T Cell Receptor (TCR) repertoire sequencing from FFPE samples

Relevance of TCR sequencing in human diseases

A large majority of T cells recognize targets of immunity through direct interaction of T-cell receptor (TCR) with antigen-derived peptide presented on the cell surface by HLA molecules. The TCR is a heterodimer constituted by α and β (or γ/δ) chains. The chains are encoded by V, D, J and C (constant region) genes. Rearrangements in the V(D)J segments by recombinases produces an incredibly large spectrum (10^9–10^{10} sequences) of complementarity-determining region 3 (CDR3) sequences that provide diversity and specificity to a wide variety of antigens. Structural studies of the TCR has revealed that the CDR3 of the Vβ chain is directly involved in the recognition/binding of antigenic peptide-HLA complexes. Consequently, the CDR3 repertoire diversity reflects clonal composition, the potential antigenic recognition spectrum, and the quantity of antigen specific T-cell responses which are critical in assessing the bodies immunity status against specific antigens viz. viral, bacterial, self/autologous and somatic mutation derived autologous antigens.

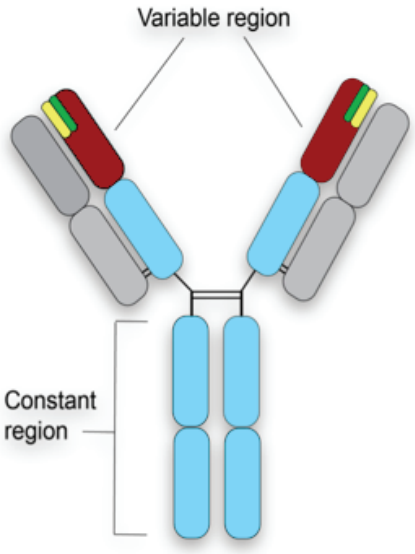

Figure 1: *The CDR3 variable region of TCR is encoded by the V(D)J junction (maroon, green, yellow) which plays an essential role in the interaction of the TCR with the antigen-MHC complex. The unique clonal diversity of the CDR3 region provides specificity to a wide array of antigens.*

In cancer immunotherapy TCR repertoire analysis is critical for:

a. disease monitoring post IO drugs treatment
b. analysis of tumor infiltrating leucocytes (TILs)
c. identification of TCR specific to cytokine-induced killer T cells
d. TCR engineered T cells as a therapeutic
e. TCR-like mAb antibodies as therapeutics

Challenges in TCR sequencing from FFPE

Tumor-infiltrating lymphocytes (TILs) are considered important in anticancer immunosurveillance. Higher prevalence of TILs in the tumor milieu have been correlated with better survival outcomes supporting TIL as an independent prognostic biomarker. The challenge however is to analyze TIL repertoire diversity from clinical FFPE samples. The technical ease and low storage cost of tissues in formalin comes at the expense of sample quality. Fixation causes cross-linking between proteins and nucleic acids resulting in irreversible damage to both DNA and RNA. RNA from FFPE samples display varying ranges of RNA degradation, covalent chemical modification and poly-A tail damage. These effects can impact reverse transcription from mRNA to cDNA and significantly impede downstream gene analysis using standard protocols.

TCR repertoire analysis method from FFPE tumor tissue samples

Despite these shortcomings, in this application note we present an optimized method to successfully analyze the TCR repertoire in RNA samples obtained from tumor FFPE specimens. RNA was isolated from PBMCs stimulated with a 9-mer peptide and from two FFPE tumor samples using QIAGEN RNeasy kit. TCR-Seq libraries were generated using SMARTer TCR Profiling Kit with modifications to the protocol. Libraries were sequenced on Ilumina's MiSeq Next Generation Sequencer and the data analyzed using the MiXCR T and B cell repertoire analysis tool.

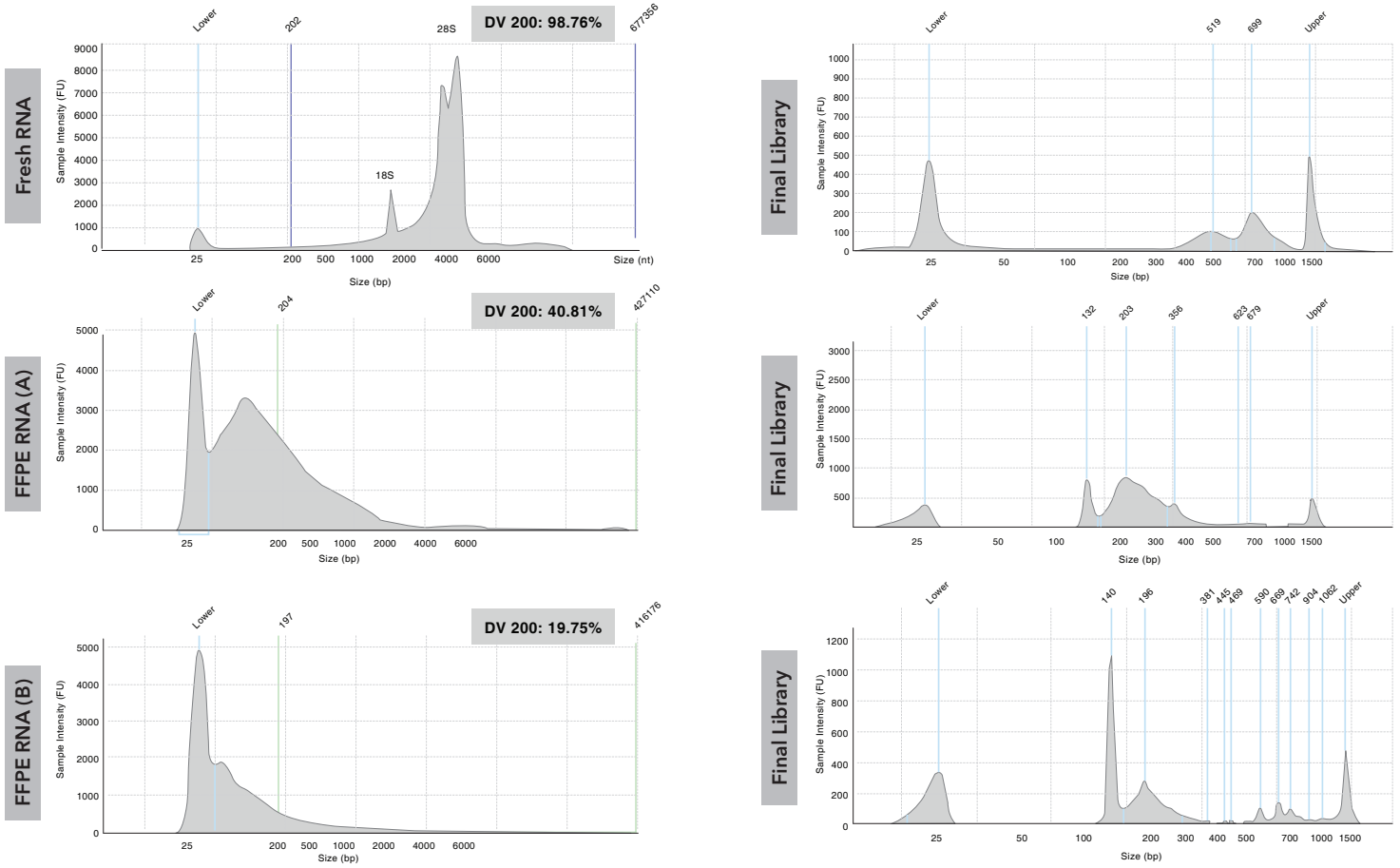

Figure 2: *TapeStation profiles to evaluate RNA quality and TCR-Seq libraries generated from FFPE tissues in comparison to fresh tissue: Three panels on the left show the RNA profiles of total RNA isolated from PBMCs (top panel) and 2 different FFPE tumor tissue samples. DV 200 values for each sample shows the RNA integrity of the samples (inset). Panels on the right shows profiles of TCR-Seq libraries generated using SMARTer TCR Profiling Kit with modifications to the protocol from the corresponding input RNAs.*

Fresh RNA PBMC DV 200: 98.76% Total unique CDR3 : 10,440		
Clonal Count	Clonal Frequency	CDR3 Sequence
6651	2.61%	CATPNDYKLSF
4672	1.84%	CALSRRYNFNKFYF
3928	1.54%	CALSDRGKLIF
3182	1.25%	CASRSGTGGSGTEAFF
2428	0.95%	CALTPLYGGATNKLIF
1876	0.74%	CAFERGSQGNLIF
1813	0.71%	CAYRGYNTDKLIF
1812	0.71%	CAVDSNTGNQFYF
1795	0.71%	CALSDRGKLIF
1461	0.57%	CASSLTPPPNTGELFF

FFPE RNA (A) DV 200: 40.81% Total unique CDR3 124		
Clonal Count	Clonal Frequency	CDR3 Sequence
48124	47.86%	CAVRSYNQGKLIF
47372	47.11%	CASSLDPGANTEVFF
1125	1.12%	CAIGHQ_TGKLTF
1113	1.11%	CAARGNRIFF
1073	1.07%	CAASTNTNTGKLTS
808	0.80%	CAASTNTNTGKLTF
456	0.45%	CAVIT_NAPRF
327	0.33%	CAVRGR_NNKLTF
15	0.01%	YAEQFF
11	0.01%	CASSLDPGANTEGGF

FFPE RNA (B) DV 200: 19.75% Total unique CDR3 : 79		
Clonal Count	Clonal Frequency	CDR3 Sequence
66727	79.93%	CASSLDPGANTEVFF
13330	15.97%	CAVRSYNQGKLIF
752	0.90%	CAMRGTGGNNKLTF
724	0.87%	CAMRDTNAYKVIF
630	0.75%	CATDAGI_NNRIFF
370	0.44%	CALSPLQ_TNKVVF
314	0.38%	CAMRGINTGNYKYVF
214	0.26%	CAASVG_NNKLTF
131	0.16%	CALIYGNEKITF
130	0.16%	CALSLWE_SGNKLIF

Figure 3: *Shows the top 10 TCR α/β CDR3 clonotypes identified in samples from fresh PBMC and FFPE tumor tissue block RNA. Tumor tissue samples display an increased frequency of top CDR3 clonotypes indicating an antigen mediated enrichment of unique T cell clones. Also, intratumoral convergence of the TCR repertoire is observed in both tumor FFPE samples indicated by a much lower number of unique CDR3 clonotypes compared to the antigen stimulated PBMC samples. This is possibly caused by antigen induced clonally expanded T cells in the tumor mileu. Furthermore, one of the top CDR3 clonotypes is shared between the two tumor FFPE samples, possibly indicating the presence of shared tumor antigens.*

Conclusion

Here we present a method for TCR sequencing and analysis of TILs from a FFPE tumor tissue block using modifications to the SMARTer® TCR Profiling Kit. We accept sample both as RNA (100 ng minimum) or 5 µm unstained FFPE tumor tissue blocks. We encourage users to confirm the presence of T cell in the tumor milieu by alternative methods such as IHC or flow-cytometry, if possible to corroborate the TCR sequencing results.