

PCR from FFPE tissues without DNA extraction using Phusion® DNA Polymerase

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Finnzymes' Phusion® High-Fidelity DNA Polymerase enables robust and efficient DNA amplification from impure or difficult starting materials. Here we present a fast and simple protocol to amplify DNA directly from disrupted formalin-fixed paraffin-embedded (FFPE) tissues without the need for time consuming and complicated DNA extraction. Optimal results are obtained when using the entire High Performance PCR solution from Finnzymes: Phusion DNA Polymerase, high-speed Piko® Thermal Cycler and ultra-thin walled UTW® reaction vessels.

Introduction

Most hospitals collect and archive tissue samples as formalin-fixed paraffin-embedded (FFPE) blocks. These samples represent an invaluable source of research material providing both molecular and clinical information. Unfortunately DNA and RNA extraction from FFPE tissues is challenging as fixation causes modifications and consequent cross-linkage of biomolecules (1,2). Furthermore, nucleic acids are often degraded into smaller fragments. Despite these challenges, damaged DNA can be extracted and analyzed with variety of standard molecular biology techniques (3,4), although the performance of subsequent assays and the maximum length of the amplifiable DNA fragments depend greatly on the quality of the FFPE tissue samples.

There are a number of different methods for DNA extraction from archival samples ranging from simple tissue disruption to laborious protocols (5,6). We show here that Phusion DNA Polymerase performs robustly and efficiently in direct PCR from disrupted FFPE tissues. In this protocol the tedious removal of paraffin is not required, and the resulting preparation can be used without any further purification steps for amplification of short DNA fragments. The unique performance of Phusion DNA Polymerase relies on a specially engineered protein structure (7), which enhances the processivity and robustness of the polymerase.

Materials and methods

- Phusion® High-Fidelity DNA Polymerase (Finnzymes Oy)
- 10 mM dNTP Mix (Finnzymes Oy)
- Proteinase K (Finnzymes Oy)
- 24-well Piko® Thermal Cycler (Finnzymes Oy)
- Ultra-thin walled Piko® PCR Plates (Finnzymes Oy)
- Arktik™ Isothermal Incubator (Finnzymes Oy)
- Primers:

221 bp fragment of highly polymorphic non-coding DNA (locus D21S11):
 F: ATATGTGAGTCAATCCCAAG 22 nt Tm = 61.9°C
 R: TGTATTAGTCAATGTTCCAGAGAC 26 nt Tm = 61.7°C

313 bp fragment of highly polymorphic non-coding DNA (locus D18S51):
 F: TTCTTGAGCCAGAAGGTTA 20 nt Tm = 61.8°C
 R: ATCTACCAGCAACAACACAATAAAC 27 nt Tm = 64.1°C

261 bp fragment of tumor suppressor gene

F: AGGCACTCTTGATGGTTAGGA 21 nt Tm = 62.9°C
 R: GATGTTAGATGGACTACAAGGAC 24 nt Tm = 63.0°C

Preparation of formalin-fixed paraffin-embedded tissue samples for PCR

FFPE sections: Single 10 µm sections of FFPE tissues were treated with 0.2 mg/ml of proteinase K in 50–200 µl of 1x Phusion HF Reaction Buffer, the volume depending on the size of the tissue section. The samples were incubated either for 1 hour or overnight at 60°C in a programmable Arktik Incubator, after which proteinase K was inactivated by increasing the temperature to 98°C for 10 min. After cooling and centrifuging (16 000 x g, 2 min), the supernatant was transferred into a new tube and stored at –20°C if not used immediately for PCR. 1–5 µl of the supernatant was used either directly for PCR, or diluted in 1x Phusion HF Reaction Buffer 1:10 or 1:100. When using more than 1 µl of supernatant, the 5x Phusion HF Reaction Buffer volume was adjusted accordingly.

FFPE blocks: A small amount of tissue was scraped with a sterile scalpel and treated overnight with proteinase K as described above. Further processing was done as with FFPE sections.

FFPE microscope slide: A small amount of tissue was scraped with a sterile scalpel or a pipette tip from a microscope slide (4 µm sections, unstained). A few microliters of 1x Phusion HF Reaction Buffer was used to transfer the dislodged tissue material from the slide to the PCR tube. Samples were treated overnight with proteinase K as described above. Further processing was done as with FFPE sections.

The FFPE samples used in preparation of this protocol were from human breast, prostate, uterus and colon tissue.

Reactions conditions for PCR

Component	20 µl reaction	Final conc.
H ₂ O	Add to 20 µl	
5x Phusion® HF Reaction Buffer	3.8 µl	1x
10 mM dNTP Mix	0.4 µl	200 µM
primer A	x µl	0.5 µM
primer B	x µl	0.5 µM
Template DNA	1 µl*	
Phusion® High Fidelity DNA Polymerase	0.2 µl	0.02 U/µl

* If more supernatant is needed as template, the 5x Phusion HF Reaction Buffer volume has to be adjusted accordingly.

Cycling protocols

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
	98°C	30 s	98°C	30 s	1
Denaturation	98°C	5–10 s	98°C	5–10 s	35–40
Annealing*	-	-	X°C	10–30 s	
Extension	72°C	30 s /kb	72°C	30 s /kb	
Final extension	72°C 4°C	5–10 min hold	72°C 4°C	5–10 min hold	1

* For primers > 20 nt, anneal at Tm +3°C of the lower Tm primer. For primers ≤ 20 nt, anneal at temperature equal to the Tm of the lower Tm primer. A 2-step protocol is applicable when primer Tm values are at least 69°C (> 20 nt) or 72°C (≤ 20 nt) when calculated with Finnzymes' Tm calculator.

Results

We found that the use of Phusion DNA Polymerase enabled direct PCR from disrupted FFPE tissue samples without DNA extraction. Sections of four different FFPE tissues (human breast, prostate, uterus and colon) were digested in proteinase K either for 1 hour or overnight. With some tissue samples as little as one hour treatment was sufficient to release enough genomic DNA for PCR (Figure 1, prostate FFPE tissue). However, with all FFPE samples the results were further improved when the digestion was continued overnight.

For comparison, we tested Phusion DNA Polymerase against *Taq* DNA polymerase in amplification from unpurified proteinase K treated FFPE samples (Figure 2). Phusion DNA Polymerase, combined with Piko Thermal Cycler and ultra-thin walled Piko PCR Plates, produced remarkably higher yields over a wider range of input DNA.

To assess the quality of the PCR results obtained with this simple tissue disruption protocol, we compared it to a commercial DNA extraction kit designed specifically for FFPE samples. Phusion DNA Polymerase efficiently amplified both breast tissue preparations producing comparable yields for a 261 bp fragment (Figure 3). Both methods were able to provide DNA suitable for PCR, but the simple tissue disruption protocol was easier and safer to use since the tedious removal of paraffin was not required.

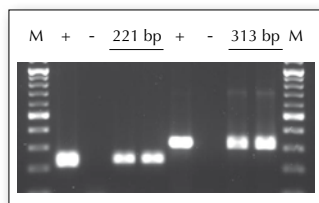


Figure 1. Direct PCR from FFPE tissue using Phusion DNA Polymerase. Single prostate tissue section (10 μ m) was treated with proteinase K for one hour. Fragments of 221 and 313 bp were amplified from different volumes of tissue supernatant as described in Materials and Methods (40 cycles).

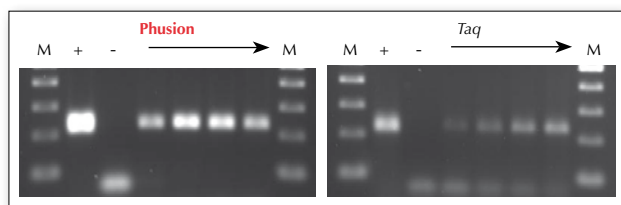


Figure 2. Comparison of Phusion DNA Polymerase against *Taq* DNA polymerase. Single FFPE prostate tissue sections (10 μ m) were incubated o/n with proteinase K. A 221 bp DNA fragment was amplified from different concentrations of each tissue supernatant (arrow indicates increasing template amount). PCR reactions with Phusion DNA Polymerase were performed as described in Materials and Methods (40 cycles). *Taq* DNA Polymerase from the leading supplier was used as recommended (40 cycles).

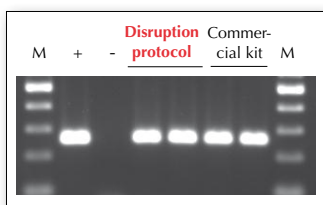


Figure 3. Phusion DNA Polymerase performs equally well from disrupted tissue samples as from DNA extracted with a commercial kit. The tissue disruption protocol was performed as described in Materials and Methods using o/n incubation in proteinase K for FFPE breast tissue sections. Commercial kit was used

as recommended by the manufacturer (paraffin removal step included). A 261 bp fragment was amplified from both sample preparations using Phusion DNA Polymerase.

Discussion

In this application note we describe a simple PCR protocol that can be used for FFPE tissues without the need for DNA extraction or paraffin removal. This protocol relies on Phusion DNA polymerase, which is an ideal choice for PCR applications using samples of variable DNA quality. When compared to *Taq* DNA polymerase, Phusion DNA Polymerase clearly produced higher yields and more consistent results over a wide range of input DNA quantities. Furthermore, because of the extremely high fidelity of Phusion DNA Polymerase, the subsequent PCR products are well suited for sequencing or other applications where high accuracy is required.

We found that the quality of the FFPE block was extremely critical for the successful use of this protocol. With some old or poorly prepared blocks the amount of amplifiable DNA was clearly lower than from blocks prepared more recently, or even negligible (data not shown). Despite the similar performance of all the different tissue types tested here, it is expected that with more condensed tissues the amount of released amplifiable DNA could be lower. In some cases, increasing the time for proteinase K treatment even longer or using more FFPE tissue as starting material may improve the results.

Because of the DNA degradation typical to FFPE tissues, only rather short DNA fragments are easily amplified from these samples. With the FFPE tissue samples tested in this application note, the maximum length of the successfully amplified PCR product was approximately 300 bp.

Acknowledgements

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Finnzymes' Direct PCR allows amplification of DNA directly from various starting materials such as blood, mouse ear and tail tissues, plants, and FFPE tissue samples. For more information about the Direct PCR products and protocols, please visit www.finnzymes.com/directpcr.