Research Posters Fall 2011

Spelman College G-STEM

Follow this and additional works at: http://digitalcommons.auctr.edu/scgstempst

Part of the Animal Sciences Commons, and the Plant Sciences Commons

Recommended Citation
http://digitalcommons.auctr.edu/scgstempst/2

This Article is brought to you for free and open access by the Enhancing Global Research and Education in STEM at Spelman College (G-STEM) at DigitalCommons@Robert W. Woodruff Library, Atlanta University Center. It has been accepted for inclusion in G-STEM Posters by an authorized administrator of DigitalCommons@Robert W. Woodruff Library, Atlanta University Center. For more information, please contact cwiseman@auctr.edu.
Potential Regulatory Region in the Mouse Brain

Taisha Blair
Mark Lee, PhD.
Biology

Objective
Two oligonucleotides were created to possibly clone a region from the mouse brain upstream from the DLX5/DLX6 genes which may be regulated by FGF signaling that could be a potential regulatory region.

Introduction
Gene expression is a crucial step in embryonic development. Regulatory elements are regions necessary for the expression of genes in specific tissues projected to be conserved evolutionarily due to recent whole genome sequencing of an ample amount of species (Courtens et al., 2005). The DLX5 and DLX6 genes are transcription factors important for craniofacial, inner ear, and limb development, believed to be regulated by a long-range enhancer (Brown et al., 2010).

Materials and Methods
Breakpoint forward and breakpoint reverse oligonucleotides went through Polymerase Chain Reaction using High- Fidelity polymerase (PCR)

The dilutions from PCR went through a .9% agarose gel to confirm the presence of a 3kb band and then cut out to be gene cleaned

Digestion of insert and vector to determine if DNA band was present and if vector was cut

Digestion of insert and vector and precipitation of DNA fragment

Transformation plates were made to create colonies to be able to inoculate cultures

Plasmid minipreps were made from the 12 inoculation cultures then digested and run through a gel to determine if insert successfully entered the cut

Results
The necessary conditions for the amplification of the DNA through PCR were found because the presence of a 3kb band was identified after gene cleaning and colonies were present during the transformation plate.

Fig. 2: This picture is showing the 3kb band of the DNA under PCR conditions 61.6°C, -DMSO, 50 genomic mouse DNA, +HF Buffer

Fig. 3: One of the transformation plates containing colonies

Conclusion
The cloning of a region in the mouse brain was attempted in this project. Although there was insufficient time to slightly alter the parameters of the minipreps to allow the plasmids to successfully contain the insert, the conditions were established for reproducible fragments for DNA to be amplified. This research will go on to successfully contain an insert allowing for the DNA to be cloned, and potentially identify the regulatory region in the mouse brain upstream of the DLX5/DLX6 genes.

References

Acknowledgements
This research was based upon work supported by the National Science Foundation (NSF) under Grant # HRD-0963629. Any opinions, findings, and conclusions expressed are those of the author and do not reflect the views of the NSF. Spelman College G-STEM program, and the University of Sussex Neuroscience Department in Brighton, England also supported this project. I thankfully acknowledge Dr. Mark Maconochie and Dr. Mark Lee.
Hypothesis: An oxidizing environment encourages the formation of an oxidized form of nitrogen (nitrate) and discourages the formation of reduced species like iron Fe(II) compounds. The opposite is true in a reducing (oxygen deficit) environment. Water and sediment samples collected from the River Leith located in England should exhibit the same behavior.

Methods and Material:

- **Fe2+** was analyzed using the spectrophotometer (it has distinctive absorption bands in certain regions of the spectrum). After it was bound to 0.1% o-phenanthroline as a ligand... Another technique that needed was the use of titrations of mixtures to accurately measure the concentration Fe0.

Data and Analysis (July 2010):

<table>
<thead>
<tr>
<th>SU1</th>
<th>SU2</th>
<th>SU3</th>
<th>SU4</th>
<th>SU5</th>
<th>SU6</th>
<th>SU7</th>
<th>SU8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nit...</td>
<td>Nit...</td>
<td>Nit...</td>
<td>Nit...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Notes:**
- Increase in concentration
- Decrease in concentration
- Consistent with hypothesis

Literature References:


Acknowledgements:

This research was based upon work supported by the National Science Foundation and Grant # HRD-0653629 (G-STEM). We acknowledge the G-STEM program for their continuing effort to encourage global study experiences.
Structure and Function of the Plant Alternative Oxidase Studied by Site-directed Mutagenesis

Chelsy Webb
Mark Lee, Ph.D.
Biology

Objective
In this project, a heme-deficient strain, FN102, of *Escherichia coli* is used in order to determine if oxygen production was due only to the AOX. Using *E.coli* as a model, the mutation Cys-172 was inserted using site-directed mutagenesis to determine the structure function relationship. Cys-172 is known to be a well conserved cysteine residue that alters the AOX’s affinity for oxygen (Crichton, 2005).

Introduction
The alternative oxidase is an integral membrane protein found in plants, protists, some fungi, pathogenic organisms, and several other organisms. AOX acts as an alternative pathway in the electron transport chain. This type of pathway is found in *Sauromatum guttatum* as well as *trypanosoma brucei* (Albury, 2002). The wild type for this project was taken from *S. guttatum*. In the electron transport chain, the flow of electrons leads to the synthesis of ATP in the mitochondria. Overall, the electron transport chain consists of four membrane bound complexes, an ATP synthase pump, coenzymes, and cytochromes (Figure 1).

Materials and Methods
Bacterial plasmid, pET15b, containing the AOX gene was inserted into FN102 *E.coli* cells. Agar plates were streaked and incubated.

Using a Clark-type electrode, oxygen consumption rate was recorded.

The amount of protein in a given membrane sample was obtained using Pierce BCA protein assay kit.

SDS-PAGE/Western Transfer was performed in order to confirm that AOX was properly expressed in the *E.coli*.

Summary of Results
1. The alternative oxidase was properly inserted and expressed. Through growth and harvest several cell samples were obtained.
2. The WT AOX was also expressed in order for comparison purposes.
3. Mutant C172 lowers the alternative oxidase’s ability to reduce oxygen and synthesize ATP.

<table>
<thead>
<tr>
<th>Date of C172 Sample</th>
<th>Rate of Consumption (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-Oct</td>
<td>37.24</td>
</tr>
<tr>
<td>2-Nov</td>
<td>42.9</td>
</tr>
<tr>
<td>17-Nov</td>
<td>32.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Rate of Consumption (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-Oct</td>
<td>87.96</td>
</tr>
</tbody>
</table>

Table 1: Rate of Consumption for C172 and WT

Results Cont’d
Using a formulated excel spreadsheet, the protein estimation was determined to be 39.9 mg/mL. The overall amount of protein for the entire sample was estimated to be 163.63 mg/mL. The spreadsheet also calculated the specific activity to be 328.00. The protein estimation for the WT was 28.9 mg/mL and was obtained from one of the post-graduates working in the lab.

![Figure 2: The western blot showed that the AOX was properly inserted into the plasmid based on the literature value for the alternative oxidase. It also shows that AOX exist as a dimer at 76 kD.](image)

Conclusion
Through site-directed mutagenesis, we determine the structure function relationship of the plant alternative oxidase by expressing AOX in a heme-deficient strain of *E. coli*. By using additives such as 5-aminolevulinic acid (ALA) and other antibiotics, the *E.coli* was able to override death. Once the ALA was washed away oxidase was used to allow *E. coli* to survive. This proved that oxygen consumption was based solely on AOX. In comparing the WT to C172, the mutant decreased the overall oxygen consumption meaning it has less affinity for oxygen. This report proved that *E. coli* can be used and yield similar results as in the published literature (Kido, 2010).

References

Acknowledgements
This research is based upon work supported by the National Science Foundation under Grant # HRD-0963629. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. National Science Foundation (NSF), Spelman College G-STEM program, University of Sussex Biochemistry Department (Brighton, England). I gratefully acknowledge Dr. Antony Mary, Dr. Mark Lee, Mary Albury, Julia Shearman, and Tom Stimpson.