



DNA extraction from mammalian samples and subsequent PCR for DNA barcoding is time consuming and laborious, limiting the potential for rapid, high-throughput analysis.

KAPA Express Extract, combined with KAPA2G Robust HotStart ReadyMix, is ideally suited for the routine extraction and amplification of DNA from a variety of mammalian samples. The novel KAPA Express Extract enzyme and buffer offer an easy and fast way to prepare PCR-ready DNA. KAPA2G Robust HotStart ReadyMix contains an engineered DNA polymerase tolerant to common PCR inhibitors, offering reliable amplification of DNA for barcoding applications.

Introduction

"DNA barcoding" is rapidly gaining support as a quick, cost-effective and broadly applicable tool for species identification. The DNA barcoding process targets a standardized fragment of the 16S rRNA or the mitochondrial cytochrome c oxidase I (COI) genes, which are amplified and sequenced to yield a reference sequence or "barcode". This rapid and inexpensive strategy has revolutionized the way species are identified and classified – in a fraction of the time required for traditional methods based on morphology or other physical characteristics. Barcoding projects are currently contributing to advances in the identification and conservation of new species, pest and disease control, food safety, research policy and many other areas of scientific research.

KAPA Express Extract offers a fast, convenient and efficient alternative for the routine extraction of DNA from a variety of animal species and sample types. The novel thermostable protease and buffer system has been designed for optimal tissue lysis and preservation of DNA extracts. The KAPA Express Extract enzyme and buffer are combined in a single tube with a ~2 mm³ tissue sample or a single hair follicle. Tissue lysis is performed in a standard thermocycler, after which the reaction product is centrifuged and the DNA-containing supernatant recovered. This protocol eliminates excessive handling, thereby generating PCR-ready DNA in as little as 15 min. The process is easily scaled to handle multiple samples in a 96-well format.

KAPA2G Robust HotStart ReadyMix is ideally suited for the routine and reliable amplification of DNA extracted from mammalian samples. This ready-to-use cocktail contains KAPA2G Robust HotStart DNA polymerase, a novel enzyme engineered for improved processivity and tolerance to common PCR inhibitors through a process of molecular evolution. Its improved tolerance to carry-over inhibition offers more reliable routine amplification of mammalian amplicons for direct sequencing. When used in combination with the KAPA Express Extract system, the turnaround times for DNA barcoding applications can be significantly reduced.

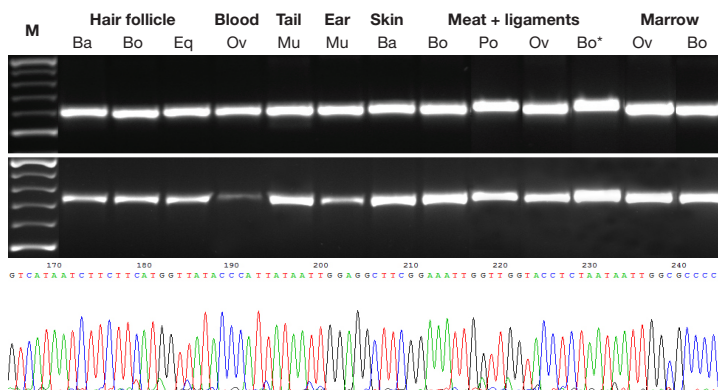


Figure 1. Amplification and sequencing of fragments the 16S rRNA and COI genes from different mammalian samples using KAPA Express Extract and KAPA 2G Robust HotStart ReadyMix.

DNA extracts were prepared using KAPA Express Extract, as outlined on the next page. Of each extract, 1 – 2 μ l was used directly (without quantification) in a PCR containing KAPA2G Robust HotStart ReadyMix and primers for the ~580 bp 16S rRNA (Palumbi, 1996) or the ~650 bp COI (Eaton *et al.*, 2009) gene fragments. Reaction products generated using the 16S rRNA primers (top panel) and COI primers (bottom panel) are shown in gel image. Animal species are abbreviated as follows: Ba = baboon, Bo = bovine (*dried meat), Eq = equine, Mu = murine, Ov = ovine, Po = porcine.

COI PCR products were subjected directly to standard Sanger sequencing using out-nested M13 primers (1 μ l PCR product per 10 μ l sequencing reaction). Sequence data was of a high quality and enabled the identification of every species. A portion of the sequence trace generated from a hair follicle of a Cape baboon (*Papio ursus*) is shown below the gel image.

Results

To demonstrate the suitability of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix for DNA barcoding of mammals, DNA was extracted from a variety of animal samples. The ~650 bp COI fragment (Eaton *et al.*, 2009) and the ~580 bp 16S rRNA fragment (Palumbi, 1996) were subsequently amplified with KAPA2G Robust HotStart ReadyMix (see **Figure 1**). PCR products

Mammalian DNA barcoding

generated with COI primers were subjected to standard Sanger sequencing. All species included in the study were successfully identified by BLAST analysis of sequence data.

DNA extraction protocol

An overview of the KAPA Express Extract protocol is given in **Table 1**. Samples were added directly to 100 µl of KAPA Express Extract Buffer and 2U of KAPA Express Extract Enzyme in a standard 0.2 ml PCR tube. Several negative (no tissue) controls were included to control for contamination. Each tube was placed in a thermocycler and the lysis protocol performed as indicated in **Table 2**. After the 15 min lysis protocol, each 0.2 ml tube was centrifuged briefly to pellet debris. Of each lysate supernatant, 80 µl was transferred to a new 0.2 ml PCR tube. DNA extracts prepared in this way do not have to be quantified and may be used directly in a PCR. One extraction typically yields sufficient template for >50 x 25 µl PCRs. DNA extracts may be diluted 1:5 in TE Buffer for long-term storage at -20 °C. The extraction process may be modified to process multiple samples in a 96 well plate.

Table 1: KAPA Express Extract protocol for mammalian samples.

Step	Description
Reaction setup	<ol style="list-style-type: none"> Transfer samples directly into individual 0.2 ml PCR tubes. Prepare a bulk lysis solution by combining KAPA Express Extract Buffer, Enzyme and PCR grade water, as outlined in Table 2. Add 100 µl bulk lysis solution to each sample.
Lysis	<ol style="list-style-type: none"> Place tubes in a thermocycler. 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	<ol style="list-style-type: none"> Centrifuge tubes for 1 min to pellet debris. Recover DNA-containing supernatant.

Table 2: KAPA Express Extract reaction setup for mammalian samples.

Reaction component	Final conc.	Per 100 µl reaction	Bulk solution (for 100 rxns)
PCR grade water	-	88.0 µl	8.8 ml
10X KAPA Express Extract Buffer	1X	10.0 µl	1.0 ml
KAPA Express Extract Enzyme (1 U/µl)	20 mU/µl	2.00 µl	0.2 ml

PCR reaction conditions and cycling parameters

The KAPA2G Robust HotStart ReadyMix reaction setup and cycling parameters for the amplification of the ~580 bp 16S rRNA and ~650 bp COI fragments from animal tissues are given in **Tables 3 and 4**. Parameters were derived from referenced literature, but adapted to the specific characteristics of the novel KAPA2G Robust enzyme. Overall cycling times were reduced considerably from those used in original protocols. With the combination of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix, reliable DNA amplification from mammalian samples can be achieved in ≤2 hours, compared to ≥1 day with conventional methods.

Table 3: KAPA2G Robust HotStart ReadyMix reaction setup for amplification of 16S rRNA or COI gene fragments from mammalian samples.

Reaction component	Final conc.	Per 25 µl reaction ¹
PCR grade water	-	Up to 25.0 µl
2X KAPA2G Robust HotStart ReadyMix	1X	12.5 µl
Primer premix	16S: 0.4 µM each primer ² COI: 0.2 µM total primers ³	16S: 1.00 µl COI-2: 0.50 µl
KAPA Express Extract DNA extract	-	2.00 µl

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

² The 16S rRNA primer premix consisted of 10 µM each of the 16S ar and 16S br primers (Palumbi, 1996).

³ A COI-2 primer premix (10 µM total primer concentration) was used. This consisted of a mixture of four degenerate forward and four degenerate reverse primers. For full details, see Eaton *et al.*, 2009.

Table 4: KAPA2G Robust HotStart ReadyMix cycling parameters for amplification of 16S rRNA and COI gene fragments from mammalian species¹.

Cycling step	Temperature & time	
Initial denaturation	3 min at 95 °C	
Denaturation	15 sec at 95 °C	
Annealing	16S: 10 sec at 50 °C COI: 30 sec at 50 °C	16S: x 35 cycles COI: x 40 cycles
Extension	16S: 15 sec at 72 °C COI: 20 sec at 72 °C	
Final extension	16S: 1 min at 72 °C COI: 10 min at 72 °C	

¹ These cycling parameters were specifically optimized for the mammalian 16S rRNA and COI DNA barcoding assays. For other primer-template combinations, please refer to the KAPA2G Robust HotStart ReadyMix Technical Data Sheet for general cycling parameters.

References

- Eaton, M. J., Meyers, G. L., Kolokotronis, S. O., Leslie, M. S., Martin, A. P. and Amato, G. (2009). Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. *Conservation Genetics*. doi: 10.1007/s10592-009-9967-0.
- Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. In: Molecular Systematics (eds. Hillis, D. M., Moritz, C. and Mable, B. K.), pp. 205–247. Sinauer & Associates Inc., Sunderland, Massachusetts.

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