Cutting Edge Tools for Phenotyping and Characterising T-Cell Subsets

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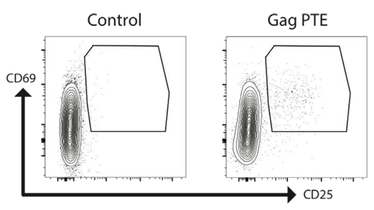
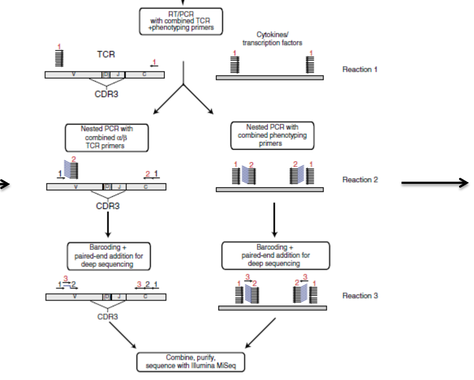
Background

Next generation Sequencing (NGS) has enabled high throughput analysis of T cell receptor (TCR) repertoires, which are useful for monitoring antigen-specific T cell responses. Integrating TCR sequencing with expression levels of genes that characterise T cell phenotype at the single cell level allows comprehensive analysis of T cell function and specificity. Single cell TCR sequencing on the Illumina platform enables the identification of paired TCR α/β chains and T cell phenotype from sorted antigen-specific T cell populations. Here we highlight novel techniques available for T cell phenotyping and TCR repertoire analysis.

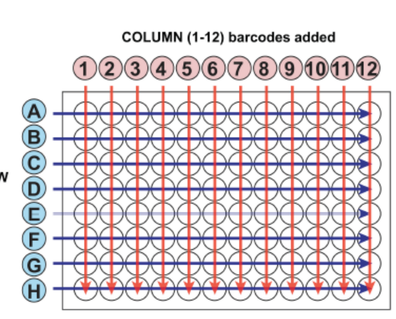
Workflow

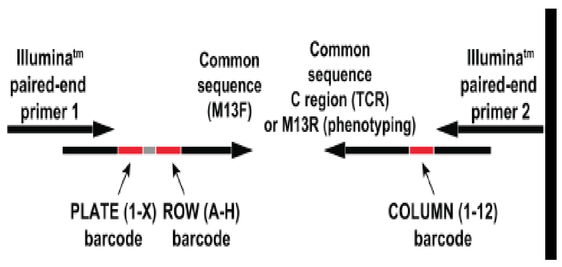


CD8 T cells that increased expression of both CD69 and CD25 on stimulation with the Gag peptide were sorted for T cell receptor repertoire analysis.



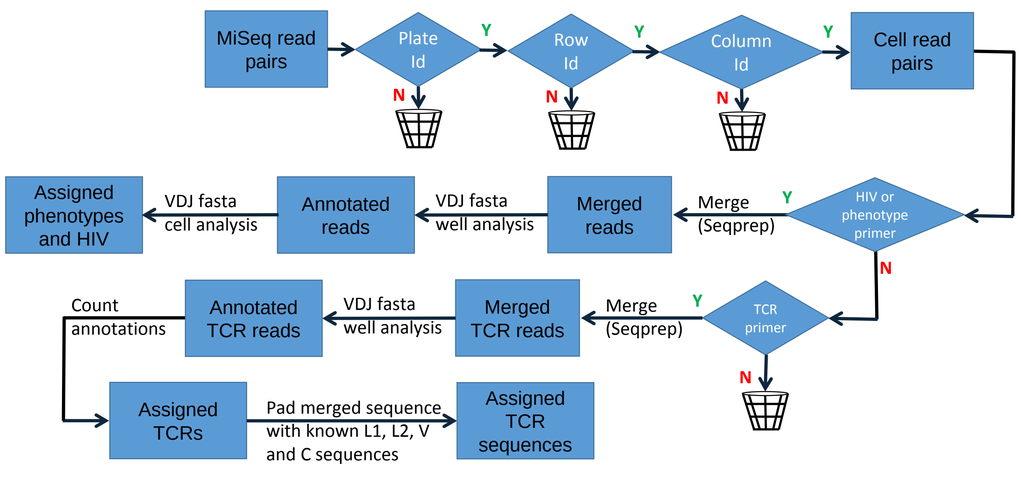
RNA from each well is reverse transcribed and amplified to obtain TCR α and β sequence and phenotype information (Han et al 2014).





Tagged primers were used during amplification to add oligos representing acting as identifiers for the plate number, and the row and column positions on the plate for the sample. Additionally library tags for the Illumina MiSeq sequencer were appended to the end of the amplified molecules. The amplified and tagged products were then pooled and sequenced on an Illumina MiSeq sequencer using a 300bp paired end sequencing kit.

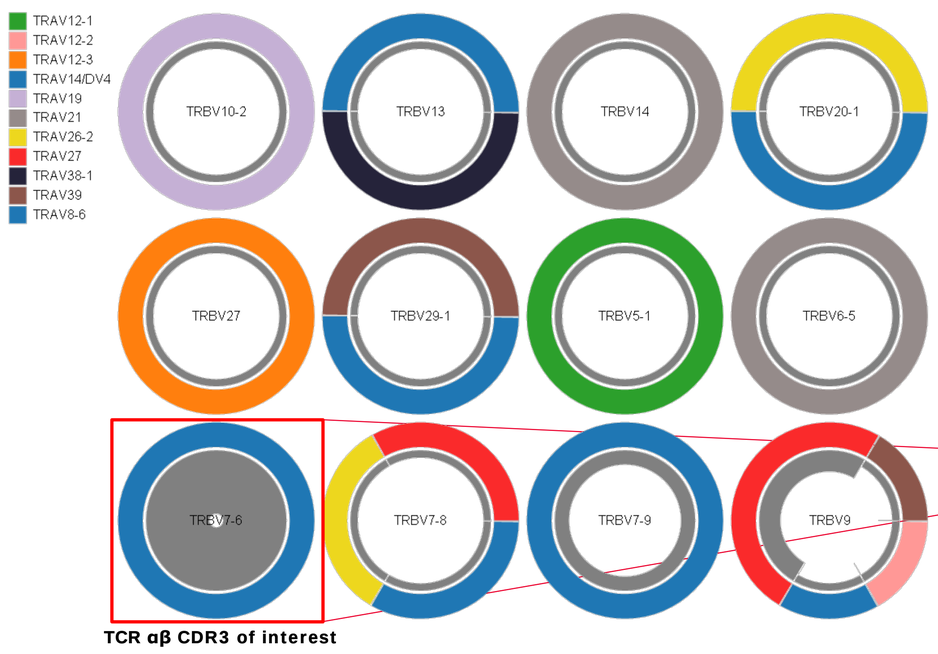
Analysis



Read pairs that did not have valid plate, row or column tags were discarded. The remaining pairs were then sorted based on the amplification primer sequences. Next the read pairs were merged using Seqprep (https://github.com/jstjohn/SeqPrep).

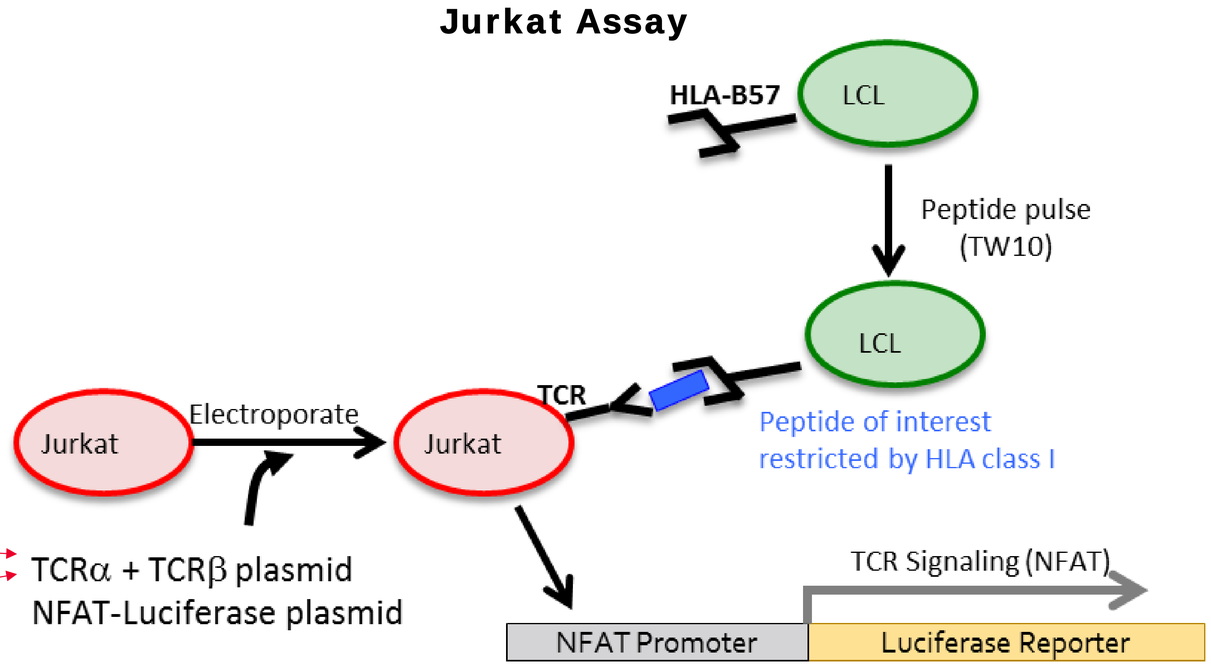
Merged read pairs were analysed with the VDJfasta analysis suite (http://www.ncbi.nlm.nih.gov/pubmed/19875695).

Full TCR sequences are then produced by combining the sequence data with reference data from IMGT V-Quest (http://www.ncbi.nlm.nih.gov/pubmed/18503082).

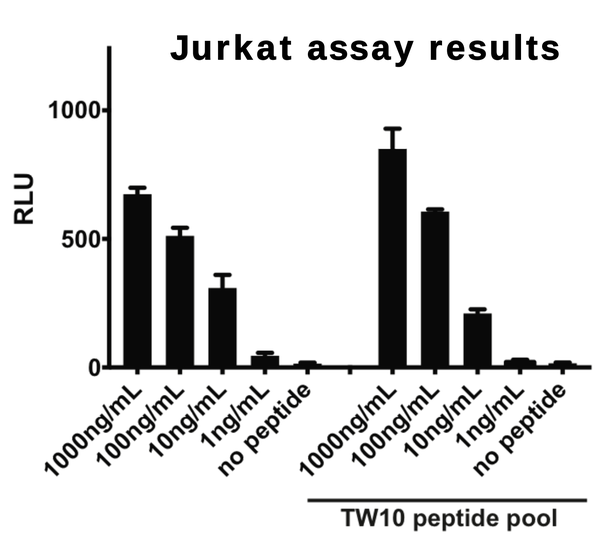


The analysed TCR cells from a subject can be visualised using the VGAS program. A suite of visualisations are provided to highlight aspects of the data, such as alpha beta pairings.

Jurkat Assay



Identified TCR α/β chains are chemically synthesised and introduced into a plasmid. Plasmids containing the TCR sequences were cloned into a Jurkat cell line with pNFAT luciferase. Jurkat cells were then added to LCLs that had been pulsed with the relevant peptide. Luciferase output from the interaction . This system can then be used to compare responses to peptides from alternative subtypes and genotypes to determine specificity.



Jurkat cells were electroporated with TCRa/b subunits specific for KF11 restricted by HLA B*57 along with plasmids for CD8 and an NFAT driven reporter. LCL from the subject were incubated with decreasing amounts of KF11 peptide in the absence or presence of a pool of 6 peptide at a constant concentration to demonstrate sensitivity and specificity.

Conclusions

Single cell TCR sequencing on the Illumina platform enables the identification of paired TCR α/β chains and T cell phenotype from sorted antigen-specific T cell populations. The transfected Jurkat cells expressing the appropriate TCR combination were then combined with autologous B-LCL and pulsed with the cognate peptide epitope. Detection of luciferase activity was used as an outcome to identify peptide-TCR combinations. This system can then be used to compare responses to peptides from alternative subtypes and genotypes to determine specificity.

References

*Han, A., et al., Linking T-cell receptor sequence to functional phenotype at the single-cell level. *Nature Biotechnology*, 2014. 32(7): p. 684-692

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