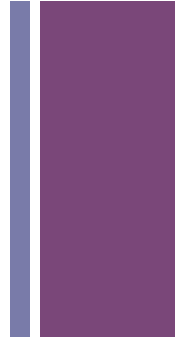


# gNarLy prom-Otors

A Review of Our Original Project

# + Project Background



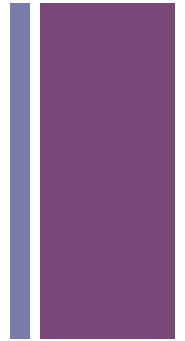
- The Nar operon is located with the E. Coli genome.
- It helps regulate nitrate concentrations under anaerobic conditions.
- The nar operon is regulated by two events that occur in it's promotor region.
  - 1. binding of the FNR protein, a common regulatory protein, under anaerobic or low oxygen conditions
  - 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



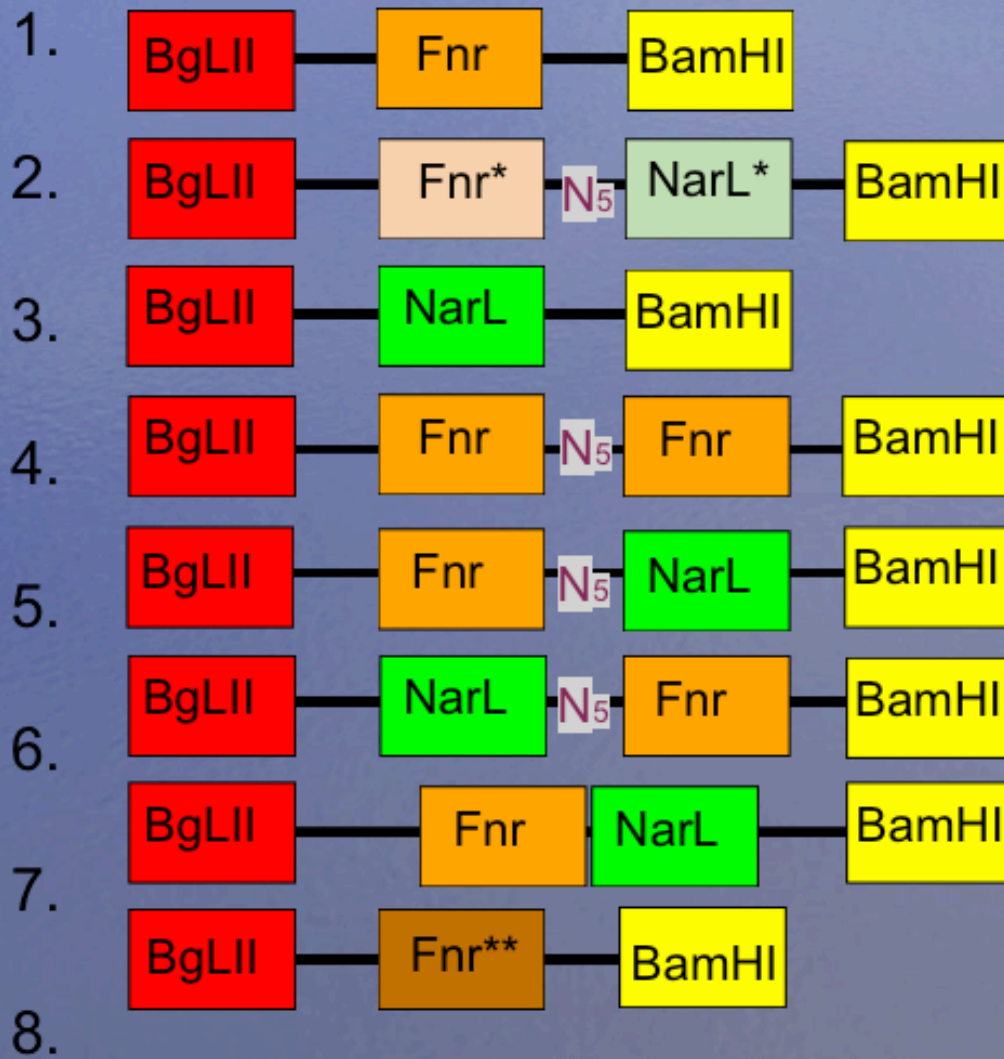
- To manipulate the sequencing of the FNR and NarL binding regions of the Nar Promoter.
- In doing so we hoped to:
  - 1. Test the effectiveness of different binding site configurations.
  - 2. Test the relative efficiencies of a wild type promotor region and a construct based promotor region.
  - 3. 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.

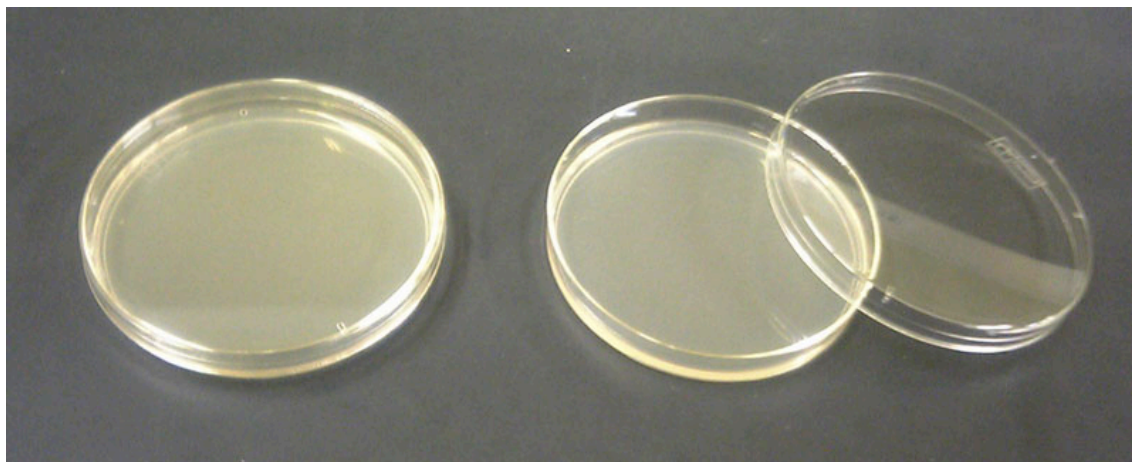




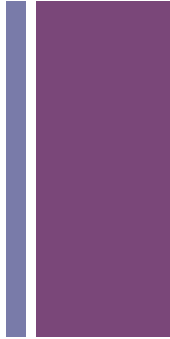
\* indicates wild type  
\*\* indicates 2 base pairs were altered

# + Our First Attempt at Transformation

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



# + Parts



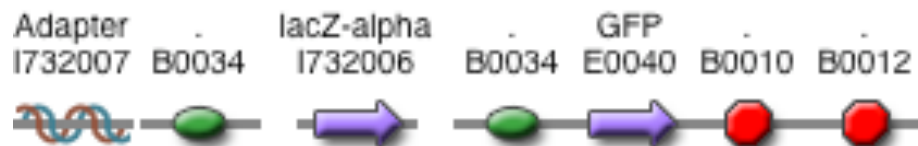
I732094



I732097



I732096



E0430



E0240



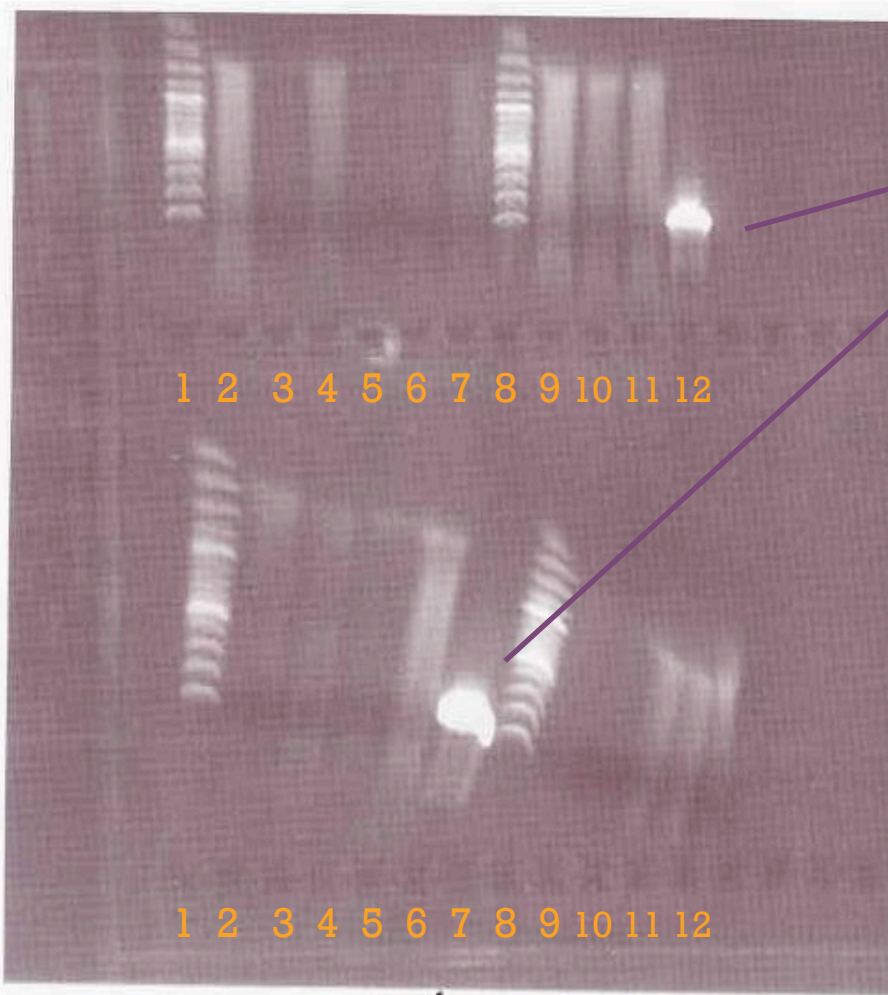
E0840





# Our Second Attempt at Transformation

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

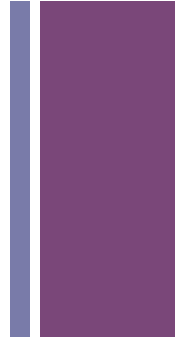


Our Positive Controls

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



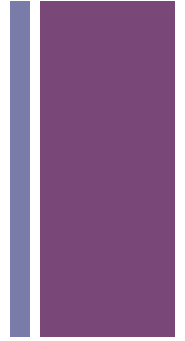
# + Results



- 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

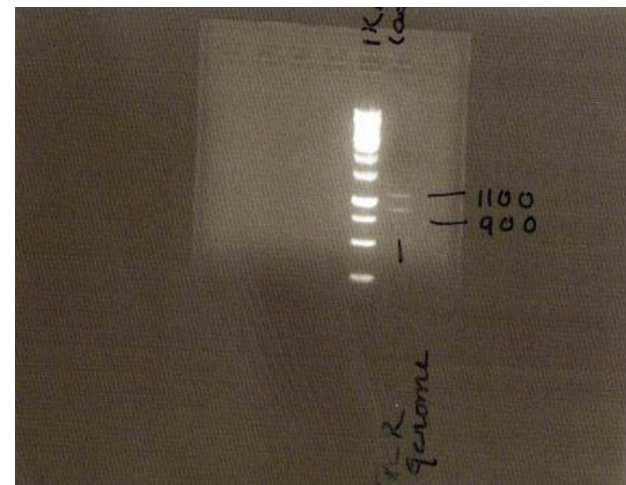
- -647 bp
- -100% match to target sequence

### ■ Reverse Read

- 241 bp
- 92% match to target sequence

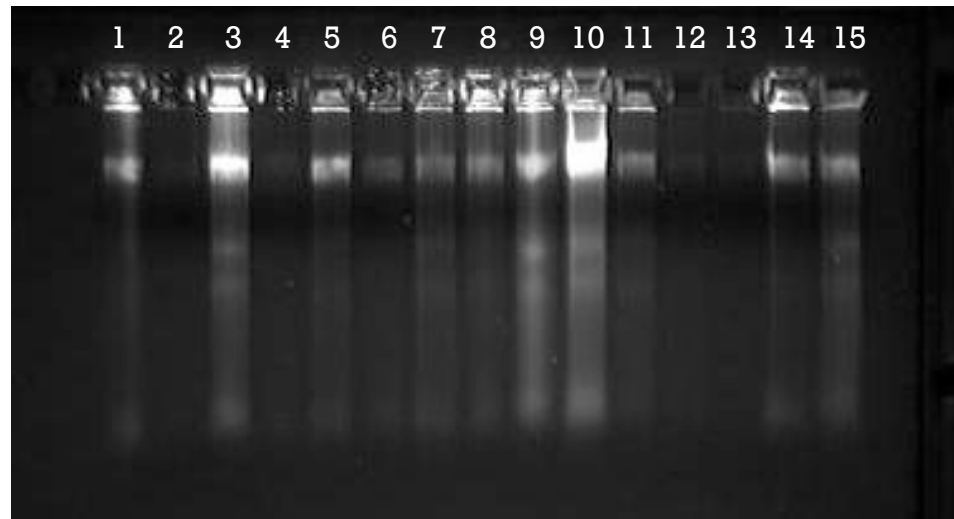
## ■ The last thing Emblazon did was a restriction digest

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

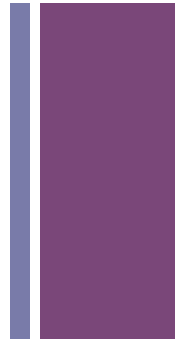


# + Emblazon

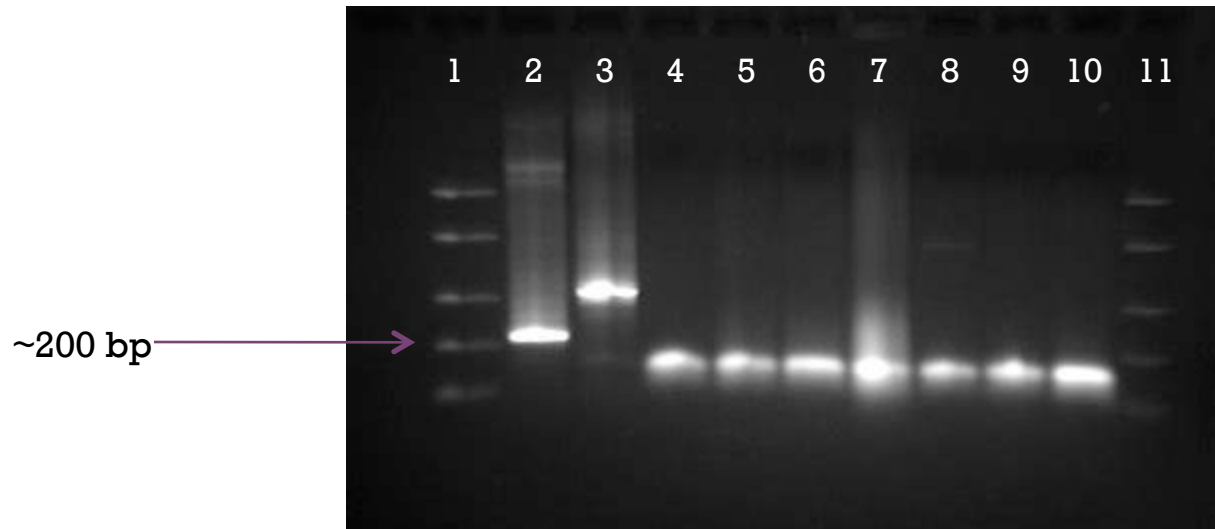
- DNA extraction from the Phalaenopsis (Orchid) plant leaf
  - 3, 5, 9, and 10 were put into the High concentration tube
  - 1, 7, 8, 11, 14, and 15 were put into the Medium concentration tube
  - 2, 4, 6, 12, and 13 were put into the Low concentration tube



# + Emblazon



- PCR of our gene, using promoters from last semester



Lane 1 and 11 = ladder

Lane 2 = pBlue

Lane 3 = pSB1A7

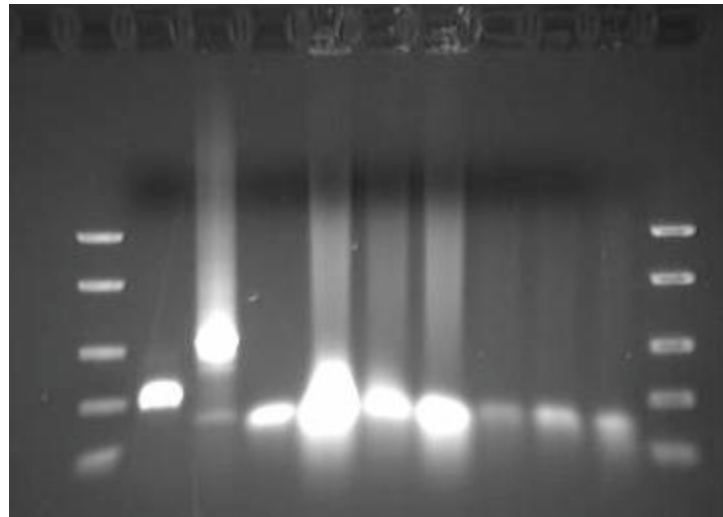
Lane 4 = negative control

Lane 5-7 = high concentration

Lane 8-10 = diluted high concentration

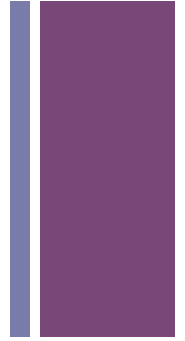
# + Emblazon

- Diluted primers and tried PCR again using same concentrations



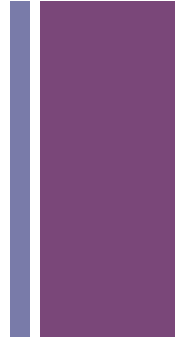
- Same results as the first time

# + 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

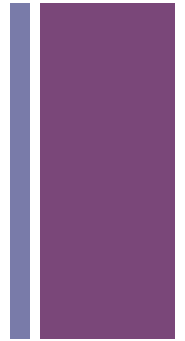
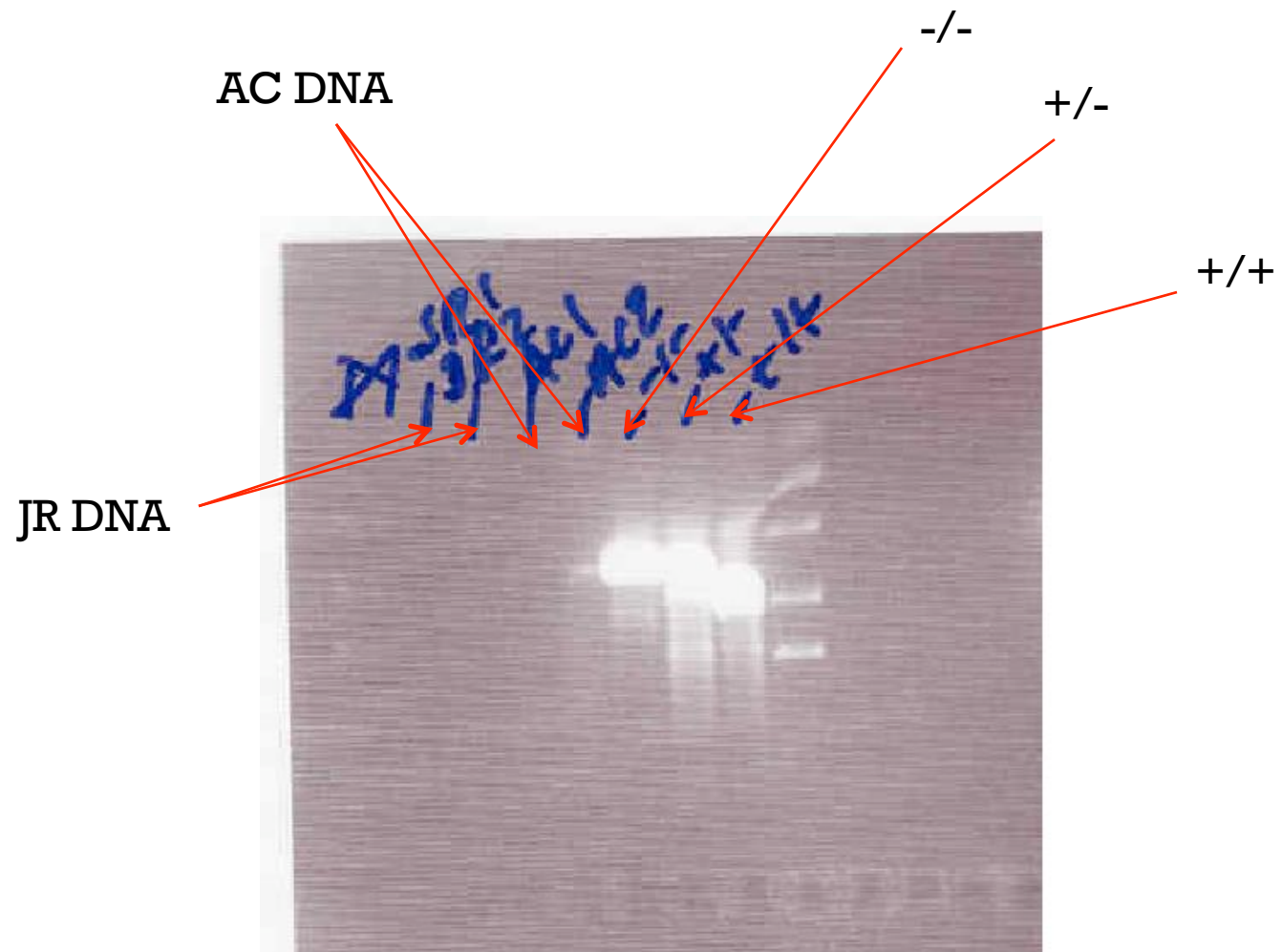
# + Alu gene



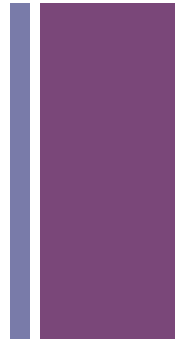
- We used the kit to extract DNA from our cheek cells and hair follicles (Alex did cheek, Jessica did hair)
- The kit was also used to run a PCR for the amplification of the alu sequence
- When we did the DNA extraction, we also made a blunt end digest of pBlue as a vector for later transformation (Eco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



# + Alu gene

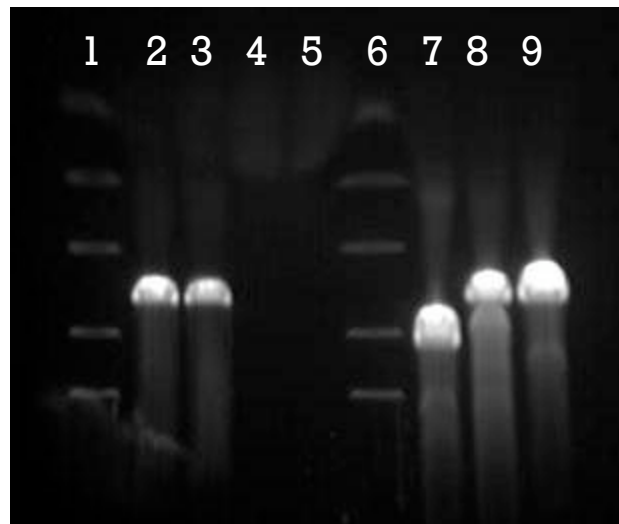


# + Alu gene



- 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= -/-

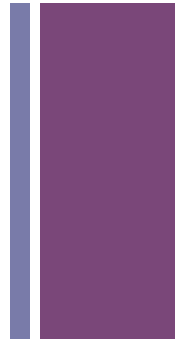
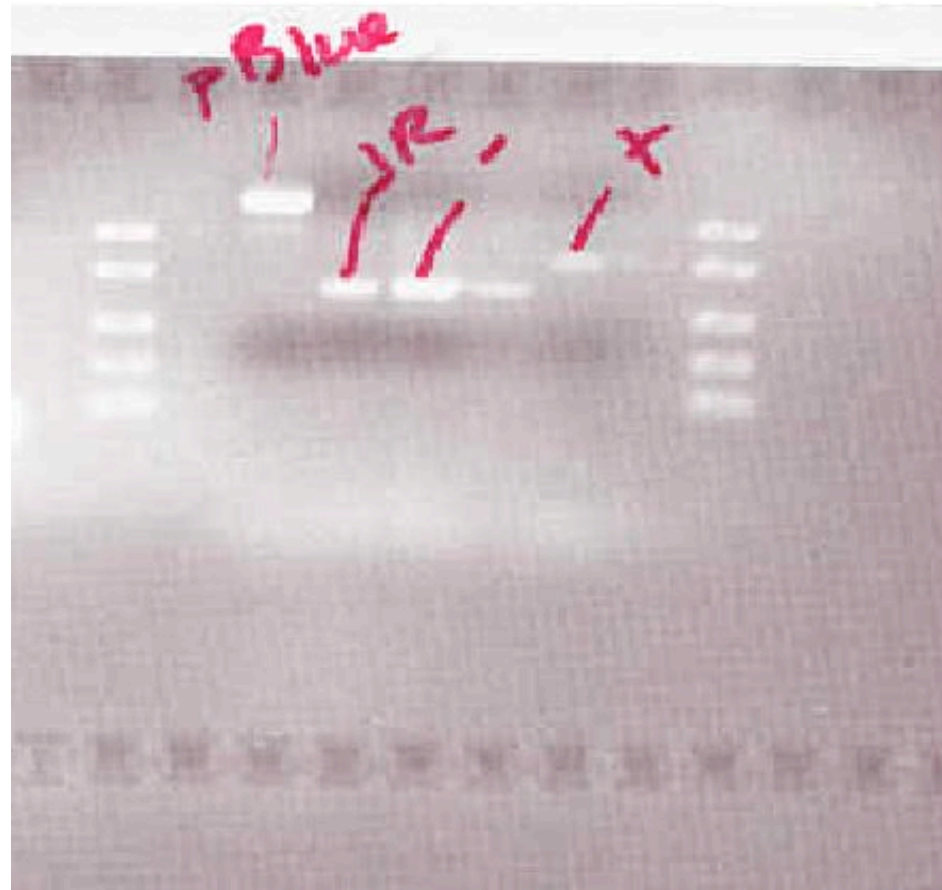


# + Alu gene

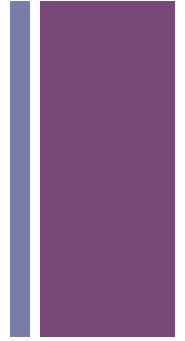


- Gel purification of PCR samples from gel
- PCR purification of blunt end digested plasmid
- Concentration check on a gel

# + Alu gene

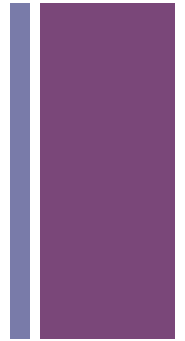


# + Alu gene



- 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

