**Abstract**

T-cells play a central part in the immune response in humans and related species. T-cell receptors (TCRs), heterodimers located on the T-cell surface, specifically bind foreign antigens displayed on the MHC complex of antigen-presenting cells. The wide spectrum of potential antigens is addressed by the diversity of TCRs created by V(D)J recombination. Profiling this repertoire of TCRs could be useful from, but not limited to, diagnosis, monitoring response to treatments, and examining T-cell development and diversification. Due to the combinatorial generation of the TCRs, analyzing the full-length genes is more informative than probing partial regions when trying to distinguish unique variants. Accurate sequences are also required to confidently identify specific clonotypes that may be critical in patient diagnosis or finding optimal binding interactions for therapy. Sequencing from genomic DNA is useful for gaining information about the representation of different T-cells in a population. However, starting from mRNA provides additional benefits by enriching for the TCR sequence as well as giving a more direct view of the functional expression of the genes. Current methods may fall short if transcript length extends beyond the instrument limits and if read quality is not high.

By combining the Takara Bio SMARTer Human TCR a/b Profiling Kit and the PacBio Sequel System, full-length TCR genes have been sequenced. Here we present results from Jurkat- and Peripheral Blood Mononuclear Cell (PBMC)-derived RNA showing high reference alignment and sequence accuracy along the entire lengths of the TCR genes. Starting with total RNA from Jurkat cells and peripheral blood leukocytes, 400-900 bp amplics containing the entire variable region and a portion of the constant region of TCR-α and/or TCR-β were generated, taken directly into PacBio SMARTbell library prep and run on the Sequel System, producing >200,000 high-quality, full-length TCR gene sequences per SMRT Cell 1M.

**Expanding Applications in Immunology**

Beyond TCR profiling, we are investigating how PacBio sequencing may provide advantages in other applications in the immunology space. Long-read sequencing for cell receptor related constructs as well as synthetic short chain variable fragments are presented below.

**Results and Conclusions**

- Combining the Takara Bio TCR a/b Profiling Kit and PacBio sequencing, full-length (650-900 bp) amplons were sequenced from Jurkat and PBMC total RNA.
- With high-quality CCS reads, nearly all reads (97.5%) align to TCR loci from Jurkat, and a wide range (>18,000) of PBMC clonotypes were detected on a single PacBio RS II SMRT Cell.
- The library preparation yield of SMARTbell DNA exceeded the amount required for a single Sequel SMRT Cell 1M, starting with 200-250 ng input DNA. Investigation into lower DNA input levels for library preparation is currently underway.
- PacBio sequencing is well-suited for immunological projects requiring high accuracy sequences >600 bp to several kb.

**References**