Otogenetics establishes QC standards for optimized completion of FFPE exome sequencing

Introduction

Although FFPE specimens are a great archive of samples for many disease states, nucleic acids from FFPE samples are often highly cross-linked and degraded due to fixation, making it difficult to perform assays such as sequencing. This article discusses the QC standards developed by Otogenetics to establish grades for FFPE samples, and the adjustment of workflow accordingly to achieve satisfactory exome sequencing data.

QC standards and classification of FFPE gDNA samples based on initial sample quality

Otogenetics uses standard gel electrophoresis and spectrophotometer assays to assess the quality of initial gDNA. DNA from fresh tissues appears as a large molecular weight band above 10Kb on the gel (Fig. 1). gDNA from FFPE samples have various appearances on the gel (Fig. 1), depending on how fixation was done, how gDNA extraction was performed, and how well gDNAs were stored after purification.

In addition to standard DNA QC assays, Otogenetics developed a PCR-based assay that examines the degree of degradation/cross-linking in gDNA from FFPE tissues. Based on both electrophoresis/spectrophotometer and PCR-based QC results, FFPE gDNA can be classified into three grades as shown (Fig. 1).

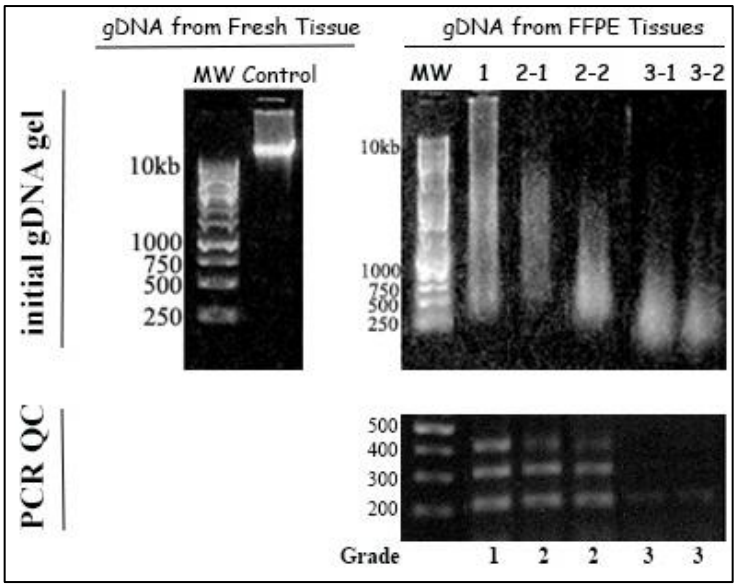

Fig 1. QC of gDNA from FFPE tissues and Grades 1, 2, and 3 based on size distribution of PCR and initial electrophoresis.

Adjustment of workflows to achieve optimized FFPE gDNA exome sequencing data

Among FFPE gDNA samples submitted for whole exome sequencing, most were classified as Grade 2. For Grade 1 FFPE gDNAs, all of the samples achieved satisfactory sequencing results. For Grade 2 FFPE gDNAs, customers opted out about 15% of further processing after failure of our internal QC steps, all the samples proceeded with adjusted workflow achieved satisfactory exome sequencing. For Grade 3 FFPE gDNAs, 75% of the samples completed sequencing with 25% opted out of processing. Adjustment of the workflow ensures the achievement of coverage; however, the duplication rate is higher in lower grade of FFPE gDNA. Sequencing data quality itself (Q30) is not affected by the quality of gDNAs from FFPE tissues.

Summary

- Otogenetics developed QC standards to classify FFPE gDNA into different grades that correlate with sequencing outcome
- Based on classification of the initial FFPE gDNA, Otogenetics adjusts workflow to achieve maximized completion of FFPE gDNA exome sequencing.