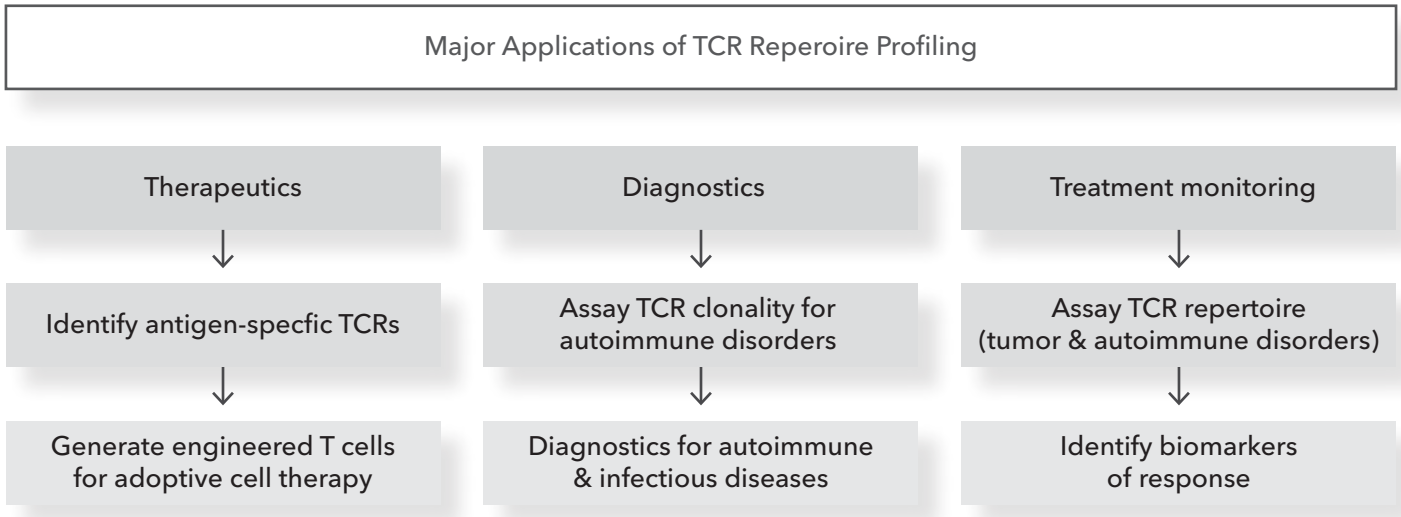
MEDGENOME

T Cell Receptor (TCR) Repertoire Sequencing

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 repertoireis 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 selective expansion of a specific CDR3 region in the repertoire, 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 .

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 MedGenome's NGS based workflows for profiling of the TCR repertoire: namely a) Bulk TCR profiling using: SMARTer TCR Profiling Kit (Takara Bio USA Inc) and modifications to the protocol for Gamma/Delta and FFPE TCR repertoire profiling, b) Single cell TCR Profiling using: 10X Genomics Chromium Immune Profiling solutions, and Takara single-cell TCR sequencing kits. We also present an overview of the types of samples we have processed in-house and application.



MedGenome's workflows for TCR-Sequencing

Table 1: Shows the sample requirements and workflows for MedGenome's TCR profiling services

Name of Offering	Input Type	Amount of Material needed	Analysis Method	Information Obtained
SMARTer TCR Profiling Kit (Takara Bio USA)	isolated cells or RNA	10 ng-3 µg/50-10,000 cells (Human, Mouse)	MiXCR	CDR3, V (D)J sequences α/β pairing (from SC Kit)
Single cell Immune profiling (10X Genomics)	isolated cells	Single-cells (Human, Mouse)	Loupe browser	CDR3, α/β pairing and clonotypes V(D)J sequences
Gamma delta TCR Profiling	isolated cells or RNA	10 ng-3 µg/1000-10,000 cellls	MiXCR	CDR3, V(D)J sequences
FFPE TCR Profiling	RNA (DV200>20)	> 10 ng total RNA	MiXCR	CDR3, V(D)J sequences

Workflows at MedGenome for TCR-Sequencing:

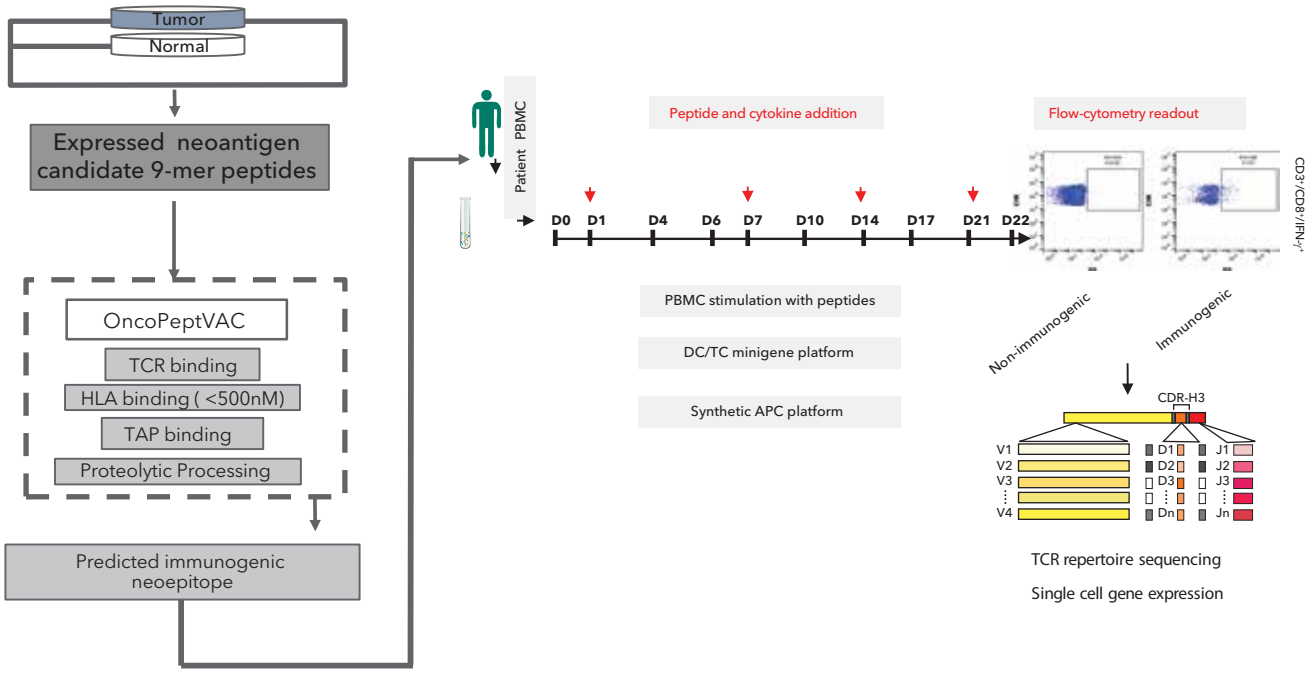
Based on the research question and the sample types available, we recommend any of the above-mentioned workflows (Table 1) for TCR repertoire sequencing. To ensure that we obtain best quality data from the samples, we perform quality control measurements of the extracted RNA and sequencing ready libraries for the Bulk TCR projects, and cell viability measurements for the single-cell TCR projects using the 10X Genomics Chromium workflow.

Table 2: Summary of validated sample types for TCR Repertoire sequencing

Source of Input Material	Approximate Number of Cells	Input Amount of RNA	Total TCR alpha/ Beta Clonotypes Identified
PBMC fresh/frozen	500,000	10 ng	> 20,000 clonotypes
T-Cell clonal cell line	NA	50 ng	> 4000 clonotypes
Mouse T cell sorted	34,000	10 ng	> 40,000 clonotypes
Sorted $CD8^+$-from lesion	10-15,000	1 ng	> 10,000 clonotypes
Tumor infiltrating lymphocytes	2000 cells	10 ng	> 3000 clonotypes

TCR α/β repertoire analysis to identify antigen specific T-cell clones in a neoantigen vaccine screen

A. Workflow of OncoPeptVAC™ prediction and OncoPeptSCRN™ validation



B. Table 3: Example of output provided of the TCR-β clonotypes

cloneId	cloneCount	cloneFraction	allVHitsWithScore	allDHitsWithScore	allJHitsWithScore	allCHitsWithScore	aaSeqCDR3
0	1736	0.007594316511513963	TRBV9*00(1308)	TRBD1*00(35)	TRBJ2-1*00(209.5)	TRBC2*00(281), TRBC1*00(244.9)	CASSVAGGDEQFF
1	1052	0.004602085812276895	TRBV28*00(1313.4)	TRBD1*00(45)	TRBJ2-7*00(224.4)	TRBC2*00(280.9)	CASGRQGAYEQYF
2	852	0.003727164555190033	TRAV12-2*00(1221.9)		TRAJ42*00(319.6)	TRAC*00(174)	CALNYGGSQGNLIF
3	721	0.0031540911317981383	TRBV12-3*00(1299.6), TRBV12-4*00(1263.8)	TRBD1*00(26)	TRBJ1-4*00(194.6)	TRBC1*00(278.9)	CASSFGQAAQLFF
4	659	0.002882865542101211	TRBV4-1*00(1312.5)	TRBD1*00(40)	TRBJ1-5*00(214.7)	TRBC1*00(279.4)	CASSLRTGDGQPQHF
5	651	0.0028478686918177363	TRBV7-9*00(1240.6)	TRBD1*00(26), TRBD2*00(25)	TRBJ2-7*00(215.4)	TRBC2*00(280.4)	CASSLIGEGFSDEQYF
6	648	0.0028347448729614333	TRBV4-3*00(1297.8), TRBV4-2*00(1251.2)	TRBD1*00(41)	TRBJ2-1*00(234.5)	TRBC2*00(279.7)	CASSQDGTGGYNEQFF
7	543	0.0023754112129908307	TRAV21*00(1308.9)	TRDD3*00(25)	TRAJ31*00(254.6)	TRAC*00(172)	CAAPGLDNARLMF
8	543	0.0023754112129908307	TRAV4*00(1310.1)		TRAJ33*00(284.6)	TRAC*00(173.8)	CLVDSNYQLIW
9	536	0.0023447889689927905	TRBV12-4*00(1292.1), TRBV12-3*00(1244.4)		TRBJ1-1*00(220)	TRBC1*00(279.6)	CASSFMVVQADSTEAFF
10	528	0.002309792118709316	TRAV26-2*00(1269.7)		TRAJ49*00(285)	TRAC*00(174)	CILRDPNTGNQFYF
11	515	0.00225292223699867	TRAV21*00(1306.8)		TRAJ15*00(250.6)	TRAC*00(172.2)	CAVKGQAGTALIF
12	490	0.0021435570798628125	TRAV21*00(1281.1)		TRAJ4*00(319.2)	TRAC*00(173.6)	CAGPMFSGGYNKLIF
13	478	0.0020910618044376007	TRBV7-9*00(1269.8)	TRBD1*00(25), TRBD2*00(25)	TRBJ2-1*00(259.7)	TRBC2*00(281.2)	CASSLIGISSYNEQFF
14	468	0.0020473157415832575	TRAV21*00(1312.7)		TRAJ52*00(329.7)	TRAC*00(173.7)	CAVMDAGGTSYGKLTF
15	466	0.002038566529012389	TRAV38-2DV8*00(1350.4)	TRDD3*00(30)	TRAJ49*00(259.6)	TRAC*00(173.9)	CAYRSPPTGNQFYF
16	466	0.002038566529012389	TRAV13-1*00(1333.7)		TRAJ11*00(264.3)	TRAC*00(174.2)	CAAHEGYSTLTF
17	431	0.001885455309022188	TRBV7-9*00(1260.2)		TRBJ2-1*00(259.8)	TRBC2*00(280.6)	CASSLIGVSSYNEQFF
18	400	0.0017498425141737244	TRAV12-2*00(1215.5)		TRAJ20*00(259.6)	TRAC*00(172.3)	CAVNINDYKLSF
19	399	0.00174546790788829	TRAV2*00(1250.9)		TRAJ18*00(309.8)	TRAC*00(172.6)	CASRGSTLGRLYF
0	1151	0.00424535261138979	TRAV21*00(1338.6)		TRAJ9*00(279.5)	TRAC*00(173.3)	CAVTTGGFKTIF
1	929	0.003426526999114783	TRAV21*00(1304.6)		TRAJ33*00(274.5)	TRAC*00(173.4)	CAVPDSNYQLIW
2	925	0.0034117733844791975	TRAV21*00(1306.1)		TRAJ4*00(309.6)	TRAC*00(173)	CALYQFSGGYNKLIF

C. Total T Cell Population (4000 clonotypes) Expanded Clone

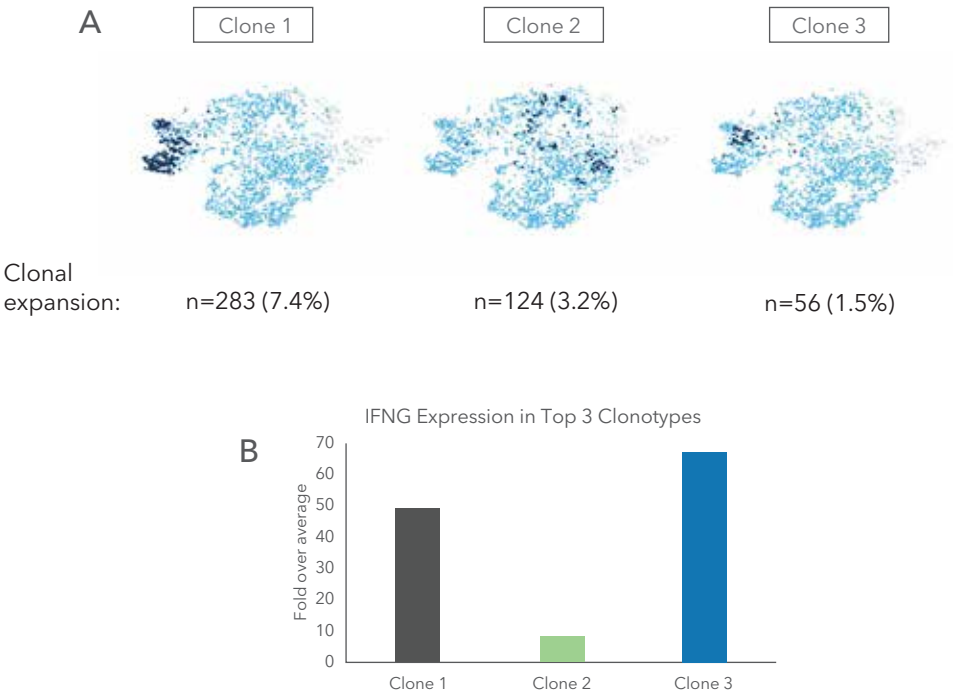


Figure 3: *Application of TCR repertoire sequencing and single cell gene expression to assay for antigen specific changes to T-cell clonality and functional T-cell response: A) Shows schematic representation of the workflow to screen for neoantigen candidate vaccines, and perform TCR repertoire sequencing to obtain clonality information of the antigen specific response.*

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. While we offer several platforms for our services, in order to select an appropriate method for TCR profiling, the scientific question, the amount of material available and the information required should be taken into consideration[4]. 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.

References

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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.

3. Stubbington, MJT, et al (2016) T cell fate and clonality inference from single-cell transcriptomes. Nat Methods.13(4):329-332.

4. Rosati , E, et al (2017) Overview of methodologies for T-cell receptor repertoire analysis. BMC Biotechnology 10;17(1):61.