DNA EXTRACTION FROM FROZEN TUMOR SAMPLES USING THE MINILYS TISSUE HOMOGENIZER

Molecular Pathology Unit, Liverpool Clinical Laboratories , UK

CONTEXT

Breast, ovarian, endometrial and lung tumor samples are **routinely homogenized** and processed for DNA in **cancer research**. In addition to local diagnostic requirements, DNA obtained from tumor samples is submitted to the **100,000 Genome Project** that aims to use **Whole Genome Sequencing** (WGS) **technique** on patients, plus their families, with a rare disease or cancer. This project imposes **high standards of DNA quantity** and **fragment length quality**.

In this study, the **Minilys tissue homogenizer** was evaluated for tumor tissue sample homogenization and results were compared to those obtained following a **manual sample homogenization method**. The DNA yield and quality as well as hands-on time required were compared between the two methods.

MATERIALS

- **Samples**: 4mm punch biopsies of frozen specimens
- **Buffer**: Proteinase K buffer
- **For Minilys Method**: Minilys homogenizer and 2mL CK28-R Precellys lysing kit.
- **For manual method**: Mini plastic disposable pestle and mortar (optional, a razor blade or scalpel)

DNA EXTRACTION FROM FROZEN TUMOR SAMPLES USING THE MINILYS TISSUE HOMOGENIZER

PROTOCOL

Manual method: The frozen biopsies were manually treated using the mini plastic disposable pestle/mortar. Samples not homogenized satisfactorily, were chopped up using razor blades/scalpels (treatment time: 5 to 10 minutes per sample). Each sample was then split in 2 tubes: one for storage and one for analysis. Tube for analysis was lysed overnight with 20 µl of **Proteinase K** (at 37°C) followed by a fluid extraction performed on the next day with a standard kit extraction.

Minilys method: The biopsies were placed into **Precellys 2ml CK28-R tubes** containing 180µl of ATL buffer. The samples were homogenized with **Minilys** for 2x20 seconds at 5,000 rpm and at the end of the run, 20µl of Proteinase K were then added directly into the tube for lysis (1h at 37°C). After lysis, each sample was split into 2 tubes: one for storage and one for analysis. Fluid extraction was then performed on the tube for analysis with a standard kit extraction.

RESULTS

- The processing time was **significantly reduced** when using **Minilys** for homogenization, as well as the post treatment time with Proteinase K (reduced to one hour vs overnight for the manual method).
- The yield of DNA recovery with **Minilys** was **higher in 81% of the samples** compared to the manual method. Nine out of eleven samples homogenized by the manual method didn't exceed the concentration of 15ng/µl while the lowest concentration found in samples homogenized by the **Minilys** was 3 times higher (49ng/µl). The average DNA yield recovery with the **Minilys** was 185.7ng/µl compared to 26.8ng/µl for the manual method. Therefore, only 1 sample needed to be treated.
- All DNA samples obtained with the **Minilys** showed **good quality**, including **excellent fragment length** (Figure 1) meeting the 100.000 genome requirements of >60% of fragments with a minimal length of 23kbp.

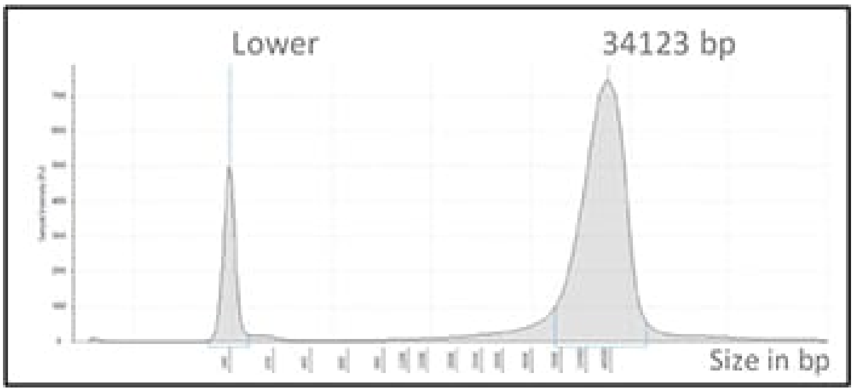

Figure 1. Fragment length characterization using the TapeStation instrument (Agilent)

CONCLUSION

The use of the **Minilys tissue homogenizer** to homogenize tumor samples **proved to be an efficient method** compared to manual sample preparation, and is now the reference method at Liverpool Clinical Laboratories:

- As DNA recovery yield is higher, DNA extraction no longer needs to be duplicated, reducing costs by half as only one DNA extraction kit per sample is needed.
- Hands-on time and total processing time were **considerably reduced**, thus saving both technical and human resources.
- The quality of the DNA samples obtained had optimal fragment length, leading to a **high likelihood of successful WGS**.

Liverpool Clinical Laboratories lcl

Dr. Alistair Reid
Consultant Clinical Scientist

DISCOVER OUR COMPREHENSIVE RANGE OF SOLUTIONS

AIR SAMPLING → **SAMPLE PREPARATION** → ASSAY KITS → TECHNOLOGY ON-DEMAND

Join the Bertin Technologies community!
1500+ documents available online on the Application center
www.bertin-technologies.com/application-center/