Type 2 diabetes and obesity

During my Scholarship I learn different things first of all I learn to design primers to run PCR and agarose gel electrophoresis and to sequencing. Also I used to with animal handling because I saw different methods like EchoMRI and Indirect Calorimetry. Also other transferable skills that I gain were that I worked as part of a group in the lab. I helped to keep the working area clean and tidy and contributed to the lab. Interaction with other staff members that I met and talked with people in other labs. Finally important thing that I learn was the self-organisation because my supervisor and the start of my Vacatin Scholarsip explain to me what I need to do during the eight weeks s after I organised my experiment during this time and run my experiment.

This project was on a new ENU model of Type 2 Diabetes and Obesity that had already been identified with 2 coding mutations The first aim of my project was to prove that there are no other mutations that account for the phenotype allowing a focus on the two candidate genes this achieved by analysis of the sequence to identify mutations and polymorphisms and validation of any new mutations y sequencing and then genotyping of affected and wildtype animals.

The second aim was to understand at the physiological levels why the mice became obese so that further mechanistic work can be carried out.

**Introduction**

There are three mouse models of obesity and type 2 diabetes has been identified from the ENU Mutagenesis project, MRC Harwell. My project was focus n BIGBOY was identified as obese and hyperglycaemic from the dominant ENU screen. BIGBOY (+/-) mice are insulin resistant, hyperleptinaemic, have an altered metabolic rate.

Genome analysis of BC1 DNA and sebsquent SNP genotyping identified linkage to Chr 13 between 93.07 and 97.42 Mb for BIGBOY (+/-). Whole genome sequencing identified 3 coding mutations of which 2 were validated. Single base changes resulting in missense amino acid substitutions were identified 2 genes in highly conserved residues. Genotyping has shown that both mutations are observed in BIGBOY (+/-).

Although a few small regions of genes within the critical interval have not yet been sequenced at adequate depth in order to rule out any other possible mutations. DNA will be prepared from heterozygous mutant and wild type mice.

**Methods: Primer design and Optimisation**

First PCR primers were designed using ExonPrimer(<http://biotools.umassmed.edu/bioapps/primer3_www.cgi>) for exons and flanking sequence in the regions of interest and the fragments amplified by PCR.

After PCR amplification was carried out on template the F1 DNA strain control and H2O as a negative control this happened because need to identify in which conditions each gene have the ability to work so we used two different concentrations of MgCl2 [2.5 μl 2.5mM MgCl2 (Applied Biosystems)and 1.5 μl 1.5mM MgCl2 (Applied Biosystems)]; in two different temperatures [WAVE 60 and WAVE 55 programme]. Made up to a 25 μl volume with d H2O.

PCR thermocycling was carried out on a G-storm thermocycler (Gene Technologies Limited, Essex,UK), using the WAVE 60 and WAVE 55 programme.

5 μl of PCR product was analysed on a 2% agarose gel containing 0.4 μg/ml ethidium bromide and visualised using a Gel Doc XR Molecular Imager (BioRad, Hertfordshire, UK). Product size was estimated next to a 100 bp ladder (100 bp-2072 bp, Invitrogen).

**Re-optimisation-PCR Gradient**

Some gene have not the ability to work in any conditions so for this genes we try the PCR gradient

Gradient PCR was used in order to determine the optimal annealing temperature. Using the gradient function of the universal block, a gradient of 53 to 67°C was set.

PCR amplification was carried out on template DNA F1 and no H2O.

Although some genes didn’t work and with PCR gradient for this reason didn’t know in which conditions have the ability to work. So re-design PCR primers using different program this time (<http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html>).

**PCR for sequencing**

So each gene that have work in one of the conditions above, we run second PCR amplification was carried out this time on different template DNA from C57BL/6J and C3H, founder F1 CBMLC/661.3e **using the conditions that each have work during optimisation.????(is correct like this)**

Made up to a 50 μl volume with d H2O.

5 μl of PCR product was analysed on a 2% agarose gel containing 0.4 μg/ml ethidium bromide and visualised using a Gel Doc XR Molecular Imager (BioRad, Hertfordshire, UK). Product size was estimated next to a 100 bp ladder (100 bp-2072 bp, Invitrogen).

**PCR Purification**

PCR products were purified using a QIAquick PCR purification kit (Qiagen, Sussex,UK) according to the manufacturer’s instuctions and eluted in 25 μl ddH2O.

**Sequencing**

Sequencing was carried out by Source Bioscience (www.sourcebioscience.com). Samples with 10 pmol/ μl of forward and reverse primer.

All sequencing files were analysed using DNASTAR Lasergene 7 SeqMan software (DNASTAR Inc, Wisconsin, USA). Μl

**Results**

Run a lot of PCR because tried to work all genes but finally send about **(number genes that send for sequencing) ????** but didn’t observe any other mutation.