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Genexus use for for NGS molecular profiling of tumors

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Manual NGS setup

FFPE Block → DNA / RNA Extraction → Library Preparation → Sequencing → Data Analysis

*Currently **5-10 working days** from sample to report*

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Oncomine™ Precision Assay for tissue and liquid biopsies

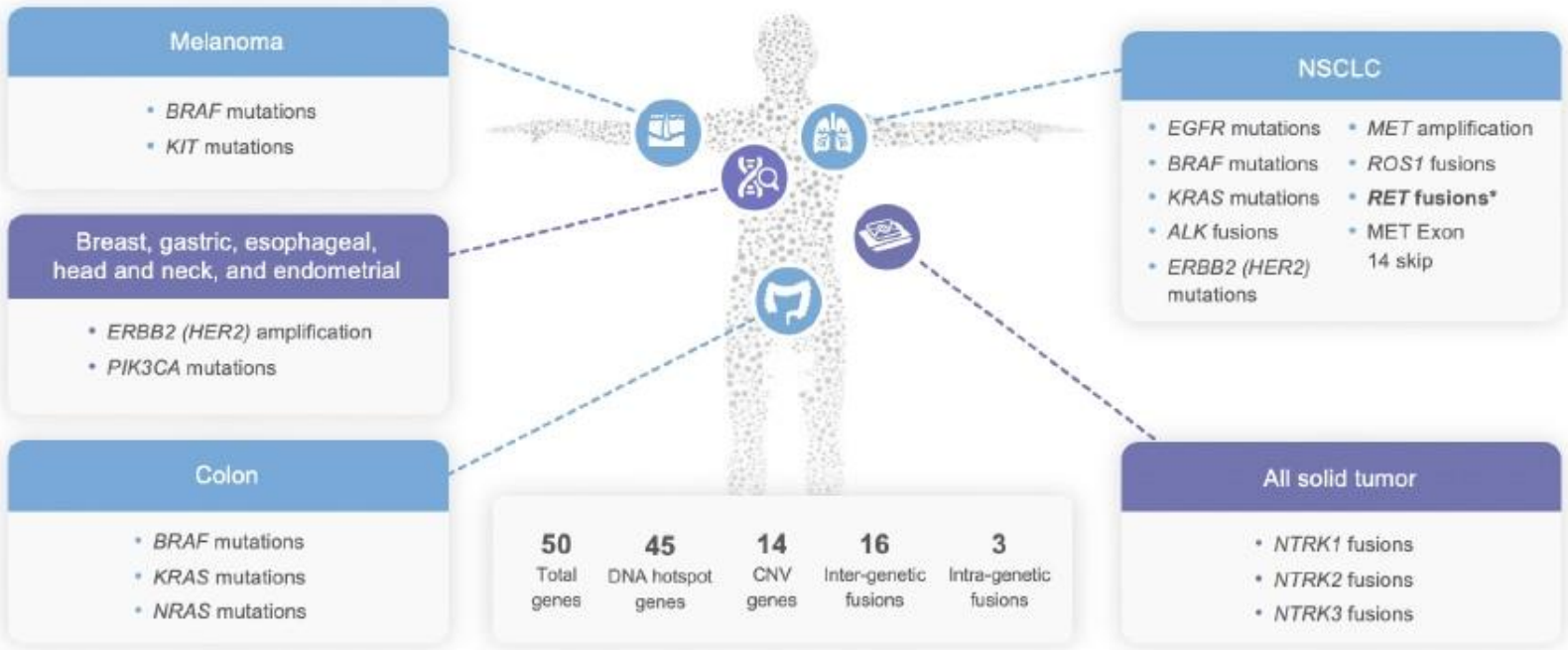
- ✓ **Fully automated** library-prep, sequencing, and data analysis
- ✓ From sample to report in ***1-2 days***
- ✓ *Up to* ***32 samples/run***
- ✓ Only **10ng DNA / RNA input** required
- ✓ Requires **minimal hands-on**

50 Actionable Genes

Tissue and Plasma

Fusion Detection

UMI-technology



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Automated NGS setup using Genexus Sequencer




The Present
5-10 days TaT

Sample → DNA / RNA Extraction → Library Preparation → Sequencing → Data Analysis



The Future
4-6 days TaT

Sample → DNA / RNA Extraction → Library Prep. / Seuqencing / Data Analysis

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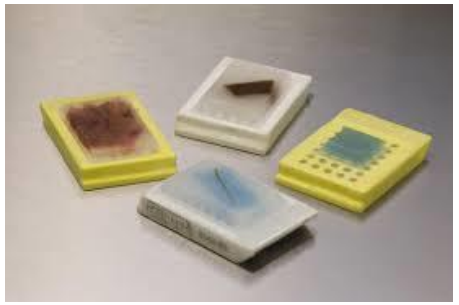
The Genexus Workflow

Step by Step



FFPE Block
Cytological Sample
Blood

Tissue microdissection

DNA / RNA Extraction

Library Prep. / Seuqencing / Data Analysis

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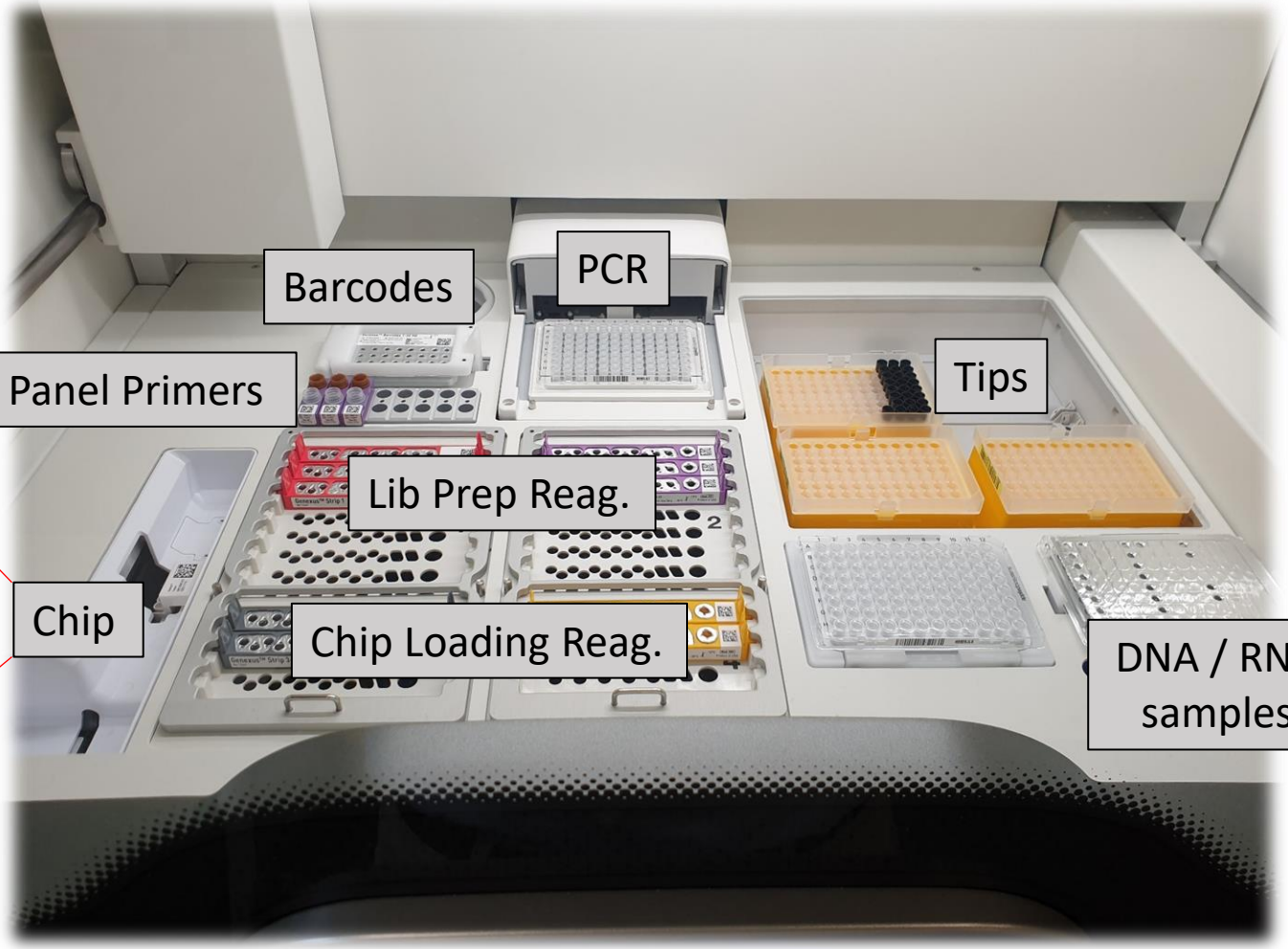
The Genexus Sequencer

Chip



DNA / RNA samples

15 min Hands-On; all reagents come in strips, only pipetting step is to fill DNA / RNA in sample plate

Runtime varies depending on no. samples from approx. 13 – 30 hours incl. data analysis

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Genexus Software – run overview

Genexus | Ion Torrent — Version: 6.2.1

Run Name:	20210727	Run Status:	Completed
Started On:	2021-07-27 12:53	Completed On:	2021-07-28 03:58
Operator:	ANATOMIA1	Sequencing Flows:	500
Instrument Name:	GNXS-0293		

Oncomine Precision - GX5 - Solid Tumor - DNA - w2.6.0

Loading	Key Signal	Total Bases	Final Reads	Mean Raw Accuracy 1x
93.08%	80	1.36G	14.586.011	99.3

Loading 93.08% — Empty Wells 6.92%

30.233.407

Wells with ISPs(30.233.407) / Total Addressable Wells(32.481.466)

Enrichment 99.93% — Unenriched 0.07%

30.211.153

Wells with Live ISPs(30.211.153) / Wells with ISPs(30.233.407)

Library 99.93% — Control ISPs 0.07%

30.188.738

Wells with Library ISPs(30.188.738) / Wells with Live ISPs(30.211.153)

Final Reads 48.32% — Filtered 51.68%

14.586.011

Final Reads(14.586.011) / Wells with Library ISPs(30.188.738)

Loading Density

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Genexus Software – run overview

Run Samples

Sample Name	Nucleic Acid Type	Barcode	Total Reads	Mean Read Length	>= Q20 Bases	Uniformity	Read Length Histogram
21-23465A2	DNA	IonHDdual_0101	1.419.328	95	122.964.867	97.22%	
21-24585A2	DNA	IonHDdual_0102	1.467.626	92	123.221.280	96.59%	
21-24870A1	DNA	IonHDdual_0103	1.487.357	93	126.342.986	98.24%	
21-27868A1	DNA	IonHDdual_0104	1.342.221	93	114.953.246	98.30%	
21-27961	DNA	IonHDdual_0105	1.327.972	96	116.047.939	98.83%	
21-28072	DNA	IonHDdual_0106	1.349.428	93	113.842.526	99.38%	
21-29577A4	DNA	IonHDdual_0107	1.615.711	95	139.915.324	96.82%	
21-30278A1	DNA	IonHDdual_0108	1.556.177	94	133.389.034	96.56%	

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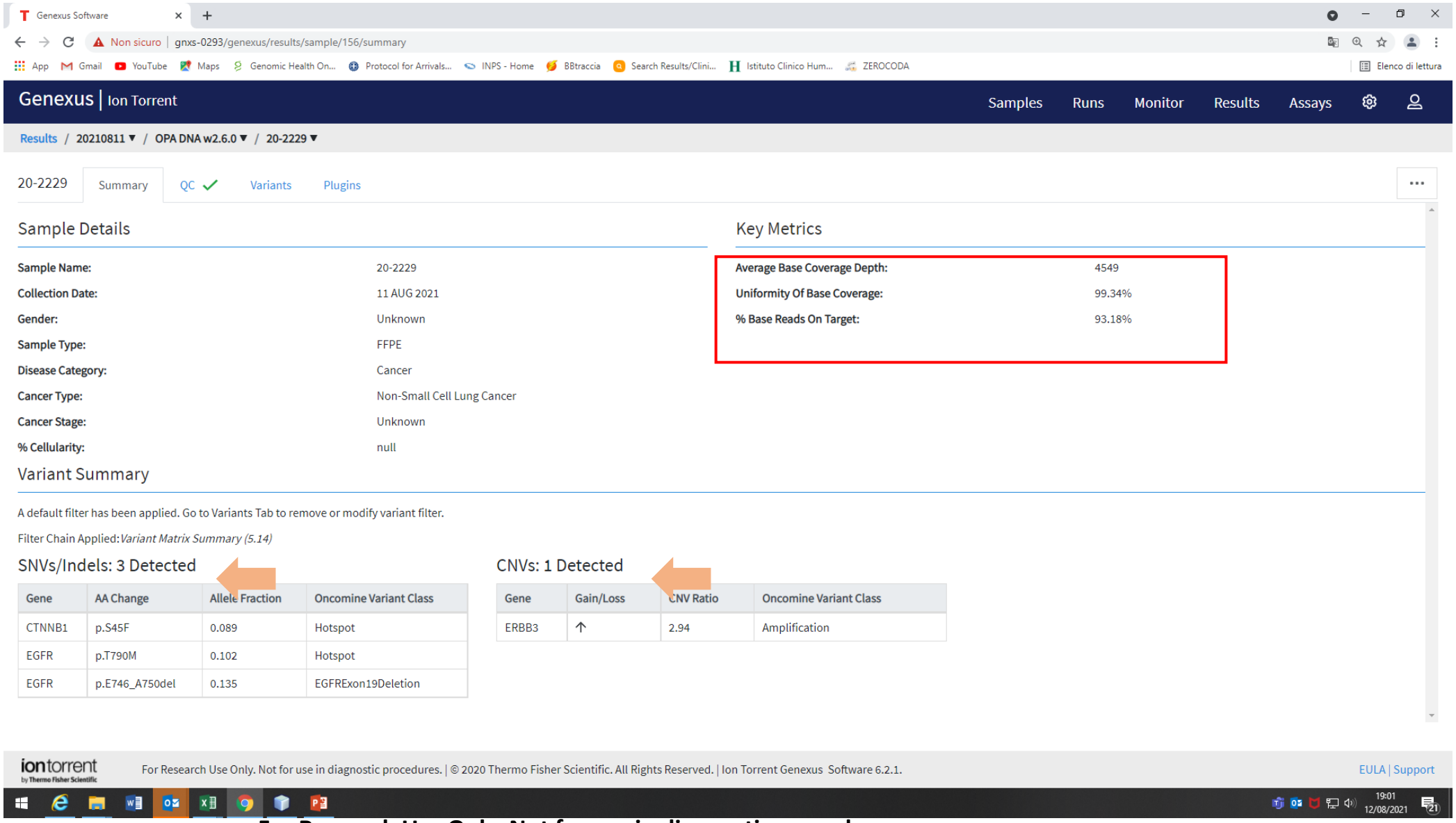
Genexus Software – DNA sample overview



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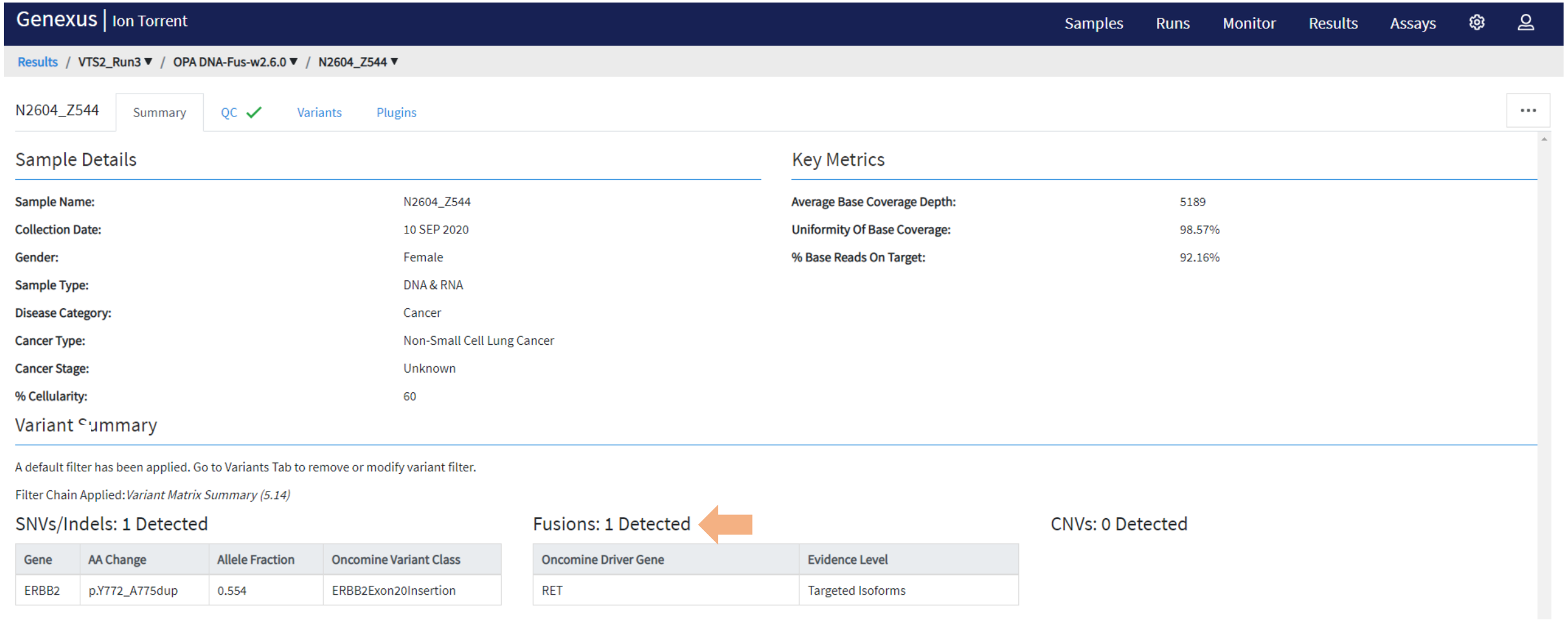
Genexus Software – DNA and RNA sample overview



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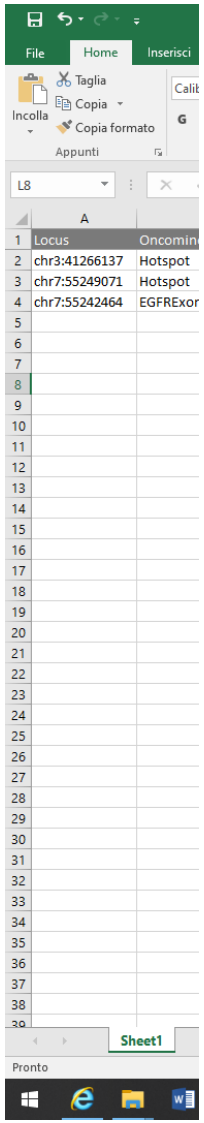
Genexus Software – download files



SAMPLE REPORT

My Lab

My Lab Address
Tel: (999)-999-9999

Sequencing Lab Report : Oncomine Precision - GX5 - Solid Tumor - DNA - w2.6.0

Reported Date: 17 AUG 2021

Draft Report

Sample Details

Sequence Variations: Detected

DNA Sequence Variants

Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	Amino Acid Change	Allele Fraction	Ref	Alt	Type	Call	Alt Allele Read Counts	Variant ID	Nucleotide Change
chr12:25398285	Hotspot	Gain-of-Function	KRAS	p.G12C	0.315	C	A	snp	PRESENT (HETEROZYGOUS)	2049	COSM516	c.34G>T
chr3:178936094	Hotspot	Gain-of-Function	PIK3CA	p.Q546K	0.247	C	A	snp	PRESENT (HETEROZYGOUS)	682	COSM766	c.1636C>A
chr17:7577538	Hotspot	Loss-of-Function	TP53	p.R248Q	0.089	C	T	snp	PRESENT (HETEROZYGOUS)	182	COSM10662	c.743G>A

Copy Number Variations

No CNV sequence variations detected

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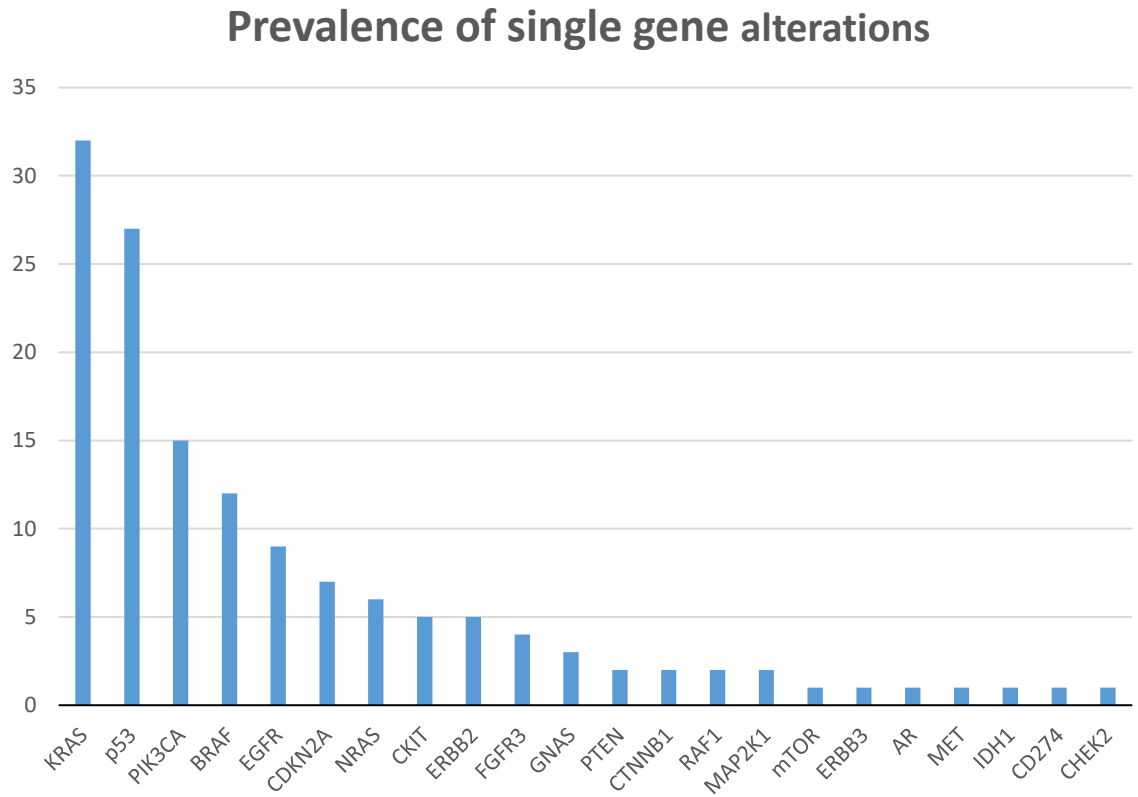
Genexus analysis in FFPE tumor samples

95 tested samples:
44 lung adenocarcinoma
28 colon adenocarcinoma
11 melanoma
4 glial tumors
4 DLBCL
3 GIST
1 thyroid carcinoma



Number of alterations detected with genexus:
- 128 mutations vs 73 mutations with canonical techniques
- 12 CNV

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Genexus analysis in FFPE tumor samples vs conventional techniques

Concordance rate: 90/95= 94,7%

Discordant cases (n=5):

1- Genexus detected BRAF V600E below threshold at 0.9% of allele frequency in lung adenocarcinoma with <10% tumoral cells.

2- Genexus detected NRAS Q61R in two melanoma samples of the same patient; conventional technique detected the mutation only in a second sample.

3- Genexus detects BRAF D594A in lung adenocarcinoma that is not detected by conventional technique, despite the mutation is covered.

4- Genexus detects BRAF G469A in lung adenocarcinoma containing low % of tumoral cells (<10%) that is not detected by conventional technique, despite the mutation is covered.

5- Genexus detects KRAS G12C in lung adenocarcinoma misinterpretated by conventional techniques as KRAS G12insGA/G12fs*3 (fw assay failed)

Minor discrepancies:

1- in 3 cases (2 lung adenocarcinoma and 1 GIST) Genexus software failed to call the correct deletion. The software calls 2 different deletions that require IGV to be correctly interpretated.

2- in 2 cases Genexus **has an advantage** in the call of correct mutation respect conventional technique (Real Time PCR) that mix two possible variant in the same codon.

We have concordance also in melanoma melanina-rich and in decalcificated-samples.

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Conclusions

- Reduction of TAT from 5-10 working days to 4-6
- Optimization of lab workflow analysing until 32 samples of different cancer type
- High concordance with the major diagnostic techniques
- Good performance in difficult samples as decalcificated bones and melanina-enriched samples
- Good sensitivity in the detection of mutations in tisssues with <10% of tumoral cells
- Instrument and Software easly to be used

Areas of improvement:

- Correct discrimination of deletions/insertions by software
- Introduce the possibility of intervention on the instrument during the run
- Implementation new panel kit

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