

gNarLy prom-Otors

A Review of Our Original Project



Project Background

- The Nar operon is located with the E. Coli genome.
- It helps regulate nitrate concentrations under anaerobic conditions.
- The nar operon is regulated by two events that occur in its promotor region.
 - 1. binding of the FNR protein, a common regulatory protein, under anaerobic or low oxygen conditions
 - 2. binding of the NarL protein, a regulatory protein specific to this operon, in the presence of nitrates
- **This gives it the unique property of being dually inducible.**



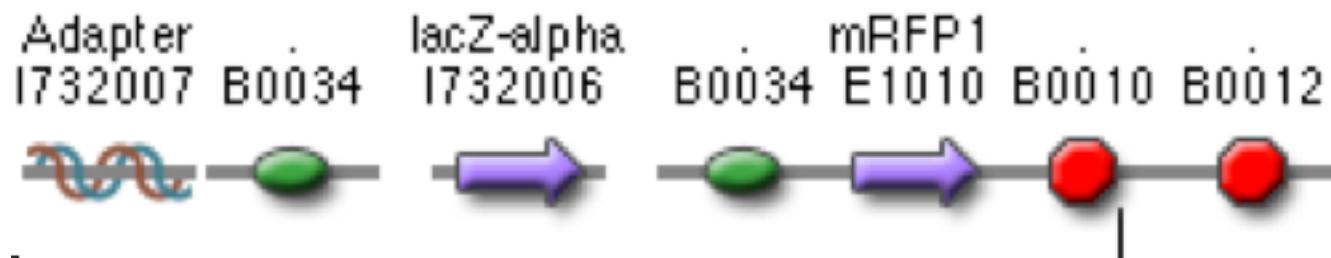
Purpose

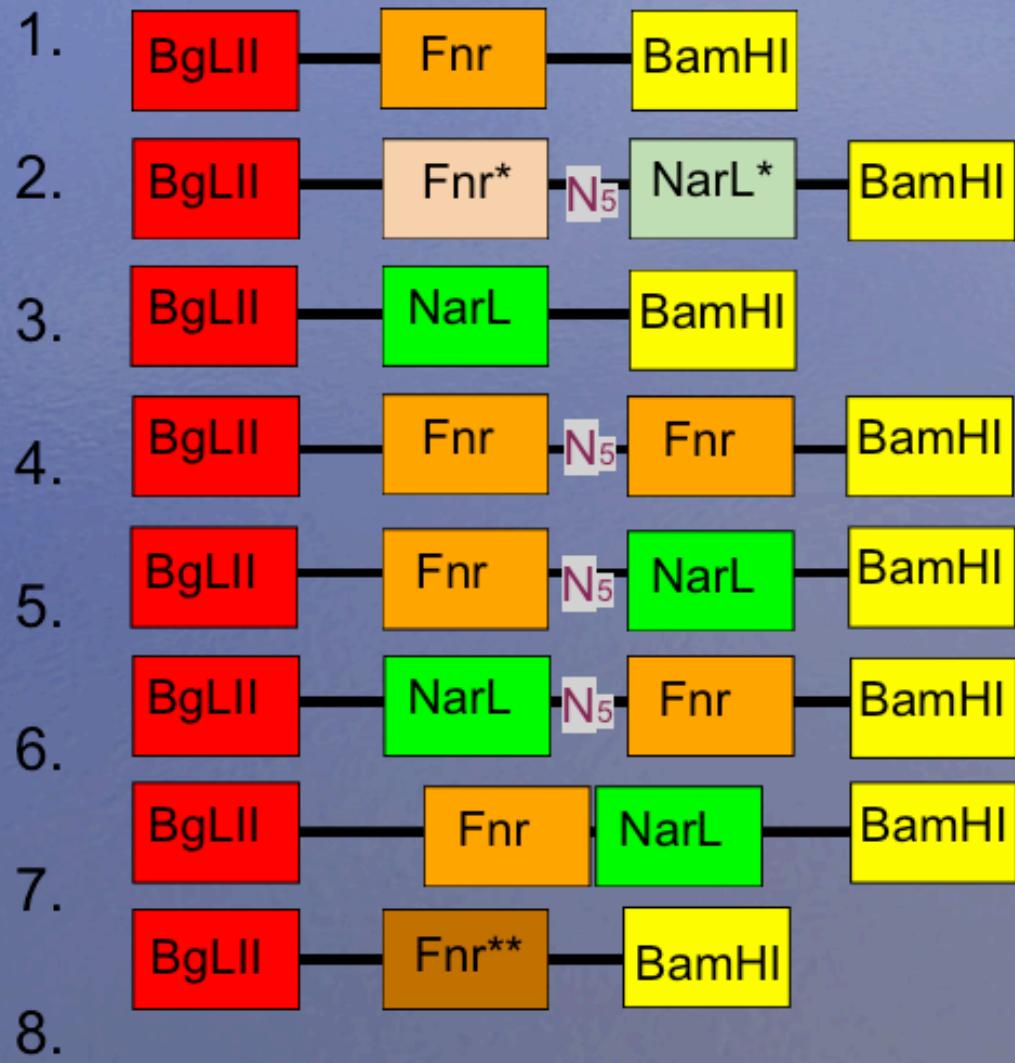
- To manipulate the sequencing of the FNR and NarL binding regions of the Nar Promoter.
- In doing so we hoped to:
 - 1. Test the effectiveness of different binding site configurations.
 - 2. Test the relative efficiencies of a wild type promotor region and a construct based promotor region.
 - 3. Create a promotor region for the parts registry that is dually inducible allowing for some control over expression.



Our Part

- We needed a very specific type of part!
- One without a promoter but containing an RBS site and gene downstream.





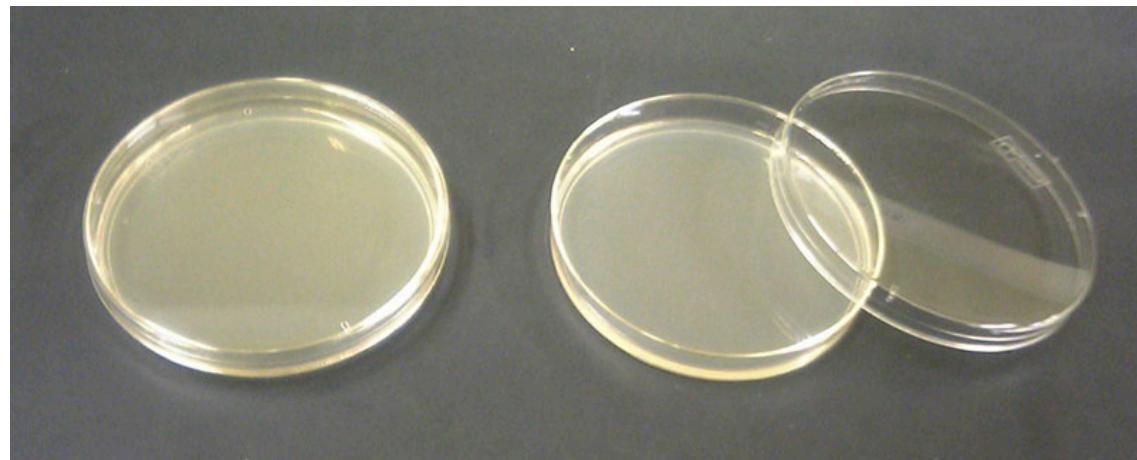
* indicates wild type

** indicates 2 base pairs were altered



Our First Attempt at Transformation

- Transforming part I732095.
- The plates didn't produce any colonies.
- Found six more parts in the iGEM Library 3 from the paper wells, 3 from the other.





Parts

I732094



I732097



I732096



E0430



E0240



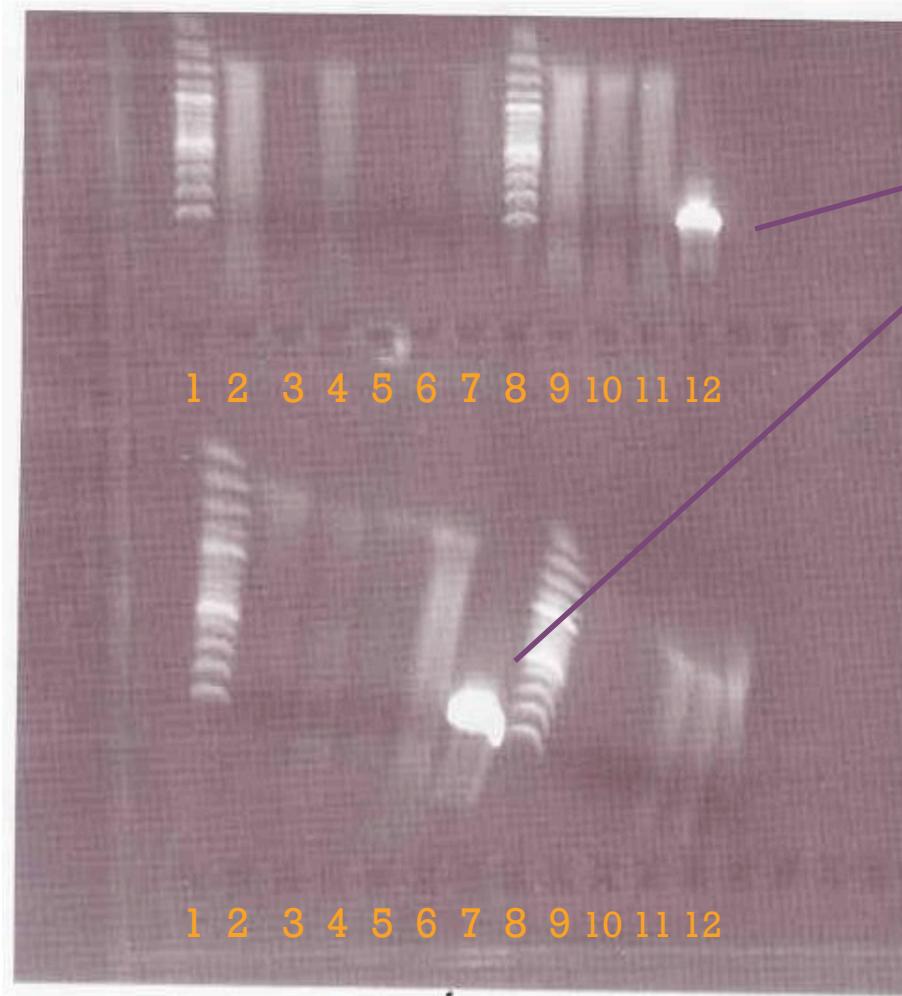
E0840





Our Second Attempt at Transformation

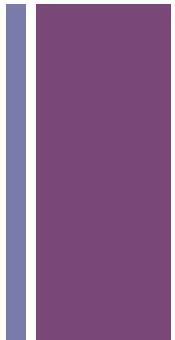
Transforming parts I732094, I732097, I732096, E0430, E0240, and E0840



Gel turned out kind of crappy but results were conclusive; none of our parts were present.



Results

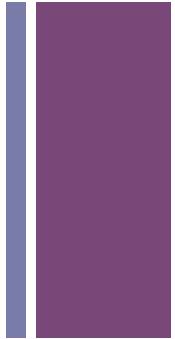


- Failed to transform any of the parts from the library.
- Because we needed a very specific type of part our project had to be abandoned.
- **QUESTIONS??**





Project Change



- Since we can't continue with our original project, we picked from last semester's projects to continue.
- Emblazon caught our interest
- Take the gene from an Orchid that turns the flower blue, and put it into E. coli
 - mRNA sequence
 - Introns?
 - DNA extraction and sequencing

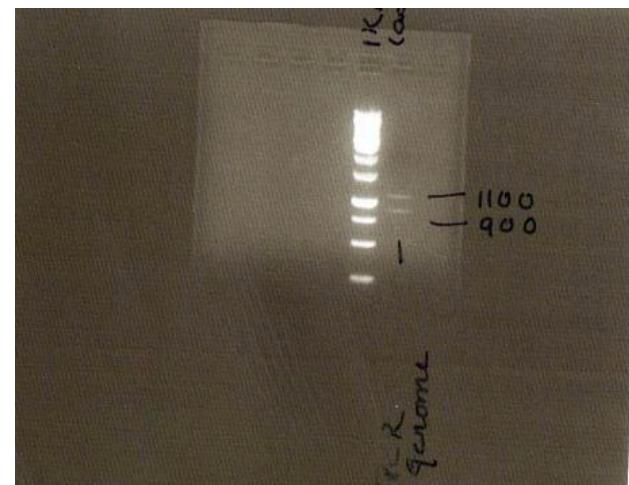
+Emblazon

■ Their results

- Forward Read
 - -647 bp
 - -100% match to target sequence
- Reverse Read
 - 241 bp
 - 92% match to target sequence

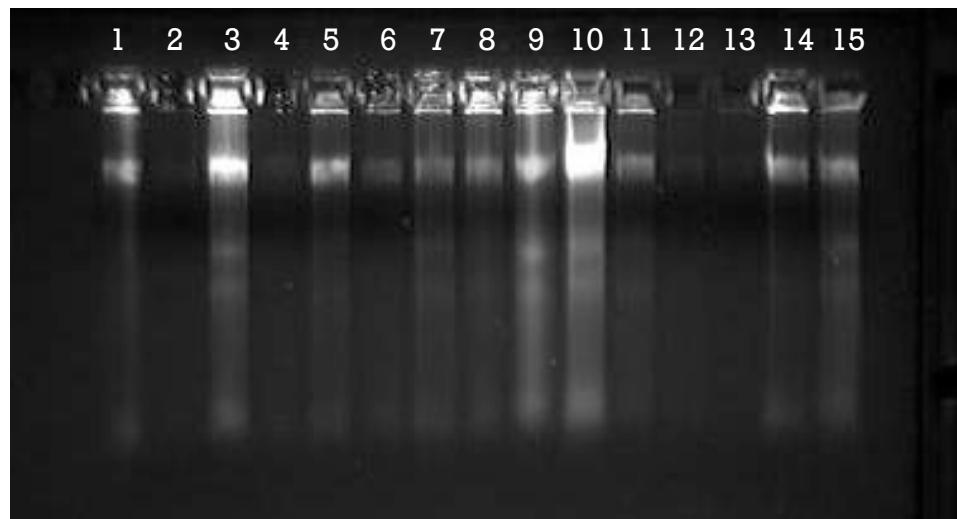
■ The last thing Emblazon did was a restriction digest

- Expected to see one band at 2,000 bp
- Saw two bands at 1,100 and 900 bp



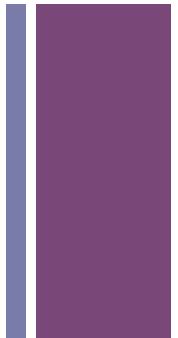
■ DNA extraction from the Phalaenopsis (Orchid) plant leaf

- 3, 5, 9, and 10 were put into the High concentration tube
- 1, 7, 8, 11, 14, and 15 were put into the Medium concentration tube
- 2, 4, 6, 12, and 13 were put into the Low concentration tube

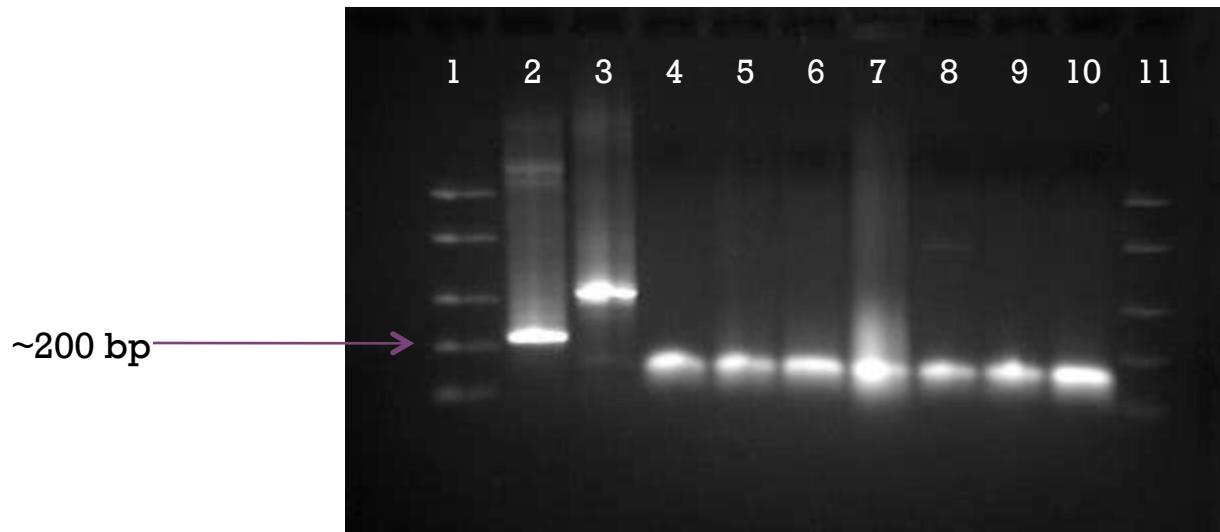




Emblazon



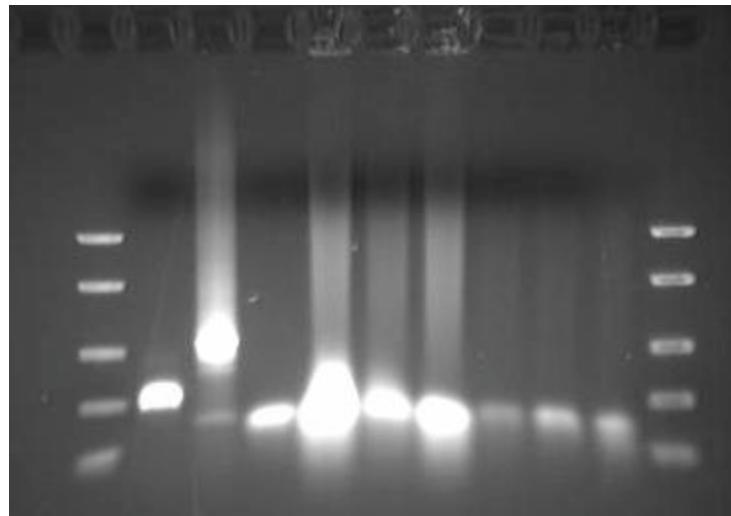
- PCR of our gene, using promoters from last semester





Emblazon

- Diluted primers and tried PCR again using same concentrations



- Same results as the first time



Project Change, again

- After class discussion, we felt we should get some other kinds of experience in the lab
- We decided to work on cloning DNA fragments
 - We used a kit to extract the Alu gene from ourselves.
 - Alu is a gene that we all have, some people have tandem repeats of it, and some people do not

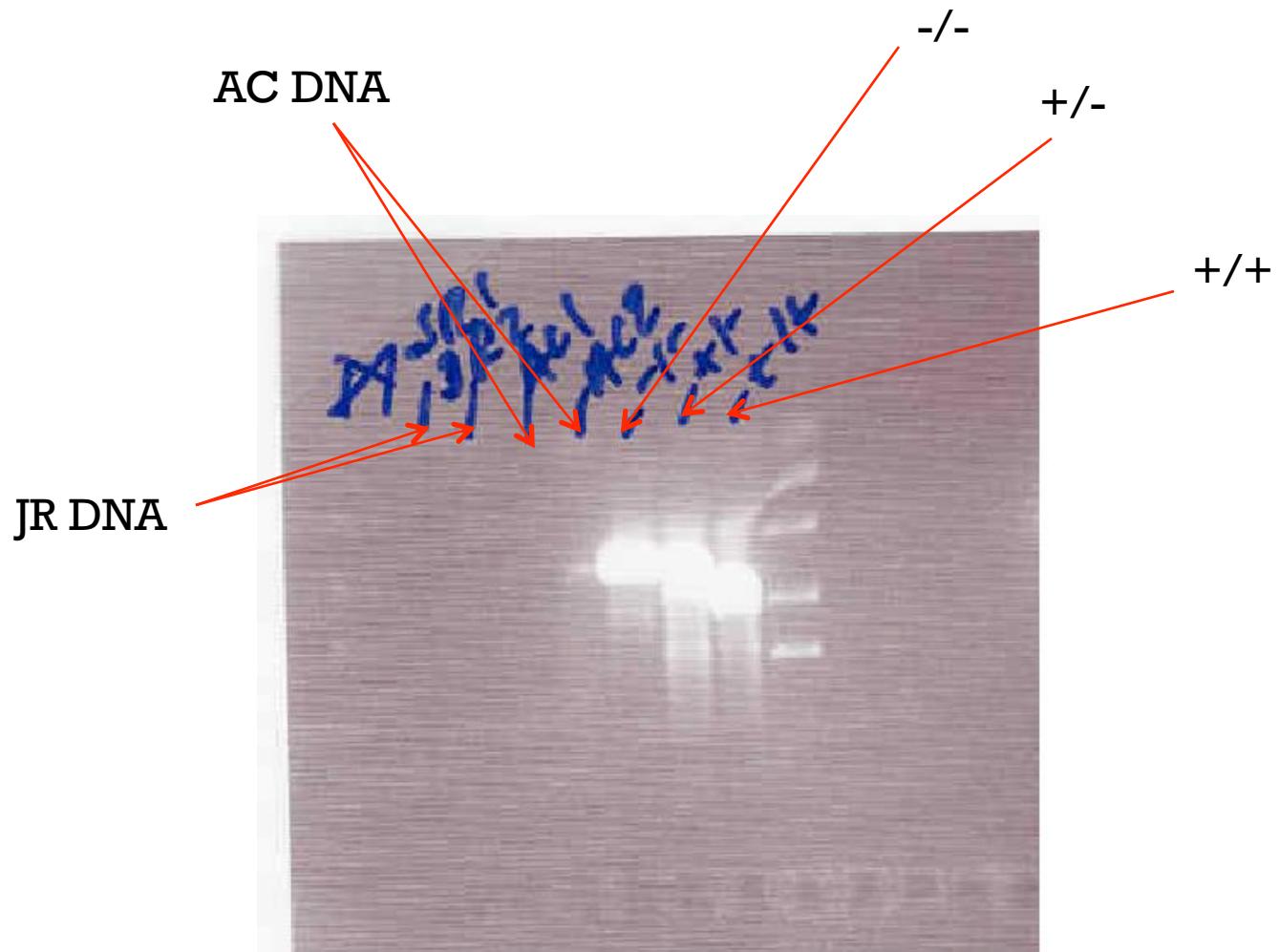


Alu gene

- We used the kit to extract DNA from our cheek cells and hair follicles (Alex did cheek, Jessica did hair)
- The kit was also used to run a PCR for the amplification of the alu sequence
- When we did the DNA extraction, we also made a blunt end digest of pBlue as a vector for later transformation (Eco RV)
- The PCR product was run on a gel

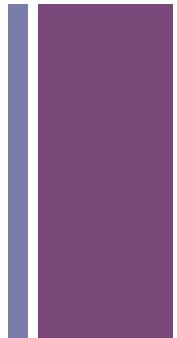
+

Alu gene

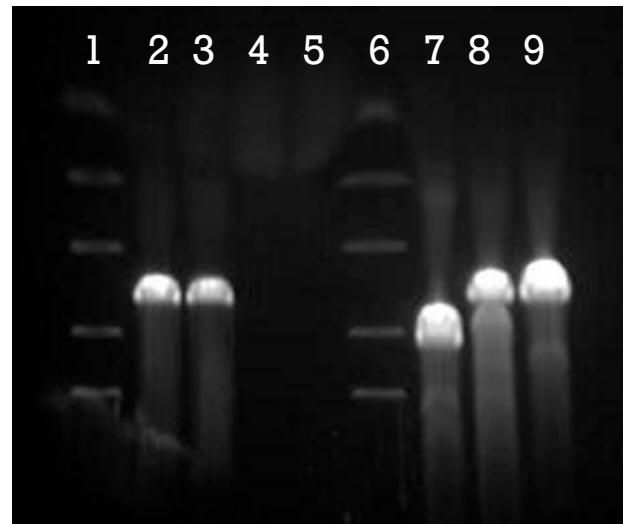




Alu gene



- Retry, made a few changes
 - Both did cheek
 - Directions from kit + handout rather than just handout



Lane 1 and 6= ladder

Lane 2 and 3= JR DNA

Lane 4 and 5= AC DNA

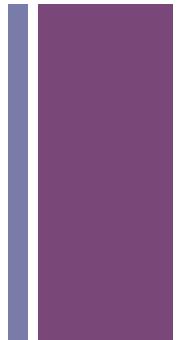
Lane 7= +/+

Lane 8= +/−

Lane 9= −/−



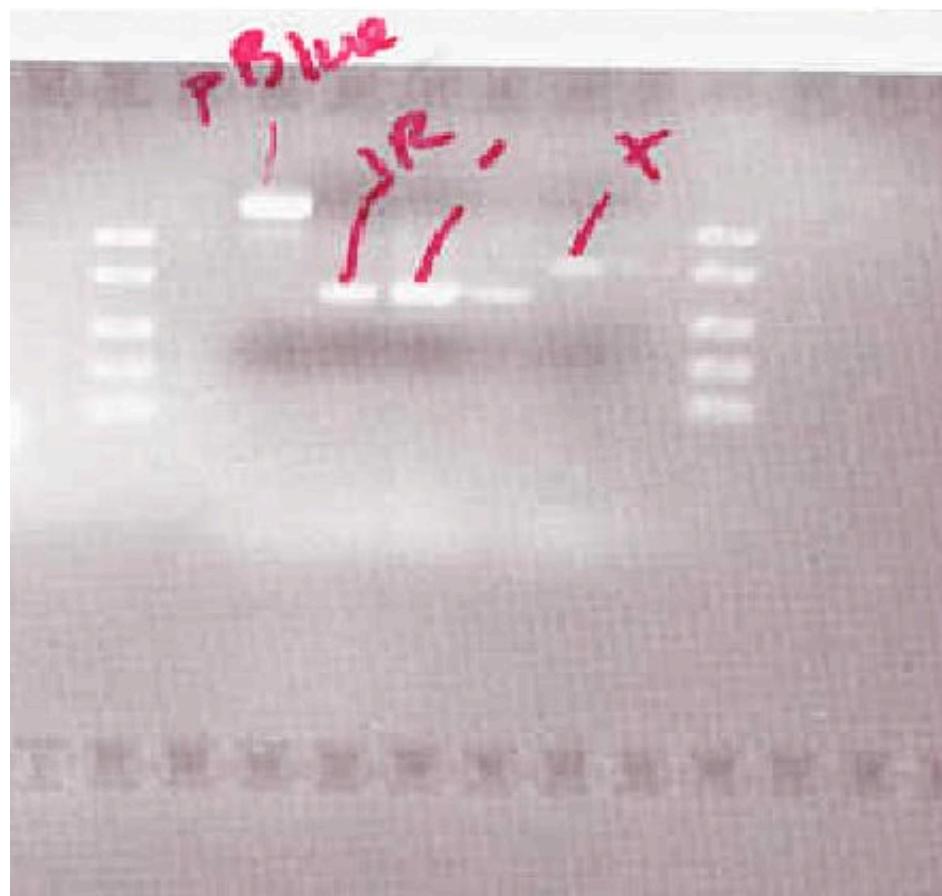
Alu gene



- Gel purification of PCR samples from gel
- PCR purification of blunt end digested plasmid
- Concentration check on a gel

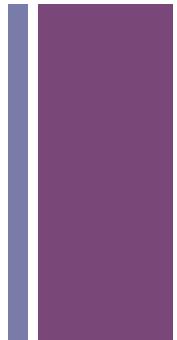
+

Alu gene





Alu gene



- Ligations of plasmid and DNA
 - 1:1, 1:3, 3:1, 0:3
- Transformations
- Overnight cultures
- Minipreps
- Digest (Eco R1)
- Gel

+

Alu gene

Lane 1, 8, 15= ladder

Lane 2-13= digested samples

Lane 14= digested pBlue

Lane 8 accidentally received both
ladder and a sample

