Genotyping: MBNL1 and MBNL2 Mice

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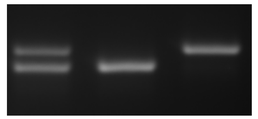
Introduction

The term genotyping comes from greek words “genos” and “-tupos”, which mean race/offspring and type. In other words genotyping is determining the genetic makeup of an individual. Through genotyping, one can determine whether a mouse is a heterozygous, wild type, or knock-out mouse. This information shows which alleles the progeny inherited. The genotype of the mouse is represented by the following sign patterns:

Heterozygous: +/-

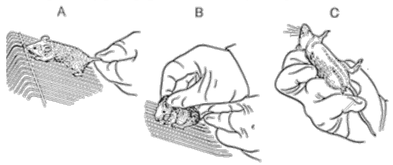
Wild Type: +/+

Knock-out: -/-



Tail Biopsy

Often times, tail samples on newborn pups are genotyped before the pups become three weeks old. After three weeks, the pups begin to develop nerves in the end of their tails and must be put under anesthesia if a tail sample is needed. The sample should be less than 5mm. To obtain the sample, the mouse should be scruffed and held like in the picture. Each individual sample is placed in a labeled tube. Once all samples are collected the genotyping process may begin.



Genotyping Process

Once the tail samples have been collected, the sample digestion process begins. The digestion is followed by the PCR (polymerase chain reaction). Subsequently, the gDNA are subjected to electrophoresis and are run in a gel to determine the sample genotype.

Digestion:

1. A digestion buffer is prepared to ensure each tube gets 500 µl of tail lysis buffer and 5 µl of Proteinase K.
2. Incubate samples on the thermomixer set at 50° C at 500rpm for 4 hours.
3. Add 500 µl of 100% EtOH (Ethanol) to each tube.
4. Centrifuge the samples on the max speed for 10 minutes.
5. Remove the supernatant by carefully pouring into a waste bottle; be sure not to disturb the pellet.
6. Add 500 µl of 70% EtOH (Ethanol) to each tube.
7. Centrifuge the samples on the max speed for 10 minutes.
8. Remove the supernatant by carefully pouring into a waste bottle; be sure not to disturb the pellet.
9. Centrifuge the samples for at least 1 minute.
10. Remove the remaining EtOH with a micropipette.
11. Add 100 µl of 1 X TE to each tube
12. Incubate samples on the thermomixer set at 80° C at 0 rpm for 1 minute with the tube caps open to remove excess EtOH from the pellets.
13. Leave the samples on the thermomixer set at 80° C at 0 rpm for 15 minutes with the tube caps closed.
14. Store the DNA at 4° C.
15. Prepare the PCR reaction using the table below.

MBNL 1 PCR (1 Sample)

DNA - 1 µl

Primers:

- 30 µM 884 - 0.5 µl
- 30 µM 1382 - 0.5 µl
- 30 µM 1383 - 0.5 µl

10 mM dNTPs - 0.5 µl

10 X HiFi Buffer - 2.5 µl

Phire II Taq - 0.25 µl

ddH_2O - 19.25 µl

Total volume of solution - 25 µl

MBNL 2 PCR (1 Sample)

DNA - 2 µl

Primers:

- 30 µM 4254 - 0.5 µl
- 30 µM 4255 - 0.5 µl
- 30 µM 4256 - 0.5 µl

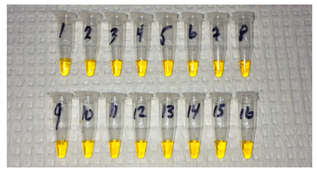
10 mM dNTPs - 1 µl

HiFi Buffer - 5 µl

Invitrogen Taq - 0.5 µl

ddH_2O - 40 µl

Total volume of solution - 50 µl



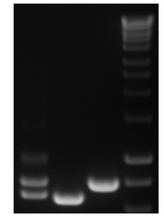
Summary

- Digest the samples
- Prepare the samples for PCR
- Place the samples in the Thermomixer under the saved files for MBNL1 and MBNL2
- Add 1/10 reaction volume of loading dye to the reactions
- Prepare an agarose gel of the appropriate percent

Electrophoresis and Results

For MBNL1 samples, a 1.5% gel is made using 2.55g of Agarose and 170 mL of 1 X TAE Buffer. The 1 X TAE Buffer is poured into an Erlenmeyer flask. Agarose is added to the flask and the solution should be heated in an approved microwave for 5 minutes. Next, allow the solution to cool for 1-2 minutes. Using the hot hands, remove the flask from the microwave and place it on the counter near. Add 8.5 uL of ethium bromide to the solution and carefully swirl the solution until the reddish of tint of the ethic bromide is no longer visible. Pour the solution into a casting tray with the desired well comb. Leave the gel to set for 25-30 minutes. Once the gel has solidified, carefully, remove the well comb. Set up the gel box and insert the casting tray holding the gel. Pour 1 X TAE Buffer into the gel box until it covers the gel by at least 2mm. Next, the samples will be loaded. The beginning well is filled with 10 uL of Hyper Ladder. The next wells are filled with the samples. Each well gets 15 uL of an individual sample. After the last sample, the last well is filled with 10 uL of hyper ladder.

For MBNL2 samples the steps remain the same, but the volumes (measurements) have slightly changed. A 2.5% gel is made using 5.00g of Agarose and 200 mL of 1 X TAE Buffer. The 1 X TAE Buffer is be poured into an Erlenmeyer flask. MBNL2 samples require 10.0 uL of ethium bromide. Each well gets 20 uL of an individual sample.



Once the gel is set, it should be run at 90 volts for 40 minutes. After the 40 minutes, the gel can be safely removed and using UV rays, a picture can be taken of the DNA bands.

Work Cited

"Components of the Human Connectome Project." Human Connectome Project. National Institutes of Health, 01 Mar. 2017. Web. 05 Apr. 2017.

Silver, Lee M. Mouse Genetics: Concepts and Applications. New York: Oxford University Press, 1995. Print.

Acknowledgments

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