ENGINEERING SARCOSINE AND URIC ACID APTAMERS VIA RIBOSWITCH-BASED DUAL SELECTION FOR BIOMARKER SENSOR

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BACKGROUND

- Riboswitches are RNA elements that regulate gene expression via ligand-binding
  - It consist of 2 parts: an **aptamer** (detect ligands) and **expression platform** (turn on/off genes)
  - Enable **low-cost, cell-based biosensors**
- Prospective biomarkers:
  - **Uric acid**: gout
  - **Sarcosine**: prostate cancer

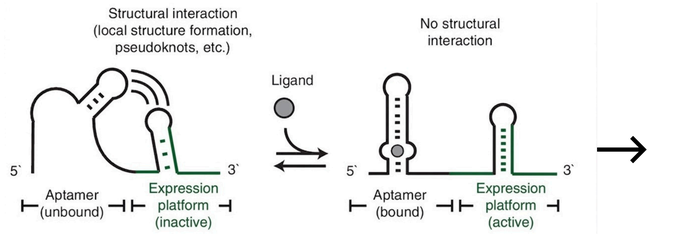


Figure 1. Riboswitch compostion and gene regulation mechanism

RESEARCH OBJECTIVE

- Engineer **low-cost, cell-based biosensor** via libraries derived from theophylline and glycine riboswitch scaffolds
  - **Uric acid**: Develop riboswitches that are triggered by uric acid via **dual selection**
  - **Sarcosine**: Evaluate the selectivity for sarcosine and responder activation of riboswitches (A6, C9) via **LacZ and GFP assays**

DUAL SELECTION

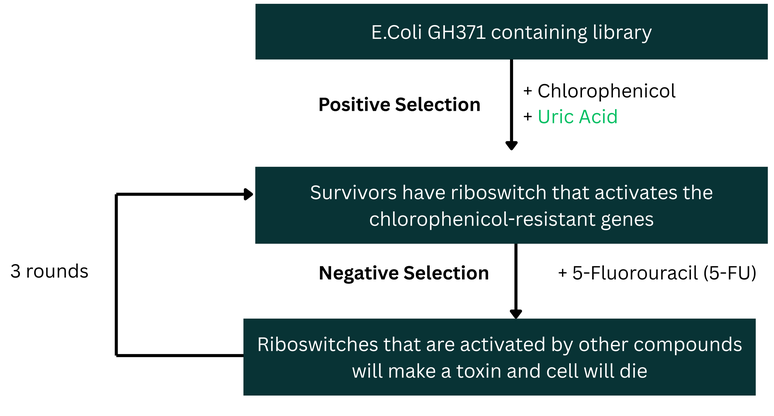


Figure 2: Dual Selection for uric acid-specific riboswitch

EVALUATE SELECTIVITY

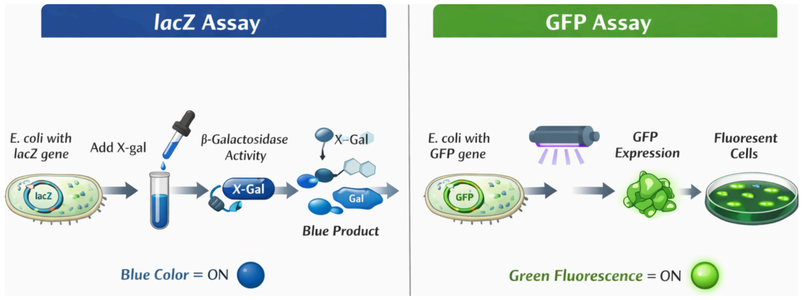


Figure 3: Protocol of LacZ and GFP assay to assess selectivity of sarcosine riboswitch

RESULTS

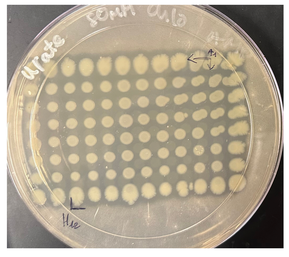
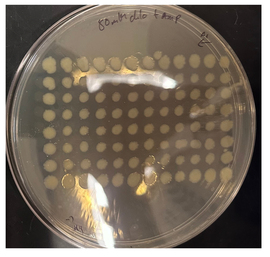
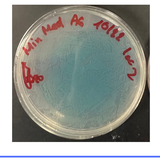
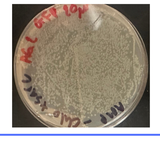
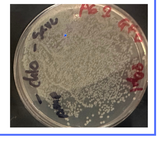
	- Uric acid	+ Uric acid
Dual Selection		

Table 1: Testing of 96 clones after 3 rounds of dual selection for uric acid-specific riboswitch. 0/96 clones showed uric-acid dependent growth.

A6	- Sarcosine	+ Sarcosine
LacZ		
GFP		

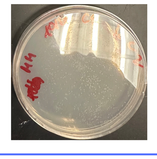
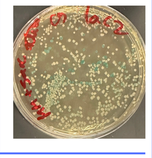
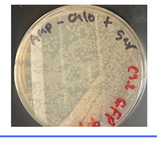
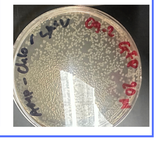
C9	- Sarcosine	+ Sarcosine
LacZ		
GFP		

Table 2: Sarcosine-induced expression of LacZ and GFP assay in A6 and C9 riboswitch. 0/2 riboswitch candidates (A6, C9) showed sarcosine-dependent activation in LacZ or GFP assays

CONCLUSIONS

- Uric acid: **No potential targets have been identified** through 3 cycles of dual selection
  - High selective pressures and more rounds of dual selection could yield targets that is selective for uric acid
- Sarcosine: **Neither A6 nor C9 riboswitches showed measurable activation** in LacZ or GFP assays in response to sarcosine.
  - This could be due to the the expression platform not being compatible with GFP and LacZ gene regulation.

FUTURE DIRECTIONS

- Uric acid: **More rounds of dual selection** can be done with higher concentration of chlorophenicol and 5-FU can be done to **increase the selective pressure.**
- Sarcosine: More re**search on the compatibility of the expression platform** with reporter genes can be done to identify suitable genes for assay.
- Future project will explore the use of **libraries of other compound** like Adenine to engineer riboswitch that are capable of detecting biomarkers of pathogens

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ACKNOWNLEDGEMENTS

TCU Department of Chemistry and Biochemistry | The Ryu Laboratory
TCU Science and Engineering Research Center Grant
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