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A New qPCR Assay to Detect Geosmin-Producing Cyanobacteria

Shane Brian Davis

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

A New qPCR Assay to Detect Geosmin-Producing Cyanobacteria

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Department of Civil and Environmental Engineering, BYU
Master of Science

Taste-and-odor (T&O) compounds are frequently produced by cyanobacterial blooms in bodies of water. Geosmin, perhaps the most common T&O compound produced by these blooms, is not effectively removed by conventional water treatment processes and frequently causes the tap water to have an off flavor. Although geosmin is not harmful when ingested, it damages the consumers' confidence in the cleanliness of their water. There are treatment options for geosmin removal, but the most common methods are often not implemented until complaints are made by consumers.

There has been an increasing amount of research on the use of polymerase chain reaction (PCR)-based methods that can detect the presence of the geosmin synthase gene which is responsible for the production of geosmin. If the geosmin synthase gene is found to be present in an emerging cyanobacterial bloom, water treatment facilities can prepare in advance to treat for geosmin. In this study, we developed a qPCR (quantitative polymerase chain reaction) assay that can detect the presence of the geosmin synthase gene in several species of cyanobacteria within the *Anabaena* genus. We tested our assay, as well as PCR assays designed by Giglio et al. (2008) and Suurnäkki et al. (2015) on extracted *Anabaena flos-aquae* DNA, biosynthesized *Anabaena ucrainica* DNA and DNA extracted from environmental samples of Deer Creek Reservoir, Strawberry Reservoir, and Utah Lake. It is important to note that the geosmin gene was not confirmed to be present in any of the environmental samples nor in the *Anabaena flos-aquae* DNA and our assay did not test positive on these samples. Our qPCR assay was very successful when used with the biosynthesized *Anabaena ucrainica* DNA. We used the results to estimate a DNA standard curve that can be used to estimate the starting concentration of the geosmin synthase gene. Because our assay was not successfully used with any extracted DNA, further testing and calibration may be necessary to produce a DNA standard curve that is representative of DNA that is extracted. Further calibration of the DNA standard curve was not done because there were no geosmin events during the course of the research.

Development of PCR-based methods of detecting geosmin-producing cyanobacteria requires genetic sequencing information of the target-organisms. Thus, further development of PCR-based methods requires that the local geosmin-producers be identified and sequenced. Our assay as well as the assay designed by Moore (2019) can assist with the identification of these species by classifying their genus.

Keywords: geosmin, water quality, Cyanobacteria, PCR, qPCR, taste and odor

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1 INTRODUCTION

Cyanobacterial blooms in drinking water reservoirs often produce secondary metabolites that affect the taste-and-odor of the water even after it has been treated and distributed to consumers. Geosmin and 2-methylisoborneol (MIB) are two of the most common taste-and-odor causing compounds produced by cyanobacteria. Geosmin and MIB have no adverse effects when ingested, however, even very low concentrations can give the water an unpleasant taste. This is a cause of concern for water treatment facilities because consumers judge the safety of their tap water based on its taste and odor and often interpret off-flavored water as unsafe (Nerenberg et al. 2000; Tsao et al. 2014).

The concentration at which humans can detect geosmin has been reported at below 10 ng/L (Tsao et al. 2014). Conventional water treatment processes (coagulation, flocculation, filtration, chlorination) usually do not effectively remove geosmin to below 10 ng/L (Howe et al. 2012; Kim et al. 1997). The most common method of removing geosmin from drinking water is powdered activated carbon (Nerenberg et al. 2000). At a high enough dose, activated carbon can remove all or nearly all geosmin from the drinking water (Drikas et al. 2009). When geosmin is detected in the system, facilities can start dosing the water with activated carbon. The disadvantage of this method is that it is usually used only after geosmin has entered the system and consumers have made complaints. Other methods of geosmin removal involve advanced oxidation such as ozone and hydrogen peroxide treatment. These methods can reliably remove

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geosmin but require the installation of expensive facilities which may not be practical for the treatment of cyanobacterial blooms that only occur periodically.

There are currently no reliable methods that can be used to predict and prepare for cyanobacterial blooms that produce geosmin. Some researchers have attempted to correlate geosmin production to light, temperature, nutrient supply, chlorophyll-a and other variables, but no clear patterns have emerged from these studies (Watson et al. 2016). Monitoring techniques often involve obtaining grab-samples near the drinking water intake and taking the samples to a lab to be tested for tase-and-odor (T&O) compounds. If a test result is positive for geosmin or another T&O compound, that usually means that it has already entered the system.

Polymerase Chain Reaction (PCR)-based methods of detection have been given an increasing amount of attention in recent years. PCR is a DNA test that is used to detect the presence or absence of a specific segment of DNA. Quantitative polymerase chain reaction (qPCR) is a variation of PCR that can be used to quantify the presence of a segment of DNA. After the discovery of the geosmin synthase gene, which is the gene responsible for the production of geosmin, researchers have started developing PCR-based methods to detect and/or quantify the presence of the geosmin synthase gene in drinking water sources. Theoretically, these methods can be used to classify a cyanobacterial bloom in its early stages and predict which secondary metabolites will be produced. Water treatment facilities can then prepare in advance to treat the water accordingly.

Currently, there is limited knowledge about which species of cyanobacteria are responsible for geosmin events in Utah's drinking water reservoirs. While there are several PCR assays designed to detect the presence or absence of the geosmin synthase gene in a wide variety of species of cyanobacteria, these tests cannot identify which species are present. Identification

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of the species responsible for geosmin production in local reservoirs is essential in the design of qPCR assays that can target these organisms.

Moore (2019), in his research, developed a qPCR assay targeting the geosmin synthase gene of several species of *Nostoc*, which is a potential geosmin producer in Deer Creek Reservoir in Utah. Assays such as his can be used during a cyanobacterial bloom to test whether a *Nostoc* species is present. If several similar qPCR assays targeting a wide variety of cyanobacteria are developed, this can help determine which species are responsible for geosmin production in local reservoirs.

In this study, we designed a qPCR assay that can target the geosmin synthase gene in several cyanobacterial species within the genus *Anabaena* that have been found in Deer Creek Reservoir. We tested this assay on several samples of DNA extracted from environmental samples, a sample of biosynthesized *Anabaena ucrainica* CHAB2155 DNA, and DNA extracted from cultured *Anabaena flos-aquae* UTEX 1444. We also tested the PCR primers designed by Giglio et al. (2008) and by Suurnäkki et al. (2015) on the same DNA samples.

Our assay worked very well for the biosynthesized *Anabaena ucrainica* CHAB2155 DNA but had negative results for most of the environmental samples and the *Anabaena flos-aquae* UTEX 1444 DNA. The *Anabaena flos-aquae* culture that was used was not confirmed to be a geosmin producer, and therefore, may not possess the geosmin synthase gene. The results for the environmental samples suggest that either there was no geosmin-producing *Anabaena* species in the samples, or that it was at a concentration that was below the limit of detection. The PCR primers designed by Giglio et al. (2008) and by Suurnäkki et al. (2015) also had negative results on the environmental samples and the *Anabaena flos-aquae* DNA, but positive results for the *Anabaena ucrainica* CHAB2155 DNA.

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The qPCR assay we designed can be used along with Moore's assay to help identify local geosmin producing cyanobacteria. The hope is that more assays targeting a wider variety of geosmin producers will be designed so that when another geosmin event occurs in a reservoir in Utah, we can use these assays to identify the species that are responsible. This will enable further development of PCR-based methods that are specially designed to target local geosmin producers and forewarn water treatment facilities so that they can prepare. This same method can be expanded to detect other T&O compounds and toxins that result from these blooms.

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2 BACKGROUND

2.1 Geosmin in Drinking Water Sources

Geosmin is an organic compound that gives water an earthy, muddy smell and flavor that is not pleasant to drink. Although there are no known detrimental effects from geosmin intake, consumers often mistake the off-flavored water as unsafe and improperly treated. Geosmin can be detected by consumers at very low concentrations. Zamyadi reports that geosmin can be smelled or tasted by consumers at concentrations of 10 ng/L (Zamyadi et al. 2015). Geosmin concentrations between 7 and 12 ng/L in drinking water have resulted in consumer complaints to water treatment facilities (Howe et al. 2012). Adding to the taste and odor problem, conventional water treatment methods do not effectively remove geosmin from source water, often resulting in complaints from consumers (Kim et al. 1997).

No dependable methods exist that can effectively predict blooms of cyanobacteria that produce T&O compounds before they begin. Water treatment plants begin treating the T&O compounds only after they have been detected in the drinking water system or consumers have filed complaints. This approach is retroactive and not preventative.

Microscopy is often used to detect the presence of the T&O-producing cyanobacteria. This process requires specialized personnel to identify cyanobacteria through a microscope making this method expensive and time consuming. Microscopy can sometimes be impractical because cyanobacterial blooms can occur in a matter of days under the right conditions (Tsao et al. 2014).

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Another disadvantage to microscopy is that it can't predict whether a strain of cyanobacteria will produce a secondary metabolite such as geosmin (Al-Tebrineh et al. 2012). Within the same species, there can be both geosmin producers and non-producers. A time-sensitive and reliable method to predict or identify a cyanobacterial bloom is necessary so that water treatment facilities can prepare in advance to treat for T&O compounds.

One factor that makes geosmin difficult to monitor is that there are very few methods that can measure the concentration in water. Perhaps the most common method, gas chromatography-mass spectrometry (GC-MS), is a test that can accurately measure the concentration of geosmin but requires experienced personnel and expensive equipment. There are currently no quick, inexpensive methods to continually monitor geosmin.

One study suggests that cyanobacterial blooms appear to be increasing in frequency, duration, and magnitude around the globe. This increase is due to eutrophication that is accelerated by human activity (Otten et al. 2016). It is becoming increasingly important to develop methods to characterize cyanobacterial blooms and the secondary metabolites that they will produce.

2.2 Current Methods of Geosmin Removal

There are currently several methods of removing geosmin from drinking water. Some of these methods include advanced oxidation, biodegradation and activated carbon. When designed properly, these treatment methods can remove all, or nearly all the geosmin present. These methods, however, are either very expensive to install, or are retroactive, meaning that they treat for geosmin only after it is in the distribution system and complaints have been made by consumers.

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Ozone treatment can very effectively remove geosmin by oxidizing it (Tchobanoglous et al. 2013). At a high enough ozone dosage, geosmin can be completely oxidized (Nerenberg et al. 2000). The disadvantage of ozone treatment is that it requires a large capital investment. Because taste-and-odor events only happen on occasion, it may not be practical to install the facilities required for ozone treatment for the sole purpose of removing occasional taste-and-odor compounds.

One method that has been the subject of several research articles is the use of biodegradation to remove geosmin. Ho et al. (2007) found that geosmin could be biodegraded using an aged sand filter and a bioreactor. They were able to remove in excess of 90% of the initial geosmin in a bench-scale analysis (Ho et al. 2007). Zhou et al. (2011), in a series of experiments using biodegradation, removed between 74% and 85% of the initial geosmin when the starting concentrations were 560 ng/L and 2000 ng/L. Doederer et al. (2019) demonstrated that geosmin could also be removed using a moving-bed bioreactor (MBBR). They concluded that the dominant mechanism for removing geosmin in an MBBR is biodegradation. Using an MBBR, they achieved removal rates exceeding 80% (Doederer et al. 2019). While these methods can be effective for geosmin removal under the right conditions, in practice they are not widely used for that purpose.

Activated carbon is the most common method of dealing with geosmin events (Nerenberg et al. 2000). One reason that activated carbon is a common solution to taste-and-odor events is that it requires a much smaller initial investment than other treatment options. Treatment plants can easily retrofit their facilities with the equipment necessary to dose the water with activated carbon when taste-and-odor events occur. Activated carbon removes geosmin by both adsorption and biodegradation. Drikas et al. (2009) experimented with geosmin removal using granular

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activated carbon (GAC) filters. They found that GAC filters that were run with pre-chlorinated water, and therefore lower bacterial cell content, had significantly lower geosmin removal, suggesting that biodegradation contributed to geosmin removal. They also found that by increasing the energy bed contact time (EBCT) of the GAC filters run with pre-chlorinated water, they could account for the lower geosmin removal. They concluded that both adsorption and biodegradation are significant in geosmin/MIB removal in GAC filters (Drikas et al. 2009).

One method of dealing with cyanobacterial blooms involves dosing the water with chemicals. The chemicals that are used to control algal blooms, such as copper sulfate, are often environmentally unfriendly (Giglio et al. 2008). This method is not very common due to its harmful nature.

2.3 Geosmin Production in Cyanobacteria

Geosmin was first identified in actinomycetes 1965 by Gerber and Lechevalier (1965) who characterized geosmin as a neutral oil that consists of hydrogen and carbon, but no nitrogen. It wasn't until 1981 that Bentley and Meganathan (1981) made the first proposition for the biosynthetic pathway of geosmin. They suggested that geosmin is likely derived from a sesquiterpenoid precursor produced via the mevalonate (MV) pathway (see Figure 2.1), based on their studies on *Streptomyces antibioticus* (Bentley and Meganathan 1981). Another pathway, known as the MEP (2-C-methyl-D-erythritol 4-phosphate) pathway was found to be the most likely means by which geosmin production takes place in cyanobacteria (Watson et al. 2016).

There are currently studies that seem to conflict regarding the exact processes that cyanobacteria use to produce geosmin. Wang and Li (2015) found that geosmin production and chlorophyll production in cyanobacteria have an inverse relationship. Farnesyl pyrophosphate

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(FPP) is the intermediate precursor in chlorophyll synthesis and is also an immediate precursor to geosmin synthesis. Wang and Lai concluded that the inverse relationship between geosmin production and chlorophyll production may be due to the competition for FPP. In ideal conditions, these FPP is used for synthesis of chlorophyll. However, when conditions become unfavorable and cell growth is inhibited, geosmin synthesis increases. Therefore, production of geosmin may reflect decreased metabolic activity (Wang and Li 2015). Other studies suggest that geosmin is produced in the growth phase and released in the death phase (Kim et al. 2018; Zamyadi et al. 2015). There is consensus, however, that regardless of when the geosmin is produced, when the cells are damaged, the geosmin is released into its surroundings.

Using *Nostoc punctiforme* PCC 73102 (ATCC 29133) as a model cyanobacterium, Giglio et al. (2008) showed that a single enzyme is used to produce geosmin. They also sequenced the gene that was responsible for the production of the enzyme (Giglio et al. 2008). This gene will be referred to the geosmin synthase gene in this paper. This finding led to the beginning of the use of DNA-based methods of detecting geosmin-producing cyanobacteria. In this paper, the DNA based methods of detection that we focus on are PCR and qPCR.

2.4 PCR Background

PCR-based methods of detection are sensitive, inexpensive and are capable of quickly detecting the presence of even a small amount of a gene, like the geosmin synthase gene (John et al. 2018). PCR is a process that can make millions of copies of a targeted segment of a DNA sequence without copying any other DNA. When designed properly, it is possible to detect the presence of very small amounts of the targeted gene. The major "ingredients" of PCR are shown in Table 2.1.

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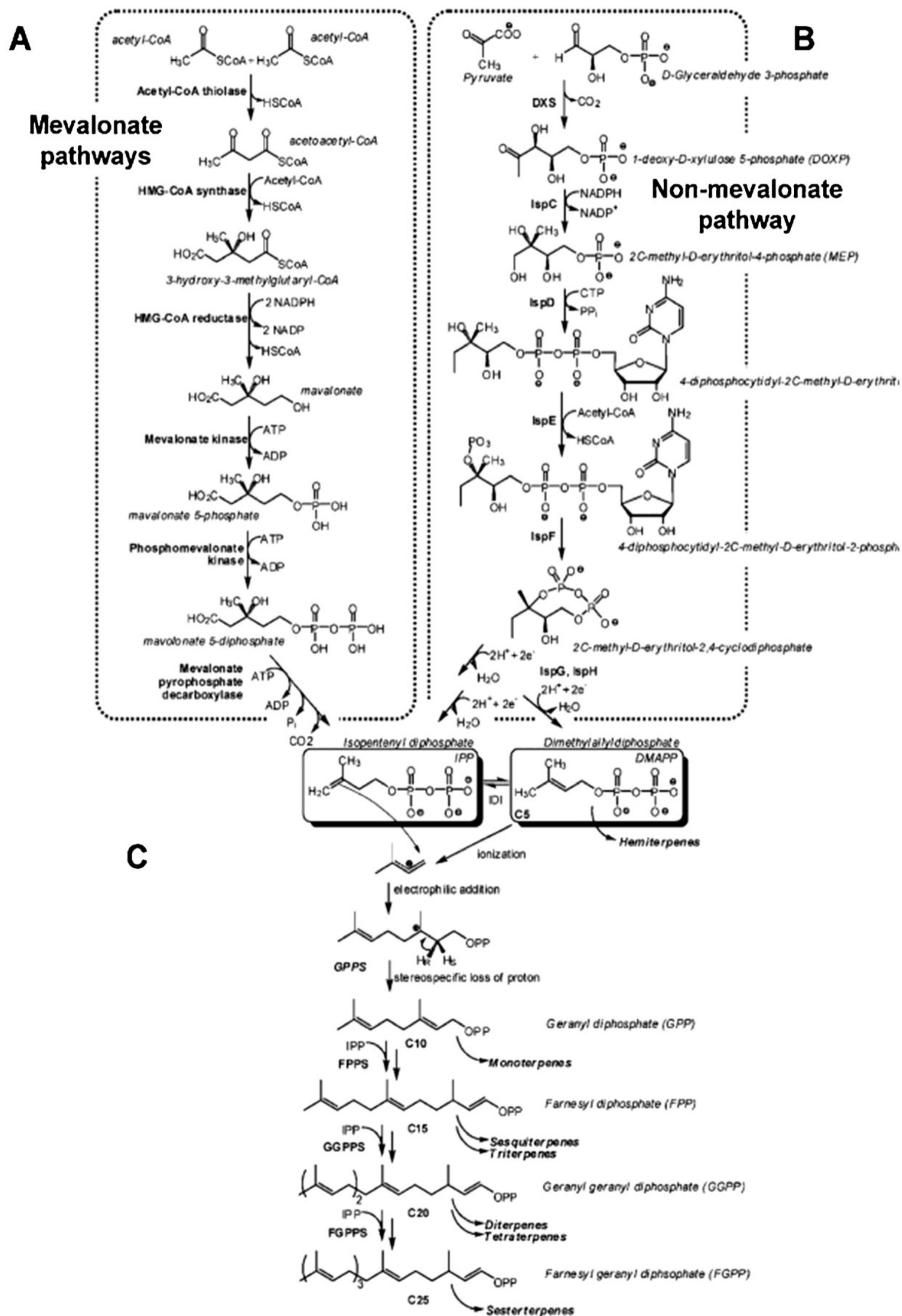

Figure 2.1: Pathways of geosmin production, from Watson et al. (2016)

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Table 2.1: The major constituents of PCR

Constituent	Description
DNA template	DNA sample that includes the targeted sequence
Primers	Short segments of synthesized DNA that are designed to bind to the beginning and end of the DNA template
DNA nucleotide bases (dNTPs)	The A's, C's, G's and T's which are the building blocks of DNA
Taq polymerase enzyme	An enzyme that puts the dNTPs together
Buffer solution	To ensure proper suspension and mixing

The PCR process consists of three major steps shown in Figure 2.2. (1) Denaturing stage: the mixture of all the ingredients listed in Table 2.1 are heated up to 94-95°C and the double stranded DNA is split into two single strands. (2) Annealing stage: the DNA is cooled to 50-65°C and the primers attach themselves to their complementary regions. (3) Extending stage: the DNA is heated up to 72°C and the Taq polymerase enzyme uses the dNTPs to make a new strand of DNA. These stages are repeated 20-40 times and the result is thousands to millions of copies of the target region of DNA (YourGenome 2016). The resulting quantity of copies of the target region of DNA depends on how well the primers are designed and the starting quantity of DNA.

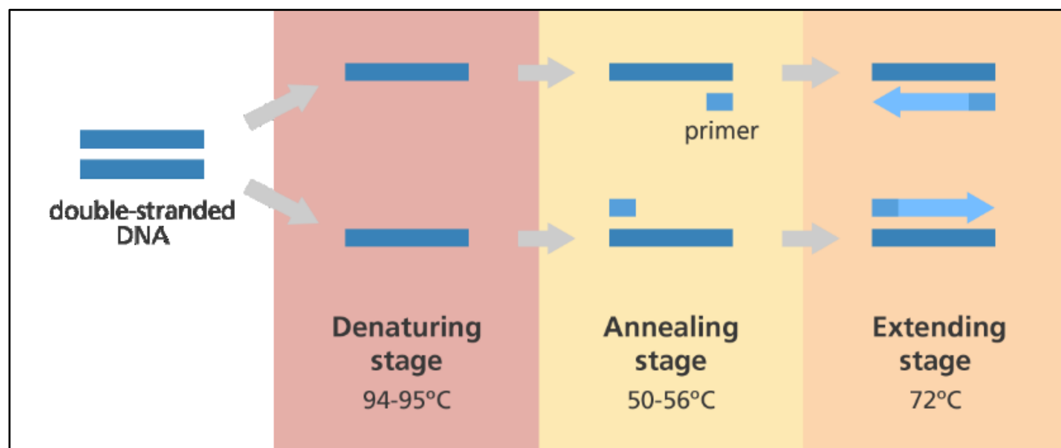

Figure 2.2. The denaturing and annealing stages are repeated 20-40 times, followed by the extending phase (YourGenome 2016).

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qPCR, also commonly called real-time PCR enables the quantification of the gene in real time. In addition to the constituents of PCR, qPCR also uses probes and/or dyes. While basic PCR can only detect the presence or absence of a gene, qPCR can also be used to measure the amplification of the DNA template and estimate the original quantity. In dye-based qPCR, a dye that fluoresces upon amplification is used to measure how much amplification occurs in each cycle. The most common dye used for this type of qPCR is called SYBR Green. The disadvantage of dye-based qPCR is that the dye is nonspecific and binds to all DNA, not just the target region. Therefore, any undesired amplification such as primer-dimers, self-dimers, or any other secondary structures will also be detected (Sciences 2017). Probe-based qPCR technology includes a probe that is designed bind only to its specific complementary. A probe is a synthesized single strand of DNA that is usually 24-30 bp (base pairs) long and has intercalating dyes that fluoresce upon amplification and then are subsequently quenched. It is designed to attach to a segment of the DNA template between the primers and fluoresce when that segment is amplified. This method is more specific and will measure only the amplification of the target region of DNA without measuring the amplification of secondary structures. The disadvantage of probe based qPCR is that both the primers and probes need to be designed and tested, requiring more time and money than dye-based qPCR (Sciences 2017).

2.5 The Use of PCR Technology

Both PCR and qPCR have been increasingly used to detect cyanobacteria that produce secondary metabolites such as toxins and taste-and-odor compounds. These methods have been given a lot of attention because they can target the specific genes that are responsible for the production of those metabolites. There are efforts being made to develop PCR technology that

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can be used with a cyanobacterial bloom in its early stages to predict which secondary metabolites will be produced, thus enabling water treatment facilities to prepare accordingly.

Nübel et al. (1997) used PCR to amplify 16S rRNA genes from cyanobacteria. This is useful when the goal is to detect the presence or absence of any species of cyanobacteria. PCR can also be used to amplify a common gene that is conserved between species. In another study, Rasmussen et al. (2008) designed a real-time PCR assay that targeted the genes that are responsible for the production of the toxin cylindrospermopsin. They used their assay on *Cylindrospermopsis raciborskii*. The assay produced positive results for strains that produced the toxin and negative results for non-producing strains (Rasmussen et al. 2008).

qPCR and PCR assays have been developed by researchers for various geosmin producers as well. Su et al. (2013) used qPCR to detect *Anabaena, Cylindrospermopsis,* and *Microcystis* species which are known geosmin producers in China and Australia. Kutovaya and Watson (2014) designed a PCR assay that was successful in amplifying the geosmin synthase gene in *Anabaena Planktonica, Anabaena Ucrainica, Anabaena Lemmermanni, Anabaena flos-aquae, anabaena variabilis, aphanizomenon gracile, colothrix* sp.*, geitlerinema splendida, lyngbya wollei, phormidium, planktothrix* (Kutovaya and Watson 2014). Suurnäkki et al. (2015) designed two PCR assays that were successful with an *aphanizomenon* species*, cylindrospermum stagnale,* several species of *Planktothix, Oscillatoria,* and *Nostoc* (Suurnäkki et al. 2015). The primers designed by Giglio et al. (2008) are perhaps the most widely referenced. Their primers were successful with several *Phormidium* species, and A*nabaena Circinalis.*

Tsao et al. (2014) developed a qPCR assay targeting several geosmin producers and successfully used the assay to measure the starting quantity of the cyanobacteria. Their qPCR assay was successful with *Anabaena aphanizomeniodes*, *Anabaena bergii*, *A. circinalis*,

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Anabaena galeata, *Anabaena oscilarioides*, *Anabaena solitaria*, *Cylindropsermopsis raciborskii*, *Microcystis aeruginosa*, *Nodularia spumigena* and *Trichodesmium sp*. *M. aeruginosa* TWNCKU11, *Pseudanabaena sp*, TWNCKU12, *M. aeruginosa* PCC7820, (Tsao et al. 2014).

Moore (2019) developed a qPCR assay that targets the geosmin synthase gene in *Nostoc Punctiforme*, which is a well-known geosmin producer. Moore obtained the gene sequence data from GenBank and used that information to design qPCR primers that target several strains of geosmin-producing *Nostoc Punctiforme* (Moore 2019).

2.6 qPCR Assays Targeting Local Geosmin-Producers

Periodic blooms of geosmin-producing cyanobacteria are common in drinking water reservoirs in Utah. qPCR assays designed to target the geosmin-producing species of cyanobacteria that are native to Utah would be beneficial in the monitoring of these blooms. Deer Creek Reservoir has periodical cyanobacterial blooms (DEQ 2018). A variety of potential geosmin-producing cyanobacteria have been discovered in Deer Creek Reservoir through microscopy of samples taken from 2011-2017 (Cram 2017). However, even though a species has been identified as a geosmin-producer in one study, this does not mean that all strains of that species will produce geosmin. Table 2.2 shows the names of the species of potential geosmin-producing cyanobacteria that were found in the samples that were studied by microscopy. Table 2.2 also shows the availability of genetic sequencing information for each species. This information is necessary for the design of primers and probes. Even with the microscopy data, there is little understanding about which species of cyanobacteria are responsible for the periodic geosmin events in Deer Creek Reservoir. Identifying the species that are responsible for geosmin

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events is one of the first steps needed to enable the creation of PCR and qPCR assays designed to target these species.

GenBank has very limited information for the species listed in Table 2.2. Genetic information for the geosmin-producing cyanobacteria native to Deer Creek Reservoir and other drinking water reservoirs in Utah is necessary for the design qPCR primers that target these species. In this study, we focused on developing a qPCR assay that can target the geosmin synthase gene in several *Anabaena* species. We selected *Anabaena* because the geosmin synthase gene for several species of *Anabaena* can be found on GenBank. Also, *Anabaena crassa, Anabaena flos-aquae,* and *Anabaena circinalis* have all been identified in Deer Creek Reservoir. In other studies, these species were found to produce geosmin (Watson et al. 2016). The aim of designing a qPCR assay to target geosmin-producing *Anabaena* species is to help identify which species are responsible for geosmin production in Deer Creek Reservoir. As more similar assays are developed that target other geosmin-producers, we hope that once another geosmin event occurs, we can use these assays to help identify the geosmin-producers so that we can better predict whether an early-stage cyanobacterial bloom will produce geosmin.

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Table 2.2: Species of potential geosmin-producing cyanobacteria found in Deer Creek Reservoir through microscopy by the Central Utah Water Conservancy District.

Name	Alternate Name (Watson et al. 2016)	Available on GenBank?
Oscillatoria agardhii	*Planktothrix agardhii*	No
Anabaena flos-aquae	*Dolichospermum flos-aquae*	No
Aphanizomenon flos-aquae		Yes
Anabaena crassa	*Dolichospermum crassum*	Yes
Anabaena circinalis	*Dolichospermum circinale*	Yes
Gloeotrichia echinulata		No
Nostoc sp.		Yes

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3 MATERIALS AND METHODS

3.1 DNA Extraction

We performed all DNA extractions using the Qiagen QIAmp Mini Kit. The steps we used are found in Appendix A. The DNA we extracted was from *Anabaena flos-aquae* UTEX 1444, and several samples from Deer Creek Reservoir, Strawberry Reservoir, and Utah Lake. DNA from all of the environmental samples were extracted previously by Moore (2019). After extracting the DNA from the samples, we measured the concentration of DNA in each using NanoDrop® ND-1000 spectrophotometer. The resulting concentrations are shown in Table 3.1.

Table 3.1: Concentrations of DNA after DNA extraction

DNA Sample	Concentration (ng/µL)
Deer Creek 10/14/2017	20.3
Strawberry	43.6
Utah Lake (# 1, 2, and 3)	5.8
Deer Creek Upper 10/25/2017	18.5
Deer Creek Mid-Upper 10/25/2017	98
Anabaena flos-aquae UTEX 1444 - #1	5.7
Anabaena flos-aquae UTEX 1444 - #2	8.9

3.2 Biosynthesized DNA

The culture of *Anabaena flos-aquae* UTEX 1444 that was used in Section 3.1 was not confirmed to be a geosmin-producing strain. Therefore, we needed a sample of DNA that would

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undoubtedly contain the geosmin synthase gene. We used biosynthesized *Anabaena ucrainica* CHAB2155 DNA from Integrated DNA Technologies. We obtained the genetic information for *Anabaena ucrainica* CHAB2155 geosmin synthesis operon from NCBI GenBank. We then ordered a Custom Gene Synthesis with a 997-bp long segment that encompassed the majority geosmin synthase gene from Integrated DNA Technology. The sequence that we used is shown in Appendix B.

3.3 Primer and Probe Design

We designed primers and probes to target a segment of the geosmin synthase gene that is completely conserved among several species of *Anabaena*. We obtained genetic sequence information on the gene from NCBI GenBank. Genetic sequence information available for the geosmin synthase gene in all *Anabaena* and *Dolichospermum* species. Names of all the species whose genetic information was used in the design of the primers and probes are shown in Table 3.2. Note that *Anabaena* and *Dolichospermum* are alternate names for the same genus and can be used interchangeably (Watson et al. 2016).

We then aligned all the sequence information using a free program called the CLC Viewer 8 by Qiagen. This sequence alignment was used in designing the primers and probes. The geosmin synthase gene was very well conserved between species. A perfectly conserved region of the gene was used for the design of the primers and probes.

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Table 3.2: Sequence information for *Anabaena* species obtained from NCBI GenBank to design the qPCR primers and probes

Sequence Name
Dolichospermum planctonicum DGUC012
Dolichospermum planctonicum DGUC006
Anabaena ucrainica CHAB2155
Anabaena ucrainica CHAB1432
Dolichospermum ucrainicum UOM24
Dolichospermum ucrainicum UOM31
Dolichospermum ucrainicum UOM25
Dolichospermum ucrainicum UOM59
Dolichospermum planctonicum DGUC012
Dolichospermum planctonicum DGUC006
Dolichospermum circinale CHAB3585
Dolichospermum ucrainicum UOM67
Anabaena planctonica SDZ-1

The following criteria were followed to design the primers:

- Primer melting temperature (T_m) that is at least 7-10°C less than that of the probe
- No hairpin T_m within 5-10°C of the primer T_m
- Amplicon length of 80 to 175 bp (base pairs)
- No more than 2 Cs or Gs in the last 5 bp
- No runs of more than 4 identical bp
- No self-dimer Delta G below -7 kcal/mol
- Primer Length: 18-24 bp
- Probe Length: 24-30 bp
- GC Content: 30-80%

All or most of these criteria were followed in both the primer and probe design. The actual design process was automated using an online tool called PrimerQuest by Integrated DNA Technologies. The resulting primers and probes were analyzed using the OligoAnalyzer tool,

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also by Integrated DNA Technologies. The primers and probe are shown in Table 3.3. The resulting assay, referred to as An_Geo 1, has an amplicon length of 84 bp.

It is fairly common for a qPCR assay to not perform well, even though it was designed according to the criteria listed above. This can occur due to factors that are not accounted for by the aforementioned design criteria. Therefore, we designed several assays in case one didn't function as expected. We also tested the primers first before ordering the complementary probe, because primers are significantly cheaper than probes. We did not want to purchase the probes if the primers did not perform well. Fortunately, the first primer/probe pair functioned properly.

Table 3.3: The primers and probe for the An_Geo 1 PCR assay that we developed

Type	Sequence	T_m (°C)	Self-Dimer Delta G (kcal/mol)	Hairpin T_m (°C)	GC Content
Forward Primer	CTGGAAATCTATAAGCG	51	-3.61	4.6	41
Probe	AAAGGAGTATCTTGACCGACTACCG	65	-3.61	32	48
Reverse Primer	GGGATAAATTGGCATAA	51	-5.36	-2.7	35

3.4 PCR

In this study, we used PCR assays that we designed, as well as assays that were designed by Giglio et al. (2008) and Suurnäkki et al. (2015) on DNA extracted from environmental water samples, biosynthesized *Anabaena ucrainica* CHAB2155 DNA, and DNA extracted from *Anabaena flos-aquae* UTEX 1444. The primers designed by Giglio et al. (2008) and Suurnäkki et al. (2015) target the geoA gene in a wide variety of cyanobacterial species. The primers we designed target the geoA gene in only the *Anabaena* species listed in Table 3.2.

The typical amounts of each constituent of PCR that we used were 2 µL primer working stock, 13 µL AmpliTaq Gold™ 360 Master Mix, 3 µL template DNA, and 7 µL ddH_2O. We mixed all reactions underneath an AirClean® 600 PCR Workstation DNase/RNase free molecular

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hood to prevent contamination. All PCR reactions were facilitated on 8-well 0.2 mL reaction tubes in a ProFlex PCR System thermal cycler by Applied Biosystems. The reactions started with a 3-minute denaturing phase at 95°C, followed by 35 cycles of the following: 30-second denaturing at 95°C, then a 30-second annealing phase at the annealing temperature specific to the primer, then a 1-minute extension period at 72°C. A 7-minute extension period was used after the 35th cycle. The annealing temperatures used for each primer are shown in Table 3.4.

Table 3.4: PCR primers and the corresponding annealing temperatures

Primers	Annealing Temperature (°C)
Giglio 250F-971R	55
Suurnäkki 78F-971R	55
Suurnäkki 78F-982R	55
An_Geo 1	52

3.5 Gel Electrophoresis

After the PCR process was complete, we used gel electrophoresis to determine if the targeted segment of DNA was successfully amplified. We used 1% agarose gel in our gel electrophoresis. 1% agarose gel is made by mixing 100 mL 1x TAE buffer, 1 g of agarose powder, and 10 µL 1% ethidium bromide. The mixture is heated until the agarose powder is completely dissolved. We then poured 25-50µL of the mixture into the gel electrophoresis unit and let it solidify completely. We put 5 µL GeneRuler #SM0333 DNA Ladder Mix from ThermoFisher Scientific into the first and the last of the 10 wells. We then mixed 2 µL of loading dye with 5 µL of each of the PCR products; 5 µL of this mixture was then loaded into each of the 8 remaining wells. The electrophoresis unit was run at 100 volts for about 45 minutes.

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The gels were then analyzed using an Amersham Imager 600 fluorescent imager. The bands shown in the resulting image show whether amplification occurred and the length of the amplified product. If no bands show, that means amplification did not occur; if bands show at the wrong locations, that means that amplification occurred due to secondary structure formations. The formation of bands that correspond to the correct length mean that the PCR process was a success. Figure 3.1 shows a key for correlating band location with the length of the amplified product.

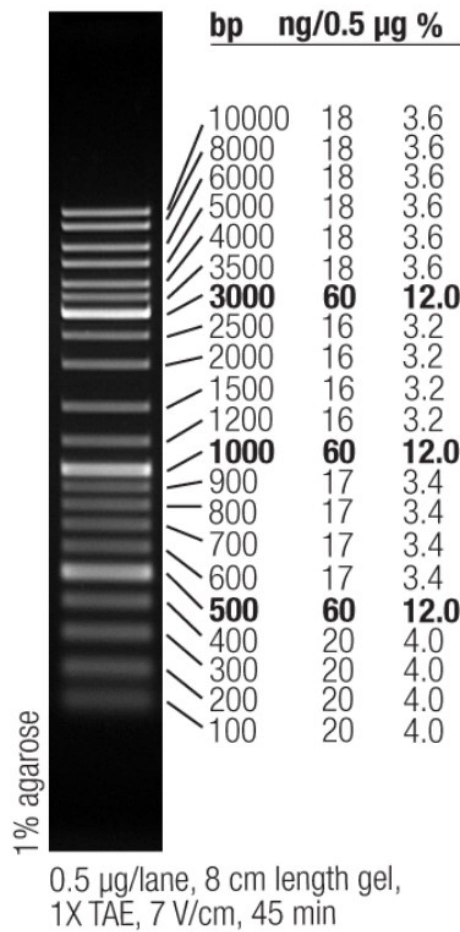

Figure 3.1: Key for GeneRuler Ladder Mix #SM0333

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3.6 qPCR

After we found that the primer pair from An_Geo 1 worked well, we then started doing qPCR using the primers and the probe. Typical quantities of the constituents in qPCR are 10 µL of 250 nm PrimeTime® Gene Expression Master Mix from Integrated DNA Technologies, 4.5 µL Real Time PCR Grade Water, 3 µL of DNA template, 1.5 µL primer working stock, and 1 µL of probe working stock (250 nm PrimeTime® 5' 6-FAM/ZEN/3' IB®FQ) from Integrated DNA Technologies in each reaction well. The constituents were placed in MicroAmp™ EnduraPlate™ Optical 96-Well Fast Clear Reaction Plates by ThermoFisher. All reactions were prepared underneath an AirClean® 600 PCR Workstation DNase/RNase free molecular hood to prevent contamination. The reactions were run on the QuantStudio5 by ThermoFisher. The qPCR begins with one cycle of 50°C for 2 minutes, then 95°C for 10 minutes, and then 40 cycles of 95°C for 15 seconds, and then the annealing temperature for 1 minute. We found the optimized annealing temperature to be 52°C.

3.7 DNA Standard Curve and Limit of Detection

The main result that the qPCR test produces is the C_T value which represents the number of cycles necessary before the readings rise above the background-noise level. During each cycle in the qPCR test, the machine measures the amount of fluorescence that is generated by the probes. Even though the probes are designed to fluoresce only upon amplification of the DNA template, there is some level of background fluorescence that is read by the machine. When the readings of fluorescence, or the ▲RN value, exceed the background noise, the background threshold is exceeded. The cycle at which the threshold is exceeded is called the C_T value. When a particular qPCR run contains more of the DNA template, it will produce a lower C_T value.

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Therefore, the C_T value can be correlated with a starting concentration of DNA template. This correlation is called the DNA standard curve.

We estimated the DNA standard curve by making dilutions of the biosynthesized *Anabaena ucrainica* CHAB2155 DNA and running the qPCR assay with the optimized annealing temperature of 52°C. In the same run, we used water as a negative control and plotted the C_T values vs the concentration.

To make dilutions of the original DNA sample. We mixed 5 μL of the DNA sample (10 ng/μL) with 45 μL of 1xTE buffer to make 50 μL of DNA sample with a concentration of 1 ng/μL. We then mixed 5 μL of the 1 ng/μL sample with 45 μL 1xTE buffer to make 50 μL of a sample with a concentration of 0.1 ng/μL. We repeated this process 10 times until we reached a DNA concentration 10^{-9} ng/μL. In one well, as a negative control, we replaced 3 μL of DNA sample with 3 μL of RT-PCR Grade Water by ThermoFisher®.

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4 RESULTS

4.1 PCR Results

The PCR primers we designed worked very well with the biosynthesized *Anabaena ucrainica* CHAB2155 DNA. The first well shown in Figure 4.1 shows a band that is well defined that is about 80 bp long. The first well is the only well with the *Anabaena ucrainica* DNA sample in it. The next 7 wells in Figure 4.1 contain the following DNA samples respectively: Upper Deer Creek Reservoir, Deer Creek Reservoir, Mid-Upper Deer Creek Reservoir, *Anabaena flos-aquae* UTEX1444 sample #1, *Anabaena flos-aquae* UTEX1444 sample #2, and water as a negative control. We also tested the primers designed by Giglio et al. (2008) and Suurnäkki et al. (2015) on all of the same DNA samples. The results for all the PCR tests are shown in Table 4.1. The only DNA sample that had positive results with any of the primers used was the biosynthesized *Anabaena ucrainica* CHAB2155 DNA. None of the environmental DNA samples had amplification. This is potentially due to one of several reasons. (1) It is possible that the geosmin synthase gene was not present in the samples that produced negative results, (2) the gene may be present, but not in a high enough concentration to provide positive results, or (3) the geosmin synthase gene may be present in other organisms that the primers were not designed to target.

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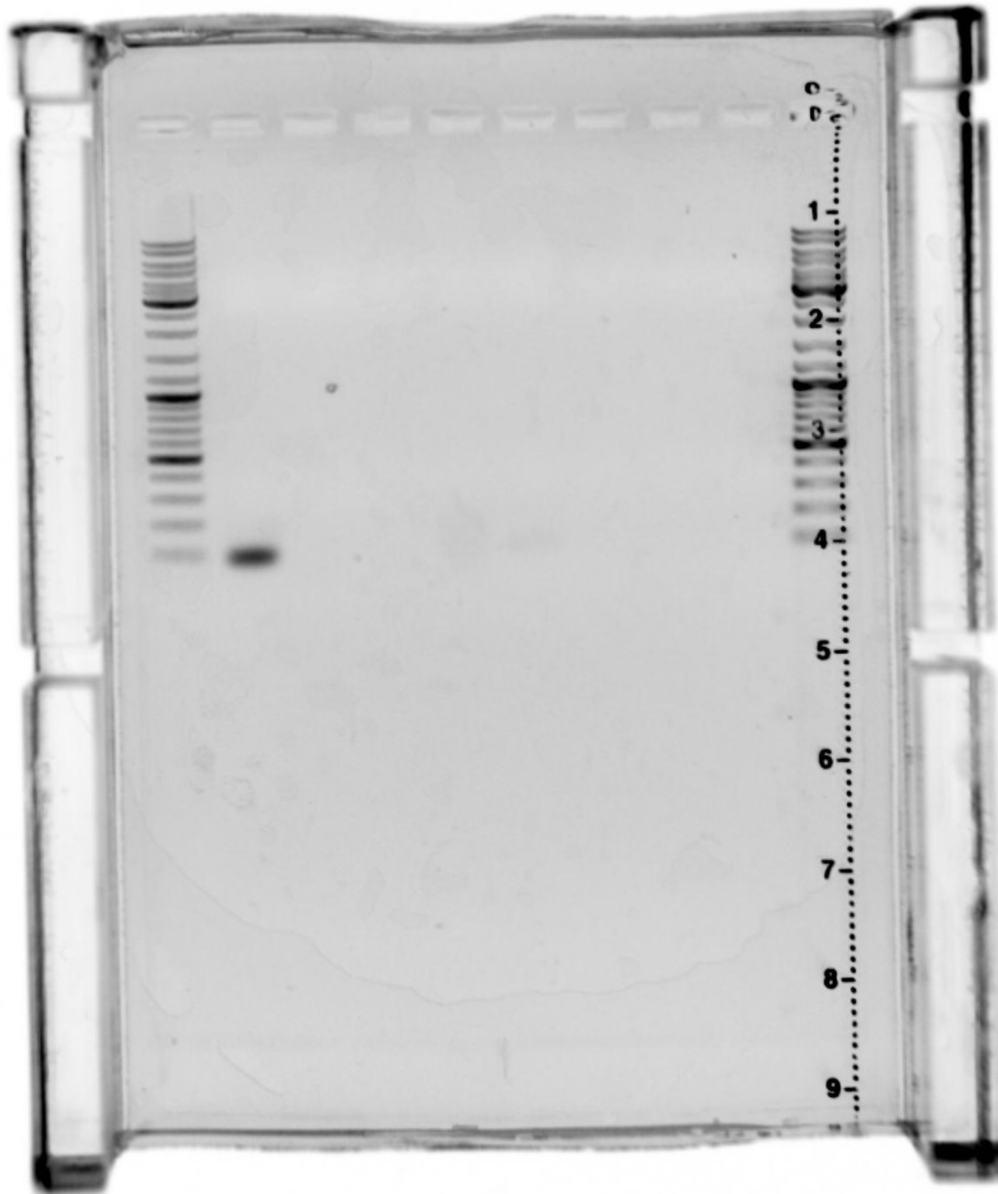

Figure 4.1: The first well shows a clearly defined band with a product length of about 80 bp

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Table 4.1: PCR results. P denotes positive and N denotes negative. The positive results are shaded green.

DNA Sample	Giglio 250F-971R	Suurnäkki 78F-971R	Suurnäkki 78F-982R	An_Geo 1
Anabaena flos-aquae	N	N	N	N
Anabaena Ucrainica CHAB2155 (biosynthesized)	N	P	P	P
Deer Creek	N	N	N	N
Upper Deer Creek	N	N	N	N
Mid-Upper Deer Creek	N	N	N	N
Strawberry Reservoir	N	N	N	N
Utah Lake Sample #1	N	N	N	N
Utah Lake Sample #2	N	N	N	N
Utah Lake Sample #3	N	N	N	N

4.2 qPCR Results

In this study, we designed a qPCR assay that targets a region of the geoA gene that is conserved among various *Anabaena* species. In the first qPCR run, we wanted to verify that the An_Geo 1 primer and probe combination worked properly. We ran it with five wells that each had 3 µL of *Anabaena ucrainica* CHAB2155 DNA with a DNA concentration of 10 ng/µL. The amplification plot is shown in Figure 4.2 The C_T values for each sample are shown Table 4.2. There was positive amplification in each of the five wells and the average C_T value was 7.1 cycles.

After we confirmed that the An_Geo 1 assay worked properly, we diluted the DNA sample as discussed in Section 3.7. We ran the qPCR assay with each of the dilutions as well as water as a negative control. The resulting amplification plot and C_T values are shown in Figure 4.3. The C_T values were plotted against DNA concentration as shown in Figure 4.4.

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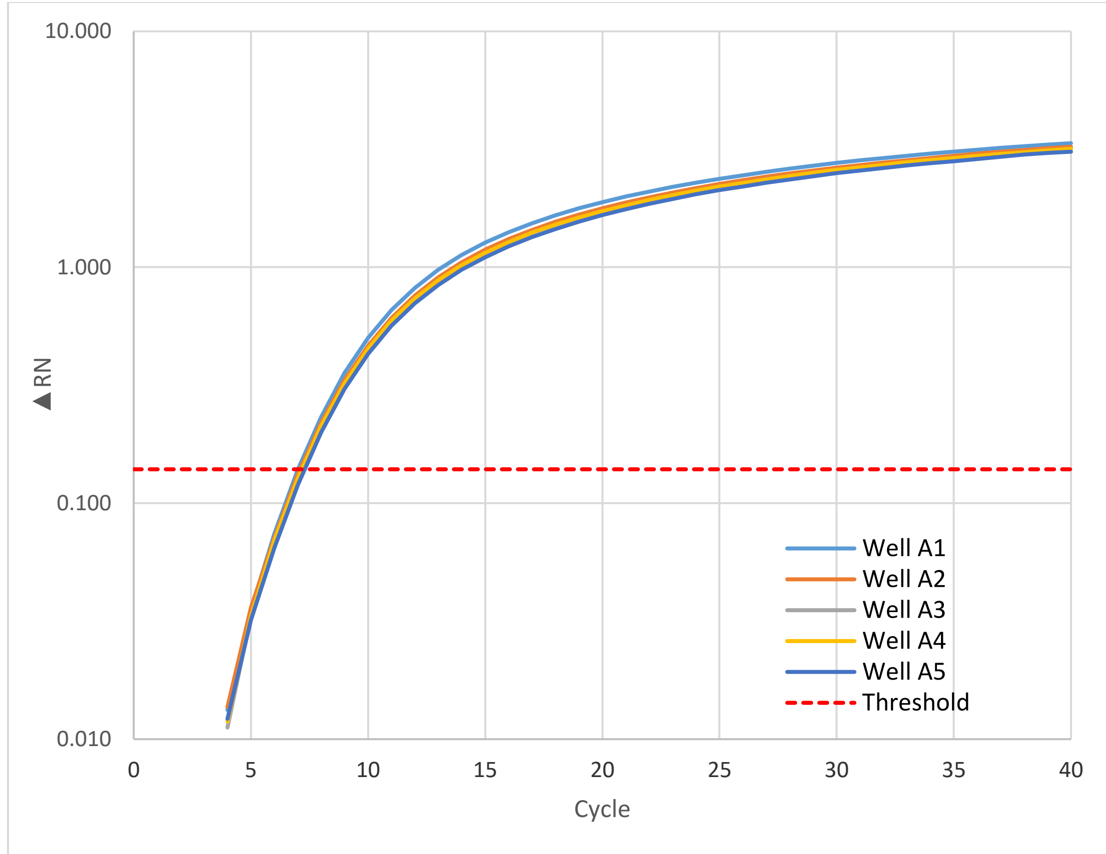

Figure 4.2: Amplification plot for 10 ng/µL of *Anabaena ucrainica* CHAB2155 DNA showing a C_T value of about 7.1.

Table 4.2: The resulting C_T values for the An_Geo 1 assay averaging at 7.1. Each well is identical with a DNA sample of *Anabaena ucrainica* CHAB2155 at 10 ng/µL

Well	C_T
A1	6.984
A2	7.072
A3	7.164
A4	7.138
A5	7.256

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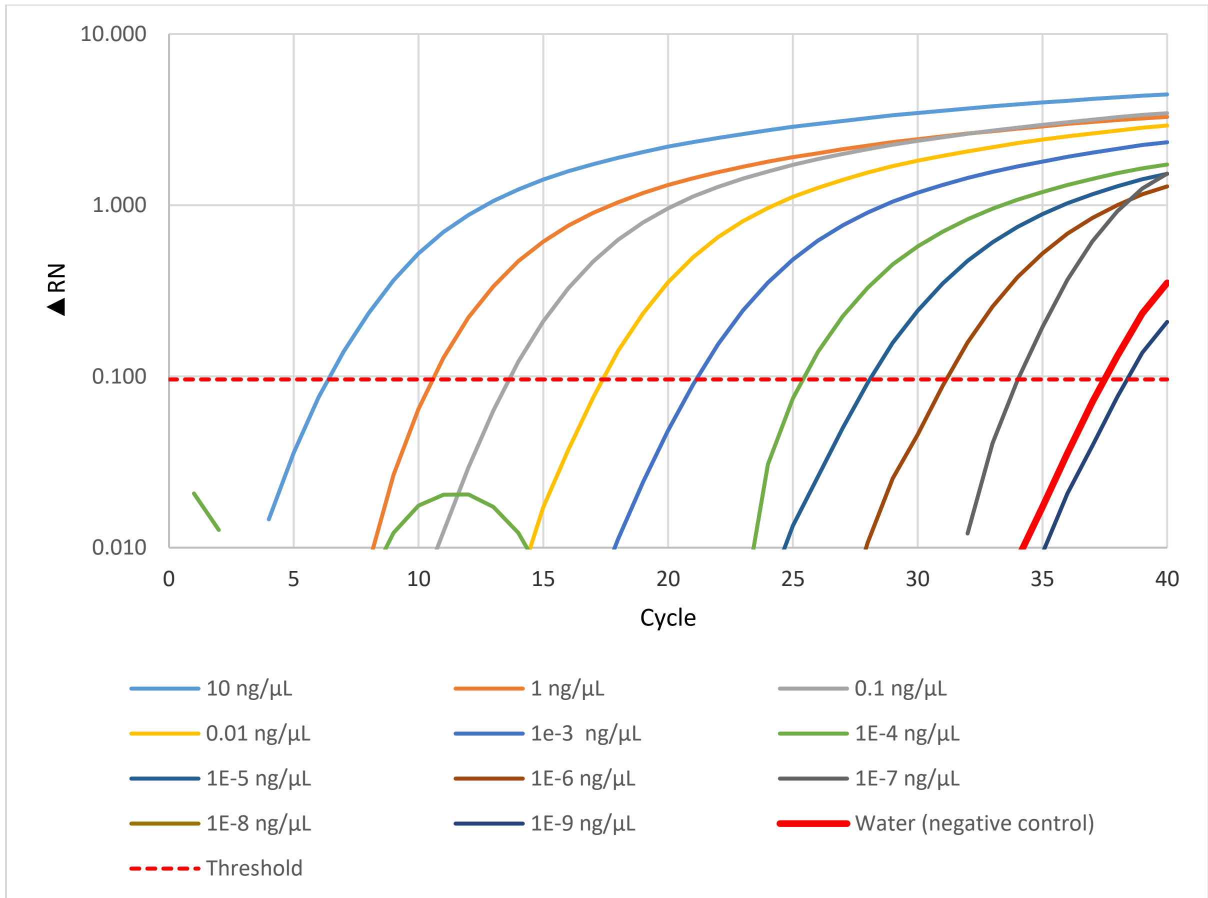

Figure 4.3: Results for each dilution of the *Anabaena ucrainica* CHAB2155 DNA sample

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Table 4.3: Resulting C_T values for each dilution

Sample Name	C_T
10 ng/μL	6.365
1 ng/μL	10.521
0.1 ng/μL	13.600
0.01 ng/μL	17.335
0.001 ng/μL	21.092
1e-4 ng/μL	25.360
1e-5 ng/μL	28.047
1e-6 ng/μL	31.100
1e-7 ng/μL	34.019
1e-8 ng/μL	Undetermined
1e-9 ng/μL	38.325
Water (negative control)	37.419

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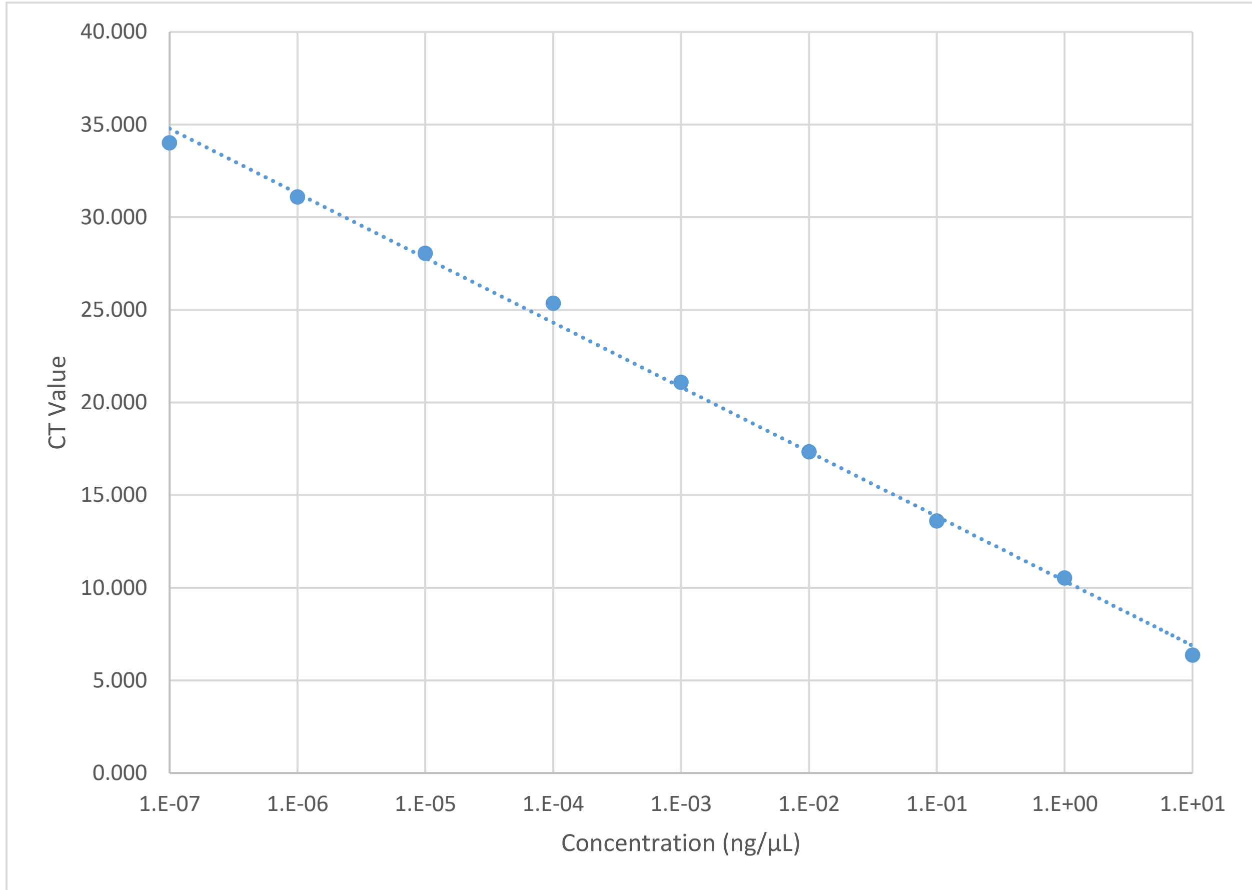

Figure 4.4: Standard Curve for An_Geo 1 qPCR assay

The DNA extracted from environmental samples showed little amplification. The resulting amplification plot and C_T values are shown in Figure 4.5 and Table 4.4 respectively. When the environmental samples were run, only the Deer Creek, Utah Lake #1 and Utah Lake #2 samples had C_T values lower than water. Of these, Deer Creek had a C_T value within 0.02% of the negative control, Utah Lake #1 was 2.6% lower, and Utah Lake #3 was 11.6% lower. All other environmental DNA samples had C_T values greater than the negative control, and

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therefore, the presence of the geosmin synthase gene in the targeted *Anabaena* species is likely below the limit of detection.

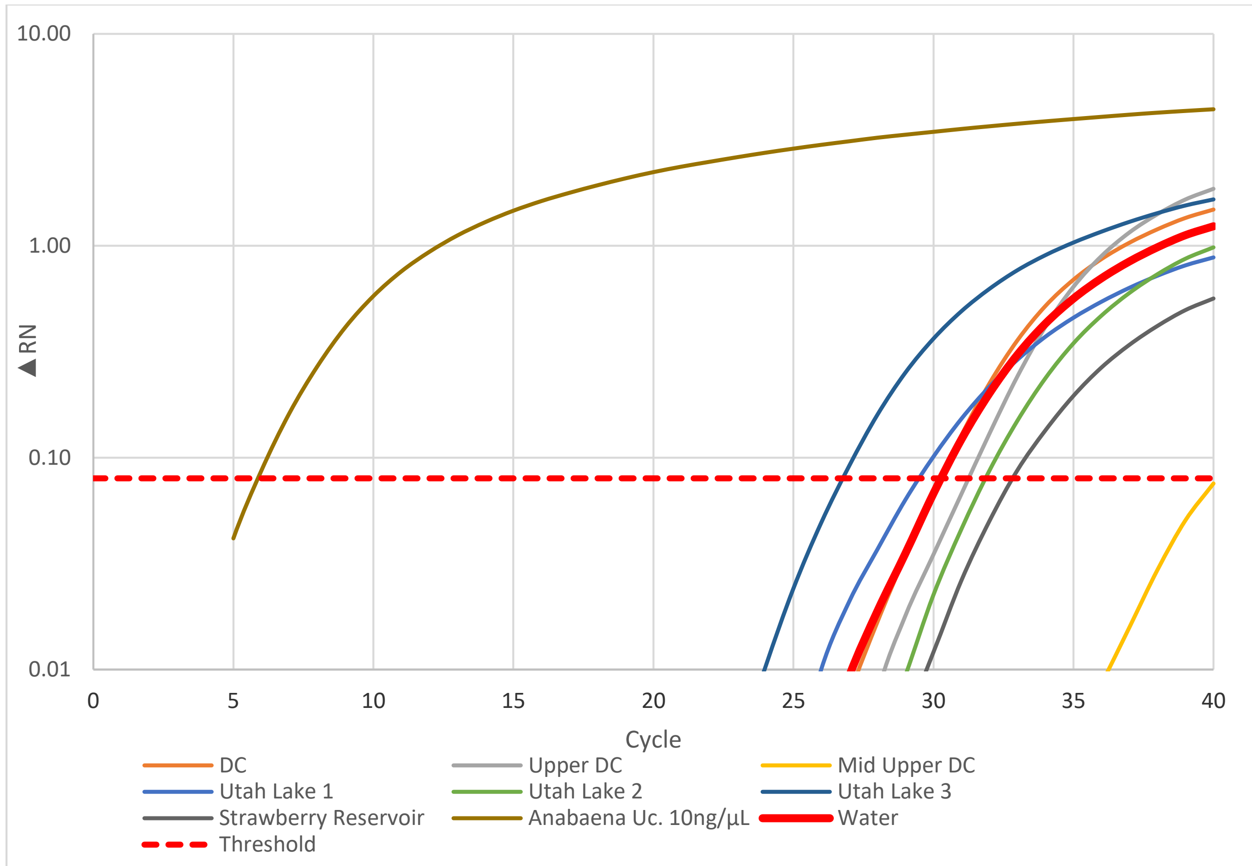

Figure 4.5: Amplification plot for environmental samples with *Anabaena ucrainica* CHAB2155 10ng/µL as a positive control, and water as a negative control.

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Table 4.4: The resulting C_T values for the test shown in Figure 4.5

Sample Name	C_T
Deer Creek Reservoir	30.238
Upper Deer Creek Reservoir	31.267
Mid-Upper Deer Creek Reservoir	Undetermined
Utah Lake #1	29.466
Utah Lake #2	31.852
Utah Lake #3	26.746
Strawberry Reservoir	32.830
Anabaena ucrainica CHAB2155 10 ng/µL (positive control)	5.861
Water (negative control)	30.243

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5 CONCLUSION

5.1 Discussion of Results

In this study we designed a qPCR assay that targets the geosmin synthase gene in several *Anabaena* species and tested PCR primers that were designed by other researchers to target the geosmin synthase gene in a variety of species of cyanobacteria. Our qPCR assay and the PCR assays designed by others were each tested on DNA extracted from environmental samples, DNA extracted from a culture of *Anabaena flos-aquae* UTEX1444, as well as a biosynthesized region of the geosmin synthase gene of *Anabaena ucrainica* CHAB2155. It is important to note that the *Anabaena flos-aquae* UTEX1444 that was used was not confirmed to be a geosmin-producing strain, and that the DNA extracted from environmental samples of cyanobacteria are not confirmed to contain geosmin.

Using PCR primers designed by Giglio et al. (2008) and Suurnäkki et al. (2015), we only had positive amplification of the biosynthesized *Anabaena ucrainica* CHAB2155 geosmin synthase gene. We did not have positive amplification of any environmental samples nor with *Anabaena flos-aquae* UTEX 1444. We had positive results when our assay was used with the biosynthesized *Anabaena ucrainica* CHAB2155 geosmin synthase gene. We made a DNA standard curve using the results (see Section 4.2), however, the standard curve we developed using biosynthesized DNA may be different than if it were developed using DNA extracted from environmental samples. Further testing of our qPCR on DNA extracted from a culture of a

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geosmin producing *Anabaena* species from Table 3.2 would produce a more reliable standard curve. However, to our knowledge, there are no cyanobacterial species from Table 3.2 for which there is a culture available for purchase.

We did not have strong positive results when we used our qPCR assay with any of the environmental samples nor with *Anabaena flos-aquae*. The amplification of the Utah Lake #1 and #3 samples were only slightly lower than that of the negative control (see Table 4.4). This may reflect the detection of the geosmin synthase gene in these samples but at a very low concentration of less than 1e-4 ng/µL.

The development of the An_Geo 1 qPCR assay will help solve the taste-and-odor problems in drinking water reservoirs by enabling the detection of geosmin-producing *Anabaena* species. At the beginning of a cyanobacterial bloom, this qPCR assay can assist in the characterization of the bloom. If a geosmin-producing *Anabaena* species is present, this assay will help identify and quantify it. The qPCR assay we designed is limited in that it is specific to the *Anabaena* species that are currently available on NCBI GenBank. Other assays will need to be designed and tested to provide more diverse detection of geosmin-producers. However, genetic sequencing information for geosmin-producing cyanobacteria is limited. qPCR assays cannot be designed to target species for which there is no genetic sequencing information available.

Several other researchers have designed PCR assays that can detect the presence or absence of the geosmin synthase gene in a variety of geosmin-producing cyanobacteria. These assays are helpful in the early classification of a cyanobacterial bloom as geosmin-producing or non-geosmin-producing. Once the geosmin synthase gene has been confirmed to be present in a water sample, our qPCR assay, as well as the assay that Moore (2019) designed to target several

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Nostoc species, can be used to test whether a geosmin-producing *Anabaeana* species and/or a *Nostoc* species is responsible. This is useful because qPCR can be used to quantify the presence of the gene using the DNA standard curve. While our assay is only capable of detecting several *Anabaena* species, and Moore's assay can only detect *Nostoc* species, the development of more qPCR assays targeting other species will expand our ability to classify and quantify more species of geosmin-producing bacteria.

5.2 Future Research Possibilities

Currently, there is little knowledge about which species of cyanobacteria are most commonly responsible for T&O-producing cyanobacterial blooms in Utah's reservoirs. There is some microscopy data available for Deer Creek Reservoir, however, microscopy data cannot discriminate between a geosmin-producing strain and a non-geosmin-producing strain. Identification of the geosmin-producers in Utah's reservoirs is crucial to early detection of geosmin events. Future research could include the retrieval of water samples during a geosmin event. Once a sample is collected, it would be necessary to get the sample tested for geosmin to confirm that it is present in the sample. After we obtain a sample that tests positive for geosmin, we can use PCR assays designed by other researchers to amplify the geosmin synthase gene (Giglio et al. 2008; Suurnäkki et al. 2015). Moore's qPCR assay as well as ours can be used to detect the presence of a geosmin-producing *Nostoc,* and/or *Anabaena* species.

We can then sequence the geosmin synthase gene that was amplified using PCR in the BYU sequencing lab and use the genetic information obtained to identify the organisms from which the gene came. This method can provide more genetic information of local geosmin-

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producers. The disadvantages of this method are that cyanobacterial blooms are unpredictable, and it can be expensive to do DNA sequencing.

Other potential research could include the development of qPCR assays similar to ours targeting other species of potential geosmin-producers from Table 2.2. Currently, the only species from Table 2.2 that has sequencing information available is *Aphanizomenon flos-aquae*. Similar research can also be done for MIB, another common T&O compound produced by cyanobacteria. As more genetic sequence data becomes available, more qPCR assays can be designed and tested, expanding our ability to create PCR-based methods that can predict what secondary metabolites will be produced by an emerging cyanobacterial bloom.

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APPENDIX A: ISOLATION OF GENOMIC DNA FROM BACTERIAL PLATE CULTURES

The following was taken from QIAamp® DNA Mini and Blood Mini Handbook by Qiagen (2016):

1. Remove bacteria from culture plate with an inoculation loop and suspend in 180 µl of Buffer ATL (supplied in the QIAamp DNA Mini Kit) by vigorous stirring.
2. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform. Note: Proteinase K must be used. QIAGEN Protease has reduced activity in the presence of Buffer ATL. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. Lysis overnight is possible and does not influence the preparation. To ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.
3. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
4. If RNA-free genomic DNA is required, follow step 4a. Otherwise, follow step 4b. Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA

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which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

a. First add 4 µl RNase A (100 mg/ml), mix by pulse-vortexing for 15 s, and incubate for 2 min at room temperature (15–25°C). Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid before adding 200 µl Buffer AL to the sample. Mix again by pulse-vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid. It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL. In most cases it will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.
b. Add 200 µl Buffer AL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid. It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL, which in most cases will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

5. Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid. It is essential that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is

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essential to apply all of the precipitate to the QIAamp Mini spin column. This precipitate does not interfere with the QIAamp procedure or with any subsequent application. Do not use alcohols other than ethanol since this may result in reduced yields.

6. Carefully apply the mixture from step 5 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.* Close each spin column to avoid aerosol formation during centrifugation. It is essential to apply all of the precipitate to the QIAamp Mini spin column. Centrifugation is performed at 6000 x g (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through. * Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.
7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
9. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

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10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.
11. Repeat step 10. A 5 min incubation of the QIAamp Mini spin column loaded with Buffer AE or water, before centrifugation, generally increases DNA yield. A third elution step with a further 200 µl Buffer AE will increase yields by up to 15%. Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation. Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield…. Eluting with 4 x 100 µl instead of 2 x 200 µl does not increase elution efficiency. For long-term storage of DNA, eluting in Buffer AE and placing at –30 to –15°C is recommended, since DNA stored in water is subject to acid hydrolysis. Yields of DNA will depend both on the amount and the type of tissue processed. 25 mg of tissue will yield approximately 10–30 µg of DNA in 400 µl of water (25–75 ng/µl), with an A260/A280 ratio of 1.7–1.9 (Qiagen 2016).

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APPENDIX B: *ANABAENA-UCRAINICA* CHAB 2155 DNA SEQUENCE

The following sequence from NCBI GenBank was used in the purchasing of biosynthesized *Anabaena ucrainica* CHAB2155 geosmin synthase gene.

5'-
CAACCATTTAAACTGCCAGCATTTTATATGCCCTGGCCAGCGCGGCTGAATCCAAAC
CTGGAAGCGGCACGAGTGCATTCCAAAGCCTGGGCTTATGAAATGGGAATACTTGG
CTCAAAAGAGGAGTCCCAAGGTGAGCCTATATGGGATGAACGTAAATTTGATGCTC
ACGACTATGCTTTACTTTGTTCTTATACTCATCCAGACACGCCCTCAACAGAGTTGAA
TCTGGTAACGGATTGGTATGTATGGGTATTCTTTTTCGATGATCACTTTCTGGAAATC
TATAAGCGCAGTCAGGATCTAATTGGGGCAAAGGAGTATCTTGACCGACTACCGGC
ATTTATGCCAATTTATCCCCAAGATAACCTTCCCTTTCCCACGAACCCAGTAGAGCG
CGGTTTAGCTGACTTGTGGTCTCGTACTGCCTTTACTAAGTCCGTAGAATGGCGGCA
AAGATTCTTTGAAAGTACCAAAAATCTTTTAGATGAGTCAATGTGGGAACTAGCAAA
TATTAATCAAAATCGTATTGCTAACCCCATTGAATACATTGAAATGCGGCGGAAAGT
TGGGGGCGCACCTTGGTCAGCAGATTTAGTCGAACACGCGGCCTTTGTGGAAGTTCC
GGCTAAAATTGCGGCAACTAGACCCATGCGGGTTTTAAAAGACACATTTGCTGATGG
TGTACATCTCCGCAATGATCTATTCTCCTACCAAAGAGAGGTGGAAGAGGAAGGTG
AAAATTCTAACTGTGTGCTTGTAGTTGAGCGTTTCTTGAATGTGAGTACCCAAGAGG
CCGCTAACCTCACTAACGAACTACTCAACTCCCGTTTATACCAATTTGACAACACTG
CTGTCACTGAATTACCCTCTCTTTTTGAGGAGTACGGAGTAGATCCAGTAGAGCGTG
TGAATGTTCTCCTTTACATTAAAGGACTTCAAGATTGGCAATCTGGTGGTCACGAGT
GGCACATGAGGTCAAGCCGCTATATGAACA -3'

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APPENDIX C: AMPLIFICATION DATA FOR AN_GEO 1 ASSAY

Amplification Data for *Anabaena ucrainica* CHAB2155 DNA at 10 ng/µL

Cycle	Well A1	Well A2	Well A3	Well A4	Well A5
1	-0.008	-0.009	-0.008	-0.012	-0.010
2	-0.006	-0.007	-0.006	-0.009	-0.008
3	0.000	0.000	0.000	0.000	0.000
4	0.013	0.014	0.011	0.012	0.012
5	0.035	0.036	0.033	0.033	0.032
6	0.074	0.071	0.069	0.070	0.065
7	0.138	0.132	0.125	0.127	0.119
8	0.232	0.219	0.209	0.212	0.200
9	0.356	0.333	0.318	0.321	0.305
10	0.502	0.465	0.449	0.452	0.429
11	0.659	0.610	0.590	0.594	0.566
12	0.819	0.759	0.736	0.739	0.704
13	0.976	0.906	0.880	0.882	0.842
14	1.129	1.051	1.018	1.020	0.977
15	1.272	1.187	1.149	1.153	1.104
16	1.408	1.317	1.277	1.279	1.226
17	1.537	1.439	1.397	1.401	1.343
18	1.659	1.558	1.512	1.515	1.451
19	1.777	1.671	1.620	1.626	1.560
20	1.888	1.779	1.726	1.730	1.664
21	1.993	1.881	1.825	1.832	1.761
22	2.094	1.978	1.921	1.928	1.857
23	2.190	2.073	2.009	2.021	1.947
24	2.279	2.167	2.102	2.110	2.037
25	2.367	2.251	2.188	2.197	2.122
26	2.452	2.339	2.267	2.275	2.199
27	2.537	2.420	2.352	2.355	2.280
28	2.617	2.496	2.432	2.435	2.357

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29	2.694	2.573	2.499	2.511	2.430
30	2.768	2.644	2.570	2.582	2.503
31	2.836	2.714	2.639	2.650	2.570
32	2.906	2.775	2.707	2.721	2.634
33	2.968	2.839	2.762	2.788	2.698
34	3.029	2.907	2.828	2.845	2.760
35	3.089	2.967	2.894	2.903	2.818
36	3.148	3.028	2.953	2.966	2.880
37	3.207	3.087	3.010	3.024	2.942
38	3.259	3.141	3.064	3.073	3.001
39	3.310	3.193	3.113	3.128	3.054
40	3.355	3.239	3.161	3.175	3.089

Amplification Data for Dilutions of *Anabaena ucrainica* CHAB2155 DNA

Cycle	10 ng/µL	1 ng/µL	0.1 ng/µL	0.01 ng/µL	1e-3 ng/µL	1E-4 ng/µL
1	-0.015	0.009	0.002	0.000	0.004	0.021
2	-0.012	0.004	-0.001	0.000	0.002	0.013
3	0.000	0.001	0.000	0.000	0.001	-0.014
4	0.015	-0.001	0.001	0.000	0.001	-0.022
5	0.036	-0.001	0.000	0.000	0.001	-0.015
6	0.076	-0.001	-0.001	0.000	0.000	-0.007
7	0.139	0.002	-0.001	-0.001	0.000	0.000
8	0.234	0.008	0.000	0.001	-0.001	0.006
9	0.364	0.027	0.001	0.000	-0.001	0.012
10	0.523	0.065	0.005	-0.001	-0.001	0.018
11	0.697	0.129	0.012	0.000	-0.001	0.020
12	0.877	0.221	0.029	0.000	-0.002	0.020
13	1.060	0.336	0.063	0.001	-0.002	0.017
14	1.238	0.471	0.122	0.006	-0.001	0.012
15	1.411	0.612	0.210	0.017	-0.001	0.007
16	1.579	0.760	0.328	0.037	0.002	0.001
17	1.737	0.902	0.470	0.075	0.005	-0.005
18	1.894	1.043	0.628	0.141	0.011	-0.010
19	2.049	1.179	0.794	0.234	0.024	-0.012
20	2.197	1.311	0.961	0.354	0.048	-0.014
21	2.334	1.437	1.124	0.496	0.090	-0.014
22	2.467	1.560	1.279	0.650	0.154	-0.009
23	2.604	1.677	1.432	0.807	0.243	0.004

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24	2.738	1.794	1.577	0.965	0.354	0.031
25	2.870	1.908	1.720	1.119	0.482	0.074
26	2.991	2.013	1.858	1.263	0.620	0.139
27	3.102	2.122	1.995	1.409	0.763	0.225
28	3.225	2.229	2.125	1.552	0.906	0.330
29	3.349	2.335	2.256	1.695	1.052	0.452
30	3.464	2.429	2.376	1.823	1.183	0.575
31	3.565	2.523	2.498	1.945	1.313	0.702
32	3.667	2.620	2.613	2.065	1.441	0.829
33	3.777	2.711	2.725	2.183	1.566	0.953
34	3.885	2.797	2.841	2.304	1.684	1.077
35	3.979	2.891	2.947	2.415	1.799	1.196
36	4.076	2.982	3.057	2.523	1.915	1.312
37	4.175	3.064	3.165	2.628	2.029	1.427
38	4.274	3.143	3.266	2.735	2.138	1.537
39	4.363	3.217	3.365	2.837	2.246	1.642
40	4.439	3.283	3.447	2.921	2.332	1.729

Cycle	**1E-5 ng/µL**	**1E-6 ng/µL**	**1E-7 ng/µL**	**1E-8 ng/µL**	**1E-9 ng/µL**	**Water**
1	0.002	-0.002	-0.001	-0.030	-0.004	-0.001
2	0.000	-0.003	0.002	-0.009	-0.004	-0.002
3	0.000	-0.001	0.003	0.002	-0.003	-0.001
4	0.000	-0.001	0.002	0.002	-0.002	-0.001
5	0.001	-0.001	-0.003	0.001	-0.001	0.000
6	0.001	0.000	-0.004	0.001	-0.001	0.000
7	0.001	0.000	-0.001	0.001	0.001	0.001
8	0.000	0.000	-0.002	0.000	0.002	0.001
9	-0.001	0.000	-0.002	0.000	0.002	0.002
10	-0.001	0.000	-0.001	0.001	0.001	0.001
11	0.000	0.001	-0.001	0.001	0.002	0.000
12	-0.001	0.002	0.000	0.001	0.002	0.000
13	-0.001	0.002	0.000	-0.002	0.001	0.000
14	-0.001	0.001	0.001	-0.001	0.000	0.000
15	0.000	0.000	0.001	-0.001	0.001	0.000
16	0.000	0.001	0.000	0.000	0.000	-0.001
17	-0.001	0.000	0.000	-0.001	0.000	-0.001
18	0.000	0.000	0.000	0.000	0.000	0.000
19	0.000	0.000	0.003	0.000	-0.002	0.000
20	0.000	0.001	0.004	-0.001	-0.001	0.001
21	0.000	0.001	0.003	-0.001	0.000	0.001

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22	0.000	0.000	0.002	0.000	-0.001	0.000
23	0.003	-0.002	0.001	0.000	-0.001	0.000
24	0.005	-0.002	0.000	-0.001	-0.001	0.000
25	0.013	-0.001	0.000	-0.001	0.000	-0.002
26	0.026	0.000	0.001	-0.002	-0.001	-0.001
27	0.050	0.004	0.002	-0.001	-0.001	0.000
28	0.092	0.011	0.002	-0.001	-0.001	0.000
29	0.157	0.025	-0.004	-0.001	-0.001	0.000
30	0.242	0.046	-0.008	-0.001	-0.002	0.000
31	0.349	0.088	-0.004	-0.002	-0.002	-0.001
32	0.474	0.158	0.012	-0.001	0.001	0.000
33	0.609	0.256	0.041	-0.002	0.002	0.003
34	0.748	0.380	0.095	-0.002	0.004	0.009
35	0.888	0.524	0.194	0.001	0.009	0.017
36	1.027	0.682	0.367	0.003	0.021	0.036
37	1.161	0.842	0.616	0.001	0.039	0.071
38	1.290	1.003	0.923	0.002	0.076	0.132
39	1.418	1.159	1.251	0.007	0.138	0.234
40	1.525	1.288	1.530	0.006	0.208	0.352

Amplification Data for Environmental Samples. This data also includes data for the positive and negative controls *Anabaena ucrinaica* 10ng/µL and water respectively.

Cycle	**DC**	**Upper DC**	**Mid Upper DC**	**Utah Lake 1**	**Utah Lake 2**	**Utah Lake 3**	**Strawberry Reservoir**	***Anabaena uc.* 10ng/µL**	**Water**
1	-0.002	-0.002	0.003	0.000	0.001	0.003	-0.003	-0.012	0.003
2	-0.002	-0.002	0.003	0.000	0.001	0.001	-0.003	-0.009	0.002
3	-0.001	-0.001	0.003	0.000	0.001	0.000	-0.002	0.000	0.002
4	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.016	0.001
5	0.001	0.000	0.002	0.001	-0.001	0.001	0.000	0.042	0.000
6	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.087	-0.001
7	0.000	0.000	0.000	0.001	0.000	0.001	0.002	0.162	0.001
8	0.001	0.000	-0.001	0.000	-0.001	0.000	0.001	0.270	0.001
9	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.413	-0.001
10	0.000	0.000	-0.001	0.000	0.000	0.000	0.000	0.579	-0.002
11	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.757	-0.002
12	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.937	0.000

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13	0.001	0.001	0.000	0.000	0.001	-0.001	0.001	1.118	0.000
14	0.001	0.000	-0.001	-0.001	0.000	-0.001	-0.001	1.294	0.000
15	-0.001	-0.001	0.000	-0.001	0.000	-0.001	0.000	1.464	0.000
16	-0.001	-0.001	-0.001	0.000	-0.001	0.000	0.001	1.625	0.000
17	0.000	0.000	-0.001	-0.001	-0.001	-0.001	0.000	1.778	0.001
18	-0.001	-0.001	-0.002	-0.002	-0.001	-0.002	-0.003	1.929	0.000
19	-0.001	0.000	-0.001	-0.002	0.000	-0.001	-0.001	2.080	-0.001
20	-0.001	0.000	-0.001	-0.001	-0.001	-0.002	0.000	2.226	-0.001
21	-0.001	0.000	-0.001	0.000	-0.001	0.000	-0.001	2.362	-0.002
22	0.000	0.000	-0.001	-0.001	-0.001	0.002	-0.002	2.490	-0.002
23	-0.001	-0.001	-0.001	0.001	-0.001	0.004	-0.003	2.619	-0.001
24	0.000	-0.001	-0.001	0.002	-0.002	0.010	-0.003	2.748	0.000
25	0.002	0.000	0.000	0.003	-0.001	0.024	-0.003	2.873	0.002
26	0.003	0.000	0.001	0.010	0.000	0.050	-0.001	2.996	0.004
27	0.007	0.002	-0.001	0.021	0.002	0.092	0.001	3.113	0.009
28	0.017	0.008	-0.001	0.037	0.004	0.160	0.004	3.235	0.019
29	0.034	0.018	-0.001	0.063	0.009	0.252	0.006	3.343	0.035
30	0.067	0.035	-0.002	0.101	0.023	0.364	0.012	3.449	0.068
31	0.128	0.067	-0.001	0.153	0.046	0.491	0.026	3.559	0.122
32	0.224	0.130	0.001	0.216	0.087	0.626	0.050	3.663	0.201
33	0.358	0.242	0.002	0.290	0.150	0.767	0.087	3.766	0.306
34	0.520	0.415	0.003	0.372	0.237	0.903	0.135	3.867	0.429
35	0.692	0.641	0.005	0.457	0.346	1.036	0.196	3.962	0.564
36	0.865	0.894	0.009	0.544	0.468	1.166	0.266	4.059	0.703
37	1.034	1.156	0.016	0.634	0.599	1.297	0.341	4.156	0.843
38	1.197	1.410	0.030	0.722	0.734	1.426	0.419	4.247	0.984
39	1.353	1.654	0.051	0.808	0.869	1.550	0.497	4.329	1.122
40	1.482	1.857	0.076	0.881	0.983	1.655	0.564	4.407	1.236

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