

NeXT V3 Updates



Precision Genomics for Immuno-Oncology

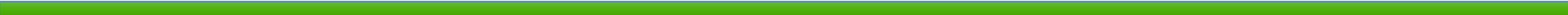
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NeXT V3 Updates

- **InfiltrateID™**
 - Quantifying the Immunocellular Content in the Tumor Microenvironment
- **RepertoireID™**
 - Characterization of the Immune Repertoire: TCR & **BCR** analysis
- **SHERPA™ Enhancements**
 - Integrating diverse and large amounts of training data for accurate neoantigen prediction
- **GRCh38 Reference Genome**
 - GRCh38 Reference Genome alignment option available



InfiltrateID™

Quantifying the Immunocellular Content in the Tumor Microenvironment



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Infiltrate ID - Customer deliverables

1. A **new tab** in the summary report (html report) showing the **ssGSEA scores** for the **eight** immune cell types.
2. A **data file** (.xlsx, .tsv) with the ssGSEA scores is available as **separate document**



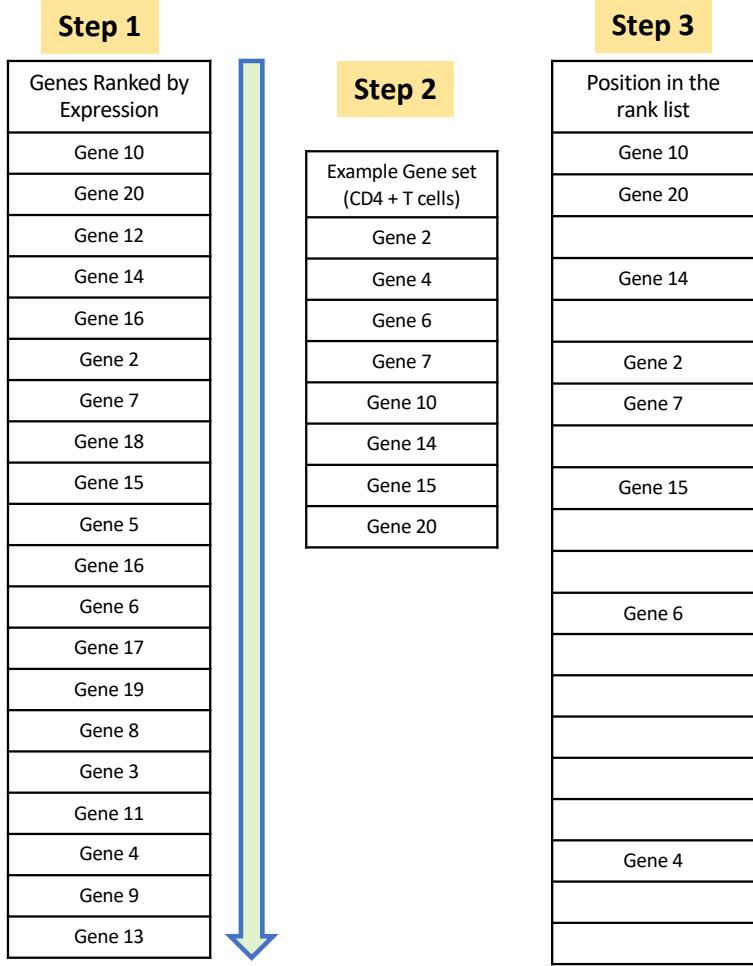
Results Summary Report for DNA_NGT-LCS-1901003T

Cancer DNA Cancer RNA MSI TCR BCR Oncovirus Immune Infiltrate

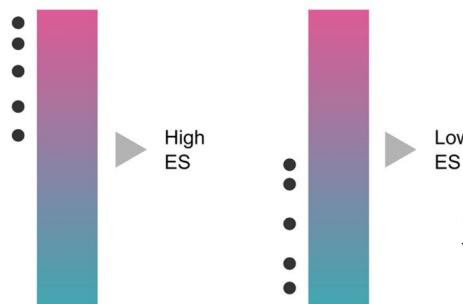
Immunocellular Quantification

Immune Cell Type	ssGSEA Score
Lymphoid Lineage	
B-cells	-60
NK cells	-5,537
T-cells – CD4+	-1,171
T-cells – CD8+	-2,644
T-cells – Tregs	2,286
Myeloid Lineage	
Dendritic cells – Myeloid (mDCs)	3,917
Macrophages	5,864
Dendritic cells – Plasmacytoid (pDCs)	98

How does the ssGSEA method compute enrichment scores?



1. Rank the genes in the dataset based on their expression level (TPM)
2. Use our **proprietary gene sets**, representative of **each immune cell population**, to assess the relative position of the genes in each gene set within the ranked list generated for a sample
3. ssGSEA score for each immune cell population (gene set) is then calculated by walking down the ranked list of genes, **increasing** a running-sum statistic when a gene is in the gene set is encountered and **decreasing** it when it is not, and the final total is **reported as the enrichment score.**
4. Enrichment score will be high if majority of the genes in a gene set for a certain type are amongst the top ranked genes in the sample or low otherwise



Finotello F, Trajanoski Z. Quantifying tumor-infiltrating immune cells from transcriptomics data. Cancer Immunology, Immunotherapy. 2018

Advantages of single-sample enrichment score

InfiltrateID allows for the **delineation of the underlying Immunocellular profile** in a given tumor sample, providing novel information that may help **stratify cohorts of patients** with similar profiles

- **Easily-interpretable** single sample level score & can be computed independently for one sample at a given time
- Ideal for **both small and large** sample-sets
- **Inter-sample comparison & cohort analysis**
 - **Longitudinal & temporal tracking of** changes in the TME of the same patient or for a subset of patients without having to recalculate scores when new data points are collected during the duration of a study

Foroutan, M., Bhuvva, D.D., Lyu, R. et al. Single sample scoring of molecular phenotypes. *BMC Bioinformatics* **19**, 404 (2018).

Concordance studies and use cases



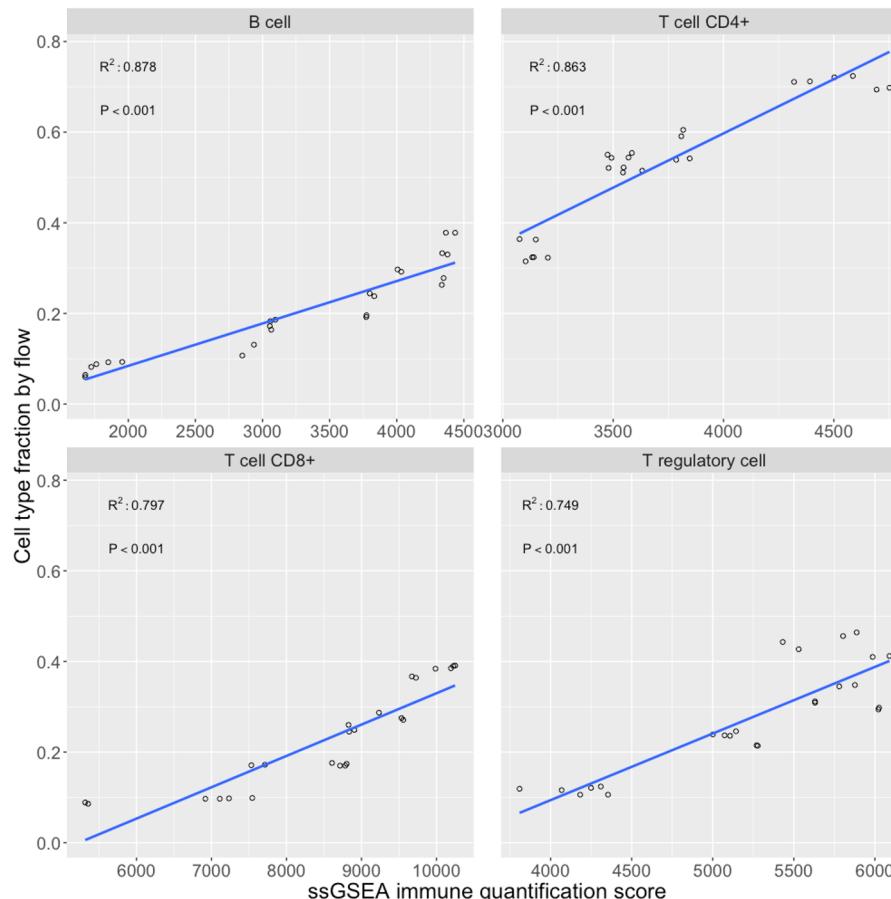
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Concordance studies with immune cell mixtures - evaluated with FACS

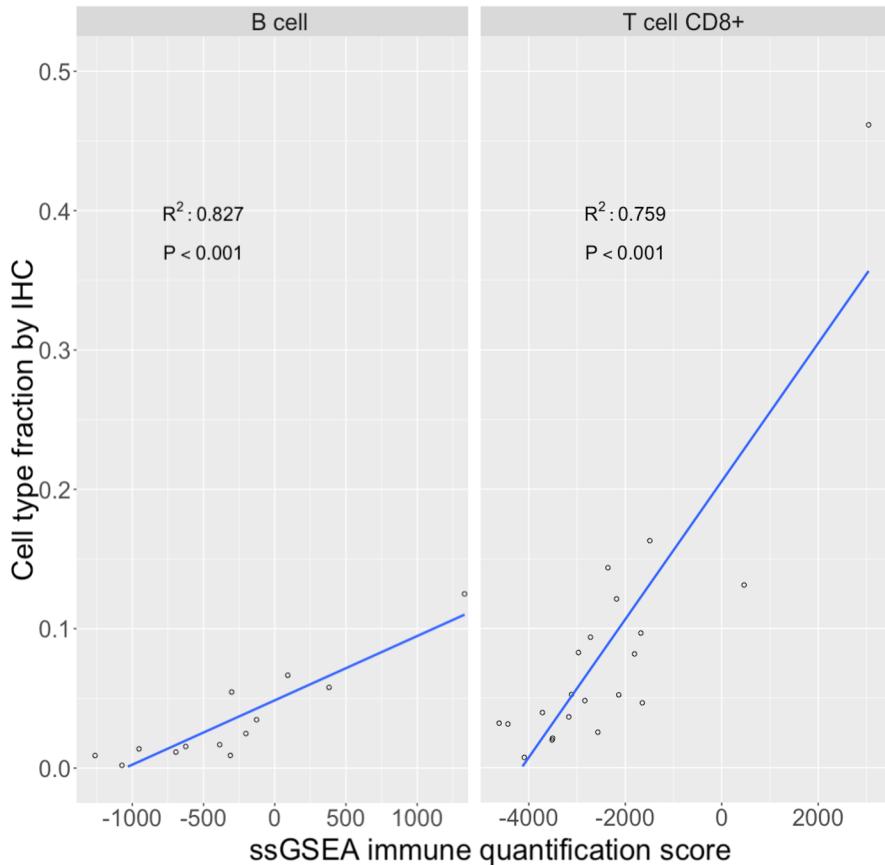


- Created set of predefined mixtures of four purified immune cell populations and quantified abundance of each immune cell type using flow cytometry
 - The results suggests that **increasing ssGSEA scores correlated with increasing abundance/ proportions of immune cell populations**

Note

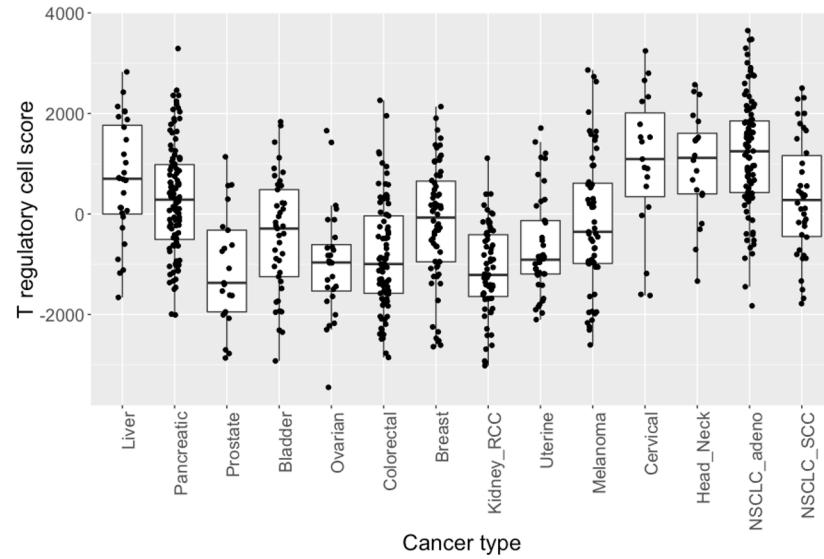
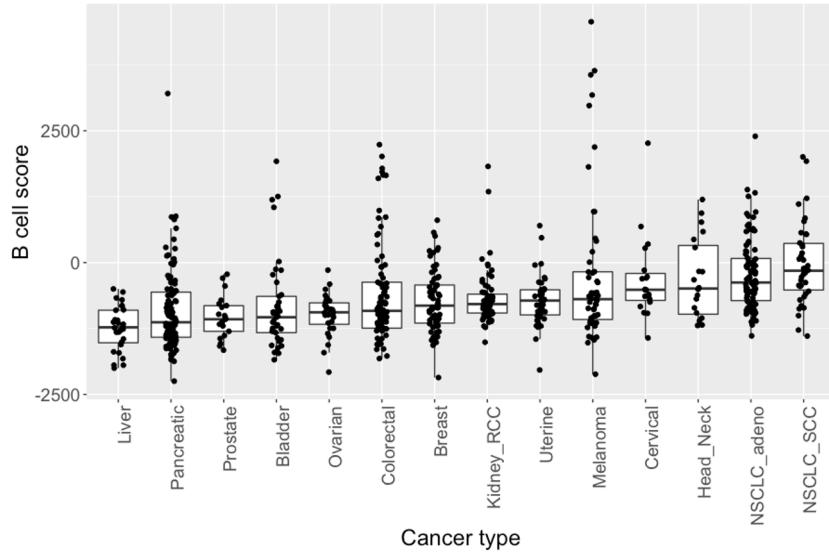
* The SSGSEA scores are in arbitrary units and the change in values shouldn't be interpreted as direct fold change (a score of 2000 for one samples doesn't mean twice the enrichment compared to sample with a score of 1000)

Concordance studies with tumor FFPE samples - evaluated with IHC



- Colorectal and lung cancer FFPE samples were utilized to compare the concordance between our transcriptome-based ssGSEA scores and the cell fractions quantified using IHC
 - The results suggests that **increasing ssGSEA scores correlated with higher infiltration** of immune cell populations measured using IHC

Sample cohort analysis- ssGSEA score distribution plots



1. The box plots shows the **distribution of ssGSEA scores for 708 samples from 14 tumor types**, representing the relative enrichment of different immune cell populations (B cells & T reg cells) in the TME in various cohorts of tumor types
2. The ssGSEA scores can be only **compared between the same immune cell population** (ex: B cell score vs B cell score) across samples
3. **Each cohort of samples should to be analyzed independently** because each cohort would have a different range of distribution of values

InfiltrateID Summary

- InfiltrateID provides a report with the enrichment scores (ssGSEA score) for eight distinct immune cell types, which could be used as a proxy to **gauge the relative infiltration/presence of various immune cell populations** within the tumor microenvironment (TME)
- The individual ssGSEA score can be used for **inter-sample comparison within a cohort of samples** to evaluate the relative enrichment of these eight distinct immune cell types
- InfiltrateID allows to **delineate the immunocellular content in the TME for patients**, providing novel information that can be simultaneously evaluated alongside tumor-specific biomarker data to facilitate patient stratification (Responders vs Non-Responders)

RepertoireID™

Characterization of the Immune Repertoire: TCR & BCR analysis



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NeXT V3 Repertoire ID module - BCR heavy chain (IgH) analysis

Profile BCR heavy chain (IgH) to characterize BCR repertoire (Clonotypes) and determine the composition of tumor-infiltrating BCR Isotypes (IgG, IgM, IgA, IgD) within the tumor microenvironment (TME) simultaneously from the same tumor FFPE sample*

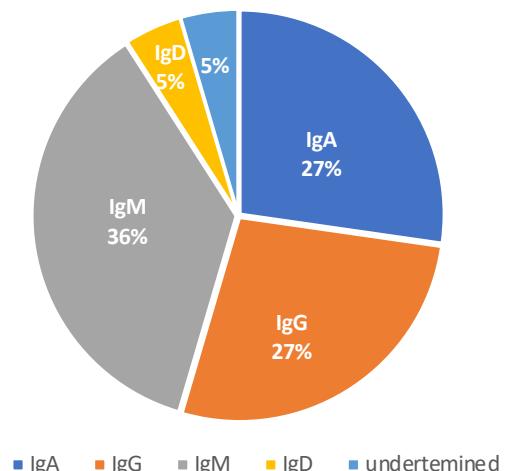
- This analytical module **does not** provide BCR Light chains (IgK & IgL) information
- Not suitable** for blood/PBMC/heme BCR applications, as these require deeper sequencing & higher sensitivity

▼ IgA

IgA Repertoire Clonality						
Clonality				0.10		
Top 10 Clones						
Clone Count	Clone Frequency	CDR3 Clonal Sequence	Top V Hits	Top D Hits	Top J Hits	CDR3 Amino Acid Sequence
182	0.08303	TGTGCCAGTAGCCTAACCGCGGGAGCTCTACATGACGAGTTCTTC	TRBV19		TRBJ2-1	CASLTSGSSYNEQFF
34	0.01551	TGTGCCAGCAGCCAAGTGCGGCGCTGGGCTAGCGGGGGGGCGAACACCGGGGAGCTTTTT	TRBV4-2, TRBV4-3	TRBD2	TRBJ2-2	CASSQVRPWG*RGGANNTGELFF
32	0.01460	TGCAGCGTGAGGGGGGGAGGTCTTATGGCTACACCTTC	TRBV29-1	TRBD1, TRBD2	TRBJ1-2	CSVRGGGLYGYTF
26	0.01186	TGTGCCAGTAGTACAACACTAGTCGCTCTACATGACGAGTTCTTC	TRBV19	TRBD2	TRBJ2-1	CASSTTSSSYNEQFF
20	0.00912	TGTGCCAGCAGCAATTCCGGCTCGGGGTATGGATAACGGCACTTTT	TRBV14	TRBD1	TRBJ2-3	CASSQFALGGMDTQYF
20	0.00912	TGTGCCAGTAGTATAACTAGCGGAACTACAATGACGAGTTCTTC	TRBV19	TRBD2	TRBJ2-1	CASSITSGNYNEQFF
16	0.00730	TGTGCCAGCAGTGAGGGGGGGACAGGGGAGGAATGACGAGTTCTTC	TRBV2	TRBD1	TRBJ2-1	CASSEGGQGRNEQFF
16	0.00730	TGTGCCAGCAGCCCTCCGGGGGGACGGAACACTGAAGCTTTTT	TRBV11-1	TRBD1, TRBD2	TRBJ1-1	CASSLRAGRNTTEAFF
14	0.00639	TGTGCCAGTAGTATCGGGGATTGAACACCGGGGAGCTTTTT	TRBV19		TRBJ2-2	CASSIGELNTGELFF
12	0.00547	TGTGCCAGCAGTTAGGGGGATCGCTTACATGACGAGTTCTTC	TRBV28	TRBD1	TRBJ2-1	CASSLGSLYNEQFF

CDR3 nucleotide and amino acid sequence (Isotype-specific as well)
 CDR3 clone frequency and length distribution
 VDJ – Gene usage and overlap

Isotype composition



Proportion of 4 Isotypes

*IgE is not reported

Role of infiltrating B cells in cancers:

- **Infiltration** of B cells observed in multiple cancers
- **Abundance of BCR clones** implicated in prognosis
 - **Increased clonality of BCR repertoire** was favorably prognostic in some cancers
- Class abundance (**Isotype composition**) varied across different cancers have prognostic implications
 - **High proportion of IgA isotype** has been shown to be associated with **negative prognosis** in melanoma

Landscape of B cell immunity and related immune evasion in human cancers

Xihao Hu, Jian Zhang, Jin Wang, Jingxin Fu, Taiwen Li, Xiaoqi Zheng, Binbin Wang, Shengqing Gu, Peng Jiang, Jingyu Fan, Xiaomin Ying, Jing Zhang, Michael C. Carroll, Kai W. Wucherpfennig, Nir Hacohen, Fan Zhang, Peng Zhang, Jun S. Liu  Bo Li  & X. Shirley Liu 

Nature Genetics 51, 560–567 (2019) | [Cite this article](#)

10k Accesses | 37 Citations | 72 Altmetric | [Metrics](#)

Genome Medicine

RESEARCH

Open Access

Prognostic value of B cells in cutaneous melanoma



Sara R. Selitsky¹, Lisle E. Mose¹, Christof C. Smith¹, Shengjie Chai¹, Katherine A. Hoadley^{1,3}, Dirk P. Dittmer^{1,2}, Stergios J. Moschos^{1,4}, Joel S. Parker^{1,3*} and Benjamin G. Vincent^{1,2,4*}

Isaeva et al. *Journal for ImmunoTherapy of Cancer* (2019) 7:279
<https://doi.org/10.1186/s13073-019-0747-1>

Journal for ImmunoTherapy of Cancer

RESEARCH ARTICLE

Open Access

Intratumoral immunoglobulin isotypes predict survival in lung adenocarcinoma subtypes



O. I. Isaeva^{1,2}, G. V. Sharonov^{3,4}, E. O. Serebrovskaya^{4,5}, M. A. Turchaninova^{3,4}, A. R. Zaretsky^{4,5,6}, M. Shugay^{1,3,4,5} and D. M. Chudakov^{1,3,4,5*} 

Antigen receptor repertoire profiling from RNA-seq data

Dmitriy A Bolotin, Stanislav Poslavsky, Alexey N Davydov, Felix E Frenkel, Lorenzo Fanchi, Olga I Zolotareva, Saskia Hemmers, Ekaterina V Putintseva, Anna S Obraztsova, Mikhail Shugay, Ravshan Ataullakhanov, Alexander Y Rudensky, Ton N Schumacher & Dmitriy M Chudakov 

Nature Biotechnology 35, 908–911 (2017) | [Cite this article](#)

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Concordance studies and use cases



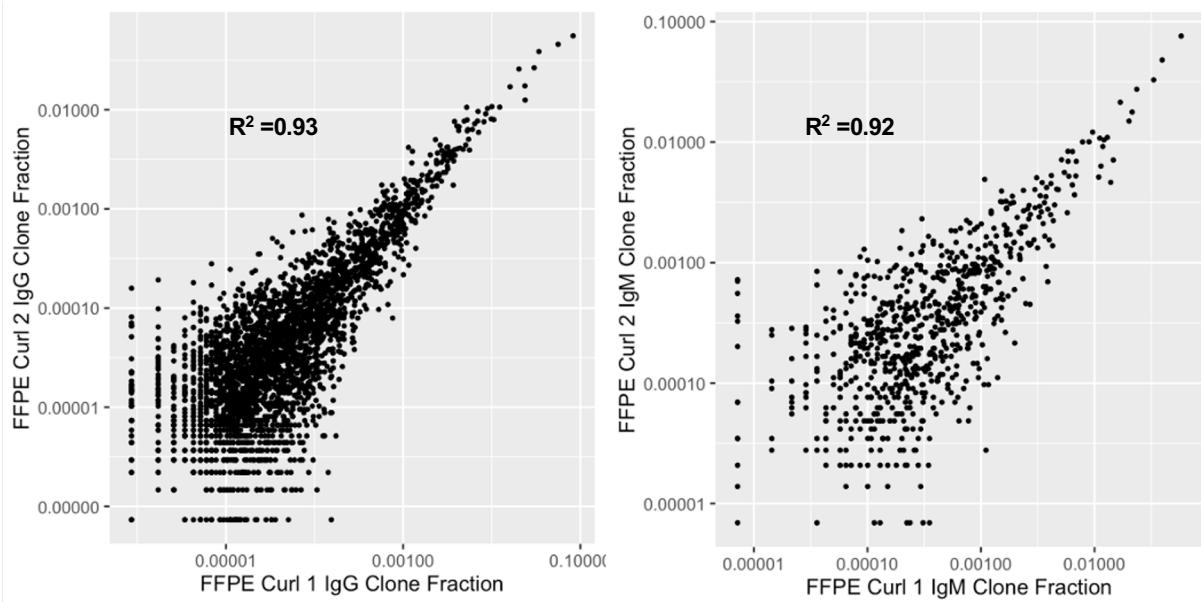
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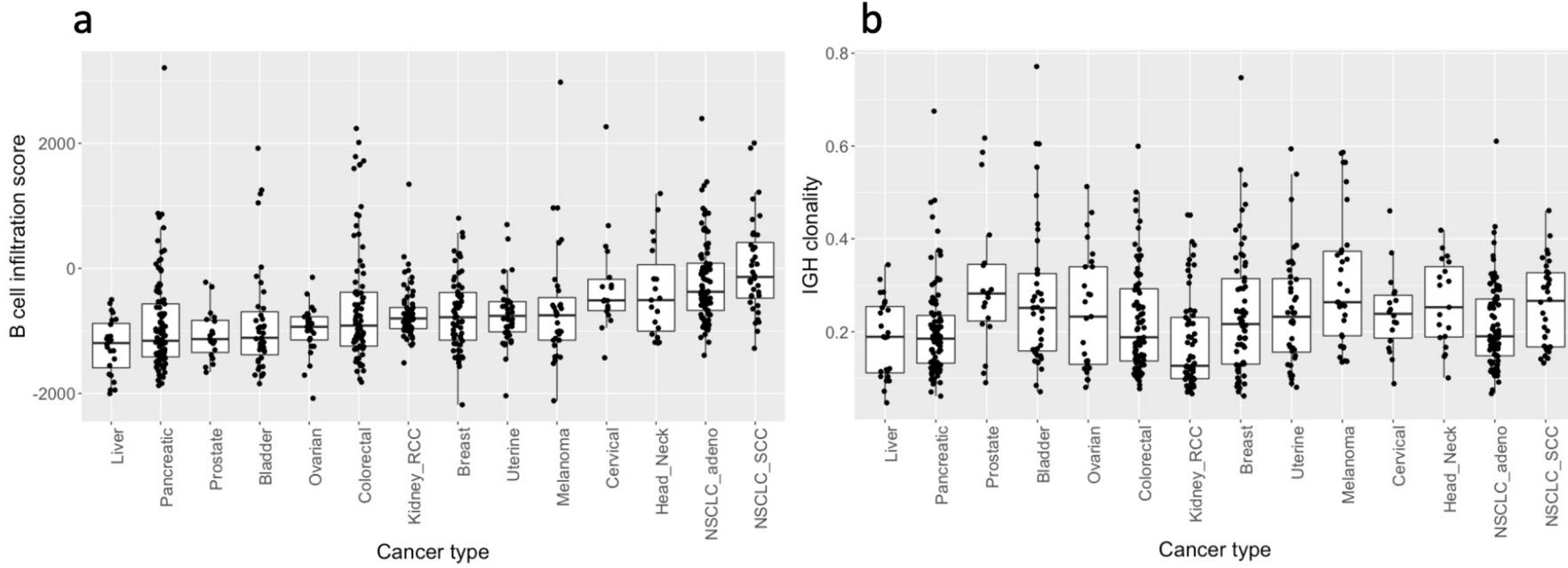


Concordance studies with tumor FFPE samples



- A patient derived FFPE sample was utilized to compare the subsequent curls of the tumor sample
 - The results demonstrates that **a high concordance of clonal abundance** was achieved, and thus showing that our approach can **robustly characterize BCR repertoires** from FFPE samples.

Sample cohort analysis - BCR repertoire clonality distribution plots



- The box plots shows **a) B cell ssGSEA score distribution b) BCR heavy chain repertoire clonality distribution**, across 647 tumor samples from a set of 14 different tumor types, providing novel and important biomarker information to help further elucidate the complex tumor-immune relationship in cancers.

RepertoireID Summary

- Repertoire ID enables **characterization of TCR and BCR Repertoire** from the same sample, along with the enumeration of the underlying **isotype make-up of BCRs** within the tumor microenvironment (TME).
- Repertoire ID provides **novel and relevant biomarker information** to help further elucidate the complex interplay between the tumor cells and immune cells of the TME.
- Repertoire ID leverages NeXT Platform's™ **boosted coverage of BCR & TCR regions** to thoroughly profile the TCR and BCR immune repertoire within the TME.

SHERPA™ Enhancements



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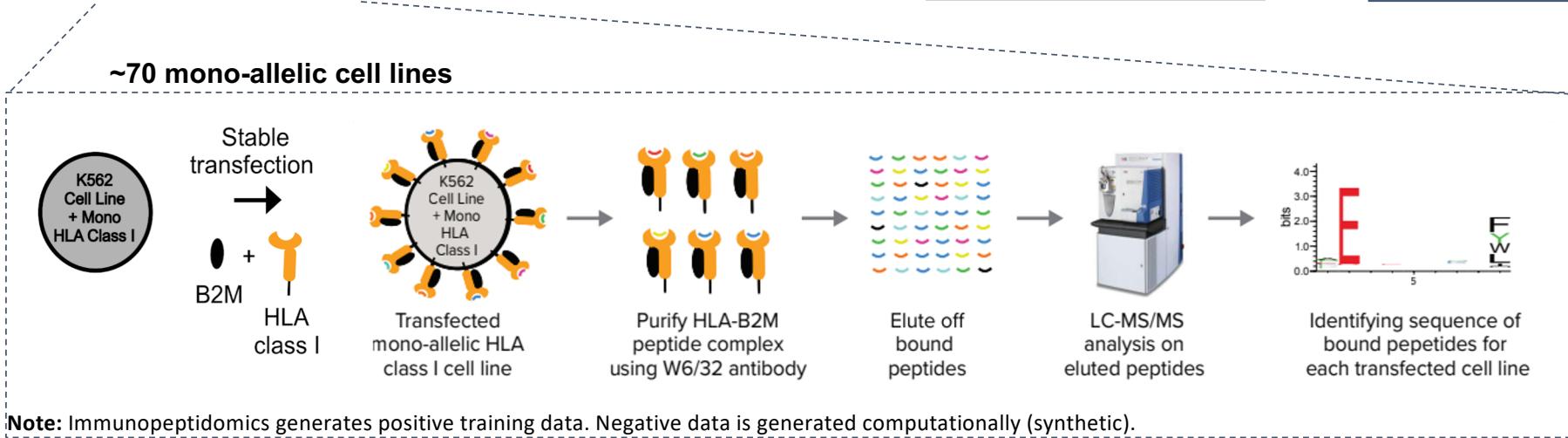
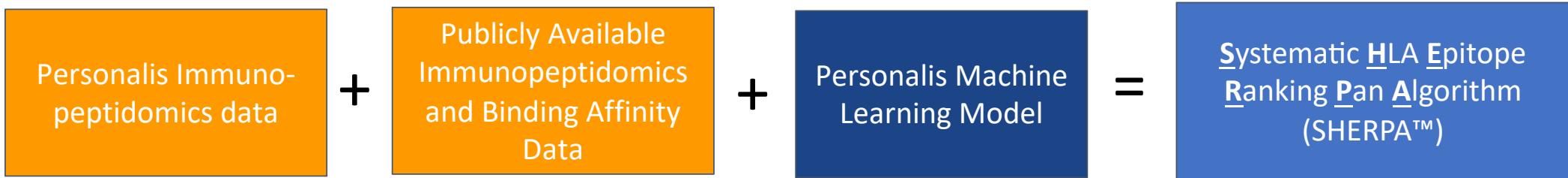
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SHERPA: Neoantigen Machine Learning Algorithm

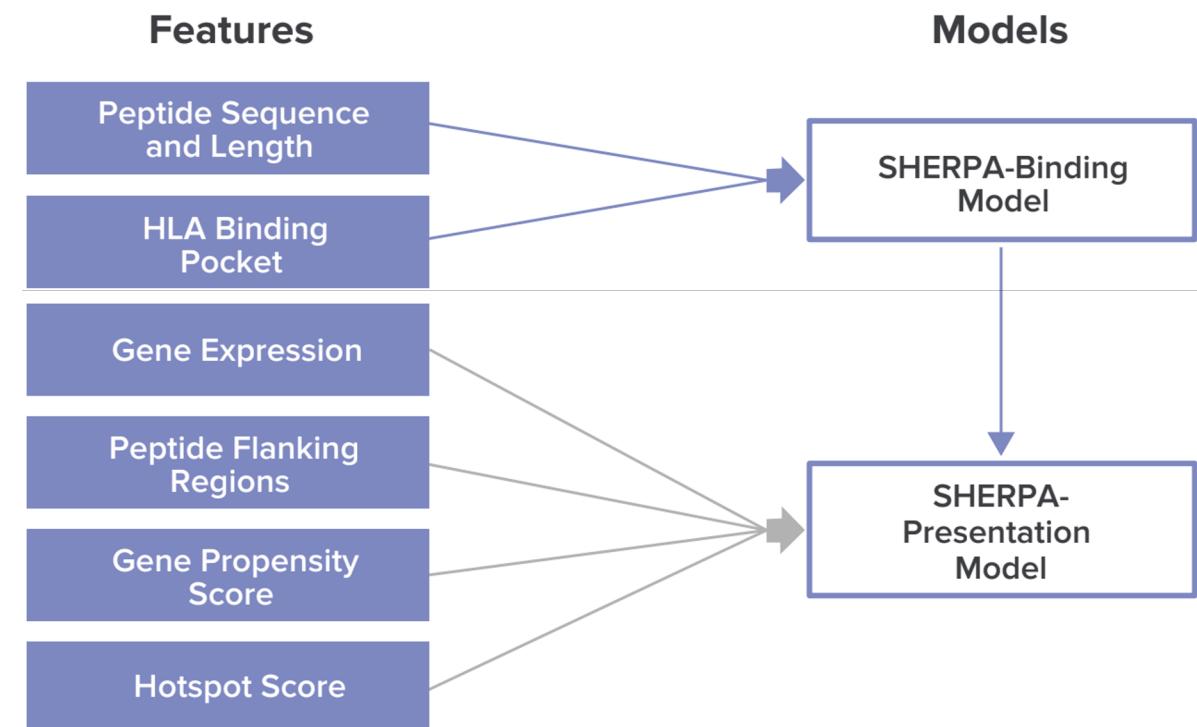
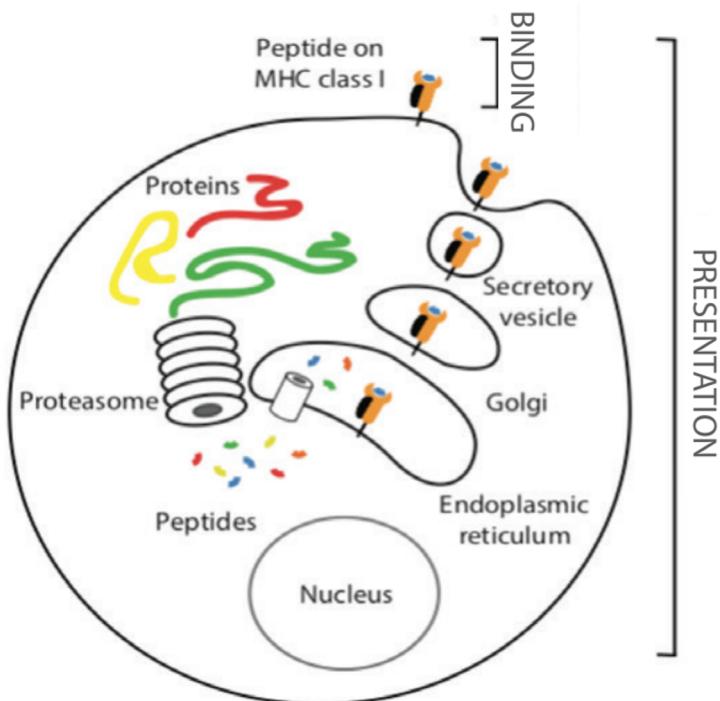
SHERPA is trained on total of 180 human Class I MHC alleles and >1.4 million positive peptides



Note: Immunopeptidomics generates positive training data. Negative data is generated computationally (synthetic).

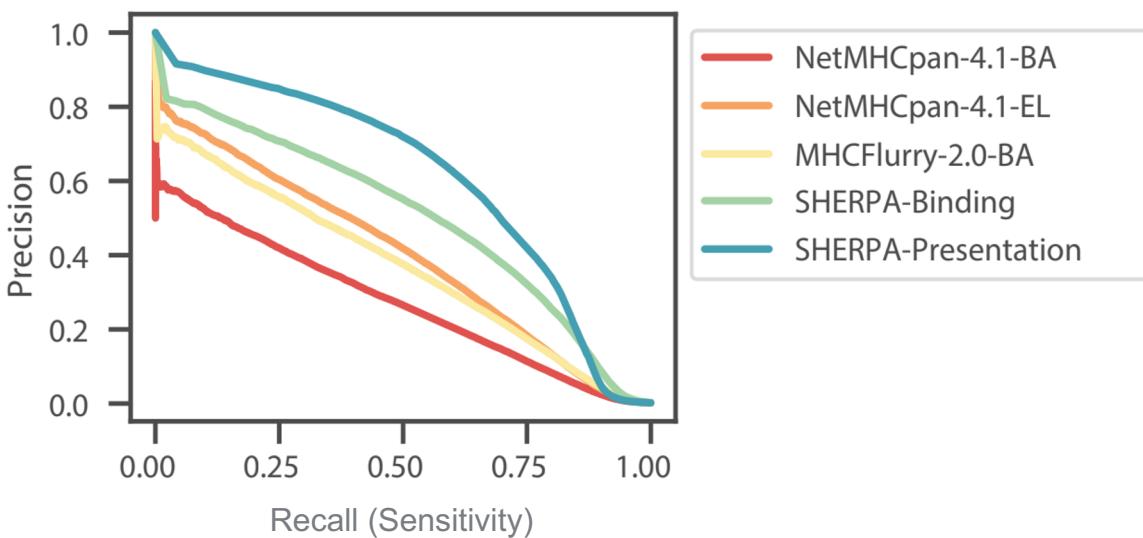
- Integrating data from diverse cell lines and tissue types improves the generalizability of SHERPA, a critically important aspect when applying these models to patient samples.

SHERPA Models Comprehensively Captures Neoantigen Prediction Features

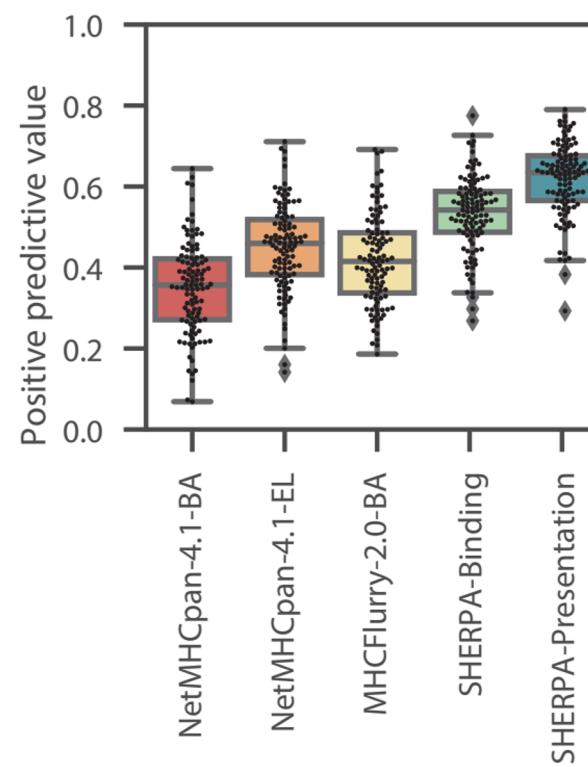


SHERPA Outperforms State-of-the-Art Public Algorithms

A Precision-recall curves
for all alleles (held-out data)



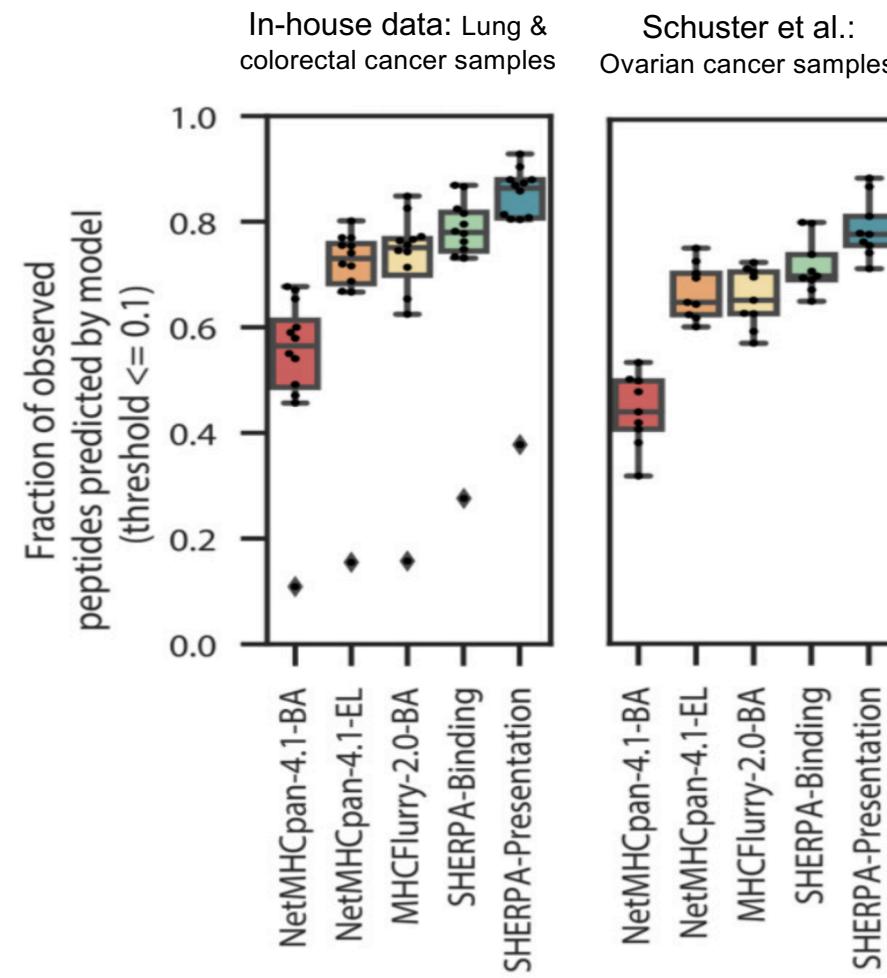
B Positive predictive value (PPV)
for top 0.1% of predicted peptides per allele



- SHERPA demonstrates higher precision at all recall values compared to NetMHCpan and MHCFlurry
- SHERPA displays better overall PPV than NetMHCpan and MHCFlurry

SHERPA has High Recall for Peptides from Independent Tissue Samples

- **Immunopeptidomics on tumor samples**
 - 7 lung cancer, 5 CRC samples
 - Robust peptide yield (4043 median)
 - Total of 46 alleles across 12 patients
 - Two alleles outside of the training dataset
- **ImmunID NeXT**
 - Assign patient specific features
- **MHC binding and presentation prediction evaluation**



SHERPA Publication Available Online



Volume 20, 2021, 100111



Technological Innovation and Resources

Special Issue: Immunopeptidomics

Precision Neoantigen Discovery Using Large-scale Immunopeptidomes and Composite Modeling of MHC Peptide Presentation

Rachel Marty Pyke^{1,‡}, Dattatreya Mellacheruvu^{1,‡}, Steven Dea¹, Charles W. Abbott¹, Simo V. Zhang¹, Nick A. Phillips¹, Jason Harris¹, Gabor Bartha¹, Sejal Desai¹, Rena McClory¹, John West¹, Michael P. Snyder², Richard Chen^{1,§}, Sean Michael Boyle^{1,§,✉}

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<https://doi.org/10.1016/j.mcpro.2021.100111>

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Highlights

- Generated 25 stably transfected monoallelic cell lines and applied immunopeptidomics.
- Harmonized 512 public immunopeptidomic samples through systematic reprocessing.
- Developed pan-allele MHC-binding algorithm (SHERPA) utilizing 167 human HLA alleles.
- SHERPA demonstrates up to 1.44-fold increased precision over competing algorithms.

Note: The commercial version of SHERPA has been trained on 180 unique human Class I MHC alleles including immunopeptidomics data from ~70 in-house generated monoallelic cell lines

SHERPA Summary

- SHERPA integrates diverse and large amounts of training data for accurate neoantigen prediction.
- SHERPA utilizes comprehensive binding and presentation features, aligned with TESLA consortium guideline, as well as other publicly available tools.
- Performance evaluation analysis shows that SHERPA has higher accuracy and superior MHC presentation prediction as compared to NetMHCpan and MHCFlurry.

GRCh38 Reference Genome



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Immunoid NeXT™ is Now Available with the Option of GRCh38 Reference Genome Alignment

- In Silico equivalency study performed on GRCh38 alignment
 - Find similar sensitivity and specificity of Immunoid Next with GRCh38 compared to GRCh37 alignment
- Currently biomarker use cases (T/N and T Only) have been prioritized for this release
 - PCT and other use cases can be prioritized depending on the need
 - NeXT Dx™ is out of scope
- Variant level data comparison between GRCh37 and GRCh38 alignment output is not supported
 - Other publications supporting this finding as well
- All analytics except InfiltrateID™ are provided with GRCh38

In Silico Equivalency Study Results – Exome Small Variants

- Immunoid NeXT Exome @ ~300X average coverage
 - Panel-grade performance at LOD as low as $\geq 5\%$ minor allelic fraction (MAF)

Analytical Sensitivity

	GRCh37		GRCh38	
Analytical Sensitivity	SNVs	Indels	SNVs	Indels
	99.00%	97.00%	98.00%	97.00%

Analytical Sensitivity = TP (True Positive) * 100 / (TP + FN (False Negative))

Limit of Detection (LOD)

	Sensitivity (PPA)				Specificity (PPV)			
	GRCh37		GRCh38		GRCh37		GRCh38	
Metric	$\geq 5\%$	$\geq 10\%$	$\geq 5\%$	$\geq 10\%$	$\geq 5\%$	$\geq 10\%$	$\geq 5\%$	$\geq 10\%$
SNVs	96.81%	98.70%	96.80%	98.68%	97.91%	99.00%	98.01%	99.03%
Indels	87.70%	93.90%	87.38%	94.46%	88.64%	93.98%	88.30%	93.40%

The only difference is alignment,
no difference in the pipeline

Equivalency Study Results – Transcriptome Small Variants and Fusions

- ImmunID NeXT Transcriptome @ 100M paired-end reads (200M total reads)
 - Optimized for detection of known/novel gene fusions
 - Expression-based confirmation of somatic small variants
 - Small variants detected in the RNA filtered against those detected in highly-specific DNA small variant detection pipeline
 - Varying gene expression levels, RNA editing, and alternative splicing commonly lead to FPs in RNA

Analytical Sensitivity

	GRCh37		GRCh38	
Analytical Sensitivity	Small variants	Fusions	Small variants	Fusions
	96.3%	100%	96%	100%

Analytical Sensitivity = TP (True Positive) * 100 / (TP + FN (False Negative))

Fusion specificity was >99% for both GRCh37 and GRCh38

Thank You!



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