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

KAPA Express Extract, combined with KAPA2G Robust HotStart ReadyMix, is ideally suited for the routine extraction and amplification of DNA for mouse genotyping. The novel KAPA Express Extract kit offers an easy and fast way to prepare PCR-ready DNA. KAPA2G Robust HotStart ReadyMix contains a highly processive, engineered DNA polymerase tolerant to common PCR inhibitors, offering 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[1]. 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 or tail biopsy samples. 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. The latter process can be simplified and shortened even more by simply heating the tissue sample in Tris-HCl buffer. 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 the concentration of amplifiable template, leading to reduced PCR success rates with wild-type DNA polymerases.

Kapa Biosystems offers a unique solution for mouse genotyping. **KAPA Express Extract DNA Extraction Kits** contain 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 a 96-well format. **KAPA2G Robust Hotstart ReadyMix** is ideally suited for the fast and reliable amplification of DNA fragments across a wide range of amplicon lengths and GC contents[2]. This ready-to-use cocktail contains KAPA2G Robust Hotstart DNA polymerase, a novel enzyme engineered for improved processivity (speed) and tolerance to carry-over inhibitors present in crude DNA extracts. Together, these reagents allow for significantly reduced turnaround times and improved success rates in mouse genotyping (**Figure 1**).

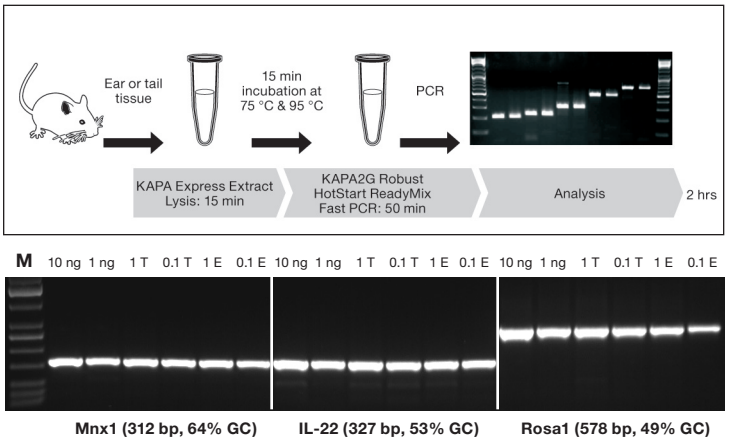

Figure 1. KAPA Express Extract and KAPA2G Robust HotStart ReadyMix allow for rapid and efficient routine mouse genotyping.

PCR-ready DNA was extracted with KAPA Express Extract from mouse tail clippings (**T**) or ear punches (**E**), and amplified with KAPA2G Robust HotStart ReadyMix using standard protocols. For each amplicon, yields obtained with the undiluted (**1**) or 1/10-diluted (**0.1**) DNA extract were compared to those obtained using the same PCR protocol, but **10 ng** or **1 ng** purified mouse genomic DNA as template. Half of each 25 µl PCR product was electrophoresed in a 1% TBE-agarose gel.

Results

To demonstrate the suitability of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix for mouse genotyping, DNA was extracted from mouse ear punches or tail clippings. Extracted DNA was used as template in eleven PCR assays targeting amplicons <1 kb, with a GC-content ranging between 39 and 64%. Selected results are shown in **Figure 1**. All fragments could be amplified successfully from undiluted or 1/10-diluted extracts. Results obtained with ear and tail samples were similar. Yields of the target amplicon and specificity of amplification were comparable to those obtained with purified mouse genomic DNA in all assays.

DNA extraction protocol

An overview of the KAPA Express Extract protocol for mouse samples is given in **Table 1**. Lysis reactions may be set up individually as outlined in **Table 2**. Alternatively, a master mix containing the KAPA Express Extract enzyme and buffer may be prepared and aliquotted into individual tubes or wells of a 96-well plate containing the ear punch or tail clipping. Punches with a diameter of 2 mm, or tail clippings of 1 – 2 mm in length are recommended. After the 15 min lysis protocol, samples should be centrifuged briefly to pellet debris. DNA extracts prepared in this way do not have to be quantified and may be used directly in a PCR (1 – 2 µl of an undiluted or 1/10-diluted extract per reaction). One extraction yields sufficient template for up to 500 x 25 µl PCRs. For multiple use and long-term storage (at -20 °C), it is recommended that cleared lysate supernatants be transferred to fresh tubes or plates.

Table 1: KAPA Express Extract protocol for mouse samples.

Step	Description
Reaction setup	1. Transfer mouse ear punches (2 mm diameter) or mouse tail clippings (1 – 2 mm) to individual tubes or wells of a 96-well plate. 2. Add the appropriate volumes of PCR grade water and KAPA Express Extract enzyme and buffer to each tube or well (see **Table 2**) **or** Prepare a bulk lysis solution by combining KAPA Express Extract Buffer, enzyme and PCR grade water, and aliquot the appropriate volume into each tube or well.
Lysis	1. Close tubes or seal plate and place in thermocycler. 2. Incubate at 75 °C for 10 min. (During this step, cells are lysed, nucleases and proteins degraded and DNA released.)
Heat-inactivation	Incubate plate at 95 °C for 5 min to inactivate the thermostable KAPA Express Extract enzyme.
Sample recovery	1. Centrifuge plate for 1 min to pellet debris. 2. Recover DNA-containing supernatant.

Table 2: KAPA Express Extract lysis reaction setup for mouse samples.

Reaction component	Final conc.	Per 100 µl reaction	Per 96-well plate
PCR grade water	–	Up to 100 µl	Up to 10 ml
10X KAPA Express Extract Buffer	1X	10.0 µl	1.00 ml
KAPA Express Extract Enzyme (1 U/µl)	20 mU/µl	2.00 µl	0.20 ml
Ear punch **or** tail clipping	–	2 mm Ø 1 – 2 mm	–

PCR reaction conditions and cycling parameters

The recommended KAPA2G Robust HotStart ReadyMix reaction setup and cycling parameters are given in **Tables 3 and 4**. Final primer concentrations and annealing temperatures may be adapted as required for specific assays. For more information on reaction setup and cycling parameters, please refer to the KAPA2G Robust HotStart ReadyMix Technical Data Sheet and other technical resources, which are available at http://www.kapabiosystems.com/products/name/kapa2g-robust-pcr-kits.

Table 3: KAPA2G Robust HotStart ReadyMix reaction setup for mouse genotyping.

Reaction component	Final conc.	Per 25 µl reaction[1]
PCR grade water	–	Up to 25.0 µl
2X KAPA2G Robust HotStart ReadyMix[2]	1X	12.5 µl
Primers (10 µM)[3]	0.5 µM (each primer)	1.25 µl (each primer)
KAPA Express Extract DNA extract	–	1.00 – 2.00 µl[4]

[1] For smaller reaction volumes, scale down all volumes proportionally. Do not perform reactions >25 µl.

[2] Contains $MgCl_2$ at a 1X concentration of 2 mM. Additional $MgCl_2$ may be added if required for a specific assay.

[3] Include the forward and reverse primers at a final concentration of 0.5 µM each. Final primer concentrations may be modified as required for specific assays, particularly those including more than one forward and/or reverse primer.

[4] Start with 1 µl of undiluted extract per 25 µl reaction. Use more (up to 2 µl of undiluted extract) or less template (1 – 2 µl of a 1/10-diluted extract) to improve yields and/or specificity, if necessary.

Table 4: KAPA2G Robust HotStart ReadyMix cycling parameters.

Cycling step	Temperature & time	
Initial denaturation	3 min at 95 °C	
Denaturation	15 sec at 95 °C	x 40 cycles
Annealing	15 sec at 60 °C[1]	
Extension	15 sec at 72 °C	
Final extension[2]	0 – 10 min at 72 °C	

[1] An annealing temperature of 60 °C is recommended for workflows involving multiple amplicon types and primer sets. The optimal annealing temperature for individual assays or subsets of assays may be determined empirically. For optimal results with KAPA2G Robust HotStart ReadyMix, annealing temperatures between 55 and 65 °C should be used as far as possible.

[2] Only required if 3'-dA-tailing is essential for analysis or cloning of the PCR product.

Conclusions

KAPA Express Extract DNA Extraction Kits and KAPA2G Robust HotStart ReadyMix are ideally suited for rapid and efficient routine mouse genotyping. With this combination, reliable results can be achieved in ≤2 hours, compared to ≥1 day with conventional methods. DNA extracts generated with KAPA Express Extract are also suitable for mouse genotyping using qPCR.

References

1. Linask, K. L. & Lo, C. W. (2005). *BioTechniques* 38: 219 – 223.
2. Application Note: KAPA2G Robust HotStart ReadyMix – Single Protocol PCR. Kapa Biosystems (2009).

Technical support: **support@kapabiosystems.com**
Ordering information: **sales@kapabiosystems.com**