

## PCR Protocol

Modified by Anna Heger

### **Chr 10 (MADM)**

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

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#### **Mastermix for 20 µl**

13,65 µl H<sub>2</sub>O

4 µl Phire Hot-Start II

0,5 µl dNTPs

0,6 µl Primermix M10 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

#### **Primer Sequences 5' – 3'**

Chr 10 for CCG GCC TAG TCT CAC AAG AG

Chr 10 rev AAG GGA GGA GCT GGA GAG TC

MADM for CCT CCC ACA ACG AGG ACT AC (new primer for MADM cassette)

#### **Results**

WT = 215bp

HOM = 607bp

#### **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

**TG**

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

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**Mastermix for 20 µl**

13,25 µl H<sub>2</sub>O

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