Bioprospector: Adding Senses to the Sensor
Team Lethbridge, Alberta, Canada
Brainstorming

- Natural resources are a very important part of Canada
  - Alberta
    - Oil, Coal, water, minerals
Wastes

- Prospecting, Extraction
- Remediation
  - Tailings ponds
Project

- Use a biological answer
  - Sense novel molecules
  - Process novel molecules

- This year
  - Focus on sensing
  - Sensor Characteristics
    - Something highly selective and customizable
Riboswitches

- RNA-encoded genetic control elements
- Ligand-dependent regulation
- Could be engineered to respond to any cell-permeable molecule
  - Ex. metals, organics

Structure

- Comprised of:
  - Aptamer domain
    - binds the ligand
  - Expression platform

- Conformational change in expression platform key to regulation
Function

- Riboswitches work in four basic ways:

Transcription Termination

Autocleavage

Translation Inhibition

Translation Initiation
Synthesis

- The aptamer is:
  - short (~ 50 nucleotides),
  - easily produced by DNA synthesis.

- Synthetic riboswitches will allow:
  - Custom design
  - Control of cellular behavior.
**Development**

- Schematic for custom riboswitch production

1. Create random riboswitches
2. Insert riboswitches into 5’ UTR of GFP
3. Grow cells in presence of target ligand
4. Using flow cytometry, sort cells into expressers and non-expressers
5. Grow in absence of ligand or presence of similar ligands
6. Grow at target concentration
7. Sequence novel custom riboswitch
Project

- Found riboswitch for proof of concept
  - Theophylline riboswitch (Topp and Gallivan, 2007)
  - Paper presented easy way of controlling chemotaxis as well

Motility

- CheZ enables run mode
- Lack of CheZ causes tumbling
Motility

- Couple the riboswitch with the CheZ gene
- Transform into CheZ knockout
- Control chemotaxis
Reporter

- Couple riboswitch (RS) with common variable reporter system (Basu et. al., 2005)

![Diagram of RS + LacI, TetR, and GFP and RFP pathways]

Difficulties

- RFP and LacI transformations failed
  - Easy fix – use $\lambda$cl, RFP intermediate, and $\lambda$cl regulated promoter
Schematic
More difficulties

- Ran out of time
  - Easy fix – slash variable reporter to single reporter
Accomplishments

- Both CheZ and riboswitch were made into biobrick format
  - Not tested

- Single reporter test construct and chemotaxis model
  - In progress
Future

- Finish proof of concept
- Develop potential chemical pathways
  - Ex. Waste remediation, metal extraction
- Synthesize riboswitch using target molecule for screening
Overall Goal

- Large scale synthesis of novel riboswitches
- General characterization of riboswitches
- Rational design of riboswitches
- Attach riboswitches to:
  - reporter systems
  - motility pathways
  - metabolic pathways
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Questions?