Gene Expression Profiling of Formalin Fixed Paraffin Embedded Colon Tumor and Normal Adjacent Samples by Next Generation Sequencing with the Ovation® RNA-Seq FFPE System


ABSTRACT

Gene expression analysis of clinical samples has long presented challenges to researchers based on sample availability, array type, and time. These challenges are even more significant for small and sparsely distributed formalin-fixed, paraffin-embedded tissues which are difficult to obtain for research and clinical diagnoses. The ability to analyze gene expression content directly in formalin-fixed paraffin-embedded (FFPE) tissue is highly desirable for several reasons, including the availability of large archival tissue collections for reanalysis. RNA extraction and analysis from FFPE tissue has been demonstrated using the WT-Ovation™ FFPE RNA Amplification System and fragmented and labeled using the Encore Biotin Module FFPE System (PN 7150) using 100 ng of total RNA input, following the protocol outlined in the User Guide. 400 ng of extraction. The donor population ranged from 44–70 years of age and were all male. The tumor samples were all nonpolyadenylated transcripts.

RESULTS

In order to evaluate the performance of the FFPE RNA-Seq system, generated four RNA-Seq libraries from four FFPE samples and four cDNA libraries from four FFPE samples, using the NuGEN Ovation RNA-Seq FFPE System. The libraries were sequenced using the Illumina Genome Analyzer IIx platform. Sequencing results obtained using the Illumina Genome Analyzer IIx platform. Table 2. Table 2 shows the number of reads generated per library and the percentage of reads aligning to the human genome assembly hg18.

CONCLUSIONS

The NuGEN Ovation RNA-Seq FFPE System enables researchers to perform RNA-Seq expression analyses directly in archival FFPE tissue, and the results are comparable to those obtained with cDNA libraries from FF tissue. Therefore, the NuGEN Ovation RNA-Seq FFPE System provides an alternative approach to FFPE tissue analysis, without the need for additional enrichment steps to select for poly(A)+ RNA or to reduce the content of ribosomal genes. A variety of methods have been used to improve library preparation, fractionation procedures which can introduce bias upon transcript profiling. The NuGEN Ovation RNA-Seq FFPE System enables researchers to perform RNA-Seq expression analyses directly in archival FFPE tissue, and the results are comparable to those obtained with cDNA libraries from FF tissue. Therefore, the NuGEN Ovation RNA-Seq FFPE System provides an alternative approach to FFPE tissue analysis, without the need for additional enrichment steps to select for poly(A)+ RNA or to reduce the content of ribosomal genes.