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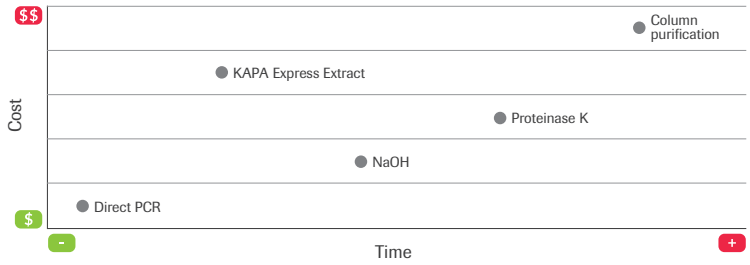
Rapid qPCR analysis from highly inhibited tissue and blood sample extractions with KAPA PROBE FORCE qPCR Kits and KAPA Express Extract

Enzymatic inhibition, caused by both sample and extraction carryover inhibitors, is a significant obstacle when optimizing crude sample workflows. KAPA PROBE FORCE qPCR Kits include a highly processive third-generation enzyme—engineered via directed evolution—which enables direct crude sample analysis without sample purification. In combination with KAPA Express Extract, KAPA PROBE FORCE offers a highly efficient and streamlined sample-to-Cq workflow solution for both low- and high-throughput genotyping and gene expression applications (<1.5 hr).

Introduction

DNA isolation presents a considerable obstacle when optimizing and streamlining crude sample workflows. Silica membrane-based column purification methods have long been employed for qPCR to remove contaminants and inhibitors. While these methods generate high-quality DNA, they impose additional time and cost burdens, and often result in sample loss. Low-cost, semi-crude extraction methods (e.g., sodium hydroxide [NaOH] and Proteinase K [PK]) reduce workflow time; however, carryover contamination and inhibition may lead to inconsistencies.

Comparison of common extraction methods



KAPA PROBE FORCE is ideally suited for direct qPCR analysis of challenging sample types without the need for purification. It contains the novel KAPA3G DNA Polymerase which was engineered via a process of directed evolution for improved tolerance to common tissue, blood and plant PCR inhibitors, crude DNA extraction carryover inhibitors, and high concentrations of salt. The unique characteristics of this evolved enzyme result in robust amplification across a wide range of sample types and methods of extraction and purification.

KAPA Express Extract is a novel thermostable protease and buffer system that allows for the extraction of PCR-ready DNA in as little as 15 minutes. It can be used on a variety of tissue types, such as buccal swabs, hair follicles, FFPE tissue, bone marrow, blood, blood spots on denim, and processed foods. Unlike existing protocols that rely on PK digestion, KAPA Express Extract reactions are conveniently performed in a single tube—without the need for hazardous chemicals and multiple washing steps—greatly reducing the risk of sample loss and contamination.

Experimental design and methods

Most commonly used extraction protocols are time consuming, have the potential for inhibitor carryover, and can impose a significant cost burden. Moreover, the addition of spin columns for the isolation of purified DNA can lead to significant sample loss.

To address these concerns, we developed a fast and highly reproducible method for crude sample qPCR using KAPA Express Extract in combination with KAPA PROBE FORCE qPCR Kits. With this method, reliable gene expression levels can be assessed directly from crude samples with improved tolerance to common PCR and carryover inhibitors.

In this experiment, four tissue types from newborn (neonate) and older (hopper) mice, and blood spots from six-year-old FTA cards were used. Sample size for each mouse tissue type and extraction method is shown in Table 1. Blood spots were prepared with a 0.5 mm Harris Uni-Core™ tool. In order to demonstrate the effect of sample size, one or two ear punches and one, two, or three blood spots per reaction were compared. Novagen Genomic Mouse DNA (EMD Millipore Cat #69239, 100 µg) was used as a purified DNA control.

Table 1. Tissue sample size used for each extraction method

Sample type	EE	NaOH	PK	Column
Tail, snip	~2 mm	~2 mm	~2 mm	~5 mm
Ear, snip	~2 mm	~2 mm	~2 mm	~5 mm
Toe, full	1	1	1	2
Hair, strands	~5	~5	~5	~15

Performance of our method was compared to DNA extracted by three additional methods: two semi-crude extraction methods, sodium hydroxide and proteinase K; and one column-based extraction method, QIAGEN® QIAmp DNA Mini Kit. Extraction methods are summarized in Table 2. Expression levels of Beta-2-microglobulin (a single-copy region of the genome) were measured with the KAPA PROBE FORCE qPCR Kit. PCR reaction conditions and cycling parameters were performed as described in the KAPA PROBE FORCE qPCR Kit Technical Data Sheet. All qPCR reactions were performed on a QIAGEN RotorGene Q instrument.

Table 2. Extraction methods used in this study

Extraction protocol	Extraction buffer	Digestion	Sample collection	Time
KAPA Express Extract	10X KAPA Express Extract Buffer KAPA Express Extract Enzyme	75ºC for 10 min 95ºC for 5 min	Centrifuge max speed for 1 min	20 min
Sodium Hydroxide	20 mM NaOH 0.2 mM EDTA	98ºC for 60 min	Add 40 mM Tris-HCl (pH 5.5) Centrifuge 4000 RPM for 3 min	75 min
Proteinase K	50 mM KCl 10 mM Tris-HCl (pH 5.5) 0.1% Triton X-100 Proteinase K (0.4 mg/mL)	60ºC for 180 min 95ºC for 10 min	Centrifuge max speed for 15 min	205 min
Qiagen QIAmp DNA Mini Kit	Buffer ATL Proteinase K	56ºC for 180 min 70ºC for 10 min	Spin column cleanup	210 min

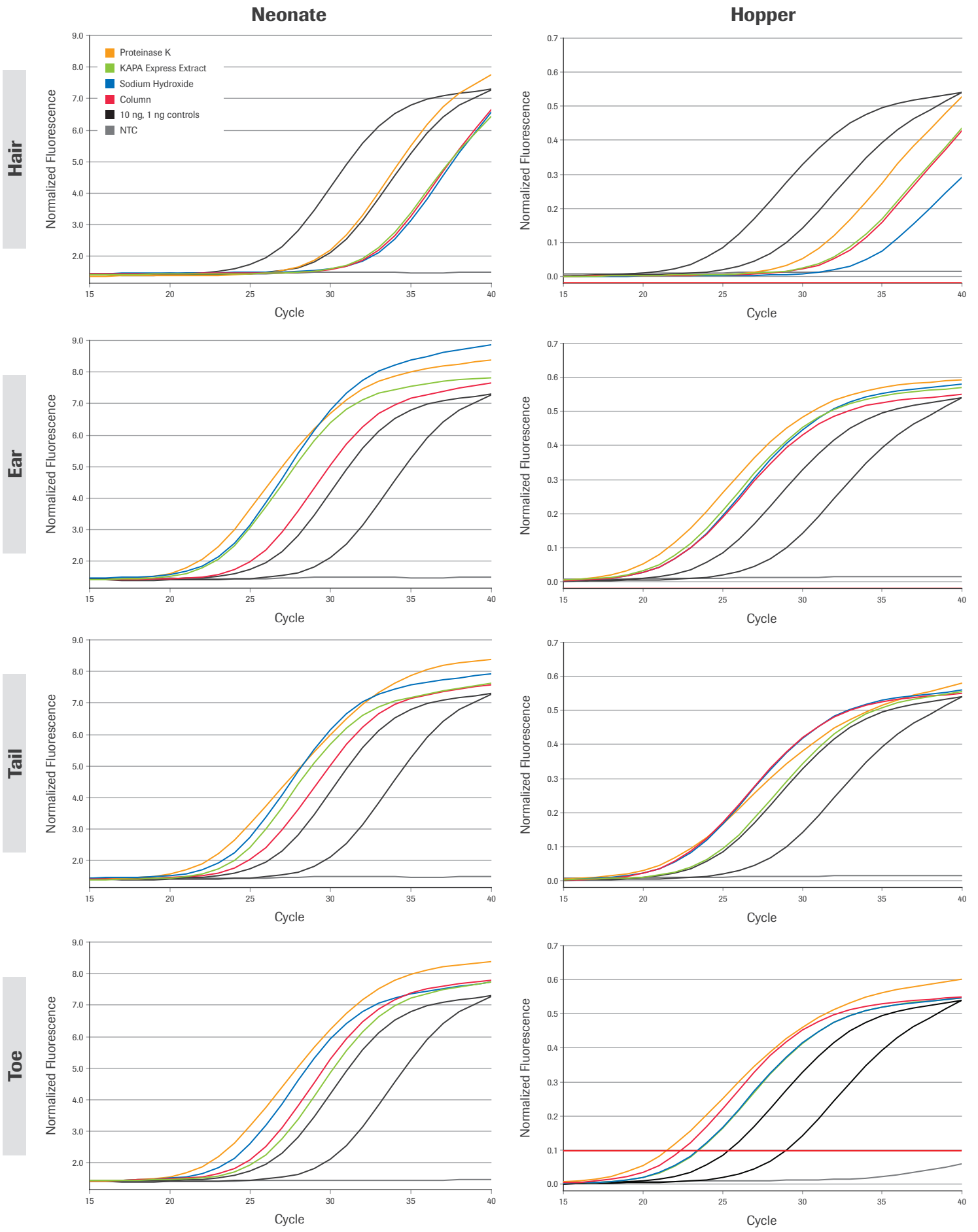


Figure 1. Extraction comparison for neonate and hopper mice

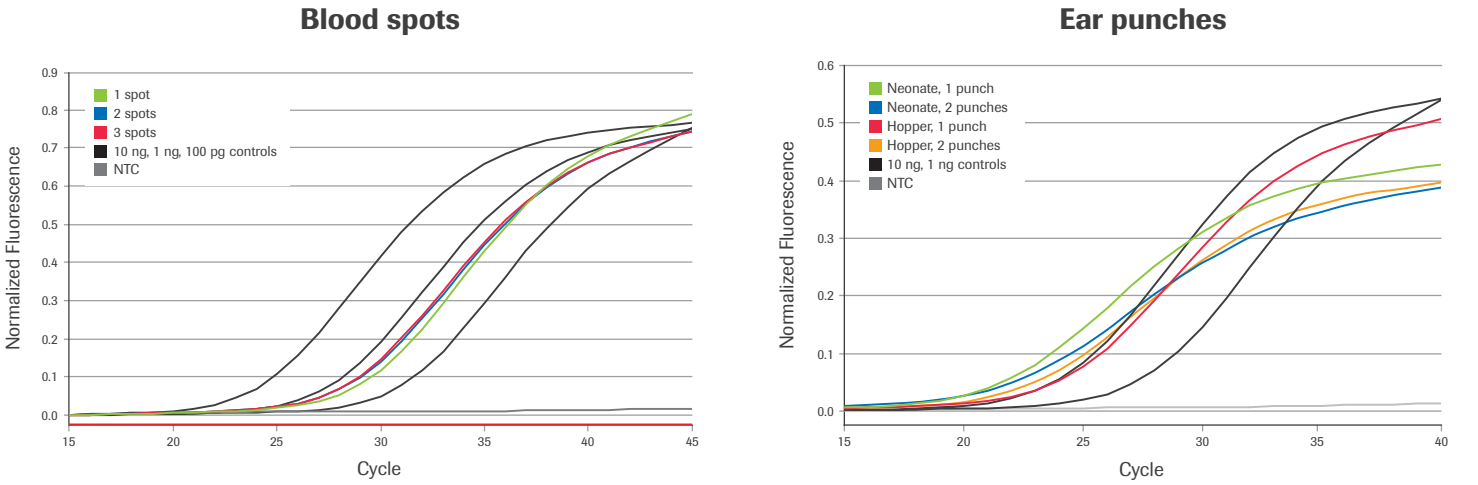


Figure 2. Direct qPCR comparison of blood spots and ear punches

Results

Neonates and hoppers were both shown to be suitable templates, with the neonates performing marginally better overall (Figure 1). For tail, ear, and toe templates, all extraction types produced yields of >10 ng/µL, whereas hair samples yielded concentrations ≤1 ng/µL. Furthermore, all reactions had high reaction efficiencies regardless of sample type or extraction method.

When comparing the extraction methods, KAPA Express Extract resulted in higher yields with less input material than column extractions; was equivalent to NaOH preparations; but was less sensitive than PK. All reactions showed no signs of inhibition and reached the same final fluorescence as the purified DNA controls.

In addition, KAPA PROBE FORCE is capable of direct amplification from six-year-old FTA blood cards and ear punches from neonate and hopper mice (Figure 2). Regardless of number, all of the blood spots produced consistent results, yielding slightly less than 1 ng of DNA. This highlights the fact that increasing the amount of blood inhibitors in the reaction mix does not adversely affect the quality of the reaction. In addition, sufficient amplification can be achieved from very low inputs. All ear punches produced yields of ~10 ng/µL, again with the neonates performing marginally better overall. One punch showed slightly better reaction efficiency than two punches. Thus, KAPA PROBE FORCE also provides high-quality results from direct qPCR of inhibitory materials, enabling an extraction-free workflow and allowing for shorter turnaround times.

Conclusion

The need for highly purified DNA for qPCR is significantly minimized by the unique formulation of KAPA PROBE FORCE qPCR Kits, which include KAPA3G DNA Polymerase, engineered for inhibitor resistance and processivity.

KAPA PROBE FORCE has been shown to perform efficiently for a range of sample types and extraction methods—whether using crude or column extractions, or direct PCR. When combined with KAPA Express Extract, sample-to-Cq workflows can be achieved in <1.5 hr, providing a unique and highly effective solution for both low- and high-throughput genotyping and gene expression applications. This allows for streamlined, cost-effective workflows designed to fit any budget without compromise.

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