMQ37512	MNAH01000003	Pseudomonas putida	alkene reductase	349		
ONH37534	MNPU01000059	Pseudomonas gessardii	alkene reductase	351		
ONH42989	MNPV01000006	Pseudomonas azotoformans	alkene reductase	351		
ONH46592	MNPV01000002	Pseudomonas azotoformans	alkene reductase	351		
ONH56488	MNPW01000002	Pseudomonas cedrina subsp. cedrina	alkene reductase	351		
SAM32360	FKLB01000037	Pseudomonas sp. 1 R 17	N-ethylmaleimide reductase	351		
SBW79344	LT599583	Pseudomonas veronii 1YdBTEX2	N-ethylmaleimide reductase	351		
SCW41458	FMTL01000001	Pseudomonas peli	2,4-dienoyl-CoA reductase	349		
SCW83011	FMTR01000011	Pseudomonas sp. NFACC05-1	2,4-dienoyl-CoA reductase	349		
SCW93541	FMTK01000013	Pseudomonas sp. NFACC56-3	2,4-dienoyl-CoA reductase	349		
SCX20837	FMUC01000003	Pseudomonas sp. NFACC25	2,4-dienoyl-CoA reductase	351		
SCX70584	FMUL01000016	Pseudomonas sp. NFACC32-1	2,4-dienoyl-CoA reductase	349		
SCY98277	FMUZ01000023	Pseudomonas sp. NFACC37-1	2,4-dienoyl-CoA reductase	349		
SCZ23436	FMVX01000002	Pseudomonas sp. NFIX46	2,4-dienoyl-CoA reductase	349		
SCZ41724	FMWA01000021	Pseudomonas sp. NFACC44-2	2,4-dienoyl-CoA reductase	349		
SCZ67909	FMWI01000003	Pseudomonas sp. NFPP17	2,4-dienoyl-CoA reductase	349		
SDA21722	FMWW01000003	Pseudomonas sp. NFPP12	2,4-dienoyl-CoA reductase	349		
SDA53725	FMXG01000001	Pseudomonas sp. NFPP33	2,4-dienoyl-CoA reductase	349		
SDA63826	FMXL01000003	Pseudomonas sp. NFPP15	2,4-dienoyl-CoA reductase	349		
SDA88071	FMXH01000017	Pseudomonas sp. NFACC51	2,4-dienoyl-CoA reductase	349		
SDA90200	FMXJ01000025	Pseudomonas sp. NFACC15-1	2,4-dienoyl-CoA reductase	349		

Table A.7. (cont'd)

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
SDB42891	FMXV01000011	Pseudomonas sp. NFACC13-1	2,4-dienoyl-CoA reductase	349		
SDB51512	FMYA01000031	Pseudomonas sp. NFACC17-2	2,4-dienoyl-CoA reductase	349		
SDB56938	FMXW01000008	Pseudomonas putida	2,4-dienoyl-CoA reductase	349		
SDC59335	FMZQ01000004	Pseudomonas chengduensis	2,4-dienoyl-CoA reductase	349		
SDD13725	FMYX01000006	Pseudomonas guariconensis	2,4-dienoyl-CoA reductase	349		
SDD61794	FNAE01000001	Pseudomonas alcaliphila	2,4-dienoyl-CoA reductase	349		
SDI29899	FNCO01000012	Pseudomonas abietaniphila	2,4-dienoyl-CoA reductase	349		
SDQ02331	FNJJ01000019	Pseudomonas guguanensis	2,4-dienoyl-CoA reductase	349		
SDQ15778	FNKJ01000002	Pseudomonas moorei	2,4-dienoyl-CoA reductase	349		
SDQ53006	FNLA01000002	Pseudomonas lundensis	2,4-dienoyl-CoA reductase	349		
SDQ86728	FNKR01000003	Pseudomonas gessardii	2,4-dienoyl-CoA reductase	351		
SDR39561	FNKM01000002	Pseudomonas grimontii	2,4-dienoyl-CoA reductase	351		
SDU06377[1]	LT629796 (677132-678181)	Pseudomonas mandelii	dienoyl-CoA reductase	349	X	A0A1H2FG62 (UniProt)
SDU07035[1]	LT629793 (1929328-1930383)	Pseudomonas yamanorum	dienoyl-CoA reductase	351	X	A0A1H2FI93 (UniProt)
SDV02422[1]	LT629802 (3538029-3539084)	Pseudomonas mucidolens	dienoyl-CoA reductase	351	X	A0A1H2NAM4 (UniProt)
SDV13310[1]	LT629803 (4140557-4141606)	Pseudomonas vancouverensis	N3_9PSED/1-349 2,4-dienoyl-CoA reductase	349	X	A0A1H2P6N3 (UniProt)
SDY29456	FNNL01000015	Pseudomonas sp. NFACC08-1	2,4-dienoyl-CoA reductase	349		
SDY38662	FNOX01000003	Pseudomonas salomonii	2,4-dienoyl-CoA reductase	351	X	SEE22002.1 (NCBI)
SDY97247	FNOA01000023	Pseudomonas sp. NFACC14	2,4-dienoyl-CoA reductase	349		
SDZ13240	FNPO01000009	Pseudomonas sp. PDC86	2,4-dienoyl-CoA reductase	351		
SEB38831	FNSU01000001	Pseudomonas marginalis	2,4-dienoyl-CoA reductase	351		
SEC04133	FNSP01000004	Pseudomonas panacis	2,4-dienoyl-CoA reductase	351		
SEC92950	FNTF01000002	Pseudomonas frederiksbergensis	2,4-dienoyl-CoA reductase	349		
SED08754	FNTJ01000002	Pseudomonas saponiphila	2,4-dienoyl-CoA reductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SED22682	FNRV01000001	Pseudomonas mohnii	2,4-dienoyl-CoA reductase	349		
SED88337	FNTR01000004	Pseudomonas proteolytica	2,4-dienoyl-CoA reductase	351		
SEE19543	FNTY01000002	Pseudomonas migulae	2,4-dienoyl-CoA reductase	349		
SEE22002	FNUA01000002	Pseudomonas palleroniana	2,4-dienoyl-CoA reductase	351	X	A0A1K2CJ75 (UniProt)
SEE30391	FNTS01000002	Pseudomonas costantinii	2,4-dienoyl-CoA reductase	351		
SEE31900	FNTT01000002	Pseudomonas kilonensis	2,4-dienoyl-CoA reductase	349		
SEF08692	FNUD01000002	Pseudomonas deceptionensis	2,4-dienoyl-CoA reductase	349		
SEJ13562	FNYJ01000002	Pseudomonas sp. NFR16	2,4-dienoyl-CoA reductase	349		
SEJ70746	FNZL01000028	Pseudomonas sp. NFACC23-1	2,4-dienoyl-CoA reductase	349		
SEJ85890	FNYG01000018	Pseudomonas sp. NFACC07-1	2,4-dienoyl-CoA reductase	349		
SEJ85899	FNZC01000053	Pseudomonas oleovorans	2,4-dienoyl-CoA reductase	349		
SEK47782	FOAR01000003	Pseudomonas agarici	2,4-dienoyl-CoA reductase	349		
SEL12555	FNZP01000003	Pseudomonas sp. NFPP18	2,4-dienoyl-CoA reductase	349		
SEL40389	FOAQ01000003	Pseudomonas sp. NFPP10	2,4-dienoyl-CoA reductase	349		
SEL93493	FOAE01000004	Pseudomonas sp. NFACC41-3	2,4-dienoyl-CoA reductase	349		
SEM36820	FOCB01000001	Pseudomonas sp. ok272	2,4-dienoyl-CoA reductase	349		
SEP06262	FOCU01000013	Pseudomonas sp. ok266	2,4-dienoyl-CoA reductase	349		
SEP82239	FOEQ01000001	Pseudomonas soli	2,4-dienoyl-CoA reductase	349		
SEQ26195	FOEY01000002	Pseudomonas sp. NFPP19	2,4-dienoyl-CoA reductase	349		
SER46881	FOEO01000032	Pseudomonas sp. NFACC02	2,4-dienoyl-CoA reductase	349		
SES68392	FOIH01000001	Pseudomonas sp. NFR09	2,4-dienoyl-CoA reductase	351		
SES82024	FOHW01000002	Pseudomonas graminis	2,4-dienoyl-CoA reductase	349		
SFA61815	FOJR01000003	Pseudomonas sp. NFPP13	2,4-dienoyl-CoA reductase	349		
SFA84395	FOKD01000002	Pseudomonas sp. NFPP24	2,4-dienoyl-CoA reductase	351		
SFB07440	FOKB01000002	Pseudomonas simiae	2,4-dienoyl-CoA reductase	351		
SFB52511	FOKH01000032	Pseudomonas sp. NFIX10	2,4-dienoyl-CoA reductase	349		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SFD28559	FOLS01000021	Pseudomonas citronellolis	2,4-dienoyl-CoA reductase	349		
SFF35749	FONJ01000028	Pseudomonas sp. NFACC06-1	2,4-dienoyl-CoA reductase	349		
SFG82382	FOPO01000003	Pseudomonas sp. NFACC45	2,4-dienoyl-CoA reductase	349		
SFH82384	FOPT01000010	Pseudomonas sp. NFACC54	2,4-dienoyl-CoA reductase	349		
SFJ05126	FOQR01000003	Pseudomonas sp. NFPP08	2,4-dienoyl-CoA reductase	349		
SFK51295	FOSO01000009	Pseudomonas sp. NFACC52	2,4-dienoyl-CoA reductase	349		
SFL26404	FOTH01000004	Pseudomonas sp. NFACC46-3	2,4-dienoyl-CoA reductase	349		
SFM37229	FOUL01000002	Pseudomonas sp. ok602	2,4-dienoyl-CoA reductase	349		
SFM67152	FOUH01000003	Pseudomonas sp. NFPP05	2,4-dienoyl-CoA reductase	349		
SFO79162	FOWT01000001	Pseudomonas sp. NFPP28	2,4-dienoyl-CoA reductase	351		
SFO92034	FOVS01000046	Pseudomonas sp. NFACC24-1	2,4-dienoyl-CoA reductase	349		
SFP15761	FOXK01000001	Pseudomonas toyotomiensis	2,4-dienoyl-CoA reductase	349		
SFP52819	FOXG01000002	Pseudomonas sp. NFPP07	2,4-dienoyl-CoA reductase	349		
SFQ74454	FOYE01000002	Pseudomonas sp. NFIX49	2,4-dienoyl-CoA reductase	349	X	A0A1K2EIT8 (UniProt)
SFS25730	FOZO01000003	Pseudomonas sp. NFACC42-2	2,4-dienoyl-CoA reductase	351		
SFS51188	FPAL01000002	Pseudomonas sp. NFACC48-1	2,4-dienoyl-CoA reductase	349		
SFT80513	FPBG01000003	Pseudomonas sp. NFPP25	2,4-dienoyl-CoA reductase	349		
SFU96342	FPCE01000007	Pseudomonas sp. OV546	2,4-dienoyl-CoA reductase	351		
SFW17210	FPJD01000001	Pseudomonas sp. NFACC19-2	2,4-dienoyl-CoA reductase	349		
SFW82632	FPJC01000034	Pseudomonas sp. NFACC04-2	2,4-dienoyl-CoA reductase	349		
SFW85607	FPIX01000031	Pseudomonas sp. NFACC16-2	2,4-dienoyl-CoA reductase	349		
SFW90883	FPIV01000029	Pseudomonas sp. NFACC09-4	2,4-dienoyl-CoA reductase	349		
SFX48187	FPJV01000003	Pseudomonas sp. NFPP09	2,4-dienoyl-CoA reductase	349		
SFX55506	FPKJ01000003	Pseudomonas sp. NFPP16	2,4-dienoyl-CoA reductase	349		
SFX60828	FPJI01000005	Pseudomonas sp. NFPP02	2,4-dienoyl-CoA reductase	351		
SFX62077	FPJJ01000002	Pseudomonas sp. NFR02	2,4-dienoyl-CoA reductase	351		

Table A.7. (cont'd)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
SFX83486	FPKG01000003	Pseudomonas sp. NFPP14	2,4-dienoyl-CoA reductase	349		
SFY08697	FPKC01000017	Pseudomonas sp. NFACC47-1	2,4-dienoyl-CoA reductase	349		
SFY10961	FPKL01000019	Pseudomonas sp. NFACC49-2	2,4-dienoyl-CoA reductase	349	X	WP_017137250.1 (NCBI)
SFY21049	FPKB01000018	Pseudomonas sp. NFACC36	2,4-dienoyl-CoA reductase	349		
SFY34800	FPKK01000014	Pseudomonas sp. NFACC43	2,4-dienoyl-CoA reductase	349	X	WP_034118542.1 (NCBI)
SHN11077	FRDA01000008	Pseudomonas asturiensis	hypothetical protein	349		
WP_017137250.1[1]	NZ_JH730961 (275353-274298)	Pseudomonas fluorescens	alkene reductase	351	X	WP_069788488.1 (NCBI)
WP_034118542.1[1]	NZ_JRYA01000006 (787835-786780)	Pseudomonas fluorescens	alkene reductase	351	X	WP_074691045.1 (NCBI)
WP_078741200.1[1]	NZ_MSDF01000022 (24368-23313)	Pseudomonas fluorescens	alkene reductase	351	X	WP_078741200.1 (NCBI)

[1] These sequences were not on FunGene so were added back in to the primer design sequence set manually.

Table A.8. FunGene and primer design sequences for *xplA*

Protein Accession No.	Nucleotide Accession No.	Organism	Definition	Size (aa)	FunGene Sequence Set	FunGene Sequence ID (Database)
AAN27917	AF449421	Rhodococcus rhodochrous	cytochrome P450-like protein XplA	552	X	AAN27917.1 (NCBI)
AAQ03207	AF406983	Rhodococcus sp. YH1	inducible cytochrome P-450	317		
ABC17843	DQ277702	Rhodococcus sp. HS1	cytochrome P450	307		
ABC17844	DQ277703	Rhodococcus erythropolis	cytochrome P450	308		
ABC17845	DQ277704	Rhodococcus erythropolis	cytochrome P450	240		
ABC17846	DQ277705	Rhodococcus erythropolis	cytochrome P450	220		
ABC17847	DQ277706	Rhodococcus erythropolis	cytochrome P450	306		
ABC17848	DQ277707	Rhodococcus sp. HS10	cytochrome P450	276		
ABC17850	DQ277709	Rhodococcus sp. DN22	cytochrome P450	236		
ABF83916	DQ487126	Rhodococcus sp. HS3	cytochrome P450	302		
ABF83917	DQ487127	Rhodococcus sp. HS6	cytochrome P450	307		
ABF83919	DQ487129	Rhodococcus sp. HS11	cytochrome P450	302		
ABF83920	DQ487130	Rhodococcus sp. HS13	cytochrome P450	294		
ABF83921	DQ487131	Rhodococcus sp. HS14	cytochrome P450	294		
ABF83922	DQ487132	Rhodococcus sp. HS15	cytochrome P450	307		
ABF83923	DQ487133	Rhodococcus sp. HS16	cytochrome P450	279		
ABF83925	DQ487135	Rhodococcus sp. HS18	cytochrome P450	306		
ABF83926	DQ487136	Rhodococcus sp. HS19	cytochrome P450	310		
ABF83927	DQ487137	Rhodococcus sp. AM1	cytochrome P450	289		
ACH54163	EU919740	uncultured bacterium	XplA	177		
ACH54165	EU919742	uncultured bacterium	XplA	177		
ACH54167	EU919744	uncultured bacterium	XplA	179		
ACO88874	FJ577793	Microbacterium sp. MA1	XplA	552	X	ACO88874.1 (NCBI)
ADK69005.2[1]	CP002112.1	Gordonia sp. KTR9	flavodoxin-cytochrome P450 XplA (plasmid)	556	X	ADK69005.2 (NCBI)
AGU90203	KF571922	Williamsia sp. EG1	RDX degrading cytochrome P450	532	X	AGU90203.1 (NCBI)
AGU90209	KF571925	Rhodococcus sp. EG2	RDX degrading cytochrome P450	121		
AGU90210	KF571926	Rhodococcus sp. EG2	RDX degradation cytochrome P450	377		
AIA70903	CP007744	Pectobacterium atrosepticum	hypothetical protein	395		
AIK14324	CP009125	Pectobacterium atrosepticum	putative cytochrome P450	395		
ALU73572	CP007257	Rhodococcus erythropolis R138	nitric oxide synthase	552	X	WP_059016415.1 (NCBI)

Table A.8. (cont’d)

Protein Accession No.	**Nucleotide Accession No.**	**Organism**	**Definition**	**Size (aa)**	**FunGene Sequence Set**	**FunGene Sequence ID (Database)**
AMU10882	CP015035	Burkholderia cenocepacia	hypothetical protein	361		
ANY22184	CP016594	Gordonia terrae	hypothetical protein	553		
CAG74973	BX950851	Pectobacterium atrosepticum SCRI1043	putative cytochrome P450	405		
EWD70452	JCML01000015	Klebsiella pneumoniae UCI 18	hypothetical protein	395		
EZP26651	JFYN01000042	Pseudomonas sp. RIT288	cytochrome P450n	392		
GAB46351	BAFD01000115	Gordonia terrae NBRC 100016	putative cytochrome P450	553	X	WP_004023245.1 (NCBI)
KFX12950	JQHK01000010	Pectobacterium atrosepticum	hypothetical protein	395		
KFX25849	JQHO01000001	Pectobacterium atrosepticum	hypothetical protein	395		
KJF70888	JWIT01000025	Agrobacterium sp. KFB 330	hypothetical protein	396		
KKA09922	JZXC01000001	Pseudomonas kilonensis	hypothetical protein	337		
KMK87888	ALIV01000006	Pectobacterium atrosepticum ICMP 1526	putative cytochrome P450	395		
KQP18769	LMMT01000008	Pseudorhodoferax sp. Leaf265	hypothetical protein	399		
KQS65700	LMQC01000021	Modestobacter sp. Leaf380	hypothetical protein	396		
KRC65821	LMIS01000001	Aeromicrobium sp. Root236	hypothetical protein	396		
KVS24837	LPCW01000072	Burkholderia cepacia	hypothetical protein	398		
KWU25137	LNCR01000033	Burkholderia cenocepacia	hypothetical protein	401		
OCB11957	MBDY01000043	Mycobacterium vulneris	hypothetical protein	551	X	WP_065514957.1 (NCBI)
SAQ11870	FLAC01000032	Klebsiella oxytoca	Biotin biosynthesis cytochrome P450	395		
SFO04715	FOUV01000049	Streptomyces sp. cf124	Cytochrome P450	403		

[1] This sequence was not on FunGene so was added back into the primer design sequence set manually.

APPENDIX B

Assay Validation

All newly designed and currently published primer sets were tested against serial plasmid dilutions on a C1000 Touch thermal cycler with CFX96 real-time platform (Bio-Rad, Hercules, CA) before proceeding with running them on the SmartChip. Reactions were run in 0.2 mL low profile, white 8-tube PCR strips (TLS0851, Bio-Rad) with optical, ultraclear flat PCR tube 8-cap strips (TCS0803, Bio-Rad). This test was to confirm the primer sets would amplify efficiently under the SmartChip’s cycling parameters.

All primers were manufactured by Integrated DNA Technologies (IDT, Coralville, IA) and tested against plasmids containing a partial gene sequence (GenScript, Piscataway, NJ). Gene copy number for the plasmids was calculated as described by Ritalahti et al. (34). Primer and plasmid sequence information is provided in Appendix C.

Individual reactions consisted of a total volume of 20 µL with 1X LightCycler 480 SYBR Green I Master (Roche Applied Sciences, Indianapolis, IN), 0.5 µM each of the forward and reverse primers, plasmid DNA, and balance PCR grade water. In the case of 2 forward and/or reverse primers, 0.25 µM was used of each individual primer for a total of 0.5 µM. Cycling was performed following the SmartChip protocol described by Wang et al. (35): initial enzyme activation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 60°C for 30 s. A melting curve analysis was also performed from 55°C to 95°C with reads every 0.5°C for 5 s following amplification.

Reactions were run in triplicate for 7 10-fold serial plasmid dilutions ($5 \cdot 10^1$ to $5 \cdot 10^7$ copies/reaction) to use later in generating a standard curve. A reaction blank was included for each trial and most runs also incorporated triplicate negative controls using a plasmid with either an *aceA* or *etnC* gene insert ($5 \cdot 10^5$ copies/reaction).

The standard curve characteristics for each assay are reported in Table B.1. Eight designed assays were removed from their respective suites based on these results. From the *xplA* suite, PS1 was eliminated due to low efficiency and PS2 for scattered replicates. PS13 from the *xenA* suite and PS12, PS13 and PS18 from the *xenB* suite were eliminated due to the linear dynamic range not extending to 10^3 copies per reaction. Also, PS3 and PS15 from the *xenB* suite were removed due to all of the reactions amplifying with or after the no template and negative controls. No assays were removed from *diaA*, *nfsI,* and *pnrB*. Three published assays also performed poorly in terms of either having low amplification efficiencies or most of the reactions amplifying with or after the no template and negative controls. These were Pub2 from *xenA*, Pub2 from *xenB*, and Pub5 from *xplA*, and they were therefore not used for the SmartChip runs.

Table B.1. Characteristics for the Standard Curves of Designed Assays. “N/A” indicates that the assay did not perform well enough to generate a standard curve.

Gene	Primer Set	Log (Linear Range)	Slope	Y-Intercept	Efficiency (%)	R^2	Use?
diaA	1	7 to 1	-3.28	38.7	102.0	0.997	Y
	3	7 to 1	-3.15	37.9	107.7	0.996	Y
	4	7 to 1	-3.28	39.2	101.8	0.996	Y
nfsI	1	7 to 1	-3.08	38.8	111.2	0.992	Y
	2	7 to 1	-3.07	38.5	111.5	0.991	Y
	3	7 to 1	-3.06	38.7	112.3	0.989	Y
	4	7 to 1	-3.05	39.2	112.6	0.983	Y
	5	6 to 3	-3.04	40.5	113.5	0.984	Y
	6	7 to 1	-3.09	39.5	110.5	0.982	Y
	7	7 to 2	-4.31	45.0	70.7	0.979	Y
	8	7 to 1	-4.05	43.1	76.5	0.994	Y
	Pub1	7 to 1	-3.17	38.1	106.9	0.996	Y
	Pub2	7 to 1	-3.50	39.5	93.0	0.992	Y
pnrB	1	7 to 2	-3.09	35.2	110.9	0.997	Y
	2	7 to 2	-3.45	40.7	95.1	0.991	Y
	3	7 to 1	-3.22	38.2	104.5	0.992	Y
	4	7 to 1	-3.21	35.5	104.7	0.998	Y
	5	7 to 1	-3.25	39.3	103.0	0.995	Y
	Pub1	7 to 1	-3.25	38.6	103.0	0.998	Y
	Pub2	7 to 1	-3.17	38.7	106.9	0.996	Y
xenA	1	7 to 3	-3.62	42.0	89.0	0.998	Y
	2	7 to 2	-3.83	47.3	82.4	0.957	Y
	3	7 to 2	-3.27	43.3	102.1	0.987	Y
	4	7 to 1	-3.37	40.7	98.0	0.993	Y
	5	7 to 2	-3.67	43.1	87.3	0.996	Y
	6	7 to 1	-3.86	43.3	81.5	0.993	Y
	7	7 to 2	-3.53	43.4	91.9	0.987	Y
	8	7 to 1	-3.52	41.2	92.4	0.996	Y
	9	7 to 2	-3.44	40.7	95.2	0.992	Y
	10	7 to 1	-3.43	40.2	95.5	0.996	Y
	11	7 to 1	-3.46	40.0	94.5	0.996	Y
	12	7 to 1	-3.38	39.9	97.5	0.962	Y
	13	N/A	N/A	N/A	N/A	N/A	N
	14	7 to 1	-3.67	43.8	87.1	0.993	Y
	15	7 to 1	-3.58	41.6	90.2	0.997	Y
	16	7 to 1	-3.51	41.2	92.6	0.998	Y
	17	7 to 1	-3.22	38.8	104.4	0.993	Y
	Pub1	7 to 1	-4.20	47.3	73.0	0.970	Y
	Pub2	N/A	N/A	N/A	N/A	N/A	N
xenB	1	7 to 1	-3.77	44.5	84.2	0.994	Y
	2	7 to 1	-3.06	40.3	112.4	0.982	Y
	3	N/A	N/A	N/A	N/A	N/A	N
	4	7 to 1	-3.59	43.0	89.8	0.994	Y
	5	7 to 1	-3.32	39.2	100.2	0.993	Y
	6	7 to 2	-3.35	46.4	98.9	0.978	Y
	7	7 to 1	-3.42	39.8	96.2	0.993	Y
	8	7 to 1	-3.58	42.2	90.4	0.993	Y
	9	7 to 1	-3.36	42.2	98.6	0.992	Y
	10	7 to 2	-3.61	47.8	89.3	0.964	Y
	11	7 to 1	-3.20	40.5	105.2	0.988	Y
	12	N/A	N/A	N/A	N/A	N/A	N

Table B.1. (cont'd)

Gene	Primer Set	Log (Linear Range)	Slope	Y-Intercept	Efficiency (%)	R^2	Use?
xenB	13	N/A	N/A	N/A	N/A	N/A	N
	14	7 to 1	-3.20	42.3	105.2	0.981	Y
	15	N/A	N/A	N/A	N/A	N/A	N
	16	7 to 2	-2.99	44.9	116.1	0.991	Y
	17	7 to 1	-3.74	45.7	85.2	0.977	Y
	18	N/A	N/A	N/A	N/A	N/A	N
	19	7 to 1	-3.13	41.3	108.7	0.988	Y
	Pub1	7 to 1	-3.83	43.9	82.4	0.957	Y
	Pub2	7 to 5	-4.91	54.4	59.9	0.997	N
xplA	1	7 to 2	-5.07	49.7	57.5	0.975	N
	2	7 to 2	-3.85	43.7	81.9	0.971	N
	3	7 to 3	-4.13	45.1	74.7	0.989	Y
	4	7 to 2	-4.24	48.7	72.0	0.995	Y
	5	7 to 1	-3.24	41.0	103.7	0.973	Y
	Pub1	7 to 1	-3.37	40.6	98.0	0.997	Y
	Pub2	7 to 1	-3.55	40.1	91.3	0.989	Y
	Pub3	7 to 2	-3.56	45.4	90.9	0.997	Y
	Pub4	7 to 2	-3.17	37.9	106.6	0.997	Y
	Pub5	7 to 1	-4.38	48.4	69.1	0.997	N
	Pub6	7 to 1	-4.18	46.4	73.4	0.995	Y
16S rRNA	16S_rRNA	7 to 3	-2.97	37.8	117.0	0.996	Y

APPENDIX C

Primer and Plasmid Sequence Information

This appendix provides the sequence information for all primers used in the qPCR experiments in Tables C.1-C.7. For the designed assays, forward and reverse end positions are specified, and these positions reference the sequence used for the plasmid that the primer targets. Also, the total number of sequences expected to be amplified by each primer set according to the EcoFunPrimer tool (design) and the SeqFilters and ProbeMatch tools (theoretical) is listed. Table C.8 provides the insert sequence accession and positions for the plasmids used in the qPCR experiments.

Table C.1. Designed assays for *diaA*

Primer Set	**Forward Sequence (5’ → 3’)**	**Forward End**	**Reverse Sequence (5’ → 3’)**	**Reverse End**	**Target Plasmid**	**Sequences Hit**	
						Design	**Theo-retical**
1	CCACAGACCTCACCAACTCATG	23,689	CACCAGAATCAGGATCTCCAACTG	23,764	CP012395	5	10
	CACCTAAGGTTGAAGCAAACATCC		CTGAGCCAGGATCTCCAACAG				
2	GGAATCTCACCTAAGGTTGAAGCA	23,682	CTGAGTCAGGATCTCCAACAGC	23,763	CP012395	3	7
3	GGTAAATCTCCACAGACCTCACC	2,798,247	TCCCAGCTCTTAGGCAAATCTTC	2,798,374	AP009049	2	3

Table C.2. Designed assays for *nfsI*

Primer Set	**Forward Sequence (5’ → 3’)**	**Forward End**	**Reverse Sequence (5’ → 3’)**	**Reverse End**	**Target Plasmid**	**Sequences Hit**	
						Design	**Theo-retical**
1	TTCTGTCGCCCTGAAACGC	3,220,727	CCACATGAGAGGCGTCCAG ACCACGTGAGATGCATCCAG	3,220,922	CP001918	401	722
2	ACTCAACCAAGGCGTTCGAC	3,220,748	TTCGCGCAGAACACCACTAC TTCGCGCAGAACACTACCAC	3,220,937	CP001918	132	721
3	ACTCGACCAAGGCGTTCG	3,220,746	CACCACCACGTGAGAAGCG CACCACCACATGAGAGGCA	3,220,927	CP001918	59	649
4	CCCTGAAACGCCACTCTACC	3,220,736	TCGCGCAGAACACTACTACG	3,220,936	CP001918	34	629
5	GCCCTGAAACGCCACTCA GCCCTGAAACGCCACTCC	3,220,733	CGCAGAACACCACCACATGA	3,220,933	CP001918	25	646
6	AACTGACCGCGGAAGAAGC	3,220,777	CAGCCAGGCGTCATCCATC	3,220,963	CP001918	7	158
7	AACGCCACTCCACGAAGG	3,220,740	CCACATGAGAGGCGTCCAG	3,220,922	CP001918	7	490
8	AAACGCCACTCCACTAAGGC	3,220,741	CACCACCACGTGAGAAGCG CACCACCACATGAGAGGCA	3,220,927	CP001918	4	643

Table C.3. Designed assays for *pnrB*

Primer Set	**Forward Sequence (5’ → 3’)**	**Forward End**	**Reverse Sequence (5’ → 3’)**	**Reverse End**	**Target Plasmid**	**Sequences Hit**	
						Design	**Theo-retical**
1	CCACCAAAGCTTACGATGCC	2,716,115	ACGATCACATGCGACGCA CACAATCACGTGCGAGGCA	2,716,291	CP010979	27	50
2	CGCCGCTACACCACCAAA CGCCGCTACACCACCAAG	321,986	GCGAGGCGTTGAGAATCTTG GGGAGGCGTTGAGGATCTTC	322,159	MCBI01000011	6	6
3	TACCGTCACCCTGGCCAA	2,716,084	TCTTCGGCGAATTGTAGGCA CTTCGGCGAGTTGTAGGCA	2,716,264	CP010979	5	22
4	ACCACCAAAGCCTACGATGC	2,716,114	GAGCAGTTTCGGCGTGTTG CGAGGAGCTTGGGTGTGTTG	2,716,270	CP010979	5	12
5	AAGCGCCGTTACACTACCAA	2,716,102	CACGATCACATGCGAGGCA	2,716,291	CP010979	4	49

Table C.4. Designed assays for *xenA*

Primer Set	**Forward Sequence (5’ → 3’)**	**Forward End**	**Reverse Sequence (5’ → 3’)**	**Reverse End**	**Target Plasmid**	**Sequences Hit**	
						Design	**Theo-retical**
1	GATGCGGGCTTTGAGTGGA CGATGCGGGCTTTGAATGGA	2,411,045	CTGCCAGAGTCTGCTCGTC CGGTGAGAGTCTGCTCGTC	2,411,242	CP004045	99	168
2	GCCGGCTTCGAGTGGATAG TGCCGGCTTTGAATGGATCG	1,370,729	CCAGCGTCTGCTCATCGC GGTGAGGGTCTGCTCATCAC	1,370,921	AP013070	38	242
3	GCCGGCTTTGAATGGATCG TGCCGGCTTTGAATGGATCG	1,370,729	TCGATTCTGCCAGAGTCTGC CGACTCTGCCAGAGTCTGC	1,370,928	AP013070	16	52
4	GCCGGTTTCGAATGGATCGA CCGGTTTCGAGTGGATCGA	2,411,049	CTCGGCAAGCGTCTGCTC ACTCGGTCAGGGTCTGCTC	2,411,245	CP004045	25	115
5	GCCGGCTTTGAATGGATCG CCGGCTTCGAGTGGATCG	1,370,729	TCGACTCTTCCAGGGTCTGC TGGATTCGGTAAGCGTCTGC	1,370,928	AP013070	22	93
6	CGTGATGCAGGCTTTGAGTG TGACGCCGGCTTTGAGTG	2,411,043	GGTGAGGGTCTGCTCGTC TCGGTGAGGGTTTGTTCGTC	2,411,242	CP004045	11	137
7	GCGGGCTTTGAATGGATCGA CCGGCTTCGAATGGATCGA	1,370,730	TGCAAGCGTCTGCTCGTC TCTTCGAGGGTTTGCTCGTC	1,370,923	AP013070	9	78
8	CCGTGATGCGGGCTTTGA	2,411,040	GAGGGTCTGCTCGTCACG GTGAGGGTCTGCTCATCACG	2,411,239	CP004045	6	113
9	CCGGCTTCGAATGGATCGA GCTGGCTTTGAGTGGATCGA	178,844	CTCCAGGGTCTGTTCGTCAC GGTCAGGGTCTGTTCGTCAC	179,035	FOEO01000004	5	91
10	GCAGGCTTTGAGTGGATCGA	2,411,049	ATCGACTCGGTGAGGGTCTG TCGACTCTGCAAGCGTCTG	2,411,248	CP004045	5	167
11	GCGGGCTTTGAATGGATCGA CGGGCTTTGAGTGGATCGA	178,844	CCAGCGTTTGCTCATCACG CCAGCGTCTGTTCGTCACG	179,034	FOEO01000004	3	21
12	GCCGGCTTTGAATGGATCG	2,411,048	GGTGAGGGTCTGCTCGTC TCTGCCAAAGTCTGCTCGTC	2,411,242	CP004045	3	212
13	GGGCTTCGAGTGGATCGAA CCGGCTTTGAGTGGATCGAG	178,845	TCGATTCTGCCAGAGTCTGC GACTCGGTCAGCGTCTGC	179,042	FOEO01000004	2	109
14	GCGAAGTCGGGTTTGAATGG TGACGCCGGTTTCGAATGG	1,370,725	CACTGAGCGTCTGCTCGTC CTTCGAGGGTCTGCTCGTC	1,370,923	AP013070	2	12
15	CCGGCTTCGAGTGGATCG	2,411,048	GATTCGGCGAGGGTCTGC GACTCGGTCAGCGTCTGC	2,411,247	CP004045	2	85
16	GCAGGCTTCGAGTGGATCG CGCTGGCTTTGAATGGATCG	2,411,048	ATGGATTCGCTGAGGGTCTG ATGGATTCGGTCAGCGTCTG	2,411,248	CP004045	2	88
17	CCCGTGAAGCAGGCTTTGAA CGCGAAGCTGGCTTCGAA	178,836	CAGCGTCTGTTCGTCACGA GAGGGTCTGCTCGTCACGA	179,033	FOEO01000004	2	31

Table C.5. Designed assays for *xenB*

Primer Set	**Forward Sequence (5’ → 3’)**	**Forward End**	**Reverse Sequence (5’ → 3’)**	**Reverse End**	**Target Plasmid**	**Sequences Hit**	
						Design	**Theo-retical**
1	GCTGGACCAGTTCCTGCA	5,114,440	GGTTCTCGTCGCCCATGTC AGGTTGTCGTCACCCATGTC	5,114,585	CP014205	179	330
2	GCTGGACCAGTTCCTGCA	5,114,440	CAGTTCACGGGCCACGTAG AGTTCACGGGCGACGTAG	5,114,620	CP014205	55	245
3	GACCAGTTCCTGCAGAGCAG	5,114,446	GGTTGGCGTCACCCATGTC GTCAGCGTCGCCCATGTC	5,114,585	CP014205	31	298
4	GCTGGACCAGTTCCTGCAA TGCTCGATCAGTTCCTGCAA	5,114,441	CGCCCATGTCGTGAGAGTC	5,114,576	CP014205	27	203
5	GCTGGACCAGTTCCTGCAA TGCTCGATCAGTTCCTGCAA	5,114,441	CAGTTCACGGGCGACGTA CAGTTCGCTGGCCACGTA	5,114,621	CP014205	18	259
6	GCTACCTGCTCGACCAGTTC	5,114,435	CGCCCATGTCGTGAGAGTC GCCCATGTCGTGGGAGTC	5,114,576	CP014205	17	295
7	GCTGGACCAGTTCCTGCA	5,114,440	CGAGCCACGTAGGTGAAGG CTGGCGACGTAGGTGAAGG	5,114,613	CP014205	12	315
8	CGACCAGTTCCTGCAAAGCA	5,114,445	CCCATGTCGTGGGAATCGG	5,114,574	CP014205	8	279
9	GCTGGACCAGTTCCTGCA	5,114,440	TGAAGGTTTCGGCCGGATTG	5,114,599	CP014205	6	44
10	TGCTCGATCAGTTCCTGCAA	1,276,288	GCCCAGTTCACGGGCTAC	1,276,471	CP002620	5	132
11	GCTGGACCAGTTCCTGCAA TGCTCGATCAGTTCCTGCAA	5,114,441	AGGTGAAGGTTTCGGACAGG	5,114,602	CP014205	4	139
12	CCTGCTCGACCAGTTTCTGC	1,276,286	GCGACGTAGCCGAAGGTC	1,276,458	CP002620	4	103
13	TGCTCGATCAGTTCCTGCAA	1,276,288	GCTCGCGGGCTACATAACC TTCACGGGCGACATAGCC	1,276,465	CP002620	4	20
14	GCTGGACCAGTTCCTGCA	5,114,440	CCAGTTCACTGGCGACGTA	5,114,621	CP014205	3	163
15	GACCAGTTCCTGCAGAGCAG	5,114,446	CGACGTAGCCGAAGGTTTCG CGACGTAGGTGAAGGTCTCG	5,114,608	CP014205	3	244
16	GCTGGACCAGTTCCTGCA	5,114,440	TCACGGGCGACGTAGGTAA	5,114,616	CP014205	2	82
17	CTACCTGGTCGACCAGTTCC	1,276,283	AGCTCACGTGCCACGTAG	1,276,467	CP002620	2	56
18	GCTCGACCAGTTTCTGCAGA	1,276,289	GCTCTCGTGCCACATAGCC TTCACGGGCGACATAGCC	1,276,465	CP002620	2	11
19	CGACCAGTTCCTGCAAAGCA CGACCAGTTCCTCCAAAGCA	5,114,445	CGAGCCACGTAGGTGAAGG CTGGCGACGTAGGTGAAGG	5,114,613	CP014205	2	308

Table C.6. Designed assays for *xplA*

Primer Set	Forward Sequence (5’ → 3’)	Forward End	Reverse Sequence (5’ → 3’)	Reverse End	Target Plasmid	Sequences Hit	
						Design	Theo-retical
1	CACGGTCGTGCTCGTCAC	1,932	TCGCTCCTGCGTCTGTCA	2,097	AF449421	23	23
2	AGGTCGCGGTGCTGATCA GAGTTGGCGGTGCTCATCA	98,438	CGAGTTCGACGAGCAGGTTC CGAGGTCGACCAGTAGGTTC	98,596	BAFD01000115	3	3
3	GCTCAACTCGCAGTCGTGA	326,330	CGCCGAGTTCGATCAGCA	326,496	CP007257	1	1
4[1]	CGGTCGTGCTCGTCACC	1,933	GCTCCTGCGTCTGTCACC	2095	AF449421	2	23
5[1]	GAAGGTCGCGGTGCTGA	98,435	AGGTTCGCCATCGTCTTGAT	98,582	BAFD01000115	1	2

[1] PS4 and PS5 were designed with the NCBI Primer-BLAST tool to replace PS1 and PS2, respectively

Table C.7. qPCR Primer Sets from Literature. The primer set(s) with the highest theoretical coverage of the relevant primer design sequence set is marked with an asterisk (*).

Gene	ID	Forward Primer (5’→3’)	Reverse Primer (5’→3’)	Target Plasmid	Sequences Hit	Reference
nfsI	Pub1*	ACACGCCGGAAGCCAAA	GGTGCATGTCGGCGAAGTA	M63808	206	(66)
	Pub2	TTCAACACGCCAGAAGCCA	AGCACTCGGTCACAATCGT	M63808	198	(67)
pnrB	Pub1	CGAGATGACTGAAGAACACCTGAAC	GTAGTGACGGCGGCTCTGG	AF532912	22	(9)
	Pub2*	CCCACGCTCACGCAAACCC	GTACTGCCTTGCTGGGTGCG	AF532912	33	(68)
xenA	Pub1*	AGCACTCCAACAAGCGTAC	ACCGACACCAGGTCCAACT	AF154061	34	(23)
	Pub2	CACCATTCCCGAGACCAACA	TTTAGATTCGGGGGCTGCTG	AF154061	5	(30)
xenB	Pub1*	TTGCTGGAAGTGACTGATG	TGCCATAGAACAGCTCAGG	AF154062	11	(23)
	Pub2	ACCTTCACCTATGTTGCTCGC	CGTTTCTAGCGTTTCATGCGGT	AF154062	2	(30)
xplA	Pub1*	CCGAGTGGGCCAAACAGT	TCCTCCTCGTCGAGTTCGAT	AF449421	23	(69)
	Pub2*	CGACGAGGAGGACATGAGATG	GCAGTCGCCTATACCAGGGATA	AF449421	23	(70)
	Pub3*	CTACGGACAGGGTGAACTG	TCCTGTTGCAGTCGCCTAT	AF449421	23	(12)
	Pub4*	GATGACCGCTGCGTCCATCGAT	CCTGTTGCAGTCGCCTATACC	AF449421	23	(71)
	Pub5	AGGCTATCGCCACGATTCTG	ATCTGTCCCGCACAGGAATG	AF449421_1139-1520	7	(30)
	Pub6	CAACAACGCGATCGACATCC	TCGAACATCGCCTCCATCAC	AF449421_300-959	5	(67)
16S rRNA	16S_rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG	KX858536	N/A	(36)

Table C.8. Plasmid sequence insert information

Gene	Sequence Accession	Start Position	End Position	Complement?
diaA	AP009049	2,798,225	2,798,397	No
	CP012395	23,659	23,831	Yes
nfsI	CP001918	3,220,709	3,220,982	No
	M63808	609	939	No
pnrB	AF532912	364	646	No
	CP010979	2,716,067	2,716,310	Yes
	MCBI01000011	321,950	322,193	No
xenA	AF154061	674	1,232	No
	AP013070	1,370,703	1,370,949	No
	CP004045	2,411,022	2,411,268	Yes
	FOEO01000004	178,817	179,063	Yes
xenB	AF154062	820	1281	No
	CP002620	1,276,263	1,276,489	Yes
	CP014205	5,113,862	5,114,088	Yes
xplA	AF449421	1,915	2,334	No
	AF449421_300-959	2,065	2,724	No
	AF449421_1139-1520	2,904	3,285	No
	BAFD01000115	98,417	98,618	No
	CP007257	326,312	326,513	No
16S rRNA	KX858536	992	1,051	No
etnC	DQ264722	362	609	No
aceA	CP000316	2,189,466	2,189,604	No

APPENDIX D

SmartChip Data and Standard Curves

Table D.1. SmartChip Ct values for *diaA* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification.

Sample	**PS1**	**PS2**	**PS3**
Plasmid Standards			
10^1 copies per rxn	0.00	0.00	26.97
	27.82	26.87	0.00
	25.65	26.21	0.00
10^2 copies per rxn	22.83	0.00	25.90
	23.19	23.20	24.35
	22.76	23.12	25.32
10^3 copies per rxn	19.99	20.06	23.27
	20.15	20.06	23.60
	19.91	19.68	23.90
10^4 copies per rxn	16.28	16.25	18.34
	16.29	16.59	18.26
	16.45	16.51	0.00
10^5 copies per rxn	13.05	12.95	15.08
	13.04	13.07	0.00
	13.01	12.82	15.13
10^6 copies per rxn	10.31	10.17	10.01
	10.57	10.46	10.13
	10.32	10.62	9.99
10^7 copies per rxn	6.97	6.88	6.05
	6.75	6.92	6.31
	6.83	6.72	6.04
Shallow Zone Aquifer			
MW32 (Pre)	0.00	34.90	0.00
	0.00	0.00	32.19
	0.00	0.00	0.00
MW32 (Post)	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
MW62 (Pre)	0.00	0.00	0.00
	0.00	0.00	32.46
	0.00	34.45	0.00
MW62 (Post)	0.00	0.00	0.00
	34.30	34.89	0.00
	0.00	0.00	0.00
Sample	PS1	PS2	PS3
Perched Zone Aquifer			
MW48 (Pre)	0.00	0.00	0.00
	33.63	0.00	0.00
	34.48	0.00	0.00
MW48 (Post)	0.00	0.00	0.00
	0.00	0.00	0.00
MW60 R (Pre)	25.47	24.89	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
MW60 R (Post)	0.00	0.00	0.00
	0.00	0.00	0.00
MW64 (Pre)	0.00	0.00	0.00
	33.04	0.00	0.00
	0.00	0.00	0.00

Sample	**PS1**	**PS2**	**PS3**
MW64 (Post)	34.90	0.00	0.00
	0.00	0.00	0.00
MW66 (Pre)	0.00	32.19	34.79
	0.00	34.89	0.00
	30.93	33.09	0.00
MW66 (Post)	0.00	0.00	0.00
	0.00	0.00	33.94
MW67 (Pre)	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
MW67 (Post)	0.00	0.00	0.00
	0.00	0.00	0.00
Red Cedar River			
Red Cedar	0.00	0.00	0.00
	0.00	0.00	0.00
Sediment			
MW71	0.00	33.54	0.00
	0.00	0.00	34.41
	0.00	0.00	31.40
Agricultural Soils			
MSU Soil E	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
MSU Soil F	0.00	33.70	0.00
	33.69	0.00	0.00
	0.00	0.00	0.00
KBS T1	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
KBS T2	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
KBS T3	28.54	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
KBS T4	0.00	0.00	27.72
	0.00	0.00	0.00
	0.00	0.00	0.00
Negative and No Template Controls			
aceA	0.00	0.00	0.00
NTC	0.00	0.00	0.00

Table D.2. SmartChip Ct values for *nfsI* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**Pub1**	**Pub2**
Plasmid Standards										
10^1 copies per rxn	0.00	0.00	0.00	26.45	27.51	27.25	0.00	26.85	27.46	25.69
	29.36	0.00	27.19	26.98	27.84	27.13	29.23	0.00	26.41	0.00
	29.56	26.88	28.13	25.91	0.00	0.00	29.33	26.73	28.43	0.00
10^2 copies per rxn	0.00	24.43	0.00	26.37	25.21	26.39	27.93	25.06	23.65	24.75
	26.56	24.49	24.28	24.57	25.98	26.03	27.97	27.13	24.35	24.99
	26.30	0.00	24.33	23.89	24.16	24.22	0.00	23.36	23.98	23.84
10^3 copies per rxn	22.86	20.68	21.12	20.55	20.92	20.91	0.00	20.50	20.92	20.55
	22.35	20.88	20.86	20.60	21.09	21.01	22.02	20.50	21.20	20.59
	21.84	19.78	20.58	0.00	20.08	0.00	21.75	0.00	21.21	20.87
10^4 copies per rxn	17.29	15.64	15.89	0.00	0.00	16.14	17.60	15.34	23.21	0.00
	17.77	16.05	16.30	16.03	16.50	16.53	17.53	16.12	0.00	22.50
	17.54	16.07	16.20	16.13	16.50	16.15	17.56	15.95	23.05	22.24
10^5 copies per rxn	13.25	11.94	12.30	11.86	12.46	12.29	13.38	11.88	13.87	12.15
	13.22	12.01	12.45	11.93	12.52	12.41	13.16	12.03	13.93	12.42
	13.40	11.92	12.54	11.89	12.59	12.36	13.61	11.97	13.93	12.25
10^6 copies per rxn	10.59	9.10	9.39	8.94	9.29	9.47	10.47	9.01	10.54	8.51
	10.56	9.22	9.51	9.12	9.41	9.63	10.62	9.23	10.64	8.67
	10.35	8.94	9.13	8.89	9.08	9.37	10.25	8.89	10.70	8.84
10^7 copies per rxn	7.09	5.60	5.80	5.67	5.94	6.11	6.89	5.60	6.92	4.72
	6.80	5.50	5.96	5.48	5.84	5.98	6.69	5.68	7.12	5.07
	6.85	5.72	5.98	5.57	5.96	6.05	6.72	5.83	7.13	5.09
Shallow Zone Aquifer										
MW32 (Pre)	24.70	0.00	27.99	0.00	31.41	25.40	0.00	0.00	0.00	29.22
	24.75	0.00	0.00	0.00	34.07	25.64	24.98	0.00	27.39	28.74
	24.80	0.00	27.60	0.00	31.70	25.49	0.00	0.00	0.00	28.90
MW32 (Post)	0.00	30.70	29.83	0.00	27.40	30.60	28.58	33.99	31.57	32.44
	0.00	0.00	0.00	0.00	29.88	29.44	0.00	34.13	0.00	34.57
	0.00	32.12	29.45	0.00	28.00	28.79	30.18	0.00	0.00	0.00
MW62 (Pre)	22.55	0.00	27.97	0.00	0.00	23.07	0.00	31.74	26.68	30.73
	22.41	0.00	29.57	0.00	0.00	23.59	0.00	0.00	26.91	31.99
	22.77	28.03	27.96	0.00	0.00	0.00	0.00	0.00	27.12	0.00
MW62 (Post)	26.91	0.00	0.00	0.00	0.00	29.50	31.15	0.00	34.30	0.00
	27.65	28.17	0.00	0.00	0.00	32.71	31.04	0.00	28.87	0.00
	26.88	0.00	33.66	0.00	0.00	0.00	33.46	0.00	30.33	0.00
Perched Zone Aquifer										
MW48 (Pre)	34.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	21.03	0.00	0.00	0.00	31.04	26.74	26.94	0.00	0.00	0.00
	20.22	0.00	29.11	0.00	0.00	0.00	25.73	0.00	27.25	30.16
MW48 (Post)	27.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	28.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.81	0.00
MW60R (Pre)	24.89	0.00	0.00	23.60	24.26	24.07	24.79	22.74	0.00	33.70
	33.93	0.00	0.00	0.00	0.00	34.30	0.00	0.00	0.00	0.00
	0.00	0.00	30.64	0.00	0.00	0.00	34.21	0.00	0.00	0.00
MW60R (Post)	23.24	0.00	31.27	0.00	0.00	0.00	28.04	0.00	0.00	28.92
	23.59	0.00	0.00	0.00	34.49	0.00	28.40	0.00	0.00	30.06
MW64 (Pre)	0.00	28.86	0.00	0.00	31.62	27.26	27.28	0.00	0.00	0.00
	0.00	33.89	0.00	0.00	33.64	28.57	29.24	0.00	0.00	0.00
	0.00	0.00	27.84	0.00	34.10	0.00	0.00	0.00	28.93	0.00

Table D.2. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	Pub1	Pub2
MW64 (Post)	26.35	29.39	28.20	0.00	31.31	0.00	26.30	32.12	0.00	29.67
	0.00	31.33	29.52	0.00	32.89	0.00	26.75	0.00	28.26	32.42
MW66 (Pre)	23.52	26.85	26.15	31.91	32.21	25.04	24.33	0.00	27.51	0.00
	24.54	28.55	27.20	0.00	31.19	0.00	25.36	0.00	0.00	30.17
	24.47	0.00	0.00	0.00	0.00	0.00	24.86	0.00	26.28	0.00
MW66 (Post)	0.00	31.25	0.00	0.00	0.00	0.00	27.55	0.00	0.00	32.38
	0.00	29.28	0.00	0.00	0.00	26.87	26.15	30.86	0.00	0.00
MW67 (Pre)	24.52	29.94	28.07	0.00	0.00	0.00	26.64	0.00	29.07	30.82
	25.01	28.55	27.79	0.00	0.00	27.59	26.86	0.00	0.00	30.36
	23.99	28.28	0.00	0.00	0.00	0.00	27.11	29.16	0.00	30.27
MW67 (Post)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Red Cedar River										
Red Cedar	25.10	0.00	28.03	0.00	0.00	0.00	25.93	31.42	27.35	30.64
	0.00	27.47	0.00	0.00	0.00	24.47	25.75	29.41	0.00	26.64
Sediment										
MW71	0.00	32.35	34.23	0.00	33.77	0.00	27.76	0.00	0.00	0.00
	29.85	34.08	0.00	0.00	29.70	28.53	27.79	0.00	0.00	31.71
	0.00	34.91	0.00	0.00	0.00	34.26	0.00	0.00	0.00	0.00
Agricultural Soils										
MSU Soil E	25.31	0.00	27.62	32.58	31.46	25.62	0.00	0.00	28.15	29.43
	26.19	29.04	29.67	0.00	0.00	27.37	0.00	0.00	0.00	28.99
	25.97	0.00	29.24	0.00	33.27	27.54	27.42	0.00	28.14	28.68
MSU Soil F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	26.90	0.00	0.00	0.00	30.35
	25.92	28.92	0.00	0.00	0.00	0.00	27.37	30.35	26.33	27.28
KBS T1	25.47	0.00	28.17	0.00	0.00	0.00	26.58	32.82	27.81	28.69
	26.51	27.73	27.64	0.00	33.20	25.79	27.40	29.67	0.00	29.99
	24.51	0.00	0.00	0.00	0.00	25.14	0.00	0.00	26.99	28.56
KBS T2	0.00	28.20	0.00	0.00	33.19	26.20	0.00	31.21	28.79	30.37
	0.00	26.73	28.10	32.62	30.93	25.66	0.00	34.11	0.00	28.93
	0.00	0.00	28.26	0.00	0.00	25.87	27.54	31.01	0.00	31.20
KBS T3	25.75	0.00	0.00	0.00	0.00	25.84	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	26.46	0.00	0.00	28.61
	24.97	26.04	27.86	0.00	32.82	26.48	26.24	0.00	27.51	28.34
KBS T4	24.93	0.00	0.00	0.00	33.27	26.22	27.09	31.96	27.88	27.08
	0.00	0.00	27.09	0.00	33.27	25.17	25.90	0.00	27.24	30.18
	24.52	0.00	0.00	0.00	31.86	25.22	25.42	0.00	26.06	28.38
Negative and No Template Controls										
aceA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.61	0.00
NTC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D.3. SmartChip Ct values for *pnrB* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification

Sample	PS1	PS2	PS3	PS4	PS5	Pub1	Pub2
Plasmid Standards							
10^1 copies per rxn	28.90	27.35	0.00	0.00	33.44	0.00	0.00
	27.85	32.24	33.96	26.72	0.00	0.00	0.00
	27.42	29.88	32.03	27.00	30.85	0.00	0.00
10^2 copies per rxn	23.90	27.47	0.00	23.63	26.80	24.60	24.30
	23.76	33.56	28.90	22.78	26.62	23.33	23.75
	24.24	0.00	29.11	23.85	0.00	23.58	24.02
10^3 copies per rxn	0.00	26.41	25.69	20.19	23.59	19.74	20.60
	20.31	26.81	25.02	18.95	0.00	21.02	21.04
	20.29	0.00	26.29	19.57	23.71	20.38	20.64
10^4 copies per rxn	17.46	19.51	22.47	17.08	19.69	20.81	19.25
	17.44	19.30	22.37	0.00	17.55	19.31	18.98
	0.00	19.00	22.67	16.89	20.44	18.72	19.98
10^5 copies per rxn	12.35	15.15	17.05	11.61	15.40	13.62	13.86
	12.38	0.00	17.48	12.03	15.41	13.81	13.97
	12.31	15.28	17.85	12.31	15.54	13.53	13.70
10^6 copies per rxn	9.19	10.58	14.34	9.05	12.49	10.07	10.00
	9.37	10.82	14.50	9.02	12.56	10.05	10.13
	8.81	10.74	14.33	0.00	12.36	9.94	10.22
10^7 copies per rxn	6.03	5.92	10.73	5.80	9.27	6.32	6.34
	5.72	6.12	10.93	5.71	9.32	6.43	6.54
	5.85	5.94	10.91	5.53	9.08	6.23	6.30
Shallow Zone Aquifer							
MW32 (Pre)	26.13	24.59	26.28	26.20	26.90	27.53	21.73
	25.47	25.13	25.77	0.00	26.58	27.11	21.50
	0.00	24.97	26.38	24.81	0.00	27.89	22.23
MW32 (Post)	29.14	34.87	25.76	29.17	0.00	0.00	0.00
	0.00	28.06	26.01	28.01	0.00	0.00	23.87
	29.05	28.10	25.85	27.60	33.67	0.00	23.61
MW62 (Pre)	25.78	23.52	22.00	0.00	0.00	25.96	0.00
	25.99	23.50	21.68	0.00	0.00	25.72	0.00
	26.14	23.64	22.01	24.00	0.00	26.50	0.00
MW62 (Post)	30.82	26.41	32.93	0.00	0.00	0.00	0.00
	28.99	24.10	32.51	33.64	0.00	0.00	0.00
	27.25	24.22	30.82	27.36	28.98	0.00	0.00
Perched Zone Aquifer							
MW48 (Pre)	32.63	32.72	0.00	33.70	0.00	0.00	29.15
	0.00	24.12	0.00	25.73	0.00	28.66	22.16
	0.00	22.57	22.62	25.37	29.08	27.67	21.95
MW48 (Post)	31.20	24.90	33.63	28.80	34.62	0.00	25.48
	31.95	25.71	0.00	28.96	0.00	32.91	26.38
MW60R (Pre)	28.55	21.55	26.69	26.73	29.36	0.00	23.16
	0.00	31.96	33.95	0.00	0.00	0.00	0.00
	0.00	24.26	0.00	29.16	0.00	0.00	28.95
MW60R (Post)	0.00	24.19	0.00	0.00	0.00	0.00	24.12
	25.90	27.19	0.00	0.00	28.24	27.70	24.54
MW64 (Pre)	0.00	0.00	0.00	0.00	0.00	0.00	24.25
	0.00	0.00	0.00	29.79	0.00	0.00	27.27
	27.38	26.49	26.06	28.48	0.00	0.00	25.18

Table D.3. (cont’d)

Sample	PS1	PS2	PS3	PS4	PS5	Pub1	Pub2
MW64 (Post)	0.00	21.06	26.90	0.00	29.73	0.00	24.58
	0.00	21.07	26.21	0.00	0.00	28.12	24.78
MW66 (Pre)	0.00	25.96	25.70	25.74	27.82	0.00	22.70
	26.23	25.57	25.23	0.00	27.45	0.00	23.13
	0.00	25.48	26.38	0.00	28.25	0.00	24.30
MW66 (Post)	28.66	23.98	0.00	0.00	31.14	0.00	0.00
	27.06	22.28	0.00	26.89	0.00	27.51	23.98
MW67 (Pre)	0.00	27.82	0.00	0.00	28.33	29.30	23.88
	0.00	25.40	26.84	0.00	0.00	28.13	23.25
	0.00	0.00	0.00	0.00	27.89	28.64	23.38
MW67 (Post)	33.75	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	29.59	0.00	0.00	0.00
Red Cedar River							
Red Cedar	0.00	0.00	26.43	25.85	0.00	0.00	22.47
	25.22	0.00	26.91	24.88	25.62	0.00	21.76
Sediment							
MW71	0.00	0.00	0.00	34.42	0.00	31.49	0.00
	0.00	33.14	0.00	28.14	31.41	31.59	27.26
	0.00	0.00	0.00	34.54	0.00	31.44	27.19
Agricultural Soils							
MSU Soil E	0.00	29.94	27.54	0.00	28.70	0.00	24.69
	0.00	28.07	0.00	28.05	29.23	0.00	0.00
	0.00	27.72	0.00	0.00	0.00	28.81	25.29
MSU Soil F	0.00	28.64	0.00	0.00	29.72	0.00	24.67
	0.00	0.00	0.00	0.00	30.19	28.81	24.67
	27.21	25.98	0.00	0.00	29.34	27.04	25.11
KBS T1	27.15	29.60	0.00	0.00	29.04	29.21	23.65
	28.21	28.88	0.00	28.82	29.59	28.04	24.76
	0.00	0.00	26.19	0.00	28.47	0.00	0.00
KBS T2	0.00	28.08	0.00	0.00	0.00	0.00	24.37
	0.00	0.00	26.12	26.31	29.36	0.00	0.00
	0.00	28.06	27.37	26.80	0.00	27.42	24.24
KBS T3	26.73	0.00	0.00	0.00	29.72	25.37	24.22
	26.47	29.12	0.00	26.00	28.27	27.71	25.22
	26.21	0.00	0.00	25.77	26.84	25.65	23.52
KBS T4	27.55	30.03	0.00	27.99	28.84	25.82	24.58
	0.00	28.89	0.00	0.00	0.00	26.63	24.30
	0.00	27.94	26.23	27.22	28.08	0.00	24.48
Negative and No Template Controls							
aceA	0.00	0.00	0.00	0.00	0.00	0.00	34.78
NTC	28.45	31.30	0.00	0.00	0.00	0.00	0.00

Table D.4. SmartChip Ct values for *xenA* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification.

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
Plasmid Standards																	
10^1 copies per rxn	0.00	0.00	0.00	33.90	27.42	0.00	31.28	28.57	27.88	32.26	32.08	27.69	0.00	0.00	29.64	27.13	29.64
	27.48	0.00	0.00	0.00	28.17	27.91	28.07	29.14	0.00	0.00	0.00	27.00	0.00	28.19	0.00	28.28	0.00
	28.66	0.00	33.65	0.00	0.00	27.86	0.00	0.00	0.00	0.00	0.00	27.18	27.81	28.30	0.00	0.00	29.53
10^2 copies per rxn	27.08	33.43	31.66	33.32	24.31	25.05	27.14	28.43	28.03	26.03	23.73	24.98	27.59	25.59	25.73	24.34	26.37
	27.93	31.75	30.58	28.11	24.35	24.96	0.00	27.63	26.86	24.66	23.51	0.00	0.00	26.35	25.38	24.90	26.48
	26.65	30.22	0.00	30.74	0.00	0.00	27.25	25.67	25.46	24.93	24.48	24.84	27.31	25.34	25.95	23.89	0.00
10^3 copies per rxn	21.72	0.00	0.00	26.45	27.71	20.91	0.00	23.39	21.09	21.96	20.78	21.22	29.76	22.67	22.41	20.55	20.31
	21.93	34.18	0.00	26.36	27.47	20.97	0.00	23.04	21.30	21.58	20.22	21.12	34.31	22.10	22.16	20.57	19.92
	21.77	0.00	0.00	0.00	0.00	21.18	29.74	23.30	21.67	22.12	20.35	21.30	28.60	21.65	22.57	20.94	20.49
10^4 copies per rxn	17.59	22.34	22.99	22.08	16.93	16.88	18.86	18.76	18.10	17.43	17.64	16.96	18.33	17.52	18.03	18.06	15.07
	17.42	22.39	22.86	21.83	16.68	16.84	18.61	18.78	18.17	17.38	17.44	16.69	18.18	17.52	17.80	17.66	14.87
	17.55	22.37	23.10	21.82	16.93	16.80	18.73	18.80	0.00	17.59	17.81	16.85	18.35	17.56	18.08	17.98	14.86
10^5 copies per rxn	14.47	18.83	19.02	18.74	13.07	13.81	14.88	15.56	13.66	14.21	12.84	13.75	14.53	14.30	14.85	12.97	11.33
	14.57	18.81	19.21	19.05	13.03	13.89	14.91	15.74	13.55	14.31	12.73	13.85	14.48	14.44	14.95	12.94	11.24
	14.40	19.17	19.31	18.68	13.23	13.76	15.00	15.62	13.69	14.26	12.57	13.75	14.51	14.35	14.84	12.88	11.41
10^6 copies per rxn	10.96	15.90	16.23	15.83	10.29	10.24	11.90	12.30	10.46	10.82	9.62	10.33	11.54	10.95	11.56	9.79	7.73
	10.82	15.89	16.24	15.40	10.27	10.26	11.77	12.19	10.45	10.74	9.55	10.29	11.64	10.91	11.40	9.75	7.49
	11.09	15.99	16.16	15.32	10.41	10.63	11.94	12.18	10.61	10.86	9.73	10.37	11.55	10.94	11.48	9.93	7.86
10^7 copies per rxn	7.42	12.04	12.38	11.64	6.28	6.40	7.88	8.44	6.80	6.96	6.08	6.58	7.57	7.10	7.68	6.18	0.00
	7.16	11.98	12.30	11.43	6.40	6.49	7.91	8.38	6.76	6.99	6.00	6.43	7.66	7.04	7.56	6.13	0.00
	7.29	12.20	12.35	11.80	6.45	6.41	7.94	8.52	6.92	7.07	6.33	6.48	7.61	7.12	7.66	6.44	0.00
Shallow Zone Aquifer																	
MW32 (Pre)	27.03	25.04	32.90	22.90	26.35	24.89	25.34	0.00	24.83	23.91	25.84	23.63	25.05	22.17	23.66	0.00	0.00
	25.88	25.13	34.45	23.38	26.99	24.21	24.70	26.07	25.64	24.65	25.75	22.97	25.15	22.16	23.97	0.00	33.91
	25.63	25.54	0.00	22.77	25.43	23.85	24.83	0.00	24.12	24.87	25.86	23.57	24.59	22.19	22.78	0.00	31.03
MW32 (Post)	0.00	0.00	0.00	24.17	28.87	27.40	28.78	30.33	29.32	31.65	0.00	0.00	30.47	0.00	28.62	0.00	0.00
	0.00	0.00	32.27	23.91	30.09	28.22	30.19	0.00	0.00	0.00	28.41	33.27	29.52	26.04	27.30	32.99	0.00
	32.56	0.00	34.91	24.29	0.00	28.72	29.06	30.26	31.18	0.00	0.00	0.00	31.64	26.27	0.00	32.99	0.00
MW62 (Pre)	26.37	29.13	0.00	23.54	0.00	27.01	27.49	24.59	28.26	0.00	27.11	0.00	0.00	21.67	0.00	0.00	34.78
	25.33	0.00	30.57	23.21	0.00	25.97	26.88	24.95	0.00	25.95	0.00	27.93	28.27	21.80	0.00	0.00	28.64
	25.82	27.43	31.20	23.40	26.71	26.25	27.59	25.02	0.00	0.00	27.76	0.00	27.42	22.39	0.00	28.47	0.00
MW62 (Post)	0.00	32.45	0.00	26.54	28.43	25.65	0.00	29.10	32.62	29.69	27.34	31.09	27.46	24.79	29.70	34.47	31.99
	30.26	0.00	32.91	26.59	27.00	25.10	29.92	29.02	29.91	0.00	28.07	27.71	26.74	25.15	28.56	0.00	0.00
	0.00	31.90	0.00	26.20	25.85	24.78	27.97	30.37	28.66	31.90	26.51	26.34	26.36	24.55	29.55	0.00	0.00

Table D.4. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
Perched Zone Aquifer																	
MW48 (Pre)	0.00	0.00	0.00	0.00	34.57	0.00	32.62	0.00	0.00	33.52	0.00	32.29	0.00	0.00	30.76	0.00	0.00
	27.17	22.41	0.00	20.15	20.44	22.58	23.34	0.00	23.06	24.42	20.90	21.51	21.59	22.00	0.00	0.00	0.00
	27.08	0.00	0.00	18.78	19.02	20.70	21.91	26.10	22.08	23.91	19.87	20.33	19.90	21.78	0.00	34.20	28.26
MW48 (Post)	0.00	0.00	0.00	0.00	27.54	31.52	0.00	0.00	33.19	33.14	26.90	0.00	31.83	0.00	0.00	0.00	0.00
	34.10	0.00	0.00	29.27	29.20	33.87	0.00	30.48	30.95	29.29	27.88	31.26	0.00	26.27	28.62	33.27	30.96
MW60R (Pre)	30.51	31.31	0.00	25.68	27.07	26.51	30.32	26.03	27.50	27.42	26.36	31.34	26.57	23.45	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	34.16	0.00	34.53	0.00	0.00	0.00	0.00	29.32	30.68	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MW60R (Post)	29.80	30.55	0.00	26.11	0.00	24.66	29.52	27.16	0.00	0.00	27.64	27.11	0.00	23.80	26.70	31.52	29.87
	28.41	0.00	0.00	25.60	0.00	25.44	28.69	26.71	30.38	28.02	28.13	26.24	0.00	23.88	26.93	28.56	0.00
MW64 (Pre)	0.00	28.31	0.00	24.29	27.92	28.08	0.00	26.56	25.60	27.15	25.96	27.50	26.99	22.74	26.43	30.13	0.00
	30.92	0.00	0.00	26.67	29.46	29.85	0.00	28.02	32.71	31.21	29.35	28.62	29.07	24.90	28.44	34.44	0.00
	31.53	29.57	0.00	25.75	29.34	26.98	28.52	27.60	27.49	29.24	27.34	29.80	28.45	24.52	26.65	31.38	30.41
MW64 (Post)	28.72	24.94	0.00	24.78	24.91	22.13	26.56	0.00	27.76	0.00	26.24	26.83	23.91	23.69	25.88	0.00	0.00
	0.00	24.30	34.96	25.03	25.51	22.47	28.03	0.00	0.00	30.40	26.08	26.28	24.10	23.87	26.65	0.00	0.00
MW66 (Pre)	26.85	24.68	0.00	22.56	25.46	24.89	27.12	0.00	24.96	26.33	23.84	0.00	24.35	21.18	0.00	0.00	0.00
	0.00	24.16	0.00	22.45	24.61	24.93	26.13	24.69	25.52	26.12	23.99	24.01	24.07	21.55	24.86	0.00	28.20
	0.00	26.66	0.00	24.03	26.63	25.93	28.55	24.90	0.00	0.00	24.38	25.89	27.13	21.99	25.93	29.88	30.48
MW66 (Post)	28.20	28.38	32.13	25.33	29.13	25.19	28.04	28.02	0.00	29.89	27.17	30.51	26.70	23.74	0.00	0.00	0.00
	27.87	0.00	33.00	25.06	26.38	24.38	0.00	25.92	26.91	0.00	26.48	25.69	26.73	22.73	0.00	31.65	0.00
MW67 (Pre)	25.51	27.28	0.00	25.65	28.62	0.00	26.09	24.22	0.00	29.94	0.00	22.67	26.30	22.89	25.40	30.74	0.00
	0.00	27.04	0.00	25.01	0.00	23.64	25.98	23.32	23.93	29.57	0.00	22.12	27.23	22.53	0.00	0.00	30.23
	26.35	26.27	0.00	24.87	27.93	23.62	24.88	22.88	23.52	32.32	23.35	21.75	29.02	23.23	0.00	0.00	0.00
MW67 (Post)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.38	0.00	0.00	0.00
	30.11	0.00	0.00	0.00	0.00	0.00	0.00	32.14	0.00	0.00	34.76	0.00	0.00	33.94	0.00	0.00	0.00
Red Cedar River																	
Red Cedar	27.59	27.02	0.00	22.56	24.99	26.35	28.02	25.74	27.97	0.00	25.18	26.82	28.11	22.18	0.00	28.66	0.00
	28.47	26.68	0.00	22.27	25.16	25.43	28.46	25.38	28.11	27.61	0.00	27.30	27.81	21.86	25.51	28.21	30.26
Sediment																	
MW71	31.82	34.25	31.30	0.00	0.00	0.00	0.00	0.00	30.97	0.00	0.00	0.00	0.00	30.33	29.31	0.00	0.00
	28.15	31.19	0.00	0.00	0.00	0.00	0.00	34.15	0.00	0.00	0.00	27.20	31.36	29.43	28.67	0.00	0.00
	28.25	29.60	0.00	29.18	30.67	0.00	30.32	31.67	28.16	0.00	0.00	0.00	31.52	29.63	27.21	0.00	0.00

Table D.4. (cont'd)

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS12**	**PS14**	**PS15**	**PS16**	**PS17**	**Pub1**
Agricultural Soil																	
MSU Soil E	27.39	26.70	32.62	24.18	26.94	0.00	27.31	25.50	28.37	0.00	0.00	26.55	0.00	21.83	0.00	29.65	0.00
	0.00	28.28	0.00	25.24	0.00	27.22	28.44	26.51	0.00	30.02	0.00	0.00	0.00	23.20	0.00	0.00	0.00
	0.00	0.00	34.50	24.84	27.78	26.90	0.00	27.21	29.28	29.30	0.00	26.13	27.28	22.91	27.51	0.00	34.35
MSU Soil F	0.00	0.00	33.61	0.00	0.00	26.99	28.22	25.57	0.00	28.01	27.12	0.00	26.67	22.05	26.14	29.80	0.00
	0.00	27.58	0.00	25.49	29.45	27.88	0.00	26.74	29.77	30.28	28.53	0.00	0.00	0.00	27.74	0.00	0.00
	29.58	28.20	0.00	0.00	29.10	28.69	0.00	27.03	29.15	29.76	0.00	0.00	0.00	23.91	27.51	0.00	29.33
KBS T1	27.18	0.00	31.64	24.25	26.66	25.87	27.19	25.43	0.00	27.49	25.54	27.52	26.37	21.55	0.00	28.28	0.00
	28.07	28.95	30.90	25.23	0.00	26.75	28.47	0.00	0.00	28.61	0.00	0.00	0.00	22.89	0.00	29.57	0.00
	28.26	27.16	0.00	23.99	0.00	0.00	0.00	25.62	27.70	27.58	26.86	23.22	0.00	22.22	0.00	0.00	28.83
KBS T2	0.00	26.79	0.00	25.11	28.31	27.00	28.68	26.73	28.47	28.86	0.00	0.00	26.18	22.43	0.00	0.00	0.00
	28.58	0.00	0.00	24.34	26.53	26.45	28.28	25.08	28.07	28.85	0.00	27.46	25.91	0.00	25.15	28.89	0.00
	28.65	27.18	0.00	24.54	27.22	26.40	0.00	0.00	28.63	27.46	0.00	27.56	26.04	22.45	0.00	29.06	31.84
KBS T3	28.68	27.59	0.00	0.00	0.00	26.70	27.15	26.48	28.44	28.69	0.00	28.66	0.00	22.44	0.00	28.94	0.00
	28.96	0.00	30.85	25.21	27.84	0.00	29.26	0.00	0.00	28.01	0.00	28.80	0.00	22.53	26.67	30.83	0.00
	0.00	0.00	33.04	23.13	26.57	0.00	0.00	24.89	29.45	0.00	0.00	26.04	0.00	22.06	0.00	28.19	0.00
KBS T4	28.05	0.00	32.43	0.00	27.28	26.34	0.00	25.58	0.00	27.48	26.49	0.00	27.39	21.96	26.30	29.92	0.00
	27.10	0.00	0.00	24.10	26.68	26.36	0.00	0.00	27.50	26.91	25.70	27.25	0.00	21.66	25.45	29.18	0.00
	27.29	25.57	0.00	23.47	0.00	25.60	27.47	25.15	26.82	26.96	26.29	25.89	26.44	21.75	25.75	27.55	35.00
Negative and No Template Controls																	
aceA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.03
NTC	0.00	0.00	0.00	34.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D.5. SmartChip Ct values for *xenB* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification.

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
Plasmid Standards															
10^1 copies per rxn	0.00	0.00	0.00	28.83	0.00	26.56	0.00	0.00	34.14	27.88	0.00	0.00	30.73	26.82	0.00
	0.00	26.88	30.77	27.34	30.47	27.71	0.00	0.00	0.00	26.15	0.00	0.00	34.78	0.00	28.53
	0.00	0.00	0.00	26.77	32.01	0.00	0.00	0.00	31.89	26.66	0.00	0.00	31.52	26.77	29.54
10^2 copies per rxn	27.53	25.29	27.47	24.63	0.00	25.37	27.64	29.22	0.00	24.98	29.00	0.00	29.27	25.59	26.64
	0.00	26.19	27.04	25.35	28.05	23.89	27.73	28.39	30.34	25.07	29.35	28.36	30.80	24.13	0.00
	27.59	25.94	29.49	26.75	27.94	26.41	27.86	27.16	29.91	0.00	28.40	30.54	27.64	25.17	0.00
10^3 copies per rxn	23.55	21.27	24.04	21.54	25.36	21.67	23.01	23.52	26.82	0.00	25.65	0.00	24.56	21.72	23.17
	24.22	21.64	25.45	22.25	26.52	22.23	24.02	25.39	26.61	22.77	25.93	28.00	24.57	22.33	22.95
	24.92	21.80	23.31	21.32	25.01	22.62	23.34	25.87	27.05	22.75	26.75	28.05	24.38	22.52	0.00
10^4 copies per rxn	19.29	17.69	20.60	18.09	20.21	17.46	0.00	20.89	21.65	17.49	21.59	0.00	19.74	17.38	18.50
	19.49	17.76	20.62	18.04	20.61	17.99	19.84	21.21	21.94	17.79	21.85	23.40	19.81	17.66	18.40
	19.67	0.00	0.00	0.00	21.05	18.43	19.80	20.63	21.64	17.82	21.59	23.56	19.53	18.13	18.44
10^5 copies per rxn	14.74	13.43	15.87	13.22	15.62	13.39	14.24	15.97	18.67	13.42	17.09	18.42	16.39	13.16	12.58
	14.82	13.60	15.93	13.67	15.83	13.54	14.53	16.24	18.47	13.69	17.36	18.65	16.20	13.33	12.74
	14.94	13.56	16.19	13.30	15.77	13.41	0.00	16.02	18.61	13.59	17.40	18.61	16.38	13.28	12.69
10^6 copies per rxn	11.64	10.52	12.92	10.53	12.32	10.70	12.12	13.46	15.22	10.75	14.52	15.77	12.92	10.51	9.41
	11.93	10.93	13.32	0.00	12.61	10.90	11.65	13.76	15.11	11.01	14.61	16.11	12.76	10.72	9.61
	11.68	10.53	12.41	10.51	11.92	10.53	11.23	12.63	15.10	10.46	0.00	15.14	12.73	10.30	9.60
10^7 copies per rxn	7.52	6.79	9.17	6.53	7.61	6.50	6.85	9.32	12.07	6.49	10.19	11.65	0.00	6.39	5.28
	7.68	6.72	8.96	6.57	7.45	6.48	6.74	9.17	11.63	6.44	9.85	11.49	8.87	6.37	5.18
	7.80	6.85	8.80	6.57	7.82	6.62	6.85	9.31	11.53	6.55	9.99	11.87	8.87	6.53	5.40
Shallow Zone Aquifer															
MW32 (Pre)	21.67	22.31	24.54	22.41	22.65	20.92	24.22	0.00	26.06	23.26	23.76	23.33	24.83	20.90	28.21
	21.88	22.10	24.15	22.43	23.37	21.13	24.43	24.39	25.64	23.74	23.55	22.54	25.25	21.33	29.43
	21.43	22.19	23.52	22.00	22.85	21.11	23.85	24.35	25.95	23.20	22.95	22.63	24.75	21.18	26.99
MW32 (Post)	27.46	0.00	31.31	27.17	33.56	29.88	30.22	0.00	31.36	26.52	30.99	0.00	31.13	26.14	29.89
	27.97	31.99	0.00	27.39	0.00	0.00	0.00	26.62	0.00	25.68	28.56	0.00	32.62	0.00	28.84
	28.62	0.00	31.03	27.61	0.00	26.08	28.14	26.86	0.00	25.44	27.69	33.79	0.00	27.43	27.74
MW62 (Pre)	22.95	25.12	28.80	25.09	22.44	23.50	27.19	22.41	25.49	0.00	25.49	25.58	26.47	26.31	32.52
	22.79	25.40	28.06	0.00	21.79	23.80	27.29	22.47	26.10	26.18	24.83	25.88	26.88	26.07	0.00
	23.04	24.92	26.73	24.77	22.66	23.82	27.29	22.68	26.14	25.59	25.16	26.44	26.64	26.26	28.05
MW62 (Post)	25.56	24.96	27.52	25.61	29.39	25.08	32.08	0.00	0.00	26.51	26.10	26.45	0.00	25.30	34.27
	27.36	25.07	27.54	25.58	26.61	24.46	29.43	0.00	0.00	27.40	25.11	25.50	33.48	24.06	30.22
	25.79	25.39	26.56	24.58	26.19	24.43	26.90	26.09	0.00	25.33	26.44	24.96	30.28	23.92	30.78

Table D.5. (cont'd)

Sample	PS1	PS2	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS14	PS16	PS17	PS19	Pub1
Perched Zone Aquifer															
MW48 (Pre)	28.81	0.00	0.00	0.00	34.16	30.43	0.00	0.00	0.00	30.56	0.00	0.00	0.00	28.64	0.00
	0.00	18.42	21.87	17.84	21.13	17.77	20.01	22.32	21.44	17.58	19.72	20.40	23.65	17.70	27.04
	14.92	17.13	20.55	16.55	19.87	16.54	0.00	21.59	20.65	16.24	18.13	18.74	22.88	16.29	26.05
MW48 (Post)	24.91	26.44	0.00	26.80	29.24	27.48	31.47	0.00	0.00	0.00	32.24	0.00	29.52	26.95	27.87
	25.55	28.76	0.00	27.88	30.40	28.91	32.19	0.00	30.48	26.09	31.74	0.00	0.00	0.00	0.00
MW60R (Pre)	23.64	23.87	25.54	24.38	23.32	22.89	25.48	24.83	0.00	25.78	24.72	26.52	26.94	22.70	25.49
	26.65	27.01	0.00	26.58	30.23	26.58	28.88	0.00	0.00	26.20	0.00	32.57	34.90	25.24	0.00
	26.08	24.51	28.66	25.46	30.35	25.62	29.99	27.92	0.00	25.61	29.64	33.80	0.00	25.49	30.13
MW60R (Post)	24.28	24.11	27.13	25.74	24.13	25.46	30.24	23.27	30.92	0.00	29.09	32.35	0.00	26.03	31.87
	24.72	24.25	29.08	26.30	25.74	26.18	29.77	0.00	34.36	29.76	29.58	34.07	0.00	27.21	33.18
MW64 (Pre)	24.12	24.53	26.97	24.36	23.86	23.13	25.60	0.00	28.68	27.53	24.70	24.81	26.15	23.36	32.01
	25.45	22.95	27.03	23.53	25.86	23.87	25.22	26.28	0.00	23.49	26.50	26.89	29.89	23.22	0.00
	25.36	25.58	26.78	26.27	24.64	24.19	26.87	0.00	32.55	0.00	26.03	27.64	0.00	24.64	32.82
MW64 (Post)	25.04	0.00	30.46	23.75	25.27	23.39	29.08	0.00	29.63	24.19	26.18	26.93	28.99	23.93	31.57
	25.10	24.44	28.68	23.91	25.39	23.10	29.13	0.00	28.78	24.06	26.91	28.33	0.00	24.08	0.00
MW66 (Pre)	22.00	22.34	24.19	22.30	23.09	21.47	23.82	0.00	27.67	23.77	22.38	22.52	26.20	21.22	27.37
	22.14	22.12	24.14	22.31	22.61	21.12	23.67	0.00	30.41	0.00	22.49	22.26	25.09	21.53	27.66
	23.43	24.70	26.60	24.68	24.28	23.68	27.35	23.82	31.63	26.31	24.99	24.91	26.23	24.62	0.00
MW66 (Post)	23.69	25.34	26.20	25.44	23.16	23.22	25.18	24.42	28.51	24.66	26.09	26.21	0.00	23.44	0.00
	22.35	23.61	25.11	23.56	21.73	22.30	25.07	0.00	27.47	24.34	25.45	24.51	0.00	23.04	0.00
MW67 (Pre)	22.37	22.61	24.49	22.42	22.14	21.65	23.24	21.80	28.13	24.30	22.56	21.89	0.00	21.16	0.00
	21.96	22.12	24.36	22.62	22.00	21.96	24.26	22.02	30.39	24.39	23.17	23.27	0.00	22.02	0.00
	21.71	21.75	23.50	21.89	21.40	21.47	22.76	21.78	28.19	22.87	22.64	21.72	24.02	20.96	0.00
MW67 (Post)	0.00	0.00	0.00	0.00	0.00	29.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	33.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.08
Red Cedar River															
Red Cedar	23.20	25.58	27.97	25.66	27.12	25.34	29.06	25.54	30.03	0.00	28.24	29.35	27.72	27.91	27.20
	22.80	0.00	29.94	24.68	25.80	24.12	33.28	24.45	28.83	0.00	27.51	27.19	28.40	26.50	30.34
Sediment															
MW71	0.00	26.91	29.86	0.00	0.00	34.39	0.00	0.00	33.27	0.00	33.31	0.00	0.00	27.55	31.84
	28.03	27.20	27.53	31.67	28.98	27.62	28.47	31.41	29.08	27.85	32.44	33.92	0.00	27.81	25.70
	28.03	26.61	0.00	27.85	32.16	27.22	0.00	31.23	0.00	33.77	29.55	29.49	0.00	27.28	31.24

Table D.5. (cont’d)

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
Agricultural Soil															
MSU Soil E	26.02	24.64	33.78	24.99	27.89	25.15	33.79	25.62	32.79	26.56	28.06	0.00	27.74	26.52	0.00
	28.39	26.66	29.81	26.55	28.76	26.10	0.00	27.06	0.00	0.00	29.67	0.00	27.90	30.43	0.00
	25.92	25.59	0.00	25.60	26.96	25.75	30.92	26.18	31.91	28.26	28.83	32.91	26.87	26.43	0.00
MSU Soil F	24.86	25.43	27.94	24.80	26.10	24.91	28.14	0.00	34.10	26.11	0.00	32.43	27.89	27.73	34.29
	26.11	26.44	32.25	26.03	26.97	25.39	0.00	27.26	28.74	28.11	0.00	30.18	26.17	27.42	0.00
	24.48	26.38	29.13	25.59	25.80	25.31	0.00	26.18	0.00	28.88	30.54	32.04	27.02	27.83	0.00
KBS T1	24.36	24.83	0.00	0.00	26.41	24.61	0.00	25.18	0.00	27.49	28.25	0.00	25.85	26.61	0.00
	23.29	24.92	28.76	26.64	25.64	22.82	26.02	26.63	0.00	23.31	27.98	32.83	27.23	23.35	0.00
	21.14	22.84	27.85	23.33	24.42	20.79	26.43	24.05	0.00	20.87	25.33	29.56	25.08	20.71	34.04
KBS T2	23.74	24.90	27.91	25.70	25.58	23.59	27.81	25.61	28.92	24.14	0.00	32.11	27.36	23.61	31.62
	26.13	25.55	28.43	25.61	28.77	24.41	28.11	25.62	31.90	26.57	0.00	0.00	26.92	25.62	34.37
	23.31	0.00	27.83	25.53	24.73	22.92	27.42	25.41	0.00	22.74	28.30	31.30	26.28	23.41	0.00
KBS T3	24.12	25.27	0.00	25.67	27.11	23.43	27.44	26.03	33.18	24.52	27.39	0.00	25.27	24.10	0.00
	25.14	25.34	30.20	25.91	25.89	25.20	27.67	0.00	0.00	24.92	28.23	0.00	27.19	25.35	0.00
	24.33	24.16	29.18	24.49	27.40	23.63	0.00	24.50	29.98	26.51	27.82	0.00	26.63	24.64	34.02
KBS T4	24.14	0.00	31.77	25.82	26.89	22.76	0.00	25.44	0.00	23.29	28.59	31.07	25.93	23.63	0.00
	24.55	24.16	29.54	25.63	26.74	23.75	0.00	25.72	0.00	26.20	27.74	32.46	25.46	25.12	0.00
	24.82	25.64	29.12	25.03	26.49	25.06	26.98	25.43	33.27	26.01	27.80	30.05	27.32	25.26	0.00
Negative and No Template Controls															
aceA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NTC	0.00	0.00	0.00	33.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D.6. SmartChip Ct values for *xplA* assays. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification

Sample	**PS3**	**PS4**	**PS5**	**Pub1**	**Pub2**	**Pub3**	**Pub4**	**Pub6**
Plasmid Standards								
10^1 copies per rxn	0.00	0.00	0.00	27.76	25.89	31.52	27.58	0.00
	0.00	29.44	29.35	27.79	27.88	0.00	26.98	29.51
	0.00	0.00	0.00	27.95	0.00	0.00	0.00	30.81
10^2 copies per rxn	30.71	26.50	0.00	24.65	23.65	28.09	23.72	0.00
	29.08	0.00	0.00	24.76	24.07	0.00	23.45	27.16
	0.00	26.25	28.85	0.00	24.25	28.50	24.68	27.24
10^3 copies per rxn	26.00	23.63	24.78	21.59	21.08	26.93	21.37	23.86
	26.42	22.77	24.77	21.65	20.90	24.67	21.51	22.62
	26.83	23.16	0.00	0.00	21.08	25.87	21.29	23.16
10^4 copies per rxn	20.69	17.64	28.65	17.14	16.61	21.37	16.60	16.94
	20.35	18.67	26.90	16.49	16.64	21.66	17.20	16.15
	20.55	18.14	26.27	0.00	16.43	20.62	16.87	18.43
10^5 copies per rxn	15.00	13.60	15.23	14.21	13.00	16.51	13.44	11.89
	14.81	13.75	15.59	14.00	13.22	16.53	13.73	12.28
	15.09	13.56	15.44	0.00	13.20	16.56	13.64	12.11
10^6 copies per rxn	10.88	11.23	10.40	11.93	10.73	14.34	11.09	8.84
	11.17	10.53	10.32	11.17	10.10	13.38	10.56	8.62
	11.31	10.70	10.45	0.00	10.52	13.66	10.92	9.08
10^7 copies per rxn	6.85	5.98	6.03	7.52	6.41	9.74	6.78	0.00
	6.64	6.06	6.42	7.38	6.18	9.73	6.71	0.00
	6.75	6.24	6.22	0.00	6.56	9.83	6.94	0.00
Shallow Zone Aquifer								
MW32 (Pre)	28.60	23.86	24.97	23.65	23.26	27.79	23.02	23.51
	0.00	23.98	24.42	0.00	23.78	28.04	23.81	24.24
	27.30	23.87	0.00	0.00	25.17	0.00	24.41	23.28
MW32 (Post)	0.00	29.01	30.64	0.00	0.00	0.00	0.00	0.00
	0.00	29.32	0.00	0.00	0.00	0.00	0.00	0.00
	32.58	27.21	0.00	0.00	0.00	34.17	0.00	0.00
MW62 (Pre)	27.17	24.80	0.00	0.00	28.59	32.19	28.48	0.00
	26.60	0.00	24.90	0.00	28.15	0.00	0.00	25.30
	27.16	0.00	25.24	28.89	28.44	34.02	28.61	25.04
MW62 (Post)	30.56	0.00	0.00	34.74	0.00	0.00	0.00	30.59
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.49
	30.49	0.00	30.10	0.00	0.00	28.56	0.00	0.00
Perched Zone Aquifer								
MW48 (Pre)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	24.07	27.33	0.00	0.00	0.00	31.15	0.00	0.00
	23.97	0.00	0.00	33.05	28.67	30.30	28.79	26.12
MW48 (Post)	34.72	0.00	0.00	0.00	0.00	0.00	0.00	30.28
	32.16	30.70	30.44	0.00	0.00	0.00	0.00	0.00
MW60R (Pre)	28.44	28.29	27.34	0.00	0.00	34.41	34.72	28.69
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MW60R (Post)	26.32	0.00	26.94	32.10	0.00	32.79	34.23	23.46
	0.00	25.81	0.00	0.00	0.00	30.52	0.00	23.67
MW64 (Pre)	29.43	26.98	0.00	0.00	0.00	0.00	34.98	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	28.77	26.85	0.00	0.00	0.00	0.00	33.60	28.91

Table D.6. (cont'd)

Sample	**PS3**	**PS4**	**PS5**	**Pub1**	**Pub2**	**Pub3**	**Pub4**	**Pub6**
MW64 (Post)	28.65	28.63	25.93	0.00	31.12	0.00	0.00	0.00
	28.86	28.25	27.27	0.00	0.00	34.52	33.28	0.00
MW66 (Pre)	27.08	24.06	0.00	26.35	0.00	30.84	33.08	0.00
	26.86	24.91	0.00	26.79	0.00	32.92	0.00	25.70
	28.28	24.74	25.38	28.51	0.00	33.31	0.00	0.00
MW66 (Post)	0.00	27.06	29.63	29.12	28.51	0.00	29.06	28.87
	27.89	25.42	0.00	26.70	0.00	32.29	0.00	0.00
MW67 (Pre)	28.81	27.75	26.99	0.00	0.00	29.20	0.00	27.57
	0.00	26.32	26.12	33.22	29.89	28.52	0.00	0.00
	0.00	26.87	26.55	0.00	0.00	29.96	29.53	27.60
MW67 (Post)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	33.29	0.00	0.00	0.00	0.00	0.00	34.20
Red Cedar River								
Red Cedar	0.00	23.26	25.44	0.00	0.00	32.00	33.45	26.62
	0.00	22.88	24.58	30.21	0.00	30.97	0.00	26.36
Sediment								
MW71	0.00	30.61	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	34.57	31.90	0.00	0.00	0.00	0.00	0.00	0.00
Agricultural Soils								
MSU Soil E	0.00	0.00	24.81	0.00	0.00	32.35	0.00	25.54
	29.30	25.12	25.32	0.00	0.00	33.56	0.00	0.00
	28.69	0.00	25.66	28.19	0.00	0.00	32.18	0.00
MSU Soil F	28.17	0.00	25.61	32.84	0.00	0.00	0.00	26.52
	29.32	0.00	26.83	31.10	0.00	34.82	0.00	0.00
	0.00	26.50	0.00	0.00	0.00	0.00	0.00	26.78
KBS T1	28.02	0.00	25.44	0.00	0.00	0.00	34.38	26.69
	28.73	0.00	25.21	29.95	0.00	30.44	0.00	26.87
	27.36	25.44	24.24	32.90	0.00	0.00	0.00	25.23
KBS T2	29.98	0.00	25.99	33.05	0.00	33.90	31.72	0.00
	0.00	0.00	25.08	30.33	0.00	30.55	30.16	25.51
	0.00	0.00	24.03	29.78	0.00	32.03	0.00	23.92
KBS T3	0.00	26.01	25.63	0.00	0.00	0.00	0.00	0.00
	28.70	0.00	25.78	0.00	0.00	0.00	0.00	0.00
	27.37	25.05	24.84	0.00	0.00	0.00	0.00	0.00
KBS T4	0.00	0.00	24.90	0.00	33.10	31.56	0.00	0.00
	0.00	25.16	24.25	31.24	0.00	0.00	0.00	0.00
	26.73	0.00	23.95	0.00	0.00	0.00	0.00	26.24
Negative and No Template Controls								
aceA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NTC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D.7. SmartChip Ct values for the 16S rRNA assay. These results correspond to the 3 chip runs performed: 1) *xenB* and *pnrB*, 2) *xplA* and *nfsI*, and 3) *xenA* and *diaA*. Green text indicates the Ct value falls within the standard curve, red text indicates it falls outside of the standard curve or is above the cycle threshold of 28 and a zero value indicates no amplification

Sample	**16S_rRNA.1**	**16S_rRNA.2**	**16S_rRNA.3**
Plasmid Standards			
10^1 copies per rxn	27.96	27.12	28.89
	27.43	27.81	27.39
	28.14	27.48	28.00
10^2 copies per rxn	24.60	25.18	27.57
	0.00	25.43	26.31
	24.55	25.62	26.97
10^3 copies per rxn	20.92	22.30	22.16
	21.13	21.99	22.20
	21.23	22.05	22.56
10^4 copies per rxn	18.76	19.42	19.68
	18.22	19.75	0.00
	18.62	19.64	19.57
10^5 copies per rxn	14.56	14.83	14.94
	15.22	15.36	15.24
	14.98	15.13	15.07
10^6 copies per rxn	11.91	12.06	12.51
	12.28	12.31	12.54
	12.16	12.17	12.65
10^7 copies per rxn	8.09	8.39	8.01
	8.65	8.46	8.29
	8.38	0.00	8.29
10^8 copies per rxn	5.72	5.55	5.34
	5.64	5.44	5.58
	5.58	5.44	5.57
Shallow Zone Aquifer			
MW32 (Pre)	13.74	13.78	13.88
	13.80	13.90	14.07
	13.45	13.67	13.72
MW32 (Post)	10.94	11.08	11.08
	10.68	10.92	10.77
	11.13	11.04	11.47
MW62 (Pre)	12.21	12.23	12.16
	12.20	12.11	12.24
	12.05	12.35	12.29
MW62 (Post)	10.29	10.26	10.41
	10.33	10.97	10.48
	10.77	10.68	10.53
Perched Zone Aquifer			
MW48 (Pre)	21.61	21.70	20.40
	13.41	13.54	13.52
	12.39	12.56	12.56
MW48 (Post)	10.50	10.85	10.50
	10.57	10.77	10.32
MW60R (Pre)	14.28	14.51	14.43
	20.02	20.12	19.98
	19.83	20.56	20.28
MW60R (Post)	12.09	11.90	11.91
	11.96	12.05	12.01

Table D.7. (cont'd)

Sample	**16S_rRNA.1**	**16S_rRNA.2**	**16S_rRNA.3**
MW64 (Pre)	15.41	15.43	15.28
	18.61	17.36	17.20
	16.00	16.21	15.88
MW64 (Post)	10.93	10.96	11.01
	11.31	11.60	11.26
MW66 (Pre)	13.35	13.13	13.02
	13.45	13.93	13.42
	0.00	13.95	13.92
MW66 (Post)	12.39	12.19	12.22
	10.60	10.72	10.74
MW67 (Pre)	14.67	14.71	14.66
	14.75	14.58	14.72
	14.40	14.49	14.41
MW67 (Post)	23.61	23.70	23.74
	22.07	22.25	22.28
Red Cedar River			
Red Cedar	13.32	13.35	13.40
	13.32	13.36	13.45
Sediment			
MW71	19.32	19.50	20.16
	18.87	19.56	19.16
	19.37	20.18	19.37
Agricultural Soils			
MSU Soil E	14.12	14.76	14.50
	15.31	14.64	14.52
	14.82	14.76	14.90
MSU Soil F	14.29	14.32	14.41
	14.75	14.98	14.94
	15.15	15.00	15.04
KBS T1	14.36	14.26	14.28
	14.19	14.97	14.36
	13.98	13.66	13.78
KBS T2	14.38	14.32	14.16
	14.46	14.51	14.51
	14.26	14.46	14.44
KBS T3	14.44	14.54	14.46
	14.33	14.48	14.37
	14.47	14.68	14.49
KBS T4	14.17	14.37	14.18
	14.28	14.40	14.38
	14.39	14.46	14.67
Negative and No Template Controls			
aceA	28.56	29.07	27.86
NTC	28.00	28.56	28.85

Table D.8. SmartChip standard curve characteristics

Gene	Assay	Plasmid	Log(Linear Range)	Efficiency (%)	R^2	Slope	Y-Intercept
diaA	1	CP012395	1 to 7	102.6	0.995	-3.26	29.6
	3	CP012395	1 to 7	102.4	0.998	-3.26	29.7
	4	AP009049	2 to 7	78.3	0.986	-3.98	34.3
nfsI	1	CP001918	2 to 7	80.5	0.991	-3.90	33.7
	2	CP001918	2 to 7	84.6	0.993	-3.76	31.4
	3	CP001918	2 to 7	86.0	0.995	-3.71	31.5
	4	CP001918	1 to 7	87.0	0.988	-3.68	31.1
	5	CP001918	1 to 7	84.1	0.994	-3.77	31.9
	6	CP001918	1 to 7	84.8	0.988	-3.75	31.9
	7	CP001918	2 to 7	76.1	0.985	-4.07	34.6
	8	CP001918	1 to 7	84.9	0.983	-3.75	31.4
	Pub1	M63808	2 to 7	91.9	0.918	-3.53	32.3
	Pub2	M63808	2 to 7	76.2	0.935	-4.06	33.6
pnrB	1	CP010979	1 to 7	87.0	0.997	-3.68	31.3
	2	MCBI01000011	3 to 7	60.1	0.987	-4.89	39.9
	3	CP010979	3 to 7	84.1	0.991	-3.77	37.0
	4	CP010979	1 to 7	90.6	0.995	-3.57	30.5
	5	CP010979	2 to 7	91.5	0.988	-3.54	33.7
	Pub1	AF532912	2 to 7	91.0	0.968	-3.56	31.7
	Pub2	AF532912	2 to 7	89.9	0.980	-3.59	31.9
xenA	1	CP004045	2 to 7	81.5	0.989	-3.86	33.9
	2	AP013070	4 to 7	97.3	0.997	-3.39	36.0
	3	AP013070	4 to 7	93.5	0.997	-3.49	36.9
	4	CP004045	3 to 7	90.8	0.995	-3.57	36.7
	5	AP013070	4 to 7	96.1	0.995	-3.42	30.5
	6	CP004045	1 to 7	89.7	0.998	-3.60	31.7
	7	AP013070	4 to 7	91.1	0.997	-3.55	32.9
	8	CP004045	2 to 7	88.5	0.995	-3.63	33.8
	9	FOEO01000004	2 to 7	83.6	0.995	-3.79	33.1
	10	CP004045	2 to 7	88.2	0.997	-3.64	32.5
	11	FOEO01000004	2 to 7	89.4	0.995	-3.61	31.3
	12	CP004045	1 to 7	92.4	0.997	-3.52	31.3
	14	AP013070	4 to 7	93.3	0.997	-3.49	32.2
	15	CP004045	2 to 7	85.8	0.996	-3.72	33.0
	16	CP004045	2 to 7	89.5	0.998	-3.60	32.9
	17	FOEO01000004	2 to 7	87.5	0.995	-3.66	31.8
	Pub1	AF154061	3 to 6	74.8	0.989	-4.12	32.1
xenB	1	CP014205	2 to 7	76.5	0.995	-4.05	35.8
	2	CP014205	2 to 7	84.1	0.996	-3.77	33.0
	4	CP014205	2 to 7	82.7	0.990	-3.82	35.6
	5	CP014205	1 to 7	88.4	0.990	-3.64	32.2
	6	CP014205	3 to 7	68.1	0.993	-4.43	38.6
	7	CP014205	2 to 7	83.8	0.991	-3.78	33.0
	8	CP014205	2 to 7	74.3	0.996	-4.14	36.0
	9	CP014205	3 to 7	80.6	0.986	-3.89	36.4
	10	CP002620	3 to 7	87.1	0.991	-3.68	37.2
	11	CP014205	1 to 7	93.0	0.986	-3.50	31.5
	14	CP014205	3 to 7	79.1	0.993	-3.95	37.7
	16	CP014205	4 to 7	83.8	0.986	-3.78	38.1
	17	CP002620	3 to 7	82.6	0.995	-3.82	35.5
	19	CP014205	1 to 7	90.1	0.988	-3.58	31.8

Table D.8. (cont'd)

Gene	**Assay**	**Plasmid**	**Log(Linear Range)**	**Efficiency (%)**	**R^2**	**Slope**	**Y-Intercept**
xenB	Pub1	AF154062	3 to 7	68.5	0.990	-4.42	35.8
xplA	3	CP007257	3 to 7	60.4	0.991	-4.88	40.3
	4	AF449421	2 to 7	75.4	0.993	-4.10	34.8
	5	BAFD01000115	4 to 7	40.2	0.930	-6.82	52.3
	Pub1	AF449421	1 to 7	97.3	0.995	-3.39	31.3
	Pub2	AF449421	1 to 7	94.5	0.994	-3.46	30.8
	Pub3	AF449421	3 to 7	79.1	0.985	-3.95	37.2
	Pub4	AF449421	1 to 7	96.3	0.995	-3.41	30.9
	Pub6	AF449421_300-959	2 to 6	61.8	0.984	-4.79	36.9
16S rRNA	16S_rRNA.1	KX858536	2 to 8	107.0	0.998	-3.16	30.9
	16S_rRNA.2	KX858536	1 to 8	103.4	0.994	-3.24	31.6
	16S_rRNA.3	KX858536	2 to 8	93.0	0.995	-3.50	33.2

Table D.9. Gene copies per mL or g of starting material for *nfsI* assays. “N/A” indicates either no amplification or false positive amplification.

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**Pub1**	**Pub2**
Shallow Zone Aquifer (gene copies per mL of groundwater)										
MW32 (Pre)	1.06E+03	N/A	N/A	N/A	N/A	2.78E+02	N/A	N/A	N/A	N/A
	1.03E+03	N/A	N/A	N/A	N/A	2.39E+02	N/A	N/A	N/A	N/A
	9.99E+02	N/A	N/A	N/A	N/A	2.64E+02	N/A	N/A	N/A	N/A
MW32 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	1.90E+04	N/A	N/A	N/A	N/A	5.87E+03	N/A	N/A	N/A	N/A
	1.85E+04	N/A	N/A	N/A	N/A	3.81E+03	N/A	N/A	N/A	N/A
	2.21E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer (gene copies per mL of groundwater)										
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	9.28E+03	N/A	N/A	N/A	N/A	N/A	3.98E+02	N/A	N/A	N/A
	1.49E+04	N/A	N/A	N/A	N/A	N/A	7.89E+02	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	6.15E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	3.91E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	7.50E+03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	7.79E+03	N/A	N/A	N/A
MW66 (Pre)	2.13E+03	N/A	N/A	N/A	N/A	N/A	1.75E+03	N/A	N/A	N/A
	1.17E+03	N/A	N/A	N/A	N/A	N/A	9.76E+02	N/A	N/A	N/A
	1.21E+03	N/A	N/A	N/A	N/A	N/A	1.29E+03	N/A	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	2.81E+02	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	1.36E+03	N/A	N/A	N/A
MW67 (Pre)	1.18E+03	N/A	N/A	N/A	N/A	N/A	4.71E+02	N/A	N/A	N/A
	8.84E+02	N/A	N/A	N/A	N/A	N/A	4.16E+02	N/A	N/A	N/A
	1.57E+03	N/A	N/A	N/A	N/A	N/A	3.51E+02	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River (gene copies per mL of river water)										
Red Cedar	N/A	N/A	N/A	N/A	N/A	N/A	3.51E+03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	4.17E+03	N/A	N/A	N/A
Sediment (gene copies per g of sediment)										
MW71	N/A	N/A	N/A	N/A	N/A	N/A	2.32E+05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	2.21E+05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils (gene copies per g of soil)										
MSU Soil E	1.05E+06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.11E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.66E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.9. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	Pub1	Pub2
MSU Soil F	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	9.17E+05	N/A	N/A	N/A	N/A	N/A	6.66E+05	N/A	N/A	N/A
	4.35E+05	N/A	N/A	N/A	N/A	2.60E+05	3.67E+05	N/A	N/A	N/A
	2.61E+06	N/A	N/A	N/A	N/A	7.16E+05	N/A	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	N/A	N/A	1.93E+05	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	3.79E+05	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.98E+05	N/A	N/A	N/A	N/A
KBS T3	6.08E+05	N/A	N/A	N/A	N/A	2.27E+05	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	6.41E+05	N/A	N/A	N/A
	1.68E+06	N/A	N/A	N/A	N/A	2.65E+05	1.10E+06	N/A	N/A	N/A
KBS T4	1.63E+06	N/A	N/A	N/A	N/A	2.94E+05	6.42E+05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	6.28E+05	1.41E+06	N/A	N/A	N/A
	2.47E+06	N/A	N/A	N/A	N/A	6.52E+05	1.98E+06	N/A	N/A	N/A

Table D.10. Gene copies per mL or g of starting material for *pnrB* assays. "N/A" indicates either no amplification or false positive amplification.

Sample	PS1	PS2	PS3	PS4	PS5	Pub1	Pub2
Shallow Zone Aquifer (gene copies per mL of groundwater)							
MW32 (Pre)	1.38E+02	7.25E+03	3.74E+03	8.23E+01	N/A	N/A	3.61E+03
	2.08E+02	5.62E+03	5.11E+03	N/A	N/A	N/A	4.19E+03
	N/A	6.06E+03	N/A	2.02E+02	N/A	N/A	2.62E+03
MW32 (Post)	N/A	N/A	1.13E+04	N/A	N/A	N/A	N/A
	N/A	N/A	2.48E+04	N/A	N/A	N/A	5.13E+03
	N/A	N/A	3.45E+04	N/A	N/A	N/A	7.65E+03
MW62 (Pre)	8.63E+02	6.06E+04	2.58E+05	N/A	N/A	N/A	N/A
	6.76E+02	5.46E+04	2.80E+05	N/A	N/A	N/A	N/A
	9.12E+02	7.55E+04	3.38E+05	N/A	N/A	N/A	N/A
MW62 (Post)	N/A	3.08E+03	N/A	N/A	N/A	N/A	N/A
	N/A	5.44E+04	N/A	N/A	N/A	N/A	N/A
	N/A	2.93E+04	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer (gene copies per mL of groundwater)							
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	9.04E+03	N/A	1.11E+02	N/A	N/A	2.74E+03
	N/A	1.88E+04	N/A	1.41E+02	N/A	N/A	3.13E+03
MW48 (Post)	N/A	1.24E+05	N/A	N/A	N/A	N/A	N/A
	N/A	9.93E+04	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	3.03E+04	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	8.47E+03	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	5.01E+05	N/A	N/A	N/A	N/A	N/A
	N/A	6.69E+05	N/A	N/A	N/A	N/A	N/A

Table D.10. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	Pub1	Pub2
MW66 (Pre)	N/A	3.80E+03	5.34E+03	N/A	N/A	N/A	1.93E+03
	N/A	4.57E+03	7.10E+03	N/A	N/A	N/A	1.47E+03
	N/A	4.78E+03	N/A	N/A	N/A	N/A	6.95E+02
MW66 (Post)	N/A	9.68E+03	N/A	N/A	N/A	N/A	N/A
	N/A	4.67E+04	N/A	N/A	N/A	N/A	N/A
MW67 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	9.10E+02
	N/A	N/A	N/A	N/A	N/A	N/A	1.36E+03
	N/A	N/A	N/A	N/A	N/A	N/A	1.22E+03
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River (gene copies per mL of river water)							
Red Cedar	N/A	N/A	N/A	5.16E+02	N/A	N/A	1.12E+04
	N/A	N/A	N/A	1.03E+03	N/A	N/A	1.89E+04
Sediment (gene copies per g of sediment)							
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils (gene copies per g of soil)							
MSU Soil E	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MSU Soil F	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.22E+05	N/A	N/A	N/A
	N/A	N/A	N/A	8.02E+04	N/A	N/A	N/A
KBS T3	1.01E+05	N/A	N/A	N/A	N/A	N/A	7.82E+05
	1.37E+05	N/A	N/A	1.15E+05	N/A	N/A	N/A
	2.42E+05	N/A	N/A	2.01E+05	N/A	N/A	2.12E+06
KBS T4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.11. Gene copies per mL or g of starting material for *xenA* assays. “N/A” indicates either no amplification or false positive amplification.

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
Shallow Zone Aquifer (gene copies per mL of groundwater)																	
MW32 (Pre)	3.21 E+02	N/A	N/A	3.80 E+04	N/A	4.18 E+02	N/A	N/A	8.06 E+02	1.20 E+03	N/A	8.03 E+02	N/A	4.39 E+03	1.91 E+03	N/A	N/A
	6.37 E+02	N/A	N/A	2.78 E+04	N/A	6.42 E+02	N/A	N/A	4.93 E+02	7.52 E+02	N/A	1.24 E+03	N/A	4.44 E+03	1.57 E+03	N/A	N/A
	7.43 E+02	N/A	N/A	4.13 E+04	N/A	8.10 E+02	N/A	N/A	1.24 E+03	6.51 E+02	N/A	8.36 E+02	N/A	4.35 E+03	3.36 E+03	N/A	N/A
MW32 (Post)	N/A	N/A	N/A	3.65 E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.10 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.24 E+03	N/A	N/A	N/A
	N/A	N/A	N/A	1.10 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.46 E+03	N/A	N/A	N/A
MW62 (Pre)	2.41 E+03	N/A	N/A	1.27 E+05	N/A	5.41 E+02	N/A	9.32 E+03	N/A	N/A	N/A	N/A	N/A	3.03 E+04	N/A	N/A	N/A
	3.99 E+03	N/A	N/A	1.40 E+05	N/A	9.40 E+02	N/A	6.62 E+03	N/A	N/A	N/A	N/A	N/A	2.49 E+04	N/A	N/A	N/A
	4.41 E+03	N/A	N/A	1.83 E+05	N/A	1.16 E+03	N/A	9.37 E+03	N/A	N/A	N/A	N/A	N/A	2.55 E+04	N/A	N/A	N/A
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	2.56 E+02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8.67 E+02	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.17 E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.12 E+03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	1.52 E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.43 E+03	N/A	N/A	N/A
Perched Zone Aquifer (gene copies per mL of groundwater)																	
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	2.97 E+02	N/A	N/A	2.25 E+05	N/A	1.83 E+03	N/A	N/A	2.35 E+03	8.66 E+02	4.04 E+03	3.22 E+03	N/A	4.89 E+03	N/A	N/A	N/A
	3.12 E+02	N/A	N/A	5.43 E+05	N/A	6.08 E+03	N/A	N/A	4.29 E+03	1.20 E+03	7.79 E+03	6.94 E+03	N/A	5.62 E+03	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.11. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
MW60R (Post)	N/A	N/A	N/A	1.17E+04	N/A	1.18E+03	N/A	8.87E+02	N/A	N/A	N/A	2.02E+02	N/A	3.93E+03	N/A	N/A	N/A
	N/A	N/A	N/A	1.27E+04	N/A	5.61E+02	N/A	9.23E+02	N/A	N/A	N/A	2.79E+02	N/A	2.91E+03	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	1.55E+04	N/A	N/A	N/A	5.31E+02	N/A	N/A	N/A	N/A	N/A	3.09E+03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8.58E+02	N/A	N/A	N/A
	N/A	N/A	N/A	1.14E+04	N/A	N/A	N/A	5.19E+02	N/A	N/A	N/A	N/A	N/A	1.95E+03	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	N/A	1.48E+05	N/A	3.20E+04	N/A	N/A	N/A	N/A	N/A	1.30E+03	N/A	2.25E+04	N/A	N/A	N/A
	N/A	N/A	N/A	1.69E+05	N/A	3.44E+04	N/A	N/A	N/A	N/A	N/A	2.50E+03	N/A	2.71E+04	N/A	N/A	N/A
MW66 (Pre)	N/A	N/A	N/A	4.72E+04	N/A	4.17E+02	N/A	N/A	7.41E+02	N/A	6.19E+02	N/A	N/A	8.12E+03	N/A	N/A	N/A
	N/A	N/A	N/A	5.06E+04	N/A	4.06E+02	N/A	1.74E+03	5.30E+02	N/A	5.61E+02	6.27E+02	N/A	6.47E+03	8.85E+02	N/A	N/A
	N/A	N/A	N/A	1.83E+04	N/A	2.14E+02	N/A	1.52E+03	N/A	N/A	4.39E+02	1.83E+02	N/A	4.90E+03	4.49E+02	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	7.92E+03	N/A	3.45E+02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.67E+03	N/A	N/A	N/A
	N/A	N/A	N/A	2.04E+04	N/A	1.26E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6.76E+03	N/A	N/A	N/A
MW67 (Pre)	7.95E+02	N/A	N/A	6.43E+03	N/A	N/A	N/A	2.34E+03	N/A	N/A	N/A	1.50E+03	N/A	2.82E+03	N/A	N/A	N/A
	N/A	N/A	N/A	9.69E+03	N/A	9.26E+02	N/A	4.12E+03	1.39E+03	N/A	N/A	2.16E+03	N/A	3.52E+03	N/A	N/A	N/A
	4.72E+02	N/A	N/A	1.03E+04	N/A	9.13E+02	N/A	5.33E+03	1.73E+03	N/A	N/A	2.67E+03	N/A	2.22E+03	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.11. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
Red Cedar River (gene copies per mL of river water)																	
Red Cedar	N/A	N/A	N/A	2.36 E+05	N/A	8.20 E+02	N/A	4.47 E+03	N/A	N/A	N/A	4.98 E+02	N/A	2.19 E+04	N/A	N/A	N/A
	N/A	N/A	N/A	3.04 E+05	N/A	1.58 E+03	N/A	5.99 E+03	N/A	N/A	N/A	3.89 E+02	N/A	2.84 E+04	N/A	N/A	N/A
Sediment (gene copies per g of sediment)																	
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soil (gene copies per g of soil)																	
MSU Soil E	N/A	N/A	N/A	2.36 E+07	N/A	N/A	N/A	1.48 E+06	N/A	N/A	N/A	1.69 E+05	N/A	7.74 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	7.82 E+06	N/A	8.77 E+04	N/A	5.12 E+05	N/A	N/A	N/A	N/A	N/A	2.18 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	1.00 E+07	N/A	1.07 E+05	N/A	3.25 E+05	N/A	N/A	N/A	1.45 E+05	N/A	2.58 E+06	N/A	N/A	N/A
MSU Soil F	N/A	N/A	N/A	N/A	N/A	1.34 E+05	N/A	1.23 E+06	N/A	N/A	N/A	N/A	N/A	5.85 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	5.70 E+04	N/A	4.39 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.54 E+05	N/A	N/A	N/A	N/A	N/A	1.35 E+06	N/A	N/A	N/A
KBS T1	N/A	N/A	N/A	2.17 E+07	N/A	3.03 E+05	N/A	1.48 E+06	N/A	N/A	N/A	8.60 E+04	N/A	8.81 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	1.00 E+07	N/A	1.51 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.36 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	4.12 E+07	N/A	N/A	N/A	2.12 E+06	N/A	N/A	N/A	2.31 E+06	N/A	9.41 E+06	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	1.04 E+07	N/A	1.23 E+05	N/A	5.39 E+05	N/A	N/A	N/A	N/A	N/A	4.27 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	2.39 E+07	N/A	2.45 E+05	N/A	2.16 E+06	N/A	N/A	N/A	1.04 E+05	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.88 E+07	N/A	2.27 E+05	N/A	N/A	N/A	N/A	N/A	8.82 E+04	N/A	5.30 E+06	N/A	N/A	N/A

Table D.11. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
KBS T3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.96 E+05	N/A	N/A	N/A	N/A	N/A	3.97 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	1.05 E+07	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.33 E+06	N/A	N/A	N/A
	N/A	N/A	N/A	6.05 E+07	N/A	N/A	N/A	2.84 E+06	N/A	N/A	N/A	N/A	N/A	8.75 E+06	N/A	N/A	N/A
KBS T4	N/A	N/A	N/A	N/A	N/A	2.90 E+05	N/A	1.73 E+06	N/A	N/A	N/A	N/A	N/A	8.81 E+06	N/A	N/A	N/A
	6.07 E+05	N/A	N/A	3.43 E+07	N/A	3.18 E+05	N/A	N/A	N/A	N/A	N/A	1.47 E+05	N/A	1.18 E+07	1.20 E+06	N/A	N/A
	5.78 E+05	N/A	N/A	5.49 E+07	N/A	5.54 E+05	N/A	2.71 E+06	N/A	N/A	N/A	3.85 E+05	N/A	1.20 E+07	1.05 E+06	N/A	N/A

Table D.12. Gene copies per mL or g of starting material for *xenB* assays. "N/A" indicates either no amplification or false positive amplification.

Sample	PS1	PS2	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS14	PS16	PS17	PS19	Pub1
Shallow Zone Aquifer (gene copies per mL of groundwater)															
MW32 (Pre)	1.64 E+04	3.59 E+03	4.23 E+03	2.51 E+03	2.03 E+04	8.35 E+03	3.64 E+03	N/A	5.59 E+03	1.18 E+03	1.72 E+04	4.25 E+04	N/A	5.84 E+03	N/A
	1.46 E+04	4.08 E+03	5.35 E+03	2.48 E+03	1.39 E+04	7.35 E+03	3.24 E+03	6.24 E+03	7.28 E+03	8.62 E+02	1.94 E+04	6.86 E+04	N/A	4.43 E+03	N/A
	1.88 E+04	3.87 E+03	7.79 E+03	3.27 E+03	1.83 E+04	7.45 E+03	4.47 E+03	6.39 E+03	6.00 E+03	1.23 E+03	2.76 E+04	6.50 E+04	N/A	4.87 E+03	N/A
MW32 (Post)	N/A	N/A	N/A	2.70 E+02	N/A	N/A	N/A	N/A	N/A	3.02 E+02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	6.01 E+02	N/A	N/A	N/A	N/A	N/A	1.34 E+03	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	6.63 E+02	N/A	N/A	N/A	N/A	N/A	2.00 E+03	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	4.02 E+04	3.28 E+03	N/A	2.33 E+03	1.14 E+05	8.75 E+03	3.53 E+03	1.02 E+05	4.04 E+04	N/A	3.17 E+04	N/A	N/A	9.14 E+02	N/A
	3.91 E+04	2.45 E+03	N/A	N/A	1.43 E+05	6.51 E+03	2.98 E+03	8.76 E+04	2.45 E+04	7.78 E+02	4.14 E+04	N/A	N/A	9.53 E+02	N/A
	5.02 E+04	4.86 E+03	N/A	3.75 E+03	1.34 E+05	9.50 E+03	4.41 E+03	1.14 E+05	3.55 E+04	1.69 E+03	5.05 E+04	N/A	N/A	1.24 E+03	N/A

Table D.12. (cont'd)

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
MW62 (Post)	1.80 E+03	7.15 E+02	7.02 E+02	3.32 E+02	N/A	6.65 E+02	N/A	N/A	N/A	1.39 E+02	4.39 E+03	N/A	N/A	3.46 E+02	N/A
	3.85 E+03	3.97 E+03	4.13 E+03	2.01 E+03	N/A	5.76 E+03	N/A	N/A	N/A	4.63 E+02	4.66 E+04	N/A	N/A	4.57 E+03	N/A
	5.39 E+03	1.86 E+03	4.25 E+03	2.17 E+03	N/A	3.35 E+03	N/A	N/A	N/A	1.03 E+03	1.23 E+04	N/A	N/A	2.85 E+03	N/A
Perched Zone Aquifer (gene copies per mL of groundwater)															
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	3.86 E+04	2.11 E+04	4.54 E+04	4.46 E+04	5.70 E+04	N/A	2.12 E+04	1.01 E+05	4.95 E+04	1.81 E+05	2.53 E+05	6.81 E+03	4.56 E+04	N/A
	N/A	8.45 E+04	4.66 E+04	1.03 E+05	8.58 E+04	1.20 E+05	N/A	3.28 E+04	1.66 E+05	1.20 E+05	4.56 E+05	6.95 E+05	1.09 E+04	1.13 E+05	N/A
MW48 (Post)	5.16 E+04	N/A	N/A	3.07 E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.22 E+04	N/A	N/A	1.83 E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	5.37 E+03	1.38 E+03	N/A	7.23 E+02	N/A	2.51 E+03	N/A	N/A	N/A	2.26 E+02	N/A	N/A	N/A	1.84 E+03	N/A
	9.72 E+02	N/A	N/A	1.79 E+02	N/A	N/A	N/A	N/A	N/A	1.71 E+02	N/A	N/A	N/A	3.60 E+02	N/A
	1.35 E+03	9.41 E+02	N/A	3.64 E+02	N/A	4.77 E+02	N/A	N/A	N/A	2.52 E+02	N/A	N/A	N/A	3.07 E+02	N/A
MW60R (Post)	9.12 E+03	2.93 E+03	N/A	7.46 E+02	2.30 E+04	1.29 E+03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	5.54 E+03	2.10 E+03	N/A	4.10 E+02	7.79 E+03	6.48 E+02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	4.08 E+03	9.29 E+02	9.77 E+02	7.33 E+02	1.08 E+04	2.18 E+03	1.69 E+03	N/A	N/A	7.11 E+01	9.92 E+03	N/A	N/A	1.20 E+03	N/A
	2.03 E+03	2.57 E+03	9.97 E+02	1.30 E+03	4.05 E+03	1.46 E+03	2.20 E+03	N/A	N/A	1.07 E+03	3.68 E+03	N/A	N/A	1.39 E+03	N/A
	3.84 E+03	9.28 E+02	2.09 E+03	4.15 E+02	1.37 E+04	2.17 E+03	1.59 E+03	N/A	N/A	N/A	8.72 E+03	N/A	N/A	1.01 E+03	N/A
MW64 (Post)	3.18 E+04	N/A	N/A	1.42 E+04	6.85 E+04	2.44 E+04	N/A	N/A	N/A	8.43 E+03	N/A	N/A	N/A	1.10 E+04	N/A
	4.13 E+04	N/A	N/A	1.71 E+04	8.59 E+04	3.91 E+04	N/A	N/A	N/A	1.23 E+04	N/A	N/A	N/A	1.33 E+04	N/A

Table D.12. (cont'd)

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
MW66 (Pre)	1.37 E+04	3.53 E+03	5.21 E+03	2.70 E+03	1.61 E+04	5.98 E+03	4.54 E+03	N/A	N/A	8.43 E+02	3.84 E+04	6.94 E+04	N/A	4.75 E+03	N/A
	1.26 E+04	4.04 E+03	5.39 E+03	2.69 E+03	2.07 E+04	7.40 E+03	4.94 E+03	N/A	N/A	N/A	3.60 E+04	8.15 E+04	N/A	3.90 E+03	N/A
	6.05 E+03	8.38 E+02	1.22 E+03	5.96 E+02	8.69 E+03	1.56 E+03	6.41 E+02	N/A	N/A	1.59 E+02	8.41 E+03	N/A	N/A	5.35 E+02	N/A
MW66 (Post)	5.22 E+03	5.66 E+02	1.55 E+03	3.69 E+02	1.56 E+04	2.05 E+03	2.13 E+03	N/A	N/A	4.72 E+02	4.42 E+03	N/A	N/A	1.15 E+03	N/A
	2.44 E+04	3.53 E+03	6.51 E+03	2.65 E+03	7.11 E+04	7.84 E+03	4.92 E+03	N/A	N/A	1.26 E+03	1.40 E+04	N/A	N/A	3.22 E+03	N/A
MW67 (Pre)	1.10 E+04	2.98 E+03	4.36 E+03	2.50 E+03	2.64 E+04	5.35 E+03	6.28 E+03	2.89 E+04	N/A	5.98 E+02	3.45 E+04	1.02 E+05	N/A	4.96 E+03	N/A
	1.40 E+04	4.04 E+03	4.71 E+03	2.21 E+03	2.85 E+04	4.43 E+03	3.57 E+03	2.54 E+04	N/A	5.61 E+02	2.42 E+04	4.41 E+04	N/A	2.84 E+03	N/A
	1.57 E+04	4.94 E+03	7.71 E+03	3.40 E+03	3.79 E+04	5.82 E+03	7.97 E+03	2.85 E+04	N/A	1.49 E+03	3.21 E+04	1.10 E+05	N/A	5.50 E+03	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River (gene copies per mL of river water)															
Red Cedar	3.44 E+04	N/A	N/A	1.60 E+03	N/A	2.83 E+03	N/A	1.59 E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.61 E+04	N/A	N/A	3.19 E+03	N/A	6.36 E+03	N/A	3.23 E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sediment (gene copies per g of sediment)															
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soil (gene copies per g of soil)															
MSU Soil E	1.98 E+06	1.23 E+06	N/A	7.01 E+05	N/A	9.05 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.25 E+05	N/A
	N/A	N/A	N/A	1.71 E+05	N/A	3.34 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.37 E+06	4.51 E+05	N/A	3.09 E+05	N/A	4.10 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.55 E+05	N/A

Table D.12. (cont'd)

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
MSU Soil F	3.31 E+06	N/A	N/A	6.83 E+05	4.19 E+06	9.09 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.23 E+06	N/A	N/A	2.35 E+05	N/A	5.10 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	3.00 E+06	N/A	N/A	3.03 E+05	3.56 E+06	5.18 E+05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	4.87 E+06	1.06 E+06	N/A	N/A	3.92 E+06	1.21 E+06	N/A	5.36 E+06	N/A	9.99 E+04	N/A	N/A	N/A	2.04 E+05	N/A
	7.82 E+06	8.72 E+05	N/A	2.05 E+05	5.12 E+06	3.13 E+06	1.60 E+06	N/A	N/A	1.37 E+06	N/A	N/A	N/A	1.44 E+06	N/A
	4.89 E+07	5.73 E+06	N/A	3.09 E+06	1.78 E+07	1.99 E+07	2.34 E+06	1.68 E+07	N/A	1.25 E+07	N/A	N/A	N/A	1.45 E+07	N/A
KBS T2	5.78 E+06	8.40 E+05	6.31 E+05	3.55 E+05	5.04 E+06	1.87 E+06	5.61 E+05	3.44 E+06	N/A	7.55 E+05	N/A	N/A	N/A	1.16 E+06	N/A
	2.08 E+06	7.94 E+05	N/A	5.29 E+05	N/A	1.59 E+06	N/A	4.82 E+06	N/A	2.14 E+05	N/A	N/A	N/A	4.50 E+05	N/A
	9.27 E+06	N/A	8.36 E+05	5.00 E+05	9.91 E+06	3.55 E+06	8.79 E+05	4.90 E+06	N/A	2.39 E+06	N/A	N/A	N/A	1.67 E+06	N/A
KBS T3	4.37 E+06	6.31 E+05	N/A	3.41 E+05	N/A	1.93 E+06	6.50 E+05	N/A	N/A	5.51 E+05	N/A	N/A	N/A	7.99 E+05	N/A
	2.82 E+06	6.96 E+05	N/A	3.37 E+05	N/A	7.60 E+05	6.56 E+05	N/A	N/A	4.89 E+05	N/A	N/A	N/A	4.11 E+05	N/A
	6.74 E+06	2.15 E+06	N/A	1.25 E+06	N/A	2.97 E+06	N/A	N/A	N/A	2.59 E+05	N/A	N/A	N/A	9.85 E+05	N/A
KBS T4	7.10 E+06	N/A	N/A	5.10 E+05	N/A	4.78 E+06	N/A	5.91 E+06	N/A	2.04 E+06	N/A	N/A	N/A	1.78 E+06	N/A
	6.28 E+06	2.29 E+06	N/A	6.44 E+05	N/A	2.93 E+06	N/A	5.59 E+06	N/A	3.35 E+05	N/A	N/A	N/A	7.61 E+05	N/A
	5.75 E+06	9.88 E+05	N/A	1.01 E+06	N/A	1.41 E+06	N/A	7.10 E+06	N/A	4.07 E+05	N/A	N/A	N/A	7.48 E+05	N/A

Table D.13. Gene copies per mL or g of starting material for *xplA* assays. "N/A" indicates either no amplification or false positive amplification.

Sample	PS3	PS4	PS5	Pub1	Pub2	Pub3	Pub4	Pub6
Shallow Zone Aquifer (gene copies per mL of groundwater)								
MW32 (Pre)	N/A	2.51E+03	5.42E+04	N/A	7.83E+02	N/A	1.09E+03	3.24E+03
	N/A	2.34E+03	6.52E+04	N/A	5.57E+02	N/A	6.39E+02	2.28E+03
	N/A	2.49E+03	N/A	N/A	2.20E+02	N/A	4.25E+02	3.62E+03
MW32 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	2.49E+05	N/A	N/A	N/A	N/A	6.17E+03
	N/A	N/A	3.29E+05	N/A	N/A	N/A	N/A	1.03E+04
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer (gene copies per mL of groundwater)								
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.14E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.19E+04	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8.13E+03
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.73E+03
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	5.14E+05	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	4.38E+05	N/A	N/A	N/A	N/A	N/A
MW66 (Pre)	N/A	2.24E+03	N/A	1.50E+02	N/A	N/A	N/A	N/A
	N/A	1.39E+03	N/A	1.11E+02	N/A	N/A	N/A	N/A
	N/A	1.53E+03	N/A	N/A	N/A	N/A	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW67 (Pre)	N/A	N/A	2.74E+04	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.67E+04	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.09E+04	N/A	N/A	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River (gene copies per mL of river water)								
Red Cedar	N/A	1.75E+04	2.31E+05	N/A	N/A	N/A	N/A	3.63E+03
	N/A	2.32E+04	3.30E+05	N/A	N/A	N/A	N/A	4.39E+03
Sediment (gene copies per g of sediment)								
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils (gene copies per g of soil)								
MSU Soil E	N/A	N/A	8.15E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	4.50E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.98E+07	N/A	N/A	N/A	N/A	N/A

Table D.13. (cont'd)

Sample	PS3	PS4	PS5	Pub1	Pub2	Pub3	Pub4	Pub6
MSU Soil F	N/A	N/A	5.39E+07	N/A	N/A	N/A	N/A	9.42E+05
	N/A	N/A	2.69E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6.03E+05
KBS T1	N/A	N/A	6.31E+07	N/A	N/A	N/A	N/A	9.56E+05
	N/A	N/A	5.96E+07	N/A	N/A	N/A	N/A	7.66E+05
	N/A	N/A	1.52E+08	N/A	N/A	N/A	N/A	3.11E+06
KBS T2	N/A	N/A	4.36E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	8.33E+07	N/A	N/A	N/A	N/A	1.98E+06
	N/A	N/A	1.07E+08	N/A	N/A	N/A	N/A	3.81E+06
KBS T3	N/A	8.02E+05	4.63E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	5.07E+07	N/A	N/A	N/A	N/A	N/A
	N/A	2.39E+06	1.05E+08	N/A	N/A	N/A	N/A	N/A
KBS T4	N/A	N/A	9.73E+07	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	1.35E+08	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	1.60E+08	N/A	N/A	N/A	N/A	N/A

Table D.14. Gene copies per mL or g of starting material for 16S rRNA assays. "N/A" indicates either no amplification or false positive amplification

Sample	16S_rRNA.1	16S_rRNA.2	16S_rRNA.3
Shallow Zone Aquifer (gene copies per mL of groundwater)			
MW32 (Pre)	1.35E+06	1.63E+06	1.69E+06
	1.29E+06	1.49E+06	1.50E+06
	1.67E+06	1.76E+06	1.88E+06
MW32 (Post)	2.26E+07	2.42E+07	2.33E+07
	7.03E+07	6.94E+07	7.32E+07
	6.39E+07	8.09E+07	5.88E+07
MW62 (Pre)	2.07E+07	2.46E+07	2.65E+07
	1.86E+07	2.40E+07	2.24E+07
	3.08E+07	3.00E+07	3.21E+07
MW62 (Post)	1.67E+07	1.98E+07	1.66E+07
	9.59E+07	7.13E+07	9.44E+07
	4.00E+07	5.00E+07	5.21E+07
Perched Zone Aquifer (gene copies per mL of groundwater)			
MW48 (Pre)	4.40E+03	5.87E+03	2.34E+04
	1.72E+06	1.93E+06	2.15E+06
	3.61E+06	3.87E+06	4.03E+06
MW48 (Post)	2.82E+08	2.58E+08	3.08E+08
	3.15E+08	3.21E+08	4.10E+08
MW60R (Pre)	9.13E+05	9.71E+05	1.18E+06
	1.40E+04	1.81E+04	3.06E+04
	1.61E+04	1.33E+04	2.51E+04
MW60R (Post)	1.10E+07	1.52E+07	1.51E+07
	9.40E+06	1.06E+07	1.10E+07
MW64 (Pre)	4.00E+05	5.04E+05	6.73E+05
	4.12E+04	1.35E+05	2.01E+05
	4.94E+05	5.50E+05	8.64E+05
MW64 (Post)	1.38E+08	1.59E+08	1.47E+08
	1.40E+08	1.35E+08	1.67E+08

Table D.14. (cont'd)

Sample	16S_rRNA.1	16S_rRNA.2	16S_rRNA.3
MW66 (Pre)	1.79E+06	2.59E+06	2.97E+06
	1.67E+06	1.47E+06	2.29E+06
	N/A	1.44E+06	1.65E+06
MW66 (Post)	3.62E+06	5.04E+06	5.04E+06
	2.88E+07	3.11E+07	2.90E+07
MW67 (Pre)	6.85E+05	8.41E+05	1.02E+06
	6.46E+05	9.25E+05	9.75E+05
	8.14E+05	9.61E+05	1.17E+06
MW67 (Post)	1.03E+03	1.43E+03	2.60E+03
	2.99E+03	3.77E+03	6.42E+03
Red Cedar River (gene copies per mL of river water)			
Red Cedar	9.13E+06	1.10E+07	1.16E+07
	9.78E+06	1.17E+07	1.20E+07
Sediment (gene copies per g of sediment)			
MW71	2.15E+07	2.60E+07	2.53E+07
	2.90E+07	2.41E+07	4.73E+07
	2.07E+07	1.60E+07	4.25E+07
Agricultural Soils (gene copies per g of soil)			
MSU Soil E	1.46E+09	1.15E+09	1.60E+09
	4.03E+08	8.27E+08	1.04E+09
	5.73E+08	7.55E+08	8.04E+08
MSU Soil F	1.12E+09	1.37E+09	1.48E+09
	6.00E+08	6.44E+08	7.84E+08
	4.37E+08	6.14E+08	7.09E+08
KBS T1	1.17E+09	1.58E+09	1.78E+09
	1.16E+09	8.33E+08	1.47E+09
	2.49E+09	3.89E+09	3.97E+09
KBS T2	9.65E+08	1.26E+09	1.60E+09
	1.28E+09	1.55E+09	1.79E+09
	1.33E+09	1.44E+09	1.68E+09
KBS T3	8.68E+08	1.02E+09	1.24E+09
	1.08E+09	1.22E+09	1.51E+09
	1.47E+09	1.59E+09	2.11E+09
KBS T4	1.73E+09	1.88E+09	2.45E+09
	1.79E+09	2.05E+09	2.39E+09
	1.77E+09	2.10E+09	2.11E+09

Table D.15. Gene copies per 16S rRNA gene copy for *nfsI* assays. “N/A” indicates either no amplification or false positive amplification.

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**Pub1**	**Pub2**
Shallow Zone Aquifer										
MW32 (Pre)	6.53E-04	N/A	N/A	N/A	N/A	1.71E-04	N/A	N/A	N/A	N/A
	6.89E-04	N/A	N/A	N/A	N/A	1.60E-04	N/A	N/A	N/A	N/A
	5.67E-04	N/A	N/A	N/A	N/A	1.50E-04	N/A	N/A	N/A	N/A
MW32 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	7.72E-04	N/A	N/A	N/A	N/A	2.38E-04	N/A	N/A	N/A	N/A
	7.71E-04	N/A	N/A	N/A	N/A	1.59E-04	N/A	N/A	N/A	N/A
	7.37E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer										
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.80E-03	N/A	N/A	N/A	N/A	N/A	2.06E-04	N/A	N/A	N/A
	3.86E-03	N/A	N/A	N/A	N/A	N/A	2.04E-04	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	4.06E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	3.67E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	4.73E-05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	5.78E-05	N/A	N/A	N/A
MW66 (Pre)	8.20E-04	N/A	N/A	N/A	N/A	N/A	6.75E-04	N/A	N/A	N/A
	7.97E-04	N/A	N/A	N/A	N/A	N/A	6.65E-04	N/A	N/A	N/A
	8.43E-04	N/A	N/A	N/A	N/A	N/A	8.95E-04	N/A	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	5.57E-05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	4.36E-05	N/A	N/A	N/A
MW67 (Pre)	1.40E-03	N/A	N/A	N/A	N/A	N/A	5.59E-04	N/A	N/A	N/A
	9.55E-04	N/A	N/A	N/A	N/A	N/A	4.50E-04	N/A	N/A	N/A
	1.64E-03	N/A	N/A	N/A	N/A	N/A	3.66E-04	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River										
Red Cedar	N/A	N/A	N/A	N/A	N/A	N/A	3.18E-04	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	3.56E-04	N/A	N/A	N/A
Sediment										
MW71	N/A	N/A	N/A	N/A	N/A	N/A	8.93E-03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	9.20E-03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils										
MSU Soil E	9.11E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.97E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	6.17E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.15. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	Pub1	Pub2
MSU Soil F	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	5.81E-04	N/A	N/A	N/A	N/A	N/A	4.22E-04	N/A	N/A	N/A
	5.22E-04	N/A	N/A	N/A	N/A	3.13E-04	4.40E-04	N/A	N/A	N/A
	6.72E-04	N/A	N/A	N/A	N/A	1.84E-04	N/A	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	N/A	N/A	1.53E-04	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.45E-04	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.08E-04	N/A	N/A	N/A	N/A
KBS T3	5.99E-04	N/A	N/A	N/A	N/A	2.24E-04	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	5.24E-04	N/A	N/A	N/A
	1.05E-03	N/A	N/A	N/A	N/A	1.66E-04	6.90E-04	N/A	N/A	N/A
KBS T4	8.65E-04	N/A	N/A	N/A	N/A	1.56E-04	3.41E-04	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	3.06E-04	6.87E-04	N/A	N/A	N/A
	1.18E-03	N/A	N/A	N/A	N/A	3.10E-04	9.41E-04	N/A	N/A	N/A

Table D.16. Gene copies per 16S rRNA gene copy for *pnrB* assays. "N/A" indicates either no amplification or false positive amplification.

Sample	PS1	PS2	PS3	PS4	PS5	Pub1	Pub2
Shallow Zone Aquifer							
MW32 (Pre)	1.02E-04	5.37E-03	2.77E-03	6.10E-05	N/A	N/A	2.67E-03
	1.62E-04	4.35E-03	3.96E-03	N/A	N/A	N/A	3.24E-03
	N/A	3.63E-03	N/A	1.21E-04	N/A	N/A	1.57E-03
MW32 (Post)	N/A	N/A	4.98E-04	N/A	N/A	N/A	N/A
	N/A	N/A	3.53E-04	N/A	N/A	N/A	7.29E-05
	N/A	N/A	5.41E-04	N/A	N/A	N/A	1.20E-04
MW62 (Pre)	4.16E-05	2.92E-03	1.25E-02	N/A	N/A	N/A	N/A
	3.64E-05	2.94E-03	1.51E-02	N/A	N/A	N/A	N/A
	2.96E-05	2.45E-03	1.10E-02	N/A	N/A	N/A	N/A
MW62 (Post)	N/A	1.85E-04	N/A	N/A	N/A	N/A	N/A
	N/A	5.68E-04	N/A	N/A	N/A	N/A	N/A
	N/A	7.33E-04	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer							
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	5.25E-03	N/A	6.45E-05	N/A	N/A	1.59E-03
	N/A	5.21E-03	N/A	3.90E-05	N/A	N/A	8.67E-04
MW48 (Post)	N/A	4.39E-04	N/A	N/A	N/A	N/A	N/A
	N/A	3.15E-04	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	3.32E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	5.25E-01	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	3.64E-03	N/A	N/A	N/A	N/A	N/A
	N/A	4.79E-03	N/A	N/A	N/A	N/A	N/A

Table D.16. (cont'd)

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**Pub1**	**Pub2**
MW66 (Pre)	N/A	2.12E-03	2.98E-03	N/A	N/A	N/A	1.08E-03
	N/A	2.74E-03	4.26E-03	N/A	N/A	N/A	8.81E-04
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW66 (Post)	N/A	2.68E-03	N/A	N/A	N/A	N/A	N/A
	N/A	1.62E-03	N/A	N/A	N/A	N/A	N/A
MW67 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	1.33E-03
	N/A	N/A	N/A	N/A	N/A	N/A	2.11E-03
	N/A	N/A	N/A	N/A	N/A	N/A	1.50E-03
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River							
Red Cedar	N/A	N/A	N/A	5.65E-05	N/A	N/A	1.23E-03
	N/A	N/A	N/A	1.05E-04	N/A	N/A	1.93E-03
Sediment							
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils							
MSU Soil E	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MSU Soil F	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	9.58E-05	N/A	N/A	N/A
	N/A	N/A	N/A	6.03E-05	N/A	N/A	N/A
KBS T3	1.16E-04	N/A	N/A	N/A	N/A	N/A	9.00E-04
	1.27E-04	N/A	N/A	1.06E-04	N/A	N/A	N/A
	1.65E-04	N/A	N/A	1.37E-04	N/A	N/A	1.44E-03
KBS T4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.17. Gene copies per 16S rRNA gene copy for *xenA* assays. “N/A” indicates either no amplification or false positive amplification.

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS12**	**PS14**	**PS15**	**PS16**	**PS17**	**Pub1**
Shallow Zone Aquifer																	
MW32 (Pre)	1.90 E-04	N/A	N/A	2.25 E-02	N/A	2.47 E-04	N/A	N/A	4.78 E-04	7.09 E-04	N/A	4.76 E-04	N/A	2.60 E-03	1.13 E-03	N/A	N/A
	4.26 E-04	N/A	N/A	1.86 E-02	N/A	4.29 E-04	N/A	N/A	3.29 E-04	5.02 E-04	N/A	8.26 E-04	N/A	2.97 E-03	1.05 E-03	N/A	N/A
	3.96 E-04	N/A	N/A	2.20 E-02	N/A	4.32 E-04	N/A	N/A	6.60 E-04	3.47 E-04	N/A	4.45 E-04	N/A	2.32 E-03	1.79 E-03	N/A	N/A
MW32 (Post)	N/A	N/A	N/A	1.57 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.51 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.06 E-05	N/A	N/A	N/A
	N/A	N/A	N/A	1.87 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.19 E-05	N/A	N/A	N/A
MW62 (Pre)	9.09 E-05	N/A	N/A	4.78 E-03	N/A	2.04 E-05	N/A	3.52 E-04	N/A	N/A	N/A	N/A	N/A	1.14 E-03	N/A	N/A	N/A
	1.78 E-04	N/A	N/A	6.22 E-03	N/A	4.19 E-05	N/A	2.95 E-04	N/A	N/A	N/A	N/A	N/A	1.11 E-03	N/A	N/A	N/A
	1.37 E-04	N/A	N/A	5.69 E-03	N/A	3.61 E-05	N/A	2.92 E-04	N/A	N/A	N/A	N/A	N/A	7.96 E-04	N/A	N/A	N/A
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	1.54 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.23 E-05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.29 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.37 E-05	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	2.91 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6.59 E-05	N/A	N/A	N/A
Perched Zone Aquifer																	
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.38 E-04	N/A	N/A	1.05 E-01	N/A	8.51 E-04	N/A	N/A	1.10 E-03	4.04 E-04	1.89 E-03	1.50 E-03	N/A	2.28 E-03	N/A	N/A	N/A
	7.75 E-05	N/A	N/A	1.35 E-01	N/A	1.51 E-03	N/A	N/A	1.06 E-03	2.97 E-04	1.93 E-03	1.72 E-03	N/A	1.39 E-03	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.17. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
MW60R (Post)	N/A	N/A	N/A	7.73 E-04	N/A	7.81 E-05	N/A	5.88 E-05	N/A	N/A	N/A	1.34 E-05	N/A	2.61 E-04	N/A	N/A	N/A
	N/A	N/A	N/A	1.15 E-03	N/A	5.08 E-05	N/A	8.36 E-05	N/A	N/A	N/A	2.53 E-05	N/A	2.63 E-04	N/A	N/A	N/A
MW64 (Pre)	N/A	N/A	N/A	2.30 E-02	N/A	N/A	N/A	7.89 E-04	N/A	N/A	N/A	N/A	N/A	4.59 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.26 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	1.32 E-02	N/A	N/A	N/A	6.01 E-04	N/A	N/A	N/A	N/A	N/A	2.25 E-03	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	N/A	1.01 E-03	N/A	2.18 E-04	N/A	N/A	N/A	N/A	N/A	8.87 E-06	N/A	1.53 E-04	N/A	N/A	N/A
	N/A	N/A	N/A	1.01 E-03	N/A	2.07 E-04	N/A	N/A	N/A	N/A	N/A	1.50 E-05	N/A	1.62 E-04	N/A	N/A	N/A
MW66 (Pre)	N/A	N/A	N/A	1.59 E-02	N/A	1.40 E-04	N/A	N/A	2.49 E-04	N/A	2.08 E-04	N/A	N/A	2.73 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	2.21 E-02	N/A	1.77 E-04	N/A	7.59 E-04	2.31 E-04	N/A	2.45 E-04	2.73 E-04	N/A	2.82 E-03	3.86 E-04	N/A	N/A
	N/A	N/A	N/A	1.11 E-02	N/A	1.30 E-04	N/A	9.22 E-04	N/A	N/A	2.67 E-04	1.11 E-04	N/A	2.98 E-03	2.73 E-04	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	1.57 E-03	N/A	6.84 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.31 E-04	N/A	N/A	N/A
	N/A	N/A	N/A	7.02 E-04	N/A	4.33 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.33 E-04	N/A	N/A	N/A
MW67 (Pre)	7.83 E-04	N/A	N/A	6.33 E-03	N/A	N/A	N/A	2.31 E-03	N/A	N/A	N/A	1.48 E-03	N/A	2.77 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	9.94 E-03	N/A	9.50 E-04	N/A	4.23 E-03	1.42 E-03	N/A	N/A	2.21 E-03	N/A	3.61 E-03	N/A	N/A	N/A
	4.05 E-04	N/A	N/A	8.88 E-03	N/A	7.83 E-04	N/A	4.57 E-03	1.49 E-03	N/A	N/A	2.29 E-03	N/A	1.91 E-03	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D.17. (cont'd)

Sample	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS12	PS14	PS15	PS16	PS17	Pub1
Red Cedar River																	
Red Cedar	N/A	N/A	N/A	2.03 E-02	N/A	7.06 E-05	N/A	3.85 E-04	N/A	N/A	N/A	4.29 E-05	N/A	1.88 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	2.55 E-02	N/A	1.32 E-04	N/A	5.01 E-04	N/A	N/A	N/A	3.25 E-05	N/A	2.38 E-03	N/A	N/A	N/A
Sediment																	
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soil																	
MSU Soil E	N/A	N/A	N/A	1.47 E-02	N/A	N/A	N/A	9.21 E-04	N/A	N/A	N/A	1.06 E-04	N/A	4.83 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	7.53 E-03	N/A	8.45 E-05	N/A	4.94 E-04	N/A	N/A	N/A	N/A	N/A	2.10 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	1.25 E-02	N/A	1.33 E-04	N/A	4.04 E-04	N/A	N/A	N/A	1.80 E-04	N/A	3.21 E-03	N/A	N/A	N/A
MSU Soil F	N/A	N/A	N/A	N/A	N/A	9.07 E-05	N/A	8.33 E-04	N/A	N/A	N/A	N/A	N/A	3.97 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	7.27 E-05	N/A	5.60 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.99 E-04	N/A	N/A	N/A	N/A	N/A	1.90 E-03	N/A	N/A	N/A
KBS T1	N/A	N/A	N/A	1.22 E-02	N/A	1.71 E-04	N/A	8.35 E-04	N/A	N/A	N/A	4.84 E-05	N/A	4.96 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	6.84 E-03	N/A	1.03 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.29 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	1.04 E-02	N/A	N/A	N/A	5.33 E-04	N/A	N/A	N/A	5.82 E-04	N/A	2.37 E-03	N/A	N/A	N/A
KBS T2	N/A	N/A	N/A	6.49 E-03	N/A	7.70 E-05	N/A	3.38 E-04	N/A	N/A	N/A	N/A	N/A	2.67 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	1.34 E-02	N/A	1.37 E-04	N/A	1.21 E-03	N/A	N/A	N/A	5.84 E-05	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.12 E-02	N/A	1.35 E-04	N/A	N/A	N/A	N/A	N/A	5.23 E-05	N/A	3.15 E-03	N/A	N/A	N/A

Table D.17. (cont'd)

Sample	**PS1**	**PS2**	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS12**	**PS14**	**PS15**	**PS16**	**PS17**	**Pub1**
KBS T3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.81 E-04	N/A	N/A	N/A	N/A	N/A	3.20 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	6.95 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.86 E-03	N/A	N/A	N/A
	N/A	N/A	N/A	2.87 E-02	N/A	N/A	N/A	1.35 E-03	N/A	N/A	N/A	N/A	N/A	4.15 E-03	N/A	N/A	N/A
KBS T4	N/A	N/A	N/A	N/A	N/A	1.18 E-04	N/A	7.07 E-04	N/A	N/A	N/A	N/A	N/A	3.60 E-03	N/A	N/A	N/A
	2.54 E-04	N/A	N/A	1.43 E-02	N/A	1.33 E-04	N/A	N/A	N/A	N/A	N/A	6.16 E-05	N/A	4.94 E-03	5.00 E-04	N/A	N/A
	2.74 E-04	N/A	N/A	2.60 E-02	N/A	2.63 E-04	N/A	1.29 E-03	N/A	N/A	N/A	1.83 E-04	N/A	5.69 E-03	4.98 E-04	N/A	N/A

Table D.18. Gene copies per 16S rRNA gene copy for *xenB* assays. "N/A" indicates either no amplification or false positive amplification.

Sample	**PS1**	**PS2**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS14**	**PS16**	**PS17**	**PS19**	**Pub1**
Shallow Zone Aquifer															
MW32 (Pre)	1.22 E-02	2.66 E-03	3.13 E-03	1.86 E-03	1.50 E-02	6.19 E-03	2.70 E-03	N/A	4.14 E-03	8.75 E-04	1.27 E-02	3.15 E-02	N/A	4.33 E-03	N/A
	1.13 E-02	3.16 E-03	4.14 E-03	1.92 E-03	1.08 E-02	5.70 E-03	2.51 E-03	4.83 E-03	5.64 E-03	6.68 E-04	1.50 E-02	5.32 E-02	N/A	3.43 E-03	N/A
	1.13 E-02	2.31 E-03	4.66 E-03	1.95 E-03	1.10 E-02	4.46 E-03	2.68 E-03	3.82 E-03	3.59 E-03	7.38 E-04	1.65 E-02	3.89 E-02	N/A	2.92 E-03	N/A
MW32 (Post)	N/A	N/A	N/A	1.20 E-05	N/A	N/A	N/A	N/A	N/A	1.34 E-05	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	8.55 E-06	N/A	N/A	N/A	N/A	N/A	1.91 E-05	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	1.04 E-05	N/A	N/A	N/A	N/A	N/A	3.14 E-05	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	1.94 E-03	1.58 E-04	N/A	1.12 E-04	5.50 E-03	4.22 E-04	1.70 E-04	4.90 E-03	1.95 E-03	N/A	1.53 E-03	N/A	N/A	4.40 E-05	N/A
	2.10 E-03	1.32 E-04	N/A	N/A	7.68 E-03	3.50 E-04	1.60 E-04	4.71 E-03	1.32 E-03	4.19 E-05	2.23 E-03	N/A	N/A	5.13 E-05	N/A
	1.63 E-03	1.58 E-04	N/A	1.22 E-04	4.36 E-03	3.08 E-04	1.43 E-04	3.71 E-03	1.15 E-03	5.50 E-05	1.64 E-03	N/A	N/A	4.03 E-05	N/A

Table D.18. (cont'd)

Sample	PS1	PS2	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS14	PS16	PS17	PS19	Pub1
MW62 (Post)	1.08 E-04	4.29 E-05	4.21 E-05	1.99 E-05	N/A	3.99 E-05	N/A	N/A	N/A	8.34 E-06	2.63 E-04	N/A	N/A	2.07 E-05	N/A
	4.02 E-05	4.14 E-05	4.31 E-05	2.10 E-05	N/A	6.00 E-05	N/A	N/A	N/A	4.83 E-06	4.86 E-04	N/A	N/A	4.77 E-05	N/A
	1.35 E-04	4.66 E-05	1.06 E-04	5.42 E-05	N/A	8.39 E-05	N/A	N/A	N/A	2.57 E-05	3.07 E-04	N/A	N/A	7.13 E-05	N/A
Perched Zone Aquifer															
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	2.24 E-02	1.23 E-02	2.64 E-02	2.59 E-02	3.31 E-02	N/A	1.23 E-02	5.89 E-02	2.88 E-02	1.05 E-01	1.47 E-01	3.95 E-03	2.65 E-02	N/A
	N/A	2.34 E-02	1.29 E-02	2.85 E-02	2.38 E-02	3.33 E-02	N/A	9.08 E-03	4.59 E-02	3.31 E-02	1.26 E-01	1.93 E-01	3.01 E-03	3.14 E-02	N/A
MW48 (Post)	1.83 E-04	N/A	N/A	1.09 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1.34 E-04	N/A	N/A	5.82 E-06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	5.88 E-03	1.52 E-03	N/A	7.92 E-04	N/A	2.75 E-03	N/A	N/A	N/A	2.48 E-04	N/A	N/A	N/A	2.01 E-03	N/A
	6.96 E-02	N/A	N/A	1.28 E-02	N/A	N/A	N/A	N/A	N/A	1.22 E-02	N/A	N/A	N/A	2.58 E-02	N/A
	8.35 E-02	5.84 E-02	N/A	2.26 E-02	N/A	2.96 E-02	N/A	N/A	N/A	1.57 E-02	N/A	N/A	N/A	1.90 E-02	N/A
MW60R (Post)	8.28 E-04	2.67 E-04	N/A	6.78 E-05	2.09 E-03	1.17 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	5.89 E-04	2.23 E-04	N/A	4.36 E-05	8.29 E-04	6.89 E-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Pre)	1.02 E-02	2.33 E-03	2.44 E-03	1.83 E-03	2.71 E-02	5.45 E-03	4.22 E-03	N/A	N/A	1.78 E-04	2.48 E-02	N/A	N/A	3.01 E-03	N/A
	4.92 E-02	6.24 E-02	2.42 E-02	3.17 E-02	9.82 E-02	3.55 E-02	5.34 E-02	N/A	N/A	2.60 E-02	8.94 E-02	N/A	N/A	3.38 E-02	N/A
	7.76 E-03	1.88 E-03	4.22 E-03	8.40 E-04	2.78 E-02	4.40 E-03	3.21 E-03	N/A	N/A	N/A	1.76 E-02	N/A	N/A	2.03 E-03	N/A
MW64 (Post)	2.31 E-04	N/A	N/A	1.03 E-04	4.98 E-04	1.78 E-04	N/A	N/A	N/A	6.13 E-05	N/A	N/A	N/A	7.97 E-05	N/A
	2.96 E-04	N/A	N/A	1.23 E-04	6.16 E-04	2.80 E-04	N/A	N/A	N/A	8.84 E-05	N/A	N/A	N/A	9.54 E-05	N/A

Table D.18. (cont'd)

Sample	PS1	PS2	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS14	PS16	PS17	PS19	Pub1
MW66 (Pre)	7.64 E-03	1.98 E-03	2.91 E-03	1.51 E-03	9.02 E-03	3.35 E-03	2.54 E-03	N/A	N/A	4.71 E-04	2.15 E-02	3.88 E-02	N/A	2.66 E-03	N/A
	7.57 E-03	2.43 E-03	3.23 E-03	1.61 E-03	1.25 E-02	4.44 E-03	2.96 E-03	N/A	N/A	N/A	2.16 E-02	4.89 E-02	N/A	2.34 E-03	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW66 (Post)	1.44 E-03	1.57 E-04	4.30 E-04	1.02 E-04	4.31 E-03	5.68 E-04	5.89 E-04	N/A	N/A	1.30 E-04	1.22 E-03	N/A	N/A	3.16 E-04	N/A
	8.45 E-04	1.22 E-04	2.26 E-04	9.18 E-05	2.47 E-03	2.72 E-04	1.71 E-04	N/A	N/A	4.38 E-05	4.84 E-04	N/A	N/A	1.12 E-04	N/A
MW67 (Pre)	1.61 E-02	4.36 E-03	6.37 E-03	3.65 E-03	3.86 E-02	7.81 E-03	9.16 E-03	4.22 E-02	N/A	8.73 E-04	5.04 E-02	1.49 E-01	N/A	7.24 E-03	N/A
	2.17 E-02	6.25 E-03	7.29 E-03	3.42 E-03	4.41 E-02	6.86 E-03	5.53 E-03	3.94 E-02	N/A	8.69 E-04	3.75 E-02	6.83 E-02	N/A	4.40 E-03	N/A
	1.92 E-02	6.07 E-03	9.47 E-03	4.18 E-03	4.66 E-02	7.15 E-03	9.78 E-03	3.50 E-02	N/A	1.83 E-03	3.94 E-02	1.35 E-01	N/A	6.75 E-03	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River															
Red Cedar	3.77 E-03	N/A	N/A	1.75 E-04	N/A	3.10 E-04	N/A	1.74 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	4.71 E-03	N/A	N/A	3.27 E-04	N/A	6.50 E-04	N/A	3.30 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sediment															
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soil															
MSU Soil E	1.36 E-03	8.48 E-04	N/A	4.82 E-04	N/A	6.22 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.55 E-04	N/A
	N/A	N/A	N/A	4.23 E-04	N/A	8.28 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	2.38 E-03	7.87 E-04	N/A	5.39 E-04	N/A	7.16 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.71 E-04	N/A

Table D.18. (cont'd)

Sample	PS1	PS2	PS4	PS5	PS6	PS7	PS8	PS9	PS10	PS11	PS14	PS16	PS17	PS19	Pub1
MSU Soil F	2.96 E-03	N/A	N/A	6.10 E-04	3.74 E-03	8.11 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	2.05 E-03	N/A	N/A	3.92 E-04	N/A	8.49 E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	6.85 E-03	N/A	N/A	6.92 E-04	8.15 E-03	1.18 E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KBS T1	4.15 E-03	9.01 E-04	N/A	N/A	3.35 E-03	1.03 E-03	N/A	4.57 E-03	N/A	8.53 E-05	N/A	N/A	N/A	1.74 E-04	N/A
	6.76 E-03	7.53 E-04	N/A	1.77 E-04	4.42 E-03	2.71 E-03	1.38 E-03	N/A	N/A	1.18 E-03	N/A	N/A	N/A	1.24 E-03	N/A
	1.97 E-02	2.30 E-03	N/A	1.24 E-03	7.16 E-03	8.01 E-03	9.42 E-04	6.77 E-03	N/A	5.03 E-03	N/A	N/A	N/A	5.85 E-03	N/A
KBS T2	5.99 E-03	8.71 E-04	6.54 E-04	3.68 E-04	5.23 E-03	1.94 E-03	5.82 E-04	3.57 E-03	N/A	7.82 E-04	N/A	N/A	N/A	1.21 E-03	N/A
	1.63 E-03	6.21 E-04	N/A	4.14 E-04	N/A	1.25 E-03	N/A	3.78 E-03	N/A	1.67 E-04	N/A	N/A	N/A	3.52 E-04	N/A
	6.97 E-03	N/A	6.28 E-04	3.76 E-04	7.45 E-03	2.67 E-03	6.61 E-04	3.68 E-03	N/A	1.80 E-03	N/A	N/A	N/A	1.26 E-03	N/A
KBS T3	5.03 E-03	7.27 E-04	N/A	3.93 E-04	N/A	2.23 E-03	7.49 E-04	N/A	N/A	6.35 E-04	N/A	N/A	N/A	9.20 E-04	N/A
	2.61 E-03	6.44 E-04	N/A	3.12 E-04	N/A	7.03 E-04	6.07 E-04	N/A	N/A	4.52 E-04	N/A	N/A	N/A	3.81 E-04	N/A
	4.59 E-03	1.46 E-03	N/A	8.52 E-04	N/A	2.02 E-03	N/A	N/A	N/A	1.76 E-04	N/A	N/A	N/A	6.70 E-04	N/A
KBS T4	4.10 E-03	N/A	N/A	2.95 E-04	N/A	2.76 E-03	N/A	3.42 E-03	N/A	1.18 E-03	N/A	N/A	N/A	1.03 E-03	N/A
	3.51 E-03	1.28 E-03	N/A	3.60 E-04	N/A	1.64 E-03	N/A	3.13 E-03	N/A	1.87 E-04	N/A	N/A	N/A	4.25 E-04	N/A
	3.25 E-03	5.58 E-04	N/A	5.68 E-04	N/A	7.95 E-04	N/A	4.01 E-03	N/A	2.30 E-04	N/A	N/A	N/A	4.22 E-04	N/A

Table D.19. Gene copies per 16S rRNA gene copy for *xplA* assays. “N/A” indicates either no amplification or false positive amplification.

Sample	**PS3**	**PS4**	**PS5**	**Pub1**	**Pub2**	**Pub3**	**Pub4**	**Pub6**
Shallow Zone Aquifer								
MW32 (Pre)	N/A	1.54E-03	3.33E-02	N/A	4.81E-04	N/A	6.67E-04	1.99E-03
	N/A	1.57E-03	4.37E-02	N/A	3.73E-04	N/A	4.29E-04	1.53E-03
	N/A	1.41E-03	N/A	N/A	1.25E-04	N/A	2.41E-04	2.06E-03
MW32 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW62 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	1.04E-02	N/A	N/A	N/A	N/A	2.57E-04
	N/A	N/A	1.10E-02	N/A	N/A	N/A	N/A	3.44E-04
MW62 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Perched Zone Aquifer								
MW48 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	5.89E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	3.08E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW48 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW60R (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.36E-04
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.38E-04
MW64 (Pre)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW64 (Post)	N/A	N/A	3.24E-03	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.25E-03	N/A	N/A	N/A	N/A	N/A
MW66 (Pre)	N/A	8.65E-04	N/A	5.79E-05	N/A	N/A	N/A	N/A
	N/A	9.50E-04	N/A	7.59E-05	N/A	N/A	N/A	N/A
	N/A	1.06E-03	N/A	N/A	N/A	N/A	N/A	N/A
MW66 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MW67 (Pre)	N/A	N/A	3.25E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.97E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	3.22E-02	N/A	N/A	N/A	N/A	N/A
MW67 (Post)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Red Cedar River								
Red Cedar	N/A	1.59E-03	2.09E-02	N/A	N/A	N/A	N/A	3.29E-04
	N/A	1.99E-03	2.82E-02	N/A	N/A	N/A	N/A	3.75E-04
Sediment								
MW71	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Agricultural Soils								
MSU Soil E	N/A	N/A	7.07E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	5.44E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	5.28E-02	N/A	N/A	N/A	N/A	N/A

Table D.19. (cont'd)

Sample	PS3	PS4	PS5	Pub1	Pub2	Pub3	Pub4	Pub6
MSU Soil F	N/A	N/A	3.94E-02	N/A	N/A	N/A	N/A	6.88E-04
	N/A	N/A	4.17E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	9.83E-04
KBS T1	N/A	N/A	4.00E-02	N/A	N/A	N/A	N/A	6.06E-04
	N/A	N/A	7.16E-02	N/A	N/A	N/A	N/A	9.20E-04
	N/A	N/A	3.92E-02	N/A	N/A	N/A	N/A	8.01E-04
KBS T2	N/A	N/A	3.45E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	5.39E-02	N/A	N/A	N/A	N/A	1.28E-03
	N/A	N/A	7.43E-02	N/A	N/A	N/A	N/A	2.65E-03
KBS T3	N/A	7.90E-04	4.56E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	4.15E-02	N/A	N/A	N/A	N/A	N/A
	N/A	1.50E-03	6.58E-02	N/A	N/A	N/A	N/A	N/A
KBS T4	N/A	N/A	5.16E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	6.59E-02	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	7.62E-02	N/A	N/A	N/A	N/A	N/A

APPENDIX E

Groundwater Pre- and Post-Biostimulation Results

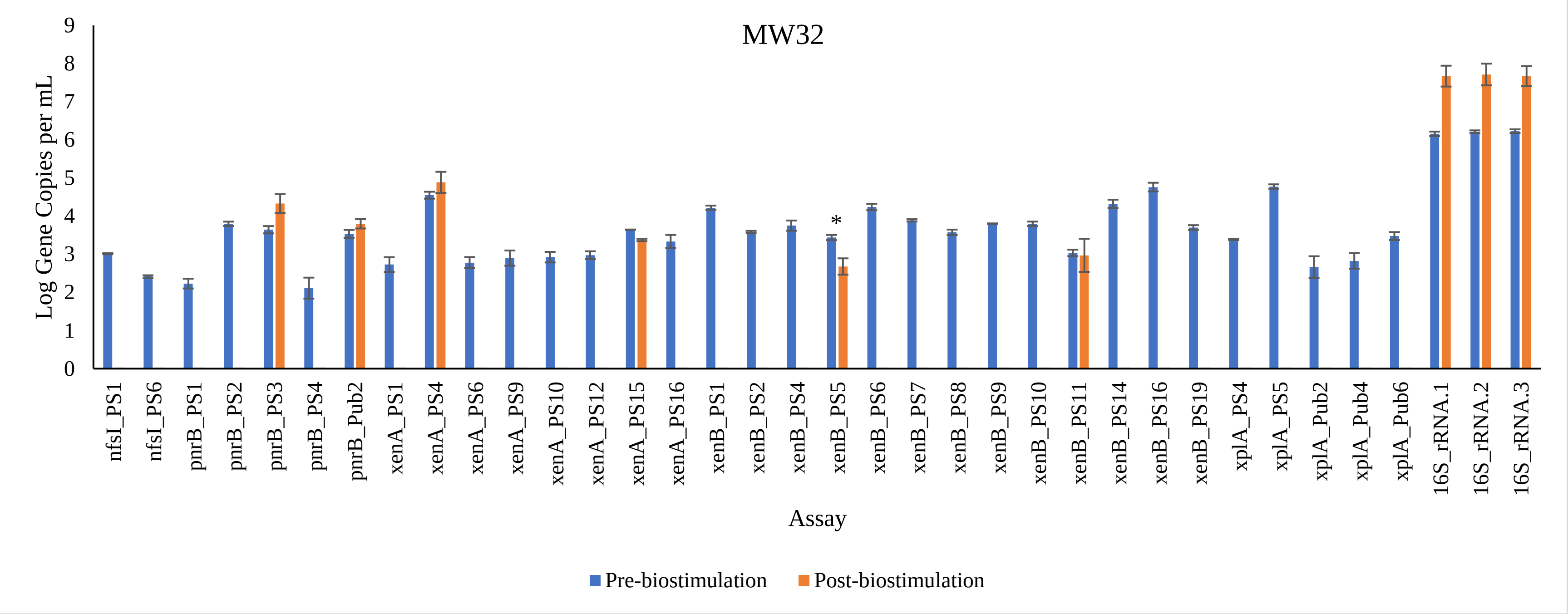

Figure E.1. Average log gene copies per mL of groundwater in shallow zone aquifer well MW62. Error bars represent the standard deviation and an asterisk (*) denotes a statistically significant difference ($p<0.05$) between the pre- and post-biostimulation levels.

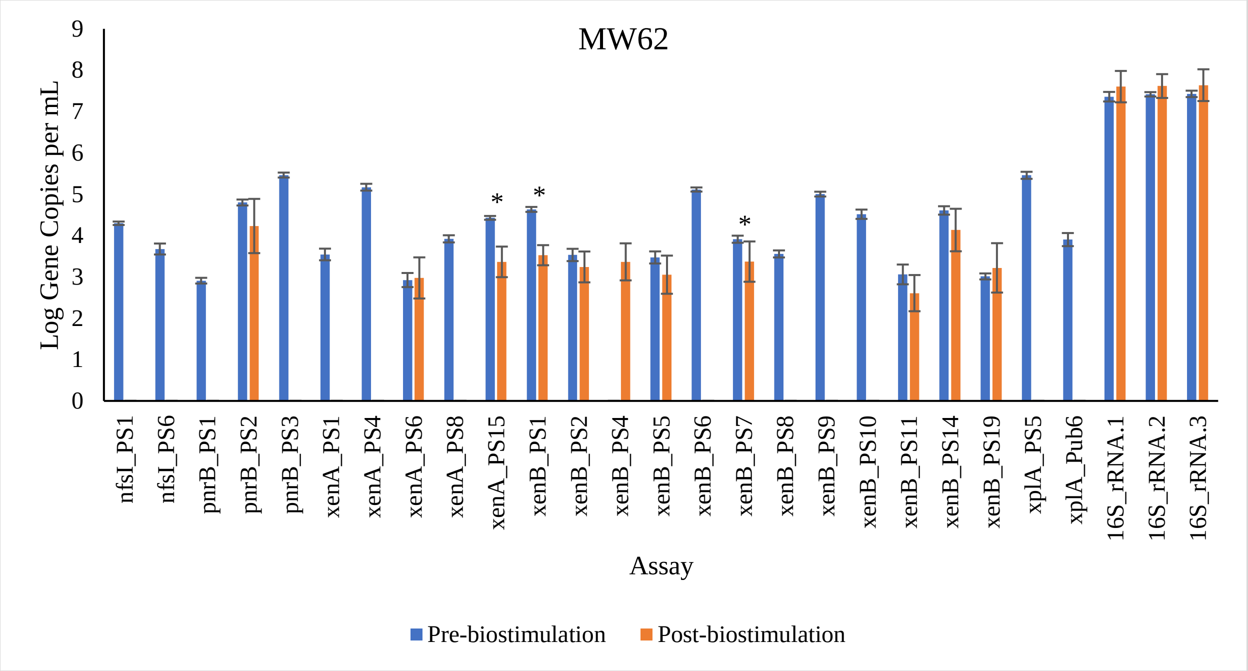

Figure E.2. Average log gene copies per mL of groundwater in shallow zone aquifer well MW62. Error bars represent the standard deviation and an asterisk (*) denotes a statistically significant difference ($p<0.05$) between the pre- and post-biostimulation levels.

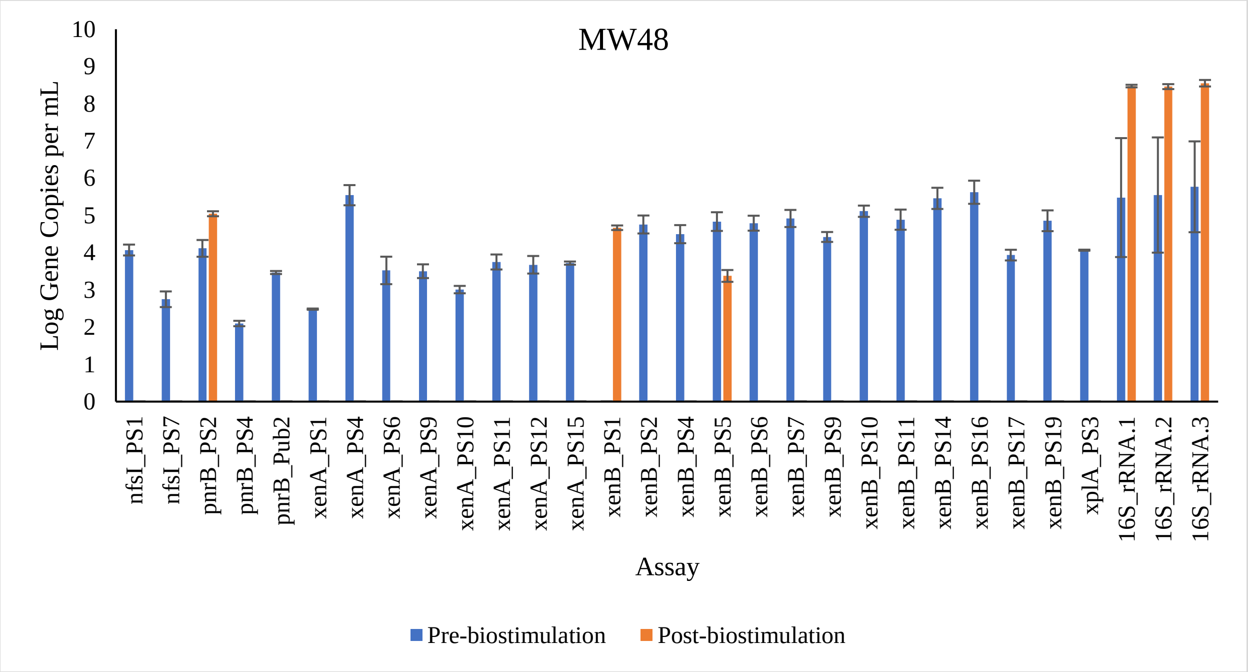

Figure E.3. Average log gene copies per mL of groundwater in perched zone aquifer well MW48. Error bars represent the standard deviation.

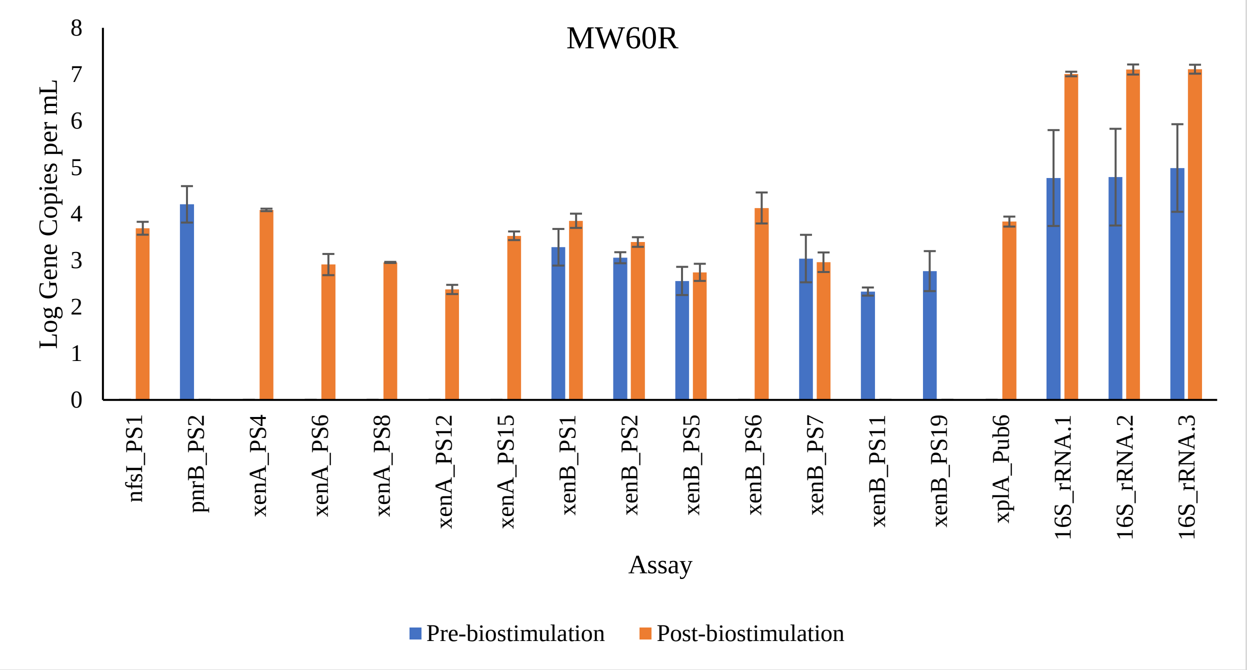

Figure E.4. Average log gene copies per mL of groundwater in perched zone aquifer well MW60R. Error bars represent the standard deviation.

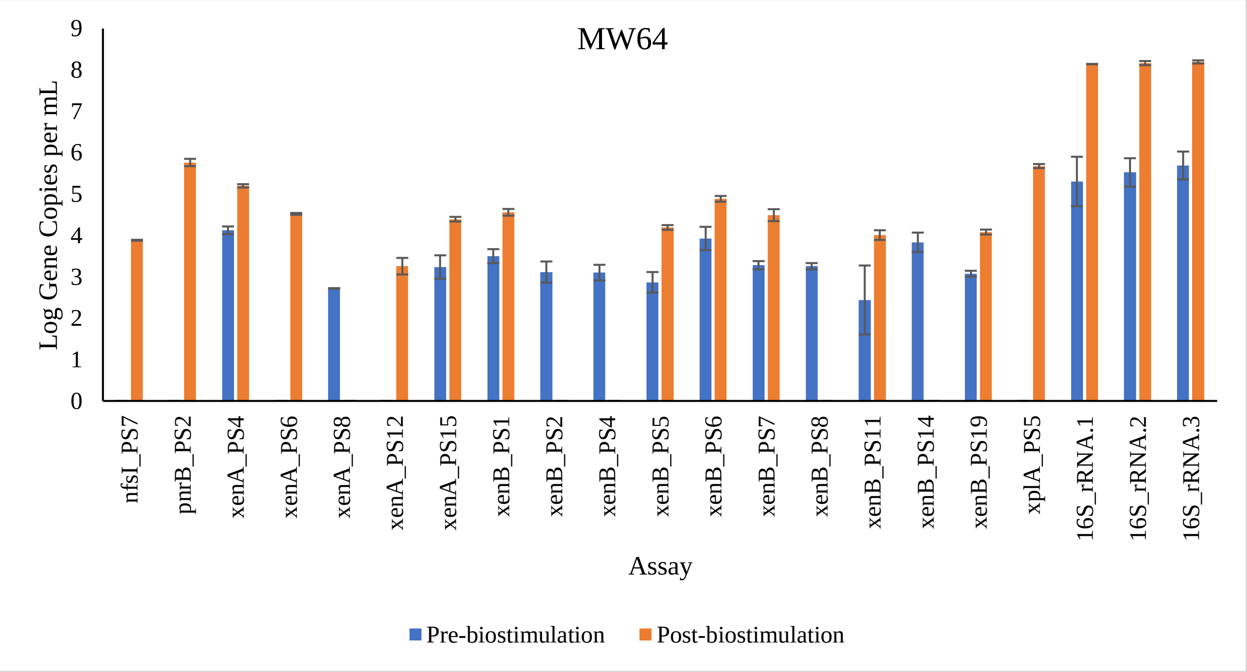

Figure E.5. Average log gene copies per mL of groundwater in perched zone aquifer well MW64. Error bars represent the standard deviation.

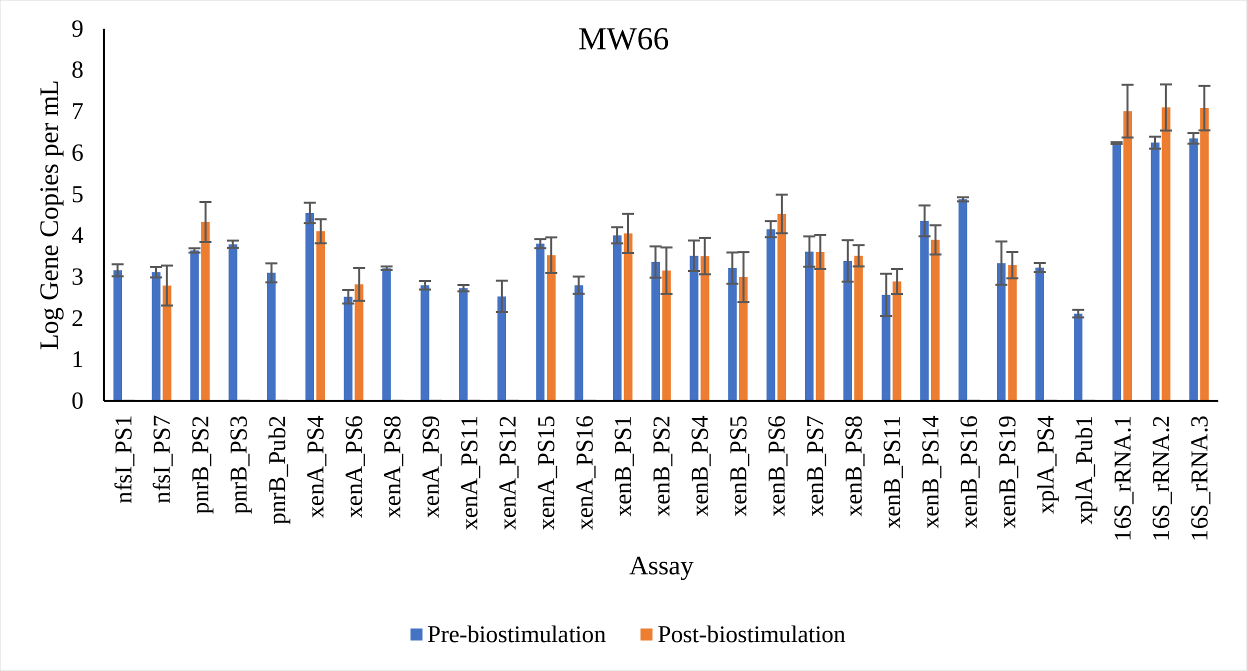

Figure E.6. Average log gene copies per mL of groundwater in perched zone aquifer well MW66. Error bars represent the standard deviation.

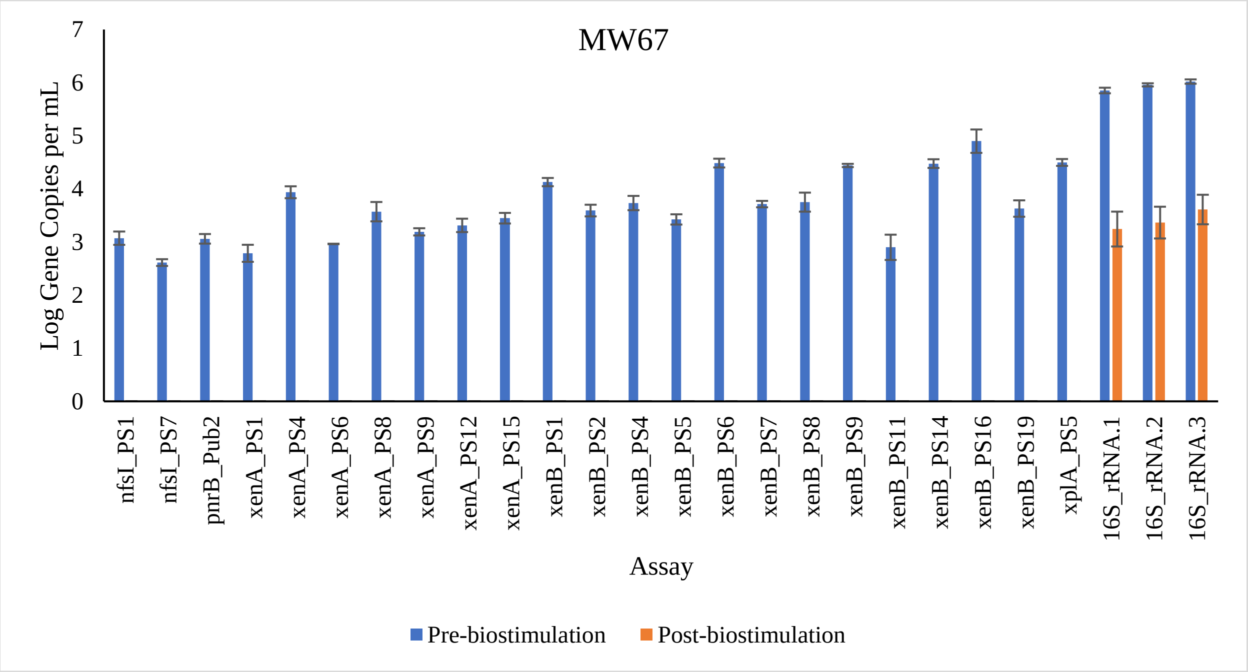

Figure E.7. Average log gene copies per mL of groundwater in perched zone aquifer well MW67. Error bars represent the standard deviation.

APPENDIX F

Statistical Analysis

The pre- and post-biostimulation gene copies per mL concentrations were tested for statistically significant difference in individual wells and for each aquifer (MW32 and MW62 samples were combined for the shallow zone aquifer and MW48, MW60R, MW64, MW66, and MW67 for the perched zone aquifer). Due to the small sample sizes ($n<30$), normal distribution could not be assumed to use the parametric statistical tests. Therefore, the Shapiro-Wilk test was used to test each sample group, pre- and post-biostimulation, for normal distribution. This test requires at least 3 data points so any assay that did not yield at least 3 detections in either the pre- or post-biostimulation samples was assumed to not meet the normal distribution requirement for the parametric tests. For assays that both the pre- and post-biostimulation sample groups had normal distributions ($p>0.05$), Bartlett's test was used to test for equal variance. The significant difference between the means was then evaluated with the Student's independent two-sample t-test for those with equal variance ($p>0.05$) and Welch's t-test for those with unequal variance ($p\leq0.05$). For assays where either the pre- or post-biostimulation sample group did not follow the normal distribution ($p\leq0.05$), the Brown-Forsythe test was used to test for equal variance. The significant difference between the medians was then evaluated with the Mann-Whitney U test for those with equal variance ($p>0.05$). The significant difference could not be determined for samples that did not follow the normal distribution and had unequal variance. The results from this analysis are reported in Tables F.1 through F.7 for the individual wells and Tables F.8 and F.9 for the shallow and perched zone aquifer, respectively.

Table F.1. P-values for statistical tests with the MW32 gene copies per mL results. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	$\text{Median}_1=\text{Median}_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
pnrB_PS3	N/A	8.21E-01	N/A	N/A	N/A	2.67E-01	2.00E-01
pnrB_Pub2	7.13E-01	N/A	N/A	N/A	N/A	1.40E-01	2.00E-01
xenA_PS4	4.58E-01	1.05E-02	N/A	N/A	N/A	4.59E-01	4.00E-01
xenA_PS15	9.29E-01	N/A	N/A	N/A	N/A	3.05E-02	N/A
xenB_PS5	6.58E-02	2.83E-01	3.69E-01	**1.38E-03**	N/A	N/A	N/A
xenB_PS11	2.47E-01	7.55E-01	1.07E-01	8.18E-01	N/A	N/A	N/A
16S_rRNA.1	2.76E-01	2.38E-01	2.70E-04	N/A	7.67E-02	N/A	N/A
16S_rRNA.2	9.99E-01	3.69E-01	1.03E-04	N/A	8.22E-02	N/A	N/A
16S_rRNA.3	9.90E-01	5.46E-01	2.40E-04	N/A	7.76E-02	N/A	N/A

Table F.2. P-values for statistical tests with the MW62 gene copies per mL results. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	$\text{Median}_1=\text{Median}_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
pnrB_PS2	5.37E-01	9.75E-01	2.99E-01	9.74E-02	N/A	N/A	N/A
xenA_PS6	6.80E-01	6.50E-01	1.89E-01	5.03E-01	N/A	N/A	N/A
xenA_PS15	2.05E-01	3.87E-01	5.06E-01	**2.54E-04**	N/A	N/A	N/A
xenB_PS1	1.69E-01	8.41E-01	1.60E-01	**4.32E-04**	N/A	N/A	N/A
xenB_PS2	6.55E-01	6.78E-01	7.06E-01	3.20E-01	N/A	N/A	N/A
xenB_PS5	N/A	1.50E-01	N/A	N/A	N/A	8.96E-01	2.00E-01
xenB_PS7	4.62E-01	9.37E-01	5.40E-01	**4.41E-02**	N/A	N/A	N/A
xenB_PS11	N/A	7.02E-01	N/A	N/A	N/A	5.01E-01	4.00E-01
xenB_PS14	9.60E-01	3.37E-01	2.97E-01	2.25E-01	N/A	N/A	N/A
xenB_PS19	2.09E-01	7.96E-01	1.68E-02	N/A	3.33E-01	N/A	N/A
16S_rRNA.1	3.17E-01	5.55E-01	5.37E-02	3.13E-01	N/A	N/A	N/A
16S_rRNA.2	1.86E-01	8.10E-01	3.53E-02	N/A	2.98E-01	N/A	N/A
16S_rRNA.3	8.27E-01	9.04E-01	3.37E-02	N/A	3.47E-01	N/A	N/A

Table F.3. P-values for statistical tests with the MW48 gene copies per mL results. “N/A” indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		**$\sigma_1=\sigma_2$**	**$\mu_1= \mu_2$**	**$\mu_1= \mu_2$**	**$\sigma_1=\sigma_2$**	**Median$_1$=Median$_2$**
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
pnrB_PS2	N/A	N/A	N/A	N/A	N/A	6.28E-32	N/A
xenB_PS5	N/A	N/A	N/A	N/A	N/A	1.68E-32	N/A
16S_rRNA.1	9.48E-01	N/A	N/A	N/A	N/A	2.76E-04	N/A
16S_rRNA.2	9.98E-01	N/A	N/A	N/A	N/A	4.66E-05	N/A
16S_rRNA.3	9.36E-01	N/A	N/A	N/A	N/A	1.19E-05	N/A

Table F.4. P-values for statistical tests with the MW60R gene copies per mL results. “N/A” indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		**$\sigma_1=\sigma_2$**	**$\mu_1= \mu_2$**	**$\mu_1= \mu_2$**	**$\sigma_1=\sigma_2$**	**Median$_1$=Median$_2$**
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
xenB_PS1	1.46E-01	N/A	N/A	N/A	N/A	8.57E-01	2.00E-01
xenB_PS2	N/A	N/A	N/A	N/A	N/A	8.30E-32	N/A
xenB_PS5	6.51E-01	N/A	N/A	N/A	N/A	9.29E-01	4.00E-01
xenB_PS7	N/A	N/A	N/A	N/A	N/A	3.30E-32	N/A
16S_rRNA.1	3.96E-03	N/A	N/A	N/A	N/A	2.80E-01	2.00E-01
16S_rRNA.2	8.43E-03	N/A	N/A	N/A	N/A	1.79E-02	N/A
16S_rRNA.3	7.87E-03	N/A	N/A	N/A	N/A	4.54E-02	N/A

Table F.5. P-values for statistical tests with the MW64 gene copies per mL results. “N/A” indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	$\text{Median}_1=\text{Median}_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
xenA_PS4	NA	NA	N/A	N/A	N/A	1.17E-32	N/A
xenA_PS15	9.72E-01	NA	N/A	N/A	N/A	4.95E-02	N/A
xenB_PS1	2.12E-01	NA	N/A	N/A	N/A	1.12E-02	N/A
xenB_PS5	6.89E-01	NA	N/A	N/A	N/A	1.20E-02	N/A
xenB_PS6	5.65E-01	NA	N/A	N/A	N/A	1.20E-01	2.00E-01
xenB_PS7	1.32E-02	NA	N/A	N/A	N/A	1.75E-04	N/A
xenB_PS11	NA	NA	N/A	N/A	N/A	6.13E-33	N/A
xenB_PS19	9.72E-01	NA	N/A	N/A	N/A	1.07E-03	N/A
16S_rRNA.1	3.81E-01	NA	N/A	N/A	N/A	8.55E-03	N/A
16S_rRNA.2	1.97E-01	NA	N/A	N/A	N/A	4.51E-06	N/A
16S_rRNA.3	5.43E-01	NA	N/A	N/A	N/A	1.38E-05	N/A

Table F.6. P-values for statistical tests with the MW66 gene copies per mL results. “N/A” indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	$\text{Median}_1=\text{Median}_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
nfsI_PS7	7.91E-01	N/A	N/A	N/A	N/A	2.08E-01	8.00E-01
pnrB_PS2	3.88E-01	N/A	N/A	N/A	N/A	9.54E-06	N/A
xenA_PS4	1.86E-01	N/A	N/A	N/A	N/A	7.25E-01	4.00E-01
xenA_PS6	9.22E-02	N/A	N/A	N/A	N/A	1.68E-02	N/A
xenA_PS15	9.68E-01	N/A	N/A	N/A	N/A	1.23E-01	8.00E-01
xenB_PS1	2.44E-01	N/A	N/A	N/A	N/A	7.54E-02	1.00E+00
xenB_PS2	2.84E-01	N/A	N/A	N/A	N/A	7.24E-01	4.00E-01
xenB_PS4	7.30E-02	N/A	N/A	N/A	N/A	5.64E-01	8.00E-01
xenB_PS5	1.32E-02	N/A	N/A	N/A	N/A	6.61E-01	4.00E-01
xenB_PS6	7.44E-01	N/A	N/A	N/A	N/A	3.43E-03	N/A
xenB_PS7	4.48E-01	N/A	N/A	N/A	N/A	6.15E-01	8.00E-01
xenB_PS8	1.59E-01	N/A	N/A	N/A	N/A	9.84E-01	1.00E+00

Table F.6. (cont'd)

Test	Shapiro-Wilk		Bartlett's test	Student t-test	Welch's t-test	Brown-Forsythe test	Mann-Whitney U test
xenB_PS11	N/A	N/A	N/A	N/A	N/A	1.94E-30	N/A
xenB_PS14	1.42E-01	N/A	N/A	N/A	N/A	6.76E-01	4.00E-01
xenB_PS19	3.68E-01	N/A	N/A	N/A	N/A	7.96E-01	8.00E-01
16S_rRNA.1	N/A	N/A	N/A	N/A	N/A	1.10E-32	N/A
16S_rRNA.2	3.99E-02	N/A	N/A	N/A	N/A	1.19E-04	N/A
16S_rRNA.3	9.73E-01	N/A	N/A	N/A	N/A	3.31E-05	N/A

Table F.7. P-values for statistical tests with the MW67 gene copies per mL results. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	Shapiro-Wilk		Bartlett's test	Student t-test	Welch's t-test	Brown-Forsythe test	Mann-Whitney U test
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	**Median**$_1$**=Median**$_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
16S_rRNA.1	4.30E-01	N/A	N/A	N/A	N/A	3.45E-01	2.00E-01
16S_rRNA.2	5.65E-01	N/A	N/A	N/A	N/A	3.05E-01	2.00E-01
16S_rRNA.3	3.87E-01	N/A	N/A	N/A	N/A	3.65E-01	2.00E-01

Table F.8. P-values for statistical tests with the shallow zone aquifer gene copies per mL results. "N/A" indicates the test was not appropriate and p-values in **bold** indicate a significant difference ($p \leq 0.05$).

Test	Shapiro-Wilk		Bartlett's test	Student t-test	Welch's t-test	Brown-Forsythe test	Mann-Whitney U test
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	**Median**$_1$**=Median**$_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
pnrB_PS2	6.62E-02	9.75E-01	7.37E-01	7.88E-01	N/A	N/A	N/A
pnrB_PS3	9.76E-02	8.21E-01	9.41E-03	N/A	9.87E-02	N/A	N/A
pnrB_Pub2	7.13E-01	N/A	N/A	N/A	N/A	1.40E-01	2.00E-01
xenA_PS4	2.03E-01	1.05E-02	N/A	N/A	N/A	1.58E-01	7.14E-01
xenA_PS6	9.37E-01	6.50E-01	3.64E-02	N/A	4.23E-01	N/A	N/A
xenA_PS15	2.88E-02	9.09E-01	N/A	N/A	N/A	3.03E-06	N/A
xenB_PS1	1.96E-01	8.41E-01	2.22E-02	N/A	**7.63E-03**	N/A	N/A
xenB_PS2	9.90E-01	6.78E-01	2.36E-01	9.83E-02	N/A	N/A	N/A

Table F.8. (cont'd)

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
xenB_PS4	5.94E-01	5.66E-02	8.99E-01	1.53E-01	N/A	N/A	N/A
xenB_PS5	2.12E-01	4.99E-02	N/A	N/A	N/A	6.68E-01	**4.33E-03**
xenB_PS7	9.26E-01	9.37E-01	1.56E-01	**4.72E-03**	N/A	N/A	N/A
xenB_PS11	5.86E-01	5.72E-01	2.00E-01	4.67E-01	N/A	N/A	N/A
xenB_PS14	7.15E-01	3.37E-01	3.59E-01	4.01E-01	N/A	N/A	N/A
xenB_PS19	8.81E-02	7.96E-01	9.32E-01	7.81E-01	N/A	N/A	N/A

Table F.9. P-values for statistical tests with the perched zone aquifer gene copies per mL results. "N/A" indicates the test was not appropriate and p-values in **bold** indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk**		**Bartlett's test**	**Student t-test**	**Welch's t-test**	**Brown-Forsythe test**	**Mann-Whitney U test**
Null Hypothesis	**The sample distribution is normal**		$\sigma_1=\sigma_2$	$\mu_1= \mu_2$	$\mu_1= \mu_2$	$\sigma_1=\sigma_2$	$Median_1=Median_2$
Data Group	**Pre-biostimulation**	**Post-biostimulation**	**Both**	**Both**	**Both**	**Both**	**Both**
nfsI_PS1	9.12E-04	N/A	N/A	N/A	N/A	6.39E-01	4.00E-01
nfsI_PS7	1.32E-01	1.20E-01	9.32E-05	N/A	1.82E-01	N/A	N/A
pnrB_PS2	4.00E-02	8.04E-02	N/A	N/A	N/A	5.62E-02	**4.66E-03**
xenA_PS4	3.45E-05	8.57E-03	N/A	N/A	N/A	6.64E-01	9.58E-01
xenA_PS6	1.76E-03	3.30E-03	N/A	N/A	N/A	1.40E-01	3.66E-01
xenA_PS8	3.02E-01	N/A	N/A	N/A	N/A	2.12E-01	5.00E-01
xenA_PS12	2.29E-01	3.39E-01	2.15E-01	2.78E-01	N/A	N/A	N/A
xenA_PS15	9.07E-01	5.69E-02	6.47E-05	N/A	1.96E-01	N/A	N/A
xenB_PS1	1.04E-01	2.87E-01	8.03E-04	N/A	**2.22E-02**	N/A	N/A
xenB_PS2	1.04E-05	7.44E-01	N/A	N/A	N/A	4.55E-01	4.12E-01
xenB_PS4	5.61E-05	N/A	N/A	N/A	N/A	6.34E-01	1.00E+00
xenB_PS5	3.04E-06	3.58E-03	N/A	N/A	N/A	4.98E-01	7.64E-01
xenB_PS6	2.41E-02	2.05E-01	N/A	N/A	N/A	8.07E-02	4.04E-01
xenB_PS7	1.18E-05	6.85E-02	N/A	N/A	N/A	8.10E-01	9.66E-01
xenB_PS8	6.96E-01	N/A	N/A	N/A	N/A	5.65E-01	1.00E+00
xenB_PS11	1.20E-05	3.69E-01	N/A	N/A	N/A	6.11E-01	2.62E-01
xenB_PS14	9.09E-06	N/A	N/A	N/A	N/A	5.45E-01	3.08E-01
xenB_PS19	3.67E-06	4.10E-01	N/A	N/A	N/A	6.38E-01	4.42E-01
xplA_PS5	7.40E-01	N/A	N/A	N/A	N/A	5.35E-04	N/A

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