PROTOCOL

FFPE DNA Extraction Protocol

Introduction

The number of archival formalin-fixed paraffin embedded (FFPE) samples is in the millions, providing an invaluable repository of information for genetic analysis. These samples can be analyzed for a wealth of applications including biomarker discovery, drug development, and cancer research.

Diagenode's FFPE DNA Extraction kit is optimized for the extraction of DNA from FFPE tissue sections in conjunction with the Bioruptor® Pico.

This protocol offers unique benefits:

- Eliminate laborious deparaffinization using organic solvents
- Remove paraffin and rehydrate tissue in just one step
- Preserve DNA integrity with a mild cross-link reversal process (no high temperature)
- Improve yield and quality of extracted DNA
- Process multiple samples with high throughput
- Prepare NGS libraries in conjunction with the Bioruptor® Pico for DNA shearing

Kit contents

Description	Quantity	Storage
FFPE extraction buffer	10 ml (2 x 5 ml)	Room temperature
Cross-link reversal buffer	220 µl	Room temperature
Precipitant	1.2 ml	Room temperature
Washing buffer (5x)*	10 ml (2 x 5 ml)	Room temperature
Elution buffer	6 ml	Room temperature
Binding buffer	55 ml	Room temperature
DiaFilter column with collection tube	50 pc	Room temperature
DiaPure DNA columns with collection tubes	50 pc	Room temperature
Additional items included in this kit:		
1.5 ml Bioruptor® Microtubes with Caps (FFPE DNA Extraction kit (Bioruptor® Pico) - Cat. No. C20000030)	50 pc	Room temperature
or 1.5 ml TPX microtubes (FFPE DNA Extraction kit (Bioruptor® Standard/Plus) - Cat. No. C20000031)	50 pc	Room temperature

*** Buffer has to be diluated 5 times with ethanol before use**

Required reagents and equipment's not supplied

- Proteinase K (Diagenode, Cat. No. C06050001 or C06050002)
- RNase cocktail (Ambion, AM 2286 A, **optional**)
- Ethanol 100%
- Bioruptor® Pico sonication device for 6 x 1.5 ml tubes (Diagenode, Cat.No. B01060002)

- **Alternatively**:
  - Bioruptor® Standard sonication device for 6 x 1.5 ml tubes (Diagenode, Cat. No. B01010001; only available in the US)
  - Bioruptor® Plus sonication device for 6 x 15 ml tubes (Diagenode, Cat. No. B01020001)
- For microvolume shearing (only applicable with Bioruptor® Pico):
  - 0.1 ml tube holder & tube adaptors for Bioruptor® Pico (Diagenode, Cat. No. B01200041)
  - 0.1 ml Bioruptor® Microtubes (Diagenode, Cat. No. C30010015)
- Thermomixer (56°C and 64°C)
- Bench top centrifuge for 1.5 ml tubes
- 1.5 ml tubes
- Vortex mixer

General remarks before starting

- Please note that the yield and quality of extracted DNA depends on the type, age of the sample and the conditions used for the fixation. DNA isolated from FFPE samples is usually of lower molecular weight than DNA from fresh or frozen samples
- This protocol is optimized to work with slices of 10 µm thickness and areas up to 2 cm^2. Adding more material to a single tube may decrease the efficiency of DNA extraction.

Required steps before starting

- Add 20 ml ethanol to the bottle containing 5 ml of ***5x Washing buffer***. Label the bottle to indicate that ethanol has been added
- Set the temperature of the ***Water cooler*** of the ***Bioruptor® Pico, Standard or Plus*** to 20 - 22°C. The water in the sonication bath should be at RT (room temperature)
- Pre-heat a thermo mixer to 56°C
- Pre-warm ***FFPE extraction buffer*** to 37°C
- Equilibrate ***Elution buffer*** to RT.

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1. Using a scalpel, trim excess paraffin off the sample block
2. Cut sections of 10 µm in thickness
3. Place the sections in ***1.5 ml Bioruptor® Microtubes*** and add 180 µl of pre-warmed (37°C) ***FFPE extraction buffer***. Ensure that sections are completely immersed in the buffer. Longer sections might be cut into 2 pieces
4. Incubate samples for 10 min at 37°C
5. Vortex samples and sonicate samples with the ***Bioruptor® Pico*** for 3 - 5 cycles for 30 sec On/30 sec Off at RT (the water in the **Water cooler** has to be set to 20 -22°C) until the paraffin is emulsified and dissociated from the tissue section. Inspect the samples visually

   The solution will turn milky due to paraffin emulsification

   Always use the shortest sonication time. Please note that additional optimization might be required depending on sample type and amount

   Settings for the **Bioruptor® Standard or Plus**: 3 - 5 cycles for 30 sec On/30 sec Off at High Power
6. Briefly spin down samples and transfer to a new 1.5 ml tube (not supplied)
7. Add 20 µl of Diagenode's ***Proteinase K***

   If Proteinase K from other suppliers is used, add a necessary volume containing 400 µg
8. Incubate for 1 h at 56°C using a thermomixer, shaking at 1300 rpm
9. Add 4 µl of ***Cross-link reversal buffer*** and incubate at 65°C for 4 h or overnight, shaking at 1300 rpm
10. Spin down samples and carefully transfer the supernatant to a ***DiaFilter column*** to remove the paraffin
11. Centrifuge for 2 min at 6000 g (8000 rpm) at RT

    Important: Do not use higher speed as it might damage the DiaFilter column
12. Discard the filter unit and keep the collection tube contaning the flow-through
13. (**Optional**) If RNA-free DNA is required, the sample can be treated with RNase cocktail. Add 1 µl of RNase cocktail and incubate for 5 min at RT
14. Add 1 ml of ***Binding buffer*** and 20 µl of ***Precipitant*** to the flow-through. Pipette carefully to mix components
15. Place a ***DiaPure DNA column*** in a collection tube, load 600 µl of the mixture from the previous step. Close the lid and centrifuge for 1 min at 6000 g (8000 rpm) at RT
16. Discard the flow-through from the collection tube and re-use the tube
17. Load the remaining mixture on the ***DiaPure DNA column***. Close the lid and centrifuge for 1 min at 6000 g (8000 rpm) at RT
18. Discard the the flow-through and re-use the collection tube
19. Wash the ***DiaPure DNA column*** twice with ***Washing buffer***: add 400 µl of ***Washing buffer*** to a DiaPure DNA column, close the lid and centrifuge for 1 min at 6000 g (8000 rpm) at RT

    Discard the the flow-through, re-use the collection tube and repeat the washing: add 400 µl of ***Washing buffer*** to ***DiaPure DNA column***, close the lid and centrifuge for 1 min at full speed (14000 rpm) at RT.

⚠ The washing buffer has to be diluted 5 times with ethanol before use.

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20. Place the ***DiaPure DNA column*** in a clean 1.5 ml tube (not supplied) and add 50 - 100 µl of **Elution buffer** (RT) to the center of the membrane

21. Incubate for 3 min at RT and centrifuge for 1 min at 14000 rpm

    The eluate contains the purified DNA. Quantify and analyze the purified DNA by a method of your choice. For long-term storage, aliquot sample and store at -20°C. Avoid multiple thaw-freezing cycles

22. (**Optional**) If DNA has to be fragmented (e.g. Next-Generation Sequencing library preparation), follow Diagenode's DNA shearing protocol: http://www.diagenode.com/en/support/protocols.php.

    DNA can be sheared in 10, 50 and 100 µl. Please contact Diagenode for more details.

Results

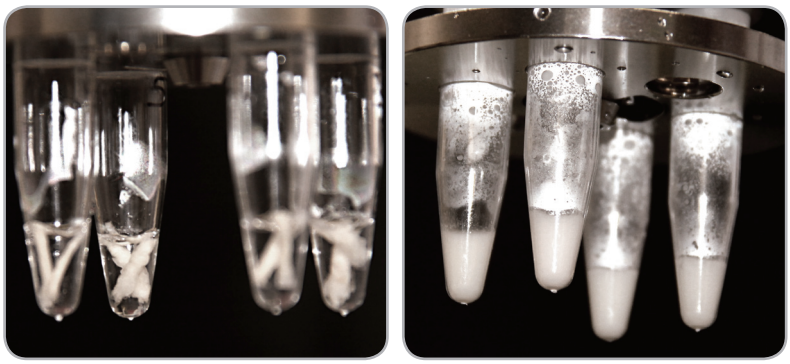

Figure 1. Efficient de-paraffinization of FFPE sections by sonication
10 µm sections were sonicated for 3 cycles (30 sec ON/OFF at RT) with the **Bioruptor® Pico**. The paraffin has been emulsified and completely dissociated from the tissue section.

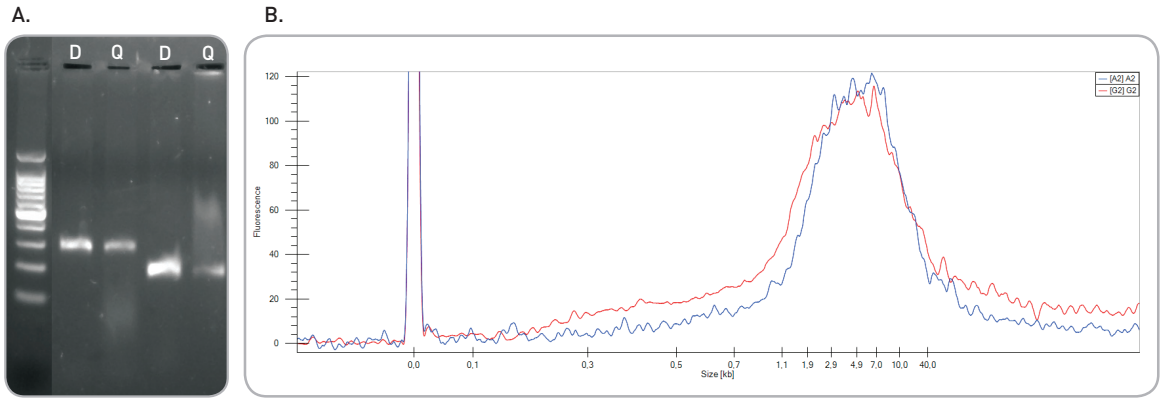

Figure 2. Large DNA fragments are extracted using Diagenode's protocol

Although the size of DNA fragments extracted from FFPE samples depends on the sample handling and storage age, Diagenode's protocol preserves DNA from further degradation seen with competitor Q DNA FFPE tissue protocol. DNA was extracted from 10 µm adjacent sections of mouse liver (10 months of storage). Panel A – agarose gel analysis of extracted DNA. Note that DNA extracted using competitor Q DNA FFPE tissue protocol shows a degradation while Diagenode's protocol results in higher molecular weight DNA. Panel B – Caliper Labchip GX traces of DNA extracted with Diagenode's protocol show fragments of 3 - 6 kb yield.

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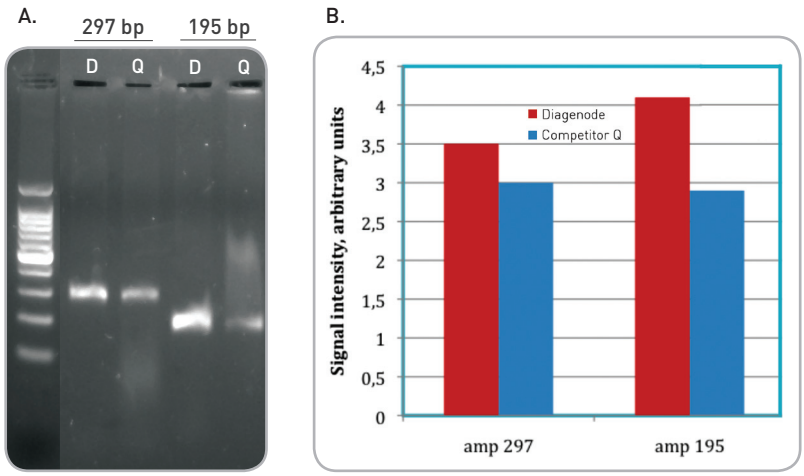

Figure 3. Quality assessment of extracted DNA by end-point PCR

DNA was extracted from 10 µm adjacent sections from mouse liver (10 months storage) using Diagenode's kit or competitor Q DNA FFPE tissue kit. Equal amount of DNA was used for end – point PCR amplification with different primer pairs as indicated in the table. The resulting amplicons were analyzed in agarose gel (panel A) and the signal intensity was quantified by ImageJ softwear (panel B). Higher yield of PCR products is obtained from the same amount of DNA extracted with Diagenode's protocol compared to competitor Q protocol.

Amplicon size	Genomic loci Assembly December 2011	Primer sequences
297 bp	chr6:125165470+125165766	TGAGAGAGGCCCAGCTACTC
		TCCCTAGACCCGTACAGTGC
195 bp	chr13:23934046-23934240	CTCCTTGCGGCATCTCTTAC
		CCGCTTCTCAACCTCAAGTC

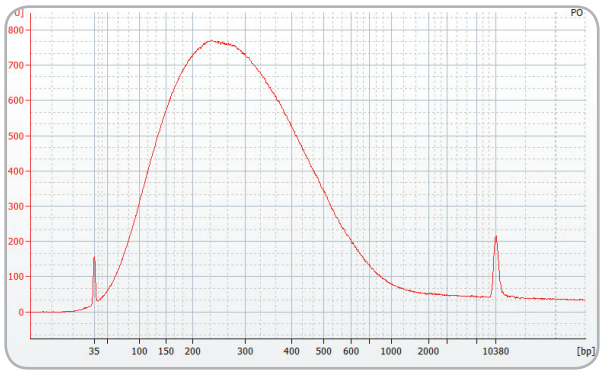

Figure 4. Extracted DNA sonicated to desired fragment size for Next-Generation Sequencing library preparation using Bioruptor® Pico

50 µl of DNA extracted from FFPE mouse liver were sonicated for 7 cycles of 30 sec ON/OFF using 0.1 ml tube holder & tube adaptors (Cat. No. B01200041) and the corresponding 0.1 ml Bioruptor® Microtubes (Cat. No. C30010015). Fragment size distribution is analyzed by Agilent Bioanalyzer (High sense kit).

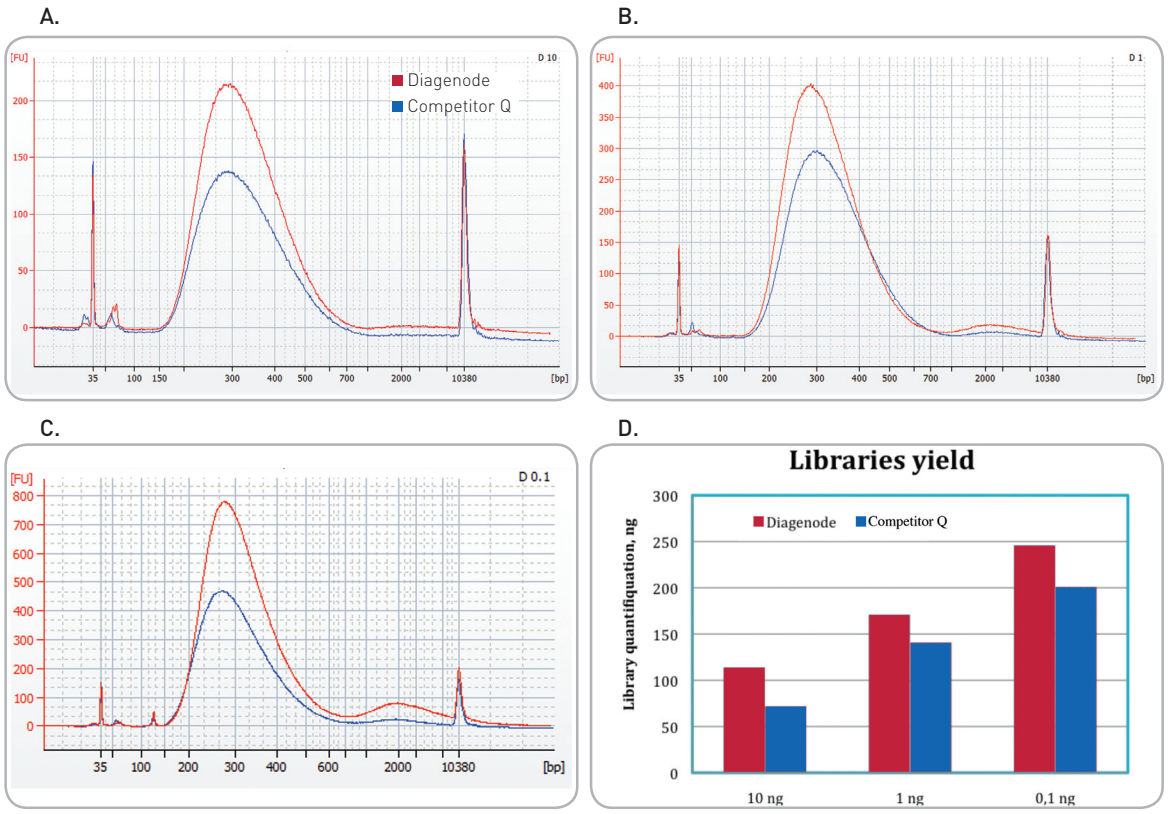

Figure 5. DNA extracted with Diagenode's protocol results in a better yield of libraries for Next-Generation Sequencing

10 (panel A), 1 (panel B) and 0.1 ng (panel C) of DNA extracted from FFPE mouse liver with Diagenode's protocol (red traces) or competitor Q DNA FFPE tissue kit (blue traces) were used for library construction with Diagenode's MicroPlex Library Preparation™ kit. Diagenode's protocol results in a higher quality DNA allowing a better yield of libraries comparing to competitor Q protocol. Panel A, B and C: BioAnalyzer traces of purified libraries. Panel D: libraries quantification by Qubit system (Invitrogen).