NGS Characterization of Multiple Immune Receptors from a Single Multiplex PCR Reaction

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INTRODUCTION

B cell repertoire analysis by next-generation sequencing (NGS) has shown utility in the field of hematological oncology research. Some advantages provided by NGS-based techniques include a lower limit-of-detection and simpler paths to standardization compared to flow-based methods, and the elimination of specifically designed primers often required for qPCR-based methods. Owing to primer-primer interactions and incompatibility of reaction conditions, most current multiplex PCR assays require separate PCR reactions to survey each immunoglobulin chain (IGH, IGK, IGL), leading to a longer time-to-answer, and increased sample usage, for samples in which no marker is initially detected. We have developed an assay for clonality assessment that eliminates specifically designed primers often required for qPCR. This highly multiplexed assay provides efficient detection of IGH, IGK, IGK, and IGL chain rearrangements in a single reaction.

MATERIALS AND METHODS

The Pan-Clonality assay includes a single primer panel targeting the framework 3 (FR3) portion of the variable gene and the joining gene region of heavy- and light-chain loci (IGH, IGK, IGL). For all alleles found within the IMGT database, enabling readout of the complementary-determining region 3 (CDR3) sequence of each immunoglobulin chain. To maximize sensitivity, we included primers to amplify IGK loci rearrangements involving kappa deletion and C intron elements. To evaluate performance, we conducted clonality assessment and limit-of-detection testing.

RESULTS

Testing used gDNA from research samples representing common B cell malignancies, including B cell lines (ATCC, DMSZ) and clinical research samples (Cureline). We included samples derived from peripheral blood, lymph node, and FFPE-preserved tissues. Sequencing was performed on the Ion GeneStudio™ S5 sequencer and analysis using Ion Reporter™ software 5.16.

Clonality Assessment (cell lines): Clonality assessment provides a means to identify the dominating clone and determine the CDR3 sequence of the clone of interest. 27 B cell lines derived from a variety of B cell malignancies (including B-ALL, CLL, Multiple Myeloma, Non-Hodgkin’s Lymphoma) were profiled in a background (1:100 dilution) of PBL gDNA using the Pan-Clonality (IGH/K/L) assay and the associated reflex assays (FR3(d)-J and FR2-J). Table 1 presents the results of the cell line clonality assessment, with the green boxes in the table reporting positive detection of clonality in the sample and the number of each rearrangement receptor detected. Positive detection of at least one rearrangement (IGH, IGK, IGL K/D/C/in) was found in 25/27 cell lines tested (93%) using the Pan-Clonality (IGH/K/L) assay (red box in Table 1).

Pan-Clonality (IGH/K/L) assay using the BDCM cell line.

CONCLUSIONS

Ion AmpliSeq library technology enables an assay for profiling B cell receptor heavy and light chains in a single library reaction. Combining receptors in a single reaction allows for a higher success rate in clonality detection while maintaining the ability to detect rare clones (down to 1:10). We expect this assay to simplify the workflow for clonality assessment and rare clone detection in B cell malignancy research.

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