Introduction

This protocol describes a method for DNA extraction from FFPE cells (we used the MCF-7 cell line). Although the quality of extracted DNA can vary greatly between different tissue types and FFPE samples of different ages, our recommendation is to use the QIAamp® DNA FFPE Tissue Kit. Out of the two kits we have tested, it gave the best results, although the output is lower than for our other extraction methods.

We have also tested extraction using the GeneRead DNA FFPE Kit, however using this kit, the DNA yield did not improve after a PCR step, and the protocol took longer than using the QIAamp kit.

Materials

- QIAamp DNA FFPE Tissue Kit
- QIAGEN Deparaffinization Solution (Cat # 19093)
- NEBNext FFPE DNA Repair Mix (Cat # M6630)
- (Optional) QIAamp MinElute Media Kit (Cat # 57414)
- Agencourt AMPure XP Beads
- Nuclease-free water
- Pure ethanol
- Freshly-prepared 70% ethanol in nuclease-free water
- Heat block at 56°C
- 1.5 ml Eppendorf DNA LoBind tubes
- Microfuge
- Scalpel
- Hula mixer (gentle rotator mixer)
- Magnetic rack
- Vortex mixer

Method

1. Remove paraffin from your sample using the QIAGEN Deparaffinization Solution: Cut one FFPE section up to 20 µm thick. If the sample surface has been exposed to air, discard the first 2–3 sections. Immediately place the section in a 1.5 ml Eppendorf DNA LoBind tube.

2. Add 320 µl Deparaffinization Solution, vortex vigorously for 10 secs, and spin down briefly.

3. Incubate at 56°C for 3 min, then allow to cool at room temperature. If too little Deparaffinization Solution is used or if too much paraffin is carried over with the sample, the Deparaffinization Solution may become waxy or solid after cooling. If this occurs, add additional Deparaffinization Solution and repeat the 56°C incubation.

4. Centrifuge at full speed for 2 minutes at room temperature.

5. Remove the supernatant by pipetting. Do not remove any of the pellet.

6. Add 1 ml pure ethanol to the pellet, and mix by vortexing. The ethanol extracts residual Deparaffinisation Solution from the sample.

7. Centrifuge at full speed for 2 min at room temperature.

8. Remove the supernatant by pipetting. Do not remove any of the pellet. Carefully remove any residual ethanol using a fine pipette tip.

9. Open the tube and incubate at room temperature (or up to 37°C). Incubate for 10 min, or until all residual ethanol has evaporated.

10. Follow the QIAamp DNA FFPE Tissue Kit protocol, starting from step10 (addition of ATL buffer). We recommend eluting your final DNA into 45 µl of ATE buffer loaded onto a QIAamp MinElute column, and incubating for 5 min before centrifugation.
11. Quantify 1 µl of extracted DNA using a Qubit fluorometer, and assess DNA integrity, e.g. using an Agilent Bioanalyzer or FEMTO Pulse.
12. Perform FFPE DNA repair:
13. Mix together:

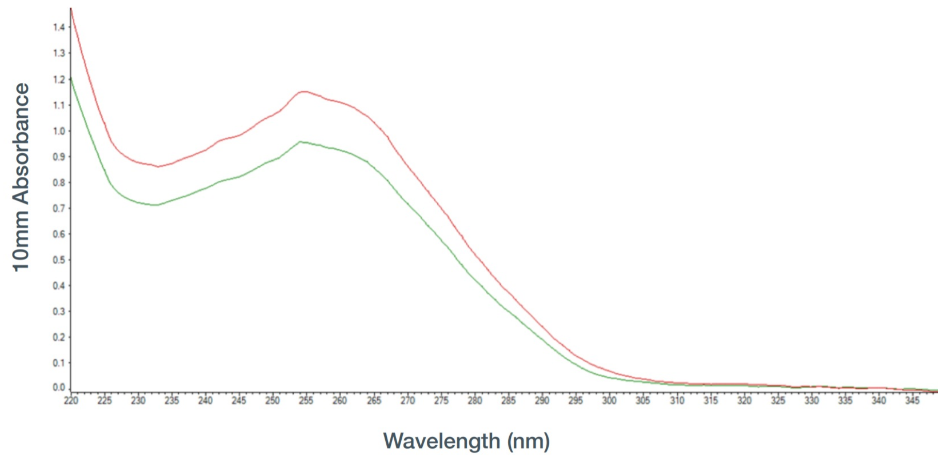
Reagent	Volume
200 ng-1000 ng FFPE DNA	x µl
Nuclease-free water	53.6-x µl
FFPE repair buffer	6.5 µl
FFPE repair mix	2 µl
Total	**62 µl**

1. Mix gently by flicking the tube, and spin down.
2. Incubate the reaction for 15 minutes at 20°C.
3. Resuspend the AMPure XP beads by vortexing.
4. Add 62 µl of the resuspended beads to the FFPE-repair reaction and mix gently by flicking the tube.
5. Incubate on a Hula mixer (rotator mixer) for 5 minutes at room temperature.
6. Prepare 500 µl of fresh 70% ethanol in nuclease-free water.
7. Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.
8. Keep on magnet, wash beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the 70% ethanol using a pipette and discard. Repeat this step one more time.
9. Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Briefly allow to dry.
10. Remove the tube from the magnetic rack and resuspend pellet in 46 µl nuclease-free water. Incubate for 2 minutes at room temperature.
11. Pellet beads on magnet until the eluate is clear and colourless.
12. Remove and retain 46 µl of eluate in a clean 1.5 ml Eppendorf DNA LoBind tube.

Results

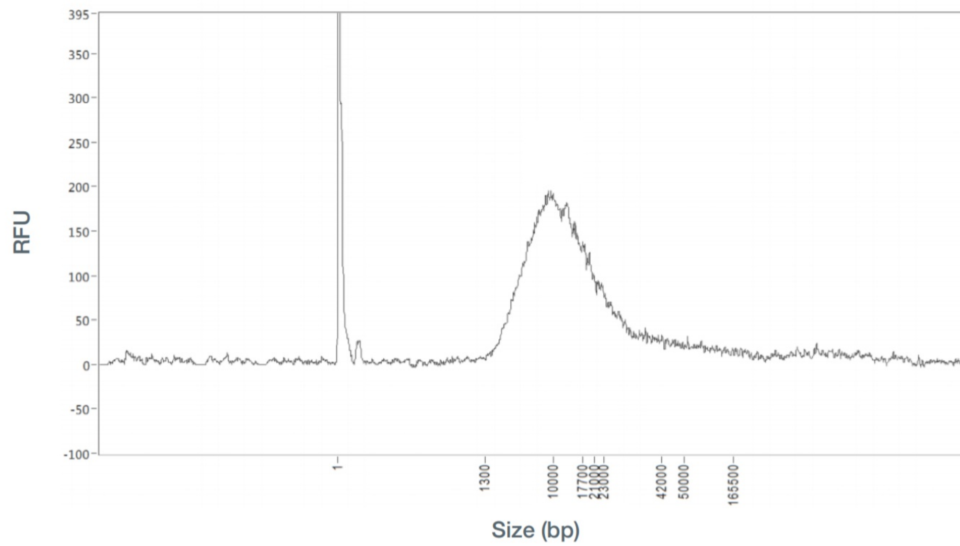
QIAamp DNA FFPE Tissue Kit

- **Yield:** 200 ng from a 10 µm FFPE curl
- **OD 260/280:** 2.16
- **OD 260/230:** 1.28



Although the 260/230 ratio is lower than expected for a pure sample, higher-quality DNA was enriched by PCR during library prep.

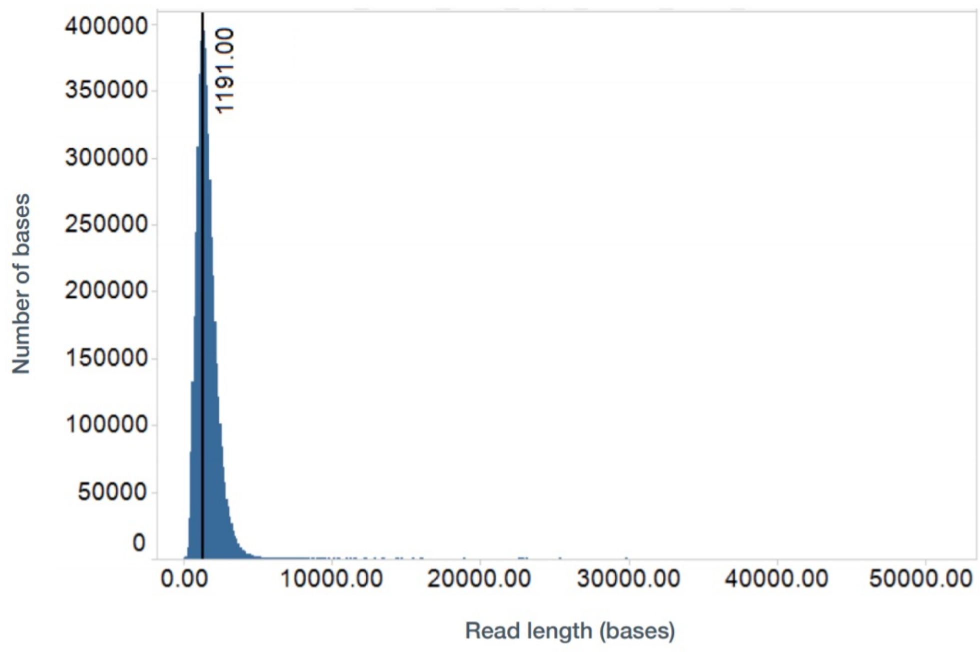
- **Fragment size (FEMTO pulse):**



Sequencing performance

Libraries for Nanopore sequencing were prepared using the Ligation Sequencing Kit, using the Low Input by PCR protocol.

- Read length profile after SPRI size selection and sequencing:



Number of bases
400000
350000
300000
250000
200000
150000
100000
50000
0
1191.00
0.00
10000.00
20000.00
30000.00
40000.00
50000.00
Read length (bases)