Application note

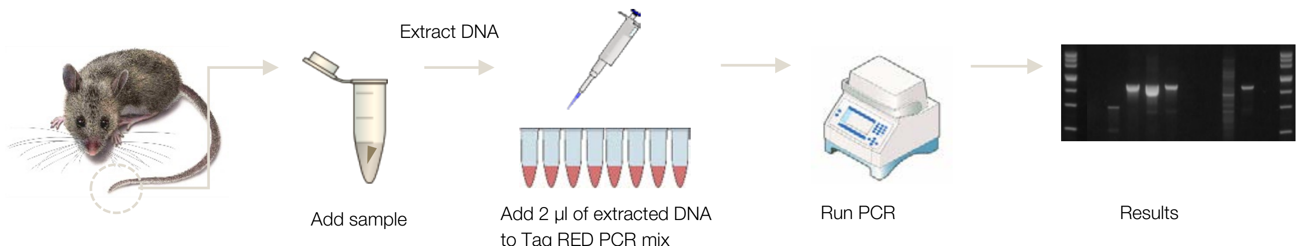
AMPLIQON
PCR ENZYMES & REAGENTS

High throughput testing of mouse genotypes using Taq DNA Polymerase Master Mix RED

This application note describes the use of Ampliqon Taq Master Mix RED for rapid amplification of genomic DNA from mouse tails. Prior to amplification genomic DNA is crude extracted from mouse tails in approximately 90 minutes using NaOH extraction: Quick and "dirty" DNA preparation. (Reference: Truett GE et al. 2000. Biotechniques 29 (1): 52-54.)

Features of application:

- Genotyping using mouse tail tissue
- Easy extraction protocol - Genomic DNA from mouse tails obtained in 90 minutes
- Results obtained 3-4 hours after sampling of mouse tails
- Ideal for high throughput testing
- DNA can be diluted 1:100
- DNA can be stored at -20 °C > 6 months
- Red dye included in Taq DNA Polymerase Master Mix RED for direct gel loading and PCR analysis during gel electrophoresis



DNA fragments from crude extracted mouse tails are amplified using Taq DNA Polymerase Master Mix RED. Final result is observed after gel-electrophoresis and obtained in 3 - 4 hours after sampling, as compared to more than 1 day using standard extraction protocols. Each undiluted crude extract allows enough template for multiple genotyping PCR reactions and can easily be handled in 96-well format for high throughput testing. Extracts can be diluted 1:100 depending on DNA concentration and assay condition. Extracts (either diluted or undiluted) are stable at -20 °C for at least 6 months.

Taq DNA Polymerase Master Mix RED is a ready-to-use master mix containing all components for reliable PCR, except primers and DNA template. The inert red dye and stabiliser are extra features of this master mix, which allow direct loading onto your agarose gel for analysis of PCR reaction during gel electrophoresis without the need for adding a loading buffer and dye. DNA fragments generated with Taq 2x Master Mix RED are 3'-dA-tailed and may be cloned into TA.

Genotyping of mice:
Genetically modified mice are frequently used for biomedical research. It is necessary to identify and/or confirm the genotype of mice, to eliminate genetic contamination and also to control the quality of the mice colony. Fast and reliable results for genotyping is most often provided by polymerase chain reaction (PCR). Successful amplification by PCR requires high-quality genomic DNA from various mice tissues, such as mouse tails.

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Simple protocol

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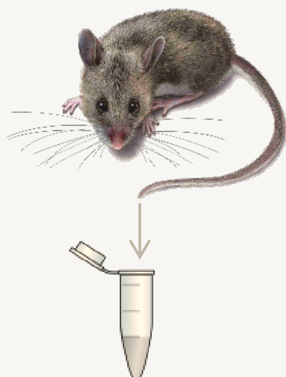


1. Preparation of the mouse tail DNA

Crude extract by NaOH extraction (quick "dirty" DNA preparation).

- → Cut 2 mm of tail and place into an Eppendorf tube or 96-well plate
- → Add 75 µl 25 mM NaOH / 0.2 mM EDTA
- → Place in thermocycler at 98 °C for 1 hour, then reduce the temperature to 15 °C until ready to proceed to the next step
- → Add 75 µl of 40 mM Tris HCl (pH 5.5)
- → Centrifuge at 4000 rpm for 3 minutes
- → Take an aliquot for PCR (use 2 µl undiluted, or 2 µl of a 1:100 dilution/reaction)

+ primers
+ PCR grade H_2O

2. PCR protocol

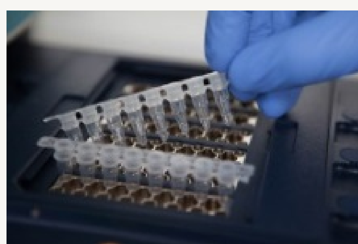
Pipet the following reaction mix.

For more than one sample, scale the volumes up and add extra 10 % volume.

Component	Vol./reaction	Final concentration
Taq 2x Master Mix RED	12.5 µl	1x
PCR-grade H_2O	9.5 µl	-
Forward primer (10 µM)	0.5 µl	0.2 µM
Reverse primer (10 µM)	0.5 µl	0.2 µM
TOTAL volume	23 µl	Final reaction volume: 25 µl

Distribute 23 µl reaction mix into each tube.

Add 2 µl of mouse tail DNA crude extract and run the PCR.



3. PCR program

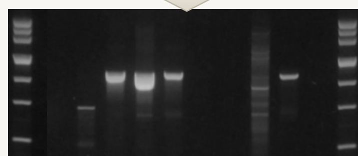
Temperature	Duration of cycle	Cycles
95 °C	5 min	1
95 °C	20 sec	
50 – 65 °C*	30 sec	25 - 35
72 °C	30 sec	
72 °C	5 min	1

* the annealing temperature depends on the primer set

4. Gel electrophoresis

Load 10 µl of the PCR product directly on an agarose gel.

The percentage of the agarose depends on the expected product size.



5. Analysis and result

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