

## Mouse genotyping



Current workflows for the extraction and amplification of DNA for mouse genotyping can benefit from improvements in throughput, turnaround time and reliability.

KAPA Mouse Genotyping Kits, consisting of KAPA Express Extract and KAPA2G Fast Genotyping Mix, are ideally suited for the routine extraction and amplification of DNA for mouse genotyping. The novel KAPA Express Extract system offers a quick and easy way to prepare PCR-ready DNA. KAPA2G Fast Genotyping Mix contains a highly processive, engineered DNA polymerase, and is designed for rapid and reliable amplification of DNA fragments from mouse samples.

### Introduction

The cost and availability of animal holding space represent a major limitation to biomedical research employing transgenic and mutant mouse models<sup>1</sup>. To optimize this valuable resource, mouse genotyping workflows are continually improved to achieve higher throughput, shorter turnaround times and better success rates. Template DNA for mouse genotyping is routinely prepared from ear, tail or toe biopsies. Typically, samples are incubated for several hours (or overnight) with proteinase K, followed by purification of the DNA to remove salts and detergents. Alternatively, DNA is released from the mouse tissue by heating the sample in an alkaline solution, followed by neutralization in a Tris-HCl buffer with an acidic pH. Proteinase K protocols yield extracts with a higher DNA concentration and quality, but are laborious and time-consuming. Heat lysis protocols allow for rapid generation of PCR-ready DNA, but samples vary in quality and quantity of amplifiable template, leading to reduced PCR success rates with wild-type DNA polymerases.

Kapa Biosystems offers a unique solution for mouse genotyping. **KAPA Mouse Genotyping Kits** contain the reagents required for the rapid extraction and amplification of DNA from mouse tissues. DNA extraction is achieved with **KAPA Express Extract**, a novel thermostable protease and buffer system designed for rapid and efficient single-tube mouse tissue lysis. Good quality PCR-ready DNA can be generated in as little as 15 min with minimal handling, thereby reducing the risk of sample loss or contamination. The process yields sufficient template for multiple assays and is easily scaled to handle samples in 96-well format. Extracted DNA is amplified with **KAPA2G Fast Genotyping Mix**, which is ideally suited for fast and reliable amplification of DNA fragments across a wide range of amplicon lengths and GC contents. This ready-to-use PCR master mix contains KAPA2G Fast DNA Polymerase, a novel enzyme engineered for improved processivity and speed. KAPA2G Fast is designed for routine, fast PCR (a genotyping assay can typically be completed in 45 min), without the need for specialized PCR consumables or thermocyclers. In addition to KAPA2G Fast Buffer, dNTPs (0.2 mM each at 1X), MgCl<sub>2</sub> (1.5 mM at 1X) and stabilizers, the KAPA2G Fast Genotyping Mix also contains two inert dyes, which allow for the analysis of reaction products by gel electrophoresis directly after completion of the PCR (i.e. without the need to add a DNA loading solution). Together, KAPA Express Extract and KAPA2G Fast Genotyping Mix allow for significantly reduced turnaround times and improved success rates in mouse genotyping.

### Important technical considerations when using KAPA Mouse Genotyping Kits:

- Due to the rapid DNA extraction with KAPA Express Extract, **tissues are not completely degraded and are still visible after the extraction protocol** (unlike extractions with proteinase K). A sufficient amount of DNA is released for amplification with the highly active, engineered KAPA2G Fast DNA Polymerase.
- The same extraction and amplification protocol may be used for all tissue types (tails, ears and toes; see Figure 1 for more details).
- Results may be improved by extending the extraction time to 15 min at 75 °C (followed by the 5 min inactivation at 95 °C). Longer extraction times may lead to template degradation and poor results.
- Centrifugation of extraction reaction products (to limit the carry-over of inhibitors into the PCR) is recommended, but not essential.
- The KAPA2G Fast Buffer system is formulated to suit the unique characteristics of the novel KAPA2G Fast DNA Polymerase and is very different to buffers used with wild-type *Taq*. **The annealing temperature for a genotyping assay may have to be re-optimized** when switching to KAPA2G Fast Genotyping Mix. This is easily done by performing an annealing temperature gradient PCR (see **Figure 2 for more details**).
- Results in multiplex assays may be improved by increasing the annealing time from 15 to 30 sec per cycle.
- If the yields obtained with KAPA2G Fast Genotyping Mix are too high to easily distinguish genotypes, the number of cycles may be reduced. Alternatively, the lysate may be diluted 1:10 prior to PCR.
- DNA extracts generated with proteinase K or alkaline lysis protocols are fully compatible with KAPA2G Fast Genotyping Mix.

## Mouse genotyping

### Methods and Results

To demonstrate the suitability of KAPA Mouse Genotyping Kits for genotyping from different mouse tissue types, DNA was extracted from mouse tail clippings (~2 mm long), toes (one toe per extraction) and ear punches (2 mm diameter) using KAPA Express Extract. Extraction mixes (100 µl) were set up in thin-walled PCR tubes and contained 1X KAPA Express Extract Buffer and 2 units KAPA Express Extract Enzyme per reaction<sup>2</sup>. Tissue samples were incubated in a thermocycler for 10 min at 75 °C, followed by deactivation of the protease for 5 min at 95 °C. Reaction products were homogenized by vortexing for 2 – 3 sec, and centrifuged for 1 min at maximum speed in a microcentrifuge. The completed extraction reactions (with residual tissue) can be stored at 4 °C for up to a week if PCRs cannot be performed immediately. PCR reactions (25 µl) contained KAPA2G Fast HotStart Genotyping Mix (12.5 µl), primers at a final concentration of 0.5 µM each, and 1 µl of supernatant from the appropriate KAPA Express Extract reaction. The recommended fast PCR cycling conditions<sup>2</sup> were used (3 min initial denaturation at 95 °C, followed by 35 cycles of 15 sec denaturation at 95 °C, 15 sec annealing at 60 °C and 15 sec extension at 72 °C). Half (12.5 µl) of each PCR product was analyzed directly in a 1% agarose TBE-gel and amplification products were visualized by ethidium bromide staining. Results obtained with DNA lysates generated with KAPA Express Extract (Figure 1) from all three tissue types were comparable to those obtained with purified mouse genomic DNA (10 ng per reaction).

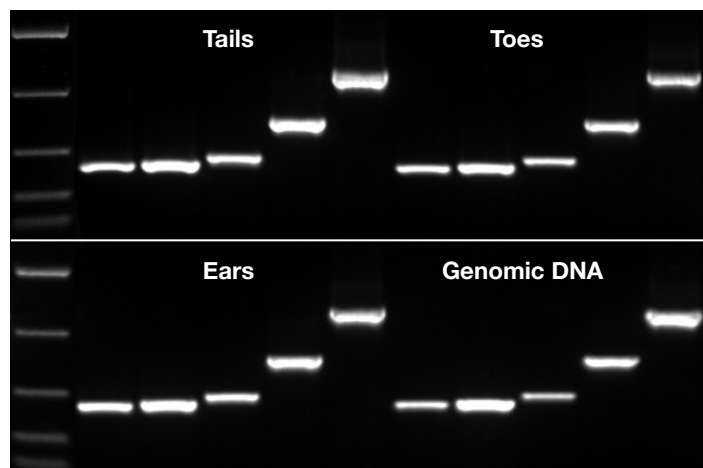
To demonstrate the importance of determining the optimal annealing temperature for a mouse genotyping assay when switching to the KAPA Mouse Genotyping Kit, DNA lysates generated from transgenic mouse ear punches were used in an annealing temperature gradient PCR (Figure 2). Each reaction contained two forward and two reverse primers, designed to amplify a 300 bp fragment from the wild-type (WT, lane 2) and a 400 bp fragment from the mutant (M, lane 11) allele<sup>3</sup>. Eight identical reactions containing the DNA of a confirmed heterozygote were performed in parallel, using the recommended KAPA2G Fast Genotyping Mix cycling parameters, but with the annealing temperature ranging between 50 and 65 °C. A conclusive assay result can only be obtained if the assay is performed at the annealing temperature that is optimal for the assay within the context of the KAPA2G Fast Genotyping Mix chemistry. If an annealing temperature gradient PCR cannot be performed, an annealing temperature between 60 and 65 °C should be used as a first approach.

### Conclusion

KAPA Mouse Genotyping Kits are ideally suited for rapid and reliable routine mouse genotyping from tail, ear or toe tissue. Results can be achieved in ≤2 hours, compared to half a day or more with conventional methods. The key to success lies in using the recommended DNA extraction and amplification protocols. The most critical factor is using the optimal annealing temperature for a new or existing assay, which should be determined empirically using an annealing temperature gradient PCR.

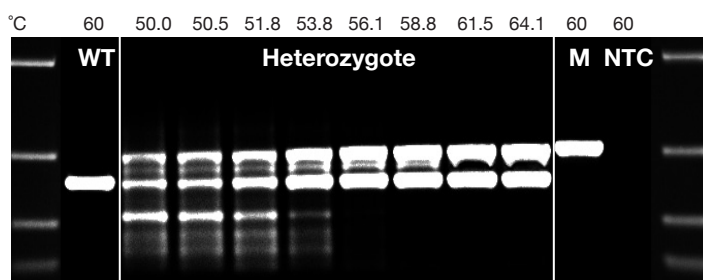
### References

1. Linask, K. L & Lo, C. W. (2005). *BioTechniques* 38: 219 - 223.
2. KAPA Mouse Genotyping Kit Technical Data Sheet.
3. P. Leyton, MD., Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA (unpublished results).



**Figure 1. KAPA Mouse Genotyping kits allow for rapid and efficient genotyping from different mouse tissue types.**

DNA extracted with KAPA Express Extract from mouse tails, ears or toes was amplified with KAPA2G Fast HotStart Genotyping Mix as outlined in Methods and Results. The total hands-on time was less than 2 hours, and results were similar to those obtained using purified mouse genomic DNA. Amplicons range from 312 to 915 bp in length, and GC content between 49 and 65%. The ladder used is KAPA Express DNA Ladder.



**Figure 2. Effect of annealing temperature on the performance of KAPA2G Fast Genotyping Mix.**

DNA extracted from transgenic mouse ear punches was amplified with KAPA2G Fast HotStart Genotyping Mix as outlined in Methods and Results. Each reaction contained four primers, designed to amplify a 300 bp fragment from the wild-type (WT) and a 400 bp fragment from the mutant (M) allele. DNA from known homozygotes (WT and M, lanes 2 and 11, respectively) was amplified using an annealing temperature of 60 °C. DNA from a known heterozygote was amplified using an annealing temperature gradient PCR (lanes 3 to 10, actual Ta for each as indicated). Lane 12 corresponds to a no template control reaction (Ta = 60 °C).

### ORDERING INFORMATION<sup>1,2</sup>

Description	Code	Kit contents
KAPA Mouse Genotyping Kit	KK7302	500 rxn
KAPA Mouse Genotyping Kit (HotStart)	KK7352	500 rxn
KAPA2G Fast Genotyping PCR Mix	KK5121	500 rxn
KAPA2G Fast HotStart Genotyping PCR Mix	KK5621	500 rxn

<sup>1</sup>To amplify difficult amplicons from lysates generated with KAPA Express Extract, KAPA2G Robust HotStart ReadyMix (KK5701 or KK5702) is recommended.

<sup>2</sup>For genotyping assays involving long fragments, purified DNA and KAPA LongRange HotStart ReadyMix with dye (KK3601 or KK3602) is recommended.

Technical support: [support@kapabiosystems.com](mailto:support@kapabiosystems.com)

Ordering information: [sales@kapabiosystems.com](mailto:sales@kapabiosystems.com)

