Efficient and sensitive high-throughput human B-cell receptor repertoire profiling using SMART technology

Genomics

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OBJECTIVE: B-cell receptor (BCR) repertoire profiling is increasingly used in health and pathogenic contexts with the goal of biomarker discovery. However, current sequencing technologies are limited in their ability to generate data accurately and reproducibly for all BCR isotypes. To overcome these limitations, we have developed a new kit to accurately profile all heavy (A, D, E, G, M) and light-chain (K, L) isotypes—an end-to-end solution, from library preparation to streamlined data analysis. Here we present data on an updated approach for efficient and high-throughput BCR repertoire profiling of human samples.

METHODS: Libraries were prepared from human peripheral blood cells (10 ng–1 µg total RNA) or from B-cells (1 ng–100 ng total RNA) using our new human BCR repertoire profiling kit (~2.5 hours hands-on time). Prepared libraries were then analyzed on the Illumina® Miseq™ benchtop sequencer using 300-bp paired-end reads.

RESULTS: For each library, >90% of sequencing reads were on-target while the most highly represented clonotype was found to remain consistent among technical duplicates across a range of input amounts. In comparison to the previous version of our BCR-sequencing kit, the new approach enabled a ~4x increase in total clonotype count observed across various RNA inputs. Furthermore, a sensitivity assay demonstrated that the cell line RNA corresponding to a single clonotype could be detected above background levels when spiked into input RNA at a relative concentration of 0.001%.

CONCLUSION: Our new human BCR repertoire profiling kit was found to accurately and reproducibly profile B-cell clones and provide information on the diversity of BCR repertoire in clinical samples.