

**Chr 7 (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 M7 (10µM)

0,25 µl Phire Hot-Start II DNA Polymerase

19 µl Lysis + 1 µl DNA

**Primer Sequences 5' – 3'**

193 GGC TGA ACT GGA GGA TTG TCT AGT TTT CC (Chr 7 wt forward)

194 GCT CTT GCT CTC ATT TCT CAG TTC AAA CCG CGT ATC (Chr 7 wt reverse)

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

**Results**

WT = 490bp

HOM = 579bp

**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

**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