Scavenger Receptor Genotyping Protocol
Mike Dorrington - Feb 12, 2011

Materials:

1. Mouse Tails
2. Extract 'n' Amp Tissue PCR Kit (Sigma)
3. Primer mix (10μM each)
4. Agarose gel

Procedure:

1. For each reaction (each tail) mix together 100μL of extraction solution and 25μL of tissue prep solution (from Extract n Amp kit)
2. In a 15mL eppendorf tube, add 125μL of mix to each tail end with the cut end facing down
    1. Room temperature for 10min
    2. Then 95˚C water bath for 3 min
3. Add 100μL of Neutralization solution B and vortex liberally
4. The tails (in solution) can either be used for PCR now or be stored at 4˚C until you want to perform the PCR reaction (can be stored this way for up to 6 months)
5. For each PCR reaction mix together:
    1. 4.5μL PCR-grade H2O
    2. 10μL PCR mix (from kit)
    3. 1.5μL primer mix (forward and back)
    4. 4μL of tissue extract solution
6. Mix gently and perform PCR cycling in a thermal cycler:
    1. Denature 3min at 94˚C
    2. Then 30 cycles of:
        1. Denature 1min at 94˚C
        2. Anneal 1min at 56˚C
        3. Extend 1min at 72˚C
    3. Final extension 10min at 72˚C
    4. Hold at 4˚C indefinitely
7. The PCR product can now be directly loaded into an agarose gel and run as per usual

Primer Sets:

SRA WT (~250bp)
F: 5' ACC TTA TAG ACA CGG GAC GCT TCC AGA A 3'
R: 5' GAC TCT GAC ATG CAG TGT TTC TGT A 3'

SRA KO (~350bp)
F: 5' ACC TTA TAG ACA CGG GAC GCT TCC AGA A 3'
R: 5' AGG AGT AGA AGG TGG CGC GAA GG 3'

MARCO WT (~500bp)
F: 5' CAG CTG GGT CCA TAC CAG C 3'
R: 5' CTG GAG AGC CTC GTT CAC C 3'

MARCO KO (~850bp)
F: 5' CCA CGC TCA TCG ATA ATT TCA C 3'
R: 5' GCC TGC AGT GGC CGT CGT TTT A 3'