Genotyping of OPN3 in Knockout & Wild-Type Mice

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Introduction & Background

Opsin 3 (OPN3) is a G protein-coupled receptor that responds to ultraviolet A affected skin by selectively activating G proteins. The OPN3 protein has 7 transmembrane segments along the cellular membrane; it has an extracellular N-terminus and an intracellular C-terminus (see **Figure 1**).In the Oancea lab, a tag called FLAG is attached to the C-terminus of OPN3 in order for us to locate the membrane protein in a cell.

We use OPN3 in conjunction with melanocortin 4 receptor (MC4R) which plays a role in homeostasis and metabolism. MC4R negatively regulates eating and energy consumption.

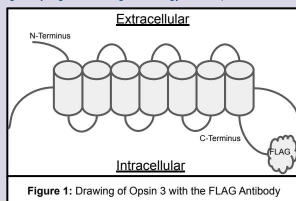


Figure 1: Drawing of Opsin 3 with the FLAG Antibody

Goals

The goal for this summer was to find a way to genotype all the mice in the lab using a reliable genotyping method. The mice all have a specific genotype in terms of the OPN3 gene. Each mouse could either have: homozygous wild-type (WT), homozygous knockout (KO), or heterozygous. A WT mouse would have the genotype (+/+) for the OPN3 gene; a KO mouse would have the genotype (-/-) for the OPN3 gene; and a heterozygous mouse would have the (+/-) genotype. It is important for the lab to have a definitive and reliable method of genotyping so that it will be efficient to genotype mice in the future. See **Figure 2** for the schematic of our lab's mouse experiment.

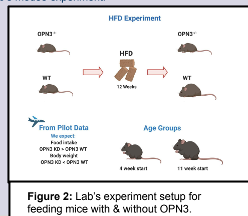


Figure 2: Lab's experiment setup for feeding mice with & without OPN3.

Genotyping & PCR Method

To genotype a mouse means to determine what genotype that mouse has. In this lab, I have experimented with a total of 4 genotyping kits: KAPA, GoTaq, Terra, and Azura.**Figure 3** includes the times and temperatures for PCR (polymerase chain reaction) needed for the Azura kit (**Figure 4**). PCR includes a total of 4 steps: melting, annealing, extending, and cooling. In the melting step, the DNA strand is untangled so that the kit contents can attach to the desired DNA sequence. Once melting occurs, the annealing step consists of sequence-specific primers attaching to the DNA sequence; there are forward and reverse primers that must contain about 40-60% GC nucleotides and must not anneal to each other. In this lab, we have a WT-specific forward primer and a KO-specific forward primer; the reverse primer is the same for both. The next step, extending, includes the extension of the DNA copy sequence, which is done by the protein Taq polymerase. After extending, the first three steps are repeated for about 32 cycles, and then the contents are cooled down to 4ºC for stability and a conclusion to the PCR.

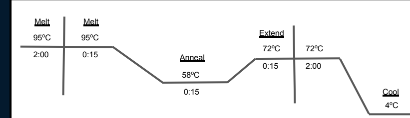
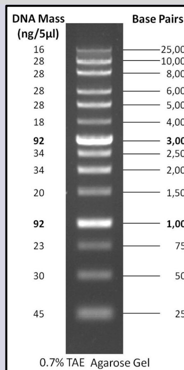


Figure 3: Times & Temperatures for PCR using the Azura kit

Figure 4: Azura Kit Contents

Gel Electrophoresis Method

Gel electrophoresis is a method use to separate the DNA fragments in terms of their size. Each miniature PCR reaction is loaded onto a gel,and an electric current pulls the DNA fragments through the gel at different lengths. The farther a fragment runs, the smaller the amount of base pairs there are in the DNA fragment. For a WT DNA sequence, the fragments should be 530 base pairs (bp) long; for a KO DNA sequence, the fragments should be 670 bp long. **Figure 5** includes an image of the ladder used to run alongside the PCR wells. This is used so that we can see at what lengths (and in turn, how many bp) a well runs. A 1kb (kilobase) ladder is used because it is easy to determine which bands are 530 bp and which are 670 bp.



Figures 5&6:DNA Ladder for 1000 base pairs

Results

In **Figure 7**, no bands we shown using the KAPA kit. This led us to find another kit to test with in case this kit did not interact well with our designed primers. This was concluded since the primers are shown as bands at the bottom of the image. In **Figure 8**, we added DMSO (dimethylsulfoxide) to see if that would prevent the primers from annealing to each other. We also lowered the annealing temperature to see if any bands would appear, and it seems that many bands appear in the form of a gradient. However, we needed to see solid bands.

Then, in **Figure 9**, we used the Azura kit, and it provided us with a 100% success rate of showing clean, solid bands in the lanes that we expected. We used control lanes 1 & 2 to show that the gel itself worked properly.

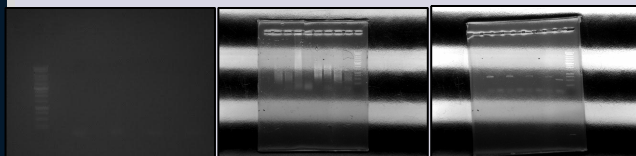


Figure 7: Blotted on 6/28/21, no bands, only can see WT primers

Figure 8: Blotted on 7/15/21, DMSO included, band gradients present

Figure 9: Blotted on 8/2/21, Azura kit, first full success, control lanes 1&2

Conclusions

According to the results shown, we have concluded that the Azura kit works the best for our lab in terms of interacting properly with our designed primers. The addition of DMSO was an important find to help us understand whether or not our primers were faulty. Although they seem to be slightly faulty, the switch to a different kit (Azura) improved our results dramatically.

A drawback of the Azura kit however is that the Taq mix includes everything other than our primers. That means that if we were to optimize anything in the experiment, it would be difficult with the little amount of materials we use.

The use of Ethidium Bromide (EtBr) in the gel mix is important because it helps brighten the bands that we need to see. However, because EtBr runs in the opposite direction of the bands, it is hard to see the bands if they are on the far end of the gel. Due to this, we can only run gels with one row of lanes.

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