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The Theophylline Riboswitch: Its Design and Implementation

Fluorescence Activated Cell Sorting, or FACS, is a method that was used to convert the theophylline aptamer into a riboswitch. This method could theoretically be used to convert other discovered aptamers into riboswitches however it is a costly method and is only available to those with these high-tech, expensive machines. The theophylline riboswitch was previously discovered by implementing the Theophylline aptamer with random sequences into a specifically-designed plasmid and using a FACS machine to sort the cells.

We can structure a new system that would select only the sequence containing the theophylline riboswitch without the use of a FACS machine. To do so, we place a pool of sequences containing the aptamer, which is linked to the Shine Dalgarno with eight random nucleotides, into a designed plasmid and transform the plasmid into bacteria cells. Then, the use of replica plating along with screening selects the cells that only contain the plasmid with the correct riboswitch sequence. By doing so, we confirm that this system is efficient in converting aptamers into riboswitches without the need for a FACS machine.

After an aptamer has been successfully converted into its riboswitch, the system of ratiometric fluorescence will allow for testing of the riboswitch's function. This is done by designing a plasmid that contains genes for red and blue fluorescence proteins, mCherry and eBFP respectively, on either side of the inserted riboswitch. A PCR product encoding for mCherry, the riboswitch, and eBFP will be inserted downstream of the lactose operon in pUC18. Ratios of the fluorescence intensities of the two florescent proteins will provide the ability to measure the riboswitch's function through fluorescence readings.

The Theophylline Riboswitch: Its Design and Implementation

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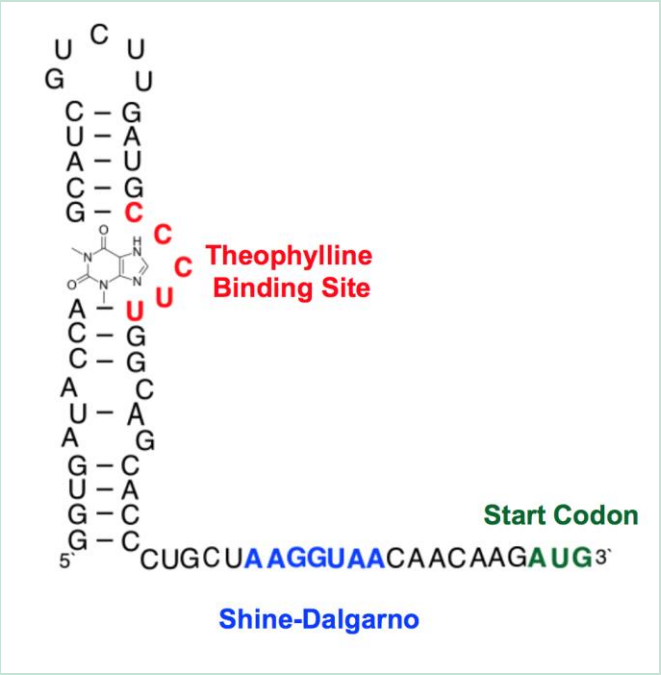


The Theophylline Riboswitch

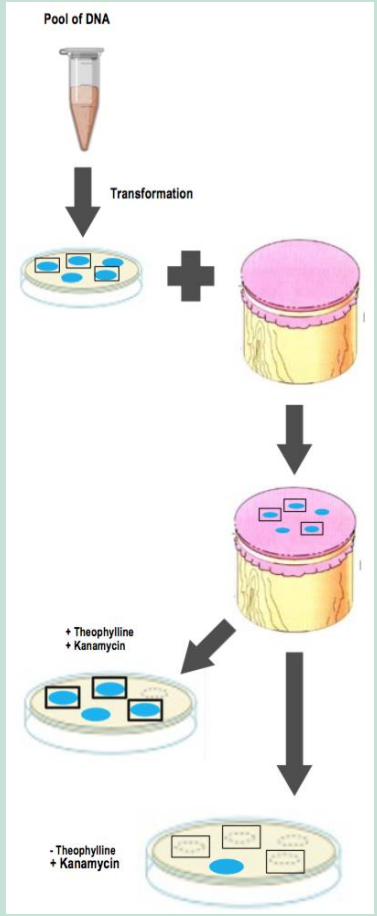
- Conversion of aptamers to riboswitches previously done by FACS
- FACS is a costly method – not attainable for small undergraduate research labs
- Converting aptamers to riboswitches allows for the potential of aptamers to be extended into the biomedical field
  - Therapies
  - Detection/Prognosis

- PART I
  - Replica Plating using Selection
    - Design and engineer a functional system to replacing FACS in converting aptamers into riboswitches
    - Using the theophylline riboswitch as a model

- PART II
  - Ratiometric Fluorescence
    - Assay for determining kinetic properties of newly discovered riboswitch
    - Negates fluorescence intensity interference from cell count

METHODS

REPLICA PLATING



PCR Product

TheoRS GFP

ApaI BpmI

Double Digestion
Ligation
Transformation

Tet-O

pHL1278

AmpR mCherry ORI GFP

ApaI T7 Term LacO BpmI Asp Term

Tet-O

pHL1720

AmpR mCherry ORI TheoRS GFP Asp Term

PCR

mCherry TheoRS GFP

KpnI SphI

Double Digestion
Ligation
Transformation

Lac-O

pUC18

LacZα ORI AmpR

Lac-O

MpTRFlac

mCherry ORI ApaI TheoRS GFP SphI Asp Term AmpR

PCR

TheoRS KanR

ApaI SphI

PCR

pJL1-eBFP2

eBFP ORI KanR

PCR

TheoRS eBFP

ApaI SphI

Double Digestion
Ligation
Transformation

Lac-O

pTRFlac

mCherry ORI TheoRS KanR AmpR Asp Term

Double Digestion
Ligation
Transformation

Lac-O

pTRFlac

mCherry ORI ApaI TheoRS eBFP AmpR SphI Asp Term

RESULTS AND FUTURE PROJECTIONS

THE FUTURE

- Troubleshoot attaching TheoRS onto eBFP in PCR
- Replace GFP with eBFP
- Determine ratiometric fluorescence efficiency
  - Induction using theophylline & IPTG
  - Comparison of literature TheoRS kinetic values
- Replace eBFP with Kan^R
- Determine success of replica plating in converting aptamers to riboswitches