TRANSGENIC MOUSE EAR BIOPSY DNA ANALYSIS v#1 23-3-2012

DNA is purified from 1 or 2 ear punch biopsies and screened by real time PCR.

DNA PURIFICATION:

Shake or spin tissue to the bottom of the tube.

Add 500 ul of tail buffer containing freshly added 1 mg/ml proteinase K.

PK = stored at –20^{o}C or -80^{o}C as 100 mg/ml in 10 mM Tris pH 7.4, 50% glycerol

Tail buffer = 100 mM sodium acetate, 50 mM EDTA pH 7.4, 0.5% SDS

Incubate tubes at 55^{o}C overnight. After overnight digestion at 55^{o}C, shake the tubes well and centrifuge for 5 min at maximum speed (~13K).

Transfer 400 ul supernatant to a new 1.5 ml tube

Add 4 ul 10 mg/ml RNaseA to give 0.1 mg/ml and stand 37^{o}C 1 hour.

Add an extra 2 ul of 100 mg/ml PK and incubate 37^{o}C for 1 hour.

PHENOL EXTRACT DNA:

Add 400 ul of equilibrated phenol and vortex.

Centrifuge for 2 min at 13 K and transfer the aqueous phase

Add 400 ul of 1:1 phenol:chloroform mixture and vortex.

Centrifuge for 2 min at 13 K and transfer the aqueous phase.

Add 400 ul of chloroform and vortex

Centrifuge for 2 min at 13 K and transfer the aqueous phase.

ETHANOL PRECIPITATE DNA:

Add 1.8 to 1.9 volumes of ethanol to precipitate the DNA and mix well.

Stand at room temperature or on ice for 5 minutes and spin for 2 min at 13K

Wash pellets in 1 ml 75% ethanol. Spin 1 min 13K, and remove all of ethanol

Air dry pellets for about 5 to 10 min.

Add 100 ul TE (10 mM Tris 1mM EDTA)

Leave DNA at room temperature to dissolve.

Store DNA samples at 4^{o}C.

DNA QUANTITATION:

Measure the DNA concentrations by Nanodrop -

Prepare ~100 to 500 ul of a 1 ng/ul stock

PCR QUANTITATION

Prepare a set of standards of 0.25, 0.5, 1 and 2 ng/ml homozygous transgenic mouse DNA to use as the standard curve.

Assay 2 ul = 2 ng of each sample in triplicate 20 ul real time PCR reactions using both transgene and control mouse primers.

Good primers to use are:

Mouse TBP promoter primers

F: TGCAGTCAAGAGCGCAACTG

R: CACCGCTACCGGACTCGAT

Human GM-CSF enhancer primers

F: GGAGCCCCTGAGTCAGCAT

R: CATGACACAGGCAGGCATTC

Anneal at 60^{o}C - 3 step PCR protocol with melt curve