Developing the Fluoride Riboswitch as a Technology to Combat Excessive Water Fluoridation

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Introduction

Fluoride is present in all bodies of water. Within the oceans, rivers, lakes, and groundwater, the levels of fluoride depend on the erosion of the sediments in the area. When fluoride concentrations are at toxic level, major health concerns arise. According to WHO, concentrations at or above 1 mg/kg of body weight are deemed poisonous and recommends concentrations of fluoride do not exceed **1.5mg/mL** in water sources. Constant exposure to 10 mg/L to 6 mg of fluoride can lead to fluorosis and developmental and reproductive concerns (**Fig 1**).

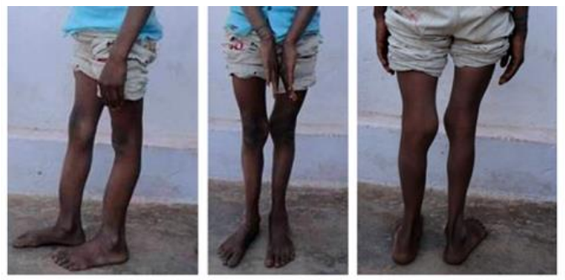


Fig. 1: A child with skeletal fluorosis
http://www.inrem.in/fluorosis/health.html

In certain areas in countries like China, India, and Sri Lanka, water sources are decentralized and residents can experience concentrations of fluoride as high as 30 mg/L (**Fig.2**).

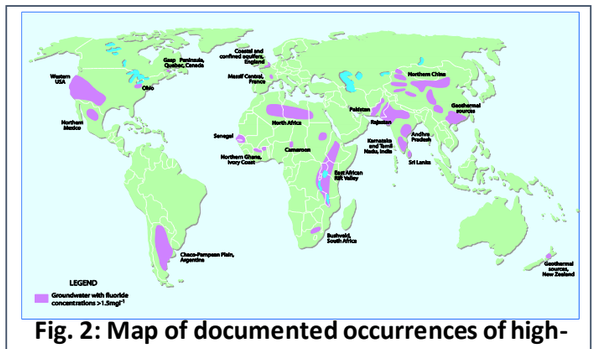


Fig. 2: Map of documented occurrences of high-fluoride groundwater

Edmunds W.M., Smedley P.L. (2013) Fluoride in Natural Waters. In: Selinus O. (eds) Essentials of Medical Geology. Springer, Dordrecht

Our Solution

We envision developing technologies utilizing fluoride riboswitches that can sequester, bioremediate, or detect fluoride in water . . . *but what is a fluoride riboswitch?*

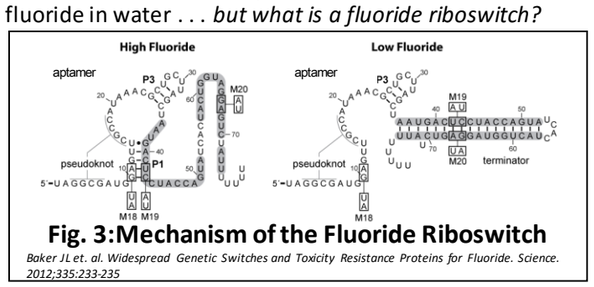
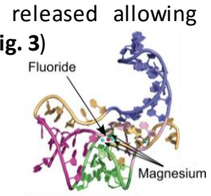


Fig. 3:Mechanism of the Fluoride Riboswitch

Baker JL et. al. Widespread Genetic Switches and Toxicity Resistance Proteins for Fluoride. Science. 2012;335:233-235

The **Fluoride Riboswitch** is an RNA transcriptional riboswitch that contains two functional elements: the aptamer, which binds to fluoride, and the terminator which regulates transcription of the downstream gene. When fluoride is present the terminator is released allowing transcription of the downstream gene (**Fig. 3**)



Crystal structure of the fluoride riboswitch aptamer domain

Ren A et. al,"Fluoride ion encapsulation by Mg2+ ions and phosphates in a fluoride riboswitch" 2012 Nature 486, 85–89

Our Design: CHOP

In order to develop technologies using the fluoride riboswitch we first need a platform to characterize the responsiveness of fluoride riboswitches that was amenable to high-throughput selection methodologies. To achieve this we developed the **fluoride riboswtich regulated chloramphenicol acetyltransferase operon (CHOP) (Figure 4).** To determine if CHOP would work we used the fluoride riboswitch from *B. cereus* which has been characterized in the literature

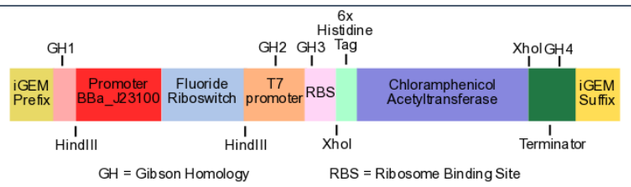


Figure 4) Architecture of CHOP

How CHOP works:

- Chloramphenicol acetyltransferase (CAT) provides resistance to the antibiotic chloramphenicol

- When sufficient fluoride enters the cell to activate the riboswitch CAT is transcribed allowing the bacteria to grow

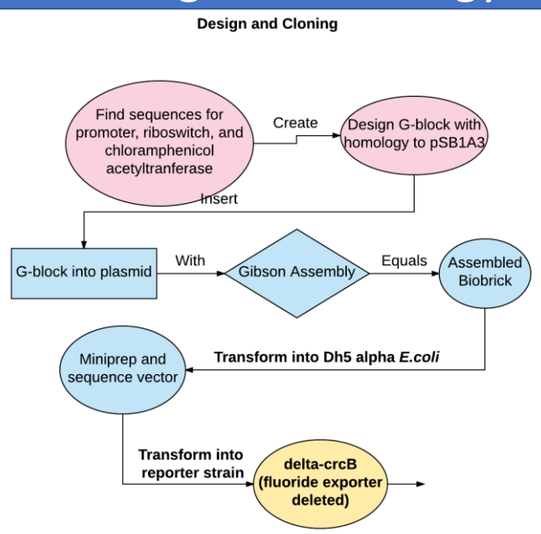
- CHOP needs to be used in *E. coli* that have the fluoride exporter gene crcB deleted (ΔcrcB) so that fluoride can accumulate inside the cell. **In the literature ΔcrcB is reported to be sensitive to fluoride concentrations above 500µM.**

How can YOU use CHOP:

- Characterize Transcriptional Riboswitches: New promoter riboswitch pairs can easily be cloned in using Gibson: order at G-block with appropriate overhangs (see the sequence for part BBa_K2290000 to find the appropriate overhangs) and linearize the vector with HindIII. This system should be amenable to screen any transcription riboswitch.

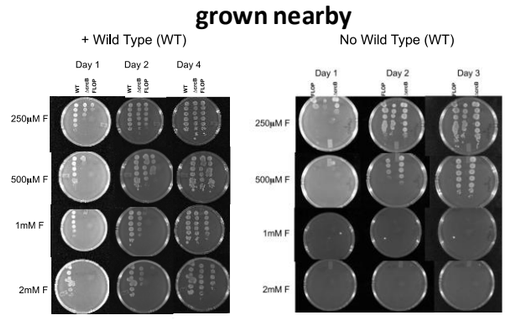
-Regulate New Genes with Transcriptional Riboswitches: New genes of interest can easily be cloned in using Gibson: order at G-block with appropriate overhangs and linearize the vector with XhoI.

Cloning Methodology

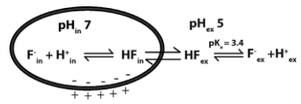


Results

WT *E. coli* protect ΔcrcB from fluoride toxicity when grown nearby

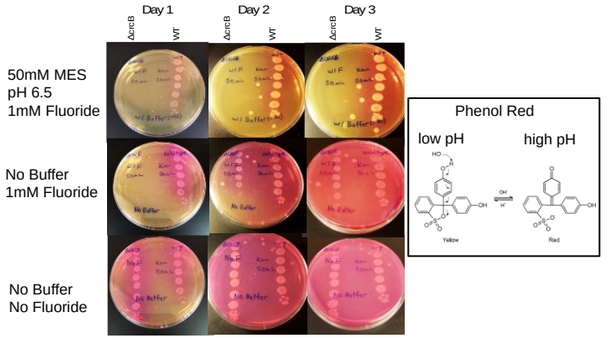


Intracellular fluoride accumulation is pH-dependent

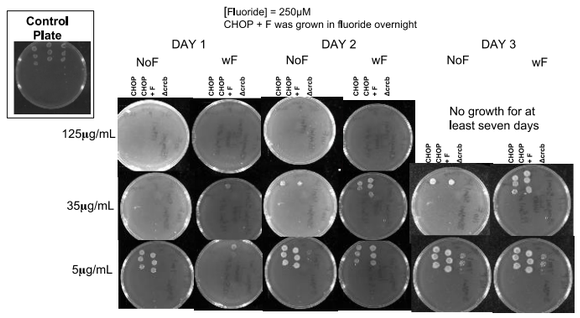


Ji C. et al. J Gen Physiol. 2014 144(3):257-61

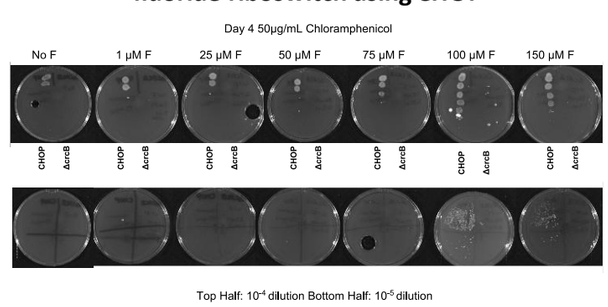
WT *E. coli* alter the pH of the agar plates to protect ΔcrcB from high concentrations of fluoride



Fluoride dependence of CHOP growth on different chloramphenicol concentrations



Characterizing the responsiveness of the *B. cereus* fluoride riboswitch using CHOP



Conclusion

- CHOP allows for characterization of the responsiveness of fluoride riboswitches
- WT *E. coli* can protect the ΔcrcB strain from fluoride toxicity by altering the pH of the plates
- The *B. cereus* fluoride riboswitch works best at 100µM fluoride concentration (1.8mg/mL). Therefore riboswitches with improved responsiveness to fluoride would be desirable for future applications.

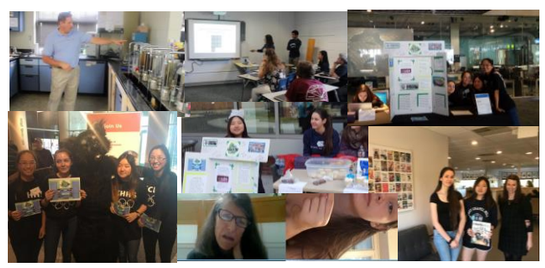
Future Directions

First, we will use CHOP to screen for native riboswitches or evolve the *B. cerues* riboswitch to find riboswithces with varied responsiveness to fluoride. With these tools we hope to:

- **Sequester** fluoride from drinking water by attaching many copies of the RNA to nano-particles.
- **Bioremediate** fluoride-contamination water by controlling the expression of enzymes that can metabolize fluoride (i.e. fluorinase)
- **Detect** fluoride-contaminated water by controlling the expression of reporter genes (i.e. fluorescent proteins) with a suite of riboswitches with differential responsiveness to fluoride.

Human Practices

- Darwin Day at the Raleigh Museum of Natural Sciences
- OWASA facility tour + documentary style video
- Collaboration in creating a collection of Tasty-Lab Videos (https://www.youtube.com/watch?v=TraYbTe7Lh8)
- Hosted Southeastern Mini iGEM Convention
- Reddit forum collaboration with Duke regarding the ethics of synthetic biology
- Rewriting the Building with Bio Malaria Kit → pertinent issue at this current point in time (the Zika Virus) with Duke and Gaston Day
- Interviews with Laura Craig (https://www.youtube.com/watch?v=qrI9hiCimz8) and Anitha Pius



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