

PCR Protocol

Modified by Anna Heger, *Preclinical Facility*

Chr 4 (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 M4 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

Primer Sequences 5' – 3'

Chr 4 for GTA CTT GCT GTG TGT CCA GAC C

Chr 4 rev ATT GAA CCC AGG TCC TTT GC

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

Results

WT = 571bp

HOM = 277bp

Cycling

98°C 30 sec

98°C 5 sec

62°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

GT

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 GT (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'

294 CCA AGC TGA AGG TGA CCA AG

295 TCT TCT TCT GCA TTA CGG GG

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

GT = 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