Studying the Effectiveness of RNA Riboswitches

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A Genetic “Light Switch” Can Help Solve Many Problems

Source: Gluten Free Living
Source: Chet
Source: ehsanaustria
Gene Regulation is not perfect, Riboswitches are “leaky”

Will An Inserted Riboswitch Work in Cells?

1. Replicate and Isolate DNA
2. Insert DNA into E. Coli Cells and Grow the Cells
3. Test Effectiveness in Cells by Measuring Fluorescence
**E. coli Will be Transformed to Test Riboswitches in Vivo**

[Diagram showing the process of transforming E. coli with a plasmid vector containing a DNA insert with a riboswitch and a fluorescent gene.]

Source: The University of Queensland

Image courtesy of Suresh Kaushik, Transformation of competent E. coli

**Fluorescence Will Be Measured to Test the Effectiveness of the Riboswitch**

[Diagram showing fluorescence levels in bacteria with and without the riboswitch insert.]

Source: News Medical Life Sciences

Source: Sartedt

Bacteria with the insert will be compared to a no cell control
Expected Results

**Riboswitch**

- Spinach

**Fluorescence**

- Time Intervals (15 Hours Total)

- Example Graph

- No Signaling Molecule for Riboswitch Present = No Gene Regulation

- Signaling Molecule for Riboswitch Present = Some Gene Regulation

- Fluorescence Present = No Fluorescence

Lower Fluorescence with Presence of Signaling Molecule

**Riboswitch**

- Spinach

**Fluorescence**

- Colony 2 Fluorescent Values Normalized to the No Cell Control

- Time Intervals (15 hours total)
Lower Fluorescence with Presence of Signaling Molecule

Fluorescence Time Intervals (15 hours total)

Colony 2 Fluorescent Values Normalized to the No Cell Control

Close to 0 when reporting molecule is not present

Moderate Fluorescence With the Riboswitch Signaling Molecule Present
Lower Fluorescence with Presence of Signaling Molecule

Colony 2 Fluorescent Values Normalized to the No Cell Control

Trend Shows a Difference in Fluorescence

Results Show a Good Start to Developing an Effective Assay

DNA Insert w Riboswitch and Fluorescent Gene was Confirmed

Tecan was Confirmed to Work with Cells

What I learned: Science does not go as planned and success can rest in the details
Testing the Effectiveness of Riboswitch Variants Using the Developed Assay

Wild-type
ATA TGA TCA ACA CTT

Variant
ATA TGA TCA ACA GTT

Wild-Type	Variant

Riboswitch Spinach

-changing Aptamers and Genes Expands Applications

Aptamer

Gene Under Regulation

Source: inhabitat
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