T-cell receptor repertoire profiling using an augmented transcriptome

#3377

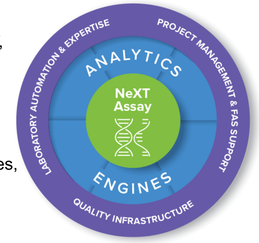
Eric Levy, Pamela Milani, Sean M. Boyle, Shujun Luo, Gabor Bartha, Charles Abbott, Rena McClory, Robin Li, John West, Richard Chen
Personalis, Inc. | 1330 O'Brien Dr., Menlo Park, CA 94025

Contact:
eric.levy@personalis.com

Introduction

Immunotherapy is growing as one of the most promising therapeutic approaches in clinical oncology practice. This brings with it an increasing need for comprehensive immuno-genomic profiling of tumors to better understand the interaction with the immune system. This includes profiling of the T and B-cell receptor repertoires (TCR/BCR), which has traditionally not been feasible with an exome/transcriptome platform.

To address these challenges, we developed ImmunoID NeXT, an augmented, immuno-oncology optimized exome/transcriptome platform designed to provide comprehensive information regarding the tumor and tumor microenvironment (TME) from limited FFPE tumor biopsies, including the TCR α, β, γ, δ, and BCR heavy and light chains. We show how this platform accurately profiles abundant clones, and can be applied to understand the diversity and activity of the adaptive immune system.

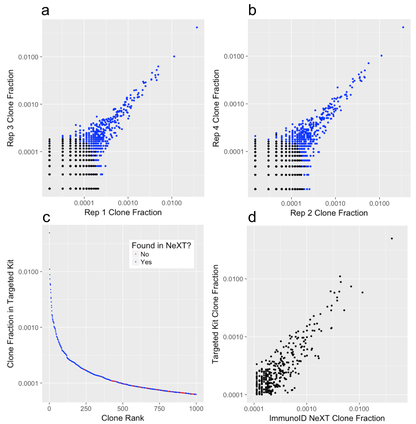


Accurate immune repertoire sequencing with NeXT

Accurate profiling of TCRβ

We first characterize the reproducibility and accuracy of ImmunoID NeXT at profiling TCRβ from RNA by analyzing the concordance of clones using replicates of PBMC samples. Abundances for clones shared between replicates have a high concordance (R^2>0.98). This shows that even with a diverse repertoire in healthy PBMCs, our TCRβ profiling provides reproducible results.

Next, we assess the concordance of top clones in ImmunoID NeXT to a commercially-available standalone TCR kit. Compared to the standalone approach, we identify 95.3% of the top 1000 clones, with highly concordant abundances across all shared clones (R^2>0.94). This shows that our approach has the capability to accurately profile top clones.



(a,b) Abundances of clones found in common between separate preps of a healthy PBMC sample. R^2 values of 0.98 for both comparisons. The top 1000 clones are in blue.
(c,d) Identification of the top 1000 clones as identified in a targeted kit, and comparison of the abundances of clones found in both the targeted kit vs. ImmunoID NeXT (clones >0.0001).

Limit of detection using known clones

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. By diluting this pool in a healthy PBMC sample and tracking the clones, we are able to reliably identify clones down to 0.00032% RNA by mass in the mixture.

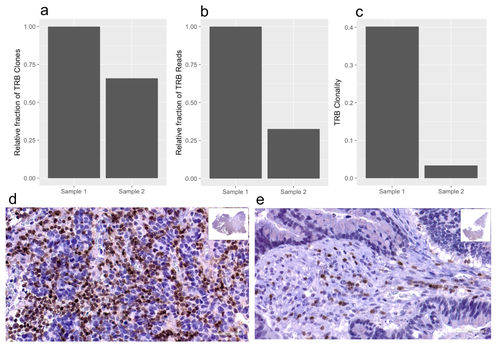
Cell line	Percentage in 1% mixture	1% rep1	1% rep2	Percentage in 0.5% mixture	0.5% rep1	0.5% rep2
P12-Ichikawa	0.097%	Detected	Detected	0.049%	Detected	Detected
MOLT-4	0.78%	Detected	Detected	0.39%	Detected	Detected
HPB-ALL	0.019%	Detected	Detected	0.0096%	Detected	Detected
CCRF-CEM	0.0016%	Detected	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

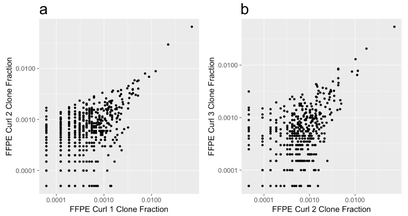
Repertoire profiling with tumor FFPE samples

Reproducibility in FFPE samples

We also analyze patient-derived colorectal cancer (CRC) FFPE tumors to characterize the profiles of tumor-infiltrating immune repertoires. First, we analyze the reproducibility of clones identified using serial sections of FFPE samples. We observe a strong concordance of the abundances for shared clones between the sections, showing that our approach is robust to degraded FFPE samples.

Clonal distributions compared to immunostaining





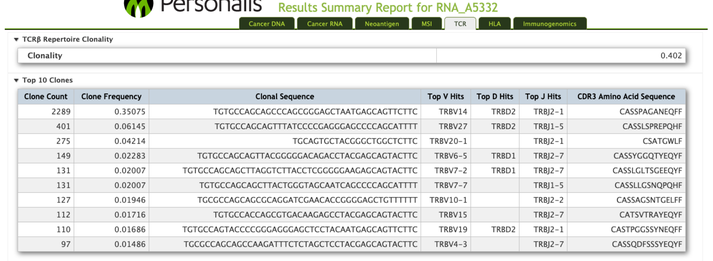
Comparison of abundances for clones found in common between serial sections of a tumor FFPE sample. R^2 values of 0.95 (a) and 0.91 (b).

We compare TCRβ profiling with immunohistochemical (IHC) staining of CD3+ cells in two additional CRC tumor tissues. In our analysis of T-cell infiltration, Sample 1 has a higher number of clones than Sample 2, and a much higher number of TRB reads and clonality than Sample 2. This is reflected in the IHC data, where Sample 1 has significant infiltration of T-cells (49%) compared to Sample 2 (23%). This shows how different metrics of TCRβ profiling provide complementary biological information.

(a,b) Comparison of Sample 2 relative to Sample 1 of fraction of TRB clones and of TRB reads. (c) TRB clonality calculated for Samples 1 and 2. (d,e) IHC of CD3 (brown) for Samples 1 and 2.

Comprehensive ImmunoID NeXT platform

Reporting

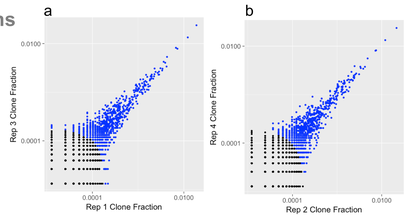


Clone Count	Clone Frequency	Clonal Sequence	Top V Hits	Top D Hits	Top J Hits	CDR3 Amino Acid Sequence
2289	0.35075	TGTGCCAGCAGCCCAGCGGAGCTAATGAGCAGTTCTTC	TRBV14	TRBD2	TRBJ2-1	CASSPAGANEQFF
401	0.06145	TGTGCCAGCAGTTTATCCCCGAGGGAGCCCCCAGCATTTT	TRBV27	TRBD2	TRBJ1-5	CASSLSPREPQHF
275	0.04214	TGCAGTCCTACGGGCTGGCTCTTC	TRBV20-1		TRBJ2-1	CSATGWLF
149	0.02283	TGTGCCAGCAGTTACGGGGGACAGACCTACGAGCAGTACTTC	TRBV6-5	TRBD1	TRBJ2-7	CASSYGCQTYEQYF
131	0.02007	TGTGCCAGCAGCTTAGTCTTACCTCGCGGGGAAGAGCAGTACTTC	TRBV7-2	TRBD1	TRBJ2-7	CASSLGLTSGEQYF
131	0.02007	TGTGCCAGCAGCTTACTGGGTAGCAATCAGCCCCAGCATTTT	TRBV7-7		TRBJ1-5	CASSLLGSNQPQHF
127	0.01946	TGCGCCAGCAGCCGCAGGATCCAACACCGGCGAGCTGTTTTTT	TRBV10-1		TRBJ2-2	CASSAGSNTGELFF
112	0.01716	TGTGCCACCAGCGTGACAAGAGCCTACGAGCAGTACTTC	TRBV15		TRBJ2-7	CATSVTRAYEQYF
110	0.01686	TGTGCCAGTACCCCCGGAGGGAGCTCCTACAATGAGCAGTTCTTC	TRBV19	TRBD2	TRBJ2-1	CASTPGGSSYNEQFF
97	0.01486	TGCGCCAGCAGCCAAGATTTCTCTAGCTCCTACGAGCAGTACTTC	TRBV4-3		TRBJ2-7	CASSQDFSSSYEQYF

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

Future directions – other chains

We are developing and testing expanded profiling capabilities of all major immune repertoire chains, including the TCR α, β, γ, δ chains and BCR heavy and light chains. Here we show early results highlighting the reproducibility of the IG heavy chain with ImmunoID NeXT.



Comparison of abundances for clones found in common between separate preps of a healthy PBMC sample. R^2 values of 0.97 (a) 0.98 (b). The top 1000 clones are in blue.

Conclusion

ImmunoID NeXT has been designed to enable sensitive detection of abundant TCR and BCR clones in addition to comprehensive biomarkers from exome/transcriptome data. We demonstrate that our platform is reproducible, sensitive, and concordant with the top-abundance clones derived from a targeted TCR method, as well as feasible with FFPE samples. Finally, we highlight how immune repertoire results from ImmunoID NeXT can be used to gain understanding about the immunological composition of the TME. 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.

Personalis