

PCR Protocol

Modified by Anna Heger, *Preclinical Facility*

Chr 14 (MADM)

Purification of tail samples with DirectPCR® Lysis Reagent Tail from Peqlab
Phire Hot-Start II DNA Polymerase

Mastermix for 20 µl

13,65 µl H₂O

4 µl Phire Hot-Start II

0,5 µl dNTPs

0,6 µl Primermix M14 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

Primer Sequences 5' – 3'

Chr 14 for CTG GGG CTA ATT TGG GCT AC

Chr 14 rev ATG CCC CCA CAG AAG GTA AC

177 TCA ATG GGC GGG GGT CGT T (MADM rev)

Results

WT = 698bp

HOM = 400bp

Cycling

98°C 30 sec

98°C 5 sec

60°C 5 sec 34x

72°C 15 sec

72°C 1 min

16°C forever

Gel Elektrophoresis

2,5% Agarose, 1x TAE, 3µl Syber Safe

Run: 110 Volt, 30min

TG

Purification of tail samples with DirectPCR® Lysis Reagent Tail from Peqlab
Phire Hot-Start II DNA Polymerase

Mastermix for 20 µl

13,25 µl H₂O

4 µl Phire Hot-Start II

0,5 µl dNTPs

0,6 µl Primermix TG (10µM)

0,4 µl Primermix Internal Control (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

Primer Sequences 5' – 3'

159 GGA CTG GGT GCT CAG GTA GTG GTT GTC G

162 GGT ACG TCC AGG AGC GCA CCA TCT TCT TCA AGG

oIMR8744 positive internal control CAA ATG TTG CTT GTC TGG TG

oIMR8745 positive internal control GTC AGT CGA GTG CAC AGT TT

Results

WT control = 200bp

TG = 279bp

(This assay will NOT distinguish hemizygous from homozygous animals)

Cycling

98°C 30 sec

98°C 5 sec

60°C 5 sec 34x

72°C 15 sec

72°C 1 min

16°C forever

Gel Elektrophoresis

3% Agarose, 1x TAE, 3µl Syber Safe

Run: 110 Volt, 30min