Determining the Structure of a Nitric Oxide Synthase (NOS)
What is Nitric Oxide?

Many functions in mammals:
• Control vasodilation
• Immune response
• Nerve signal transmission

Produced by an enzyme – Nitric Oxide Synthase
Mammalian NOS vs *Synechococcus* pcc7335 NOS (spNOS)

- Most bacterial NOSs have only oxygenase domain
- spNOS and mammalian NOS are very similar
  - Heme domain, reductase domain
Project Stages & Goals

1. **Clone Sequence**
2. **Protein is Expressed, Soluble, & Purified?**
3. **Grow Protein Crystals?**
4. **Collect & Process Diffraction Data?**

Options:
- **GO**
- **SLOW**
- **STOP**

*Maybe Solve Structure*
Synthesis of DNA

Polymerase Chain Reaction (PCR)

DNA Electrophoresis Gel
Restriction Enzyme Digestion of DNA

- Forward Primer NdeI
  5’…CA*TATG…3’
  3’…GTAT*AC…5’

- Reverse Primer EcoRI
  5’…G*AATTC…3’
  3’…CTTAA*G…5’
Restriction Enzyme Digestion of DNA

- Forward Primer NdeI
  5’…CA*TATG…3’
  3’…GTAT*AC…5’

- Reverse Primer EcoRI
  5’…G*AATTC…3’
  3’…CTTAA*G…5’

- Higher bands (PET) at ~6kbp
- Lower bands (insert) at ~0.5kbp
Ligation of Protein DNA to Bacterial Vector

Restriction enzymes cut out the DNA of interest and open the vector.

DNA ligase joins the two DNA sequences together.

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Transformation

• Vector is inserted into E. coli cells through transformation

• Bacteria colonies grow only if insert is present in bacteria
Confirm DNA Insertion

- Bright and glowing bands (PET) at ~6kbp
- Lower bands (insert) at ~0.5kbp
Where we are now
Future Work

- Clone Sequence
  - Protein is Expressed, Soluble, & Purified?
    - Grow Protein Crystals?
      - Collect & Process Diffraction Data?
        - Maybe Solve Structure
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