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 immune receptor analysis based on Ion AmpliSeq™ technology to circumvent these issues, allowing the effective use of sample production when using primers in a single reaction. This highly multiplexed, BCR Pan-Clonality NGS assay provides for efficient detection of IGH, IGK, and IGL chain rearrangements in a single reaction.

RESULTS

Table 1. Detection of clonality in cell line samples diluted 1:100 in PBL gDNA using the BCR Pan-Clonality Assay — columns outlined in red, as well as the FR3(d)-J and FR2-J assays. Green boxes indicate positive detection with the variable gene identity of the rearrangements detected. The BCR Pan-Clonality Assay shows an overall 93% positive detection rate.

Table 2. Detection of clonality in clinical research samples (MM – Multiple Myeloma, CLL – Chronic Lymphocytic Leukemia, B-ALL – B cell Acute Lymphoblastic Leukemia, DLBCL – Diffuse Large B cell Lymphoma) using the Pan-Clonality (IGH/KL) assay. Green boxes indicate positive detection with the rearrangements detected.

Figure 2. Linearity/Limit-of-detection evaluation of the single reaction Pan-Clonality (IGH/KL) assay using the BDCM cell line. Cell line gDNA was serially diluted in PBL gDNA from 1:10 to 1:10^6. Each of four rearrangements for BDCM detected by the Pan-Clonality assay shown in Table 1 show linear response to dilution and two of the four rearrangements are detected at 1:10^6.

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 1:10^6). We expect this assay to simplify the workflow for clonality assessment and rare clone detection in B cell malignancy research.

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