Design of a biosensor to detect *Enterotoxigenic E. coli* in drinking water

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Executive Summary

The objective of this project is to design an effective, inexpensive, and portable water-testing method that can be used for longer periods than ones currently available. This method would be for application in developing nations, such as South Sudan, where the necessity of such a technology would be extremely useful to the health of the population. The system will mainly be initially primarily used by non-governmental organisations. The reusability of the design is intended to reduce the cumulative cost and the subsequent polluting factor of disposable materials. In order to meet these criteria, a biosensor will be designed, using non-pathogenic bacteria, *Bacillus subtilis* that will be genetically engineered to express a specific colour in the presence of the target pathogen (ETEC).

In order to achieve this novel detection mechanism, the quorum sensing system (bacterial communication) of ETEC will be used. However, since this system is not known, a comprehensive experiment will be designed to determine the gene sequence of the quorum sensing system. Transposon mutagenesis was used to find the location of the quorum sensing operon.

Once the promoter from the quorum sensing system of ETEC is known, the promoter will be used in the biosensor, to allow for the detection of ETEC in drinking water. The design of the biosensor consists of two modules: adhesion and detection. The adhesion module is necessary to keep the biosensor in the vessel during water discharge. Polyhistidine tags will be attached to surface proteins of the *B. subtilis* biosensor cells. The tags will bind to metal ions in resin beads, allowing for the biosensor cells to remain in the vessel.

The detection module is designed to have a two coloured output system. Therefore, the violacein pathway, an enzymatic reaction, will be exploited. This pathway can be manipulated to either output green or purple coloured molecules. The expression of green will indicate that the system is active, and the expression of purple will indicate that there is ETEC contamination in the water sample. The kinetics of the colour expression was modelled using Matlab to quantify the biosensor behaviour. Based on the modelled results, the biosensor was found to have a fast response and be portable enough to be used in the field.

However, the initial cost analysis indicated that the base cost of the biosensor is much too high for feasible use, solely because of the resin bead cost. Therefore the adhesion module needs to be modified.

The successful application of this water contaminant biosensor would improve the quality of life in developing countries where potable water is a precious commodity. Further applications of the ETEC quorum sensing can be explored in the drug delivery branch of the pharmaceutical/medical industry. Additionally, the detection module was found to be robust, but the adhesion module must be improved in order for it to be feasible for use in developing nations by NGOs.
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1.0 Introduction

Access to clean drinking water is paramount for human life. In developing countries, waterborne diseases are the leading cause of death and illness. Fecal matter from sewage and agricultural runoff contaminate natural water supply and cause diarrhoeal disease problems in humans (Othman et. al., 2010). This is a highly preventable disease that affects people disproportionally; it is not as critical in developed countries, but extremely dangerous and severe in developing countries especially where medication is not as accessible (Othman et. al., 2010).

*Enterotoxigenic Escherichia coli* (ETEC) strains are a major cause of diarrhoea in developing nations, specifically in South Sudan (World Health Organization, 2009). Disease caused by ETEC ingestion (from contaminated food or water) causes dehydration and malnutrition, and affects young children more significantly (World Health Organization, 2009). ETEC, gram negative bacteria, can cause infections that can be persistent and repeated in humans. For example, infants living in the Nile delta area have been observed to have experienced between 4.6 to 8.8 diarrhoeal episodes per year (World Health Organization, 2009). ETEC is the root cause of 66% of those episodes (World Health Organization, 2009).

ETEC strains produce two types of enterotoxins that cause the disease: heat-labile enterotoxins (LT) and heat-stable enterotoxins (ST) (Czirok et. al., 1992). These enterotoxins have been fully characterized, cloned, sequenced, and studied (Qadri et. al., 2005), so there is a great wealth of information available.

Some decontamination methods are not completely effective, which may result in users unknowingly drinking contaminated water. A reliable and convenient way to detect contamination is needed. For the majority of the current water testing methods, an incubation period of at least 24 hours is usually required. The incubation and fluorescence detection are usually done in the laboratory with expensive and bulky heating/agitation devices and UV light. It would be more ideal to have a contamination detection method that is user-friendly, fast and reusable yet still effective. A novel water quality testing device can be created by using the developing field of synthetic biology.

To address the shortcomings in the current water contamination testing methods, this project utilizes the implementation of a biosensor, which is a biological system that will be able to detect ETEC contamination in water. The biosensor will exploit the quorum sensing mechanism that is associated with ETEC virulence. Since the quorum sensing system of ETEC is not known, an experiment to locate the quorum sensing operon of ETEC was designed and incorporated into the project for its implementation in the biosensor.
2.0 Objective

The overall objective of this project is to design a biosensor to detect ETEC by exploiting the quorum sensing operon of ETEC. To do so, the following strategy will be used:

a) An experiment will be designed in order to find the quorum sensing operon of ETEC using transposon mutagenesis to knock out genes related to the expression of virulence in the chromosome.

b) A biosensor system with a *B. subtilis* chassis that generates two colour outputs upon the detection of ETEC contamination in water will be designed using the ETEC quorum sensing operon.

The biosensor system will be a successful if it achieves the following success criteria:

1. Reusable.
2. Portable.
3. Easy to use.
4. Self Sufficient.
5. Inexpensive.

3.0 Background

3.1 ETEC Virulence

ETEC virulence is characterized by diarrhoea. The number of infecting bacteria present affects the ability to cause inherent disease. Virulence factors of ETEC coded for by genes in the plasmids (Sahl et. al., 2009). ETEC virulence is caused by the production of two plasmid encoded enterotoxins: the heat stable (ST) and heat labile (LT) enterotoxins. Virulence can be caused by either enterotoxin or a combination of both. ETEC uses the mechanism of quorum sensing to synchronize the release of the virulence-inducing enterotoxins (Sahl et. al., 2009). Recent research into the evolution of these virulence plasmids and the future applications of their procurement are now better understood and characterized. The plasmid genome sequences for the virulence expression are also readily available, allowing for the application of these virulence plasmids in the further research.

Since all virulence factors in ETEC are found on the plasmids, it has been found that the chromosome of ETEC is very similar to the chromosome of non-pathogenic *E. coli* (Johnson et. al., 2009).
3.2 Quorum Sensing

Quorum sensing is a form of bacterial communication that used by bacteria to coordinate gene expression (Miller, et. al, 2001). It is an induction-response system related to the population density (Miller, et. al, 2001). For example, bacteria will coordinate to express a certain gene only when a specific population density has been achieved. Pathogenic bacteria, such as ETEC, use quorum sensing to coordinate the expression of virulence. The quorum sensing operon of ETEC is used for implementation into the biosensor cells in order to detect the presence of ETEC.

Common non-pathogenic *E. coli* communicate using the LuxI/LuxR quorum sensing circuit, where LuxI synthesizes the AHL (acyl-homoserine lactone) autoinducers that induce the LuxR receptors (see Figure 1).

![Figure 1: LuxI/LuxR quorum sensing circuit (Miller et. al, 2001)](image)

The autoinducers (hexagons in Figure 1) are produced constitutively, so when the cell population density increases, the concentration of autoinducers increases intracellularly and extracellularly (Miller, et. al, 2001). At a critical concentration, when there are enough bacteria in the environment, an autoinducer from another cell will bind to the LuxR protein, activating transcription of the desired gene (*luxICDABE* in Figure 1) (Miller, et. al, 2001). At the same time, a positive feedback loop is activated, where the expression of the autoinducer is amplified, thus increasing the rate of binding to LuxR.

Therefore, the designed biosensor will be able to detect ETEC if it contains the ETEC quorum sensing receptor, or promoter.
3.2.1 Quorum sensing in ETEC

Even though there is a high incidence of death resulting from ETEC infection in developing countries, the mechanisms of ETEC virulence are still relatively unexplored on a genomic scale (Sahl et. al., 2009). The quorum sensing unit of ETEC has not been previously located, therefore an experiment is to be devised to locate and obtain the gene sequence for implementation into the ETEC detection biosensor.

3.3 Transposon Mutagenesis

Transposons are genetic sequences that have the ability to move from one DNA site to another (Dale et. al., 2007). For example, transposons can jump from the plasmid to the chromosome and are sometimes referred to as “jumping genes” (Dale et. al., 2007). The transposon is inserted into its new position by a special form of recombination by the production of an enzyme (transposase) that cuts the sequence and inserts itself. Transposons generally insert at random (Dale et. al., 2007).

Tn5 is a commonly used transposon; its insertion site requirements are non-specific and therefore inserts randomly into a wide probability of positions. The transposon will be able to insert itself within a coding sequence and as a result, inactive that operon (Dale et. al., 2007). This makes it easy to identify the site of mutation (Dale et. al., 2007). Tn5 also has an encoded antibiotic resistance (tetracycline resistance), for screening purposes (Dale et. al., 2007). This experimental design to determine the location of the ETEC quorum sensing system will utilize this genetic tool.

4.0 Discovery of Quorum Sensing Operon

To detect the presence of ETEC, the location of the quorum sensing operon must be discovered. A genetic tool called transposon mutagenesis will be utilized.

4.1 Design of Plasmids

A novel plasmid will be designed, denoted pTrans, to introduce the transposon into the system. ETEC has two virulent plasmids, pETEC948 and pETEC666, which will be modified to add selection markers.

In pTrans, the Tn5 gene is placed under control of pLac, a lactose induced promoter. Therefore transposition will not be triggered unless there is lactose in the media. Note that the Tn5 operon contains a gene for tetracycline antibiotic resistance. Finally, pTrans does not contain an origin of replication, meaning that it will not replicate in the cell. This ensures that there will only be one transposon in each cell, as the transposon will be used as a marker (see 4.3 Transposon Mutagenesis).
SacB is a negative selection marker; if expressed in sucrose-rich media, it inhibits cells from growing. By placing the sacB gene after the enterotoxin genes (eltAB expressing LT enterotoxins; sta1 and sta2 expressing ST enterotoxins), this ensures that sacB will be expressed if virulence is triggered. Conversely, sacB will not be expressed if virulence expression is interrupted. As well, pETEC 938 and pETEC 666 contain genes for ampicillin and kanamycin antibiotic resistance, respectively. Adding these antibiotic resistances is important for the screening purposes which will be discussed in the next section.

Figure 2: Design of plasmids

4.2 Tripartite Mating Conjugation

Once the plasmid constructs are designed and produced, they need to be inserted into the recipient cell (ETEC). However, typical transformation cannot be performed because the recipient cell has not been made competent, and therefore is not readily transformed (Glick, 2010).

Instead, tripartite mating conjugation will be used to insert the constructs into the recipient cell, using competent helper and donor DH5α E. coli cells (Glick, 2010).
Conjugation is the transfer of plasmid DNA between bacterial cells through a cell to cell junction (Glick, 2010). In order for these junctions to be formed, the plasmid must contain genes that encode conjugative and mobilizing factors (Glick, 2010).

However, most plasmids used in recombinant DNA research, such as the plasmids designed in the previous step, are specifically designed to lack conjugative functions to avoid the transfer of DNA between cells (Glick, 2010).

If conjugative factors are supplied by another plasmid in the cell, non-conjugative plasmids can be made motile and transfer into the recipient target cell (Glick, 2010). Thus, this method makes it possible to transfer the plasmid constructs into a cell that is difficult to transform.

Three cells will be used: helper, donor, and recipient (see Figure 3). The DH5α E. coli helper cell contains a plasmid with chloramphenicol resistance, conjugative, and mobilizing factors. The DH5α E. coli donor cell contains the three designed plasmid constructs. The recipient cell is a cured form of prototypical ETEC (O78:H11:K80 strain H10407), meaning the cell contains no plasmids. This is necessary because the virulence expression needs to be marked by our designed plasmids.

Conjugation will begin when the three strains of cells are mixed together, close enough for cell to cell junctions to form. The plasmid in the helper cell will move to the donor cell. Here, it can supply conjugative factors to allow the three plasmid constructs to transfer into the recipient cell.

Figure 3: Tripartite mating conjugation

However, most plasmids used in recombinant DNA research, such as the plasmids designed in the previous step, are specifically designed to lack conjugative functions to avoid the transfer of DNA between cells (Glick, 2010).
To screen for the target recipient cell containing the correct plasmids, the cells will be grown on minimal media with ampicillin, kanamycin, and tetracycline antibiotics. Since the helper and donor cells are DH5α *E. coli*, they will not grow in minimal media. Therefore, only cells containing the three plasmid constructs will grow, since they contain the appropriate antibiotic resistances.

On the rare occasion that the conjugative, mobilizable plasmid from the helper cell transfers into the recipient cell, a secondary test can be conducted. Cells that survive the initial screening are further grown on media with chloramphenicol resistance. The target recipient cells that do not contain the plasmid will not grow.

### 4.3 Transposon Mutagenesis

Once the designed plasmids are in the target ETEC cell, transposition can occur. The cells will be grown in special media to allow for virulence expression (DMEM-F-12 medium supplemented with 10% FBS). Recall that in the pTrans plasmid, the *Tn5* gene is placed under *pLac*, a lactose inducible promoter. Thus, transposition is triggered with the presence of lactose in the media. Additionally, recall that *sacB* is used as a negative selection marker for virulence expression (see 4.1 Design of Plasmids). Therefore, the addition of lactose and sucrose to the media is necessary.

![Figure 4: Transposon Mutagenesis](image)

Figure 4: Transposon Mutagenesis
The transposon will jump from the plasmid to a random location in the chromosome. There are three possible outcomes:

a) **Transposon does not interrupt virulence regulation**

Virulence regulation is not interrupted, and is allowed to occur. Virulence factors, and therefore *sacB* will be expressed, preventing cell growth in the media containing sucrose.

b) **Transposon interrupts quorum sensing**

The transposon has been interrupted by the transposon, therefore virulence factors and *sacB* are not expressed, allowing or the cell to grow in the presence of sucrose.

c) **Transposon interrupts virulence regulation unrelated to quorum sensing**

The transposon interrupts an operon unrelated to quorum sensing, but related to virulence regulation. This is most likely a rare case, as it has been found that there are no cargo genes for virulence found on the chromosome.

### 4.4 Selection and Testing

The potential candidates from the previous step can now be screened. A portion of the chromosome from each cell will be amplified starting from *Tn5* using polymerase chain reaction (PCR) and sequenced.

Bioinformatics will be used to predict the protein structure based on genetic sequence. Quorum sensing is an induction/response communication system between cells, and therefore the associated proteins must be transmembrane in order to produce and receive the signal.

It is hypothesized that the quorum sensing operon of ETEC will be similar to the quorum sensing operon in non-pathogenic *E. coli* K12, since it has been found that the chromosomes are largely co-linear. Thus, all candidates will be compared to known quorum sensing operons.

Unknown sequences will be compared to known QS sequences and tested experimentally in media containing ETEC AHL signals.

### 4.5 Safety in Experimental Design

Biosafety levels describe the microbiological techniques, lab practices, safety equipment and lab facilities that aim to protect the environment and the workers that handle the material of concern. The guidelines stratify the use of recombinant DNA/RNA in research and clinical applications (see Table 1).
Table 1: Biosafety Level Breakdown (FHCRC, 2012)

<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Associated Agents</th>
<th>Special Considerations</th>
<th>Operating Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not responsible for disease in healthy human adults</td>
<td>None</td>
<td>Standard lab/management practices, appropriate medical surveillance programs/procedures</td>
</tr>
<tr>
<td>2</td>
<td>Can be responsible for human diseases but preventive</td>
<td>No concern for inhalation issues</td>
<td>Biosafety level-1 practices plus: Limited access, Biohazard warning signs, Sharps precautions, Biosafety manual, Decontamination of all infectious wastes prior to disposal</td>
</tr>
<tr>
<td>2/3</td>
<td>Associated with human disease; special precautions for certain agents</td>
<td>Level 2 Lab with some level 3 controls</td>
<td>Biosafety level 2 practices plus; controlled access, decontamination of clothing and equipment</td>
</tr>
</tbody>
</table>

This experimental design falls under biosafety level 2, as seen in Table 1. For context, it is worth noting that HIV-AIDS testing labs operate at level 2 equipment and procedures with some limited level 3 precautions.

4.5.1 Precautions

Biosafety Level 2 is built upon level 1 (see Table 1). Thus, in the implementation of this experiment, there should be easy access to sinks for hand washing and hazard showers. Additionally, extreme caution should be taken with contaminated material and instruments. Primary level containment equipment and a bio-safety cabinet/safety centrifuge should be readily available. Personal protective equipment (PPE) should be worn depending on the work (splash shields, face protection, disposable gowns, and gloves) (FHCRC, 2012).

4.5.2 Ethics and Safety Clearance

The ethical question of ‘should we be altering DNA?’ is still a very controversial one. There are many benefits of gene therapy, for instance, better understanding leading to certain cures, biosensors, environmental applications and a countless range of application. But there is also the real possibility of
mutation and inherent problems with synthetic (recombinant) organisms being released into the environment, causing control issues or disease that native organisms are not equipped to deal with.

There are currently no official global requirements mandating alterations of unicellular organisms. However, there are many instances of practitioners in this field attempting to self-regulate. The International Association of Synthetic Biology has proposed self-regulation as a valid option for managing the science (Schmidt, 2008). Some scientists do not believe that self-regulation is effective enough to ensure future bio-security, and advocate for more stringent approaches for safety and security improvement that compounds upon the physical containment, which is the current norm (Bernauer et. al., 2008). They recommend regulating the profession by allowing only licensed professionals to perform recombinant gene therapy.

5.0 DETECTOR Biosensor

Using the quorum sensing unit operon discovered in the previous section, a biosensor system, named DETECTOR, will be designed to detect the presence of ETEC in drinking water.

*Bacillus subtilis*, a common, non-pathogenic, gram positive, bacterium, was chosen to be the chassis for the biosensor system. The biggest advantage of using *B. subtilis* is in its ability to sporulate and form endospores in adverse conditions (O'Hara, 1990). These endospores are able to survive with minimal nutrients and tolerate harsh environmental conditions (O'Hara, 1990). This would facilitate the storage and transportation of the DETECTOR biosensor; when the DETECTOR biosensor is not being used, the cells will sporulate and go into hibernation mode. The endospores will be re-activated by submerging them in sugar water, making the conditions more ideal with the addition of nutrients. As the application was designed for the use in developing nations, easy storage and transportation are important aspects of the system.

The detection system will have three modes:

**Hibernation Mode**

Initially, cells would be sporulated, forming a dormant endospore form and would not require moisture or nutrients. Endospores are essential for making the DETECTOR biosensor system

Endospores enable the biosensor to be stored and transported easily.

In order to activate the biosensor cells, some nutrient such as sugar water solution needs to be added.

Figure 5: Hibernation Mode
Mechanism of DETECTOR biosensor
Active Mode

Once some nutrients are provided, the biosensor cells will be reactivated. Then the cells will start to produce green coloured molecules, indicating that the system is ready for use.

Uncontaminated water samples will cause the biosensor to continue to produce green output without any change.

Detection Mode

Contaminated water samples will cause the biosensor to turn from green to purple as ETEC triggers the production of purple coloured molecules.

After testing, the effluent is discharged. To test another sample, the user must wait until the system is active (green) before proceeding.

These three modes will be achieved by the design of two modules: an adhesion and a detection module.

5.1 Adhesion Module

The adhesion module is necessary to contain the biosensor cells within the vessel when draining the post-tested water. This will allow the biosensor cells to test additional water samples. Protein binding mechanisms to surface proteins on the biosensor cells can be used to keep the cells in the vessel. An analysis of different protein adhesion options was performed to find the most optimal method of adhesion.
5.1.1 Adhesion Options

5.1.1.1 Polyhistidine-tags

A polyhistidine-tag, or his-tag, is an amino acid compound in proteins that has histidine residues at the N- or C-terminus. These affinity tags can be placed on proteins using recombinant genetics utilizing site-directed mutagenesis, polymerase chain reaction (PCR), or by using commercially available cloning vectors (Bornhorst et. al., 2010).

His-tags have a specific affinity to transition metal ions (Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$); the his-tag binds to the metal with micromolar affinity (Hochuli et. al., 1988). These metal ions are immobilized on agarose resins through N-nitrilotriacetic acid (NTA) chelating functional groups (Hochuli et. al., 1988). In general, nickel has a higher binding capacity, while cobalt offers higher specificity (Hochuli et. al., 1988). The resins are able to regenerate once the bond is broken, and therefore is ideal for repeated use.

Interaction between the M$^{2+}$-NTA complexes and the His-tag is compatible with a large number of biochemical reagents, and His-tags rarely interfere with the structure and the function of the recombinant protein, allowing this method to be a general and powerful biotechnological device (Balavoine et. al., 2007).

5.1.1.2 Glutathione S-Transferase (GST)

Integration of a Glutathione S-Transferase (GST) DNA sequence into an expression vector similar to his-tags results in the production of a functional GST (amino acid protein) at the N-terminus of the surface protein. The specific substrate for this GST is the tripeptide glutathione, which is immobilized on a resin (Scott et. al., 2006).

This method exploits the enzyme-substrate mechanism to selectively bind the GST-tagged protein; however it is only ideal for short term binding because GST is easily degraded and promotes weak interaction. Thus, this method is not reusable. Additionally, the binding capacity of GST tagging is low compared to his-tags (8 mg/mL of resin compared to 10-25 mg/mL of resin (Pierce, 2012).

5.1.1.3 AviTags

AviTags work in a similar manner to the other options considered: the surface protein is modified to expresses the AviTag that further adheres to a substrate (biotin) suspended in a resin. This enzyme-substrate mechanism leads to similar disadvantages as GST; it is difficult to maintain the adhesion for extended periods. Additionally the cost of this resin is over 6 times that of the cheapest metal-agarose histidine tag resin considered in this design (GeneCopOeia, 2012) (see Table 2).

5.1.1.4 Dynabeads

'Dynabeads' are superparamagnetic polystyrene beads that contain streptavidin which can combine with any target molecule producing biotin; essentially exploiting the high steptavidin-biotin interaction.
(as with AviTags above). The major disadvantage is the extremely high cost of the Dynabeads. It is 32 times the cost of the cheapest metal-agarose histidine tag resin (Dynal Biotech, 2003) (see Table 2).

### 5.1.1.5 Selection of Adhesion Method

Some other affinity tags that may also be taken into consideration are Argtag, calmodulin-binding peptide, cellulose-binding domain, DsbA, c-myctag, FLAGtag, maltose-binding protein, NusA, Stag, SBPtag, Streptag, and thioredoxin. However, these affinity tags are used mostly for short-term tagging and/or detection rather than for extensive pull-down applications (Terpe, 2002) (see Table 2).

Associated costs as well as the mechanism effectiveness were analysed. Table 2 details the breakdown in the costs for all the adhesion options.

Table 2: Summary of adhesion mechanism options (Pierce, 2012), (Qiagen, 2012).

<table>
<thead>
<tr>
<th>Option</th>
<th>Details</th>
<th>Resin Affinity Mechanism</th>
<th>Resin Cost/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyhistidine tags</td>
<td>Amino acid compound with His residues</td>
<td>Metal ions</td>
<td>$5.96</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione S-Transferase</td>
<td>Tripeptide glutathione</td>
<td>$16.70</td>
</tr>
<tr>
<td>AviTags</td>
<td>Recombinant streptavidin tag</td>
<td>Biotin</td>
<td>$37.23</td>
</tr>
<tr>
<td>Dynabeads</td>
<td>Targets enzyme biotin</td>
<td>Streptavidin</td>
<td>$194.00</td>
</tr>
</tbody>
</table>

Through analysis, the selection of polyhistidine tags with a nickel ion resin for the adhesion module was made based on long-term performance, high binding capacity, ease of reproducibility and the lowest relative cost of the nickel-suspended resin. The his-tags will be coded within the genetic sequence of the biosensor cells, and the resin can theoretically persist indefinitely; thus this method is extremely reusable. This ease of reproducibility is one of the major reasons why this form of containment was selected for the DETECTOR biosensor system, as the system must be reusable to meet the success criteria. Most of the other options (including cobalt for his-tag binding) focus on very specific purification as the main operating criteria, making it more expensive but less effective for the purpose of this design.

### 5.1.2 Adhesion Mechanics

Once the surface proteins are modified to produce his-tags, the *B. subtilis* biosensor cells can be easily grown. Each generation of the recombinant biosensor cells will continually express the his-tag tail on a surface protein, making it ready to bind with suspended metal ions in the resin media. The resins are also more widely available on a commercial scale through a greater variety of providers.
His-tags are able to be used in multiaffinity fusion systems. These multiaffinity fusion systems consist of two or more affinity tags attached to the same protein (i.e., more his-tags)), providing greater flexibility during adhesion by allowing for stronger protein immobilization. This allows for the operation under more abrasive conditions. Additionally, these multiaffinity tags utilize purification procedures that result in more efficient adhesion of target protein than using individual affinity tags (Bornhorst et. al., 2010).

Both di- and tri-histidine, both multiaffinity fusion systems, showed very fast association rates, but this significantly increased with peptide length (His4 to His10) (Knecht et. al., 2009). Note that the di-and tri-His nomenclatures refer to the actual number of His bodies present per peptide. The binding affinities of the multiaffinity fusion systems increased when the number of His residues increases, up to a certain threshold (Knecht et. al., 2009). It was found that hepta-histidine to deca-histidine in fact decreased its affinity. Therefore, hexahistidine is the optimal number of multi fusions (Knecht et. al., 2009).

Also, the pH should be kept above 4 for nickel-histidine binding to persist; note that the pH of water is 7. Compared to cobalt, nickel is able to operating at a wider range of pH, as well as having a higher binding capacity (Sahl et. al., 2012).

5.2 Detection Module

The detection module is responsible for the detection of ETEC in drinking water. The detection module was designed to exploit a biosynthesis process called the violacein pathway.

5.2.1 Violacein Pathway

The violacein pathway is well characterized and widely used in various applications. The final product in the pathway is purple molecules named violacein, but intermediate products of the pathway produce different coloured molecules (Sánchez, C, 2006). This makes the violacein pathway ideal for the DETECTOR biosensor, as it requires two colour outputs.
The violacein pathway is an enzymatic reaction that uses L-tryptophan as a substrate. The reaction involves 5 enzymes: VioA, VioB, VioC, VioD and VioE. Depending on the combination of these enzymes, the final product of the pathway can be proviolacein (dark green), deoxyviolacein (light green) and violacein (dark purple) (Sánchez, C, 2006). For the DETECTOR biosensor, the colour outputs were chosen to be light green and dark purple. The desired violacein pathway can be simplified as shown in Figure 9.

L-tryptophan is an amino acid that is naturally produced in the cell; when reacted with the enzymes VioA, VioB, VioC and VioE, light green molecules (deoxyviolacein) are produced (Sánchez, C, 2006). When this is further reacted with enzyme VioD, purple molecules (violacein) are produced (Sánchez, C, 2006). By controlling the presence of VioD, the output colours can be manipulated to be either light
green or purple. The detection module is designed in such a way that the DETECTOR biosensor outputs green when it is active and outputs purple when ETEC is detected in the water. To achieve this, the production of VioD enzymes should be triggered by the presence of ETEC.

5.2.2 Detection Genetic Insert

![Detection module gene sequence](image)

Figure 10: Detection module gene sequence

Figure 10 details the simplified detection module gene sequence to be implemented in the biosensor cells. The 5 enzymes are placed under two different promoters; constitutive promoter Pconst, and quorum sensing promoter P-QS. Recall that P-QS was obtained in the experimental procedure to locate the quorum sensing operon in the ETEC chromosome. Pconst is an unregulated promoter that allows for continuous transcription of its associated genes. By placing vioA, vioB, vioC and vioE genes under Pconst, the enzymes VioA, VioB, VioC and VioE will be continuously produced.

In contrast, the vioD gene is placed under the P-QS, which is induced by the presence of ETEC quorum sensing signals

5.2.3 Detection Mechanics

The gene insert allows the biosensor cells to always produce green molecules as long as the cells are living and active (i.e., not in endospore form). This serves as a safety indicator to when the DETECTOR biosensor is ready to test water. For instance, when the biosensor cells are in endospore form (Hibernation Mode), the cells will not output green (see Figure 5). Once sugar water is added to the biosensor cells to activate the spores, the cells will begin to produce the green output continuously, signifying to the user that the DETECTOR biosensor is ready to accurately test water (Active Mode) (see Figure 6).

When the clean water sample is being tested, the violacein pathway will continue to produce green molecules since there is no ETEC in the water to trigger the quorum sensing promoter (see Figure 6).

However, when the water sample contaminated with ETEC is introduced to the system, the quorum sensing promoter (P-QS) will be induced by ETEC and start to express the vioD gene, producing VioD enzymes. This catalyzes the green molecules (deoxyviolacein) to drive the violacein pathway further to produce purple molecules (violacein). Users will know that the water sample has an unacceptable ETEC concentration when the DETECTOR biosensor colour changes from green to purple.
After testing, the tested water will be drained and the user must wait for the DETECTOR biosensor to revert back to the active mode (see Figure 6) in order to test another water sample.

The ideal volume of sample water needs to be determined through experimentation.

For short time storage, the DETECTOR biosensor should be kept in sugar water to keep the biosensor viable. For long term storage, the biosensor cells can be allowed to sporulate by not providing any sugar water.

### 5.2.4 Modelling

In order to model the behaviour of the DETECTOR biosensor and perform feasibility analysis, rudimentary simulations were performed using Matlab (see Appendix B: Matlab Code for Modelling). Since no experimental data was available, parameters were estimated based on similar systems. For example, some properties of ETEC were assumed to be similar to other strains of *E.coli* that were better known and well characterized. As the chromosome of ETEC is very similar to the chromosome of non-pathogenic *E. coli*, this assumption is most likely valid.

It was assumed that the violacein pathway followed Michaelis-Menten kinetics. Tryptophan was considered to be the substrate of the enzymatic reactions, and the substrate concentration was assumed to be significantly greater than the enzyme concentration. As well, the enzyme concentrations were set to be constant as they are not consumed in the pathway.

For simplicity, VioA, VioB, VioC and VioE are grouped into the term VioABCE, as the genes are under the same Pconst promoter, generating the enzymes at the same rate.

Since there was no data on the quorum sensing operon of ETEC (which is the reason why an experiment was needed to determine the location of the quorum sensing operon), the relationship between the ETEC quorum sensing signal and the activation of the P-QS promoter was assumed to be proportional. Since the production of VioD enzymes is under the P-QS promoter, this means the ETEC quorum sensing signal is proportional to the production of VioD enzyme.

The violacein pathway can be summarized into two kinematic reactions:

\[
\begin{align*}
TRP + Vio \text{ABCE} & \xrightarrow{k_1,k_2} ES1 \xrightarrow{k_3} G + Vio \text{ABCE} \\
G + D & \xrightarrow{k_4,k_5} ES2 \xrightarrow{k_6} P + D
\end{align*}
\]

TRP is tryptophan and VioABCE is the combination of the 4 enzymes: VioA, VioB, VioC and VioE. The enzyme VioD is denoted by D. ES is the enzyme-substrate complex that occurs in both reactions. G refers to green molecules (deoxyviolacein) and P refers to purple molecules (violacein).
Modelling was performed by solving a system of ordinary differential equations. The change in concentration of each species was expressed in terms of concentrations of other species, rate constants and the total enzyme concentration ( \([\text{Enzyme}] = [\text{Total enzyme}] - [\text{Substrate complex}]\) ). The embedded Matlab function ODE23 was used to solve the differential equations, and concentration vs. time plots were generated.

5.2.4.1 Case 1: Clean Water Detection

The first case is when the DETECTOR biosensor tests clean water with no ETEC (see Figure 11).

![Figure 11: Biosensor testing the water with no ETEC](image)

Since the constitutive promoter allows the transcription of 4 genes (\(\text{vio A, B, C, E}\)) required for the deoxyviolacein production, the concentration of green increases until steady-state. The concentration at which the purple pigment can be seen by the naked eye is approximately 0.48 mM (Imperial College, 2009), so it was assumed that the green pigment visibility occurred at the same concentration. Figure 11 indicates that the green molecules are produced in high enough concentration for visibility. In contrast, the concentrations of VioD and the purple molecules are both at zero since the violacein pathway stopped at the deoxyviolacein step, not triggering quorum sensing promoter. The concentration of enzymes VioA, VioB, VioC and VioE are constant while the concentration of tryptophan has a slight
decreasing slope. The decrease in tryptophan is very small because it was assumed to exist in excess within biosensor cells.

5.2.4.2 Case 2: Contaminated Water Detection

The second case is when the water sample contaminated with ETEC is tested (see Figure 12):

![Figure 12: Biosensor testing the water contaminated with ETEC](image)

The concentration of green molecules starts off at the concentration it had reached from Case 1, but it starts decreasing as ETEC is introduced to the system at time 0. Violacein concentration starts increasing as ETEC induces quorum sensing promoter to produce VioD. The production of VioD was assumed to be proportional to the quorum sensing signals from ETEC. Recall, the concentration at which the purple pigments can be seen by the naked eye is approximately 0.48mM (See Appendix). The time that it takes for the DETECTOR biosensor to reach that concentration is about 70 seconds. As it can be seen on the plot, the concentration of violacein greatly exceeds the 0.48mM, so it can be concluded that the visibility is not a problem.

5.2.4.3 Sensitivity Analysis

The result from the sensitivity analysis indicates that the model is reasonably stable but not completely robust. By brief visual inspection, the overall behaviour of the system seems to vary quite drastically when parameters such as rate constants, rate of production and degradation were changed. However it
is important to note that this change in behaviour does not affect the performance of the biosensor. For example, in Figure 13, the plot was generated with small changes in two parameters. The shape may look different but the basic performance traits such as response time, detection concentration of ETEC, required amount of biosensor cells do not change noticeably, as the main concern is how the system behaves in the first few minutes.

![Figure 13: Biosensor testing the water contaminated with ETEC (different parameters)](image)

It is reasonable to assume that all of the forward reaction rate constants were similar while the reverse reaction rate constants were similar to each other. These parameters did not affect the system behaviour significantly, as long as the change was within an order of magnitude.

However, because the estimated rate of production and degradation were so small in the initial modelling run, a slight change in these parameters had a big impact on the system behaviour. The important rate of production and degradation factors used in this model included the ones for the green products and purple products. These values were obtained from the study discussing extinction coefficients of violacein (DeMoss and Evans, 1959). The parameters were estimated to be similar in the *B. subtilis* chassis.
5.3 Design Specifications

The preliminary modelling discussed previously can be used in system performance analysis and design specification.

The known concentration of violacein that is visible to the naked eye (0.48M) was set as the desired concentration in our system. Once ETEC is introduced to the system, violacein production starts and the concentration reaches 0.48M at about 70 seconds according to Figure 12 (similar to the varied parameter system in Figure 13). This means the quality of water can be tested in less than two minutes which is very rapid compared to current water testing methods. Therefore, the DETECTOR biosensor can be used in field work where time is an important factor. The maximum concentration of coliform in drinking water is 0/100mL (Warrington P.D., 2001); therefore experimentation is required to determine the absolute minimum concentration that can be detected.

The required amount of biosensor cells was determined by first finding the concentration of tryptophan from the graph at 70 seconds (the time it takes to visibly see the colour output). Using a typical tryptophan concentration value in B.subtilis as the proportionality factor (Shasaltaneh, 2010), the mass of surface proteins necessary was found.

The resin chosen for this application was Ni-NTA magnetic agarose beads from Qiagen (Qiagen, 2012) and it was found that 130 to 325 mL of resin would be capable of binding to all the surface proteins. There is a range of volume because two different capacities (optimistic and pessimistic) of the resin were considered. Based on the volume of resin, the whole system will be approximately 520mL to 1300mL in volume. This size is reasonable and would allow the biosensor to be portable and available for use in the field.

5.4 DETECTOR Biosensor Safety Implications

Of utmost importance is the direct human, as well as the environmental, safety factor of the DETECTOR biosensor system. The system must be reliable and the results must be consistently reproducible. This will be achieved by employing the two different coloured outputs from the violacein pathway (green for functional and clean and purple for contamination). The existence of a different coloured protein for both the activity and the presence of ETEC decrease the likelihood of false negatives, and ensure a reliable safety mechanism as it lets the user know that the system is working prior to use.

Another major concern is the impact of the system on health and environment of the community. B. subtilis is not a pathogenic bacterium and hence will not be harmful if it comes into contact with the user. The biosensor cells are expected to remain in the system (adhered to the appropriate resin) once viable, only minor release of the engineered organism is expected. To address the issue of releasing a genetically engineered organism in the environment the post-tested water will be treated with chlorine in order to ensure that no bacteria engineered or otherwise, are discharged into the environment. Chlorine is a readily available chemical in the developing world. This is very important as the indirect
repercussions (such as reproduction and persistence) may result in eco-system changes, due to the fact that *B. subtilis* is very resistant to environmental factors such as heat and/or acidity and will therefore persist for a long time (utilizing the same endospore function that makes the bacteria favourable for the integrity of the DETECTOR biosensor). The chlorine treatment is also necessary to avoid the release of other contaminants, such as ETEC.

The system is intended to be reusable and as a result, reduce the amount of waste that current water detection systems produce. This also reduces the level of care needed in dealing with biological waste that the current methods employ.

### 5.4.1 Training and production safety

Similar precautions as above and those outlined in the biosafety level in the previous section should be followed in the manufacture of the DETECTOR biosensor in order to ensure that there is no release of the genetically altered bacteria into the environment.

For the actual use of the system by humanitarian bodies, NGOs or communities, very little training would be necessary, as the DETECTOR biosensor is very easy to use. A training manual highlighting the major mechanisms of the DETECTOR biosensor would also be accompanied with the unit highlighting the previous information, as well as more details on the operation and procedures for re-seeding, trouble-shooting and general contact information of providers.

### 5.5 Implementation

This application was originally designed to be used alongside a biofilter to test the quality of biofilter treated water. The DETECTOR biosensor can be extended to other applications such as field research, but during initial pilot testing, it would be implemented with the biofilter. As well, during pilot testing, the DETECTOR biosensor will be utilized by trained people in the field. If that is successful, it can be expanded to be used by the consumer directly.

The effluent from the biofilter is expected to be free of contaminants after a certain period of time when the biolayer is being accumulated on top of the biological zone (see Figure 14) (CAWST, 2012). The plateau section seen in Figure 15 is where the water is clean enough to drink without further treatment.

![Figure 14: Biofilter Design (CAWST, 2012)](image-url)
However, the treatment efficiency of the biofilter (as seen in Figure 15) is not standard, as the behaviour depends on many different variables, such as the condition of water being treated, frequency of usage and more (CAWST, 2012). Therefore it is difficult to determine when the water can be consumed without further treatment. As a safety precaution, biofilter users are recommended to treat the effluent water with chlorine at all times (CAWST, 2012). Although small doses of chlorine are not harmful to human health, it is possible that users may be overzealous in the chlorine treatment, thus increasing the chlorine concentration in the water to unacceptable levels. Chlorine intake should be minimized to ensure consumer safety. The DETECTOR biosensor ensures that the effluent water is safe to drink by generating colour outputs when contamination is detected. If the DETECTOR biosensor indicates no contamination, the user will know that the water is clean enough to drink without the post treatment of chlorine.

6.0 Feasibility Analysis

Recall that the DETECTOR Biosensor is aimed for philanthropic use by non-governmental organisations (NGOs), and will not be sold for profit. Therefore the cost associated with the device will only be based on the cost of materials and production.

As well, all approximated biological costs are calculated to be very pessimistic. Many of the genetic parts are planned to be synthesized, but it is possible that these parts could be available for little to no cost, as it has academic/non-profit applications.
6.1 Cost of Quorum Sensing Discovery

The cost and time of the experiment is a one-time expense. This estimated cost is approximate, as it depends on how many trials it will take to discover the location of the quorum sensing operon.

The DH5α E. coli, cured ETEC and virulence plasmids will be easy to obtain from most research labs.

For the creation of the foreign DNA inserts in the Detection Module, synthesis will be performed by GeneArt (Gene Synthesis by GeneArt, 2012) (see Table 3).

To test for the quorum sensing signals, special media that allows for the expression of virulence must be used. D-MEM/F-12 media from Invitrogen will be used (DMEM/F-12 Media, 2012).

Finally, the standard biological costs for testing in a lab will still exist, with the additional cost of sequencing each trial. Sequencing will be performed from the Centre for Applied Genomics (The Centre for Applied Genomics, 2012).

Table 3: Quorum Sensing Discovery Associated Costs

<table>
<thead>
<tr>
<th>Quorum Sensing Discovery Associated Costs</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part</strong></td>
<td><strong># bp</strong></td>
</tr>
<tr>
<td>Plac</td>
<td>200</td>
</tr>
<tr>
<td>Tn5</td>
<td>5818</td>
</tr>
<tr>
<td>SacB (1)</td>
<td>100</td>
</tr>
<tr>
<td>AmpR</td>
<td>943</td>
</tr>
<tr>
<td>SacB (2)</td>
<td>100</td>
</tr>
<tr>
<td>KanR</td>
<td>967</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Approximate Standard Biological Lab Costs</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>Cost</strong></td>
</tr>
<tr>
<td>Cells</td>
<td>$0.00</td>
</tr>
<tr>
<td>Reagents</td>
<td>$200.00</td>
</tr>
<tr>
<td>LB Media</td>
<td>$50.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional Testing Costs</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>approx. # trials</strong></td>
</tr>
<tr>
<td>Sequencing</td>
<td>10</td>
</tr>
<tr>
<td>D-MEM/F-12 media</td>
<td>10</td>
</tr>
<tr>
<td>PCR Primer</td>
<td></td>
</tr>
</tbody>
</table>

| Total Cost: | $3,638.64 |
6.2 Cost of DETECTOR Biosensor Base Unit

The cost of one biosensor unit, based on volume obtained from the results (see 5.3 Design Specifications) is estimated to be approximately $775 to $1940, depending on the volume of resin. The majority of the base materials cost is from the resin, which is the most expensive part of the design (see Table 4)

Table 4: DETECTOR Biosensor Base Associated Costs

<table>
<thead>
<tr>
<th>Item</th>
<th>$ per mL</th>
<th>mL</th>
<th>Cost</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin</td>
<td>$5.96</td>
<td>130-325 mL</td>
<td>$1,355.90</td>
<td>*averaged cost, (Qigen, 2012)</td>
</tr>
<tr>
<td>Vessel</td>
<td></td>
<td></td>
<td>$8.94</td>
<td>*Nalgene Polypropylene bottle (will need modifications), (US Plastic Corp., 2012)</td>
</tr>
<tr>
<td><strong>Total Cost:</strong></td>
<td></td>
<td></td>
<td><strong>$1,364.84</strong></td>
<td></td>
</tr>
</tbody>
</table>

6.3 Cost of DETECTOR Biosensor Modules

The creation of the adhesion and detection modules of the biosensor is a one-time expense; once the foreign DNA is inserted in the cell, it is just a matter of allowing cells to replicate. As well, *B. subtilis* will be easy to obtain from most research labs. The estimated biological cost is approximate, as there are many uncertainties in biological experimentation.

In the adhesion Module, polyhistidine tags will be added to surface proteins of *B. subtilis*, by using histag vectors. The surface protein sequences will be amplified using PCR and inserted into the vectors (see Table 5).

Again, for the creation of the foreign DNA inserts, synthesis will be performed (Gene Synthesis by GeneArt, 2012) (see Table 6).

With access to a well-equipped lab, the standard biological testing costs will be minimized. These costs are approximate, as it depends on the result of the biological experiment.
Table 5: Adhesion Module Associated Costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misc. Reagents</td>
<td>$100.00</td>
<td></td>
</tr>
<tr>
<td>LB Media</td>
<td>$10.00</td>
<td></td>
</tr>
<tr>
<td>pAB-6xHis™</td>
<td>$295.00</td>
<td>*his-tag vectors, 50 μl, 0.5 μg/μl, (AB Vector - pAB-6xHis™, 2012)</td>
</tr>
<tr>
<td>PCR Primer</td>
<td>$15.00</td>
<td>(Custom Oligo Price List, 2012)</td>
</tr>
</tbody>
</table>

Total Cost: $420.00

Table 6: Detection Module Associated Costs

<table>
<thead>
<tr>
<th>Part</th>
<th># bp</th>
<th>$/bp</th>
<th>Cost</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violacein operon</td>
<td>7345</td>
<td>$0.38</td>
<td>$2,791.10</td>
<td>(Part:Bba_K274002, 2012)</td>
</tr>
<tr>
<td>Pconst</td>
<td>35</td>
<td>$0.38</td>
<td>$13.30</td>
<td>(Part:Bba_J23119, 2012)</td>
</tr>
<tr>
<td>Plux</td>
<td>55</td>
<td>$0.38</td>
<td>$20.90</td>
<td>(Part:Bba_R0062, 2012)</td>
</tr>
</tbody>
</table>

**Approximate Standard Biological Lab Costs**

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
<th>Reference/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>$0.00</td>
<td>*obtained from other research labs</td>
</tr>
<tr>
<td>Misc. Reagents</td>
<td>$100.00</td>
<td></td>
</tr>
<tr>
<td>LB Media</td>
<td>$10.00</td>
<td></td>
</tr>
</tbody>
</table>

Total Cost: $2,935.30

6.4 Recurring Biological Maintenance cost for DETECTOR biosensor

To ensure that the biosensor cells are well populated in the vessel, it will be recommended to occasionally re-seed, or add more cells, to the system approximately every 4 months. Although the biosensor cells will reproduce naturally in the system, re-seeding will guarantee that the viable cells are in great quantity. This time period depends on how often the biosensor is used, and how viable the cells are. More experimental testing will be needed to determine a better time estimate. As well, to ensure that the cells remain viable, in between uses, the cells in the system should be kept in sugar water. This is a safety precaution and one way to ensure that the DETECTOR biosensor is operating at optimal performance levels.
The system can be re-seeded from a stock of sporulated biosensor cells. As stated previously, once the cells are created, it is very simple to allow them to reproduce. So if the stock of cells becomes low, the user can simply grow more.

Overall, this maintenance cost will be negligible, as it will only require sugar and water.

6.5 Lifespan and Mutation Rate

The lifespan of the DETECTOR biosensor will be considered over when one of the foreign inserted genes mutates. Since the mutation rate of *B. subtilis* is quite slow, and the number of foreign genes introduced to the system is small compared to the chromosome (Crossman, 2010), it was determined that the expected time it will take for one of the biosensor genes to mutate is approximately 200 years (see Appendix A: Mutation Rate of Biosensor).

Clearly, mutation will not be a concern. Additionally, since the system will be occasionally re-seeded with new cells (generation: 1), this will mean that the expected time will increase and vary over time. As well, the resin can be constantly regenerated.

Thus, the lifespan of the DETECTOR biosensor is indefinite as long as the biosensor cells remain viable.

6.6 Summary of Costs

<table>
<thead>
<tr>
<th>DETECTOR Base Cost</th>
<th>Experimental One-time Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector Biosensor</td>
<td>Adhesion Module</td>
</tr>
<tr>
<td>Biological Maintenance</td>
<td>Detection Module</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Quorum Sensing Discovery</td>
</tr>
<tr>
<td>$1,364.84</td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Total w/o synthesis</strong></td>
</tr>
</tbody>
</table>

The DETECTOR biosensor is theoretically reusable indefinitely, as long as the cells are maintained properly.

Notwithstanding the one-time experimental biological cost, the base cost of the DETECTOR biosensor is still quite high (see Table 7). This makes it infeasible for use by NGOs.

The experimental cost can be considered quite high as well, but this approximated value is quite conservative. It is likely that many of the genetic parts will not have to be synthesized, due to the non-profit application. Excluding the synthesis cost, the experimental cost becomes more reasonable (see Table 7).
7.0 Achievements and Conclusions

7.1 Design of Experiment to Discover ETEC Quorum Sensing Operon

A method to determine the location of the quorum sensing operon in *Entertoxigenic Escherichia coli* was designed. Further characterizing the ETEC genome will be extremely beneficial to the field of synthetic biology, even beyond the DETECTOR biosensor application. Knowing more about how ETEC virulence works, and how ETEC communicate with each other, can potentially allow for the eradication of the disease.

The following are a few examples of how the ETEC quorum sensing system can be used in further applications, such as pathogen/pest management. The QS signals can be inhibited to control the virulence expression. This could be used in the treatment of bacterial infections in humans. This can also be used to change the characteristics of the contaminated water (March, 2004). Additionally, the quorum sensing system can be used in recombinant gene expression. Novel genes can be put under the quorum sensing to express some sort of inhibitor of virulence. Therefore the inhibitor will be expressed at the same time as the virulence factors, blocking them from fully expressing virulence (March, 2004).

7.2 DETECTOR Biosensor System

The DETECTOR biosensor was designed to detect the presence of ETEC in drinking water, using the quorum sensing operon discovered in the previous design of experiment. The system consists of two modules: adhesion and detection.

The DETECTOR biosensor was found to be reusable almost indefinitely, as long as the cells are maintained properly. It is also self-sufficient, since it does not require the use of any hard to obtain external equipment, only requiring the occasional re-seeding of additional cells and maintenance with sugar water. It is also designed to be portable (vessel size is approximately 1000 mL), and has a fast response (based on preliminary modelling), making it feasible for field analysis.

For pilot testing, it will be recommended only for use by trained personnel, but it is believed that the system is so easy to use, that it can be. Thus, 5 out of 6 success criteria have been met. The last success criterion (inexpensive) was not attained. The base cost of the DETECTOR biosensor was calculated to be very expensive.

The major cost of the system is the cost of the resin. Thus, the adhesion module should be modified or replaced in order for the system to meet all 6 success criteria.
8.0 Recommendations

In order to make the DETECTOR biosensor more feasible, it is recommended to seek sponsorship from NGOs to subsidize the initial biological cost for the discovery of the quorum sensing operon and the creation of the detection module of the DETECTOR biosensor.

As well, it is recommended to seek sponsorship from resin providers to provide the resin at a discount, so they could receive incentives for charitable discounts through federal tax breaks.

More realistically, the current adhesion module must be changed to decrease the price of the DETECTOR biosensor, and to meet the final success criteria. The current histidine tag method requires expensive resins but a different type of resin that is cheaper could be considered with a more detailed cost benefit analysis. Alternative mechanisms for keeping the biosensor cells within the vessel should also be explored.

Finally, experimental testing and implementation is needed to obtain more accurate results of system behavior.
9.0 Appendices

Appendix A: Mutation Rate of Biosensor

(Crossman, 2010), (Ross, 2010)

\[
E(X) = E(X|F)P(F) + E(X|N)P(N)
\]

\[
E(X|F) = \frac{1}{T} = \frac{1}{(\text{mutation rate})^{-1}} = \frac{1}{\left(2.5 \times 10^{-6} \text{ mutations/min}\right)} = 400,000 \text{ mins mutation}
\]

\[
E(X|N) = E(T + X) = T + E(X)
\]

\[
E(X) = TP(F) + (T + E(X))P(N)
\]

\[
E(X) = \frac{TP(F) + TP(N)}{1 - P(N)}
\]

\[
P(F) = \frac{13 \text{ foreign genes}}{13 \text{ foreign genes} + 4100 \text{ native genes}} = 0.003
\]

\[
P(N) = 1 - 0.003 = 0.997
\]

\[
E(X) = 253.678 \text{ years}
\]

Appendix B: Matlab Code for Modelling

**M. File - Parameters**

\[
\begin{align*}
k1 &= 1.3; & \%(1/s), \text{TRP + ABCE } \rightarrow \text{ ES1} \\
k2 &= 1e-2; & \%(1/s), \text{ES1 } \rightarrow \text{ TRP + ABCE} \\
k3 &= 3e2; & \%(1/s), \text{ES1 } \rightarrow \text{ G + ABCE + D} \\
k4 &= 1e3; & \%(1/s), \text{G + ABCE + D } \rightarrow \text{ ES2} \\
k5 &= 1e-1; & \%(1/s), \text{ES2 } \rightarrow \text{ G + ABCE + D} \\
k6 &= 3e3; & \%(1/s), \text{ES2 } \rightarrow \text{ P + D} \\
E_{\text{tot}} &= 5.5e-4; & \%(M), \text{Total concentration of Initial} \\
kp_{\text{trp}} &= 7.2802e-4; & \%(1/s) (\text{Fooladi, Fig3}) \\
kd_{\text{trp}} &= -1.94e-5; & \%(1/s) (\text{O'Hara General kd for protein}) \\
b &= 6.6; & \%\text{Hill function parameters} \\
n &= 1.6; \\
kd &= 5000; \\
s &= 0;
\end{align*}
\]
kd_d = -5e-5; %Degradation rate of VioD
kd_g = -8.5e-4; %Degradation rate of Green
kd_p = -1.94e-7; %Degradation rate Purple

% y0(1)=4.7e-4; %Initial amount of L-Tryptophan
% y0(2)=0; %Initial amount of ES
% y0(3)=0; %Initial amount of P

tspan = 0 : 0.01 : 5000;
[t y] = ode23 (@FYDP_mm6_graph1_FINAL, tspan, [1e-3 0 0 0 0 0], [], k1, k2, k3, k4, k5, k6, E_tot, kp_trp, kd_trp, kg, kd, kd_p, b, n, kd, s);

Trp = y(:, 1)*1000;
W_ABCE = y(:, 2)*1000;
ABCE = E_tot*1000 - W_ABCE;
G = y(:, 3)*1000;
D = y(:, 4)*1000;
G_D = y(:, 5)*1000;
P = y (:, 6)*1000;

plot(t,Trp,'k', t,ABCE,'b', t,G,'g',t,P,'m', t,D,'--r', 'LineWidth',3)
xlabel('Time (Seconds)'), ylabel('Concentration (mM)')
legend('Trp', 'Vio ABCE', 'Green', 'P', 'Vio D')

M. File - ODE

function dpdt = FYDP_mm6_graph1_FINAL(~,y, k1, k2, k3, k4, k5, k6, E_tot, kp_trp, kd_trp, kg, kd, kd_p, b, n, kd, s)

%y(1)=[Trp],
%y(2)=[W-ABCE]=[ES1],
%y(3)=[G],
%y(4)=[D],
%y(5)=[G-D]=[ES2],
%y(6)=[P]

dpdt =[-k1*E_tot*y(1) + (k1*y(1)+k2)*y(2) + kp_trp * y(1) + kd_trp * y(1)
    k1*E_tot*y(1) - (k1*y(1)+k2+k3)*y(2);
    k3*y(2) - k5*y(5) - k4*y(3)*y(4)* (E_tot-y(2)) + kd_g*y(3); 
    (k5*k6)*y(5) - k4*y(3)*y(4)* (E_tot-y(2)) + kd_d*y(4) + b*(s^n)/(kd^n+s^n);
    k4*y(3)*y(4)* (E_tot-y(2)) - (k5+k6)*y(5);
    k6*y(5) + kd_p*y(6)];

Appendix C: Concentration of violacein visible to the naked eye

A = εcl

Where,

ε, Extinction Coefficient = 17 × 10^6 cm^2/mol

A, Absorbance = 0.47
Concentration of violacein = 0.48mM

Appendix D: Resin Volume
At $5.96/mL resin for histidine-tagged protein.

Upper Estimate Volume of Resin
\[\text{Upper Estimate Volume of Resin} = \left( \frac{1\text{mL resin}}{10\text{mg protein}} \right) \times (3250 \text{ mg protein or biosensor cells necessary})\]
\[= \frac{3250}{10} \text{ mL resin} = 325\text{mL resin}\]

Lower Estimate Volume of Resin
\[\text{Lower Estimate Volume of Resin} = \left( \frac{1\text{mL resin}}{25\text{mg protein}} \right) \times (3250 \text{ mg protein or biosensor cells necessary})\]
\[= \frac{3250}{25} \text{ mL resin} = 130\text{mL resin}\]

Appendix E: Resin Cost
\[\text{Cost of resin} = \frac{$5.96}{\text{mL resin}} \times \left( \frac{3250}{10} \right)\]
10.0 References


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