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ILLINI IGEM 2008

Introducing RFP to E.Coli via Transduction

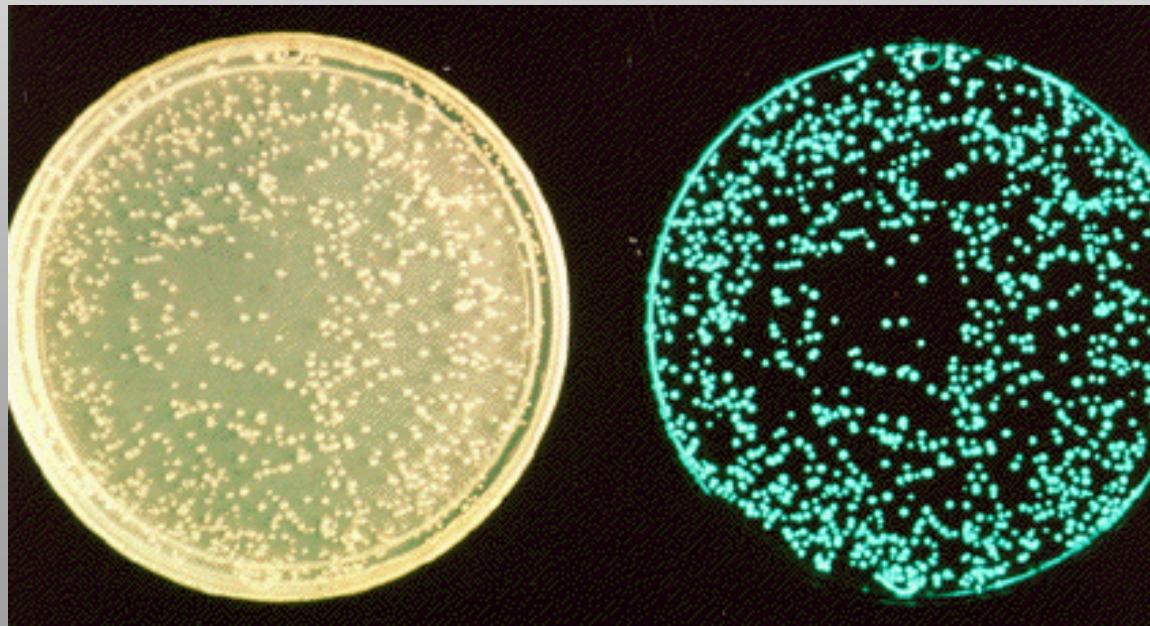


Procedure Overview

- Plasmid Selection
 - Extraction from Paper
- E.Coli Culturing
 - Colony Spreading
- Sterile Technique
- Transduction by Heat Shock
 - Protocol and Principles
- Results Quantification

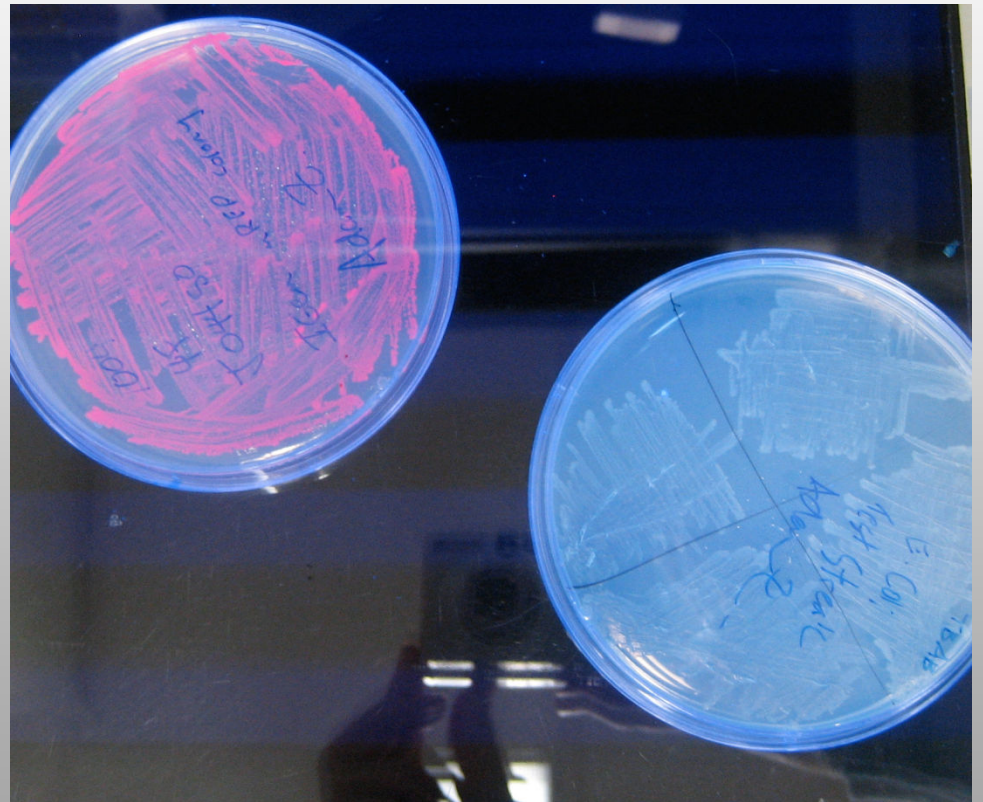
Plasmid/Culturing

- Selected BioBricks were 'Reporters'
- 'RFP' – Red Fluorescent Protein generating Dna, isolated from Coral
- Each vector includes unique antibiotic resistance i.e. confers protection from Ampicillin
- Culturing
- Grown on Auger Nutrient
- Spreading Technique to seed new Petri Dishes
- Individual Colonies rather than Smear



Plasmid/Culturing

- Tested 3 Different Biobricks in 4 Trials
- 3 Trials Failed due to:
 - ▣ Introducing Incorrect antibiotic
 - ▣ Insufficient Bacteria Population?
 - ▣ Human Error; Poor Lab Technique?
- Successful Trial produced insignificant
- Next day – retrial completely successful
- Compare Fluorescence under UV
- Blue (without the vector) contrasted with Red (with the vector)



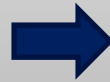
Transduction Protocol

- Registry Booklet of Plasmid DNA
 - ▣ Sterilize Puncher with Diluted Bleach and Ethanol Each Use
 - ▣ Approx. 4 uses each Biobrick Dna



Transduction Protocol

- Soak Plasmid and mix with Bacteria in microcentrifuge as directed by Booklet
- Conduct Heat Shock



- Reculture
- Try Electroporation?

Heat Shock Procedure

- Idea:
 - Cool Bacteria and then suddenly warm, cool once more
 - Opens pores due to cell repair proteins
 - Allows Plasmid DNA to enter
 - Re-cool to trap plasmid within
 - Transduction complete!
 - 99% Bacteria death – w.e.
- Electroporation
 - Maybe more efficient cell-wise
 - Costs-More

Quantification and Measurement

- Can measure optimal light transmittance input and output wavelengths for Fluorescent E.Coli Strains
 - ▣ Set specific input wavelength and scan for broad range output
 - ▣ Find peak output wavelength
 - ▣ Set peak output and scan for broad range input

