

## PCR Protocol

Modified by Anna Heger

### **Chr 9 (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 M9 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

#### **Primer Sequences 5' – 3'**

Chr 9 for GGC CAA ACT AAC CCA AGC AG

Chr 9 rev TAG AGC CTC CTC CCA ACA CC

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

#### **Results**

WT = 464bp

HOM = 289bp

#### **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

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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 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