DNA extraction from FFPE tissue and subsequent PCR is challenging and time consuming, often resulting in variable results or complete reaction failure.

KAPA Express Extract, combined with KAPA2G Robust HotStart ReadyMix, is ideally suited for the routine extraction and amplification of DNA from FFPE tissue. The novel KAPA Express Extract enzyme and buffer offers a fast and convenient way to prepare PCR-ready DNA extracts. KAPA2G Robust HotStart ReadyMix contains an engineered DNA polymerase tolerant to common PCR inhibitors, offering reliable amplification of DNA extracted from FFPE tissue.

Introduction

Formalin-fixed paraffin embedded (FFPE) tissues are valuable clinical samples that are typically generated from human biopsies, collected for histological analysis or cancer detection. There are believed to be over 1 billion FFPE tissue samples archived worldwide. Once fixed, the tissue is encased in a block of wax and can be stored for long periods of time. FFPE tissues are fixed in formaldehyde, which cross-links nucleic acids and amino acids. This presents problems when DNA has to be recovered for genetic testing or sequencing. Current methods use xylene to dissolve the paraffin, followed by ethanol washes and digestion of the tissue using proteinase K. DNA is subsequently purified by phenol-chloroform extraction and ethanol precipitation. These multi-step protocols are laborious, result in the cumulative loss of DNA and pose a high risk of sample contamination.

KAPA Express Extract offers a fast, convenient, and efficient alternative for the routine extraction of DNA from FFPE tissue for subsequent PCR analysis. The novel thermostable protease and buffer system have been designed for optimal tissue lysis and preservation of DNA extracts. The KAPA Express Extract enzyme and buffer are combined with the sample in a single tube. Tissue lysis is performed in a standard thermocycler, after which the reaction product is centrifuged and the DNA-containing supernatant recovered. This protocol is fast (typically completed in 15 min), eliminates excessive handling and opportunity for sample contamination, and generates PCR-ready DNA extracts that can be used for multiple analyses over a period of time.

KAPA2G Robust HotStart ReadyMix is ideally suited for the routine amplification of DNA extracted from FFPE tissue. This ready-to-use cocktail contains the novel KAPA2G Robust HotStart DNA Polymerase, engineered for improved processivity and tolerance to common PCR inhibitors through a process of molecular evolution. This allows convenient and reliable amplification of fragments up to 1 kb from DNA extracted with KAPA Express Extract from FFPE tissue using a fast cycling protocol.

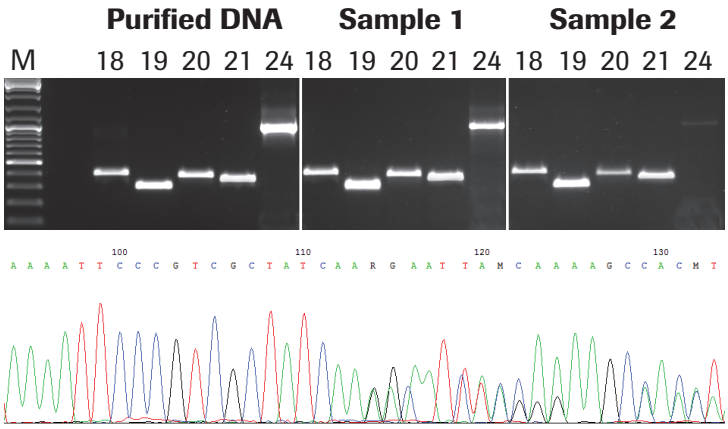

Figure 1. Amplification of EGFR fragments from FFPE tissue using KAPA Express Extract and KAPA2G Robust HotStart ReadyMix, and confirmation of a cancer-associated deletion by DNA sequencing. DNA extracts were prepared from two different FFPE samples using KAPA Express Extract according to the protocol outlined in Table 1. Sample 1 (top middle) was archived for >6 months and Sample 2 (top right) for >1 year. Each extract was used directly (without quantification) in multiple PCRs (1 µL DNA extract per 25 µL reaction), containing KAPA2G Robust HotStart ReadyMix and primers for five different fragments (293 bp – 1 kb) of the epidermal growth factor receptor (EGFR) gene (corresponding to exons 18 – 21 and 24). Results were compared to those obtained using the same reaction and cycling conditions (Tables 3 and 4), but using 1 ng purified human genomic DNA as template (top left). With the exception of the 1 kb exon 24 fragment from the older sample (Sample 2), yields and reaction efficiencies were comparable between the FFPE DNA extracts and purified genomic DNA. The lane marked M contain DNA Ladder. PCR products generated from Sample 1 were diluted 1:10 and used directly in standard Sanger sequencing reactions. Sequence data (bottom panel, Sample 1 exon 19 fragment) was of a high quality. The mixed sequence starting at the position marked with the arrow confirmed the presence of a 15-nt deletion associated with non-small cell lung carcinoma,[1] diagnosed in the patient from whom Sample 1 was collected.

Results

Figure 1 demonstrates the success of combining KAPA Express Extract and KAPA2G Robust HotStart ReadyMix for the reliable amplification of DNA from FFPE samples.

DNA extraction protocol

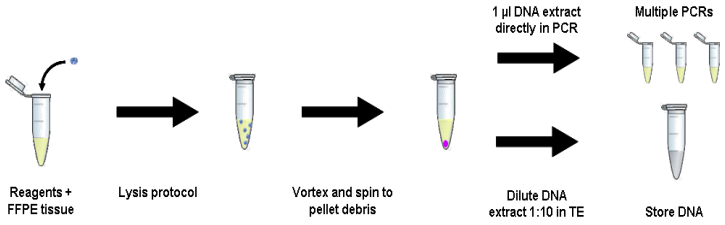

Figure 2. Overview of the KAPA Express Extract DNA extraction protocol.

It is important to use thin (approximately 10 µm) sections of the FFPE sample and trim away excess wax surrounding the tissue to obtain a fragment of approximately 2 mm² that is added to the KAPA Express Extract Buffer and enzyme in the reaction tube (Table 2). The lysis protocol is performed as outlined in Table 1. A standard thermocycler and thin-walled PCR tubes are recommended for the lysis reaction, but a heating block with appropriately sized wells may also be used. Lysis is followed by a quick spin to pellet debris. During centrifugation, residual wax may accumulate on the sample surface. The DNA-containing liquid underneath the wax layer must be carefully removed with a sterile pipette tip and transferred to a fresh tube for subsequent use and storage. It is important to limit wax carryover to a minimum. DNA extracts do not have to be quantified, but may be used directly in PCR. One extraction reaction typically yields sufficient template for 100 x 25 µL PCRs. DNA extracts may be diluted 1:5 in TE Buffer for long-term storage at -20°C.

Table 1. KAPA Express Extract protocol for FFPE tissue

Step	Description
Reaction setup	Combine KAPA Express Extract Buffer, enzyme and FFPE section in a thin-walled PCR tube.
Lysis	Incubate at 75°C for 10 min. During this step, cells are lysed, nucleases and proteins degraded and DNA released.
Heat-inactivation	Incubate at 95°C for 5 min to inactivate the thermostable KAPA Express Extract enzyme.
Sample recovery	Vortex reaction product for 2 – 3 sec. Centrifuge for 1 min to pellet debris. Recover DNA-containing supernatant.

Table 2. KAPA Express Extract reaction setup for FFPE tissue

Reaction component	Final conc.	Per 100 µL reaction
PCR grade water	–	Up to 100.0 µL
10X KAPA Express Extract Buffer	1X	10.0 µL
KAPA Express Extract Enzyme (1 U/µL)	20 mU/µL	2.00 µL
FFPE tissue (10 µm section)	–	2 mm²

PCR reaction conditions and cycling parameters

KAPA2G Robust HotStart ReadyMix reaction setup and cycling parameters for the amplification of DNA extracted from FFPE tissue are given in Tables 3 and 4. These conditions are recommended as a starting point for the routine amplification of amplicons ≤600 bp. DNA from older FFPE tissues is likely to be more cross-linked and degraded. Reaction efficiency therefore typically decreases with sample age, particularly for longer amplicons. Amplicons >1 kb are not recommended. With KAPA Express Extract and KAPA2G Robust HotStart ReadyMix, DNA extraction and amplification from FFPE tissue can be achieved in ≤2 hours, as compared ≥1 day with conventional methods.

Table 3. KAPA2G Robust HotStart ReadyMix reaction setup for FFPE PCR

Reaction component	Final conc.	Per 25 µL reaction1
PCR grade water	–	Up to 25.0 µL
2X KAPA2G Robust HotStart ReadyMix[1]	1X	12.5 µL
Forward primer (10 µM)	0.5 µM	1.25 µL
Reverse primer (10 µM)	0.5 µM	1.25 µL
100% DMSO (for amplicons with a GC content >70%)	5%	1.25 µL
KAPA Express Extract FFPE DNA extract	–	1.00 µL

[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 needed.

Table 4. KAPA2G Robust HotStart ReadyMix cycling parameters for FFPE PCR

Cycling step	Temperature and time	
Initial denaturation	3 min at 95°C	
Denaturation	15 sec at 95°C	x40 cycles
Annealing[1]	15 sec at 60°C	
Extension[2]	15 – 45 sec at 72°C	
Final extension	1 min at 72°C	

[1]The annealing temperature may be varied between 55°C and 65°C to achieve optimal yields with a specific primer set.

[2]Use 15 sec for short amplicons and DNA extracted from relatively fresh FFPE tissue. For longer amplicons and older samples yields may be improved by increasing the extension time to 30 or 45 sec per cycle.

References

1. Paez, J.G, et al. (2004). EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304: 1497 – 500.

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