  Type 2 diabetes and obesity

**Research Background**

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

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 in a mouse model called “BIGBOY” that have been identified as obese and hyperglycaemic from the dominant ENU screen.

BIGBOY (+/-) mice are insulin resistant, hyperleptinaemic and have an altered metabolic rate.

Genome analysis of BC1 DNA and subsequent SNP genotyping identified linkage to Chromosome 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 (+/-).

**Design Primers**

The first step of my project was to design primer and optimisation. 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.

Because each gene has the ability to grow in different concentrations for these reason we used two different concentrations of MgCl2.

GENE

MgCl2 [2.5 μl 2.5mM] MgCl2 [1.5 μl 1.5mM]

In WAVE 55 and 60 programme

5 μl of PCR product was analysed on a 2% agarose gel containing 0.4 μg/ml ethidium bromide.

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 for sequencing**

So for each gene that have worked in one of the conditions above, a second PCR  amplification was carried out this time on different template DNA.

**PCR Purification**

PCR products were purified using a QIAquick PCR purification kit (Qiagen, Sussex, UK) according to the manufacturer’s instructions 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.

**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.