

1. Overview

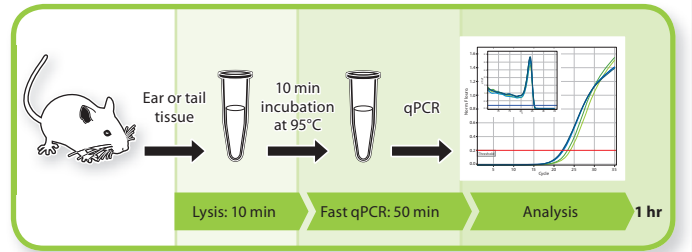
Current workflows for mouse genotyping involve laborious and time-consuming steps including upstream DNA extraction and downstream agarose gel electrophoresis analysis. KAPA SYBR® FAST qPCR Master Mixes, containing the novel KAPA SYBR® DNA Polymerase, can be used efficiently to genotype mice directly from crude lysates. qPCR-ready lysates can be prepared in as little as 10 minutes. Combined with KAPA SYBR® FAST qPCR protocols, mouse genotyping workflows may be significantly streamlined and turnaround times reduced to 1 hour. In addition, the engineered KAPA SYBR® DNA Polymerase is capable of amplifying much longer targets than wild-type Taq included in competitor qPCR kits, thus offering more flexibility in primer design.

2. Typical Results

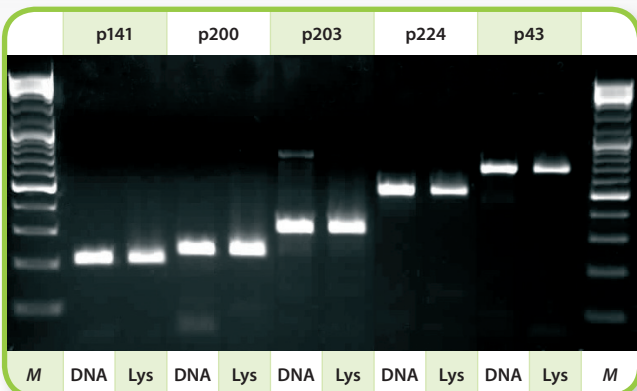
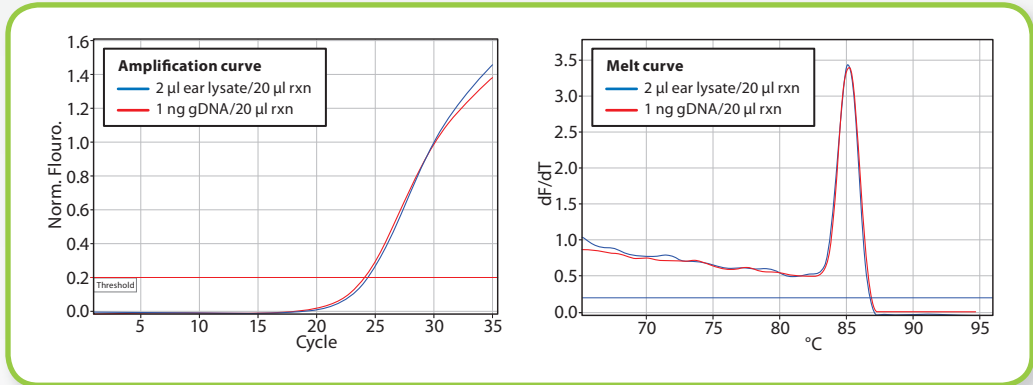
With KAPA SYBR® FAST qPCR Kits, DNA fragments may be amplified from crude lysates as efficiently as from purified genomic DNA. Five amplicons, ranging from 200 – 700 bp in size were amplified in 20 µl reactions from crude mouse ear lysates and results compared to those obtained with 1 ng purified mouse genomic DNA as template. All five amplicons were amplified with the same efficiency and specificity from ear lysates as from genomic DNA, as seen in amplification and melt curves and when products were electrophoresed in agarose gels. Using KAPA SYBR® FAST qPCR Kits with crude tissue lysates, results were achieved in 1 hour. In contrast, turnaround times for standard protocols employing wild-type Taq exceed 6 hours due to the prerequisite for DNA purification and longer cycling times.

Key features

- Perform qPCR-based mouse genotyping directly from crude mouse tail or ear lysates.
- PCR-ready lysates may be prepared in 10 min, without the need for specialized lysis buffers.
- Results comparable with those obtained using purified DNA as template.
- Streamline mouse genotyping workflows and reduce turnaround times to 1 hour.
- The ability to amplify longer targets offer flexibility in primer design.



Amplification and melt curves for the p43 amplicon (700 bp), amplified directly from 2 µl crude mouse ear lysate (blue) or 1 ng purified genomic DNA (red) in 20 µl reactions. Please refer to Section 3 for details of tissue preparation, reaction setup and cycling parameters.



Products generated with KAPA SYBR® FAST Universal qPCR Mix from purified mouse genomic DNA (DNA) or Tris-HCl crude mouse ear lysates (Lys), visualized in a 1.5% TBE-agarose gel. Amplicon details are given in the table below.

Amplicon	Amplicon size	Forward Primer sequence	Reverse Primer sequence
p141	207 bp	TCTTCCCCCTGGAGATCTTT	CTGGGAGAAAGGAGACCACA
p200	213 bp	GCTGCGGGCAAAAATCTCC	GGCAGCCCCCTTCTCCAGT
p203	315 bp	CCTCACTGACTCGGCATA	GGCCTCAAACCTCACAGAG
p224	519 bp	GCACTCTGCAATGCCACTTT	GGAAAGCACCCGATTCAGCA
p43	700 bp	GCCCTTTCACCTCATCGC	CAGCTTGCCGTACCGAC



3. General protocol

3.1 Preparation of PCR-ready mouse tissue lysates

- Take a 1 – 2 mm mouse ear clipping and transfer to an appropriately labeled thin-walled PCR tube.
- Add 50 µl Tris-HCl (10 mM, pH 8.0) to each PCR tube and vortex well for ≥15 sec.
- Incubate samples at 95°C for exactly 10 minutes in a heating block, waterbath or thermocycler.
- Remove the samples and vortex again for ≥15 sec. **Do not spin down.**
- Transfer each lysate to a fresh PCR tube. Avoid transferring cellular debris with the lysates.
- For future use, lysates may be stored at 4°C for up to 10 days, or indefinitely at -20°C.
- PCR-ready lysates may also be prepared from mouse tails, but DNA quality is generally poorer, which may affect yields and reaction efficiency.

3.2 Reaction setup and cycling parameters

Reaction component	Final conc.	Per 20 µl rxn
PCR grade water	-	Up to 20 µl
2x KAPA SYBR® FAST qPCR Master Mix*	1x	10.0 µl
Forward primer (10 µM)	0.2 µM	0.4 µl
Reverse primer (10 µM)	0.2 µM	0.4 µl
Tissue lysate	-	1 – 3 µl

*Please select the correct KAPA SYBR® FAST qPCR Master Mix (Universal, ABI Prism, Bio-Rad iCycler or LightCycler 480) for your qPCR instrument.

Cycling step	Temperature & time	
Initial denaturation	5 min at 95°C	
Denaturation	3 sec at 95°C	35 cycles
Annealing and extension	30 sec at 60°C	
Melt	Ramp from 65°C to 95°C	

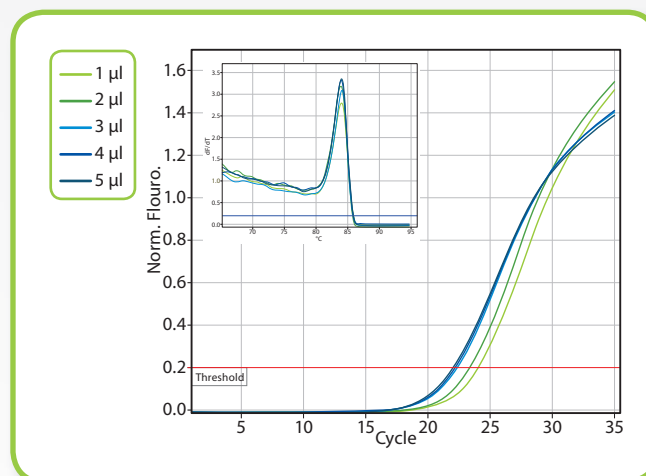
*This cycling protocol was optimized for the Corbett RotorGene 6000 HRM. Adjustments may be needed for different instruments.

Tips:

- To reduce pipetting errors, a 2x primer mix may be prepared. Of this 2x primer mix, 9 µl is added to 10 µl 2x KAPA SYBR® FAST Master Mix, after which 1 µl of crude lysate is added.
- If more than 1 µl of lysate is required per reaction (see Section 3.3 below), a 4x primer mix may be prepared. Add 5 µl of 4x primer mix to 10 µl 2x KAPA SYBR® FAST Master Mix. The desired amount of template (lysate) is added and reactions made up to 20 µl with PCR grade water.
- Always include a positive (purified genomic DNA) and negative (no template) control.

3.3 Optimization of the volume of crude tissue lysate used per reaction

The effective concentration and quality of template in crude mouse tissue lysates is likely to vary from one sample to another. For this reason, it is recommended that the optimal volume of individual lysates to be used per reaction be determined empirically, by performing a series of parallel reactions with different volumes of lysate (1 – 5 µl) per 20 µl reaction.



Amplification of the 315 bp p203 fragment with the KAPA SYBR® FAST Universal qPCR Kit, using different volumes (1, 2, 3, 4 or 5 µl) of crude ear lysate per 20 µl reaction. An initial increase in lysate volume led to an earlier Ct score. However with ≥3 µl lysate per reaction, no significant difference in Ct score or the shape of amplification curves were observed, indicating that the reaction may be lagging due to inhibition. Melting curves (inset) indicate that the same specific product is being amplified despite the amount of sample added.