Targeted T-cell receptor beta immune repertoire sequencing in several FFPE tissue types — applications in profiling the tumor microenvironment.

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ABSTRACT

T-cell receptor beta (TCRβ) immune repertoire analysis by next-generation sequencing is a valuable tool for studying the tumor microenvironment and potential immune responses to cancer immunotherapy. Here we describe a TCRβ sequencing assay that leverages the low sample input requirements of MultiplexSeq library preparation technology to extend the capability of targeted immune repertoire sequencing to include FFPE samples which can often be degraded and in short supply.

To evaluate chunk assay, we sequenced libraries including known numbers of 29 well-studied T-cell lymphoma rearrangements, as well as samples comprised of sorted T cells. T-cell repertoires were successfully evaluated from as low as 5 x 10⁶ to as large as 1 x 10⁷ copies of input samples of varying T-cell repertoire diversity, such as sorted T cells, peripheral blood leukocytes, fresh-frozen tissue, and FFPE tissue from a variety of normal and cancerous tissues including lung, colon, brain, spleen, lymph node, and thymus. In addition, we demonstrated use of a qPCR assay for quantification of sample T-cell content to guide sample input and repertoire sequence experiments.

These data represent a T-cell immune repertoire sequencing solution for application in a wide range of sample types, in particular, challenging FFPE preserved samples. We find that the assay is capable of profiling repertoire metrics from FFPE samples over a large range of input amounts from several normal and tumor tissue types.

INTRODUCTION

The Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, Catalog No. Q32823) is used to quantify and evaluate RNA integrity. Due to FFPE quality and variation in T-cell content in different tissue types, standardized inputs lead to inconsistent assay performance. For RNA samples with biologically variable or low T-cell content, or for samples that may be degraded, we developed a functional CD3 RNA qualification assay to determine the minimum acceptable input amount. For DNA samples, we use the TaqMan® RNase P assay to check for sample degradation.

Here we present the Oncomine® TCR-Beta-RA assay. A high-throughput next-generation sequencing (NGS) assay that interrogates the complementarity determining region 3 (CDR3) of the gene that codes for the T-cell receptor beta chain, and is optimized for convenient but difficult to sequence formalin-embedded paraffin-embedded (FFPE) tissue samples. These assays identify unique T-cell clones through interrogation of the diverse complementarity determining region 3 (CDR3) of the T-cell receptor (TCR) gene locus in germline DNA or RNA. The nucleotide sequence of the CDR3 region is unique to each T-cell clone and codes for the part of the TCR beta chain that is involved in antigen recognition.

MATERIALS AND METHODS

The Ion Oncomine® TCR-Beta-RA Assay leverages Ion AmpliSeq™ technology to profile the TCR repertoire through the unique highly diverse CDR3 of the TCR beta gene. By utilizing multiplex primers to target the framework 3 (FR3) region and the joining (J) region that flank the CDR3, this method produces an 80bp amplicon thus enabling the use of both germline DNA and RNA templates and high-throughput sequencing on Ion S5™, S500™ and S550™ chips. The Ion Oncomine® TCR-Beta-RA Assay is compatible with the new Ion Torrent® Dual-Index Kit 1-6, which significantly increases the assay specificity to enable deep TCR sequencing with multiplexed samples.

Figure 1. Assay Design

Figure 2. Assay Linearity

Figure 3. CD247 TaqMan® Gene Expression Assay

Figure 4. Components used in CD247 TaqMan® Gene Expression Assay

Figure 5. Total RNA was extracted from various FFPE tissue samples with high (toral), medium (thymus), and low (lung and placental) levels of T-cell content. Two potential methods were compared in this experiment: Recovery™ Total RNase Acid Kit for FFPE and MagMax™ FFPE DNA RNA Ultra Kit. (A) Extraction kits were compared by measuring amplifiable T-cell content using CD247 TaqMan® gene expression assay. (B) We observe similar RNA yields [not shown] and amplifiable T-cell content between extraction kits. (B-C) Productive read percentage and clone richness are highly correlated in replicate experiments using FFPE RNA from spleen, thymus and thymus tissue. The productive read percentage and number of clones detected also follow the same trend predicted from the CD247 TaqMan® gene expression assay.

RESULTS

While a valuable source for retrospective studies of archival tissues, the modifications that occur during the fixation-process of formalin-fixed paraffin-embedded (FFPE) tissues poses challenges for next-generation sequencing applications. NGS based TCRβ profiling in FFPE tissue has the additional difficulty of the biological variability of T-cell recruitment and tissue infiltration. RNA quality and relative T-cell content in a tissue sample significantly affects assay input requirements. To address this need, we developed a qPCR assay that guides the template input for the Oncomine TCR-Beta-RA assay by taking into account sample quality in the context of T-cell content.

Figure 6. To evaluate the linearity of the Oncomine® TCR-Beta-RA Assay, we sequenced control samples with known TCRβ rearrangement sequences spiked into peripheral blood leukocyte DNA.

Figure 7. (A) 100ng of genomic DNA extracted from primary CRC FFPE tissue, liver metastases FFPE tissue, and lymph node adjacent to the primary tumor was sequenced. We observed 26 clones in the lymph node tissue, 663 clones in the primary tumor, and 211 clones in the liver adjacent to the primary tumor. We observe greater evenness in the lymph node tissue than either the primary tumor or the metastasis. (B) Pot showing the degree of clonal overlap between the replicates. We observe 34 clones that are detected in both the primary tumor and liver metastasis (at elevated frequency). These shared clones may be at an increased probability to have arisen from a tumor neanotumor, and further study, given their detection within both primary and metastatic

CONCLUSIONS

To summarize, the Oncomine Immune Repertoire-Seq assay profiles the T-cell repertoire by enumerating the CDR3 region of the TCR beta gene in both RNA and DNA samples. An assay designed for difficult FFPE tissue samples of variable T-cell content, the Oncomine TCR-Beta-RA Assay is a sensitive assay for the unbiased profiling of immune repertoire sequences. We have also developed the TaqMan® CD247 qPCR assay to help guide assay input for particular sample types and research objectives.

REFERENCES


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