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Research Posters Fall 2011

Spelman College G-STEM

Potential Regulatory Region in the Mouse Brain

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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).

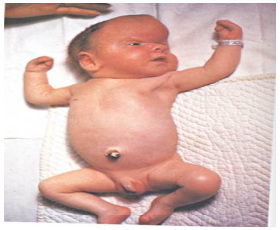

Fig.1: A baby with craniofacial and hand deformities

Previous studies suggest chromosomal rearrangements upstream of the transcription factors caused embryo developmental defects, such as craniofacial deformities (Brown et al., 2010). Using the breakpoint oligonucleotides, the dependence of the development of an embryo will aid in verifying where the regulatory region is located and if it is indeed regulated by FGF signaling.

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.

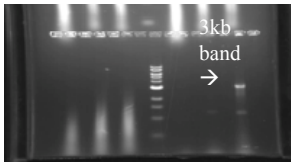

Fig. 2: This picture is showing the 3kb band of the DNA under PCR conditions 61.6° C, -DMSO, 50 genomic mouse DNA, +HF Buffer

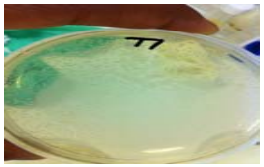

Fig. 3: One of the transformation plates containing colonies

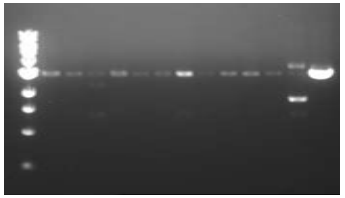

Fig. 4: 12 plasmids that could possibly contain inserts

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

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Analysis of Oxidized and Reduced Compounds *in the River Leith*

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G-STEM

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:

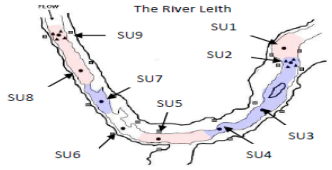


Figure 1: The River Leith and the 9 pizometer either pool sites (pink) or riffle sites (blue). The water flows from upstream [SU9] to downstream [SU1].

Spectrophotometric determination of iron(II)

- Fe^{2+} 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 to improve selectivity. Another technique that needed was the use of titrations of mixtures to accurately measure the concentration Fe^{2+}.

- The procedure was to prepare intermediate solution from stock solution 10,000 mg/L Fe solution to make 50ml of 50 mg/L Fe solution. Next, to prepare a calibration standard of $Fe^{2+}$and each standard needed, the following reagents were added before dilution to 100ml:

✓4mL of Hydroxylammonium hydrochloride solution
✓7mL of sodium acetate buffer
✓2mL of 0.1% o-phenanthroline
✓ The required amount of Fe intermediate solution
(Rep

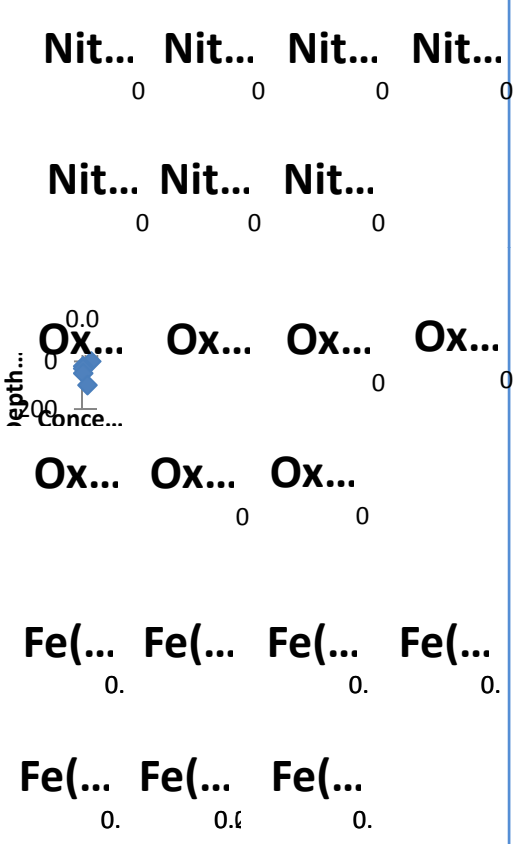
Standard #	[Fe(II)] (mg/L)	Final Volume (mL)	Volume of intermediate (mL)
1	0	100	0
2	0.05	100	0.1
3	0.1	100	0.2
4	0.5	100	1.0
5	1.0	100	2.0

$C_1V_1=C_2V_2$

- The absorbance was measured for each calibration standard in triplicate at a wavelength of 510nm. (Make sure to zero the spectrophotometer standard 1). A calibration curve was then constructed by using a trendline to see how well it matched to the standard as well as previous calibration curves. (Detection range of concentration must be between 0-2.0 mg/L)

- The instrument used to indentify species such as NO_3^- and O_2 was the Dionex high performance liquid chromatograph. This instrument is programmed to be a time and self-regenerating instrument that was useful for sediment-free samples. The Dionex HPLC was typically used for chloride analysis. Other species with very low detection levels and small sample sizes were analyzed by a Skalar continuous flow analyzer. This instrument was generally used for the analysis of nitrates, phosphates, nitrites, oxygen, sulphates and ammonium. It also uses absorption to identify and quantify pollutants, but works well for species that are sedimentary samples. This instrument focused more upon the denitrification process.

Data and Analysis (July 2010):



Results

- Data analysis of SU sites Oxidized vs. Reduced Environment (Groundwater)

	O_2	NO_3^-	Fe^{2+}	
SU1	↑	↓	↓then ↔	
SU3	↓	↑	↓then ↔	
SU4	↓	↑	↓	
SU5	↑	↔		↓
SU6	↑	↑		↑
SU7	↔	↔		↑
SU8	↑	↔	↔	

↑= increase in concentration
↓= decrease in concentration
↔ = consistent concentration
←,↔,→= inconsistent w/ hypothesis

Literature References:

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Structure and Function of the Plant Alternative Oxidase Studied by Site-directed Mutagenesis

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Objective

In this project, a heam-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 mitochondira. Overall, the electron transport chain consists of four membrane bound complexes, an ATP synthase pump, coenzymes, and cytochromes (Figure 1).

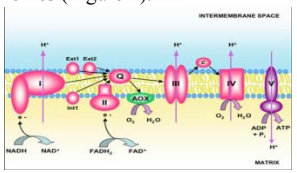


Fig. 1: The structure of the electron transport chain showing the placement of AOX.

AOX is found between complex II and III as seen in Figure 1. In this respiratory process energy is produced as electrons are transferred down the chain. Complex I and II transfer electrons to ubiquinone, which acts as an electron carrier. Ubiquinone then passes these electrons to complex III, which then passes them along to cytochrome c (Lehninger, 2005). In the alternative respiratory pathway, electrons are carried from ubiquinone to the AOX, bypassing complexes III and IV and cyctochrome c. This alternative pathway is a one-step process that donates four electrons directly to the oxygen which is then reduced to water, making the alternative oxidase the terminal electron acceptor.

Materials and Methods

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

The transformed cells were grown in 5 liter quantities allowing the AOX gene to be expressed. Cells were harvested to extract membrane samples.

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.

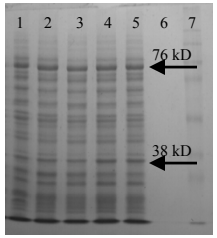
Date of C172 Sample	Rate of Consumption (nmol/min)
13-Oct	37.24
2-Nov	42.9
17-Nov	32.94
Date WT of Sample	Rate of Consumption (nmol/min)
21-Oct	87.96

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.



1 and 2-Mutant C172A
3-Wild Type
4 and 5- not relevant to this lab report
6-blank
7- Rainbow marker

Conclusion

Through site-directed mutagenisis, 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).

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Acknowledgements

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