Gene Expression Profiling

Evaluating RNA Quality from FFPE Samples

Gene expression profiling is a powerful tool that can be used to evaluate potential drug candidates in preclinical in vivo experiments. The first step in any gene expression profiling experiment is RNA isolation and quality assessment. Formalin-fixed paraffin-embedded (FFPE) samples pose a particular challenge to gene expression profiling since these samples usually contain a high amount of fragmented RNA. The nCounter platform does not require amplification, a necessary step for many gene profiling assays, making it ideal for targeted gene profiling from FFPE samples.

1. What is gene expression profiling?

RNA can be used for gene expression profiling, which is the study of the way genes are transcribed to create functional gene products. Gene expression profiling can be performed at any level: transcriptional, post-transcriptional, translational, and post-translational protein modification. RNA expression, specifically, can be measured with DNA microarrays or real-time PCR. It can also be measured using the more popular next-generation sequencing technologies, including bulk RNAseq, single-cell RNAseq, and nCounter® platform from NanoString®. From there, differential gene expression analysis helps to understand the biological differences between samples (e.g., healthy vs. diseased). Whatever the method, the first step for gene expression analysis is RNA isolation.

2. How is RNA isolated?

RNA can be isolated from fresh frozen (FF) tissues, formalin-fixed paraffin-embedded (FFPE) tissues, or blood. The process generally consists of cell lysis, quenching of biological processes, nucleic acid partitioning, RNA retrieval, and assessing RNA quality. There are special considerations and protocols for RNA isolation depending on sample preservation method.

For example, FFPE is an ideal method for preservation because samples can be stored at room temperature and are quite stable long-term. However, the process involves high heat and harsh detergents, resulting in fragmentation and degradation of the RNA isolated from such samples. Unfortunately, methods for nucleotide isolation can further fragment the RNA. Platforms for gene expression profiling that recognize the clinical importance of FFPE samples account for this by optimizing protocols for these samples.

3. How is RNA quality assessed?

Measuring the quality of the RNA is a critical step that must be performed prior to gene expression profiling. RNA quality can be represented in several ways. The Bioanalyzer 2100 platform from Agilent employs an RNA Integrity Number (RIN) which measures the ratio of 28S and 18S rRNAs. Illumina invented the DV200 method for RNAseq which measures the percentage of RNA fragments that are >200 nucleotides. Canopy Biosciences uses this method because it is more suitable for measuring RNA quality from FFPE samples.

RNA isolated from FFPE samples may have DV200 values of ~50%, indicating a high amount of fragmented RNA and other low-quality RNA in the sample. While these values are normal for FFPE samples, they are by no means ideal for most gene expression platforms. This is because most gene profiling assays require an amplification step, which only increases the amount of low-quality RNA.

4. Why is nCounter ideal for FFPE samples?

The nCounter platform from NanoString uses target-specific digital barcodes to profile 850+ gene targets using pre-validated panels. The data is collected, gene counts are normalized, and differential gene expression analysis performed. While amplification is a necessary step for many gene profiling assays, the nCounter workflow does not rely on RNA amplification. The fact that nCounter is amplification-free means that it is not subjected to the same amplification errors caused by RNA polymerase that other gene profiling assays are. This feature also makes nCounter a more competitive option with low-quality RNA that may come from a variety of samples including FFPE tissues, FF tissues, and blood.

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