Developing ARG1 and ODC Standards for a Microfluidic ELISA Device

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Chemistry

Funded by EPSCoR

Overview

- Project aims
  - Develop standards of arginase 1 and ornithine decarboxylase
  - Use the standards in tests of microfluidic enzyme-linked immunosorbent assays (ELISA)

Background

- What is Western Blotting?
  - An analytical technique used to separate and detect proteins from a tissue extract
  - Limit of detection in picograms

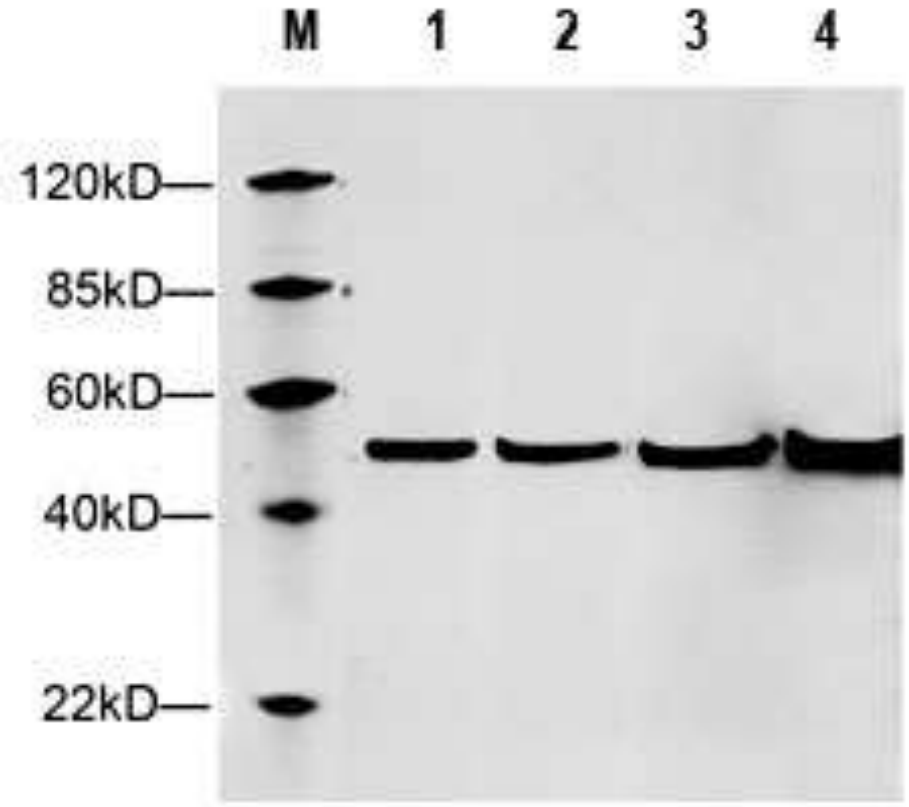
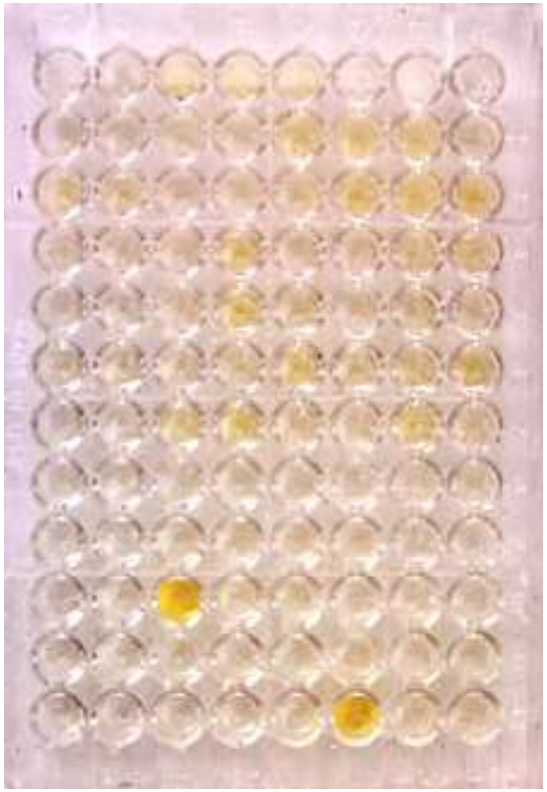


Photo from GenScript

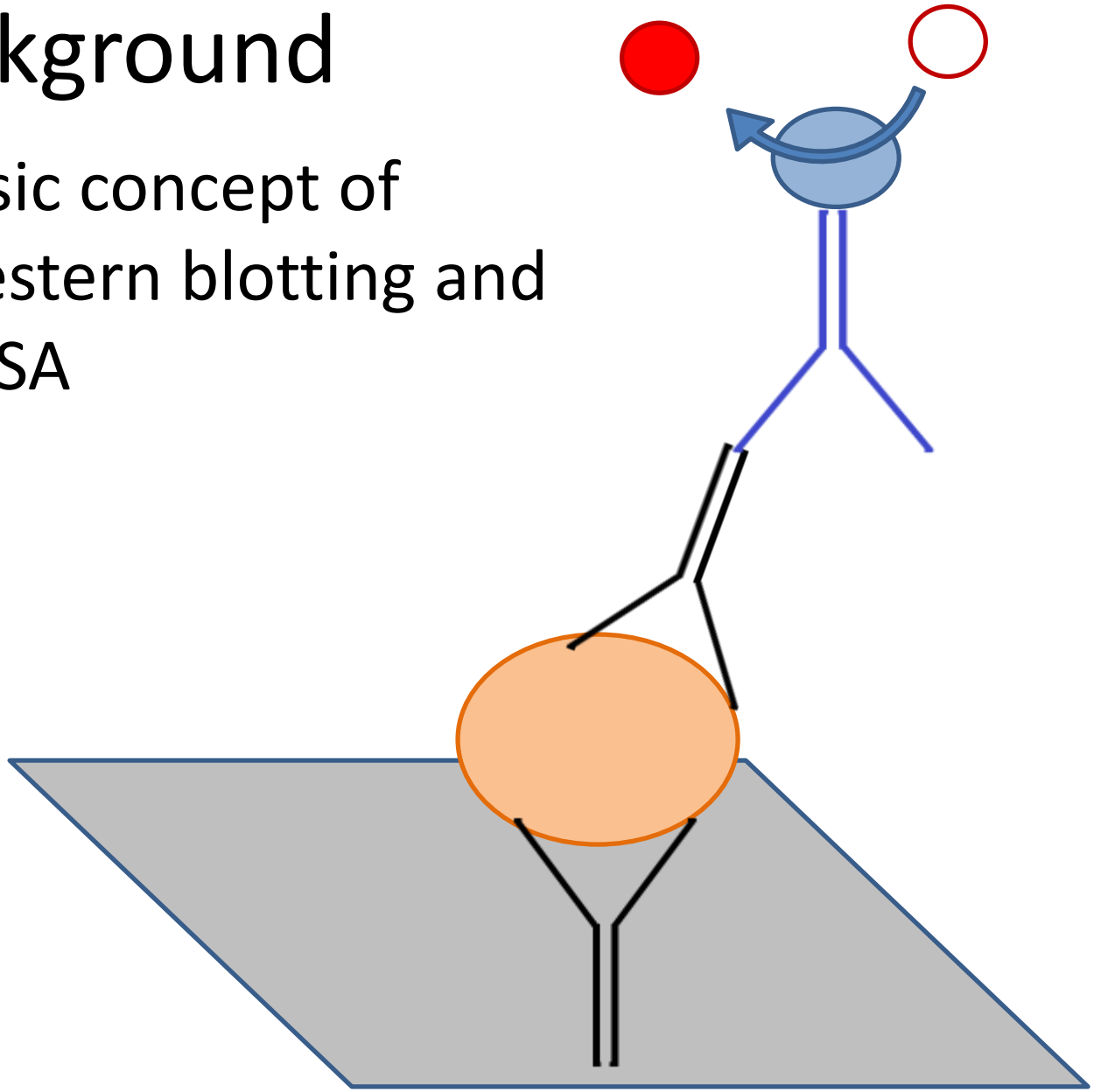
Background

- What is an Enzyme-Linked Immunosorbent Assay (ELISA)
  - An analytical technique used to detect antigens or antibodies in a sample
  - Limit of detection in nanograms.



Background

- Basic concept of Western blotting and ELISA



Background

- Western blot
  - Advantages: rapid method of protein detection
  - Drawbacks: non-quantitative

- ELISA
  - Advantages: quantitative method of protein detection
  - Drawbacks: tedious and requires large sample size

Background

- What are microfluidics?
  - The small-scale manipulation of liquids
  - Multidiscipline field of study
  - Can be used to conduct microELISA's

Background

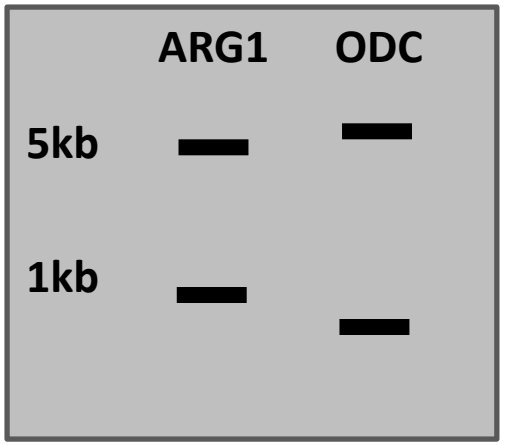
- Microfluidic ELISA
  - Advantages: Rapid analysis time, small sample sizes, quantitative, can detect multiple antigens from a single sample

Materials and Methods

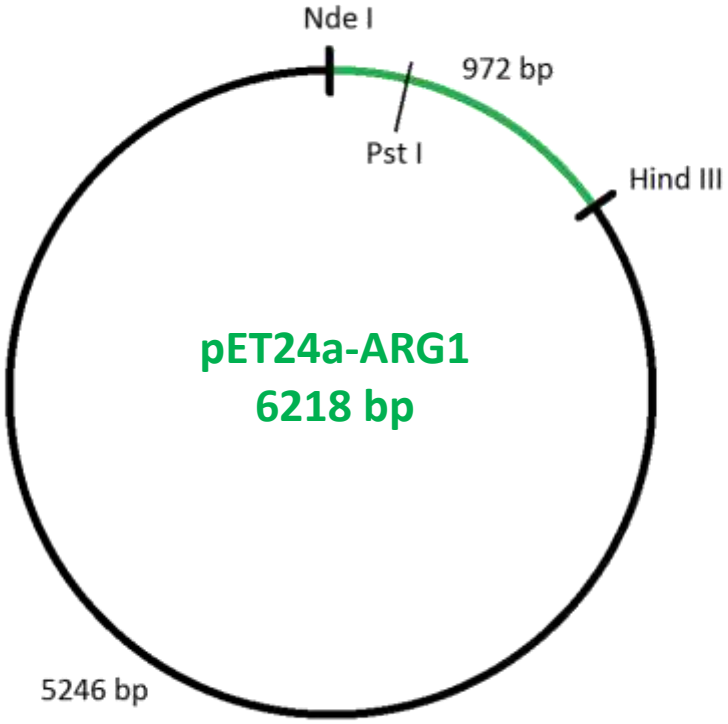
- Plan for preparation of standards
  1. Grow *E. coli* containing the genes for the desired proteins
  2. Overproduce and harvest the proteins
  3. Purify the proteins by Nickel column
  4. Assay the proteins for standardization

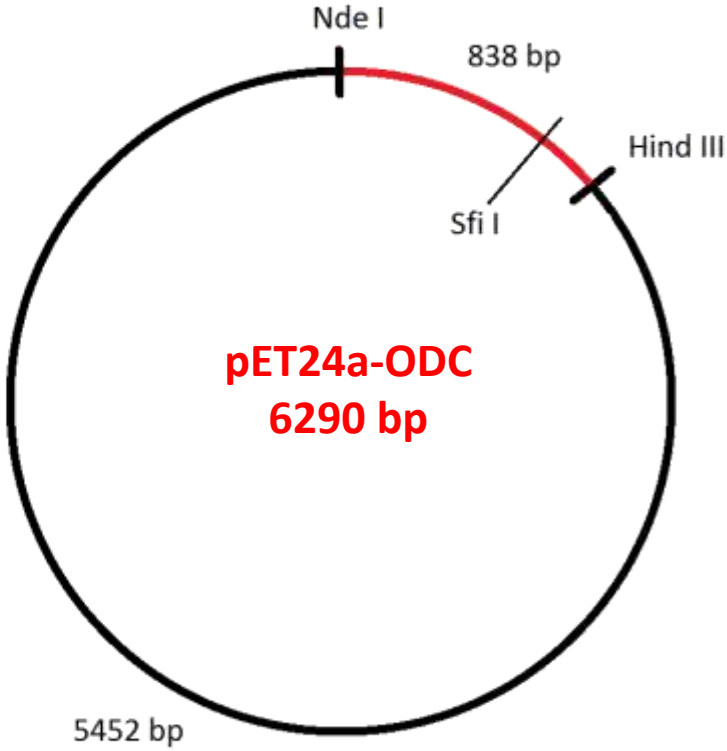
What I actually did

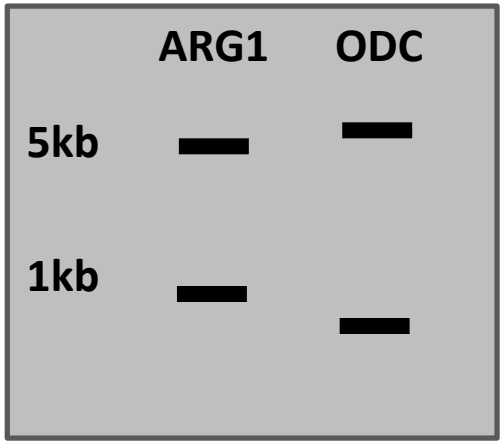
- Grew *E. coli* thought to contain the proteins of interest
- Did miniprep analysis of the bacterial DNA
- Did a restriction digest with the restriction enzymes *HindIII* and *Nde1* expecting to see:



Plasmids

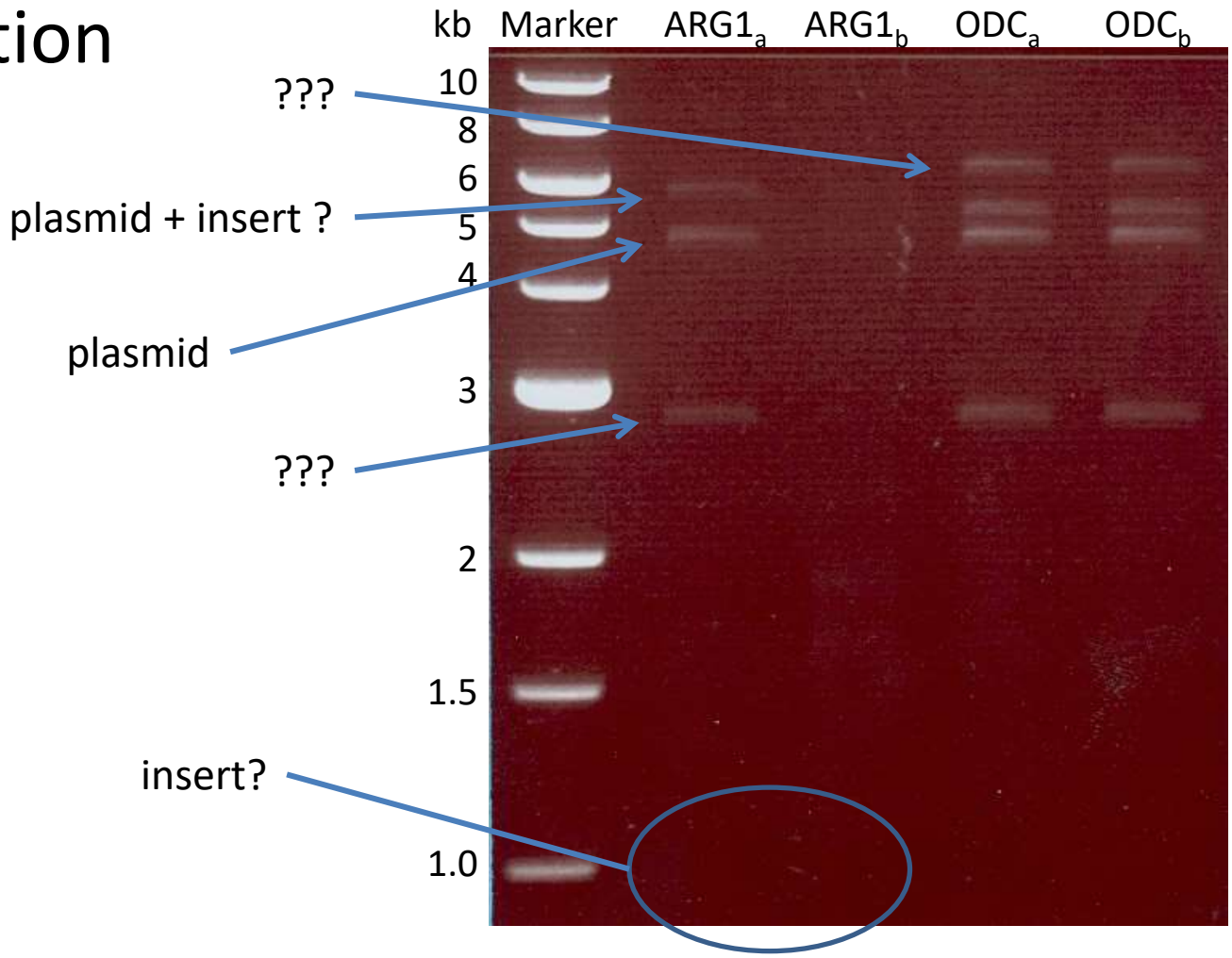

Nde I
972 bp
Pst I
Hind III
pET24a-ARG1
6218 bp
5246 bp


Nde I
838 bp
Hind III
Sfi I
pET24a-ODC
6290 bp
5452 bp

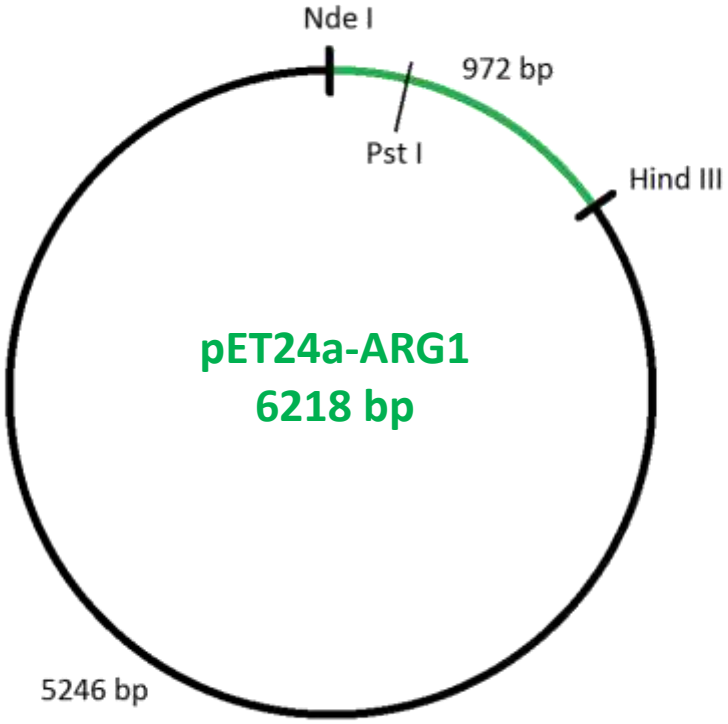

ARG1
ODC
5kb
1kb

Results

- Restriction digest



Plasmids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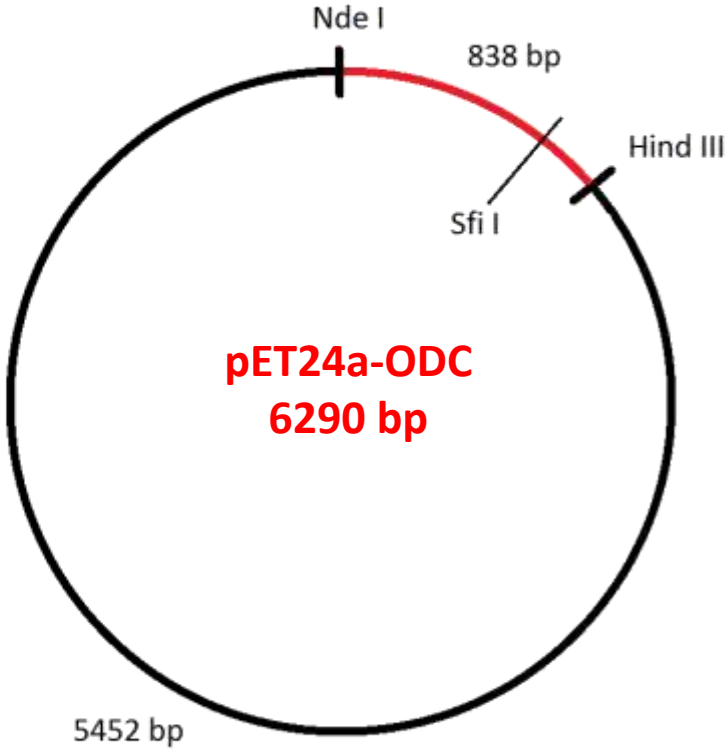
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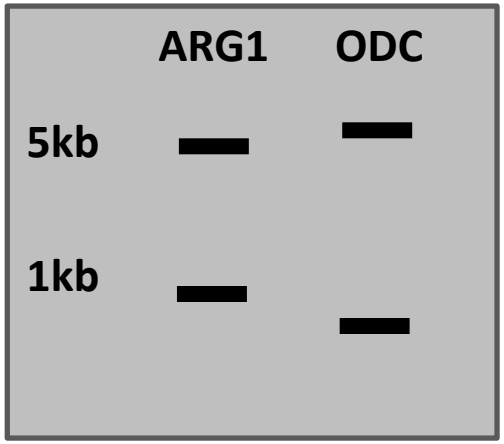
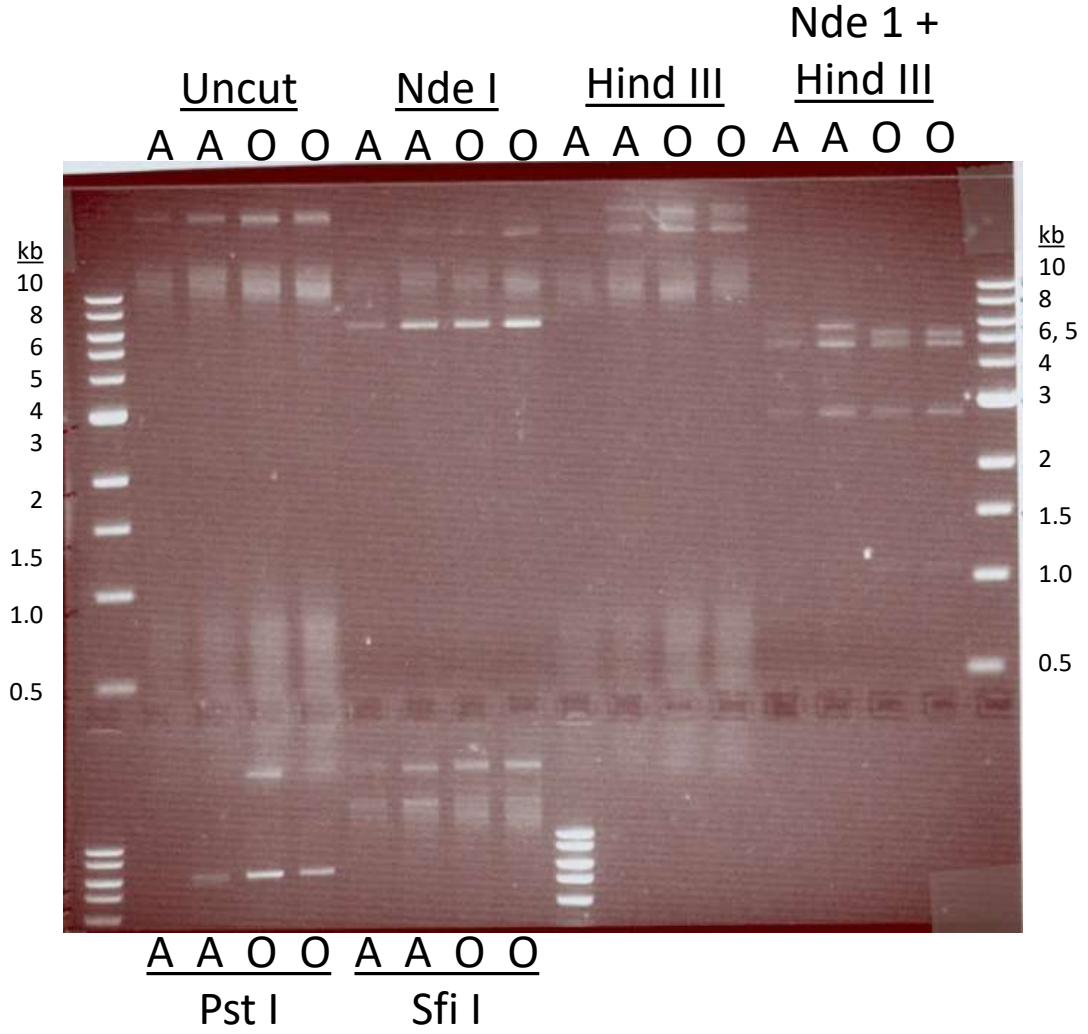
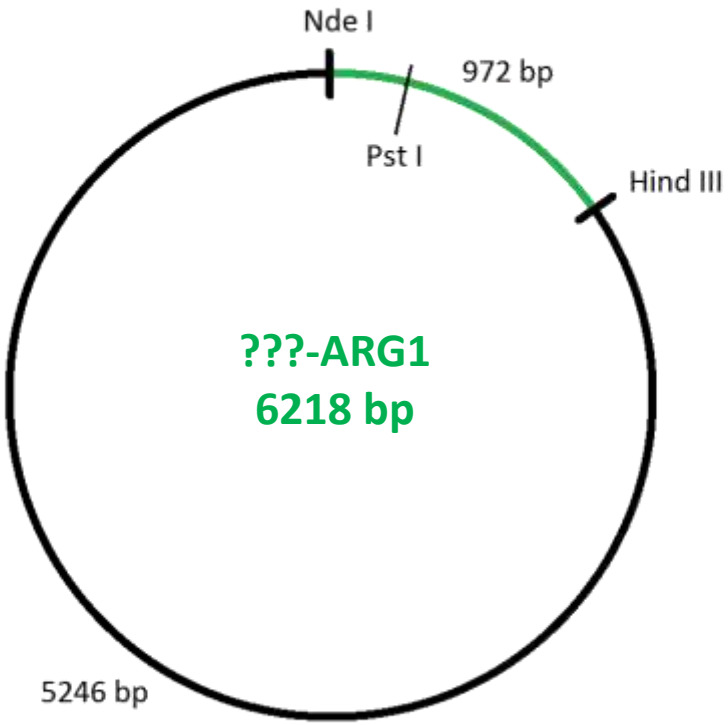

ARG1
ODC
5kb
1kb

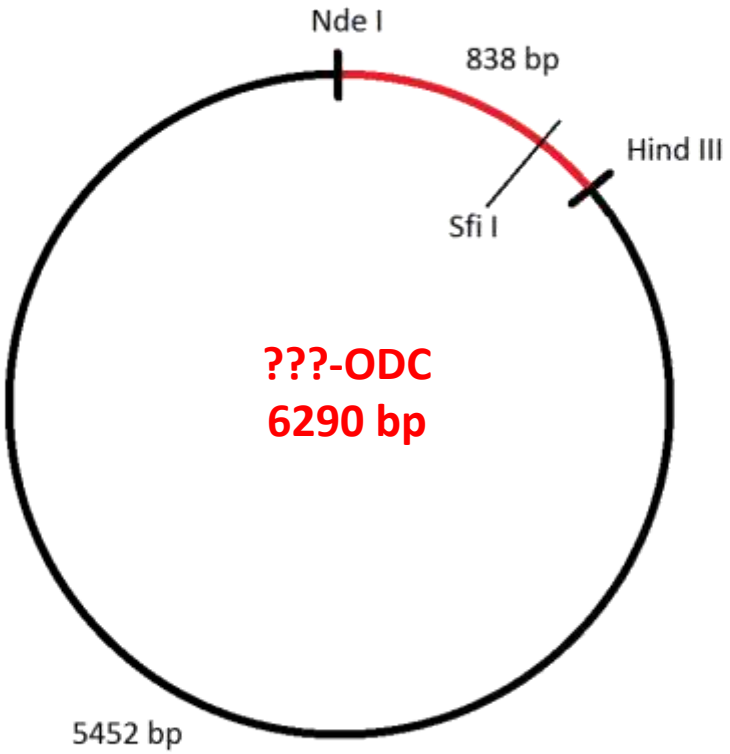
Second Attempt Results

- Restriction digest



Plasmids


Nde I
972 bp
Pst I
Hind III
???-ARG1
6218 bp
5246 bp


Nde I
838 bp
Hind III
Sfi I
???-ODC
6290 bp
5452 bp

Further Analysis

- Selective plating experiments
  - Unfortunately, the strains were poorly marked
  - Selective plates were used to further determine the identity of the stains

Discussion

- The identity was determined to be a NovaBlue TOPO plasmid
  - This accounts for the extra bands observed in the restriction digests

Conclusion

- Selected *E. coli* cultures thought to contain ARG1 and ODC
- Did restriction digests of the DNA
- But ran out of time to produce standards

Acknowledgements

- WY EPSCoR
- Dr. Debashis Dutta
- Dr. Mark Stayton
- Dr. Jacque Keele
- Dr. Mark Harpster