

PHOSPHOLAMBAN KNOCKOUT PCR GENOTYPING PROTOCOL

Extraction of genomic DNA from mouse tail.

-always in 2.0 ml tube in order to mix completely

1. Add 0.5 ml lysis solution (Stock solution and proteinase K)
 - a. Incubate overnight at 55° in incubator
2. To lysed tubes from incubator add 0.75 ml CHCl₃ (Chloroform)
 - a. Mix thoroughly. Make sure chloroform does not erase the labels on top of the tubes
 - b. Chloroform is used to extract the proteins
 - c. Centrifuge for 5 min @ 10,000 rpm
3. Transfer upper layer to a new tube. The bottom layer is the chloroform and waste so dump in liquid waste jug in lab hood
4. To new 2.0 ml tube add the upper layer and 1 ml of ethanol
 - a. Shake well, then centrifuge for 5 min @ 10,000 rpm
5. After centrifugation there should be a precipitate (DNA) on the tube
6. Pour off the supernatant making sure the precipitate is not poured and turn tube upside down to let it dry
7. Add 250 µl of dil. H₂O, then incubate at 55° overnight to dissolve the DNA

PCR

INGREDIENTS FOR MASTER MIXTURE

10X PCR BUFFER	2 µl
2.5 µM dNTP	2 µl
HLT 7 (1 ug/ul)	0.125 µl
NEO 3' (1 ug/ul)	0.125 µl
JS940 (1 ug/ul)	0.125 µl
Taq polymerase	0.2 µl
Dil H ₂ O	13.425 µl

-mix after each addition and add amount times the number of tails plus little extra!

*10 X Buffer we use is from Perkin Elmer with 15 mM MgCl₂ in 1.5 mL

1. Take 18 µl of master mixture and add to each tube along with 2 µl of DNA
 - a. Mix well
 - b. Include 2 positive control using DNA from homozygous phospholamban knockout and one endogenous PLB (any WT) plus 1 negative control with no DNA added
2. Take tubes and place in robocycler and link programs 1, 7 and 8*

ELECTROPHORESIS

1. Make gel using 100 ml of TAE buffer and add approximately 1.2 grams of agarose to buffer. Heat in microwave on medium power for 1 to 2 minutes.
2. Add 3 μ l of EtBr to the mixture.
3. Pour mixture in tray and allow to harden for 20 minutes
4. Add 1 μ l of dye containing sucrose and mix with DNA/ each sample
5. Add 3 μ l of 100 bp ladder to first well then the negative and positive controls and so on...
6. Take 10 μ l of this and add to each well
7. Set power pack around 80 mV and run for about 1 to 1.25 hours
8. Take gel to developer and expose the film

Information on Primers:

NEO 3' and HLT7: PLB knockout band (450 bp) (only one band for
PLB-KO homozygous)
JS940 and HLT7: Endogenous PLB (550 bp)

NEO 3' TCCTCGTGCTTTACGGTATC
HLT7 TGTGGGTTGCAAAGTTAGGC
JS940 CTTGGTCACTACTGTCAGAAC

*PCR Reaction (' = minutes)

- A. Cycle 1 - 94 degrees- 3 '
- B. Cycle 30 times - 94 degrees - 1 '
53 degrees - 1 '
72 degrees - 1 '
- C. Cycle 1 - 94 degrees - 1 '
55 degrees 1 '
72 degrees 10 '