



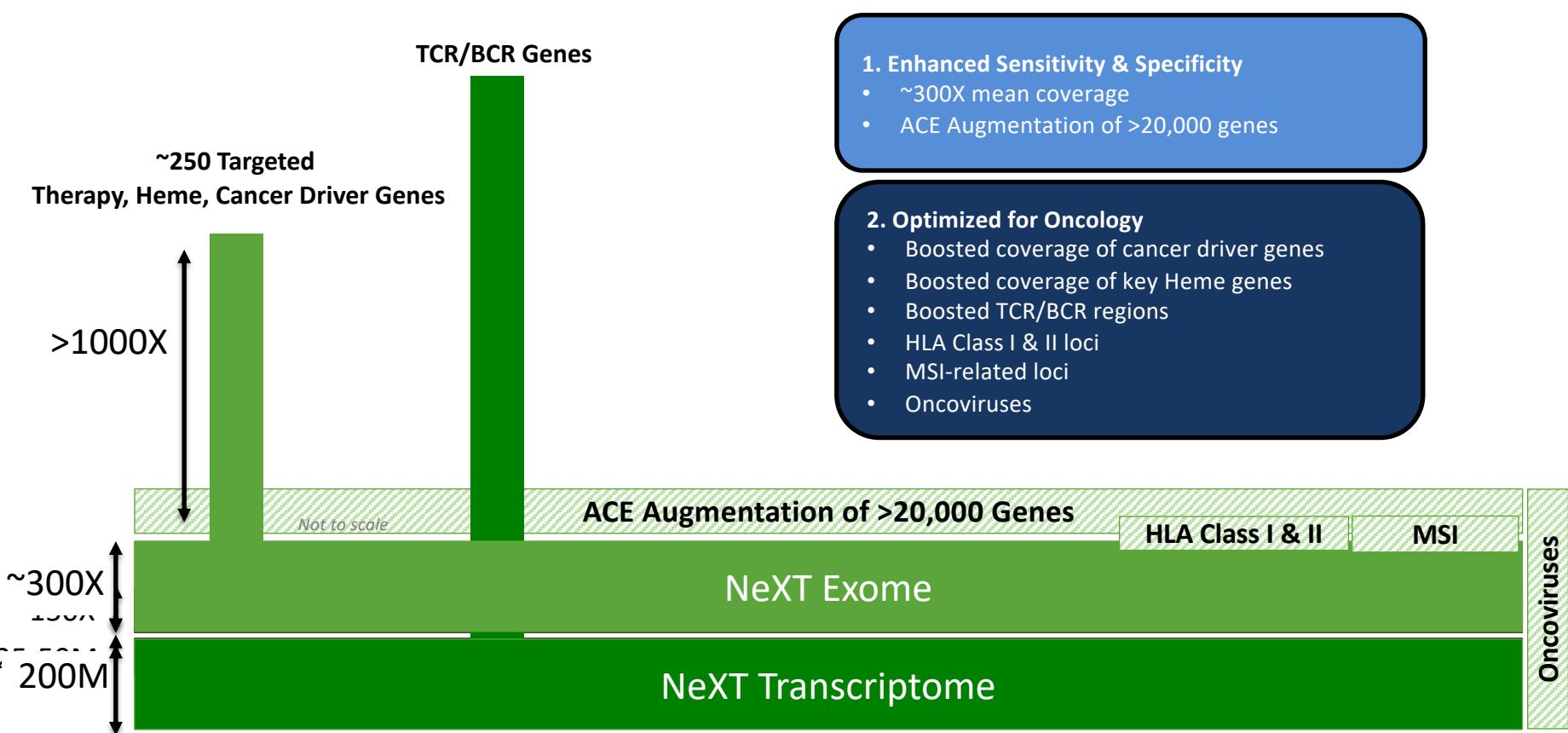
ImmunoID NeXT Example Data Set

Precision Genomics for Immuno-Oncology

© 2019 Personalis, Inc. All rights reserved. | Confidential and proprietary



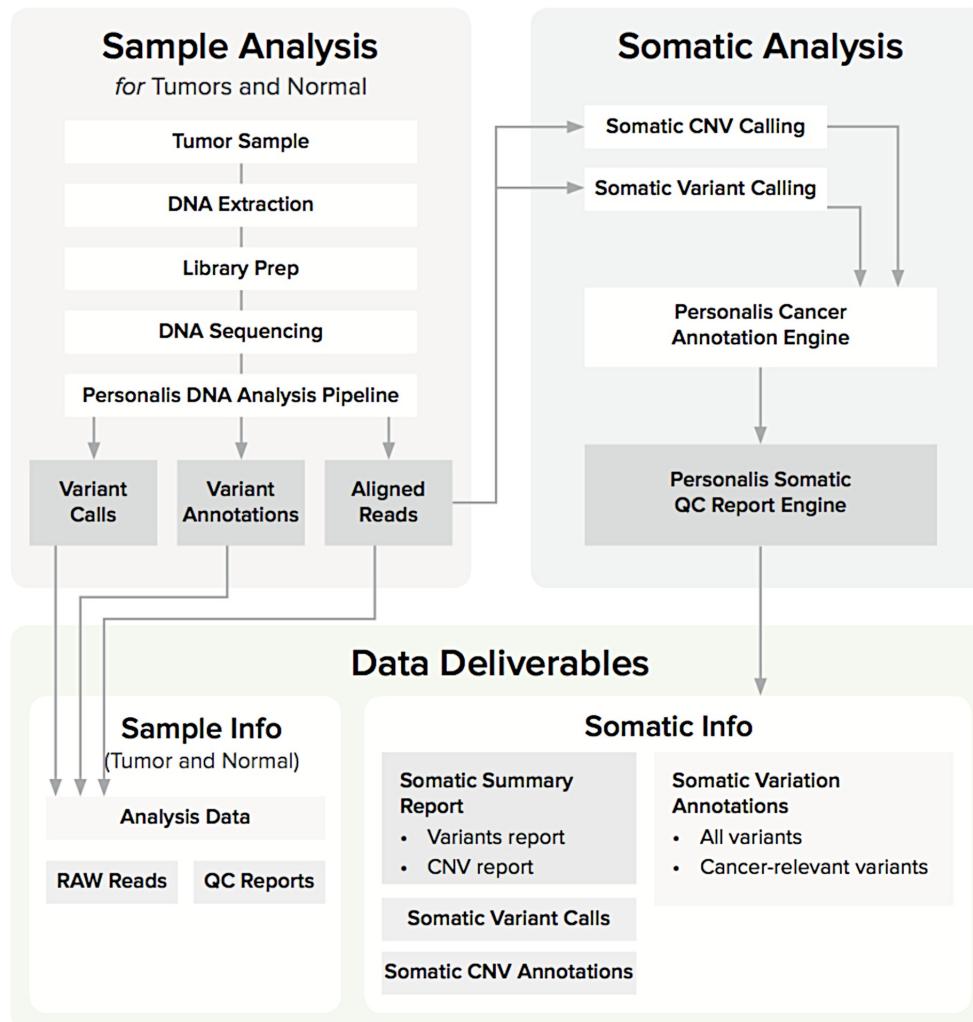
Immunoid NeXT: Optimized for Oncology & Immuno-oncology



Sample Information

- Procured FFPE NSCLC Sample (Stage IA, Adenocarcinoma)
- ImmunOID NeXT Configuration (NovaSeq; 2x150 bp)
 - ImmunOID NeXT Exome @ 35G of Tumor ~ 300X
 - ImmunOID NeXT Exome Normal @ 20G of Normal ~ 150X
 - ImmunOID NeXT Transcriptome @ 100 M paired reads (200 total reads)

Cancer DNA Workflow and Data Deliverables



Main File Structure

- The main file structure has various subfolders as shown below:

Name

 Report_Neoantigen_125443

 Report_RNA_TCR_125446

 Report_RNA_125436

 Report_QC_125437

 Report_DNA_125500

DNA QC Report



Name



Report_Neoantigen_125443



Report_RNA_TCR_125446



Report_RNA_125436



Report_QC_125437



Report_DNA_125500

Name



QC_REPORT

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

■■■ Sample and Run Information

Analysis mode	Tumor/Normal
Tumor sample	DNA_ILS44558PT6-0
Matched normal sample	DNA_ILS44558PA1
Pipeline versions	CAN_DNA_v2.7.0, CORE_DNA_v1.10.0
Annotation version	4
Platform version	ACE v4
Reference assembly	hs37d5

- Provided as both .html and .tsv format

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

Cancer DNA

Cancer RNA

Neoantigen

MSI

TCR

Oncovirus

HLA

Immunogenomics



Sequencing Information

	Normal DNA	Tumor DNA
Read length (bp)	150	150
Total reads	166,670,728	333,371,002
Total bases	25,000,609,200	50,005,650,300
Average base quality	29.71	29.46
Sex chromosome count	XY	N/A
Predicted sex	M	N/A
Predicted blood type	B	N/A
Percent contamination in Normal	< .5%	N/A
Percent contamination in Tumor	N/A	< .5%

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

Cancer DNA

Cancer RNA

Neoantigen

MSI

TCR

Oncovirus

HLA

Immunogenomics

Alignment Information

	Normal DNA	Tumor DNA
Average read depth	169.61	305.66
Percent mapped reads	99.90%	99.89%
Average mapping quality	53.48	53.08
Percent duplicate read pairs	7.04%	14.69%
Capture specificity	0.68	0.66
Insert size	200.98 +/- 72.16	197.24 +/- 74.28

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

Somatic Variant Calling and Annotation

Summary Small Variants

	SNVs	Indels	Total
Somatic variants	339	7	346
Somatic variants per Mb	4.58	0.09	4.67
Ti/Tv ratio	1.55	N/A	1.55

Mutational Burden

	SNVs	Indels	Total
Non-synonymous Somatic Variants	99	7	106
Non-synonymous Somatic Variants per Mb	1.33	0.09	1.42

SNV Statistics

Reference Base	Alternate Base			
	A	C	G	T
A	0	6	25	5
C	14	0	32	86
G	74	46	0	15
T	7	21	8	0

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

Cancer DNA

Cancer RNA

Neoantigen

MSI

TCR

Oncovirus

HLA

Immunogenomics

▼ Functional Annotation

	SNVs	Indels	Total
Nonsense	7	0	7
Missense	100	0	100
Silent	42	0	42
None	190	7	197

HTML Summary of DNA QC Reports



Personalis®

Results Summary Report for DNA_ILS44558PT6-0

Cancer DNA

Cancer RNA

Neoantigen

MSI

TCR

Oncovirus

HLA

Immunogenomics

▼ Effect Annotation

	SNVs	Indels	Total
Downstream	12	0	12
Frame shift	0	3	3
Intragenic	4	0	4
Intron	136	2	138
Nonsynonymous coding	99	0	99
Other	14	1	15
Splice site acceptor	2	0	2
Splice site region	16	0	16
Start gained	1	0	1
Stop gained	7	0	7
Synonymous coding	42	0	42
Upstream	3	0	3
UTR 3 prime	15	0	15
UTR 5 prime	6	1	7

Main File Structure

- The main file structure has various subfolders as shown below:

Name

 Report_Neoantigen_125443

 Report_RNA_TCR_125446

 Report_RNA_125436

 Report_QC_125437

 Report_DNA_125500

Main File Structure

- The main file structure has various subfolders as shown below:

Name

	Report_Neoantigen_125443
	Report_RNA_TCR_125446
	Report_RNA_125436
	Report_QC_125437
	Report_DNA_125500

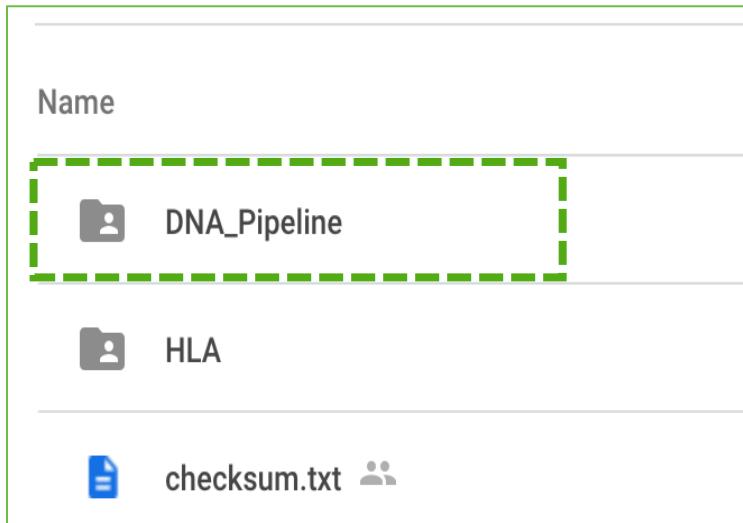
Name

	DNA_Pipeline
	HLA
	checksum.txt

DNA Pipeline



- The DNA report folder contains:
 - HLA-related analytics
 - DNA Pipeline output

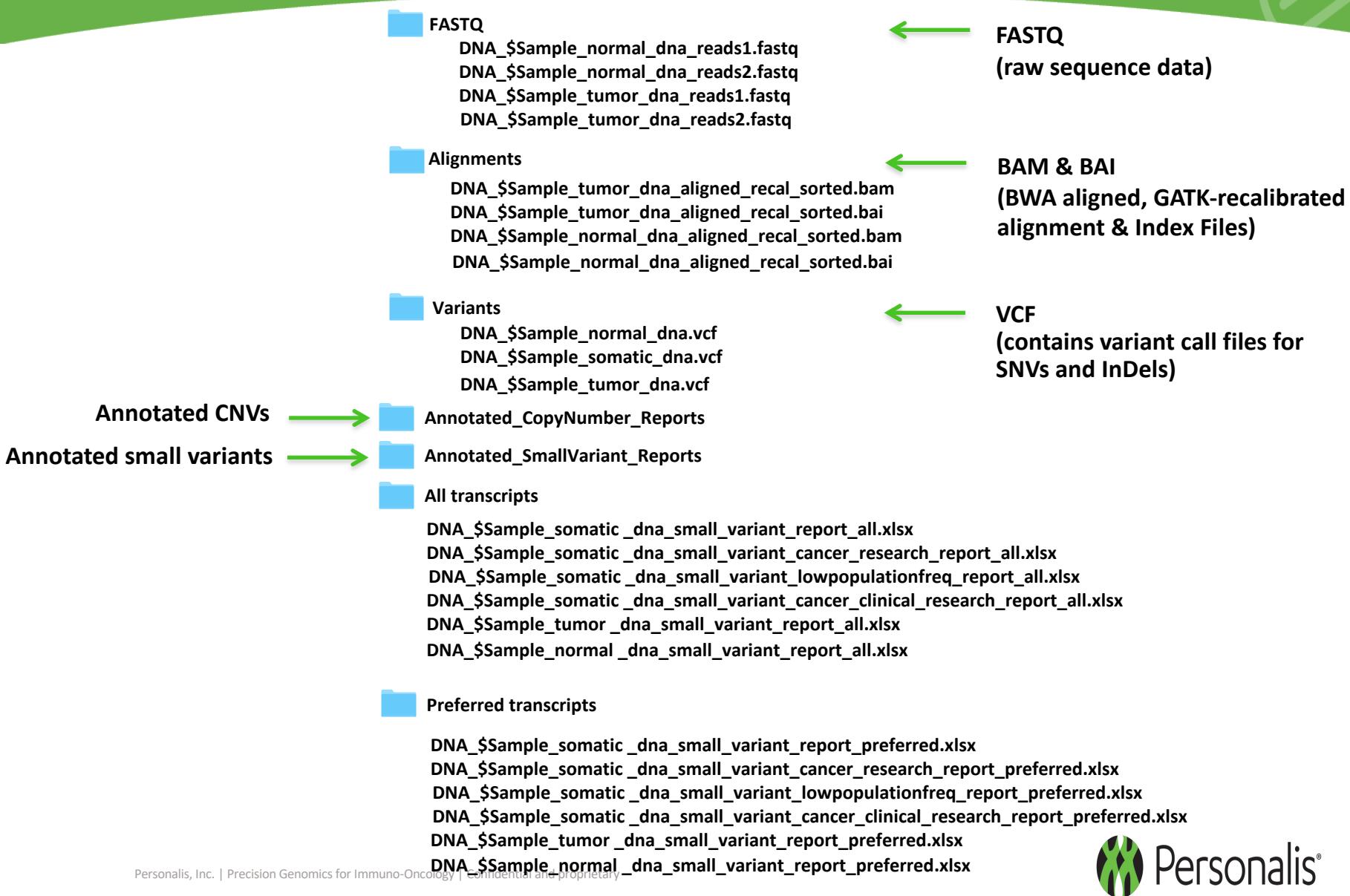


DNA Pipeline

- The DNA report folder contains:
 - HLA-related analytics
 - DNA Pipeline output



Format of DNA Data Deliverables





Variants

DNA_ILS44558PA1_normal_dna.vcf.gz

Contains all SNVs and InDels called by Personalis Pipeline in the Normal (vs. Reference Genome)

DNA_ILS44558PT6-0_tumor_dna.vcf.gz

Contains all SNVs and InDels called by Personalis Pipeline in the Tumor (vs. Reference Genome)

DNA_ILS44558PT6-0_somatic_dna.vcf

Contains all SNVs and InDels called by Personalis Somatic Pipeline and filtered using Personalis analysis tool

DNA Small Variant Annotation



Small Variants

All transcripts

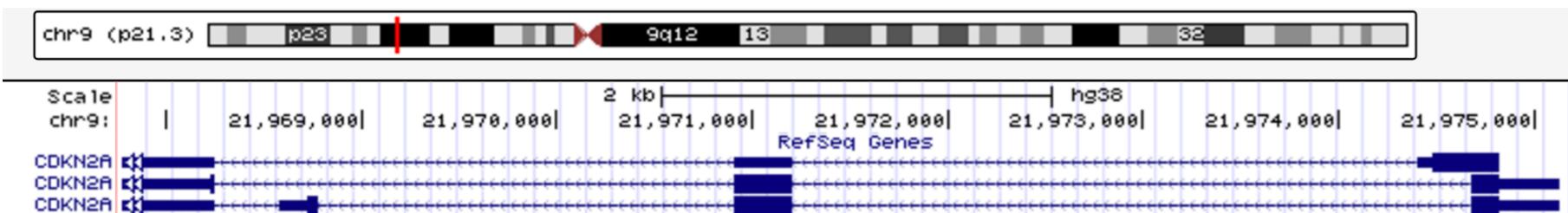
DNA_\${Sample}_somatic_dna_small_variant_report_all.xlsx
DNA_\${Sample}_somatic_dna_small_variant_cancer_research_report_all.xlsx
DNA_\${Sample}_somatic_dna_small_variant_lowpopulationfreq_report_all.xlsx
DNA_\${Sample}_somatic_dna_small_variant_cancer_clinical_research_report_all.xlsx
DNA_\${Sample}_tumor_dna_small_variant_report_all.xlsx
DNA_\${Sample}_normal_dna_small_variant_report_all.xlsx

Preferred transcripts

DNA_\${Sample}_somatic_dna_small_variant_report_preferred.xlsx
DNA_\${Sample}_somatic_dna_small_variant_cancer_research_report_preferred.xlsx
DNA_\${Sample}_somatic_dna_small_variant_lowpopulationfreq_report_preferred.xlsx
DNA_\${Sample}_somatic_dna_small_variant_cancer_clinical_research_report_preferred.xlsx
DNA_\${Sample}_tumor_dna_small_variant_report_preferred.xlsx
DNA_\${Sample}_normal_dna_small_variant_report_preferred.xlsx

All Transcripts and Preferred Transcripts definition

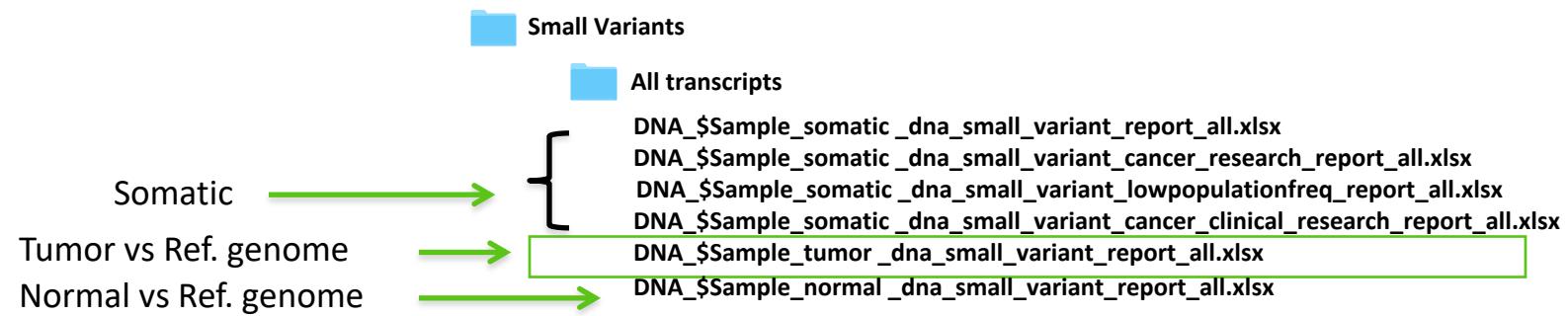
Genes often have many overlapping transcripts



Which transcript should be chosen for variant annotation?

- We select the transcript in which the called variant has its maximal effect impact on the protein
- This is followed by most cancer associated (according to COSMIC) and then longest CDS

DNA Small Variant Annotation



DNA Somatic Small Variant Annotation Report Filtering

- somatic_dna_small_variant_report.xlsx
 - AF ≥ 0.05 for exome
-
- **_lowpopulationfreq_report**
 - *Population AF across pop. columns <1%*
-
- **_cancer_research_report**
 - *Population AF across pop. columns <1%*
 - Gene in ACE Cancer Panel Gene List (>1400 genes)
 - Effect Impact = HIGH | MODERATE
-
- **_cancer_clinical_research_report**
 - *Population AF across pop. columns <1%*
 - Gene in ACE Clinical Cancer List (~250 genes)
 - Effect Impact = HIGH | MODERATE

Variant Annotation Report Content

Variant ID	Unique identifier within a sample	16
Sequence	Chromosome # where variant was identified	1
POS	Chromosome Position of the variant as defined by the VCF	24406588
REF	Reference sequence for this variant	C
ALT	Alternate sequence for this variant	T
Quality score	Base quality score for reads that pass filter	32
Total read depth	Number of high quality reads at this position	343
Reads supporting REF	Number of high quality reads supporting the REF	305
Reads supporting ALT	Number of high quality reads at this position supporting the ALT	37
Allelic fraction	Allelic fraction of variant relative to reference allele	0.108
Genomic variant	HGVS nomenclature for the genomic variant	g.24406588C>T
Gene symbol	Gene associated with the variant based on HUGO gene nomenclature	MYOM3
NCBI gene	Gene ID provided by NCBI	127294
Variant type	SNV, InDel	SNV
Transcript ID	Refseq accession version for the transcript used for variant analysis	NM_152372.3
Preferred Transcript	Yes/No	Y
Transcript biotype	Protein-coding, ncRNA	Protein-coding
Transcript Variant	Description of variant at the transcript level	c.2504G>A
Protein ID	Refseq accession version for the protein used for variant analysis	NP_689585.3
Protein variant	Description of variant at the protein level	p.G835D
Variant effect	Effect a variant has on the associated protein sequence	missense
Variant Effect Impact	Predicted impact of this variant	moderate
Functional Class	Based on snpEFF (None, silent, missense, nonsense)	missense
Codon Change	The variant in the context of the codon (e.g.	gGc/gAc
Exon Number	Exon number the variant is found in with respect to the transcript	20
Databases (columns Z-AO)	COSMIC, CGC, Population DB, Protein	Additional information on variant

Copy Number Report

- Two complementary strategies to detect somatic copy number alterations in cancer exomes:
 - ✓ Read-depth and minor allele frequency
- The Pipeline features two tools:
 - CNAsate (Personalis in-house proprietary algorithm; annotated reports)
 - Sequenza (Open source; plots)
- Regions of hyper-amplification (CN >=6) or complete deletion (CN=0)
- The Annotated Copy Number Reporting consists of 3 files as detailed below:



Annotated_CopyNumber_Reports

DNA_ILS44558PT6-0_somatic_dna_gene_cna_cancer_clinical_research_report.xlsx

DNA_ILS44558PT6-0_somatic_dna_gene_cna_cancer_research_report.xlsx

DNA_ILS44558PT6-0_somatic_dna_gene_cna_research_report.xlsx

HTML Summary of Copy Number Results



Somatic Copy Number Information

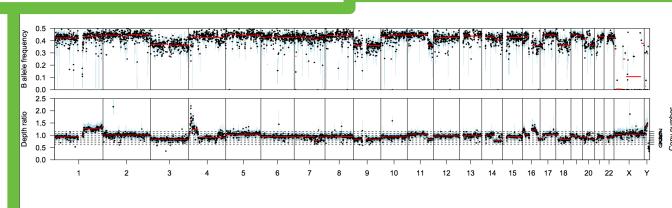
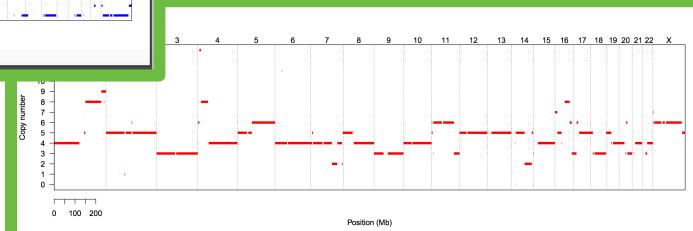
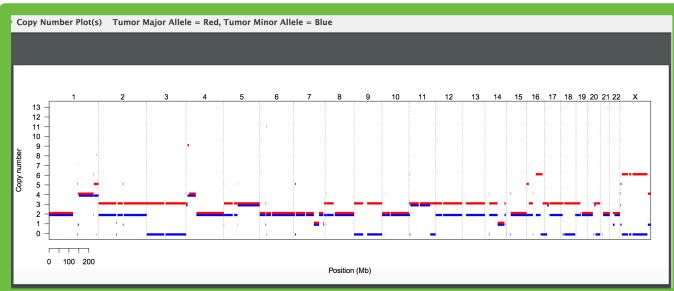
Tumor purity 0.20

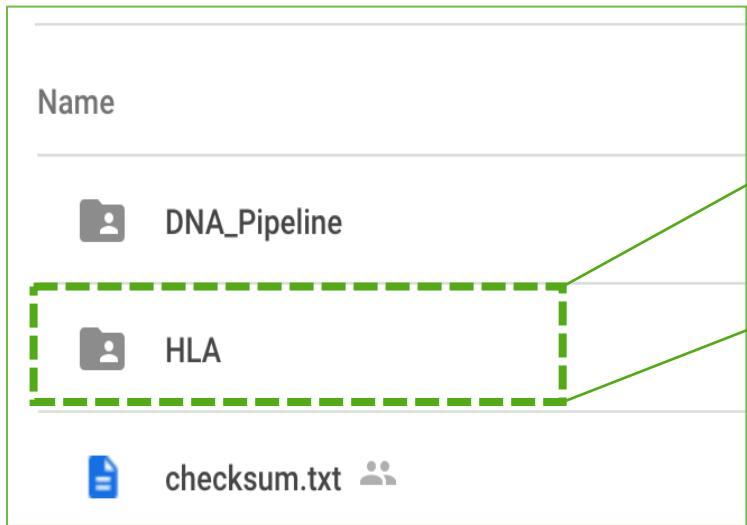
Tumor ploidy 5

Regions with complete deletions (CN=0) 0

Regions with amplification (CN>=6) 165

Personalis Cancer Genes in CN altered regions 0





- DNA_ILS44558PT6-0_HLA_somatic_mutation_report.xlsx
- DNA_ILS44558PA1_HLA.xlsx
- DNA_ILS44558PA1_HLA_allele_specific_deletions.xlsx

HLA folder contains the following:

- HLA Typing from normal specimen (HLAssign)
- Somatic HLA calling
- HLA LOH (proprietary in-house tool DASH: Deletion of Allele-Specific HLAs)

HTML Summary of HLA Analytics: HLA Typing



Personalis® Results Summary Report for DNA_ILS44558PA1

Cancer DNA Cancer RNA Neoantigen MSI TCR Oncovirus **HLA** Immunogenomics

HLA Germline Typing

Class I

Gene	Alleles
HLA-A	34:01:01,24:02:01:01
HLA-B	15:02:01,51:01:02
HLA-C	14:02:01,08:01:01

Class II

Gene	Alleles
HLA-DPA1	01:03:01:01,02:02:02
HLA-DPB1	03:01:01,05:01:01
HLA-DQA1	06:01:01,01:01:01
HLA-DQB1	03:01:01:01,05:02:01
HLA-DRB1	15:02:01,12:02:01

HTML Summary of HLA Analytics: HLA Somatic Variant Calling



Personalis®

Results Summary Report for DNA_ILS44558PA1

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

HLA Somatic Mutations

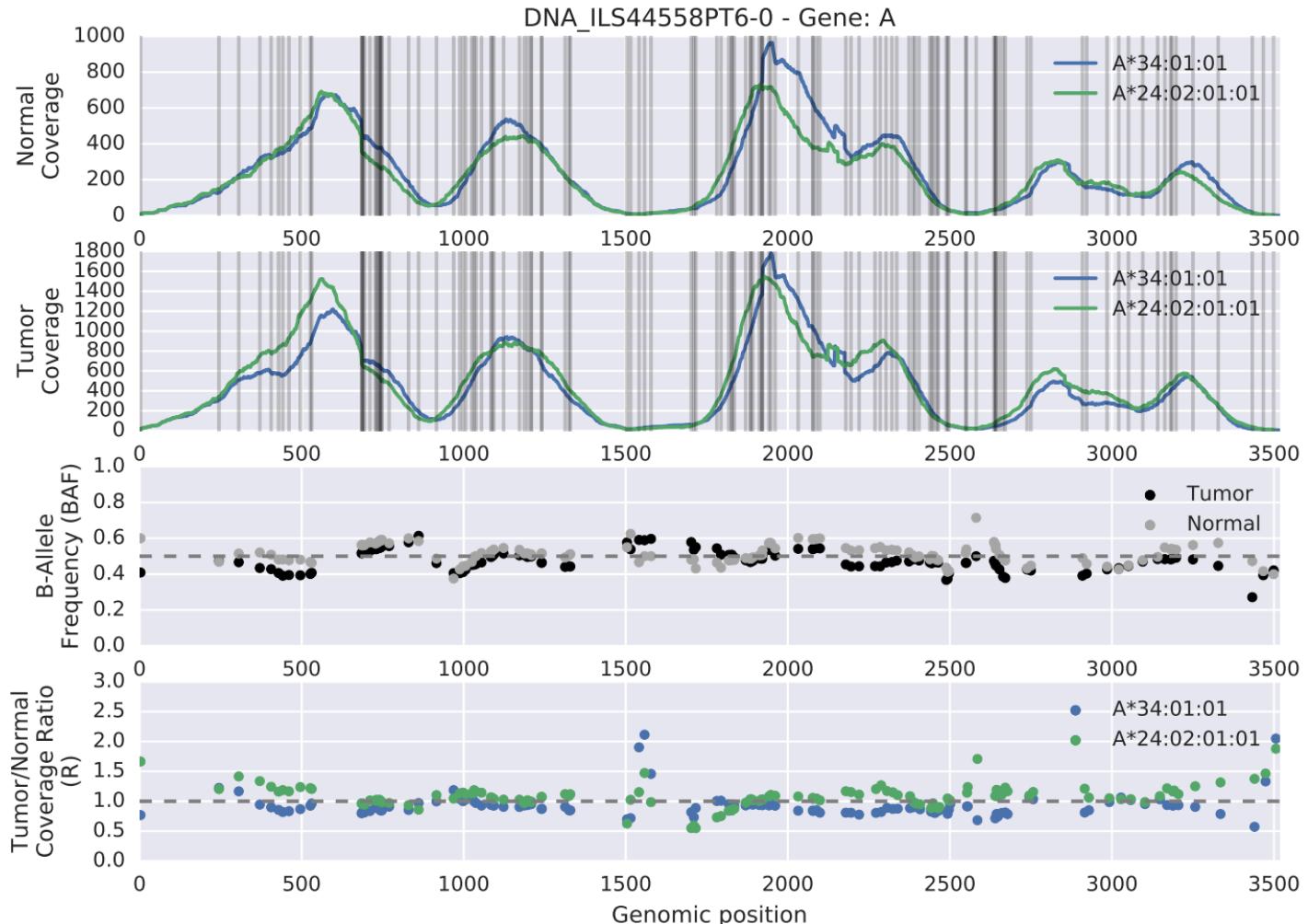
Gene Symbol	Common Symbol	General Category	RNA Gene Expression (TPM)	Variant Detection Status
B2M	B2M	Antigen Presenting Machinery	2,937.76	No Variant detected
HLA-A	HLA-A	Antigen Presenting Machinery	338.26	Refer to HLA Somatic Mutation data file for variants
HLA-B	HLA-B	Antigen Presenting Machinery	463.24	Refer to HLA Somatic Mutation data file for variants
HLA-C	HLA-C	Antigen Presenting Machinery	336.36	Refer to HLA Somatic Mutation data file for variants

HTML Summary of HLA Analytics: HLA LOH Calling

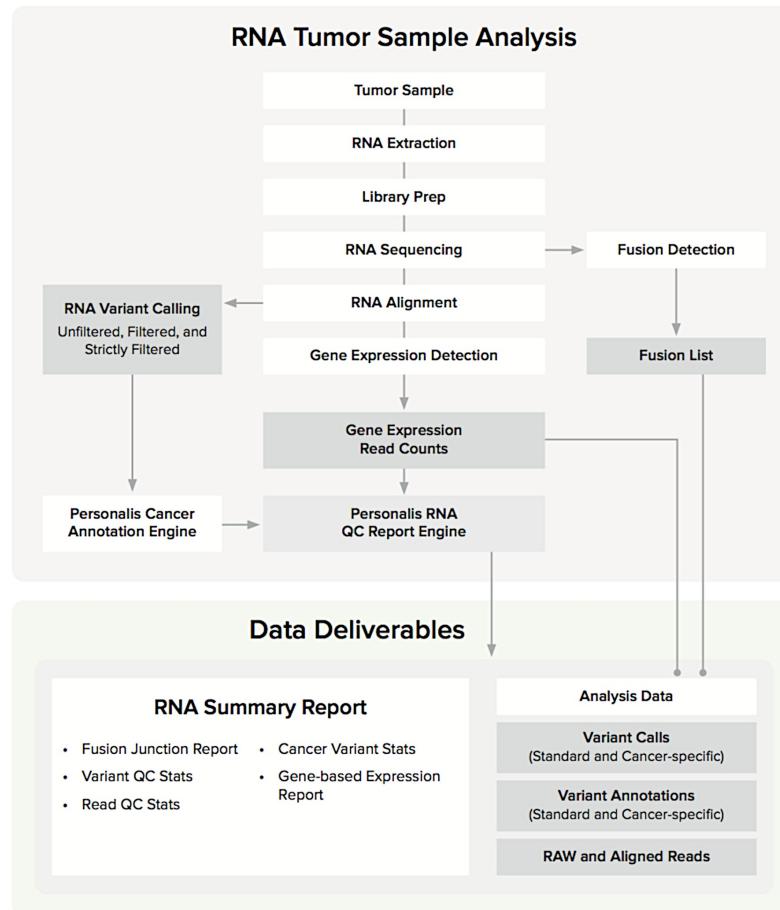


Results Summary Report for DNA_ILS44558PA1

Cancer DNA Cancer RNA Neoantigen MSI TCR Oncovirus **HLA** Immunogenomics



Cancer RNA Workflow and Data Deliverables



RNA Deliverables



Name



Report_Neoantigen_125443



Report_RNA_TCR_125446



Report_RNA_125436



Report_QC_125437



Report_DNA_125500

RNA QC Report



Name



Report_Neoantigen_125443



Report_RNA_TCR_125446



Report_RNA_125436



Report_QC_125437



Report_DNA_125500

Name



QC_REPORT

HTML Summary of RNA QC Report



Sequencing Information

Read length (bp)	150
Total reads	200,002,214
Total bases	30,000,332,100
Average base quality	36.31

HTML Summary of RNA QC Report



Personalis®

Results Summary Report for RNA_ILS44558PT6-0

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

Alignment Information

Number of remote sites

Number of reads mapped to transcript

Percentage of reads mapped to transcript

Average length of mapped reads

Number of unique genomic sites

Number of unique genomic sites

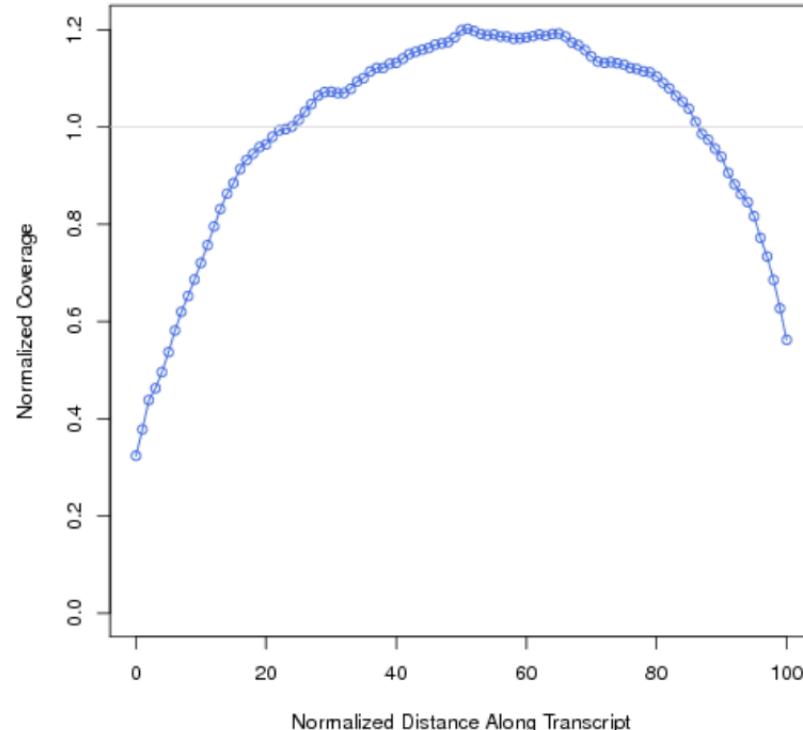
▼ Mapping Occurrence Metrics

	Counts
Reads mapped 1 time	139,56
Reads mapped 2 times	4,37
Reads mapped 3 times	1,63
Reads mapped 4 times	54
Reads mapped >5 times	74

▼ Mapping Gene Element Metrics

	Counts
Exon	117,102,880
Intron	6,950,112
Intergenic	17,947,228
Promoter	473,966
UTR-3	3,134,780
UTR-5	1,257,194

Transcript Coverage Plot



RNA Deliverables



Name



Report_Neoantigen_125443



Report_RNA_TCR_125446



Report_RNA_125436

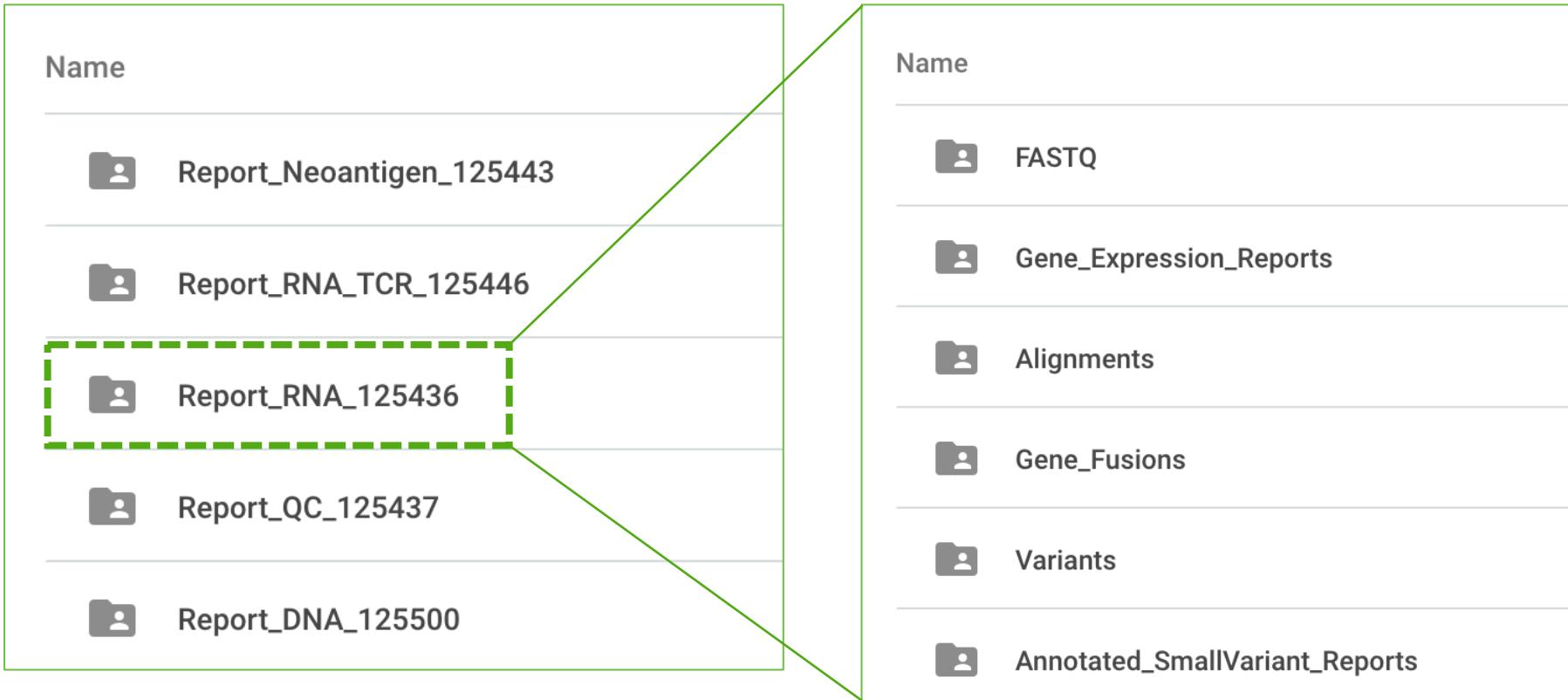


Report_QC_125437

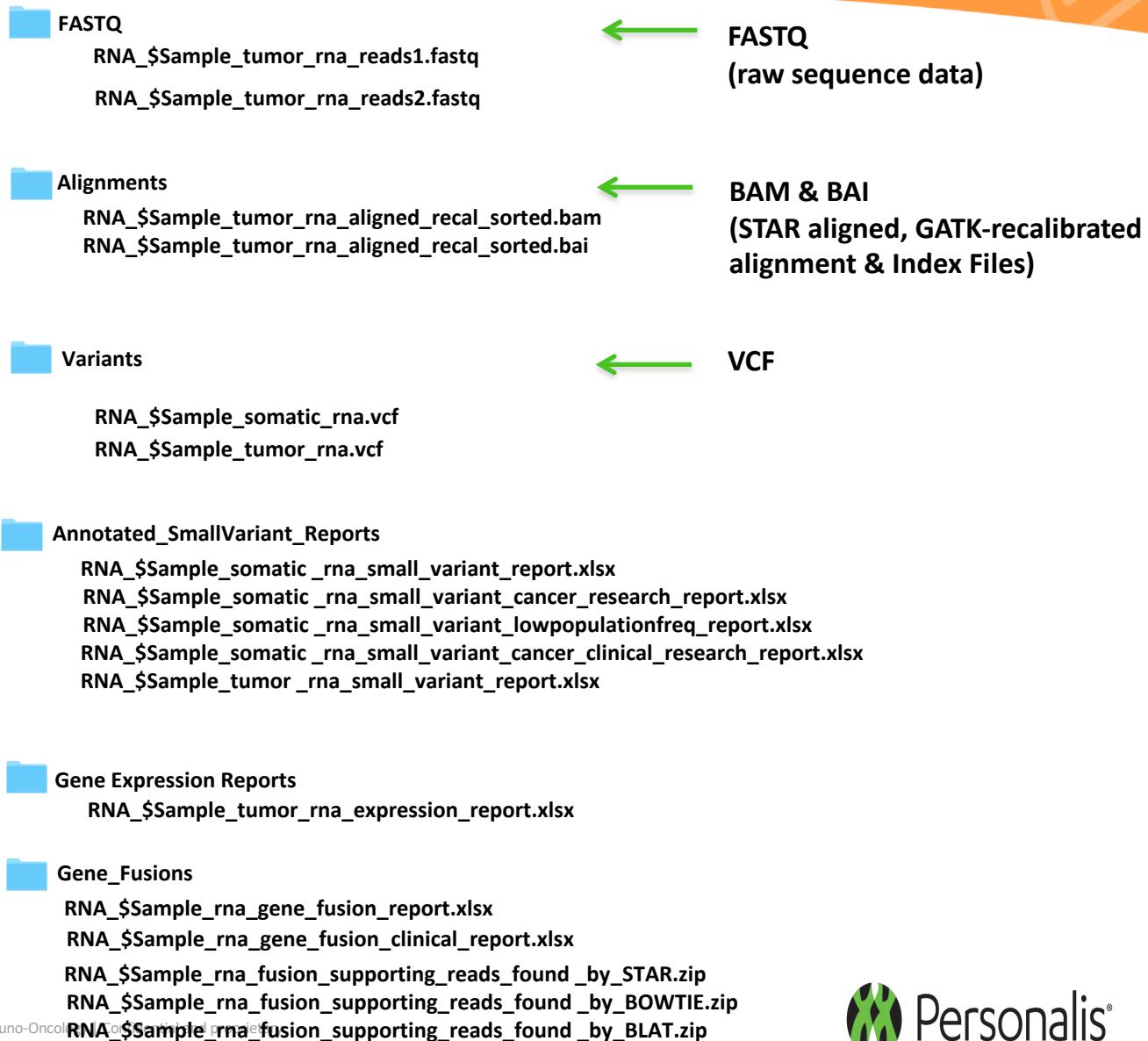


Report_DNA_125500

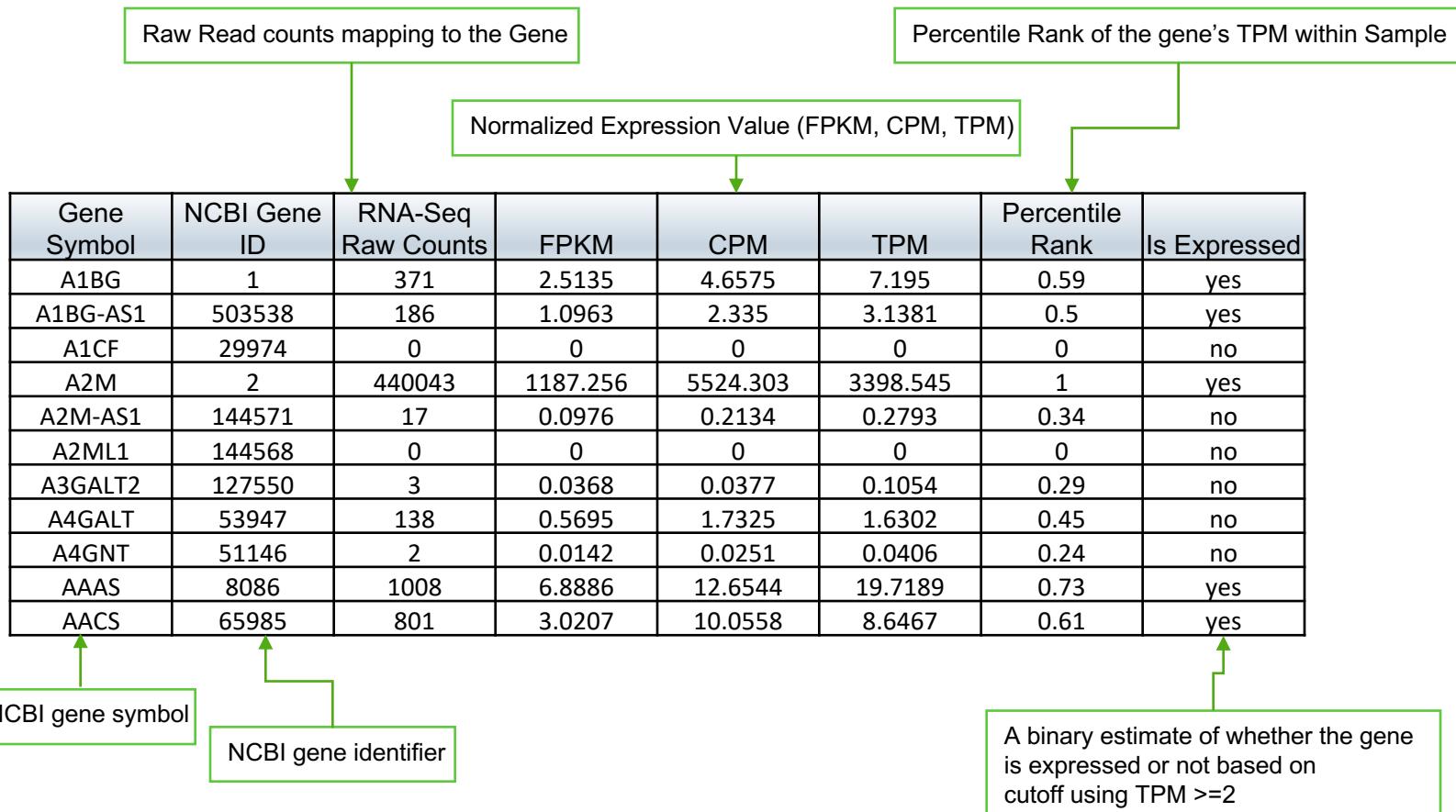
RNA Deliverables



Format of RNA Data Deliverables



Gene Expression

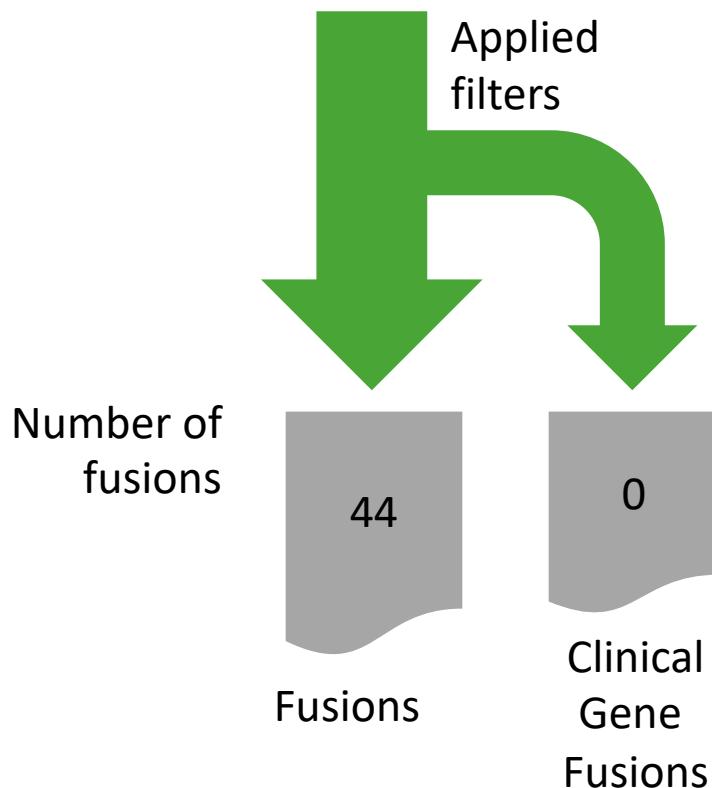


Gene Fusions



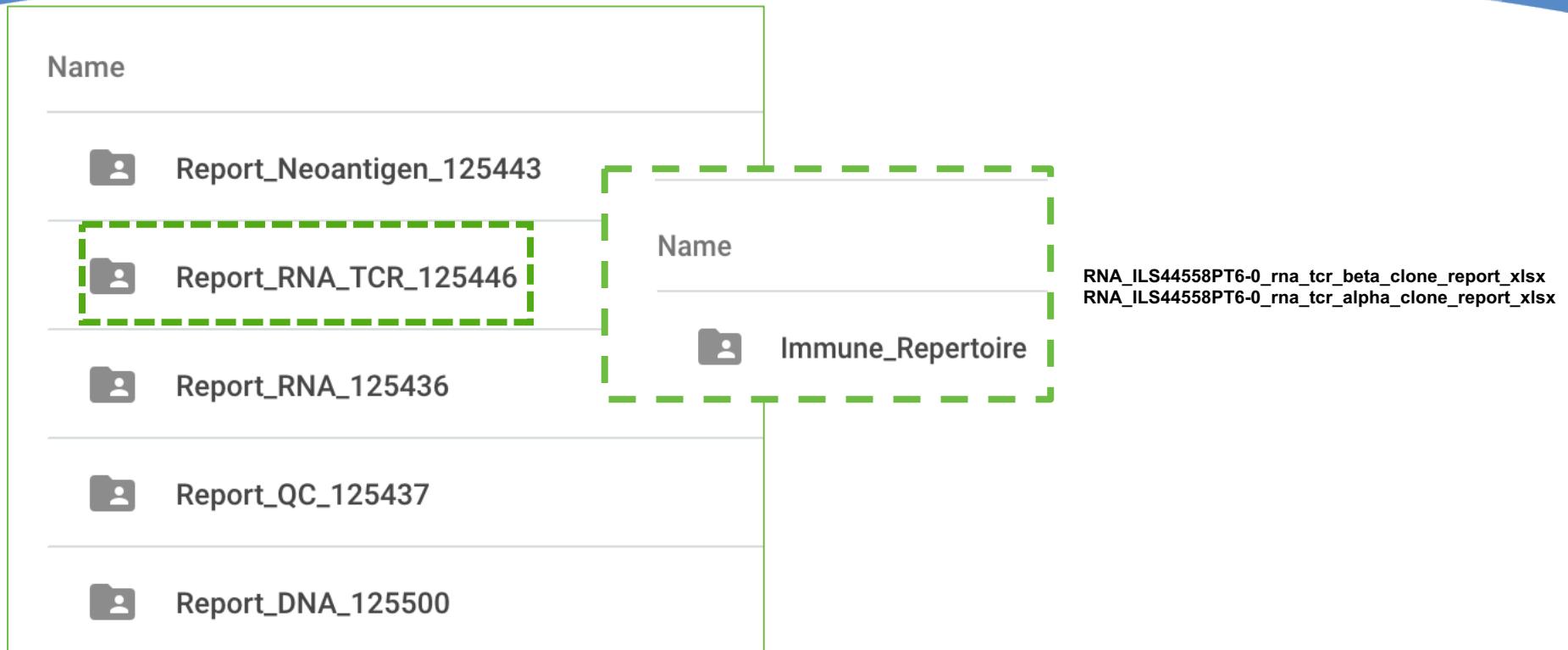
Gene_Fusions

RNA_ILS44558PT6-0_rna_gene_fusion_report.xlsx
RNA_ILS44558PT6-0_rna_gene_fusion_clinical_report.xlsx
RNA_ILS44558PT6-0_rna_fusion_supporting_reads_found_by_STAR.zip
RNA_ILS44558PT6-0_rna_fusion_supporting_reads_found_by_BOWTIE.zip
RNA_ILS44558PT6-0_rna_fusion_supporting_reads_found_by_BLAT.zip



A single gene fusion event can be represented by multiple lines of evidence:

- Multiple splicing events with different fusion points (5' and 3' end)
- Different methods of detection (BOWTIE, BLAT, STAR)
- Different number of spanning unique reads



- TCR repertoire analysis utilizing MIXCR
 - Default settings and downstream filters applied at reporting levels to remove false positives
- Excel file format for both TCR β and TCR α report
- Further information on clone count, clone frequency, Top V, J and D (only for B) hits, and CDR3 AA sequence provided

HTML Summary of RepertoireID



Results Summary Report for RNA_ILS44558PT6-0

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

Filters have been applied to the data shown in the tables and plots below to remove all non-productive clonotypes and clonotypes that have an average read alignment quality score of <80 (for TCR α) and <70 (for TCR β) in either their top V gene hit or top J gene hit. However, information relating to all detected clonotypes is provided as part of the TCR supplementary data files for further analysis.

▼ TCR α

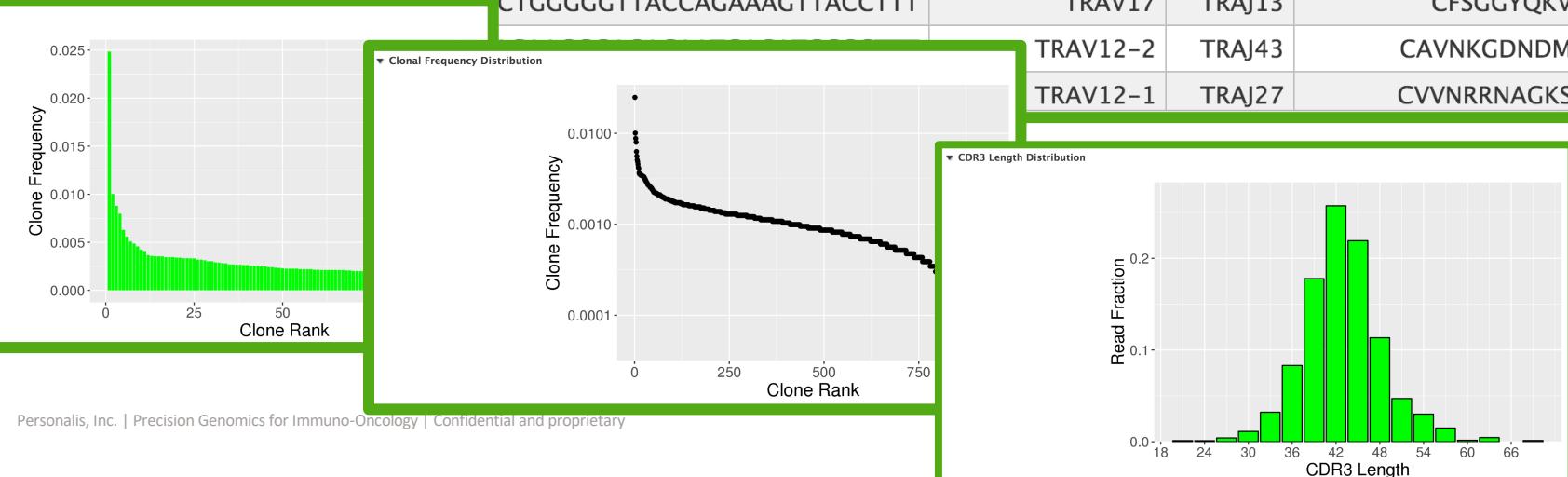
▼ TCR α Repertoire Clonality

Clonality

0.0475

▼ Top 10 Clones

Clone Count	Clone Frequency	Clonal Sequence	Top V Hits	Top J Hits	CDR3 Amino Acid Sequence
386	0.01172	TGTGCTCTAACTAACTATGGTCAGAATTTGTCTTT	TRAV6	TRAJ26	CALTNYGQNFVF
1	0.00027	CTGGGGTTACCAGAAAGTTACCTTT	TRAV17	TRAJ13	CFSGGYQKVTF
1	0.00027	CTGGGGTTACCAGAAAGTTACCTTT	TRAV12-2	TRAJ43	CAVNKGDNMDRF
1	0.00027	CTGGGGTTACCAGAAAGTTACCTTT	TRAV12-1	TRAJ27	CVVNRRNAGKSTF

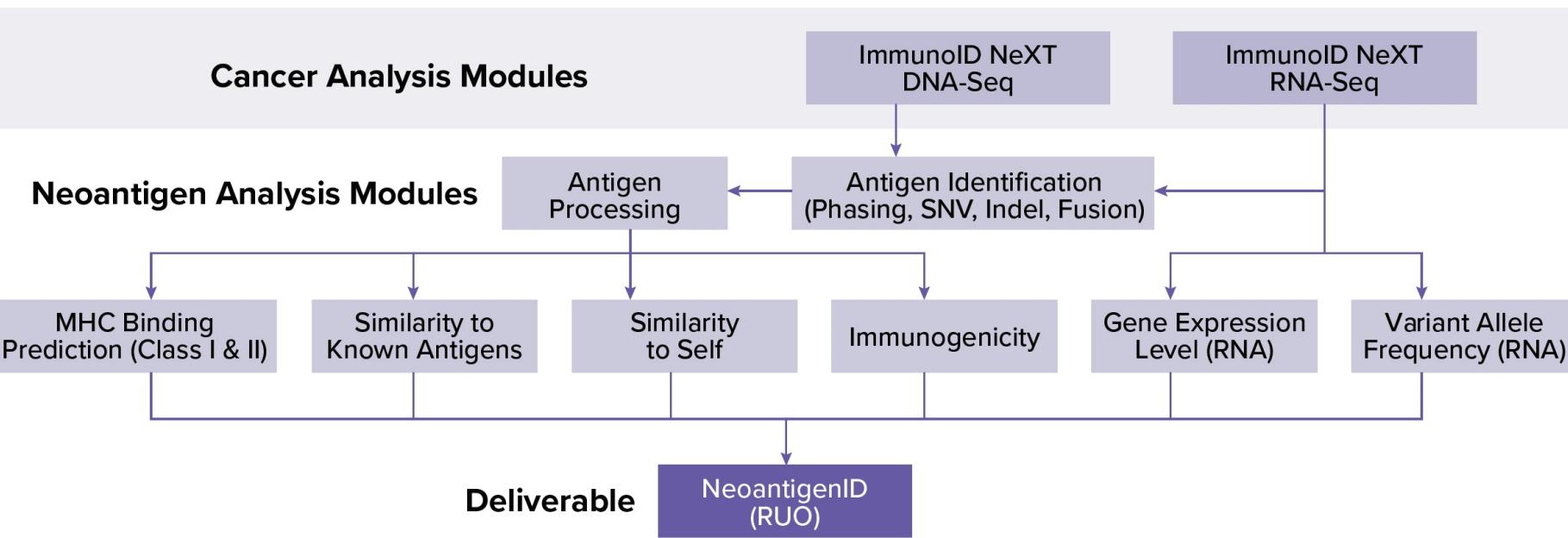


NeoantigenID and ImmunogenomicsID

Name
 Report_Neoantigen_125443
 Report_RNA_TCR_125446
 Report_RNA_125436
 Report_QC_125437
 Report_DNA_125500

Name
 Neoantigen
 Immunogenomics

:
DNA_ILS44558PT6-0_neoantigen_class_I_report_xlsx
DNA_ILS44558PT6-0_neoantigen_class_II_report_xlsx



[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

■■■ Sample and Run Information

Pipeline versions	NEO_v1.3.0
Annotation version	4
Platform version	ACE v4
Reference assembly	hs37d5



Overview Metrics

Mutation Burden

	SNVs	Indels	Total
Non-synonymous Somatic Variants	99	7	106
Non-synonymous Somatic Variants per Mb	1.33	0.09	1.42

Neoantigen Burden

Total Neoantigens	146
Neoantigens per Mb	1.97
Peptide Binders with allelic fraction >= 5% in DNA and RNA	19

	Prediction Bound (<500 nM)	Observed Expressed	Affinity Range	Median Affinity
Number of peptides binding to any one HLA type	146	104	4.50–499.43	212.19
Number of peptides binding to any two HLA types	35	26	4.50–489.93	135.71
Number of peptides binding to any three HLA types	5	3	11.40–470.90	174.08

NeoantigenID and ImmunogenomicsID

Name
 Report_Neoantigen_125443
 Report_RNA_TCR_125446
 Report_RNA_125436
 Report_QC_125437
 Report_DNA_125500

Name
 Neoantigen
 Immunogenomics

• DNA_ILS44558PT6-0_immunogenomics_report_xlsx

HTML Summary of ImmunogenomicsID



Results Summary Report for DNA_ILS44558PT6-0

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

Tumor Mutational Burden

	SNVs	Indels	Total
Non-synonymous Somatic Variants	99	7	106
Non-synonymous Somatic Variants per Mb	1.33	0.09	1.42

Checkpoint Modulators

Gene Symbol	RNA Gene Expression (TPM)	Variant Type	DNA Allelic Fraction	RNA Allelic Fraction	Transcript Variant	Protein Variant	Variant Effect	Variant Effect Impact
EGFR	253.2980	INDEL	0.168	0.430	c.2236_2250delGAATTAAGAGAAAGCA	p.Glu746_AlA750del	conservative_inframe_deletion	MODERATE

Adaptive Immune Response

Gene Symbol	RNA Gene Expression (TPM)	Variant Type	DNA Allelic Fraction	RNA Allelic Fraction	Transcript Variant	Protein Variant	Variant Effect	Variant Effect Impact
FAS	141.3056	SNV	0.121	0.096	c.809C>T	p.T270I	missense_variant	MODERATE

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

■■■ Microsatellite Instability Summary

▼ Updated Bethesda Consensus Panel

Microsatellite locus	Stability status
BAT-25	Stable
BAT-26	Stable
NR-21	Stable
NR-24	Stable
NR-27	Stable

▼ Total MS loci

Percent of unstable MS loci	Number of unstable MS loci	Total number of MS loci
0.0036	1	27609



Personalis®

Results Summary Report for DNA_ILS44558PA1

[Cancer DNA](#)[Cancer RNA](#)[Neoantigen](#)[MSI](#)[TCR](#)[Oncovirus](#)[HLA](#)[Immunogenomics](#)

Virus	DNA Read Count	RNA Read Count
MCV	2	N/A



Thank you!