Preface

Next-generation (high-throughput) DNA sequencing of immunoglobulin variable region and T-cell receptor gene repertoires is providing critical information for understanding adaptive immune responses and for diagnostic and therapeutic applications. However, existing immune repertoire sequencing technologies yield data on only one of the two chains of immune receptors and thus cannot provide information on the identity of immune receptor pairs encoded by individual B or T lymphocytes. This work directly addressed these limitations by developing two new technologies for sequencing the complementary DNA (cDNA) of multiple mRNA transcripts from isolated single cells with very high throughput. In these methods, cells are sequestered into individual compartments and lysed in situ to capture single-cell mRNA onto magnetic beads, and the magnetic beads are then used as template for RT-PCR reactions inside emulsion droplets that physically link cDNA of multiple transcripts for subsequent analysis by high-throughput DNA sequencing. We demonstrated experimental throughput of over 2×10^6 cells in a single day, with antibody heavy and light chain pairing accuracy greater than 97% as measured with in vitro expanded human B cells. These new single-cell sequencing technologies were then applied for rapid discovery of new human antibodies and for analysis of the human immune response to vaccination. Finally, we applied the techniques developed here to gain new insights regarding the development of the antibody repertoire using a high-throughput and high-resolution examination of naïve and memory B-cell compartments in healthy human donors.

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Decoding the Antibody Repertoire
High Throughput Sequencing of Multiple Transcripts from Single B Cells

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