

A match.com for T cell receptor α and β chains

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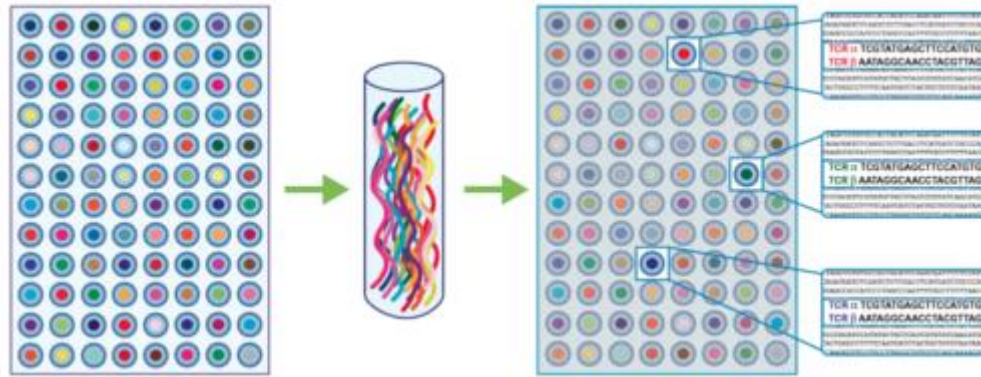


Diagram of pairSEQ experiment. A pre-determined number of T cells are distributed between wells of a 96-well plate. The mRNA in each well is then extracted and cDNA is synthesized and amplified by primers that are specific to the T-cell receptor genes. Well-specific barcodes allow for pooled sequencing and a computational framework maps each TCRA or TCRB sequence to its original well. Pairs of TCRA and TCRB sequences that share occupancy patterns are inferred to have come from the same T-cell clone.

Image from the publication.

T cells, a major subtype of white blood cells, are key to harnessing the potential of the immune system to fight cancer and other diseases. Current cancer immunotherapies seek to expand or reengineer T cells with the best cancer-fighting prowess. However, identifying the right T cell clones (a T-cell and all of its descendants) can be time and labor-intensive. T cells recognize their target cancer cell or pathogen through a T cell receptor (TCR), composed of two polypeptides, an α chain and a β chain. To identify T cell lineages it is important to know the identity of both the α and β sequences and which α and β chains pair together. Previous work from Dr. Harlan Robins (Public Health Sciences and Human Biology Divisions), and his collaborators at Adaptive Biotechnologies, developed a high throughput assay called immunoSEQ to sequence individual α and β chains. However, without resorting to single-cell methods, which are not easily scalable, it had not been previously possible to know which α and β chains occur together. Dr. Robins' team has now developed and validated a clever high-throughput way of identifying α and β chain pairs, which enables reconstitution of specific TCRs for functional assays and therapeutic applications. This study was recently published in *Science Translational Medicine*.

"We have spent many years sequencing single chains of the T cell receptor, which has allowed us to track individual T cells and develop diagnostics. With this new technology, we can sequence the full

TCR (both chains paired together) at high throughput. This allows us to recreate the TCR for functional studies as well therapeutic use," said Dr. Robins. The α and β chains are encoded by two separate genes: *TCRA* (α) and *TCRB* (β). Because both *TCRA* and *TCRB* experience genetic rearrangements during T-cell development, their mRNA sequences are unique for each T-cell clone. The investigators thus reasoned that the *TCRA* and *TCRB* from a T-cell clone could be identified by first dividing a T-cell sample into different subsets, and then by tracking which *TCRA* and *TCRB* sequences co-occur in the same subsets. Each subset harbored a barcode, which enable the amplified receptor to be pooled for high-throughput sequencing. To validate their new method, called pairSEQ, the authors sequenced the *TCRA* and *TCRB* from 1 million peripheral blood mononuclear cells (PBMCs) from two healthy adults. The two samples were mixed in a single plate and the pairSEQ computational framework correctly assigned more than 3200 *TCRA/TCRB* pairs with less than 30 errors. Next, the authors asked whether pairSEQ could identify rare T-cell clones by increasing the number of T-cells per well (160,000), and they identified more than 362,528 TCR pairs at a false discovery rate of 1%, including 26,667 with frequencies of 1 in a million or lower. Finally, the researchers asked whether pairSEQ could be used to interrogate samples other than blood, such as tumor-infiltrating lymphocytes (TILs) present in solid tumors. To this end, they obtained samples from nine solid tumors and matched blood samples. As before, the authors first sequenced the *TCRA* and *TCRB* repertoires from each sample to determine how many cells to use for the pairSEQ assay. They then ran the pairSEQ assay on dissociated tumor cells as well as the PBMCs. The pairSEQ algorithm assigned 6172 pairs from the tumor sample and 14,123 from PBMCs, while maintaining an FDR of 1%. In summary, pairSEQ can accurately identify TCR pairs in multiple sample types and across a range of clonal frequencies using standard laboratory equipment, and therefore will have broad applicability in biomedical research.

[Howie B, Sherwood AM, Berkebile AD, Berka J, Emerson RO, Williamson DW, Kirsch I, Vignali M, Rieder MJ, Carlson CS, Robins HS](#). 2015. High-throughput pairing of T cell receptor alpha and beta sequences. *Sci Transl Med*. 7(301):301ra131. doi: 10.1126/scitranslmed.aac5624.