Direct PCR from Mouse Tails ("HotShot") using Opti*Taq* DNA Polymerase

Contributed Protocol
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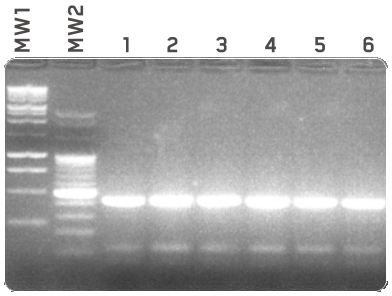

Figure 1: Direct PCR from mouse tail samples of homozygous mice. MW1: EURx 1 kb Plus Perfect Ladder (Cat. no. 3131), MW2: EURx 100 bp Perfect Ladder (Cat. no. 3134), lanes 1 to 6: direct PCR from different samples of homozygous mice, demonstrating high reproducibility of PCR band patterns upon amplification from non-purified, "dirty" mouse tail template DNA with Opti*Taq* DNA polymerase (Cat. No. E2600).

Buffers:

Alkaline Lysis Reagent pH = 12, no further pH adjustment required		
Reagent	Final conc.	Amount per 200 ml
NaOH	25 mM (MW=39,997 g mol $^{-1}$)	200 mg
EDTA	0.2 mM (MW=292,24 g mol $^{-1}$)	14.88 mg

Neutralization Buffer pH = 5 no further pH adjustment required		
Reagent	Final conc.	Amount per 200 ml
Tris-HCl	40 mM (MW=157,60 g mol $^{-1}$)	1.3 g

This protocol describes genotyping by direct PCR amplification from mouse tails or mouse ear clips, without any prior DNA purification. Based on previously described methodology (1), this quick protocol may as well be modified for other tissue samples.

High throughput genotyping of mice by PCR analysis of DNA isolated from mouse tail snips is hampered by both high costs and laborious efforts for DNA purification from tissue. Complete purification of genomic DNA from mouse tails is time-consuming, and, in a high-throughput setup, is a considerable cost factor. For many routine screening procedures, isolation of pure DNA is often not required, as long as the PCR enzyme of choice is capable of amplifying PCR products from impure DNA raw lysates. Opti*Taq* is a suitable pure and performant enzyme formulation, able to cope with template DNA impurities and tolerant against the presence of potent PCR inhibitors.

The protocol described below allows quick preparation of DNA raw lysates from mouse tail snips without costly and time consuming kit-based DNA purification. DNA is quickly available, but is impure and contains various PCR inhibitors as well as high salt concentrations, both negatively affecting PCR. Therefore, usage of only small template DNA amounts is highly recommended. Additionally, obtained template fragments are heavily fragmented, are relatively small and thus are not suitable as starting material for several non-PCR techniques, such as Southern Hybridization.

DIRECT MOUSE TAIL PCR / MOUSE EAR CLIP PCR : DNA QUICK LYSIS AND PCR PROTOCOL

1. Transfer tissue samples to plastic reaction tubes.
   - Use mouse tail snips with a length of 1 - 2 mm each. Length of tail snips: ↔
   - Use a small, thin-walled (0.65 ml) reaction tube when performing heating within a thermocycler.
   - It is possible to scale up the procedure to 96-well plate format.
2. Add 50 µl Alkaline Lysis Reagent.
3. Heat samples to 95°C for 30 minutes (minimum: 10 min) * **.
4. Cool to 4°C (optional).
5. Add 50 µl Neutralization Buffer.
6. Use DNA immediately. Else, store at +4°C (short term) / -20°C (long term).

*** Note 1:** Maximum DNA yield is usually obtained after 30 min. Extended periods of incubation neither increase nor decrease DNA yield.
**** Note 2:** DNA extraction proceeds successfully, even if there remain pieces of visibly non-digested, floating tissue. Tail snips do often not display any optical change in their visual appearance following 30 min incubation in lysis buffer at 95°C.

HotShot PCR Protocol using Opti*Taq* DNA Polymerase
Concentrations are given as final concentrations.

Template DNA	x µl (0.5 – 5 ul)*
Reverse Primer	5 pmol (200 nM)
Forward Primer	5 pmol (200 nM)
10x PCR Buffer B or C ** [1.5 mM $MgCl_2$ final]	2.5 µl
$MgCl_2$ solution [25 mM]	to 3 mM (1.5 µl) **
dNTPs	0.2 mM each (1 µl 5 mM dNTP solution)
Opti*Taq* DNA Polymerase	0.2 U- 0.5 U
H_2O sterile	ad 25 µl

* Do not use large (excess) amounts of impure template DNA to prevent pronounced inhibition of Opti*Taq* DNA polymerase by PCR inhibitors and by high salt concentration. Guideline: Use max. approx. 0.15 vol. of template DNA per total volume of PCR assay. The less template DNA, the better.
** High amounts of organic material remain present in the raw lysate, thus effectively reducing the concentration of free available Mg^{2+}-ions. Reduction of Mg^{2+}-ion concentration reduces overall activity of DNA polymerase and may result in the failure to reliably amplify certain PCR bands. Therefor, elevated $MgCl_2$ concentrations are required. Adding 0.5 µl of a 25 mM $MgCl_2$ solution will add 12.5 nmol $MgCl_2$ and thus, within a total reaction volume of 25 µl, will increase total $MgCl_2$ reaction concentration in 0.5 mM.
For buffer A (no $MgCl_2$ preadded), add 3 µl [25 mM] $MgCl_2$ solution.

References:

1. *Truett, G.E. et al. (2000), BioTechniques 29:52-54*