THE APPLICABILITY OF DNA TRACERS IN HYDROGEOLOGY

by

KIMBERLEY NICOLE COKE

(Under the Direction of John Dowd)

ABSTRACT

Hydrogeologic tracers have the ability to help explain the complexities of the unseen subsurface in order to begin to understand the network of flow patterns and pathways throughout. The tracer introduced in this thesis is based on the premise that DNA will lead to the development of a more streamlined tracer. A DNA tracer offers user specific unique capture markers where PCR is used for detection, and concentration is not a factor in order to have success. Streptavidin Coated Beads (SCB) serve as the medium for which the DNA markers are attached and moved throughout the subsurface as the DNA is more stable once it has a medium of transport.

INDEX WORDS: DNA, PCR, Tracer, Streptavidin Coated Beads, Paramagnetic, Biotin

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KIMBERLEY NICOLE COKE

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Major Professor:	John Dowd
Committee:	Doug Crowe
	Adam Milewski

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

DEDICATION

This thesis is dedicated to my Mom (Marion) and Dad (Bernett) … you guys waited way too long for this! Also, to my sister Krystal and my brother Jason, you both were inspirations to me without ever realizing it. Last but not least, I would like to thank the love of my life, Jasen, for your push and support.

:o)

Thank you all for believing that I could finish this and sticking with me.

I love you immeasurably

God Almighty has brought me;

The love of family has supported me;

*The **Joy** of friendship has uplifted me,*

And with guidance given by Jesus Christ,

I have arrived!

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CHAPTER 1

INTRODUCTION

Used in hydrogeology, a groundwater tracer is matter or energy carried by groundwater which will give information concerning the direction of movement and/or velocity of the water and potential contaminants which might be transported in the water. Tracers can also help determine flow paths of water throughout a system. If enough information is collected, the use of tracers can also help with the determination of hydraulic conductivity, porosity, dispersivity, chemical distribution coefficients, and other hydrogeologic parameters (Davis S. N., 1985) (Sabir, 1999). The use of tracers has increased our understanding of groundwater flow (Holmbeck-Pelham, 2000).

Tracers can be accidentally introduced (spilled contaminants), naturally occurring (heat from hot water springs), or introduced intentionally. With accidental or intentional release of pollutants to groundwater, regional problems for distinguishing pollutant sources are critical parts of contaminant studies and often require the use of tracer experiments.

A few of the conservative tracers used are inert gases, salt, chloride, and stable isotopes (hydrogen-deuterium to hydrogen (D/H) and oxygen-18 to oxygen-16 ($^{18}O/^{16}O$)). In addition, non-conservative tracers are used. For example, multi-colored dyes, bacteriophages, and other isotopes. Dye is a commonly used tracer, although large volumes are often required. In addition, colored tracers, where there exit is unknown,

may cause public panic. The advantages of dyes include their very high detectability, rapid field analysis, relatively low cost and low toxicity. Dyes are retarded due to adsorption, and are generally not as conservative as an ionic or radioactive tracer (Davis S. N., 1985).

It was not until the 1970s that bacteriophages were used as groundwater tracers in subsurface microbial tracer studies and have also been used as groundwater tracers in hydrologic studies (Harvey, 2004). Bacteriophages often have been the tracer of choice in field studies designed to assess subsurface transport behavior from a public health perspective because of their small size, non-pathogenic nature, and structural similarities with some of the pathogenic viruses that are of groundwater quality concern (Martin, 1974). Unfortunately, many of the common tracers, including bacteriophages, often raise environmental and public health concerns.

Contaminant transport is of interest in groundwater because a) subsurface migration is an important determinant of subsurface ecology, b) mobile bacteria or enzymes introduced into contaminated aquifers is a potential *in situ* cleanup strategy, c) contamination can contribute to aquifer clogging, and d) contamination of drinking-water wells is a potential public health concern (Kinoshita, 1993).

The use of tracers as an investigative method is often considered in planning regional ground-water studies. Tracer experiments are conducted by injecting water or a similar liquid miscible with water containing a readily identifiable compound in solution into an aquifer through an injection well. The pattern of flow is determined by the periodic sampling of nearby observation wells. The distribution of the tracer helps define the hydrologic properties of the aquifer.

Tracers should be nontoxic, relatively inexpensive to use, and easily detected with widely available and simple technology. They should be present in concentrations well above background of the same constituent in the natural system which is being studied. Finally, the tracer itself should not modify the hydraulic conductivity or other properties of the same medium being studied (Davis S. N., 1985). The use of non-conservative tracers led to the evolution to DNA tracers. There are limitations to non-conservative tracers, including decomposition, dilution, and degradation.

DNA tracers are a cost effective way to avoid the negative reputation of the more common tracers, such as bacteriophages, viruses, and radioactive. The DNA tracers are able to carry highly user specific information that can be easily identified by their sequence, and visual confirmation is not necessary.

In its most basic form, DNA is non-pathogenic, and does not pose a threat to the environment as it is found naturally. This is one of this tracer's most distinctive features. The DNA tracers can be any sequence the user chooses, including synthetic sequences. DNA tracers, unlike soluble tracers, by regulating the size distribution of beads on which the DNA is attached. When very small beads are used, they do not settle or sorb to the formation thereby mimicking water.

A DNA sequence as a marker for this tracer is unique and specific to its creator because any sequence of DNA can be applied to the beads. However false positives can occur if DNA fragments from the environment are annealed to your target DNA.

While Foppen (2011) used single-stranded RNA, in this thesis double stranded DNA is used, which has great mechanical rigidity due to short double helices (Niemeyer, 2001). Each strand of the DNA is about 2 nanometers wide and composed of a linear

chain of four possible bases (adenine, cytosine, guanine, and thymine) on a backbone of alternating sugar molecules and phosphate ions. The specific binding through hydrogen bonds between adenine (A) and thymine (T), and cytosine (C) and guanine (G) can result in the joining of two complementary single-stranded DNA to form a double-stranded DNA. Due to its unique recognition capabilities, physicochemical stability, mechanical rigidity, and high precision processibility, DNA is a promising material for "biomolecular nanotechnology" (Sun, 2007).

This DNA tracer also involves manipulating the almost irreversible streptavidin and biotin bond, which is highly specific to the sequence it has been prepared for. Streptavidin (CAS No. 9013-20-1) is a biotin binding protein found in the culture broth of the bacterium Streptomyces avidinii. Like its namesake avidin, streptavidin binds 4 moles of biotin per mole of protein with a high affinity virtually unmatched in nature (dissociation constant (Kd) ~10^{-15}). (PROzyme, 2013) Streptavidin will also be discussed later on in this thesis.

The power of DNA as a molecular tool is enhanced by the ability to synthesize virtually any DNA sequence by automated methods. The method used for the research presented in this thesis is the Polymerase Chain Reaction (PCR). This allows for DNA to be amplified from microscopic to macroscopic quantities (Niemeyer, 2000). PCR is a chain reaction that provides very accurate and reproducible quantitative target gene copies. This quantification is based on the use of fluorescent reporter molecules directly or indirectly binding to DNA, allowing fluorescence to be converted to gene copy numbers (Lindberg, 2007).

The objective of this thesis is to demonstrate the potential of DNA coated super paramagnetic beads (referred to as beads from here on) to be used as a hydrogeologic tracer with a unique identifiable marker, while also showing that these tracers are sustainable in the environment with no significant degradation. It also demonstrates that DNA can be annealed to paramagnetic beads, introduced to a sand core, retrieved, and then amplified using PCR.

In the first part of this thesis, the PCR experiment shows that the DNA used for this thesis can be amplified independent of the beads, the successful adhesion of biotin labeled DNA to streptavidin coated beads, and the amplification of the DNA coated beads using real time PCR.

The second part of this thesis involved applying the DNA coated beads to a sand core of quartz sand. This phase determined that the same DNA coated beads could be put through a sand column simulating a small scale tracer experiment. Once retrieved from the sand core, PCR successfully "found" the tracer.

The third part of this thesis involved a degradation study where the beads were incubated at room temperature with locally obtained A-horizon soil. Over three weeks beads were sampled for degradation

.

CHAPTER 2

LITERATURE REVIEW

In an investigation into the use of DNA tracers by Sabir (1999), DNA was used as a qualitative tracer. It was detected at greater depth than the paired chloride tracer, giving the appearance that DNA was transported faster than the ionic tracers. In fact, the detection limit of DNA is many orders of magnitude lower than chloride, so it is detected earlier.

Paramagnetic beads as hosts to the DNA, and slow the degradation process, have been used by a number of researchers (Lorenz, 1987) (Khanna, 1992) (Romanowski G. L., 1991) (Ogram, 1994), (Aardema, 1983). Foppen (2011) recently presented results via cyber seminar that involved the application of DNA coated paramagnetic beads in surface water. Synthetic single stranded DNA tracers and PCR were used in an injection experiment in two small surface waters the Netherlands. The DNA mass transport in the stream was not substantially retarded, so that a synthetic DNA marker can be used in stream tracer injection tests.

Genetic material (free DNA) is not immediately destroyed in soil but can be found weeks or months after introduction (Romanowski G. W., 1993). However, many organic and inorganic compounds can inhibit the DNA. Thus binding the DNA helps to minimize degradation (Prodělalová, 2004).

PCR is a process based on a specialized polymerase enzyme that can synthesize a complementary strand to a given DNA strand in a mixture containing the four DNA bases, adenine (A), thymine (T), cytosine (C) and guanine (G) and two DNA fragments (primers, each about 20 bases long) flanking the target sequence and creating multiple amounts of DNA strands. (Casey, 1992)

PCR is a technique where any piece of DNA can be quickly amplified, and can replicate fragments by a billion fold. When the double-stranded DNA is heated above a certain temperature (around 95^{o}C) the two strands will start to dehybridize and eventually separate into single strands (**Figure 2.1**). The temperature at this transition is called the melting temperature (Tm), and is a sensitive function of environmental conditions such as ionic strength, pH, and solvent conditions. Melting temperature is a unique property dependent on product length and nucleotide composition (Valasek, 2005).

While the strands of DNA are separated (1) the primers find and bind to their complementary sequences on the separated strands and (2) the polymerase to extend the primers into new complementary strands. As the temperature is reduced, the two strands will eventually come together by diffusion and rehybridize to form the double-stranded structure. Primers are single stranded DNA molecules complimentary to the ends of a defined sequence of DNA template

Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by a million fold.

PCR has been used to amplify DNA from fragments of DNA from a 40,000 year old frozen woolly mammoth; scenes of violent crimes; DNA from a single embryonic

cell for rapid prenatal diagnosis of genetic disorders, along with many other applications. (Campbell, 1999)

Qualitative PCR is able to detect a single DNA molecule in a sample solution and is used for detecting a specific DNA segment. It provides more information than the detection of DNA because it indicates also how much of a specific DNA segment is present while providing a continuous observation of the reaction. (Roche, 2007)

Real time/Quantitative PCR refers to the amplification of DNA (by PCR) that is monitored while the amplification is occurring. It allows the researcher to determine the amount of starting DNA in the sample before it underwent amplification. During the amplification, how quickly the fluorescent signal reached a threshold level correlates with the amount of the original target sequence, enabling quantification. (Valasek, 2005). The emission of fluorescence during amplification will increase in direct proportion to the amount of amplified product. This phase provides the most useful information about the reaction. The higher the initial amount of DNA the sooner the accumulated product is detected in the PCR process (Dorak, 2006).

The threshold cycle or the C_T value is the cycle of the PCR reaction at which an increase in the fluorescence of the reporter is first detected above a baseline. This cycle is where the system begins to detect the increase in the signal associated with an exponential growth of PCR product. Therefore, the Ct values decrease linearly with the increasing target input quantity, and the Ct values are a quantitative measure of the input target quantity.

Real time PCR has several advantages over conventional PCR techniques that require gel-electrophoresis (Vega, 2002). Major advantages include no post-PCR

manipulation required; the results are available as soon as PCR is completed (within 2 hours) decreasing the turnaround time and additionally reducing the risk of PCR contamination; the TaqMan® probe is complementary to the target therefore the assay is target specific with a high sensitivity.

One way to measure only the desired DNA product during PCR is to use TaqMan® probes, short DNA fragments that anneal to a middle region of the template DNA. The probes bear a reporter dye (R) at one end and a quencher (Q) at the other (see **Figure 2.2**). Quenchers are molecules that quench the fluorescence of dyes in their proximity. The polymerases in the PCR solution are able to break down the TaqMan® probes during the doubling of the DNA template. In so doing they free the reporter dye, which then migrates away from the influence of the quencher. Hence the fluorescence of the dye is measurable only if the polymerase has in fact copied the desired DNA strand. Each freed molecule of reporter dye represents a DNA strand that has been formed. TaqMan® probes can therefore be used to measure the amount of DNA formed at any given time (Schaad, 2002). An amplification plot represents a graph of the normalized reporter signal (Rn) versus cycle number during PCR (**Figure 2.3**).

The Streptavidin-Biotin bond is manipulated in this thesis in order to form a strong bond between the DNA and beads that will be used for the DNA tracers. Streptavidin is a protein purified from the bacterium Streptomyces avidinii, and Biotin is a water-soluble B-complex vitamin (vitamin B7). The association constant, Ka for the binding of Biotin to Streptavidin is among the highest for any known non-covalent structure at 10^{15} M^{-1} (González, et al., 1997). The bond is the strongest noncovalent biological interaction known and as a result is one of the most widely used affinity pairs

in molecular, immunological, and cellular assays (González, et al., 1997) (Holmberg, et al., 2005) . The bond formation is very rapid and, once formed, is unaffected by wide extremes of pH, temperature, organic solvents and other denaturing agents. Very harsh methods are required to dissociate the biotin from streptavidin which will denature the streptavidin (Anonymous, 2012). Biotin binding creates an increase in protein tightness leading to a higher thermostability (González, et al., 1997). "This further demonstrates that binding DNA to the beads will build a stronger tracer. When appended to particles, DNA persisted in the environment for greater periods of time (Aardema, 1983), (Greaves, 1970), (Lorenz, 1987), (Romanowski G. L., 1991) than without being bound to anything."

The complex is generally disrupted only by conditions which lead to irreversible denaturation of the protein, which would be PCR in this work (PROzyme, 2013) (Thompson, 2006). Streptavidin has been observed at different pH values (4.5 and 7.5) and there were no major differences in the protein structures caused by the difference in pH (Freitag, 1997). This is important to note that this tracer can be introduced to groundwater with varying pH ranges.

The DNA used to coat the beads is *Acidovorax avenae* subspecies *citrulli* (AAC) which is a pathogen found in cucurbits (any of the various, mostly climbing or trailing plants of the family Cucurbitaceae, which includes the squash, pumpkin, cucumber, gourd, watermelon, and cantaloupe) (Language, 2003) (Ha, et al., 2009).

A disadvantage of real-time PCR is that it does not provide size determination of the PCR products, therefore where multiple samples are amplified there is no way to compare sizes between samples. Also, the assay requires that the size of the target to be

amplified be limited to no more than 150 base pairs for maximum efficiency (Vega, 2002)

The largest limitation of real-time PCR is not in the technology but rather human error. Improper assay development, lab preps aeration errors, incorrect data analysis, or wrong conclusions (Valasek, 2005).

The high specificity of PCR and the production of product make PCR an ideal method for isolation and amplification of a particular DNA fragment. It is sensitive enough to amplify a single DNA molecule distinctly out of a complex mixture of DNA.

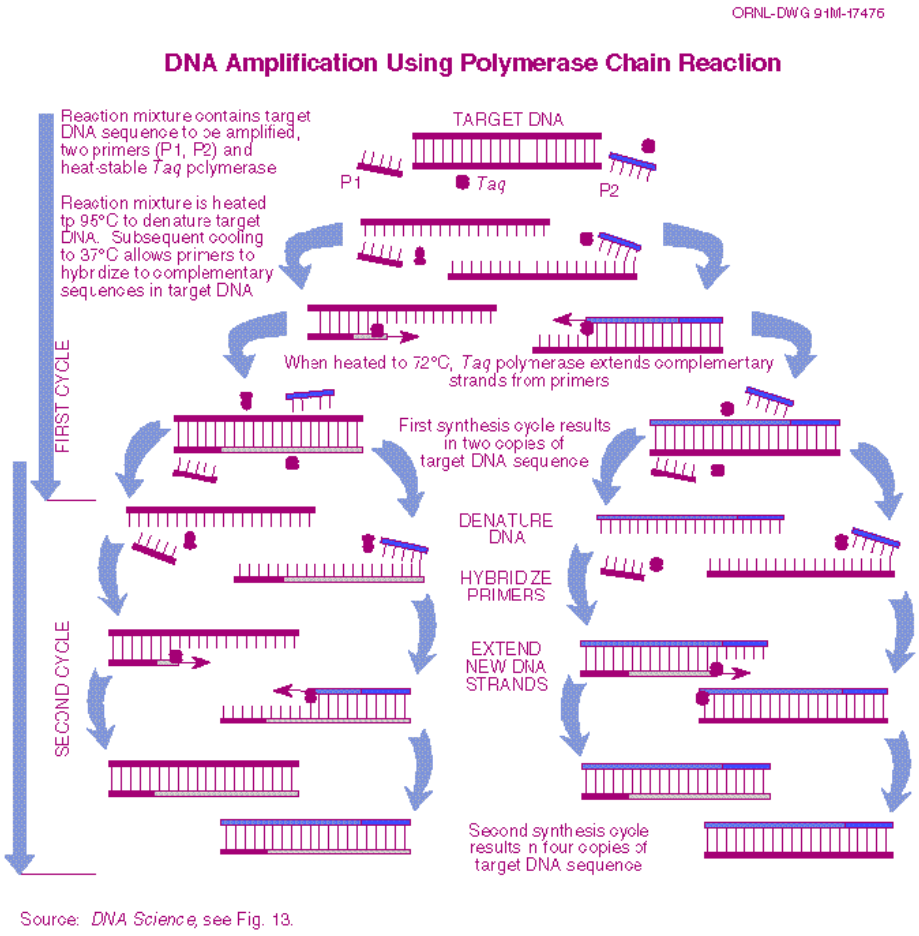

Figure 2.1. Polymerase Chain Reaction (Casey, 1992)

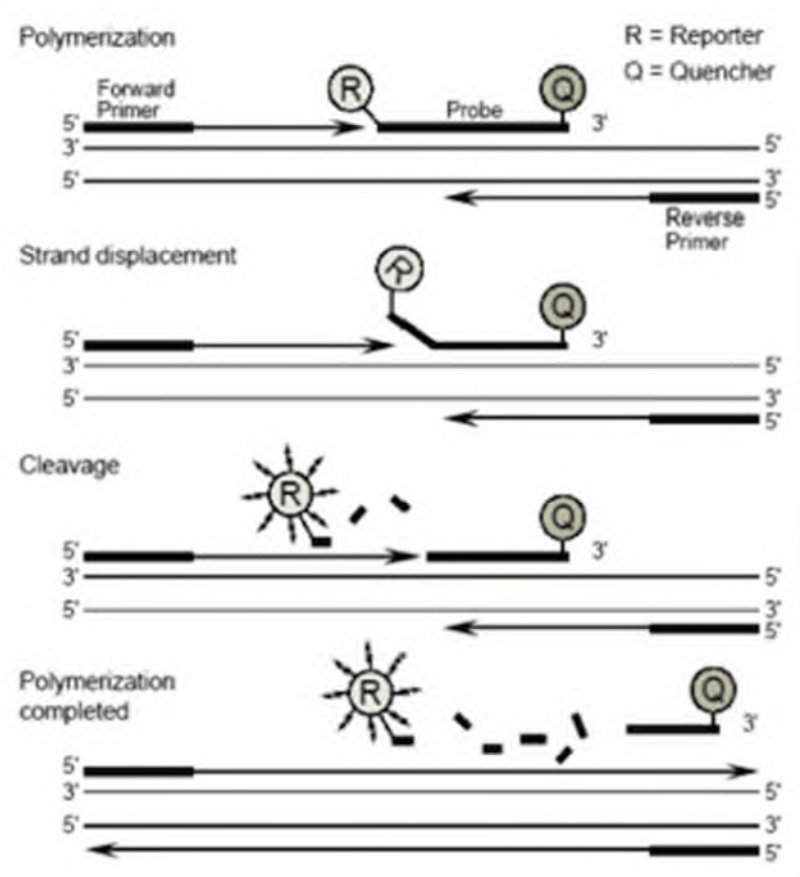

Figure 2.2. TaqMan® Probes (Florida, 2012)

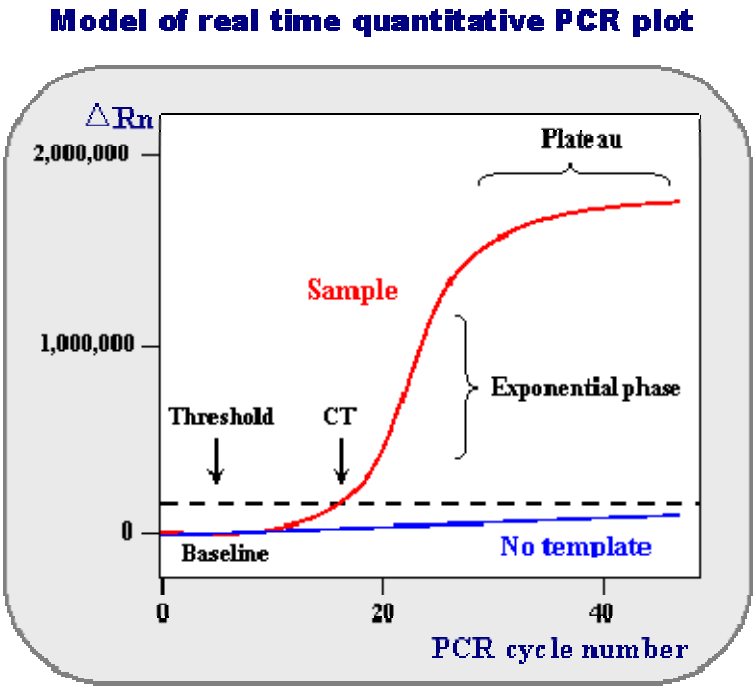

Figure 2.3. Example PCR plot (NCBI, 2013)

CHAPTER 3

MATERIALS & METHODS

Paramagnetic Streptavidin Coated Beads

The beads used for this thesis are DynaBeads® M-280 Streptavidin from Invitrogen. Dynabeads® are uniform, super paramagnetic beads, 2.8 μm (micrometer) in diameter with a streptavidin monolayer covalently coupled to the surface (Anonymous, 2012). Paramagnetism indicates that the beads are attracted to an external magnetic field however once that field is removed there are no other magnetic properties retained. Monodispersed nanoparticles of various size ranges (between 10 and 100 nm) are highly stable in aqueous systems against agglomeration (Roy, 2004). The amount of DNA that can be added to one mL (milliliter) of beads used for this thesis is about10 μg (microgram) of double stranded DNA. There are also 10 mg of beads per mL, which further simplifies to 600,000 beads per μL (micro liter) of stock beads.

Lastly, collection of these beads is simply done with magnets. The magnets secure the beads in place while excess water or sediment can be washed. These beads are very turbid in the stock form and visually can be seen once collected together.

DNA

For this thesis, Biotin labeled *Acidovorax avenae* DNA was needed, and in order to produce them, a small amount of primers were ordered with the biotin label on them and put through PCR with template DNA (121 base pairs). The result of this action was the multiplication of the primers that now contain the biotin labels on them. **Figure 3.1** shows the amplification results of the primers/DNA. The PCR product was then retrieved and stored in a freezer at -20^{o}. Due to the high concentration of the PCR product it was diluted before use to cut down on waste. This DNA was then incubated with the beads and appended to streptavidin on the beads. The beads were also washed of any residue post incubation.

The primers ordered to produce copies of the DNA are:

BOXAACF (forward primer) 5"- Bio-TEG-GCGTATGAGTCCCGAAGAAAT

BOXAACR2 (reverse primer) 5"- Bio-TEG-GCATGCCTTGTATTCAGCTAT

The conserved 121 base pair DNA sequence used for this thesis are from the 16S-23S internally transcribed spacer region of the ribosomal gene for *Acidovorax avenae* (Walcott, 2003) (Bahar, 2008).

DNA sequence of *A. avenae subsp. citrulli* is:

GCGTATGAGTCCCGAAGAAATCCGCGATCTGCCCTACCGCGCACGTAGGGC

CATTTCTGCCCCGGTCCCGAAATCCGTATTGGACGGATCGAAGGCCGCG**ATA**

GCTGAATACAAGGCATGC

(Note: The bold color indicates the primer sequences. Also, this DNA sequence is conserved meaning that it is and has remained consistent in all Acidovorax species)

In order to check the DNA from the PCR product that was amplified and collected gel electrophoresis was completed to visually verify it. **Figure 3.2** shows a photo of the 121 base pair DNA falling in between the DNA ladders 100 and 200.

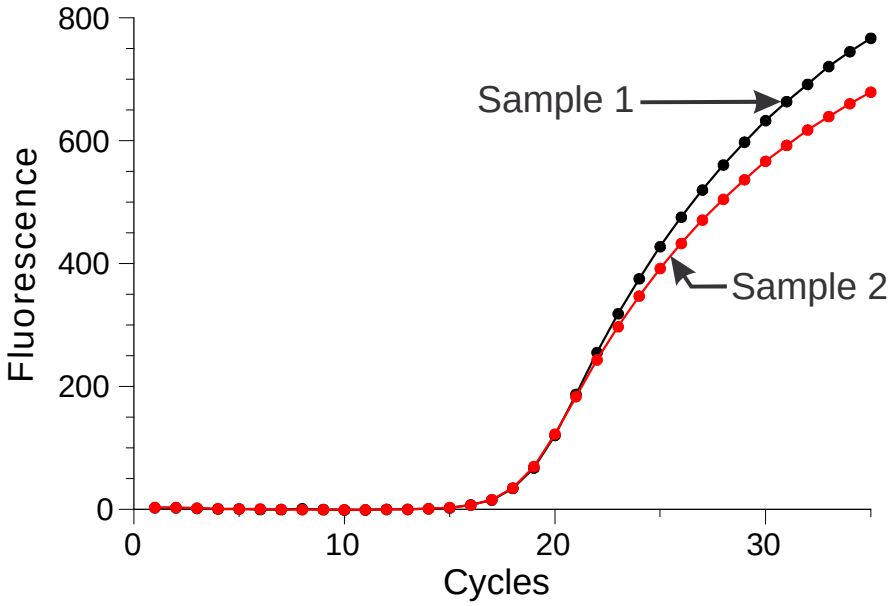

Figure 3.1. Primer amplification results

Figure 3.2. Visual confirmation of DNA product

PCR Machine

The PCR machine used for this thesis is the Cepheid SmartCycler® system (**Figure 3.3**). This machine contains 16 independent reaction sites and can run multiple experiments with different protocols and different times. The SmartCycler System software optically monitors each reaction site as the fluorescent signals develop. Growth curves are displayed in real time as amplification occurs, and the presence of amplified product is confirmed when the fluorescent signal crosses a user-determined threshold.

Figure 3.3. Cepheid SmartCycler®

It has been previously demonstrated that M-280® silica beads do not inhibit the smart cycler to a concentration of 75-100 µg of beads/50 µL PCR reaction. A concentration over 100 µg will inhibit PCR completely. (Anonymous, 2012)

Primers, which are nucleotides in the free form, are used to replicate the DNA sample found in a PCR reaction. The Probe that is used is a combination of oligonucleotides that are labeled with a fluorescent reporter. Fluorescence analysis allows for precise recognition of the target DNA from the bead it is adhered to, once a simple separation of the DNA from the bead has been performed at temperatures of 95^{o}C during PCR. Fluorescence occurs only after hybridization with its complementary DNA target. SuperMix is a ready-to-use mixture of DNA polymerase, salts, magnesium, and Deoxynucleoside triphosphates (dNTPs) for efficient PCR amplification. All that needs to be added are the aforementioned primers and probe. The super mix used was a TaqMan® based Supermix provided by Bio-Rad which contains dNTPs, 6 mM MgCl2, 50 U/ml iTaq™ DNA polymerase. Taq DNA polymerase (from Thermis aquaticus) can generate new strands of DNA using a DNA template and primers, and is heat resistant. This entire mixture (without the actual DNA sample) is called a master mix.

Each PCR reaction tube (**Figure 3.4**) had a total of 25 µL of solution inside of it. 20 µL of the master mix and five µL of the sample are then added for a total of 25µL per tube. The master mix is comprised of 12.5 µL of super mix, 0.3 µL of 25 µM concentration forward primer, 0.3 µL of 25 µM concentration reverse primer, 0.5 µL of 25 µM probe and 6.4 µL of water per sample. If there are ten samples for example, then each reagent is multiplied by 10. Due to loss while pipetting, it is common practice to always make enough master mix for an additional sample. For example, if you have ten samples you make enough mix for 11, as was done in this thesis.

Figure 3.4. PCR Reaction Tubes

The primer sequences used are:

BOXAACF (forward primer) 5”- GCGTATGAGTCCCGAAGAAAT

BOXAACR2 (reverse primer) 5”- GCATGCCTTGTATTCAGCTAT

The probe sequence is:

AACPROBE 56-FAM /CCGAAATCCGTATTGGACGGATCGAA/3BHQ_1

PCR Protocol

The profile used for amplification during PCR was denaturation of the DNA at 95^{o}C for 180 seconds, followed by 35 cycles of denaturation at 95^{o}C for 15 seconds, and annealing and elongation at 60^{o}C for 40 seconds. This protocol was developed for the primers that were used in this thesis (Ha, et al., 2009).

Washing Buffer (Anonymous, 2011)

A washing buffer was used to clean the beads once they were retrieved from the stock solution and also to help the DNA adhere to the beads. This was used to make sure that all residual reagents were removed and once amplification occurred that it could be concluded that the DNA that was amplified was that from the beads and not what may have been in solution.

The 2x buffer is made up of:

10 mM Tris-HCL [pH 7.5]

1 mM EDTA

2 M NaCl

Coating Beads

To coat the beads, the DNA and beads were incubated at room temperature and slowly mixed for 30 minutes on a rotating mixer. Once the DNA was attached to the beads they were washed of residue using an automated washer using the 2x washing buffer for three washes and a wash using HPLC grade water. Once any aliquot of beads were washed they were then put through PCR. Results showed amplification of the target DNA, proving that the DNA was successfully attached to the beads. There were negative and positive controls that checked for false positives. The process was repeated to ensure reproducibility and stored in the refrigerator.

Serial Dilutions

To determine the amplification efficiency of the A. avenae subsp. Citrulli TaqMan® assay, serial dilutions of DNA coated beads were generated ranging from $3x10^2$ to $3x10^6$ beads in sterile deionized water. Dilutions of the silica beads were done to create standard Ct values for various concentrations to compare and determine the DNA concentrations throughout the thesis.

Gel Electrophoresis

An agarose gel uses electricity to separate nucleic acids by length and is a qualitative way to determine the presence of a DNA sequence. The presence of DNA as well as the base pair totals was confirmed by using a gel.

Instructions for making the gel are as follows:

1. Weigh out 0.5 grams of agarose into a 250 mL conical flask.
2. Add 50mL of 0.5XTBE, swirl to mix.
3. Microwave for about 1 minute to dissolve the agarose. The agarose boils over quickly so pay careful attention to it. It is good to stop it after 45 seconds and give it a swirl. It can become superheated and NOT boil until you take it out whereupon it boils out all over you hands. So wear gloves and hold it at arm's length.
4. Leave it to cool on the bench for five minutes down to about 60°C (just too hot to keep holding in bare hands).
5. Add 1μL of ethidium bromide (10 mg/mL) and swirl to mix. The reason for allowing the agarose to cool a little before this step is to minimize production of ethidium bromide vapor. Ethidium Bromide is mutagenic and should be handled with extreme caution.
6. Pour the gel slowly into the tank. Push any bubbles away to the side using a disposable tip. Insert the comb and double check that it is correctly positioned.

7 Leave to set for at least 30 minutes, preferably 1 hour, with the lid on if possible.
8 Pour 0.5x TBE buffer into the gel tank to submerge the gel to 2–5 mm (millimeter) depth. This is the running buffer.
9 Add 0.2 volumes of loading buffer (bromophenol blue), e.g. 5µL into a 25µL sample.
10 Load the first well with marker that will show the varying DNA band lengths.
11 Load the remaining wells with DNA sample (25 µl)
12 Continue loading the samples and finish off with a final lane of marker
13 Close the gel tank, switch on the power-source and run the gel at five Volts/cm.
14 Stop the gel when the bromophenol blue has run 3/4 the length of the gel.

Miscellaneous lab equipment

Lab equipment included pipettes, pipette tips, DI water HPLC grade.

Sand Core

A Schedule 40 PVC pipe was used to construct a sand core. The core was 2 feet in length with a 4 inch inside diameter. It was filled with medium to course grained quartz sand.

The steps for making a sand core were as follows:

1 A 4 inch diameter PVC pipe 2 feet long was cut
2 PVC attachments were used to size down the PVC core diameter to allow for a ½ inch tube to be attached to the bottom of the sand core
3 A circular plastic petri dish was drilled with numerous holes to allow water to freely flow through. The diameter is smaller than the core.
4 Glue cheese cloth to the petri dish
5 Glue the petri dish to the inside of the PVC attachment.
6 Sand filled to 15 inches from bottom of the core

7 Drill hole into the side of the PVC core about 16 inches from the bottom of the core for water to flow out. (One inch above sand level)
8 Set up a 20 L water container (Marriott bottle) above the core to allow for controlled constant head flow
9 Once everything is glued and set, slowly add sand to the core and secure to a workbench.

Volume of the water at the bottom of the 2 foot core = ~75 mL (2.5 fluid ounces; 29.573 mL/1 ounce). Total amount of sand added to core was 6441.4 grams.

In the second core a total of 6638.1 grams of sand was added, all other measurements stayed the same.

Quartz Sand

Medium/course grained quartz sand was locally purchased in 50 pound bags.

Core Experiment

The sand core was secured to a lab bench and water was introduced into it from the bottom upwards slowly. This procedure lessened the chance of air pockets trapped in the core. Two sand cores were built in order to repeat the experiment.

Porosity was determined by obtaining a large plastic bottle with a discharge valve to serve as the water source for the core (**Figure 3.5 & 3.6**). It was placed alongside the core and the head difference between the two structures was 5.5 inches. Water was slowly added in increments of 200 mL to the source container and ceased once water was visible at the top of the sand core (**Figure 3.7**). The pore volume was calculated to be 1275 mL. The porosity obtained was consistent with reported values for sand.

The calculated porosity was:

Radius (r) = 2 in

Height (h) = 18 in

Area of Core = $\prod r^2 = 12.56\ in^2$

*Volume of Core = Area * h = 3.707L*

Pore Volume = Porosity/Volume of Core = 1.275L/3.707L = 0.34

Figure 3.5. Sand Cores

Figure 3.6. Water Source and Sand Core

Figure 3.7. Water in Sand Core

Hydraulic Conductivity (K) was also determined for the core. A Mariotte Bottle (**Figure 3.8**), which allows for a constant head flow system, was used to perform a steady-state Darcy experiment:

Quantity (Q) of water discharged/unit time (t) = 500 mL/30min

Length (dl) between Mariotte Bottle and sand core = 36 inches

Head (dh) = 0.5inches

h = (dh/dl)

Area of Core = $\prod r^2 = 12.56\ in^2$

Hydraulic Conductivity (K) = Q/Ah

The conductivity was then calculated to be *1.5977mL/min*

A chloride tracer experiment was conducted using:

Calcium Chloride

Calcium – atomic weight = 40.078

*Chloride – atomic weight = 35.453 *2 (Cl_2) = 70.906*

Molar Mass of CaCl2 = 40.078+70.906 = 110.984g

A specific conductivity meter was used to measure the outflow of water from the sand core for this tracer to determine the breakthrough curve. In order to make sure there was a distinct difference between the regular source water and the tracer, a 4M solution was made.

The chloride tracer was applied to the core initially in a concentration of 1000 mS (milli Siemen). A later chloride tracer used was about 300 mS, which appeared to flow more easily. The concentration of the water used was about 125 mS. The 300 mS tracer showed up in approximately 45 minutes (one pore volume) and was back to background in another 45 minutes.

Once the parameters for the chloride tracer were determined, PCR was run on a sample of source water to ensure that there was nothing in the system that was amplifiable with the reagents used. The beads with no DNA also underwent PCR for the same reason.

Figure 3.8. Mariotte Bottle

Figure 3.8 shows a picture of the designed Mariotte siphon that was built using a 20-liter container. A rubber stopper was used to seal the top of the container with a small hole drilled into the top just big enough to allow a small glass straw can fit through it (**Figure 3.9**). The plastic container was situated 47 inches in length away from the sand core with a difference in head of 0.5 inches. This constant head method allowed for constant slow flow through the core.

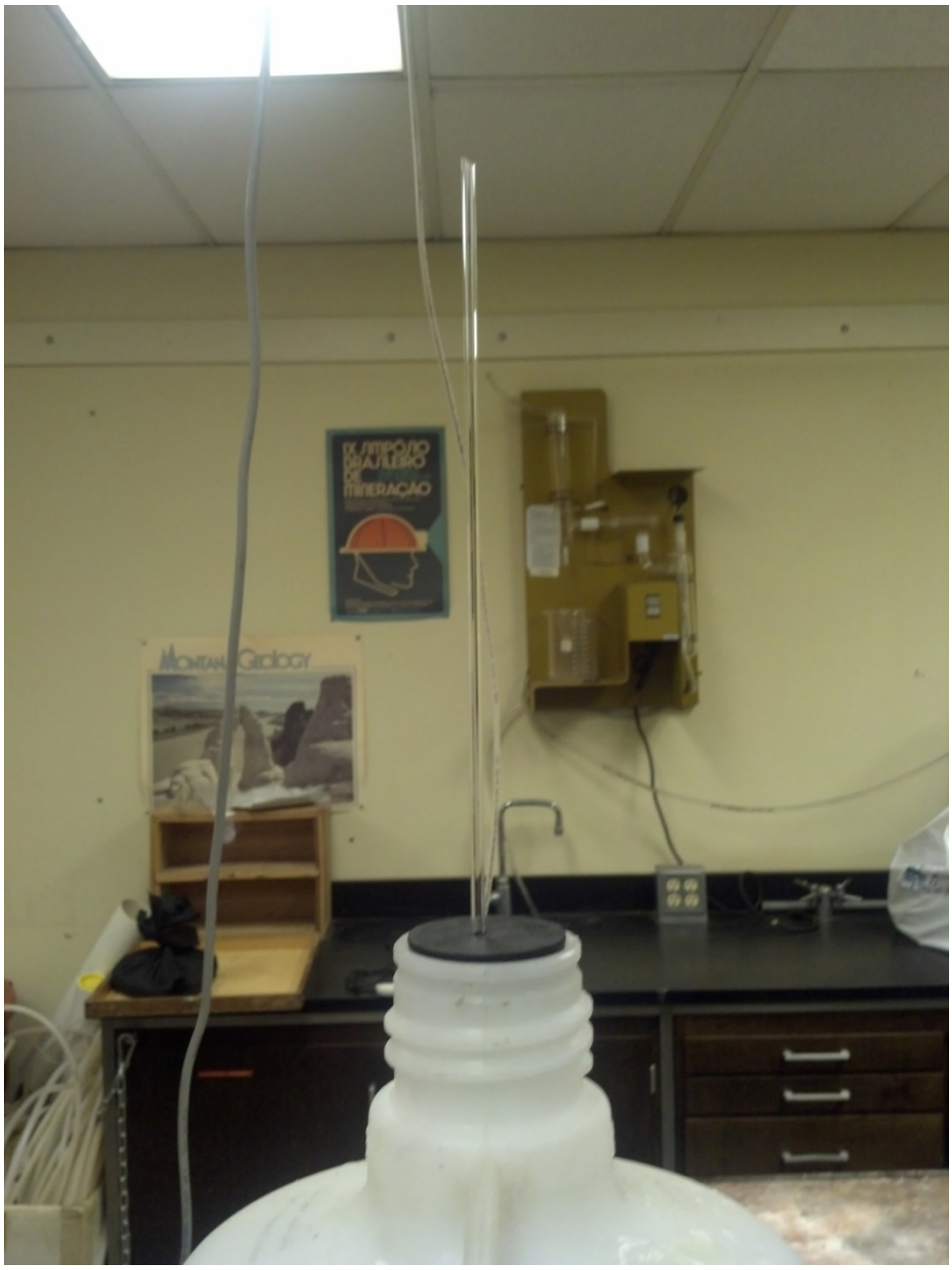

Figure 3.9. Mariotte Bottle rubber stopper and glass tubing

PCR – Core Experiment

250 µL of beads was mixed into one L of water and applied to the sand core that already contained water. Water was collected in 50 mL aliquots. The aliquots were centrifuged for 10 minutes at a speed of 10,000 RPM. Once the samples were centrifuged, a magnet was attached to the bottom of the collection tube. The supernatant was drawn from the sample tube leaving the beads immobilized at the bottom, which were collected upon removal of the magnet. The beads were amplified by PCR/fluorescence analysis with the SmartCycler®. Positive amplification demonstrates that the DNA was successfully annealed to the beads.

Degradation Study

The beads have a shelf life of three years, and the DNA may persist in the environment for months before degrading. This study was done in order to see if the DNA while attached to the beads suffered any significant degradation. A sample of soil was collected from the A horizon from Riverbend Labs in Athens, GA and placed in a glass jar with 300 µL of DNA coated beads (**Figure 3.10**). A sample was collected starting on Day 0, then cleaned with a KingFisher mL automated washer (**Figure 3.11 and Figure 3.12**). It starts with a strip- of 5 tubes, three tubes contain washing solution, the fourth has DI water, and the final tube contains water for resuspension. The sample was then collected from the fifth tube. This washer showed no evidence of cross contamination or reagent carry over.

Figure 3.10. Degradation study glass tube

Figure 3.11. Automated Washer

Figure 3.12. Automated Washer

CHAPTER 4

RESULTS

The first task of this thesis was to demonstrate that the *Acidovorax avenae* DNA was a quantifiable and reliable DNA sequence that could be amplified when attached to the beads using PCR. **Figure 4.1** illustrates this amplification, and serves as a positive control.

Following the successful amplification of the naked DNA, the next issue was ensuring that there was no amplifiable DNA on the beads or in the water. This test also showed that the beads mixed with DNA would successfully amplify, and that the beads do not interfere with PCR. Water was introduced to the sand core, then flushed for three pore volumes. This was followed by a chloride tracer, and then flushed again. Sampling was done by volume flushed from the core, with smaller volumes taken during breakthrough. PCR demonstrated that a sample of water and naked beads did not amplify the target DNA (**Figure 4.1**).

Following this experiment, a second chloride tracer test was conducted, and PCR was conducted on the flush water that contained no beads. **Figure 4.2** shows the results of that tracer experiment, and that there was no amplification except for the positive control of DNA.

Four serial dilutions of beads with DNA were run through PCR to obtain average of Ct values for a range of bead volumes. These values served as a source of comparison

for PCR amplifications throughout the thesis. **Table 4.1** shows the average values for the serial dilutions, and **Appendix A** contains the figures and PCR results reports for each of the serial dilutions. The average Ct values are shown below for the difference volume of beads.

Table 4.1. Average Ct values for serial bead dilutions coated with DNA

Ct Value:	3X10^5	3X10^4	3X10^3	3X10^2	3X10^1	Million fold DNA	Water
Average:	12.14	13.64	17.18	20.47	24.23	14.08	32.65

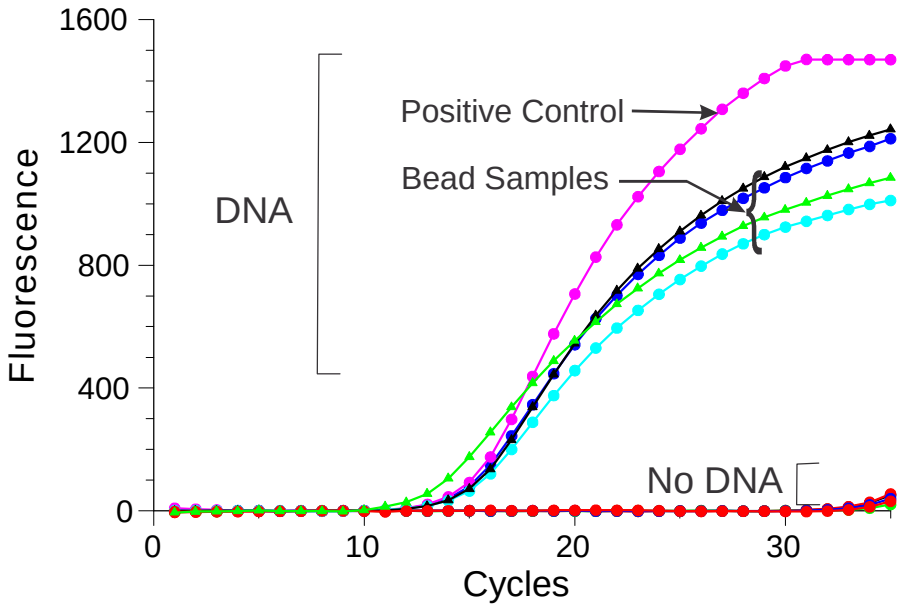

Figure 4.1. Amplification of *Acidovorax avenae* DNA and beads

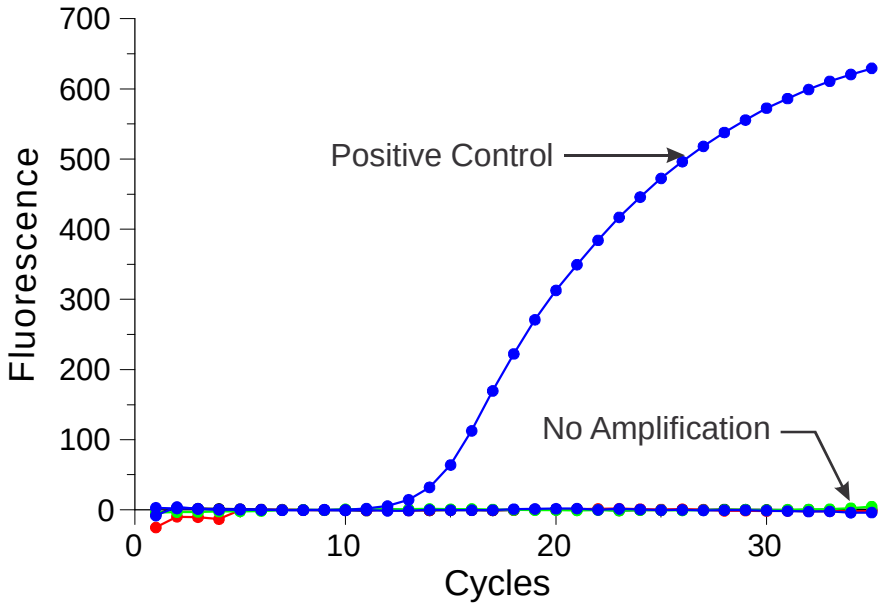

Figure 4.2. Amplification of *Acidovorax avenae* DNA and no amplification (inhibitors) in applied water or applied tracer

A chloride tracer experiment was conducted to determine the hydrologic characteristics of the sand core that would be used for the DNA tracer. The chloride tracer confirmed the pore volume of about 1200 mL. An electrical conductivity meter was used to determine the measure of elevated chloride levels. Tap water contained a background electrical conductivity of 125 µS (micro Siemens), and the tracer was 300 µS and 1000 µS. The higher the concentration of the tracer however, interacted with the sand and interfered with the flow. The experiment was repeated with 300 µS. **Figure 4.3** and **Figure 4.4** are the breakthrough curves for both experiments. Calculations for Figures 4.3 and 4.4 are found in **Appendix B**. These curves show that breakthrough occurred after about one pore volume and then returned to background levels. The second chloride tracer experiment run on the second core is shown on **Figure 4.5.** It behaved similarly to the first experiment.

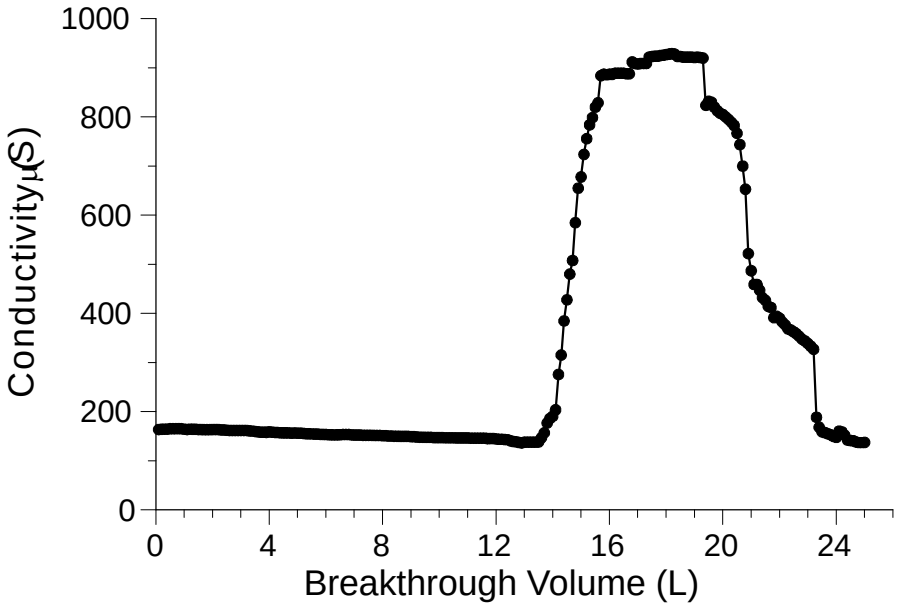

Figure 4.3. Chloride Tracer breakthrough curve 1st run 1st core

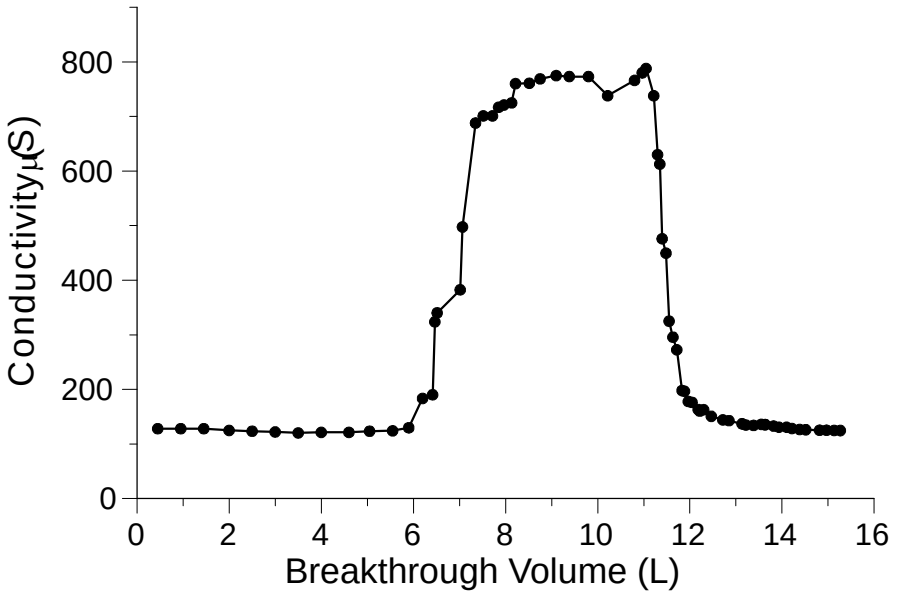

Figure 4.4. Chloride Tracer breakthrough curve 2nd run 1st core

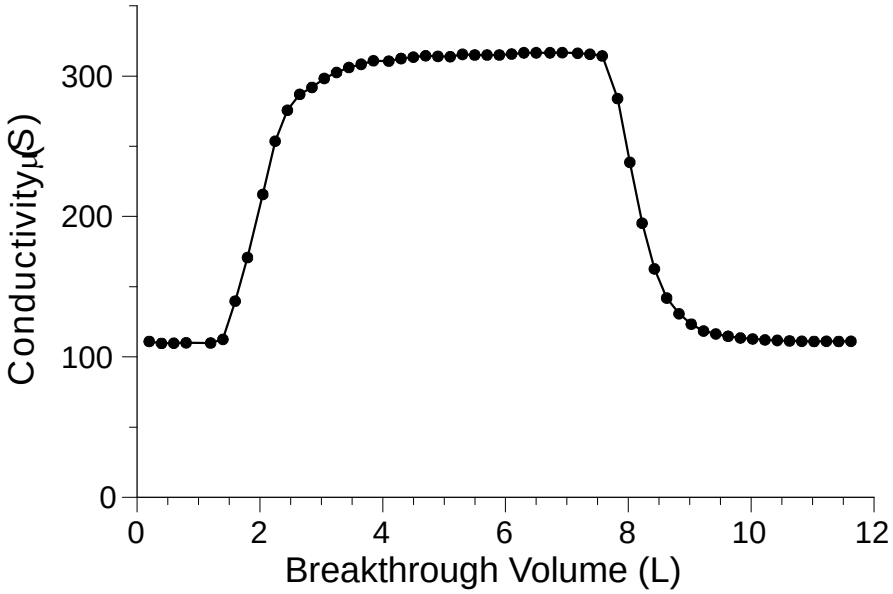

Figure 4.5. Chloride Tracer breakthrough curve 1st run 2nd core

Following the chloride tracer tests, the beads that were washed after DNA application were added to the flush water. Water collected from the core was amplified with PCR. This showed that the DNA was not lost in the core. Samples were collected every 50 mL, then spun down in a centrifuge to ensure efficient collection of the beads. The bottom 5µL were then added to the PCR and amplified.

The results showed that DNA coated beads were retrieved and were amplifiable. The DNA tracer breakthrough curves for the first run and the second run on the same core can be seen on **Figure 4.6** and **Figure 4.7** respectively. It should be noted that several data points in both tests were outliers, where less than expected amplification occurred. This may have happened where beads were lost in collection. The breakthrough curve for the DNA tracer closely resembles the chloride tracer breakthrough. This experiment was repeated twice in two separate sand cores with similar results. The breakthrough curves for the second core can be found on **Figure 4.8 and Figure 4.9**. The

tracer experiment on the second core run used half the amount of beads compared to the first experiment.

The results of the DNA beads application show a high Ct value before breakthrough. Once the beads make its way through the core, the Ct value lowers showing that there is more DNA in the water flowing out of the core, therefore less time (lower Ct value) is needed for fluorescence. The results in the second run of the second core show that the beads were fully flushed from the core at the end of the experiment. This can be attributed to using fewer beads for this run. The PCR results reports for the tracer experiments can be found in **Appendix C**.

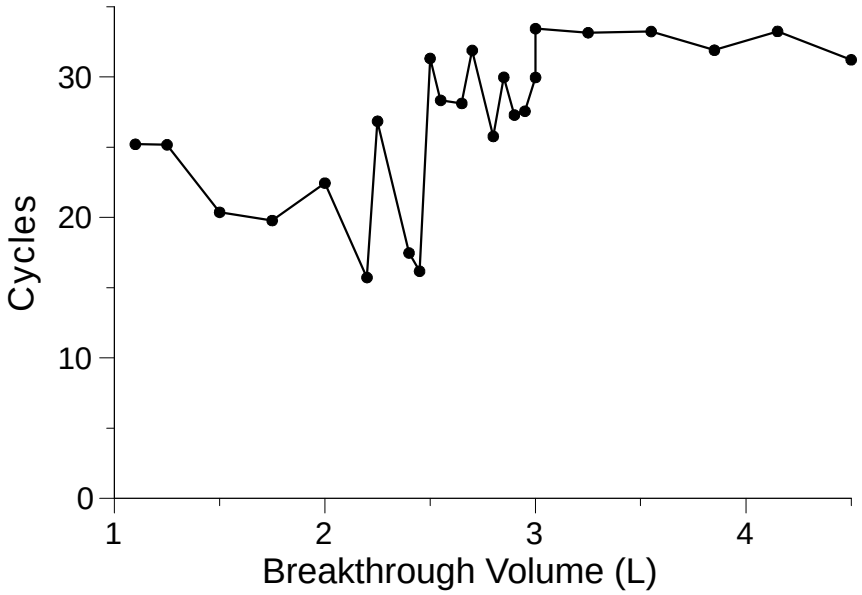

Figure 4.6. DNA tracer breakthrough curve Core 1 Run 1

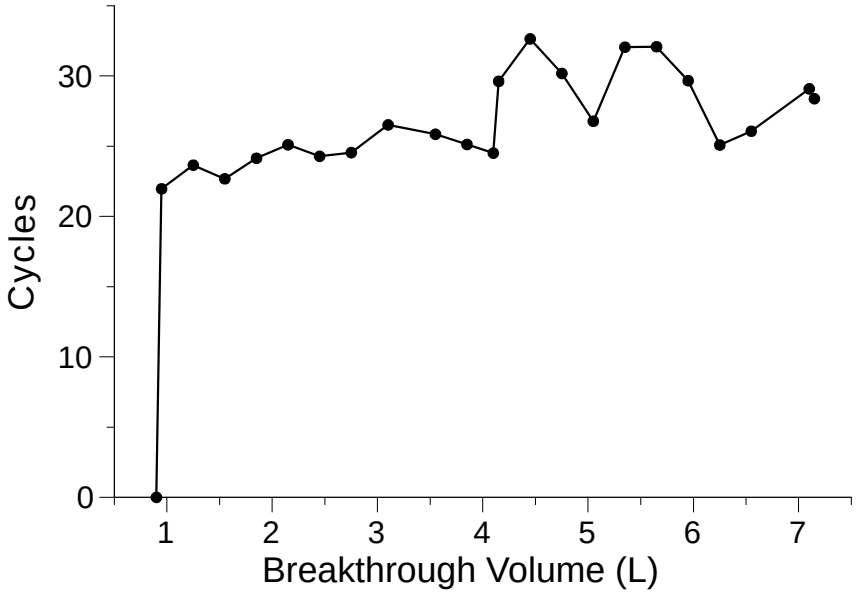

Figure 4.7. DNA tracer breakthrough curve Core 1 Run 2

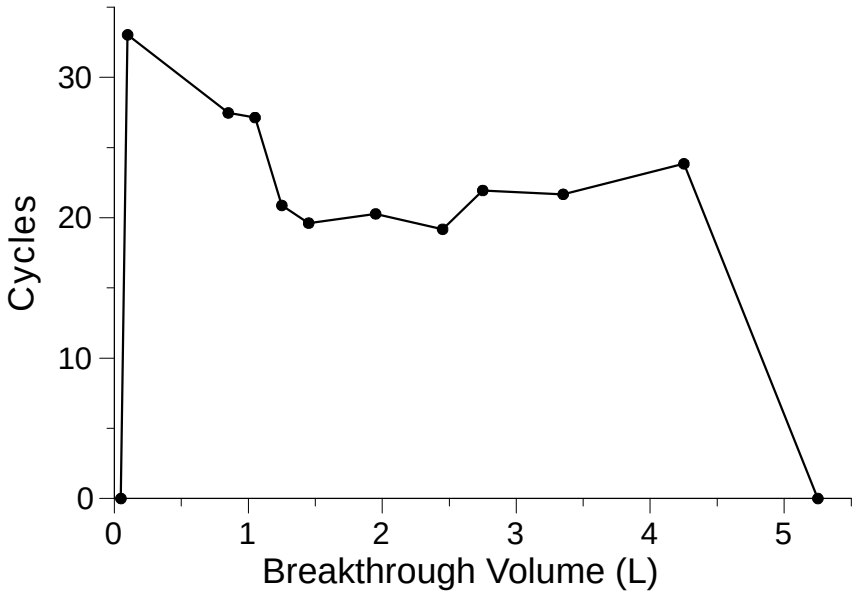

Figure 4.8. DNA tracer breakthrough curve Core 2 Run 1

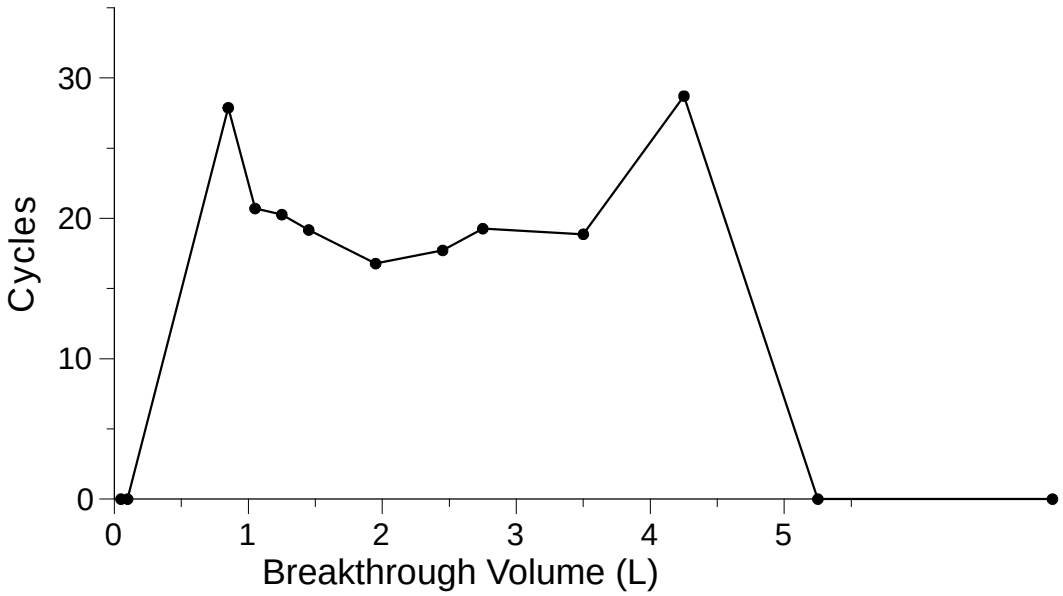

Figure 4.9. DNA tracer breakthrough curve on Core 2 Run 2

The degradation experiment showed that the DNA persisted over three weeks. **Figures 4.10-4.15** show the PCR results for different sampling days. No significant degradation occurred. **Appendix D** contains all PCR results reports.

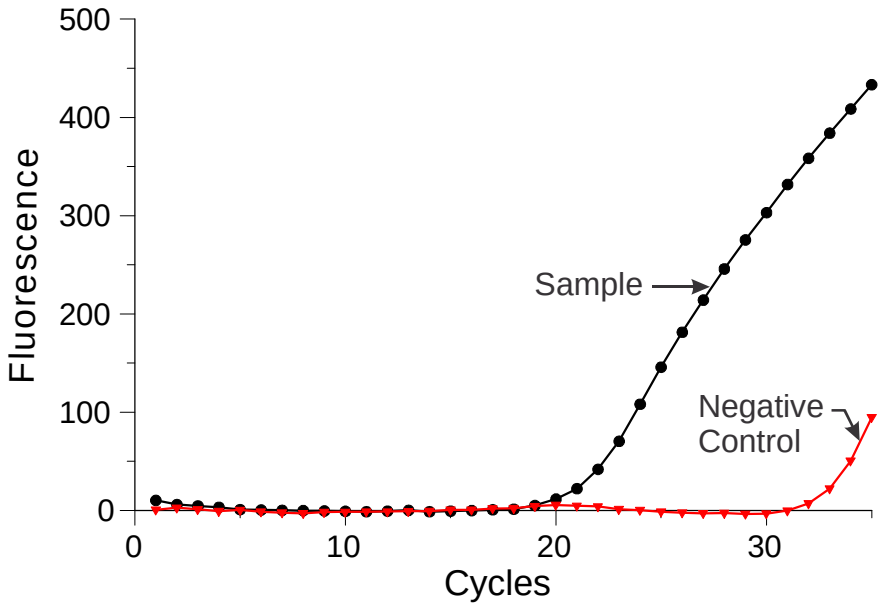

Figure 4.10. Degradation Experiment Day 0 (Ct value = 21.39)

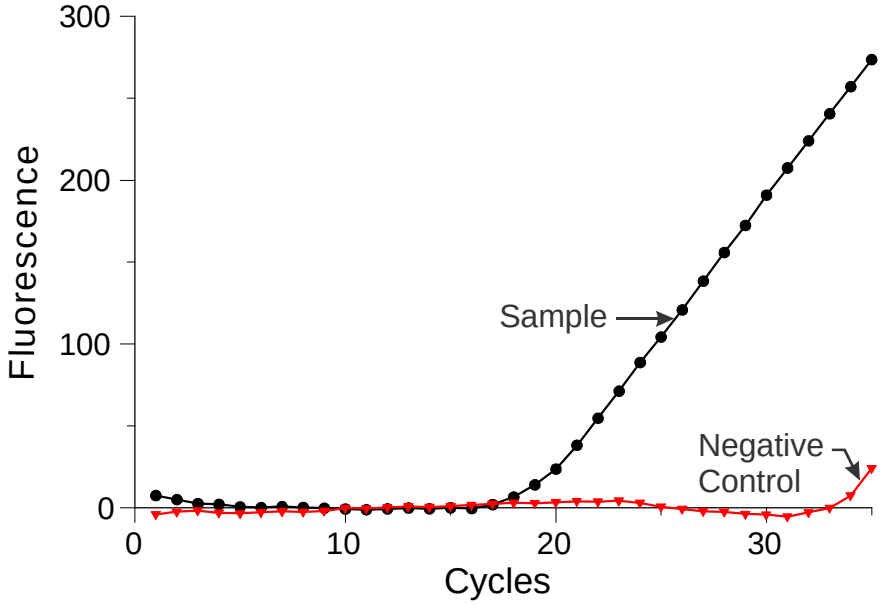

Figure 4.11. Degradation Experiment Day 3 (Ct value = 20.44)

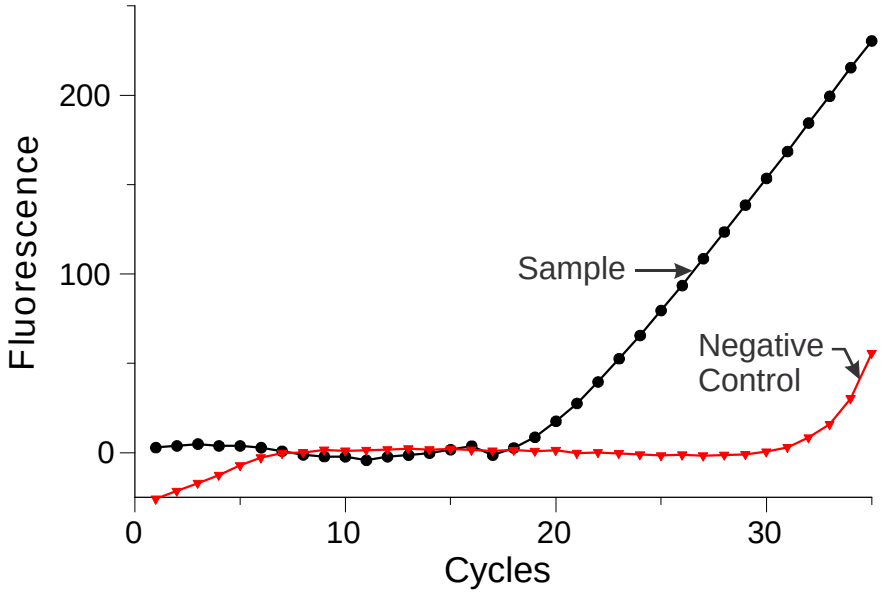

Figure 4.12. Degradation Experiment Day 10 (Ct value = 21.20)

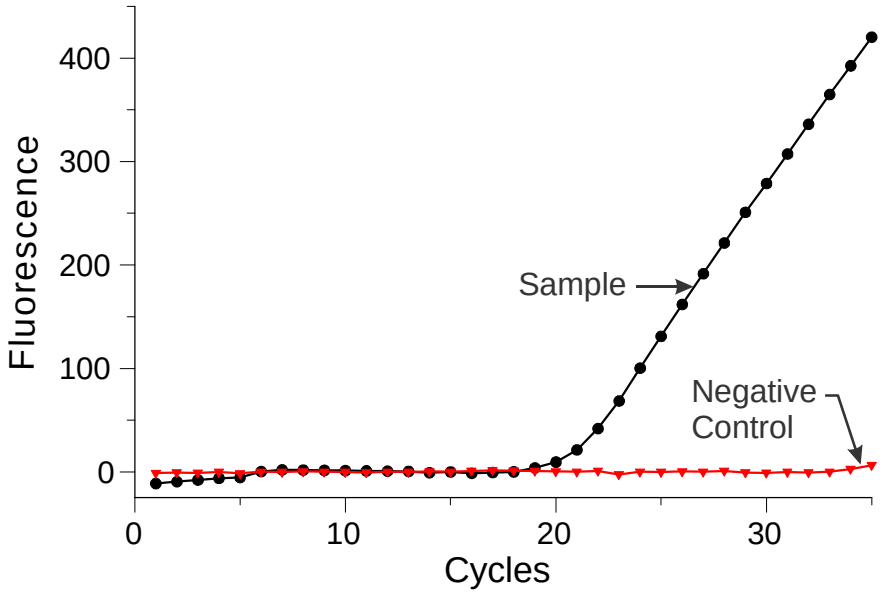

Figure 4.13. Degradation Experiment Day 12 (Ct value = 21.43)

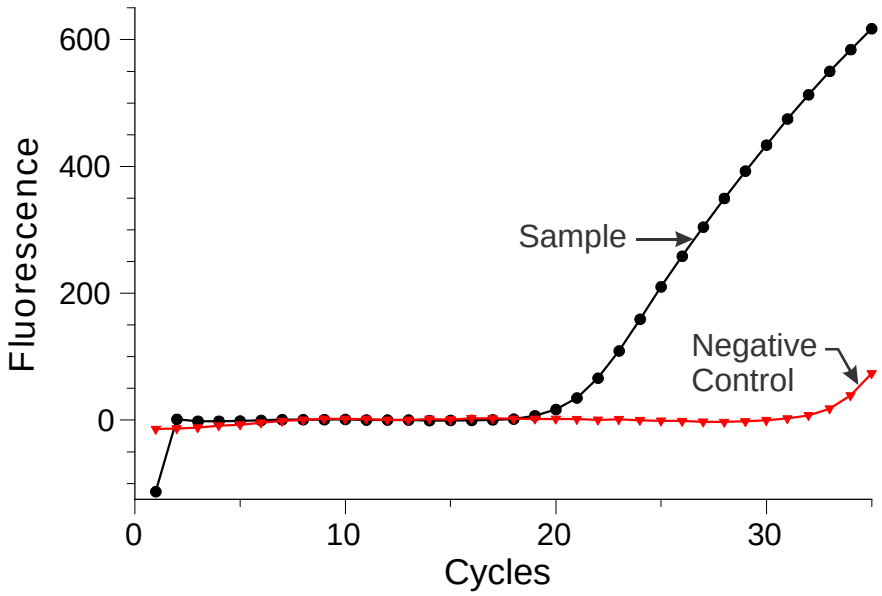

Figure 4.14. Degradation Experiment Day 14 (Ct value = 20.73)

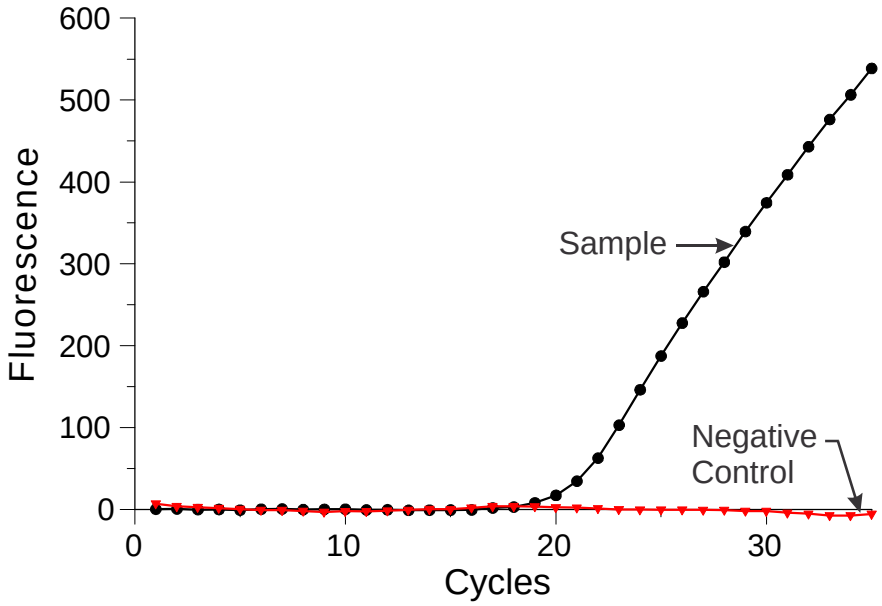

Figure 4.15. Degradation Experiment Day 20 (Ct value = 20.73) – Same Ct as Day 14

CHAPTER 5

DISCUSSION of RESULTS

It was important to ensure that nothing used in the experiment interfered with PCR. Consequently, bead volume and DNA concentrations were kept below the inhibitory amounts. The water and beads were tested to make sure they had no amplifiable parts, and the DNA was checked to make sure that there was amplification as a standalone. All sampling was done to show that any amplification would be from the presence of the target DNA and not a false positive from one of the methods or materials used in this thesis.

The negative PCR control was water, and this was used to demonstrate that the sample results are not false positives. All samples that undergo PCR have the same master mix which cannot amplify DNA without DNA being present in the sample. Once water is added to this master mix nothing from that sample should amplify.

Unfortunately, in some instances there was a slight amplification towards the end of the 30 cycles, although this does not mean that the entire reaction has failed. A small amount of cross contamination is expected given the laboratory procedures used. The negative samples were examined in an effort to minimize this affect. Ct values were used to determine if the tracer DNA was in samples. PCR was repeated on suspect samples to validate Ct results. Because the negative control showed slight amplification at 34 cycles, positive results were considered only for Ct values less than 30 cycles.

This thesis used a large amount of beads in a small volume of water. There was no way to physically calculate and confirm the amount of beads that were being used.

Consequently the manufacturer's calculation was used. Although this was given as a range, for the purposes of this research the lower value was used for all calculations. When sampling from collection tubes it is impossible to ensure that all of the beads in solution were retrieved. For this reason, any calculation of percent recovery is uncertain. The tracer residence time of the DNA tracer seems to be longer than that of the chloride tracer. In any case, the DNA experiment demonstrated that DNA coated paramagnetic beads can be used for tracer experiments.

Although, samples were collected every 50 mL and spun down in a centrifuge to make sure all beads were at the bottom, there are 30-40 samples that did not undergo PCR. These samples were not considered informative, and were discarded for cost reasons. A magnet was used to keep the beads immobile at the bottom of the sample vial. It was not possible to know if all beads were immobilized. Once the magnet was removed the bottom 5µL was collected using the residual water in the sample tube, being sure to sweep the pipette tip along the bottom of the container to retrieve all beads. The 5 µl was added to the PCR and amplified. This procedure may not have collected all of the beads.

The Ct value did go up however as time elapsed and more water passed through the core. This means, in theory, that there were fewer amount of beads getting collected as the experiment progressed. A high Ct value indicated that there was a smaller amount of DNA available for amplification, thus fewer beads were being collected and sampled.

It is thought that there were too many beads introduced to the sand core in the initial experiment. Considering that not all beads were collected per sample and there were still high Ct values in the latter part of the experiment indicated that many of the beads were available for later flushing. When the tracer experiment was repeated for the

second core, the amounts of beads were decreased by half. This caused a slight difference in the breakthrough curve: it was more apparent and did not plateau right away as in earlier runs. Also, the entire breakthrough of the DNA tracers was more visible in the second core experiments, and the decline in PCR amplifications was apparent. The residence time, however, was still longer than the chloride tracer.

The tracer results showing that the Ct values were not largely diverse showed that the beads were flowing throughout the core evenly and not in clusters. Tracers do plateau for a while before they decline as seen in the chloride tracer and that was also slightly seen in the two sand core experiments.

The DNA used for this thesis were the biotin labeled primers which themselves are small DNA fragments. Because the concentration of the PCR product was extremely high, dilutions were made down to a million fold **(Figure 5.1)** in order to determine an acceptable Ct value to be used to identify the presence of the target DNA. The Ct value for this is dilution is 13.26. This value was also used for positive control. The million fold samples were consistent throughout the experiment. Therefore this Ct value was used to determine background for all samples. The low Ct value demonstrates that an adequate amount of DNA is in the million fold dilution. If the Ct value was at 30 for example, any amplification in the negative controls at that point would make all results unreliable.

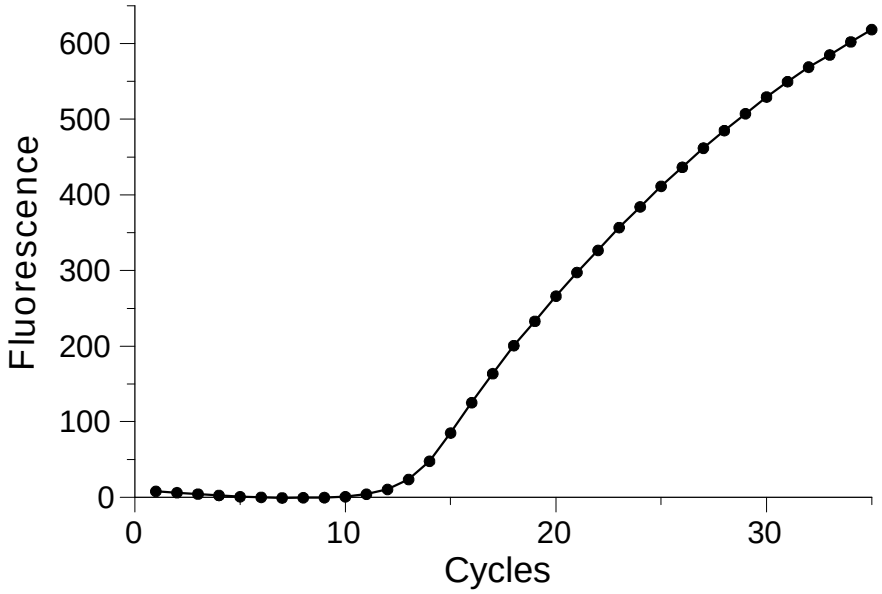

Figure 5.1. Million fold DNA amplification (Ct value = 13.26)

The degradation experiment was also promising. After nearly three weeks of interacting with soil, there was no major degradation to the beads, as there was no increase in the Ct value. This was a laboratory experiment and should be repeated in the field, where the DNA coated beads would be exposed more to potential degradation agents. Another experiment would be to run the degradation experiment paired with the DNA in water. A comparison of Ct values would indicate potential degradation. These findings do reinforce the belief that binding the DNA to a host will aid in DNA longevity. It should be emphasized that resampling of negative results (i.e. high Ct values) may be necessary to ensure accurate results.

Large amounts of DNA also cause PCR problems. Consequently it is important to determine the optimal amount of beads for a tracer experiment.

CHAPTER 6

SUMMARY & CONCLUSIONS

The goal of this thesis was to determine if the use of streptavidin coated beads coupled with DNA is a practical alternative to the common tracers presently in use. My findings show that they can be. These streptavidin coated magnetic beads have the ability to bind DNA and maintain that bond throughout a tracer experiment. The ability of this tracer to withstand some of the more harsh environmental conditions that it underwent also shows that with additional research and development a more complex network of beads can be used effectively.

The application of the beads to a simple sand core demonstrated that the overall proposal of using silica colloids as a tracer was possible in its most elementary application. These tracers are potentially very useful and cost-effective where in situ tracing is required.

A common problem with tracers is dilution in a water body. In some cases, it may not be permissible to introduce sufficient tracer to overcome the dilution problem. DNA tracers are less susceptible to dilution as a small of beads are potentially detectable. Using different strands of non-native DNA, a better understanding of the soil type and flow type can be obtained without an overload of any one tracer. Using different DNA at different times, flow paths can be determined.

One of the bigger advantages of the application of this tracer is that multiple tracer experiments can be simultaneously run. The DNA that will be bound to the beads

have their own unique “code”, therefore applying various DNA tracers with different sequences simultaneously is possible. Multiplex PCR is the amplification of more than one target in a single reaction tube (Brisson, 2004). Although multiple tracers can be used, there is a maximum amount of dyes and tracers that can be used simultaneously (Foppen, 2011). The use of multiple PCR amplification could accommodate the unlimited number of DNA tracers. There is no inhibition of one DNA sequence on the other if PCR were run for multiple targets. Multiplexing need only be done in order to save money, time, and sample handling. Multiplexing would allow for multiple targets to be amplified and quantified in the same reaction tube showing that the results from the one reaction is comparable to the results from the multiple reactions. If a connected flow path exists at a site that was being investigated, the use of these tracers would be able to determine that particular feature based on the amplification of both of the markers during PCR.

With the development of synthetic DNA the amount of potential markers is infinite. A “BLAST” (Medicine, 2009) can be conducted to make sure tracer and primers are unique to the research and cannot be found in your area. BLAST can also be used to locate DNA to use.

The flow patterns the DNA beads go through are highly relatable to the actual water flow pattern as the DNA tracers do not disturb the properties of the water they are traveling with. At low concentrations, they do not influence the viscosity and density of water, do not adsorb to the soil particles, are not rapidly degraded, and are not harmful to the environment. The DNA tags can be produced in large quantities and applied to the groundwater in a short time.

When investigating the flow of groundwater it is important to understand the way in which solutes, in this case the DNA beads, are physically transported in groundwater. Advection is the process by which solutes are transported by the bulk motion of the flowing groundwater. Overall solutes are carried at an average rate equal to the average pore water velocity of the water. At higher velocities dispersion, or the smearing of the contaminant front is increased (Freeze, 1979).

The aperture of the pathway as well as the actual pathway that the DNA beads travel also can affect the breakthrough. Dispersion can be caused by three mechanisms (Freeze, 1979): 1) the drag can be exerted on the fluid due to the roughness of the pore surfaces. 2) The difference in pore sizes along flow paths cause different velocities. The smaller the pore sizes a pathway the slower the solutes will travel and vice versa. The breakthrough curve of the DNA tracers in this thesis could vary depending on the size of the pathway it is flowing through. And 3) there is dispersion where the tracer would flow in the direction of bulk flow (longitudinal dispersion) and dispersion perpendicular to flow (transverse dispersion). In general, longitudinal dispersion is 10 times transverse dispersion. The mechanics of groundwater flow is dependent on many variables of the pathway they take and therefore those variables must also be considered when applying this tracer.

One of the places this DNA tracer would be great to study in the field would be in a Karst environment, where the groundwater flow is fast and the detection of the tracers would be a lot more rapid than in dealing with a less conductive site. This would be a good starting point to the "real world" applicability of these tracers and lead to their development.

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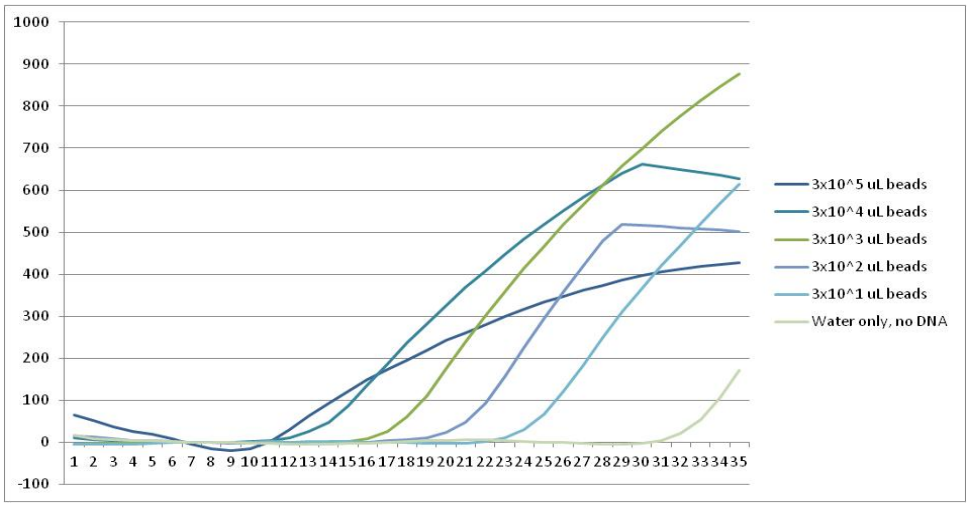
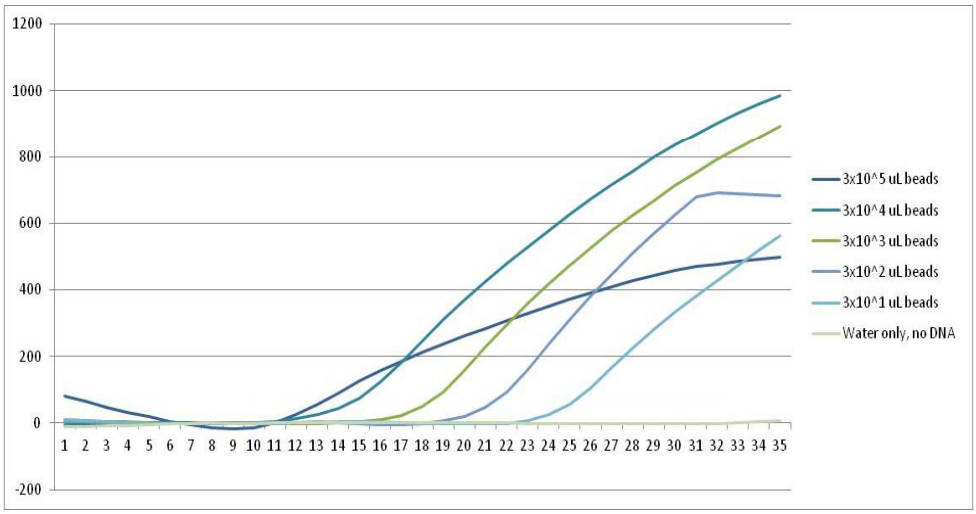
APPENDIX A

SERIAL DILUTION RESULTS

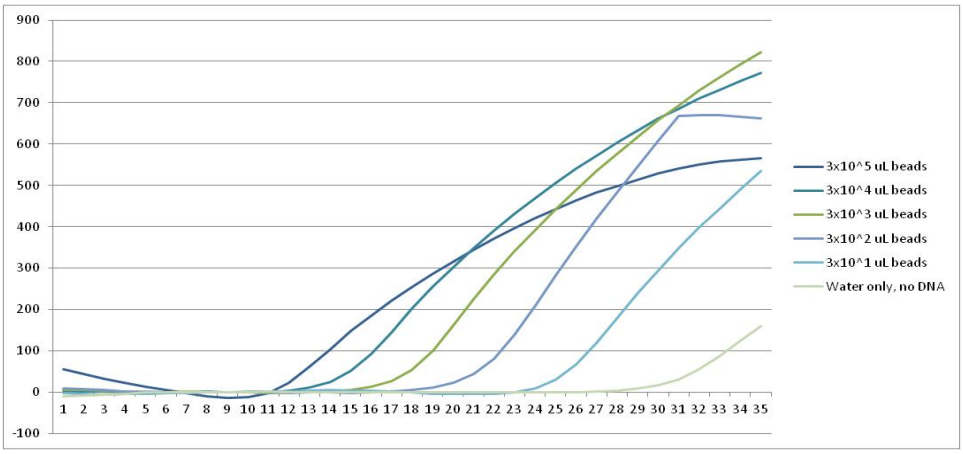
3/14/13 SD



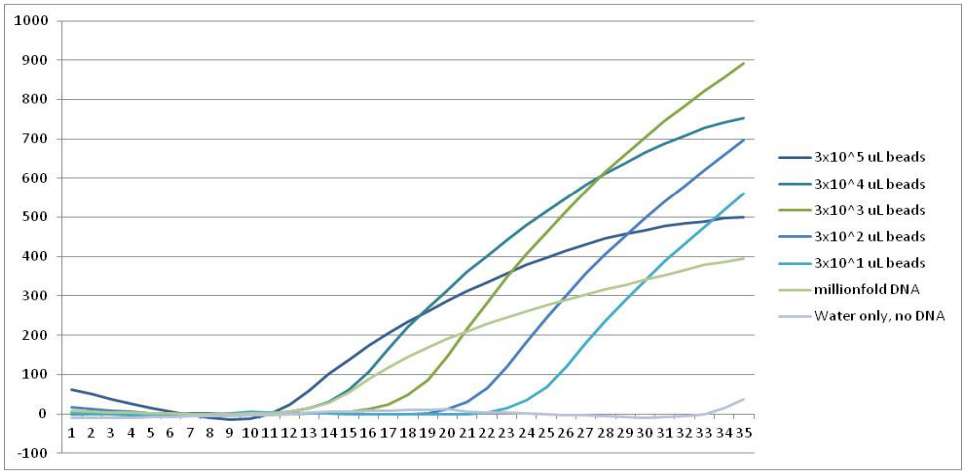
3/15/13 SD



3/18/13 SD



3/20/13 SD



	uL beads						
Ct Value:	3X10^5	3X10^4	3X10^3	3X10^2	3X10^1	Millionfold DNA	Water
3/14/2013	12.03	13.22	17.12	20.23	23.96	NA	32.25
3/15/2013	12.13	13.19	17.24	20.37	24.16	NA	0.00
3/18/2013	12.19	14.19	17.11	20.32	24.97	NA	30.99
3/20/2013	12.19	13.95	17.23	20.94	23.81	14.08	34.72
Average:	12.14	13.64	17.18	20.47	24.23	14.08	NA

Run Report

Run Information

Run Name: KC-Serial Dilution 3_14_13 Beads
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: New washed beads set 3/7/13 take 2, diluted samples 1-3

Started: 2013/3/14 04:15 PM
Finished: 2013/3/14 05:21 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 6
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	KC new beads 3 x 10^5	OK	POS	12.03
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	KC new beads 3 x 10^4	Warning	POS	13.22
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	KC new beads 3 x 10^3	OK	POS	17.12
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	KC new beads 3 x 10^2	Warning	POS	20.23
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	KC new beads 3 x 10^1	OK	POS	23.96
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	Water	OK	POS	32.25

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	POS	30.98	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00

Error(s)

Site ID	Code	Error	Value	Time
A2	3079	Warning: Fluorescence Signal Too High	0	05:12 PM
A2	3079	Warning: Fluorescence Signal Too High	0	05:14 PM
A2	3079	Warning: Fluorescence Signal Too High	0	05:15 PM
A2	3079	Warning: Fluorescence Signal Too High	0	05:17 PM
A2	3079	Warning: Fluorescence Signal Too High	0	05:18 PM

Error(s)

A2	3079	Warning: Fluorescence Signal Too High	0	05:20 PM
A2	3079	Warning: Fluorescence Signal Too High	0	05:21 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:11 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:12 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:14 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:15 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:17 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:18 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:20 PM
A4	3079	Warning: Fluorescence Signal Too High	0	05:21 PM

Instrument(s)

Instrument	**A**
Serial Number	**900811**

Run Report

Run Information

Run Name: KC-Serial Dilution 3_14_13Beads2
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Started: 2013/3/15 08:40 AM
Finished: 2013/3/15 09:46 AM
SW Version :2.0d
Notes: Repeat of yesterdays - New washed beads set 3/7/13 take 2, diluted samples 1-3
Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 6
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	KC new beads 3 x 10^5	OK	POS	12.13
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	KC new beads 3 x 10^4	Warning	POS	13.19
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	KC new beads 3 x 10^3	OK	POS	17.24
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	KC new beads 3 x 10^2	Warning	POS	20.37
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	KC new beads 3 x 10^1	OK	POS	24.16
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	Water	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	POS	33.81	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00

Error(s)

Site ID	Code	Error	Value	Time
A2	3079	Warning: Fluorescence Signal Too High	0	09:43 AM
A2	3079	Warning: Fluorescence Signal Too High	0	09:45 AM
A2	3079	Warning: Fluorescence Signal Too High	0	09:46 AM
A4	3079	Warning: Fluorescence Signal Too High	0	09:40 AM
A4	3079	Warning: Fluorescence Signal Too High	0	09:42 AM

Error(s)

A4	3079	Warning: Fluorescence Signal Too High	0	09:43 AM
A4	3079	Warning: Fluorescence Signal Too High	0	09:45 AM
A4	3079	Warning: Fluorescence Signal Too High	0	09:46 AM

Instrument(s)

Instrument	**A**
Serial Number	**900811**

Run Report

Run Information

Run Name: KC-Serial Dilution 3_7_13Beads
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes:

Started: 2013/3/18 10:16 AM
Finished: 2013/3/18 11:22 AM
SW Version : 2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 6
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	KC new beads 3 x 10^5	OK	POS	12.19
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	KC new beads 3 x 10^4	Warning	POS	14.19
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	KC new beads 3 x 10^3	OK	POS	17.11
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	KC new beads 3 x 10^2	Warning	POS	20.32
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	KC new beads 3 x 10^1	OK	POS	24.97
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	Water	OK	POS	30.99

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	POS	33.15	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00

Error(s)

Site ID	Code	Error	Value	Time
A2	3079	Warning: Fluorescence Signal Too High	0	11:20 AM
A2	3079	Warning: Fluorescence Signal Too High	0	11:22 AM
A4	3079	Warning: Fluorescence Signal Too High	0	11:16 AM
A4	3079	Warning: Fluorescence Signal Too High	0	11:17 AM
A4	3079	Warning: Fluorescence Signal Too High	0	11:19 AM

Error(s)

A4	3079	Warning: Fluorescence Signal Too High	0	11:20 AM
A4	3079	Warning: Fluorescence Signal Too High	0	11:22 AM

Instrument(s)

Instrument	**A**
Serial Number	**900811**

Run Report

Run Information

Run Name: KC-Serial Dilution 3_7_13Beads 2
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: 3rd Serial Dilution aliquot for comparison

Started: 2013/3/20 10:44 AM
Finished: 2013/3/20 11:50 AM
SW Version : 2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 7
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	KC new beads 3 x 10^5	OK	POS	12.19
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	KC new beads 3 x 10^4	OK	POS	13.95
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	KC new beads 3 x 10^3	OK	POS	17.23
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	KC new beads 3 x 10^2	OK	POS	20.94
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	KC new beads 3 x 10^1	OK	POS	23.81
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	Millionfold DNA Dilution	OK	POS	14.08
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	Water	OK	POS	34.72

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A

Instrument(s)

Serial Number	**900811**

APPENDIX B

CHLORIDE TRACER RESULTS

Core 1 - Chloride Tracer Experiment #1

water = 160.2 uS

chloride tracer = 925 uS

time	*uS*	*Notes*
18:02	163.8	
18:07	164.3	
18:09	164.4	
18:12	164.4	
18:15	165.3	
18:18	165.4	
18:21	165.4	
18:23	165.6	
18:24	165.2	
18:27	164.4	
18:30	163.9	
18:34	164.5	
18:36	164.3	
18:41	164.2	
18:43	163.9	
18:45	163.6	
18:48	163.4	
18:51	163.3	
18:55	163.4	
19:00	163.8	
19:04	163.9	
19:06	163.9	
19:09	163.1	
19:14	163.1	
19:17	162.2	
19:19	161.9	
19:21	161.7	
19:25	161.7	
19:28	161.7	
19:30	161.7	
19:33	161.7	
19:37	161.7	
19:42	161	
19:45	160.5	
19:50	159.8	
19:54	158.9	
19:57	158.4	
20:00	158.3	
20:05	158.1	
20:07	159	
20:10	158.2	
20:12	157.8	
20:15	157.4	
20:18	157	
20:20	156.9	
20:23	156.9	
20:25	156.7	
20:28	156.5	
20:30	156.6	
20:33	156.5	
20:35	156.2	
20:38	155.8	

20:42	155.3
20:45	155.1
20:50	154.9
20:55	154.5
20:58	154.2
21:00	154
21:03	153.8
21:05	153.7
21:08	153.3
21:10	153
21:13	152.9
21:15	153
21:18	153.3
21:20	153.5
21:23	153.6
21:25	153.5
21:30	152.7
21:33	152.5
21:35	152.5
21:42	152.3
21:45	152.2
21:48	152.1
21:50	152
21:53	151.9
21:55	151.7
21:58	151.7
22:00	151.6
22:03	151.4
22:05	151.1
22:08	150.5
22:10	150.3
22:13	150.2
22:15	150.2
22:18	149.9
22:20	149.8
22:23	149.9
22:25	149.8
22:28	149.4
22:30	149.1
22:33	148.6
22:35	148.2
22:38	148.1
22:40	147.9
22:43	147.8
22:45	147.7
22:48	147.4
22:50	147.4
22:53	147.3
22:55	147.3
22:58	146.8
23:00	146.9
23:05	146.8
23:08	146.8
23:10	146.7

23:13	146.6	
23:15	146.5	
23:18	146.5	
23:20	146.4	
23:23	146.3	
23:25	146.3	
23:28	146.3	
23:30	146.2	
23:33	146.1	
23:35	146.1	
15:45	145.2	
15:50	145.3	
15:53	145.3	
15:55	144.6	
15:58	143.8	
16:00	143.9	
16:03	143.5	
16:05	143.2	
16:10	140.8	
16:15	139.6	
16:18	138.6	
16:20	137.6	
16:23	136.4	
16:26	137.9	
16:28	137.9	
16:30	137.9	
16:31	138	
16:32	138.1	
16:33	138.2	
16:34	147.1	
16:35	157	
16:36	176.9	
16:37	185.9	
16:40	190.8	changed collection cup so change in co
16:41	203.8	
16:43	275.7	
16:45	315.4	
16:48	385	
16:50	427.9	
16:52	480	
16:55	508	
17:00	585	
17:05	655	
17:10	678	
17:17	724	
17:20	756	
17:23	784	
17:25	799	
17:28	820	
17:30	829	
17:35	884	
17:38	887	
17:40	886	
17:43	887	

17:45	887	
17:48	889	
17:50	889	
17:53	889	
17:55	889	
17:58	888	
18:00	888	switch to flush water system off
18:05	912	
18:08	909	
18:10	908	
18:13	909	
18:15	909	
18:18	909	
18:19	922	
18:20	923	
18:23	924	
18:25	924	
18:28	925	
18:30	926	
18:33	927	
18:36	928	
18:38	929	
18:40	928	
18:43	923	
18:45	923	
18:48	922	
18:50	922	
18:53	922	
18:55	922	
18:58	921	
19:00	922	
19:02	921	
19:05	920	
19:06	824	
19:08	832	
19:10	830	
19:13	820	
19:14	813	
19:15	808	
19:16	805	
19:18	800	
19:19	795	
19:20	789	
19:21	783	
19:23	767	
19:26	744	
19:30	700	
19:33	653	
19:35	522	
19:38	487	
19:41	459	
19:43	459	
19:45	447.4	
19:48	432.1	

19:50	426.6
19:53	414.5
19:55	412.2
19:58	391.5
20:00	394.1
20:05	390.1
20:08	382.4
20:10	376.6
20:13	368.5
20:15	366.1
20:18	362.7
20:20	358.8
20:25	353.3
20:28	347.4
20:30	344
20:33	338.6
20:35	333.3
20:38	327.2
20:41	188.7
20:43	168.6
20:46	159
20:48	157.3
20:50	155.1
20:53	153.1
20:55	149.6
20:58	147.6
21:02	160.5
21:03	158.7
21:05	152.1
21:11	142.3
21:13	141.3
21:15	140.6
21:23	138.2
21:25	137.3
21:27	137.1
21:30	137.6

Core 1 - Chloride Tracer Experiment #2

water = 115.1 uS

time	*uS*	*Notes*
10:25	128.1	
10:35	128.1	
10:50	128.1	
11:02	124.9	
11:14	123.5	
11:27	122.0	
11:39	120.4	
11:52	121.3	
12:04	121.4	system off
12:56	123.6	12:20 system on
13:19	124.1	
13:44	129.8	
14:14	183.6	
14:27	190.3	
14:30	323.9	
14:33	340.5	
15:03	382.7	
15:06	497.7	
15:23	688.0	
15:33	701.0	
15:45	701.0	
15:53	717.0	
16:00	721.0	
16:10	725.0	
16:15	760.0	
16:33	761.0	
16:47	769.0	
17:08	775.0	
17:25	773.0	
17:50	773.0	system off
18:15	738.0	18:05 restart system with fresh water
18:50	766.0	
19:00	780.0	
19:05	788.0	
19:15	738.0	
19:20	630.0	
19:23	613.0	
19:26	476.2	
19:31	449.7	
19:35	325.1	
19:40	295.6	
19:45	272.5	
19:52	198.1	
19:55	196.6	
20:00	178.0	

20:05	176.2	
20:13	163.3	
20:15	160.3	
20:20	162.7	
20:30	150.7	
20:45	144.1	
20:53	142.8	
21:10	137.0	
21:!5	134.8	
21:25	133.9	
21:35	136.2	
21:40	135.6	system off
9:12	135.5	09:07 system on
9:23	133.0	
9:30	130.9	
9:40	130.7	
9:47	128.4	
9:57	126.5	
10:05	126.1	
10:23	125.3	
10:32	125.1	
10:42	124.8	
10:50	124.7	system off

Core 2 - Chloride Tracer Experiment #1

water = 121.5 uS	*time*	*uS*	*Breakthrough Volume (mL)*	*Notes*
chloride tracer = 332.4 uS	14:40	111	200	
	14:50	109.7	400	
	15:02	109.8	600	
	15:13	110.1	800	
	15:16	NR	1000	
	15:18	110	1200	
	15:24	112.6	1400	NR because was going to overflow
	15:32	139.7	1600	reduced head to 2" from 3"
	15:39	170.8	1800	
	15:47	215.8	2050	
	15:53	253.7	2250	
	16:00	275.6	2450	
	16:06	287	2650	
	16:13	291.9	2850	
	16:19	298.3	3050	
	16:26	302.6	3250	
	16:34	306.1	3450	
	16:40	308.4	3650	
	16:46	310.9	3850	
	16:55	310.6	4100	
	17:01	312.5	4300	
	17:08	313.5	4500	
	17:14	314.5	4700	
	17:21	314.1	4900	
	17:28	313.8	5100	
	17:35	315.5	5300	
	17:41	315	5500	
	17:48	315	5700	
	17:55	315	5900	
	18:01	315.7	6100	
	18:03	316.6	6300	
	18:05	316.6	6500	
	18:08	316.6	6725	
	18:10	316.7	6925	
	18:13	316.3	7175	
	18:17	315.6	7375	
	10:33	314.3	7575	
	10:35	284	7825	
	10:38	238.6	8025	
	10:40	195.2	8225	
	10:41	162.7	8425	
	10:43	141.9	8625	
	10:45	130.7	8825	
	10:47	123.3	9025	

10:48	118.4	9225
10:50	116.4	9425
10:52	114.7	9625
10:53	113.5	9825
10:55	112.8	10025
10:56	112.1	10225
10:58	111.8	10425
11:00	111.5	10625
11:02	111.2	10825
11:03	111.1	11025
11:05	111.2	11225
11:07	111.1	11425
11:09	111.2	11625

APPENDIX C

DNA TRACER PCR RESULTS

Run Report

Run Information

Run Name: KC - Thesis - Run 1
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: First Core run with 2/14 beads 300 uL

Started: 2013/3/15 02:08 PM
Finished: 2013/3/15 03:14 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 7
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	1050-1100 uL	OK	POS	25.21
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	1200-1250 uL	OK	POS	25.18
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	1450-1500 uL	OK	POS	20.37
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	1700-1750 uL	OK	POS	19.79
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	1950-2000 uL	OK	POS	22.45
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	Water	OK	NEG	0.00
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	2200-2250 uL	OK	POS	26.86

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A

Instrument(s)

Serial Number	**900811**

Run Report

Run Information

Run Name: KC - Thesis - Run 2
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: First Core run (continued) with 2/14 beads 300 uL

Started: 2013/3/18 04:08 PM
Finished: 2013/3/18 05:13 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 7
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	2400-2450 uL	OK	POS	16.18
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	2450-2500 uL	OK	POS	31.33
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	2600-2650 uL	OK	POS	28.13
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	2750-2800 uL	OK	POS	25.78
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	2850-2900 uL	OK	POS	27.29
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	2950-3000 uL	OK	POS	29.97
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	Water	OK	POS	33.22

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A

Instrument(s)

Serial Number	**900811**

Run Report

Run Information

Run Name: KC - Thesis - Run 3
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: First Core run (continued) with 2/14 beads 300 uL

Started: 2013/3/18 06:39 PM
Finished: 2013/3/18 07:43 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 7
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	2150-2200 uL	OK	POS	15.72
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	2350-2400 uL	OK	POS	17.47
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	2500-2550 uL	OK	POS	28.34
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	2650-2700 uL	OK	POS	31.90
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	2800-2850 uL	OK	POS	29.98
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	2900-2950 uL	OK	POS	27.57
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	Water	OK	POS	34.05

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A

Instrument(s)

Serial Number	**900811**

Run Report

Run Information

Run Name: KC - Thesis - Run 4
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Started: 2013/3/22 01:57 PM
Finished: 2013/3/22 03:03 PM
SW Version :2.0d
Notes: Fourth Core run (continued) with 2/14 beads 300 uL. Last run before introduce flush water (still may be beads in core however)
Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 8
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	2950-3000 uL	OK	POS	33.45
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	3200-3250 uL	OK	POS	33.16
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	3500-3550 uL	OK	POS	33.24
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	3800-3850 uL	OK	POS	31.91
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	4100-4150 uL	OK	POS	33.25
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	4450-4500 uL	OK	POS	31.23
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	Water	OK	NEG	0.00
A8	BoxvsSchaad_ACC primProbeJan3_04	8	UNKN	Water (not full mix)	OK	POS	33.75

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00
A8	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: KC - Thesis - Run B3
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Started: 2013/4/8 04:06 PM
Finished: 2013/4/8 05:11 PM
SW Version : 2.0d
Notes: Third Core Run on first soil core for the first 1000 uL of sample (Forgot to bring it to the lab with first run)

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 2
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A15	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	0-500 mL	OK	POS	33.67
A16	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	500-900 mL	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A15	NEG	0.00	NEG	0.00	NEG	0.00
A16	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: KC - Thesis - Run B1
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Second Core run with 3/26 beads 250 uL

Started: 2013/4/2 04:45 PM
Finished: 2013/4/2 05:52 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 13
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	900-950 mL	OK	POS	21.97
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	1200-1250 mL	OK	POS	23.66
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	1500-1550 mL	OK	POS	22.69
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	1800-1850 mL	OK	POS	24.15
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	2100-2150 mL	OK	POS	25.11
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	2400-2450 mL	OK	POS	24.29
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	2700-2750 mL	OK	POS	24.55
A8	BoxvsSchaad_ACC primProbeJan3_04	8	UNKN	3050-3100 mL	OK	POS	26.53
A9	BoxvsSchaad_ACC primProbeJan3_04	9	UNKN	3500-3550 mL	OK	POS	25.86
A10	BoxvsSchaad_ACC primProbeJan3_04	10	UNKN	3800-3850 mL	OK	POS	25.13
A11	BoxvsSchaad_ACC primProbeJan3_04	11	UNKN	4050-4100 mL	OK	POS	24.52
A12	BoxvsSchaad_ACC primProbeJan3_04	12	UNKN	Water	OK	NEG	0.00
A13	BoxvsSchaad_ACC primProbeJan3_04	13	UNKN	Millionfold DNA	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00
A8	NEG	0.00	NEG	0.00	NEG	0.00
A9	NEG	0.00	NEG	0.00	NEG	0.00
A10	NEG	0.00	NEG	0.00	NEG	0.00
A11	NEG	0.00	NEG	0.00	NEG	0.00
A12	NEG	0.00	NEG	0.00	NEG	0.00
A13	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: KC - Thesis - Run B2
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Started: 2013/4/4 02:43 PM
Finished: 2013/4/4 03:50 PM
SW Version :2.0d
Notes: Second Core run with 3/26 beads 250 uL. flush water and end of the run to see when do not get any amplification
Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 13
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	4100-4150 mL	OK	POS	29.63
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	4400-4450 mL	OK	POS	32.65
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	4700-4750 mL	OK	POS	30.19
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	5000-5050 mL	OK	POS	26.78
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	5300-5350 mL	OK	POS	32.06
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	5600-5650 mL	OK	POS	32.09
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	5900-5950 mL	OK	POS	29.67
A8	BoxvsSchaad_ACC primProbeJan3_04	8	UNKN	6200-6250 mL	OK	POS	25.09
A9	BoxvsSchaad_ACC primProbeJan3_04	9	UNKN	6500-6550 mL	OK	POS	26.07
A10	BoxvsSchaad_ACC primProbeJan3_04	10	UNKN	6800-7100 mL	OK	POS	29.09
A11	BoxvsSchaad_ACC primProbeJan3_04	11	UNKN	7100-7150 mL	OK	POS	28.38
A12	BoxvsSchaad_ACC primProbeJan3_04	12	UNKN	Water	OK	NEG	0.00
A13	BoxvsSchaad_ACC primProbeJan3_04	13	UNKN	Millionfold DNA	OK	POS	13.56

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00
A8	NEG	0.00	NEG	0.00	NEG	0.00
A9	NEG	0.00	NEG	0.00	NEG	0.00
A10	NEG	0.00	NEG	0.00	NEG	0.00
A11	NEG	0.00	NEG	0.00	NEG	0.00
A12	NEG	0.00	NEG	0.00	NEG	0.00
A13	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: KC - Thesis - Run C1
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: First Core run with 3/26 beads ~100 uL2nd Core

Started: 2013/4/8 02:57 PM
Finished: 2013/4/8 04:03 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 14
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	12	UNKN	flush water	OK	NEG	0.00
A2	BoxvsSchaad_ACC primProbeJan3_04	14	UNKN	50-100 mL	OK	POS	33.04
A3	BoxvsSchaad_ACC primProbeJan3_04	16	UNKN	800-850 mL	OK	POS	27.47
A4	BoxvsSchaad_ACC primProbeJan3_04	17	UNKN	1000-1050 mL	OK	POS	27.14
A5	BoxvsSchaad_ACC primProbeJan3_04	18	UNKN	1200-1250 mL	OK	POS	20.88
A6	BoxvsSchaad_ACC primProbeJan3_04	19	UNKN	1400-1450 mL	OK	POS	19.62
A7	BoxvsSchaad_ACC primProbeJan3_04	21	UNKN	1900-1950 mL	OK	POS	20.28
A8	BoxvsSchaad_ACC primProbeJan3_04	23	UNKN	2400-2450 mL	OK	POS	19.19
A9	BoxvsSchaad_ACC primProbeJan3_04	24	UNKN	2700-2750 mL	OK	POS	21.95
A10	BoxvsSchaad_ACC primProbeJan3_04	26	UNKN	3300-3500 mL	OK	POS	21.67
A11	BoxvsSchaad_ACC primProbeJan3_04	29	UNKN	4200-4250 mL	OK	POS	23.85
A12	BoxvsSchaad_ACC primProbeJan3_04	3B	UNKN	5200-5250 mL	OK	NEG	0.00
A13	BoxvsSchaad_ACC primProbeJan3_04	6B	UNKN	6100-6150 mL	OK	POS	24.53
A14	BoxvsSchaad_ACC primProbeJan3_04	W	UNKN	Water	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00
A8	NEG	0.00	NEG	0.00	NEG	0.00
A9	NEG	0.00	NEG	0.00	NEG	0.00
A10	NEG	0.00	NEG	0.00	NEG	0.00
A11	NEG	0.00	NEG	0.00	NEG	0.00
A12	NEG	0.00	NEG	0.00	NEG	0.00
A13	NEG	0.00	NEG	0.00	NEG	0.00
A14	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: KC - Thesis - Run D1
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Second Core run with 3/26 beads ~100 uL2nd Core

Started: 2013/4/11 03:06 PM
Finished: 2013/4/11 04:13 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 16
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	flush water	OK	NEG	0.00
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	50-100 mL	OK	NEG	0.00
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	800-850 mL	OK	POS	27.88
A4	BoxvsSchaad_ACC primProbeJan3_04	4	UNKN	1000-1050 mL	OK	POS	20.70
A5	BoxvsSchaad_ACC primProbeJan3_04	5	UNKN	1200-1250 mL	OK	POS	20.26
A6	BoxvsSchaad_ACC primProbeJan3_04	6	UNKN	1400-1450 mL	OK	POS	19.17
A7	BoxvsSchaad_ACC primProbeJan3_04	7	UNKN	1900-1950 mL	OK	POS	16.78
A8	BoxvsSchaad_ACC primProbeJan3_04	8	UNKN	2400-2450 mL	OK	POS	17.72
A9	BoxvsSchaad_ACC primProbeJan3_04	9	UNKN	2700-2750 mL	OK	POS	19.27
A10	BoxvsSchaad_ACC primProbeJan3_04	10	UNKN	3300-3500 mL	OK	POS	18.87
A11	BoxvsSchaad_ACC primProbeJan3_04	11	UNKN	4200-4250 mL	OK	POS	28.71
A12	BoxvsSchaad_ACC primProbeJan3_04	12	UNKN	5200-5250 mL	OK	NEG	0.00
A13	BoxvsSchaad_ACC primProbeJan3_04	13	UNKN	6100-6150 mL	OK	POS	33.72
A14	BoxvsSchaad_ACC primProbeJan3_04	14	UNKN	~7000	OK	NEG	0.00
A15	BoxvsSchaad_ACC primProbeJan3_04	15	UNKN	Millionfold	OK	POS	12.91
A16	BoxvsSchaad_ACC primProbeJan3_04	16	UNKN	Water	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00
A4	NEG	0.00	NEG	0.00	NEG	0.00
A5	NEG	0.00	NEG	0.00	NEG	0.00
A6	NEG	0.00	NEG	0.00	NEG	0.00
A7	NEG	0.00	NEG	0.00	NEG	0.00
A8	NEG	0.00	NEG	0.00	NEG	0.00
A9	NEG	0.00	NEG	0.00	NEG	0.00
A10	NEG	0.00	NEG	0.00	NEG	0.00
A11	NEG	0.00	NEG	0.00	NEG	0.00
A12	NEG	0.00	NEG	0.00	NEG	0.00
A13	NEG	0.00	NEG	0.00	NEG	0.00
A14	NEG	0.00	NEG	0.00	NEG	0.00
A15	NEG	0.00	NEG	0.00	NEG	0.00
A16	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A

Instrument(s)

Serial Number	**900811**

APPENDIX D

DEGRADATION RESULTS

Run Report

Run Information

Run Name: Degradation Experiment_Day0
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/3/22 02:23 PM
Finished: 2013/3/22 03:29 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 3
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A9	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Soil Sample	OK	POS	21.39
A10	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water	OK	POS	33.27
A11	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	Water (not full mix)	OK	POS	32.81

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A9	NEG	0.00	NEG	0.00	NEG	0.00
A10	NEG	0.00	NEG	0.00	NEG	0.00
A11	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: Degradation Experiment_Day3_2
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/3/25 06:52 PM
Finished: 2013/3/25 07:58 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 3
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Soil Sample	OK	POS	20.44
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water	OK	NEG	0.00
A3	BoxvsSchaad_ACC primProbeJan3_04	3	UNKN	Soil Sample - more soil in tube	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00
A3	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: Degradation Experiment_Day10
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/4/1 12:20 PM
Finished: 2013/4/1 01:24 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 2
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Soil Sample	OK	POS	21.20
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water (newer)	OK	POS	33.98

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: Degradation Experiment_Day12
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/4/3 09:33 AM
Finished: 2013/4/3 10:38 AM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 2
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A15	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Beads	OK	POS	21.43
A16	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A15	NEG	0.00	NEG	0.00	NEG	0.00
A16	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: Degradation Experiment_Day14
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/4/5 04:21 PM
Finished: 2013/4/5 05:27 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 2
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Soil Sample	OK	POS	20.73
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water (newer)	OK	POS	33.58

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811

Run Report

Run Information

Run Name: Degradation Experiment_Day20
User Name: Default User
Run Status: Done
Dye Set: FCTC25
Notes: Put beads in soil and took a sample

Started: 2013/4/11 04:36 PM
Finished: 2013/4/11 05:42 PM
SW Version :2.0d

Analysis Settings:

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting
1	FAM	Assay	ON	5	40	Primary Curve	Manual
2	Cy3	Assay	ON	5	40	Primary Curve	Manual
3	TxR	Assay	ON	5	40	Primary Curve	Manual
4	Cy5	Assay	ON	5	40	Primary Curve	Manual

Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxcar Avg Cycles	Target
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	
30.0	NA	5	10	3	60	0	

Protocol(s):
Name : BoxvsSchaad_ACCprimProbeJan3_04
Lot Number: None
Stage 1: Hold 50.0ºC for 120 seconds
Stage 2: Hold 95.0ºC for 600 seconds
Stage 3: 2-Temperature Cycle repeat for 35 times.
95.0ºC for 15 seconds
60.0ºC for 60 seconds with Optics ON

Number of Sites: 2
Results Table:

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct
A1	BoxvsSchaad_ACC primProbeJan3_04	1	UNKN	Soil Sample	OK	POS	20.73
A2	BoxvsSchaad_ACC primProbeJan3_04	2	UNKN	Water	OK	NEG	0.00

Site ID	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct
A1	NEG	0.00	NEG	0.00	NEG	0.00
A2	NEG	0.00	NEG	0.00	NEG	0.00

Error(s): None

Instrument(s)

Instrument	A
Serial Number	900811