Generation of Expression Constructs to Elucidate Phage Gene Function

PRESENTER
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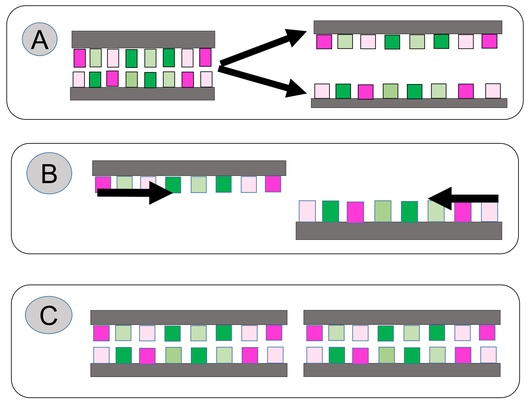
Background

Generation of an inducible construct is required to test whether phage genes are cytotoxic to *M. smegmatis*

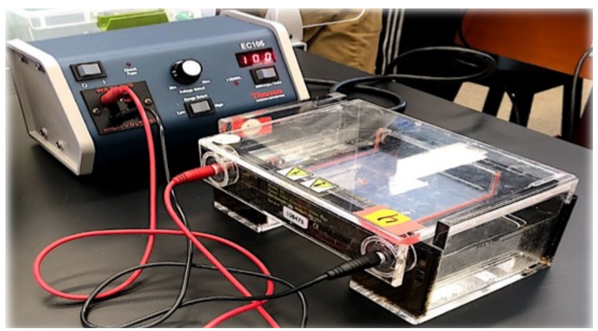
Methods

Polymerase Chain Reaction:

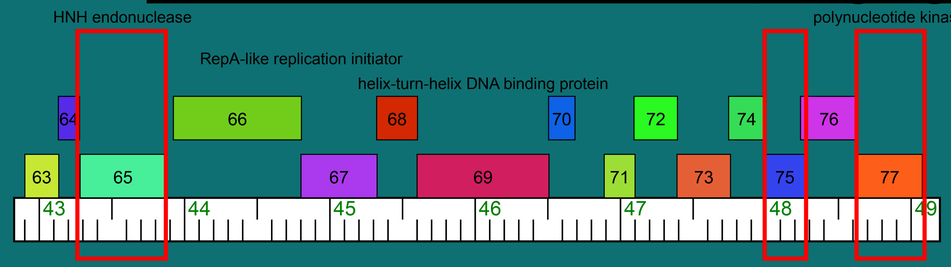
a. Heat ***denatures*** DNA
b. Gene specific primers ***anneal*** near the start and stop codons
c. DNA polymerase ***extends*** DNA to amplfy region of interest



Agarose Gel Electrophoresis
Separate DNA fragment by size



Start with an annotated phage genome:



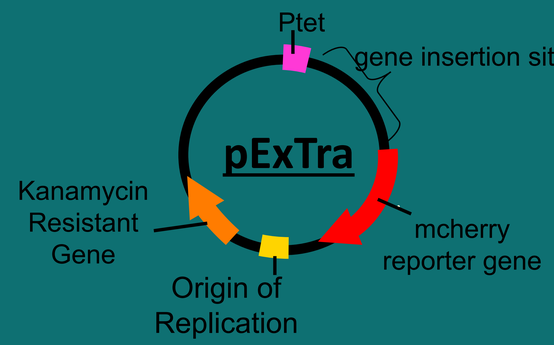
Right end of Phayonce (P5)

1. Preparing the insert:

- Amplify your gene of interest using gene specific primers
- Verify using gel electrophoresis
- Gel 1

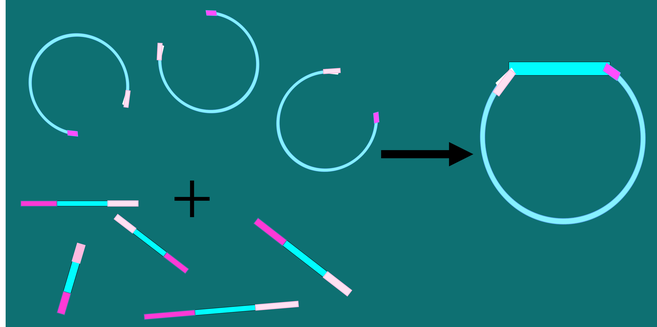
2. Inducable expression vector:

- pExTra
- Tetracycline induced expression of phage genes in *M. smegmatis.*



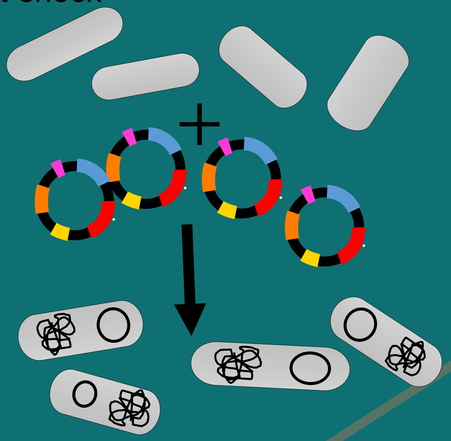
3. Isothermal Assembly

- Mix PCR product and linearized vector
- Assemble into desired plasmid



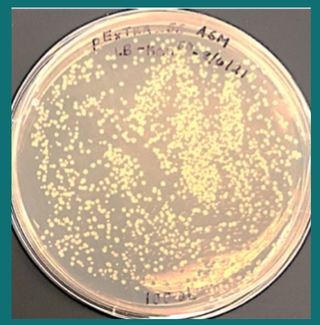
4. Inducable expression vector:

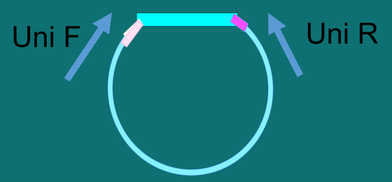
- Plasmid and competent *E. coli*
- Heat-shock



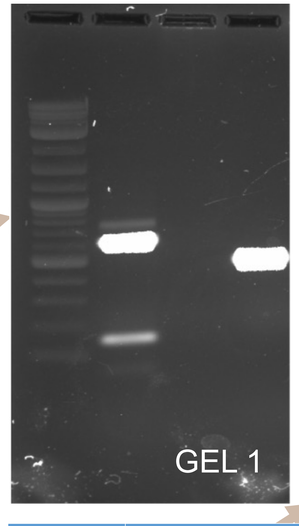
5. Plate and PCR Verifiction

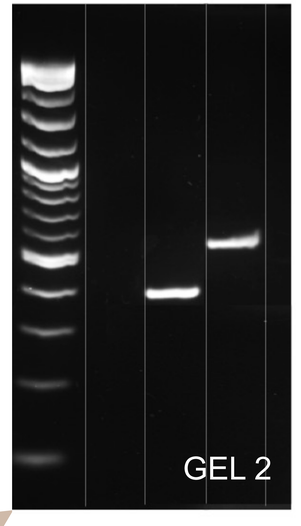
- Transformed cells plated on Kan media
- Colony verification with universal primers
- Gel 2





Results



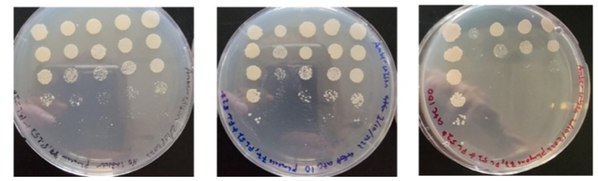


Lane	Gel #1 (gene amplification)
1	DNA Ladder
2	Phayonce 65 (637bp)
3	empty
4	Phayonce 77 (496bp)

Lane	Gel #2 (clone verification)
1	DNA Ladder
2	No template control
3	pExTra:Phayonce 75 (393bp)
4	pExTra:Phayonce 77 (558bp)

Future work!!

Gene #77 Cytotoxic Assay:



no aTc | aTc 10 | aTc 100

Authors

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