More sensitive identification of T-cell receptor beta rearrangements with an augmented transcriptome method

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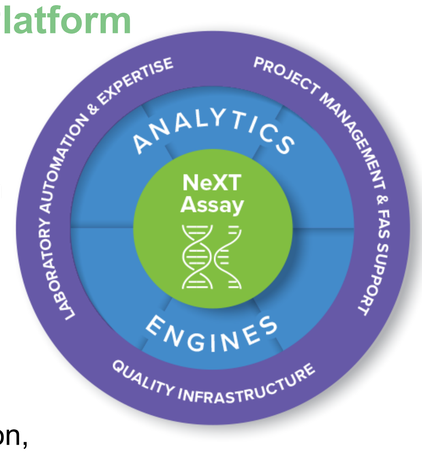
Introduction

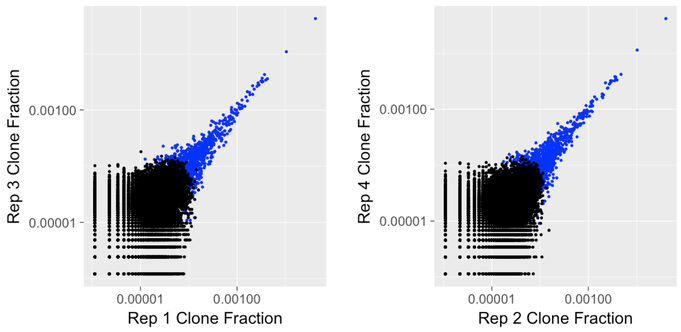
With the increase in development and use of immunotherapies, there is also an increasing need for comprehensive immuno-genomic profiling of tumors to identify new potential biomarkers. This includes profiling of the T-cell receptor (TCR), which has traditionally not been feasible with an exome/transcriptome scale platform. To solve this problem, we have developed comprehensive TCR & BCR profiling as part of the ImmunoID NeXT platform. Here, we show how this can be applied to understand the diversity and activity of the adaptive immune system.

ImmunoID NeXT Platform



Biomarkers of critical importance in immuno-genomic profiling include TCR profiling, neoantigen identification, HLA typing, expression of immuno-modulators, and composition of the tumor microenvironment. However, limited sample amount, FFPE degradation, and cost of many assays pose significant barriers to conducting such comprehensive characterization in clinical trials. To address these challenges, we have developed an augmented, immuno-oncology optimized exome/transcriptome platform (ImmunoID NeXT) that can also identify abundant TCR clones, from limited FFPE tumor biopsy samples.

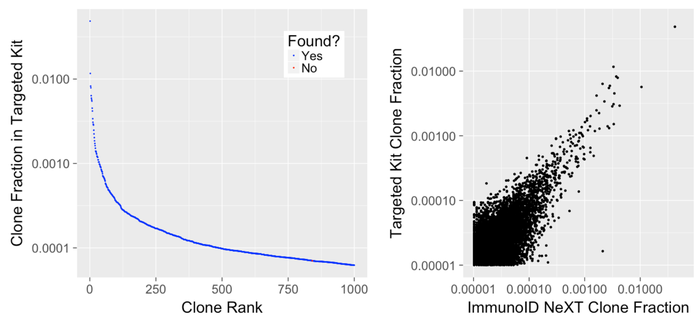
Reproducibility of clonal abundances



Abundances of clones found in common between separate preps of a healthy PBMC sample. R^2 values of 0.99 for both comparisons. The top 1000 clones are in blue.

To first test the reproducibility of ImmunoID NeXT, we sequenced replicates of RNA from the same healthy donor PBMC sample. Abundances for clones found in common had very high concordance, showing that even with a diverse repertoire in healthy PBMCs, our TCRB profiling provides reproducible results.

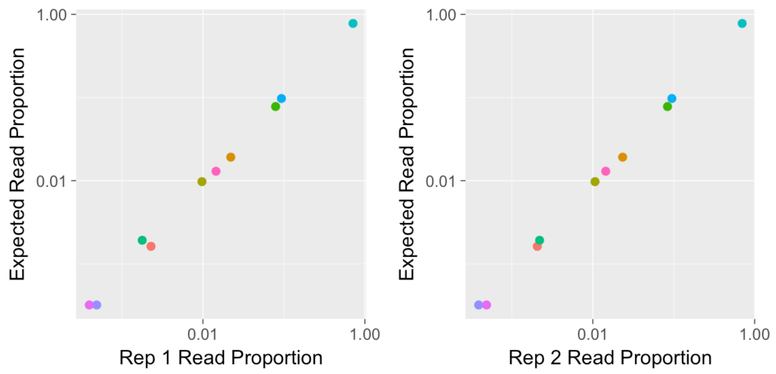
Verification of top clones across methods



Identification of the top 1000 clones as identified in a targeted kit, and comparison of the abundances of clones found in the targeted kit vs. ImmunoID NeXT (clones >0.00001).

To evaluate the capability of ImmunoID NeXT to identify the highest-abundance clones in a sample, we compared our results to the top 1000 clones as identified in a commercially-available TCR kit. We were able to pick up 99.9% of the top 1000 clones, showing that our approach has the capability to profile high-abundance clones. In addition, the estimated abundances of the top clones were highly concordant between the targeted approach and our own.

Limit of detection using known clones

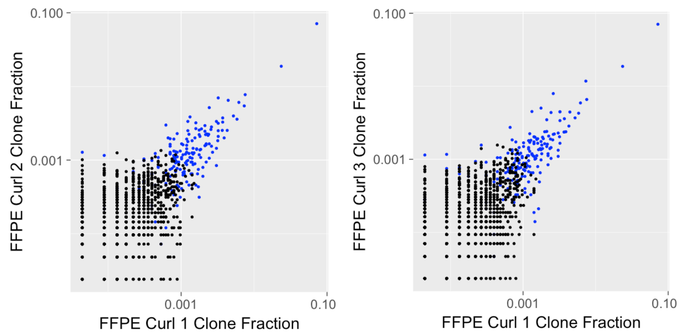


Expected vs. observed read proportions for pools of 10 T-cell lines mixed at known proportions, ranging from 78% to 0.3%, to capture a dynamic range of known clones for further dilutions.

To test the limit of detection of known clones using ImmunoID NeXT, we created a pool of 10 T-cell lines with known T-cell receptor rearrangements. Through diluting this pool in a healthy PBMC sample and tracking the clones, we were still able to reliably identify clones down to 0.00032% RNA by mass in the mixture.

Cell line	Percentage in 1% mixture	1% rep1	1% rep2	Percentage in 0.5% mixture	0.5% rep1	0.5% rep2
P12-Ichikawa	0.097%	Detected	Detected	0.049%	Detected	Detected
MOLT-4	0.78%	Detected	Detected	0.39%	Detected	Detected
HPB-ALL	0.019%	Detected	Detected	0.0096%	Detected	Detected
CCRF-CEM	0.0016%	Detected	Detected	0.00081%	Detected	Detected
TALL-1	0.013%	Detected	Detected	0.0065%	Detected	Detected
PF-382	**0.00032%**	Detected	Detected	0.00016%	Not Detected	Detected
SUP-T1	**0.00032%**	Detected	Detected	0.00016%	Detected	Not Detected
MOLT-16	0.0019%	Detected	Detected	0.00096%	Detected	Detected
Jurkat	0.078%	Detected	Detected	0.039%	Detected	Detected
HuT 78	0.0097%	Detected	Detected	0.0049%	Detected	Detected

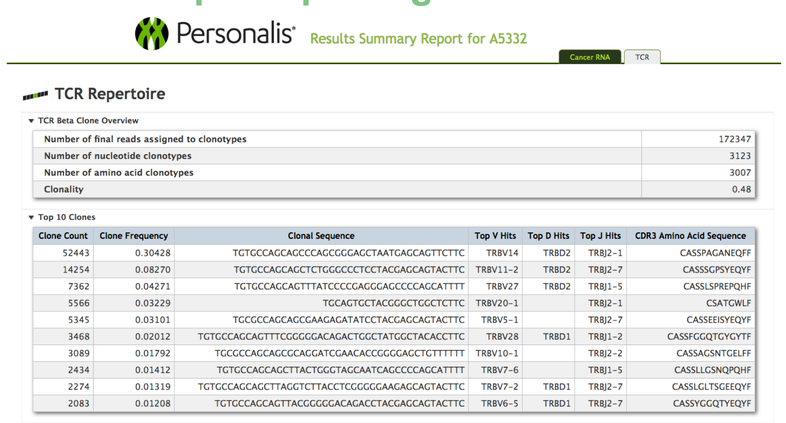
Reproducibility of clones found in a tumor FFPE sample



Comparison of abundances for clones found in common between separate curls of a tumor FFPE sample. R^2 values of 0.97 and 0.96, respectively. The top 250 clones are in blue.

To evaluate ImmunoID NeXT's ability to profile clones in real tumor FFPE samples, we profiled subsequent curls of a real clinical sample. We observed strong concordance of the abundances for clones found between the curls, showing that our approach is robust to degraded FFPE samples.

Clone output reporting


Personalis Results Summary Report for A5332

Cancer RNA | TCR

TCR Repertoire

TCR Beta Clone Overview

Number of final reads assigned to clonotypes	172347
Number of nucleotide clonotypes	3123
Number of amino acid clonotypes	3007
Clonality	0.48

Top 10 Clones

Clone Count	Clone Frequency	Clonal Sequence	Top V Hits	Top D Hits	Top J Hits	CDR3 Amino Acid Sequence
52443	0.30428	TGTGCCCAGCAGCCCAGCCGCGAGCTAATGAGCAGTTCTTC	TRBV14	TRBD2	TRBJ2-1	CASSPAGANEQFF
14254	0.08270	TGTGCCAGCAGCTCTGGGCCCTCCTACGAGCAGTACTTC	TRBV11-2	TRBD2	TRBJ2-7	CASSSGPSYEQYF
7362	0.04271	TGTGCCAGCAGTTTATCCCCGAGGAGCCCCAGCATTTT	TRBV27	TRBD2	TRBJ1-5	CASSLSPREPQHF
5566	0.03229	TGCAGTGCTACGGGCTGGCTCTTC	TRBV20-1		TRBJ2-1	CSATGWLF
5345	0.03101	TGCGCCAGCAGCGAAGAGATATCCTACGAGCAGTACTTC	TRBV5-1		TRBJ2-7	CASSEEISYEQYF
3468	0.02012	TGTGCCAGCAGTTTCGGGGGACAGACTGGCTATGGCTACACCTTC	TRBV28	TRBD1	TRBJ1-2	CASSFGGQTGYGYTF
3089	0.01792	TGCGCCAGCAGCGCACGATCGAACACCGGGGAGCTGTTTTTT	TRBV10-1		TRBJ2-2	CASSAGSNTGELFF
2434	0.01412	TGTGCCAGCAGCTTACTGGGTAGCAATCAGCCCCAGCATTTT	TRBV7-6		TRBJ1-5	CASSLLGSNQPQHF
2274	0.01319	TGTGCCAGCAGCTTAGGTCTTACCTCCGGGGAAGAGCAGTACTTC	TRBV7-2	TRBD1	TRBJ2-7	CASSLGLTSGEEQYF
2083	0.01208	TGTGCCAGCAGTTACGGGGGACAGACCTACGAGCAGTACTTC	TRBV6-5	TRBD1	TRBJ2-7	CASSYGGQTYEQYF

Finally, we have integrated a TCR analysis module into our reporting framework, providing useful sample-level metrics, clonotype outputs, and clonal distribution plots.

Conclusion

Our ImmunoID NeXT platform has been designed to enable sensitive detection of abundant TCR clones in addition to comprehensive biomarkers from exome/transcriptome results. Here we demonstrate that our platform is highly reproducible, sensitive, and has high concordance with the top-abundance clones derived from targeted TCR methods. We also show that our method is feasible with FFPE samples, making it practical for clinical trial use. In summary, by combining exome/transcriptome sequencing with TCR characterization into a single assay, our ImmunoID NeXT platform enables comprehensive immuno-genomics characterization of a tumor sample while reducing overall sample requirements and cost.

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