

PCR Protocol

Modified by Anna Heger, *Preclinical Facility*

Chr 17 (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 M17 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

Primer Sequences 5' – 3'

Chr 17 for AGC ACC TGT GCC ATC CTG

Chr 17 rev AGC CAC ACA ATT ATC GAA GAG C

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

Results

WT = 490bp

HOM = 224bp

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