

1. Overview

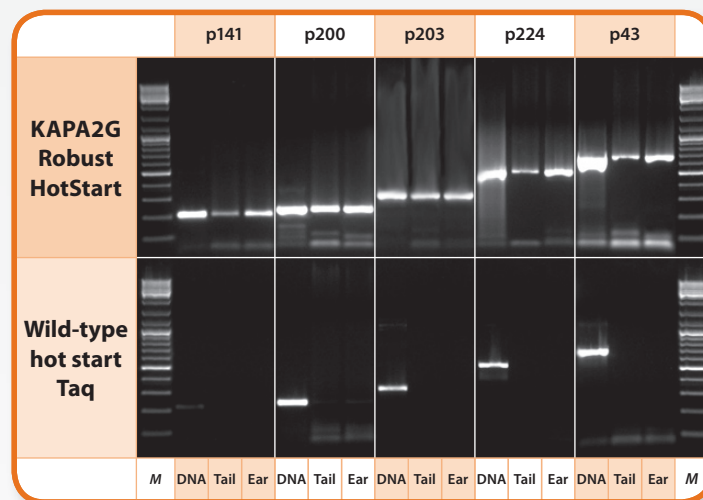
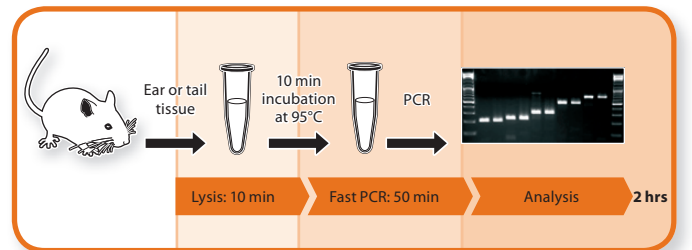
Current workflows for mouse genotyping involve laborious and time-consuming upstream DNA extraction steps. KAPA2G Robust HotStart DNA Polymerase, a highly robust and versatile second-generation enzyme derived through a process of molecular evolution, exhibits high performance across a range of crude sample PCR applications. With KAPA2G Robust HotStart, DNA fragments ≤ 1 kb may be amplified directly from crude tissue lysates, prepared from mouse tail or ear clippings in as little as 10 minutes. Combined with the faster extension rate of KAPA2G Robust HotStart, mouse genotyping workflows may be significantly streamlined and turnaround times reduced to 2 hours.

2. Typical Results

Five amplicons, ranging from 200 – 700 bp in size were amplified from crude mouse tail or ear lysates and results compared to those obtained with 10 ng purified mouse genomic DNA as template. All five amplicons were successfully amplified from both lysate types with KAPA2G Robust HotStart. Yields were generally higher with ear lysates. Wild-type Taq yielded significantly poorer results with purified DNA as template, and failed to amplify any of the fragments directly from the crude tissue lysates. Using KAPA2G Robust HotStart with crude tissue lysates, results were achieved in 2 hours. 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

- Amplify DNA fragments up to 1 kb 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 2 hours.



PCR products generated with KAPA2G Robust HotStart (top) or wild-type hot start Taq (Competitor I, below) using either 10 ng genomic DNA (**DNA**), mouse tail lysate (**Tail**) or mouse ear lysate (**Ear**) as template. Tissue lysates were prepared as described in Section 3. Standard 3-step cycling profiles (40 cycles) were used. The extension time per cycle was 30 sec for KAPA2G Robust HotStart and 1 min for wild-type hot start Taq. PCR products were electrophoresed in a 1.5% TBE-agarose gel and visualized by ethidium bromide staining.

Amplicon details are as follows: **p141** (207 bp; F primer: 5'-TCTTCCCCCTGGAGATCTTT, R primer: 5'-CTGGGAGAAAGGAGACCACA); **p200** (213 bp; F primer: 5'-GCTGCGGGCAAAAATCTCC, R primer: 5'-GGCAGCCCCTCCTCCAGT); **p203** (315 bp; F primer: 5'-CCTCACTGACTCGGCATA, R primer: 5'-GGCCTCAAACCTCACAGAG); **p224** (519 bp; F primer: 5'-GCACTCTGCAATGCCACTTT, R primer: 5'-GGAAAGCACCGATTCCAGCA) and **p43** (700 bp; F primer: 5'-GCCCTTTCACCCCTCATCGC, R primer: 5'-CAGCTTGCCGTACCGAC).



3. General protocol

3.1 Preparation of PCR-ready mouse tissue lysates

- Transfer a 1 - 2 mm mouse tail cut or a 2 mm diameter ear punch to an appropriately labelled thin-walled PCR tube.
- Add 50 µl of 10 mM Tris-HCl (pH 8.0 - 8.5) to the tube and vortex for ≥15 seconds.
- Incubate samples at 95°C for 10 minutes.
- Vortex tubes again for ≥15 seconds.
- *Optional:* collect cellular debris by centrifugation and transfer supernatants to fresh PCR tubes. Avoid transfer of any cellular debris.
- Use 2 µl of crude lysate as template for PCR with KAPA2G Robust HotStart. Optimal results are achieved when lysates are used immediately.

3.2 Reaction setup and cycling parameters

Reaction component	Final concentration	Per 25 µl rxn
PCR grade water	-	Up to 25 µl
5x KAPA2G Buffer A	1x	5.0 µl
10 mM KAPA dNTP mix	0.2 mM each dNTP	0.50 µl
Forward primer (10 µM)	0.5 µM	1.25 µl
Reverse primer (10 µM)	0.5 µM	1.25 µl
KAPA2G Robust HotStart (5 U/µl)	1 U/25 µl rxn	0.20 µl
Tissue lysate*	-	2.0 µl

*For each primer set, include a positive control reaction with 1 – 20 ng genomic DNA as template, as well as a negative (no template) control.

Cycling step	Temperature & time	
Initial denaturation	3 – 5 min at 95°C	
Denaturation	15 sec at 95°C	40 cycles
Annealing and extension	15 sec at optimal Ta	
Extension*	30 sec at 72°C	
Final extension (optional)	1 min at 72°C	

*For amplicons <500 bp or when tissue lysates are prepared with commercial lysis reagents, extension times may be reduced to 15 sec per cycle.

For technical support with these or other applications, please contact: support@kapabiosystems.com

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