T Cell Receptor (TCR) Repertoire Sequencing

Background

A functional adaptive immune system in humans consists of a diverse population of T & B cells, which mount an immune response upon exposure to a foreign antigen (derived from pathogens, or mutated proteins expressed by cancer cells). These antigens are presented on the surface of antigen-presenting cells (APCs) as a peptide bound to HLA proteins and engage T cells via T cell receptors (TCRs). When a naive T cell engages productively with an antigen, it proliferates and undergoes a functional transformation into a cytotoxic T cell (CTL), which then has the ability to eliminate target cells bearing the foreign antigen. TCRs are typically composed of two subunit chains (α- and β-). Each T cell and its clones have a unique combination of α/ β heterodimeric TCRs contributing to the diversity and high selectivity in binding to specific antigens presented on the surface of APCs. To detect a wide variety of antigens from natural and un-natural sources, there are 10^9 – 10^{10} unique TCRs in humans. The TCR diversity also referred to as T cell repertoire is generated through extensive recombination between different V, D and J gene segments followed by junctional diversity that arises due to site-specific hyper-mutations during T cell development. The region of TCR-β chain that spans the V-D and D-J junctions, is referred to as the complementarity-determining region 3 (CDR3), which is unique to each TCR-β chain and dictates antigen specificity. The diversity of the TCR repertoire is analyzed by enumerating the unique number of CDR3 sequences present in a T cell pool. When a specific T cell with its unique TCR expands by binding to an antigen, there is selecive expansion of a specific CDR3 region in the reperotoire, resulting in one specific T cell clone dominating the repertoire. The expanded T cell clone is referred to as the clonotype. In addition to the diversity from the TCR-β sequences, unique expression of TCR-α and TCR-β pairs on individual T cell also drives specificity in antigen binding and can dictate functional diversity of the TCR repertoire[3].

TCR profiling holds great potential not only for understanding the mechanisms of development of the normal immune response, but also in providing insights into disease mechanisms and development of new therapeutics and treatment modalities in infectious diseases, autoimmunity and in immuno-oncology[1]. However identification of all potential clonotypes in a diverse repertoire of TCRs requires sensitive methods of detection[2,3]. Next-generation sequencing (NGS) technologies have recently enabled accurate detection of TCRs, and in combination with other assays allow for the assessment of the TCR repertoire in patients- which in turn is a proxy for patient prognosis and response. Shown in the figure below is a workflow using TCR Profiling for Biomarker discovery, Immunotherapy and treatment monitoring. In this whitepaper, we present three NGS based methods of profiling TCRs, which are offered as services by MedGenome: immunoSEQ™ assay (Adaptive Biotechnologies), SMARTer® TCR Profiling Kit (Takara Bio USA Inc), and Single-cell V(D)J Immune Profiling solution (10X™ Genomics Inc.) respectively. In addition to the NGS services, MedGenome offers solutions for tumor microenvironment analysis,: OncoPeptTUME™ and prioritization of vaccine candidates: OncoPeptVAC™.

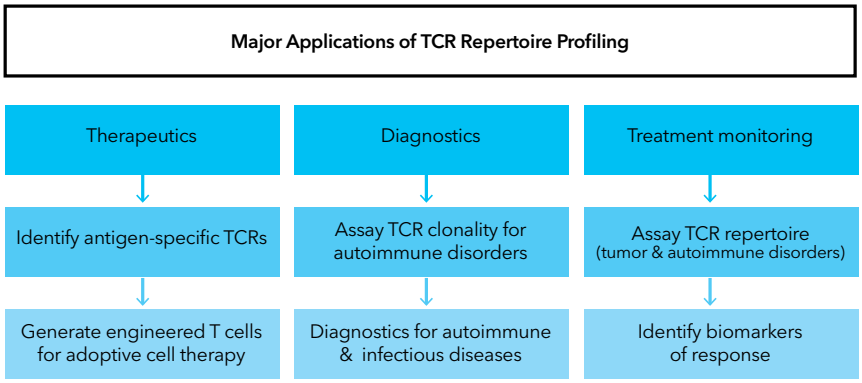

Figure 1. *Overview of major applications of TCR Repertoire Profiling: in a) Therapeutics application: TCR Sequencing of lymphocytes to identify antigen specific TCRs to generate engineered T cells for Adoptive cell therapy b) Diagnostics assay for clonality for autoimmune disorders c) Treatment monitoring: to identify biomarkers of response.*

TCR Sequencing using Adaptive Biotechnologies immunoSEQ™ assay

The immunoSEQ Kit is designed to analyze TCR repertoire in a high-throughput 96-well plate format, allowing for the assessment of many samples in parallel. For this assay, a pool of genomic DNA from each sample of interest is used as input. Multiplexed PCR primers are used to amplify the CDR3 region of TCR-β. From the sequencing results the TCR-β profiles for each sample is annotated and the clonal amplification is assessed.

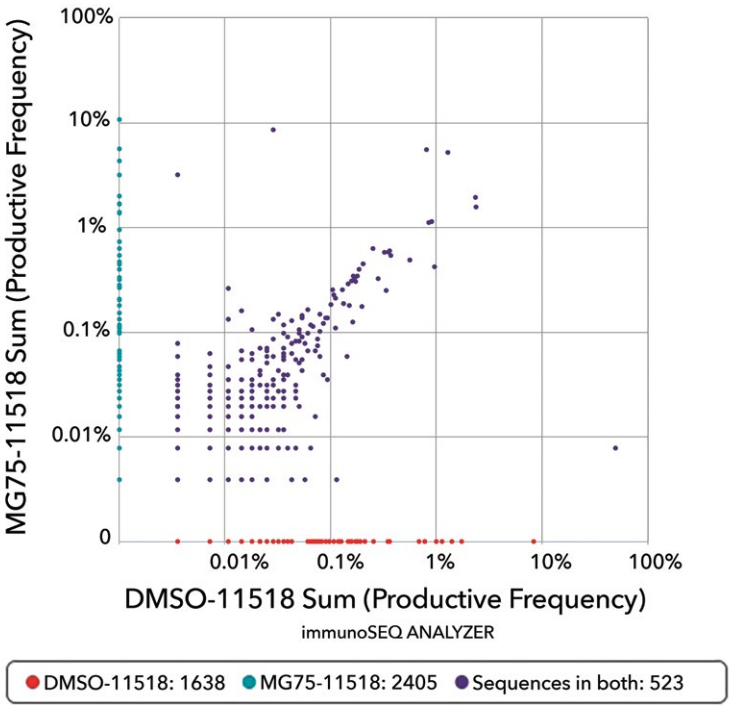

Figure 2. *Antigen specific CD8 clonal amplification (TCR-β chains) measured in PBMC samples post exposure to an immunogenic peptide in an immune cell co-culture assay. Y-axis shows antigen specific TCR expansion in comparison to DMSO on the X-axis.*

TCR α/β sequencing with 10x Genomics Single Cell Immune Profiling Solution

The 10x™ Genomics platform uses a single cell approach, where an input of highly viable single cells in suspension is used for TCR sequencing. The 10x GemCode™ technology is used to separate each cell into a Gel Bead-in-Emulsion (GEM), so that cDNA from each cell is generated with the same barcode. The end product of the assay is full-length V, D, and J-gene segments from each input cell, which are used for a clonotype analysis. The platform can run a maximum of 8 samples in a single run. For all 8 samples, the sequencing is done at a single cell level, and paired α- and β- chain information is obtained, leading to a high-resolution profiling of the TCR repertoire.

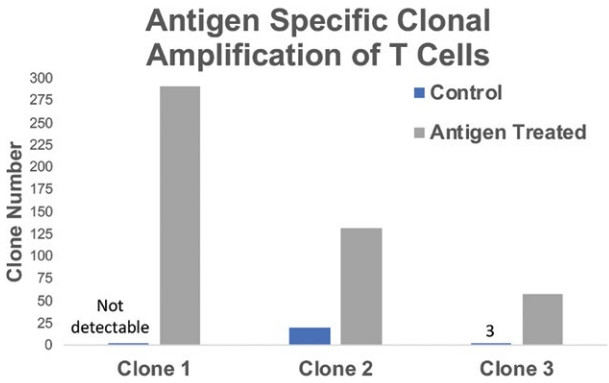

Figure 3. *Antigen specific CD8 clonal amplification (alpha and beta TCR chains) measured by single cell immune profiling. Immune cell co-culture assays were performed in presence of antigenic peptides. A maximum input of 17,000 cells per sample were used in this assay.*

TCR Sequencing using SMARTer ® Human/Mouse TCR α/β Profiling Kits from Takara Bio USA

The SMARTer Human & Mouse TCR α/β Profiling Kits generate NGS libraries from RNA inputs from purified T cells or total RNA (10ng-3ug) to provide complete V(D)J information for TCR α and β chains. The data generated using these kits can provide insights into TCR repertoire diversity and allow for sensitive identification of clonotypes. A single-cell version is also available to perform TCR profiling from 96 or 480 sorted cells and learn about TCR α/β pairing. To analyze the data generated using these kits we use the MiXCR software as recommended by the provider.

Table 1: Overview of the service offerings from MedGenome

Name of offering	Input type	Amount of material needed	Analysis method	Information obtained
immunoSEQ Kit (Adaptive Biotechnologies)	gDNA	100ng gDNA (Human, Mouse)	immunoSEQ Analyzer	CDR3, clonotypes
Single cell Immune profiling (10X Genomics)	Isolated cells	Single-cells (Human, Mouse)	Loupe browser	CDR3, α/β pairing and clonotypes V(D)J sequences
SMARTer TCR Profiling Kit (Takara Bio USA)	Isolated cells or RNA	10ng-3ug/ 50-10,000 cells (Human, Mouse)	MiXCR	CDR3, V(D)J sequences, α/β pairing (from SC Kit)

Conclusion

Here we present three methods for TCR sequencing commonly utilized and discuss the features and benefits of each of the methods. We present data generated and analyzed in-house of clonal amplification after T cell stimulation with an antigen. We have tested the Adaptive immunoSEQ and the 10X V(D)J solutions, and are conducting tests to implement the Takara Bio kits into our workflow. For selecting an appropriate method for TCR profiling, the scientific question, the amount of material available and the information required should be taken into consideration. For example, while using gDNA allows for quantification of single TCR clones, the level of expression of the TCR as well as the full-length V(D)J information can't always be obtained due to intronic retention, making RNA a preferred input for read-out of the V(D)J and getting a complete picture of a TCR repertoire diversity[4]. Another critical difference in various approaches is in multiplex PCR vs RACE-based methods. While multiplex PCR is commonly used for gDNA template, the RACE based chemistries (such as those in the Takara and the 10x Genomics Single Cell Immune profiling) can lead to the detection of novel clonotypes, and sensitive identification of full-length V(D)J and gives high resolution TCR-α and TCR-β pairing information.

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2. Six, A., et al. (2013) The past, present, and future of immune repertoire biology–the rise of next-generation repertoire analysis. Front. Immunol. 4(413):1–16.
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4. Rosati , E, et al (2017) Overview of methodologies for T-cell receptor repertoire analysis. BMC Biotechnology 10;17(1):61.