Cutting Edge Tools for Phenotyping and Characterising T-Cell Subsets

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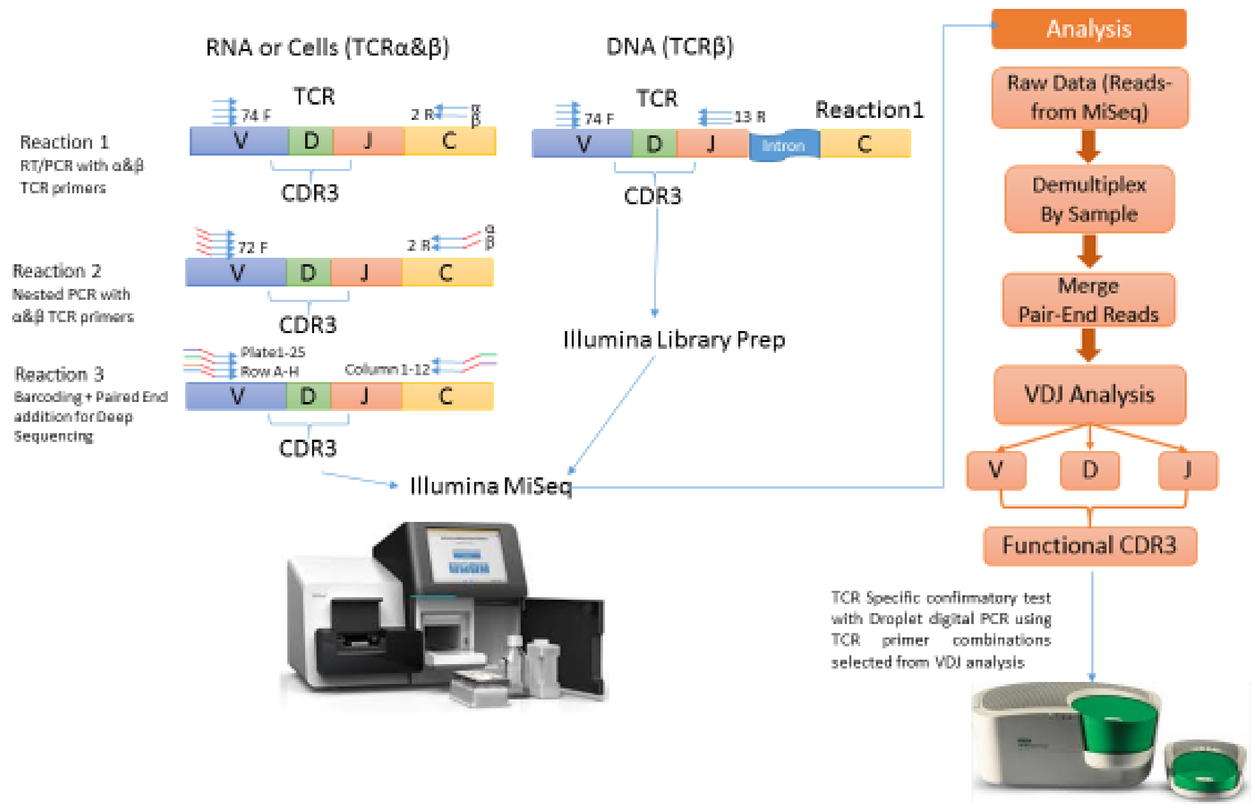
Introduction:

- T-cell receptors (TCR) recognise specific antigens presented by the major histocompatibility (MHC) molecules
- TCR are highly variable, consisting of an α (VJ) recombination and β (VDJ) recombination chain, the intersection region corresponds to CDR3.
- Clonal TCRs are indicative of malignant, infectious and autoimmune diseases.
- TCR repertoire analysis could lead to the identification of disease-associated TCRs in cancer, autoimmunity, infectious diseases and drug hyper-sensitivity.
- TCR profiling can be performed from sorted single cell, DNA or RNA depending on the questions asked.
- Next generation sequencing (NGS) allows high throughput of 1000+ samples simultaneously.

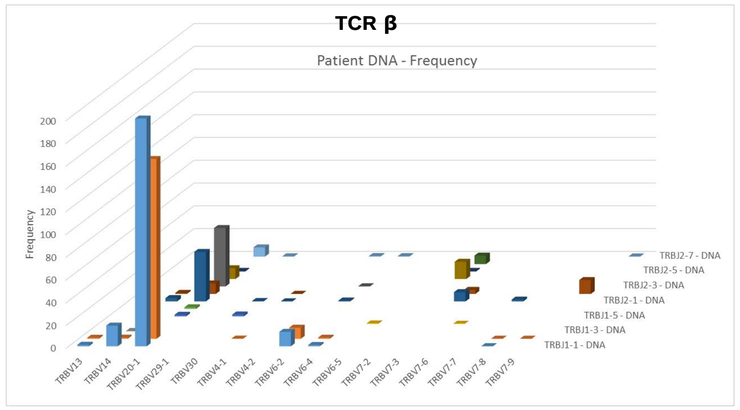
Method:

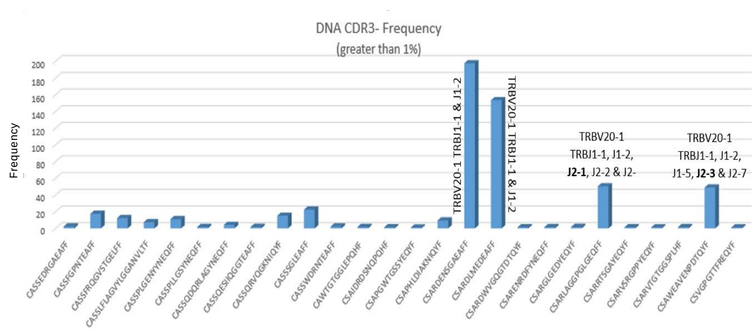
- Proof of concept experiment was performed adapted from Han *et al** using RNA, DNA and cell assay on Illumina Miseq platform.



DNA TCR Results

Multiple TCRβ and functional CDR3 were successfully obtained from DNA.

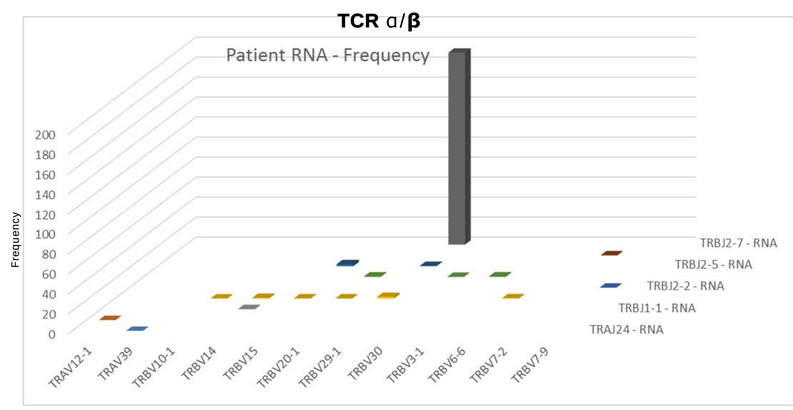


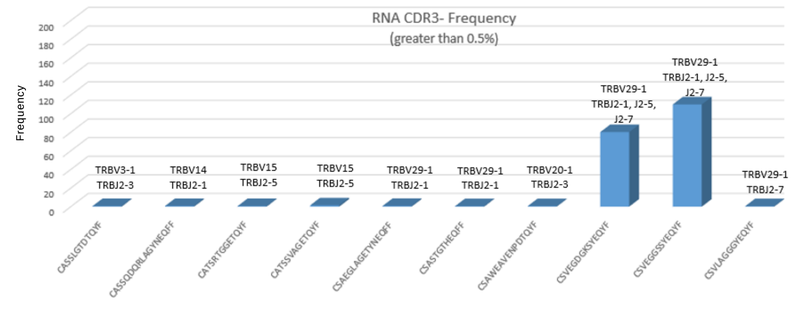


- Advantages: One step PCR setup, DNA easy to obtain.
- Disadvantages: Frequency skewed due to amplification bias.

RNA TCR Results

TCRα, TCRβ and functional CDR3 were successfully obtained from RNA.

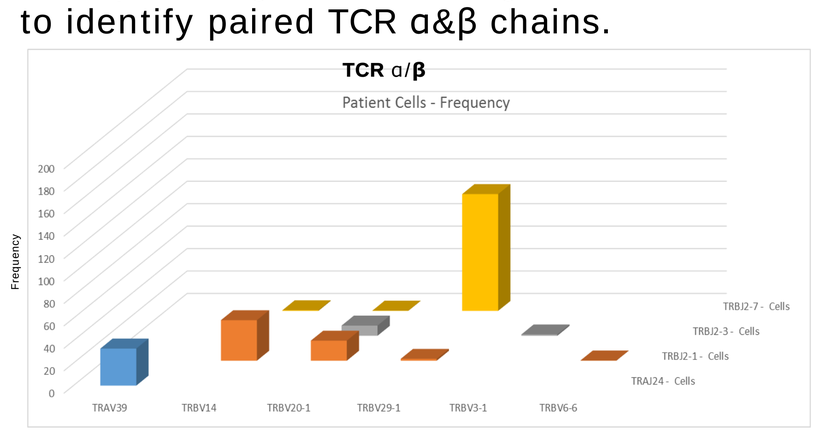


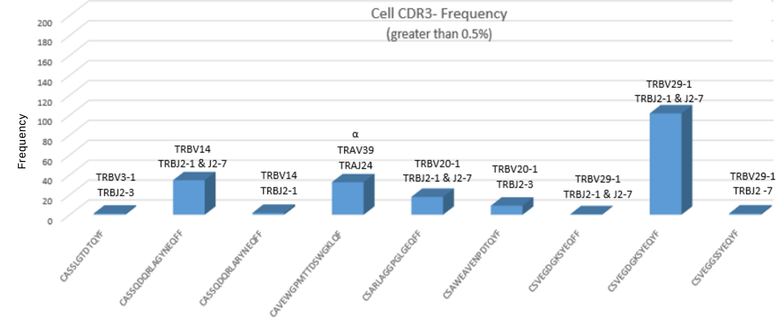


- Advantages: Expressed TCR
- Disadvantages: Time point dependent, frequency skewed due to amplification bias

Cell TCR Results

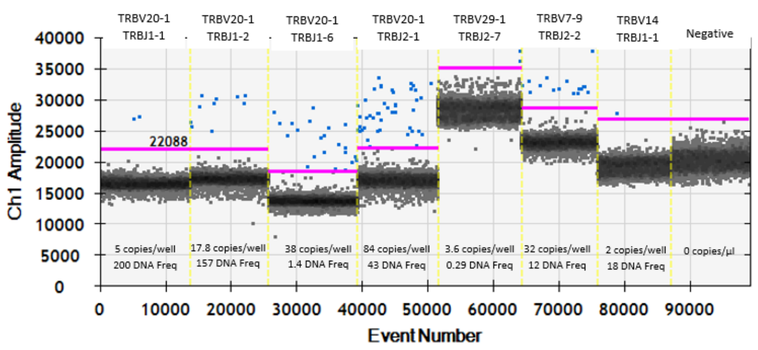
PBMCs were diluted such that 5-10 cells per reaction accounting for multiple TRβ in this example. Single Cell Sorting is recommended to identify paired TCR α&β chains.





- Advantages: Possible to identify paired TCR α&β chains. If assay performed in conjunction with phenotypic primers, enables to correlate phenotypic markers.
- Disadvantages: Expensive, need to be able to sort cells

Digital PCR Results



Digital PCR on the DNA sample did not confirm the quantitation shown by sequencing, suggesting there is likely PCR amplification bias.

Conclusions

- TCR sequencing using NGS is a powerful technique to identify TCR repertoire.
- Amplification bias was observed with DNA and RNA samples.
- Therefore it is useful to confirm the results using the single cell TCR assay of selected cell populations.
- We have also evaluated the commercially available kit from "Adaptive Biotechnologies" for DNA and RNA sequencing at our Vanderbilt lab, this kit helps to minimise the amplification bias (data not presented here).
- As T cells are implicated in many diseases such as cancers, infections and autoimmune diseases, Possessing correlated information on the TCR and the phenotype of the T cell will likely be important in the diagnosis, treatment and prevention of a wide range of conditions.

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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http://iiid.murdoch.edu.au/