The Journal of Immunology

ABSTRACT | MAY 01 2023

HLA-PopSeq: High throughput, multiplexed six-locus Human Leukocyte Antigen typing for population-scale T cell immune profiling using rapid long-read nanopore sequencing FREE

Oliver Kask; ... et. al

J Immunol (2023) 210 (1_Supplement): 250.10.
https://doi.org/10.4049/jimmunol.210.Supp.250.10

Related Content

Identification of splice isoforms in T helper cells using Nanopore-based cDNA sequencing

J Immunol (May,2020)

Single-Molecule DNA Methylation Reveals Unique Epigenetic Identity Profiles of T Helper Cells

J Immunol (January,2024)

The role of MATRIN3 (MATR3) in innate immune response

J Immunol (May,2023)

HLA-PopSeq: High throughput, multiplexed six-locus Human Leukocyte Antigen typing for population-scale T cell immune profiling using rapid long-read nanopore sequencing

Oliver Kask[1], Mark Knappenberger[1], Heewook Lee[1], Alicia Van Stone[1], and Karen Anderson[1]
[1]Arizona State University

A major technical hurdle for T cell immune profiling is the time and cost to accurately genotype the Human Leukocyte Antigen (HLA) loci from peripheral blood. Here, we developed a rapid, highly multiplexed approach for HLA typing using RNA from <100,000 peripheral blood mononuclear cells with the Oxford Nanopore Technology (ONT) Minion sequencer. This method uses selective reverse transcription of mRNA of six HLA loci (A,B,C, DRB1, DQB1, DPB1), followed by PCR amplification. The individual amplified HLA cDNA was multiplexed in a single sequencing pool using primers with unique molecular identifiers, designed to permit sequencing errors for enhanced data capture. Pooled HLA amplicons were sequenced using the ONT Minion MK1B and R10.4 flowcells, with sequence Q scores> 20. Total RNA was extracted from PBMC samples from 12 individuals, reverse transcribed and amplified using the designed HLA loci specific primers. The pooled, amplified cDNA was then sequenced for 16 hours on the ONT Minion sequencer. The resulting sequencing data was analyzed and an average depth of coverage of 6000x was observed per sample. An average per loci depth of coverage of 1000x was observed. This method is designed to permit rapid (<24h), low-cost, portable HLA sequencing for T cell immune monitoring and epitope identification for immunologic studies.