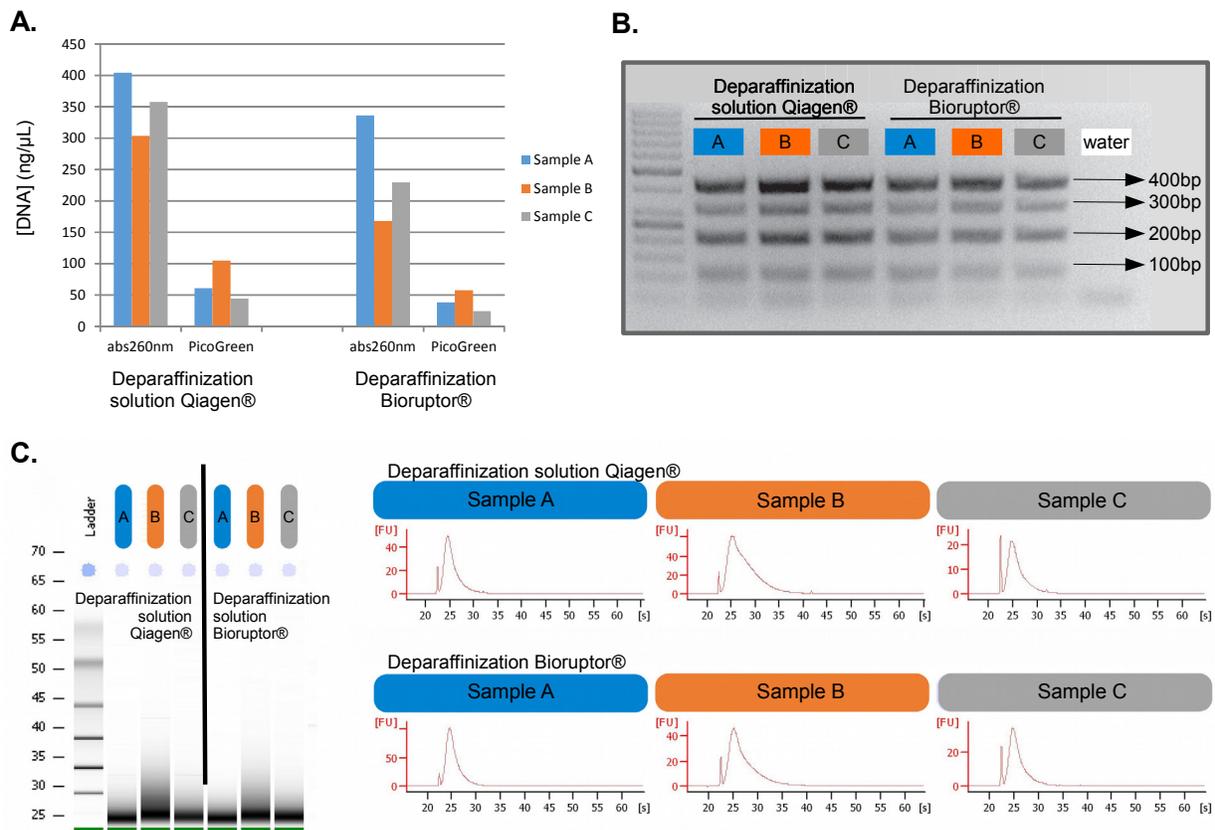


# BIORUPTOR® DEPARAFFINIZATION

## PREPARATION OF FFPE SAMPLES FOR NEXT-GENERATION SEQUENCING USING THE BIORUPTOR® PICO AND QIAGEN® ALLPREP FFPE DNA/RNA KIT

Deparaffinization of FFPE samples is typically performed using a non-polar solvent, such as xylene, or a mineral oil-based method which can be time consuming and messy. Claire Josse and colleagues from the Human Genetic Laboratory at the GIGA - University of Liège have developed a new protocol combining the Bioruptor® Pico with the AllPrep FFPE DNA/RNA kit from Qiagen® to efficiently prepare FFPE samples for Next-Generation Sequencing. The Bioruptor® Pico removes the paraffin and rehydrates the tissue in just one solvent-free step followed by a mild crosslink reversal to preserve DNA and RNA integrity. The nucleic acids are then extracted with the AllPrep FFPE DNA/RNA kit and sheared using the Bioruptor® Pico to the desired length for sequencing library preparation.



**Figure:** Comparison of genomic DNA and total RNA yield and quality obtained when deparaffinization is performed with the Bioruptor or the deparaffinization solution from Qiagen®. Three samples are deparaffinized by both methods, extracted with AllPrep Qiagen® columns, and quantified **A.** by absorbance @260nm or by the the intercalating agent PicoGreen. **B.** DNA integrity is stated by amplification of fragments of 100, 200, 300 and 400 bp in a multiplex PCR of the GAPDH gene. **C.** RNA profiles are performed on a Agilent 2100 Bioanalyzer.

### 1. Material required

- 20 tissues sections of 4 μM thick mounted on glass slides are used for extraction
- Sterile needle
- Bioruptor® Pico (Cat. No. B01060001) with 1.5 ml tube holder
- 1.5 ml Bioruptor® Pico Microtubes with Caps (Cat. No. C30010016)
- Allprep FFPE DNA/RNA kit, Qiagen® (Cat. No. 80234)



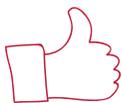
## 2. Procedure

### Day 1

- Use 2 g of cells / purification. If starting from cell pellets, thaw quickly in a water bath at 37 °C.
- Optional : In order to maximize tumoral content, a pathologist should examine one tumour haematoxylin-eosin stained section to determine the tumour area. To isolate nucleic acids, the annotated stained section is superposed with 19 unstained sections; the tumor area is manually macro-dissected and scraped with a sterile needle.
- Scrape tissue from 10 tissue slices and harvest in 1.5 ml Bioruptor® Pico Microtubes containing 250 µL PKD buffer (Qiagen®). This operation is performed in duplicate for each sample, 10 tissues sections/tube.
- Place the 2 tubes inside the Bioruptor® and sonicate for 6 cycles (sonication cycle: 30 sec ON, 30 sec OFF). It is important to not have more than 10 tissues sections/tube in order to obtain optimal emulsification of the paraffin.
- Briefly spin down tubes and transfer the samples into new 1.5ml tubes (Qiagen®).
- Add 50 µL proteinase K (Qiagen®) and mix by vortexing.
- Incubate at 56°C for 15 min.
- Incubate on ice for 10 min. Complete cooling is important for efficient precipitation.
- Centrifuge for 15 min at 20.000 x g.
- Carefully transfer the supernatant, without disturbing the pellet, to a new RNase free 1.5 ml tube for RNA purification. Keep the pellet for DNA purification.
- Proceed with the Qiagen® protocol for RNA and DNA purification.
- Elution step is performed with 2 x 20 µl RNase free water for RNA purification, and with 2 x 20 µl ATE buffer (Qiagen®) for DNA purification.
- The DNA and RNA can then be analyzed by traditional methods or can be sheared with the Bioruptor® Pico for downstream NGS library preparation. Please refer to the DNA Shearing Guide for additional information. <https://www.diagenode.com/en/dna-shearing-guide>.

## 3. Selected publications

1. Boukerroucha M, Josse C, Segers K, El-Guendi S, Frères P, Jerusalem G, Bours V. **BRCA1 germline mutation and glioblastoma development: report of cases.** *BMC Cancer* 2015, **15**:181.
2. Boukerroucha M, Josse C, El-Guendi S, Boujemla B, Frères P, Marée R, Wenric S, Segers K, Collignon J, Jerusalem G, Bours V: **Evaluation of BRCA1-related molecular features and microRNAs as prognostic factors for triple negative breast cancers.** *BMC Cancer* 2015, **15**:755.



Using Bioruptor® in a creative way?

Share your protocol with us at [info@diagenode.com](mailto:info@diagenode.com)

