Development and Benefits of an Automated Protocol for DNA Extraction from Formalin-Fixed, Paraffin-Embedded Tissues (FFPE) for Use in Solid Tumor Genomic Analyses

Promega Corporation

Automated Nucleic Acid Extraction for Increased Throughput and Improved Assay Performance

In oncology, tissue biopsies are commonly fixed in formalin and embedded in paraffin (FFPE). These FFPE samples can be used with immunohistochemical and/or molecular analysis for identifying biomarkers that guide the diagnosis and therapeutic management of patients.

This fixation technique allows long-term storage of samples but impacts the integrity of nucleic acids. This makes extracting DNA and RNA from FFPE tissues in sufficient quantity and quality for molecular analysis techniques such as NGS analyses challenging for molecular oncology laboratories.

Dr. Alexandra Lespagnol, Technical Manager of the Molecular Genetics of Cancer core lab at the University Hospital of Rennes, shares her experience implementing the Maxwell® HT DNA FFPE extraction chemistry on the Hamilton STAR robotic platform.

"At Rennes University Hospital, we receive many lung cancer samples with little material available, or samples of poor quality. Nucleic acid extraction step is therefore critical to get good yield. We have seen that it had a direct impact on the success of downstream analysis." - Dr. Lespagnol

To address the increasing number of samples that needed to be analyzed, the Molecular Genetics of Cancer core lab of the University Hospital of Rennes initiated an automation project. In addition to increasing throughput, the lab wanted to improve sample tracking and reproducibility of the results.

Taking advantage of the Hamilton STAR liquid handler that the laboratory already owned, the project goal was to develop a DNA extraction protocol that would meet their expectations without interfering with the activity of other users.
Automated DNA FFPE Extraction Workflow

Designed to be compatible with a variety of laboratory automation platforms, the Maxwell® HT extraction solutions offer flexible and efficient workflows for DNA isolation. The lab evaluated the performance of the Promega Maxwell® HT DNA FFPE Isolation System (Cat.# A6372) using common FFPE biopsy samples and NGS analysis.

**CASE STUDY**

30 Minutes 2 Hours Overnight

Sample deparaffinization without solvent 10 minutes at 80°C.

Lysis step 8 hours at 56°C.

Decrosslinking for 4 hours at 80°C.

Automated extraction of 96 samples on the Hamilton STAR.

“The protocol has been smartly designed and optimized. It is very fast; in less than 2 hours, nucleic acid from 96 samples can be extracted. The protocol is also flexible and can process from 1 to 96 samples with optimal usage of reagents and plasticware.” - Dr. Lespagnol

**Validation Data**

Improved Success Rate in NGS Analysis (>15%)

**Consistent and Proportional DNA Concentration**

![Graph showing DNA concentration is proportional to sample input volume. Batch lysate from 10 paraffin curls were aliquoted in triplicate as 5, 25 or 125µl of lysate in lysis buffer (qsp 200µl) and extracted using the Maxwell® HT DNA FFPE Isolation System on the Hamilton STAR instrument.]

**Figure 1.** Comparative NGS analysis success rate with DNA isolated using the in-house protocol and the Maxwell® HT DNA FFPE Isolation System protocol. DNA isolation was performed using melanoma, colorectal and lung cancer tumor samples (~500), using the in-house lab protocol and the Promega Maxwell® HT DNA FFPE Isolation System protocol on the Hamilton STAR instrument.
Development and Benefits of an Automated Protocol to Extract DNA from FFPE Tissues

Repeateble Results with No Cross-Contamination

Figure 3. Results are consistent with no cross-contamination. Eight FFPE samples and 3 blanks were extracted using the Maxwell® HT DNA FFPE Isolation Kit.

“After we got the first results from samples extracted with the Promega Maxwell® HT FFPE System, we realized that sample quality with our in-house technique was suboptimal. Initially, ovarian tumor samples were tested with the Promega protocol; then we extended its use to all tumor types. Quality improvement is quite phenomenal; we can reduce the number of retesting and reduce time to results.” - Dr. Lespagnol

Future Projects

The Molecular Genetics of Cancer core lab plans to expand automated extraction protocols to other sample types, including liquid biopsy (circulating cell-free DNA from plasma) and RNA from FFPE. Testing for these applications has already begun.

“A Promega team perfectly understood our needs and translated it into solutions. Project qualification and cost evaluation were established together. Nans came in November 2020 to install the script and test the protocol, between two [Covid] lockdown periods. The week after, I was already extracting FFPE samples with the new method. We switched to routine on April 1. [...]. After several weeks, we were able to make small adjustments to make everyone feel comfortable with the instrument and the protocol.” - Dr. Lespagnol

To learn more about preconfigured and custom automated nucleic acid extraction solutions, visit:

www.promega.com/HighThroughputExtraction

To learn more about the system described here, visit:

www.promega.com/HighThroughputFFPEDNA

Maxwell is a registered trademark of Promega Corporation. Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.