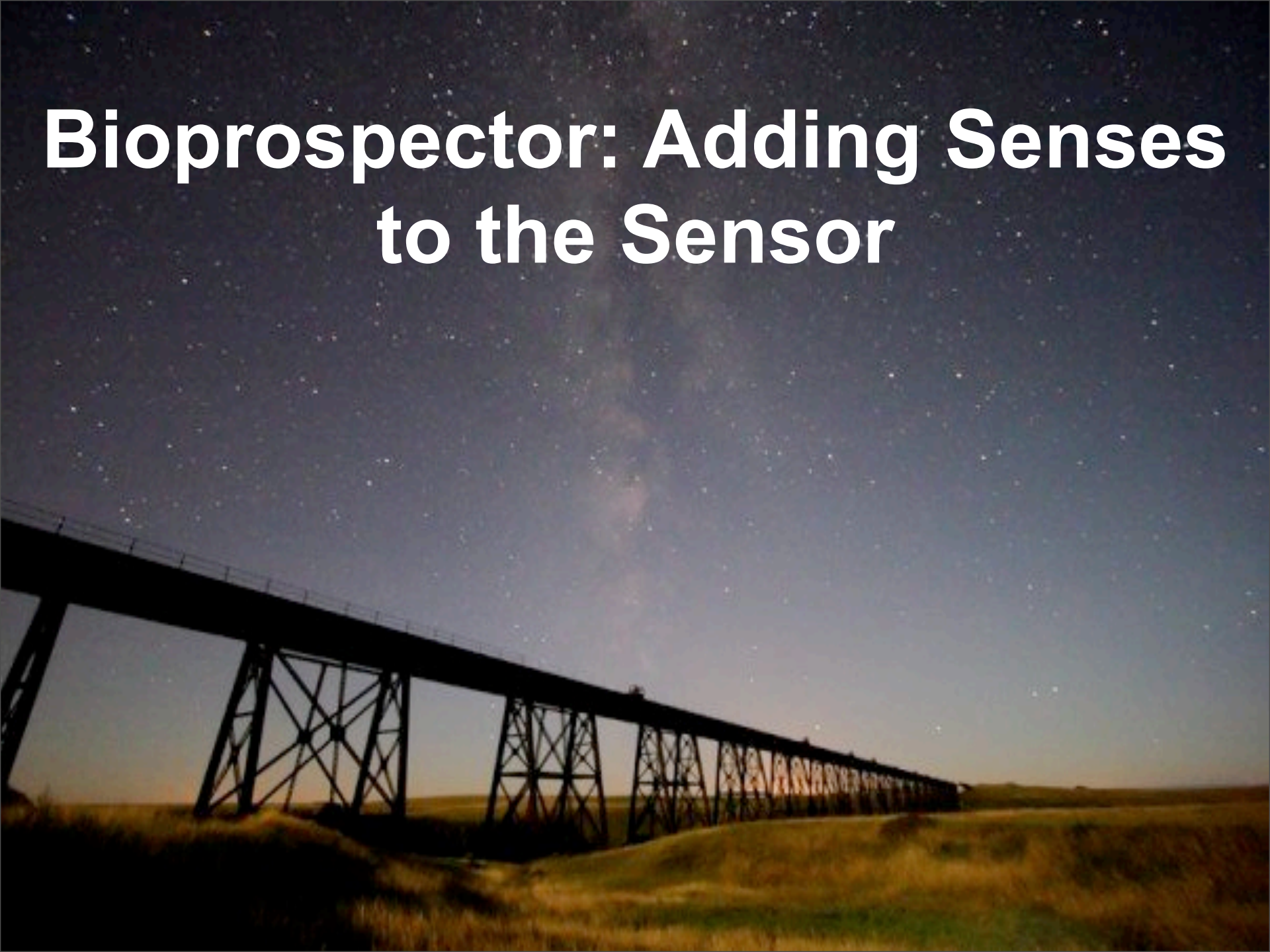
Bioprospector: Adding Senses to the Sensor



Team Lethbridge, Alberta, Canada

University of Lethbridge

Brainstorming

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

University of Lethbridge

Wastes

- Prospecting, Extraction
- Remediation
  - Tailings ponds

University of Lethbridge

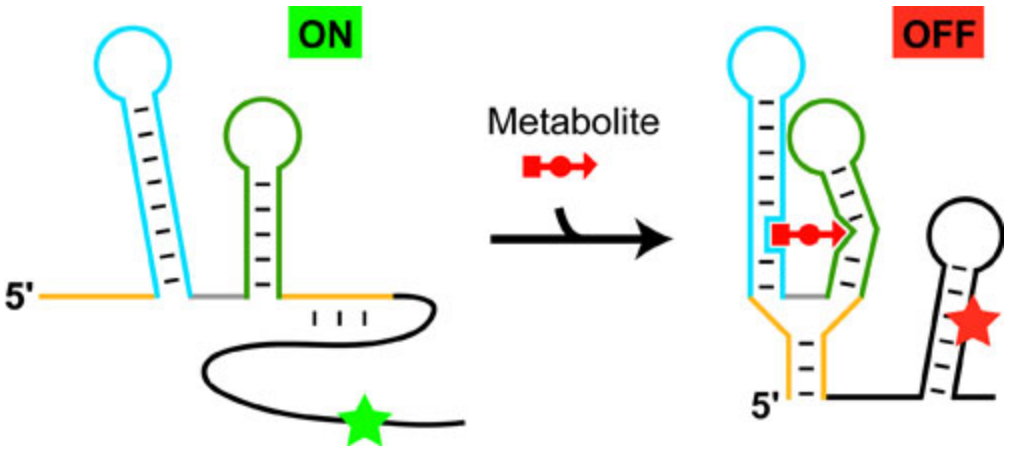
Project

- Use a biological answer
  - Sense novel molecules
  - Process novel molecules
- This year
  - Focus on sensing
  - Sensor Characteristics
    - Something highly selective and customizable

University of Lethbridge

Riboswitches

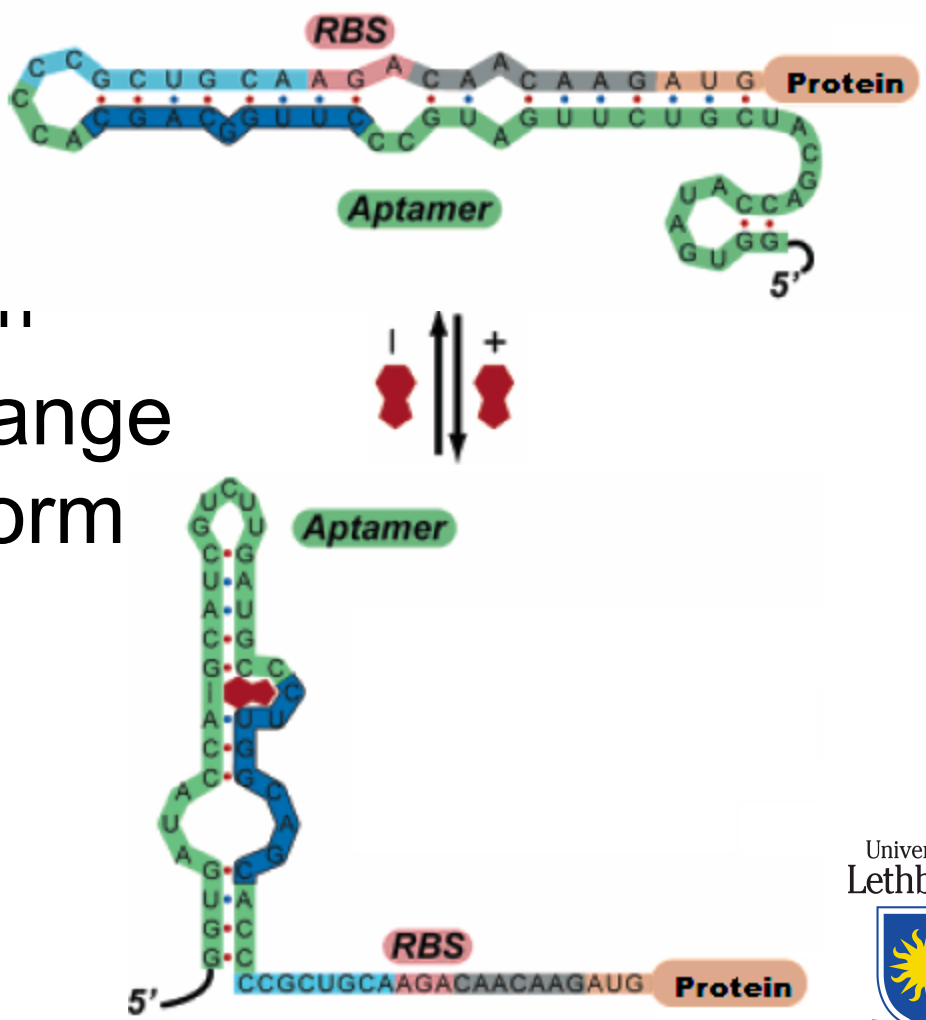
- RNA-encoded genetic control elements
- Ligand-dependent regulation
- Could be engineered to respond to any cell-permeable molecule
  - Ex. metals, organics



Serganov, A., Polonskaia, A., Phan, A.-T., Breaker, R.R., Patel, D.J., "Structural basis for gene regulation by a thiamine pyrophosphate-sensing riboswitch," *Nature* **441**, 1167-1171 (2006).

Structure

- Comprised of:
  - Aptamer domain
    - binds the ligand
  - Expression platform
- Conformational change in expression platform key to regulation

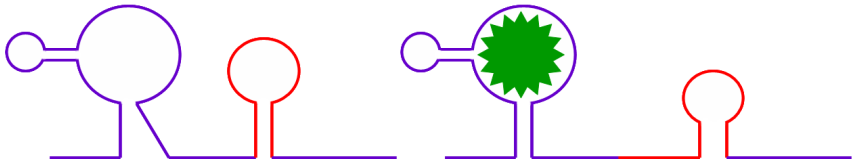


University of Lethbridge

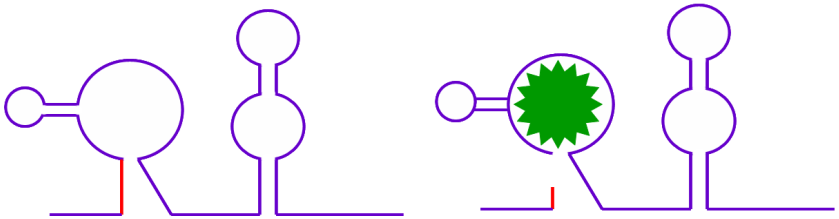
Function

- Riboswitches work in four basic ways:

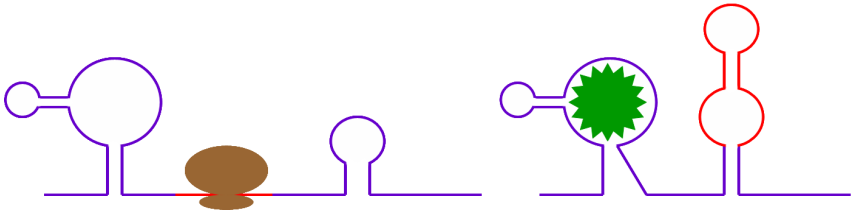
Transcription Termination



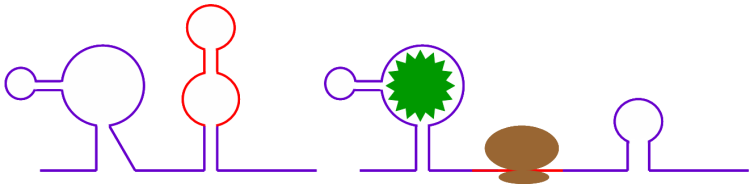
Autocleavage



Translation Inhibition



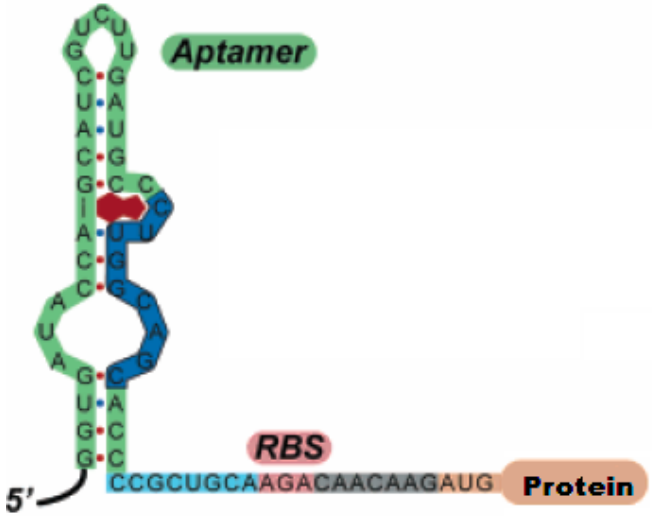
Translation Initiation



University of Lethbridge

Synthesis

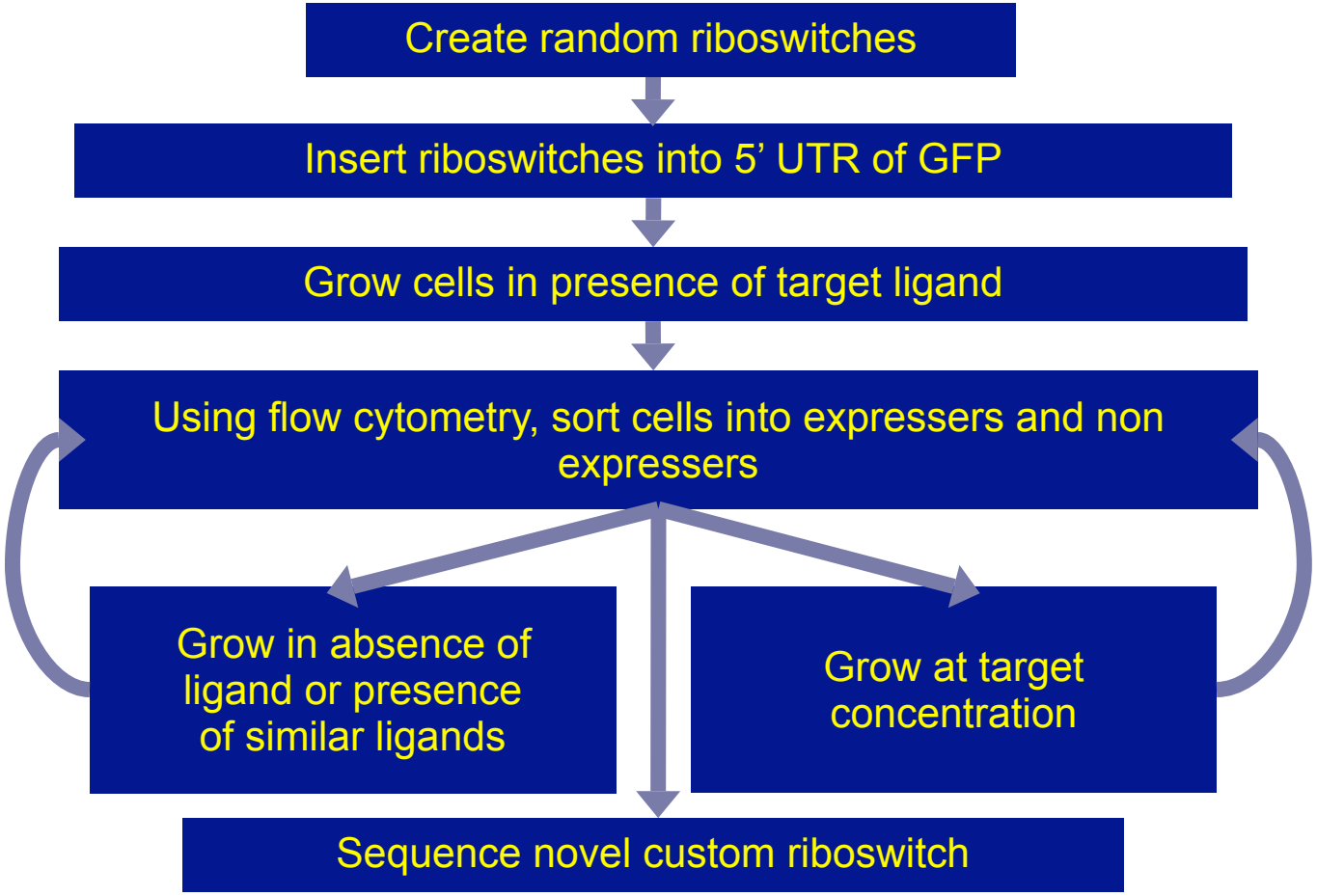
- The aptamer is:
  - short (~ 50 nucleotides),
  - easily produced by DNA synthesis.
- Synthetic riboswitches will allow:
  - Custom design
  - Control of cellular behavior.



University of Lethbridge

Development

- Schematic for custom riboswitch production



University of Lethbridge

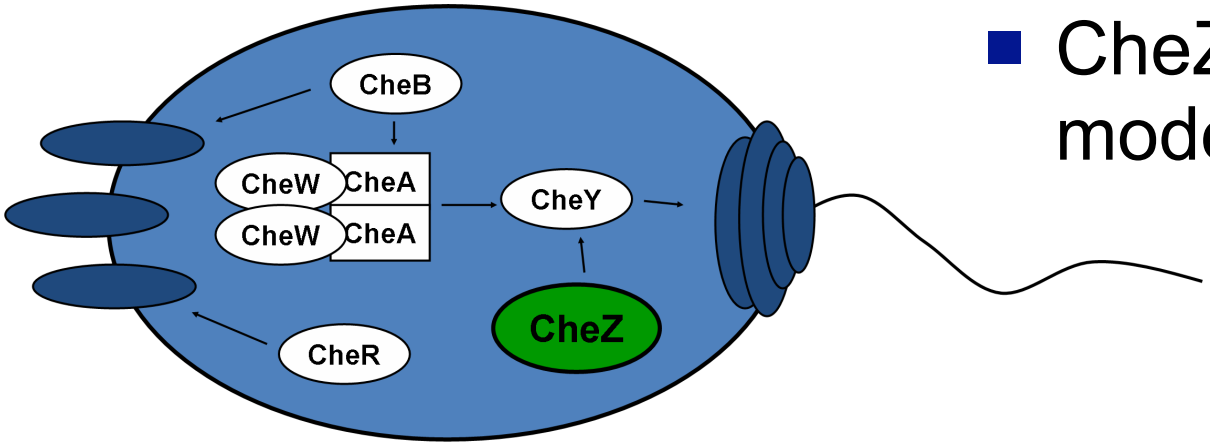
Project

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

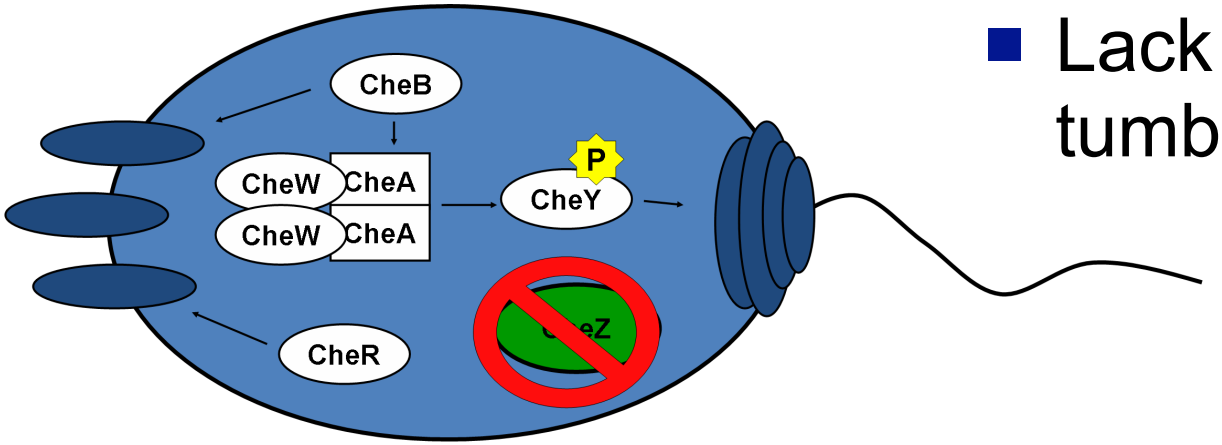
Topp, S and J. P. Gallivan. 2007. Guiding bacteria with small molecules and RNA. J. AM. Chem. Soc. 129:6807-6811.

University of Lethbridge

Motility



- CheZ enables run mode

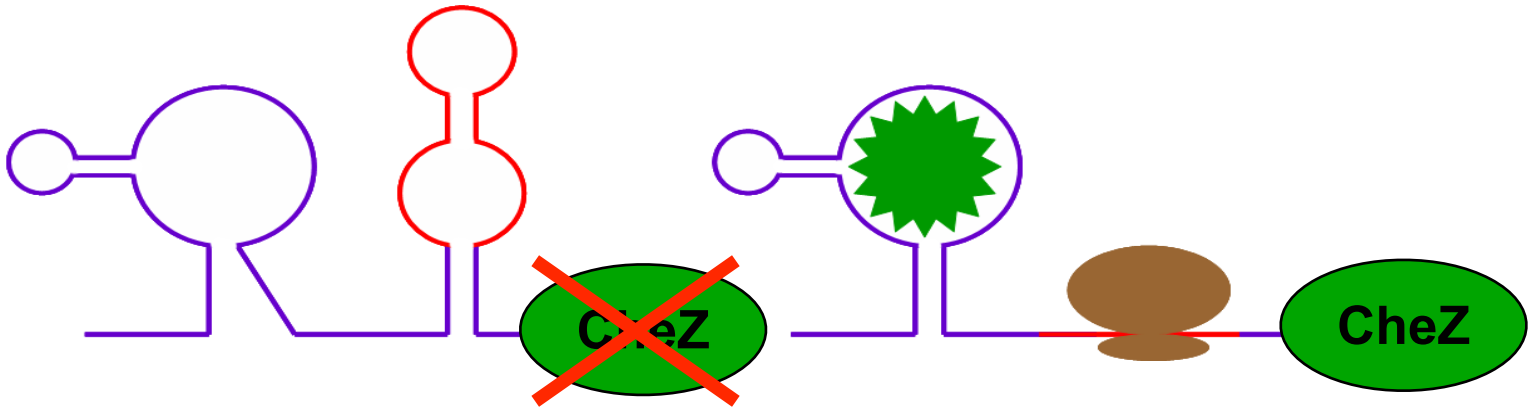


- Lack of CheZ causes tumbling

University of Lethbridge
FIAT LUX

Motility

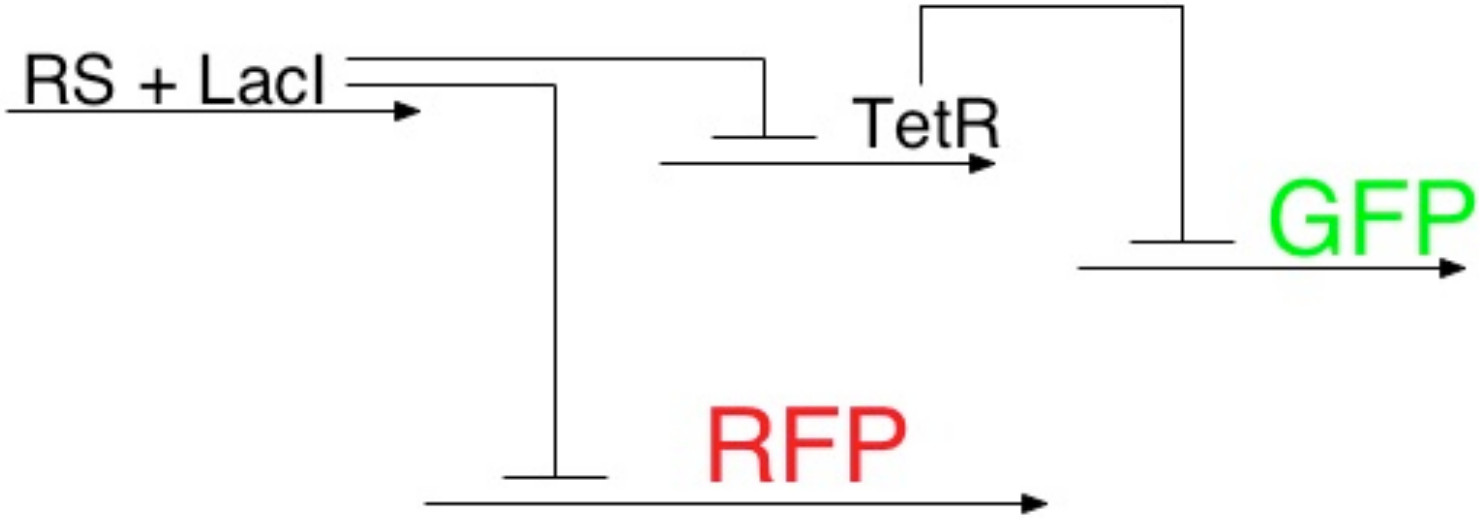
- Couple the riboswitch with the CheZ gene
- Transform into CheZ knockout
- Control chemotaxis



ersity of
Lethbridge
FIAT LUX

Reporter

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

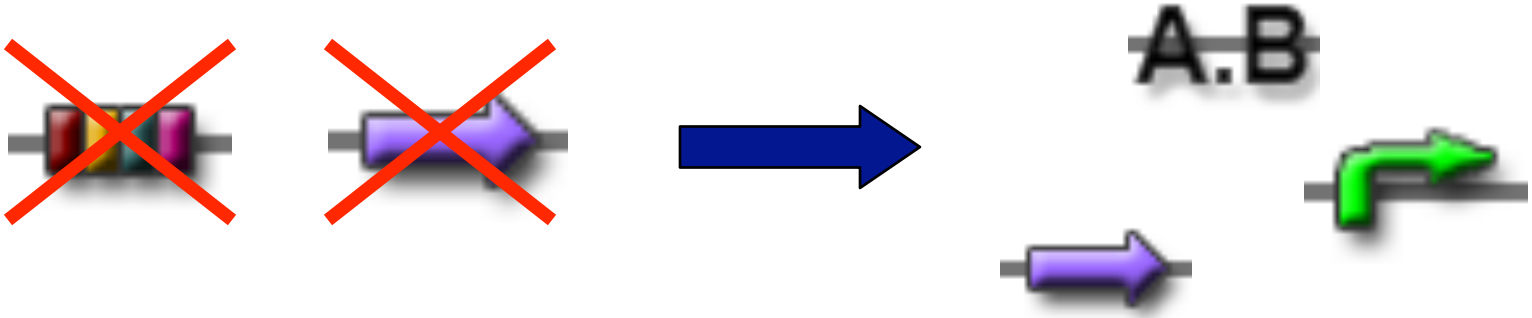


Basu S., Y. Gerchman, C. H. Colins, F. H. Arnold, & R. Weiss. 2005. A synthetic multicellular system for programmed pattern formation. Nature. 434:1130-1134.

University of Lethbridge

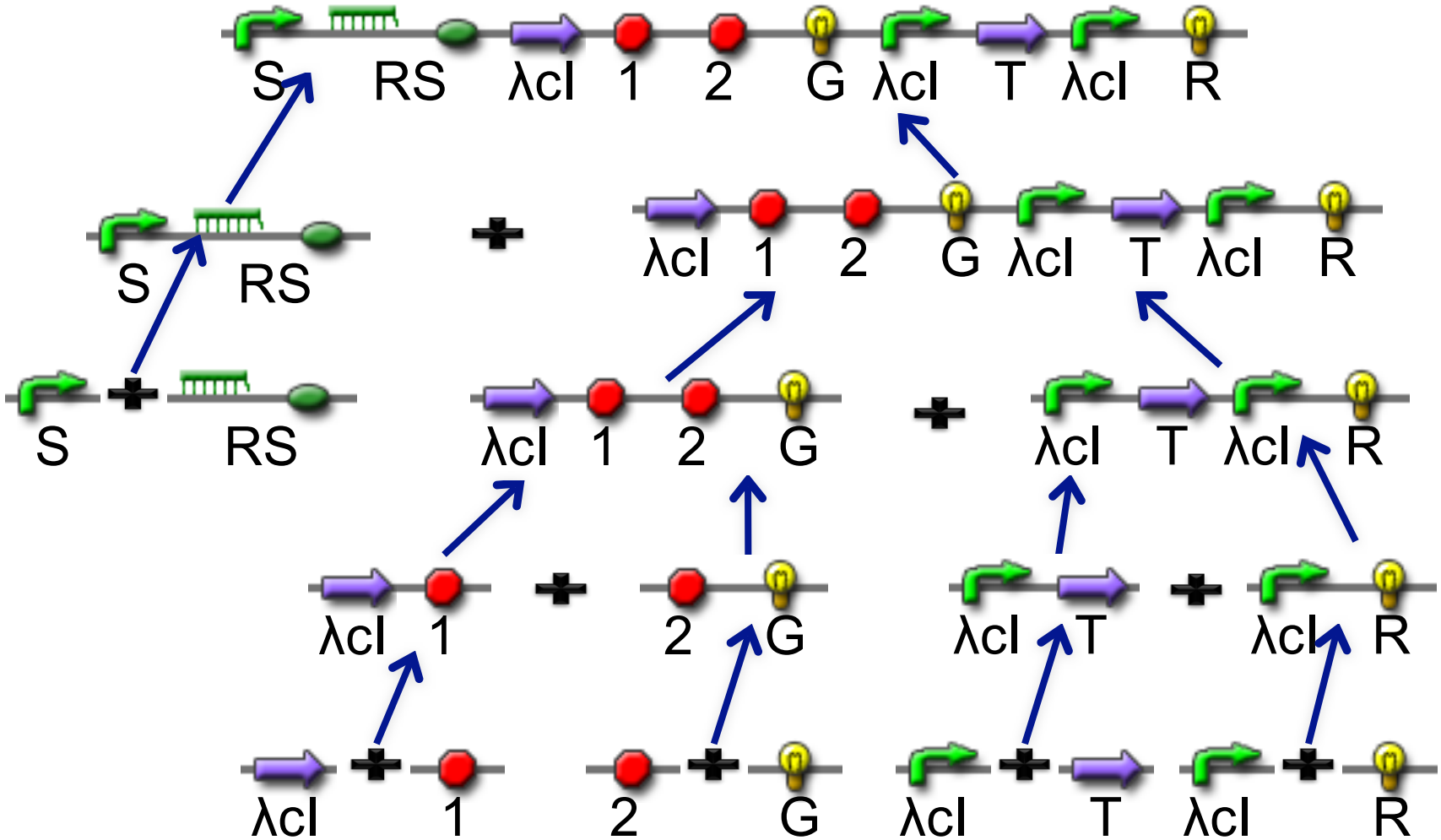
Difficulties

- RFP and LacI transformations failed
  - Easy fix – use λcI, RFP intermediate, and λcI regulated promoter



University of Lethbridge

Schematic



University of Lethbridge

More difficulties

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

University of Lethbridge

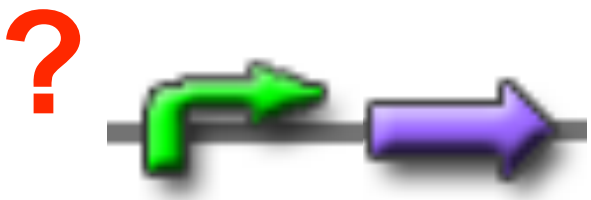
Accomplishments

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

I752000

I752001

- Single reporter test construct and chemotaxis model
  - In progress



University of Lethbridge

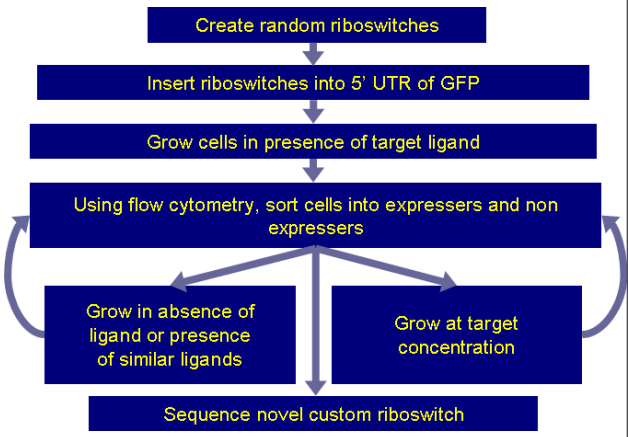
Future

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

University of Lethbridge

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



University of Lethbridge

Acknowledgements

- Dr. Justin Gallivan, Emery University for DNA and cells
- Dr David Simonovich, Dr Marc Roussel
- Department of Chemistry and Biochemisty, and Department of Biology and Faculty of Arts and Science

Innovation & Precision In Nucleic Acid Synthesis

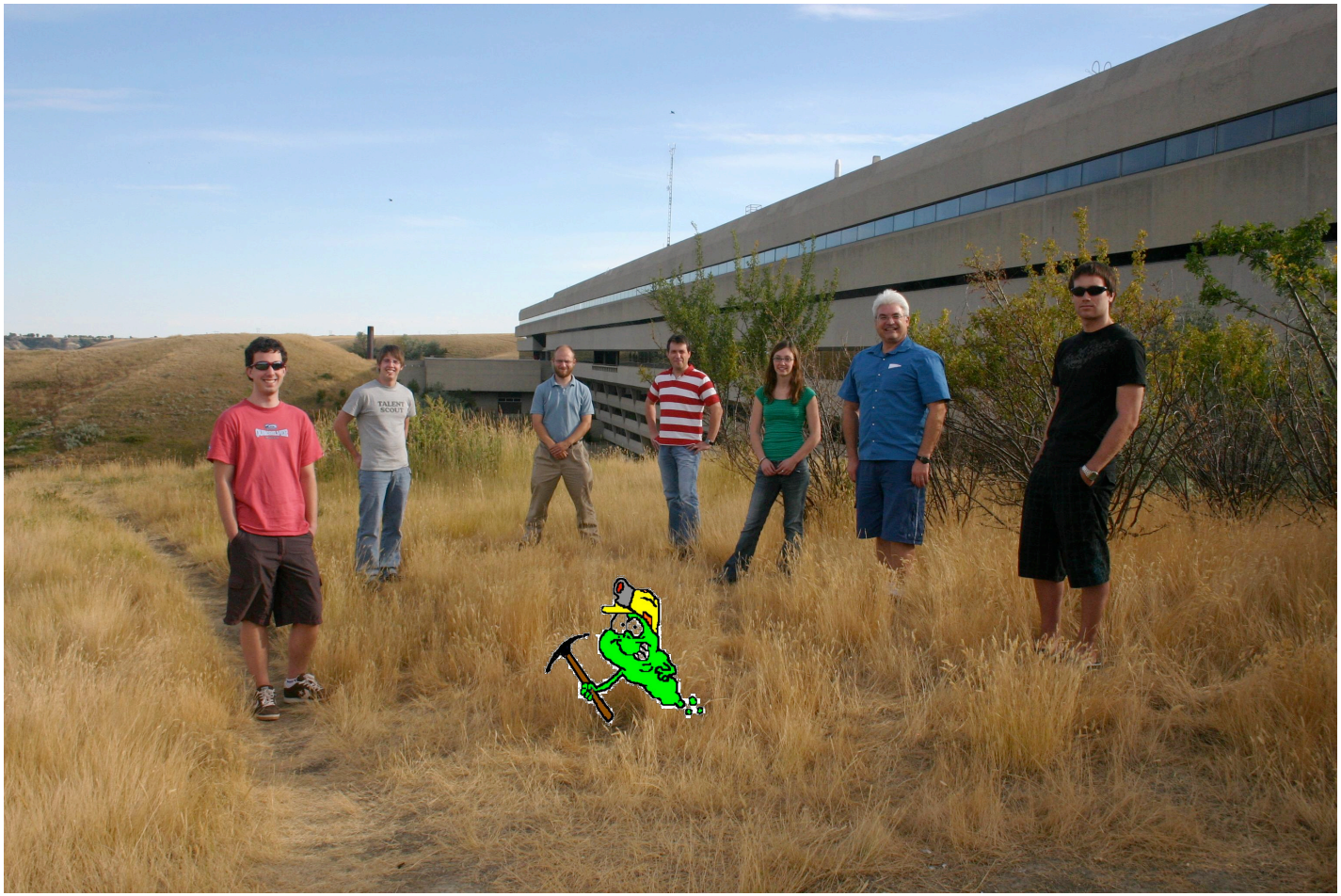
IDT® INTEGRATED DNA TECHNOLOGIES

ALBERTA INGENUITY FUND

University of Lethbridge

FIAT LUX

Team Lethbridge



University of Lethbridge

Questions?