

Genexus use for NGS molecular profiling of tumors

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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



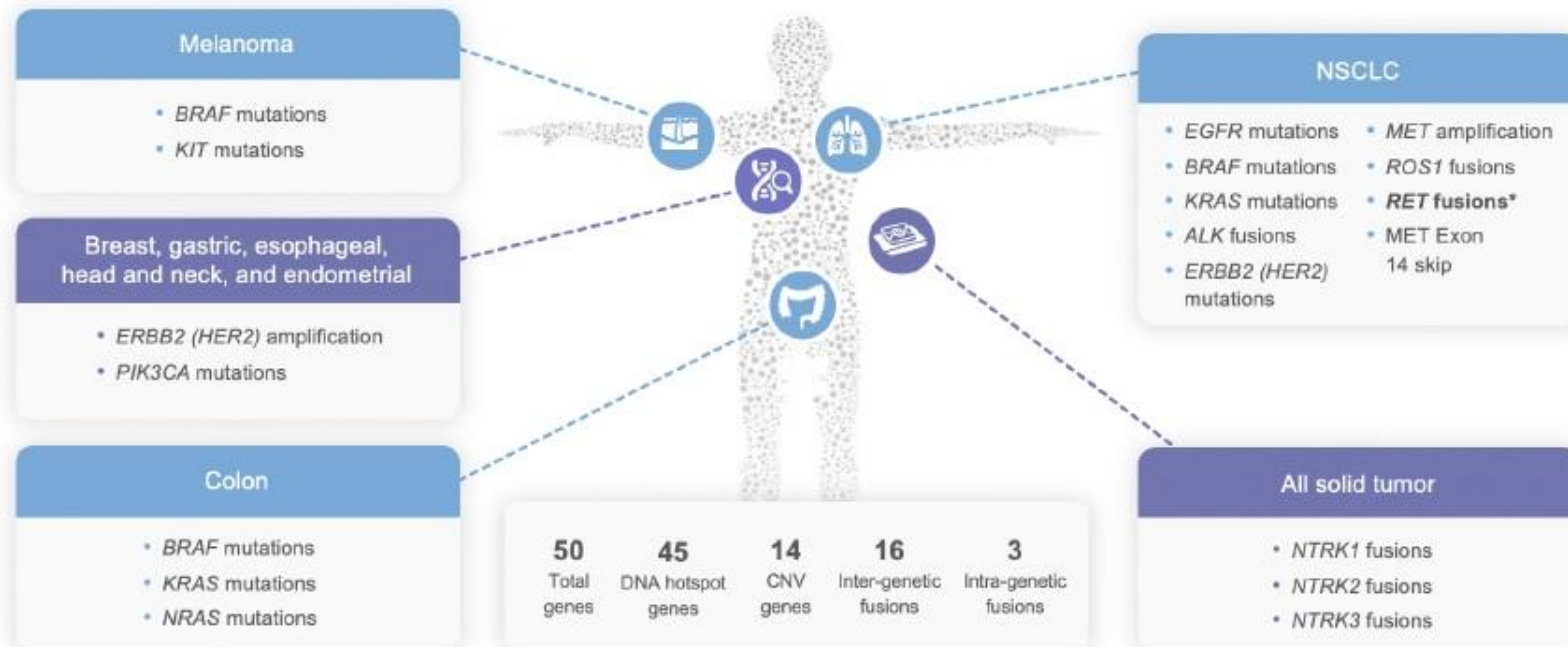
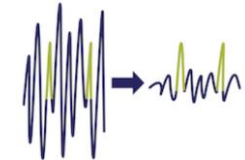
Fusion Detection



Tissue and Plasma



UMI-technology



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Automated NGS setup using Genexus Sequencer

The Present
5-10 days TaT



Sample	Gene	Variant	RefSeq	Impact	Filter	Frequency	Allele	Genotype
Sample 1	BRCA1	c.1234G>A	rs12345	Missense	Pathogenic	0.001	G	GA
		c.567C>T	rs67890	Stop	Pathogenic	0.0001	C	CT
		c.890A>G	rs11111	Missense	Benign	0.01	A	AG
		c.1122G>C	rs22222	Missense	Benign	0.005	G	GC
Sample 2	BRCA2	c.345T>G	rs33333	Missense	Pathogenic	0.0005	T	TG
		c.678A>C	rs44444	Missense	Pathogenic	0.0002	A	AC
		c.901G>T	rs55555	Missense	Benign	0.002	G	GT

Sample

DNA / RNA
Extraction

Library
Preparation

Sequencing

Data Analysis



The Future
4-6 days TaT

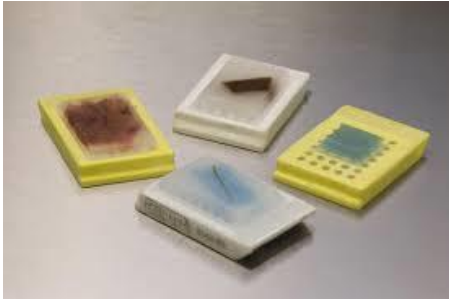
Sample

DNA / RNA
Extraction

Library Prep. /
Seuqencing /
Data Analysis

The Genexus Workflow

Step by Step



FFPE Block
Cytological Sample
Blood



Tissue
microdissection

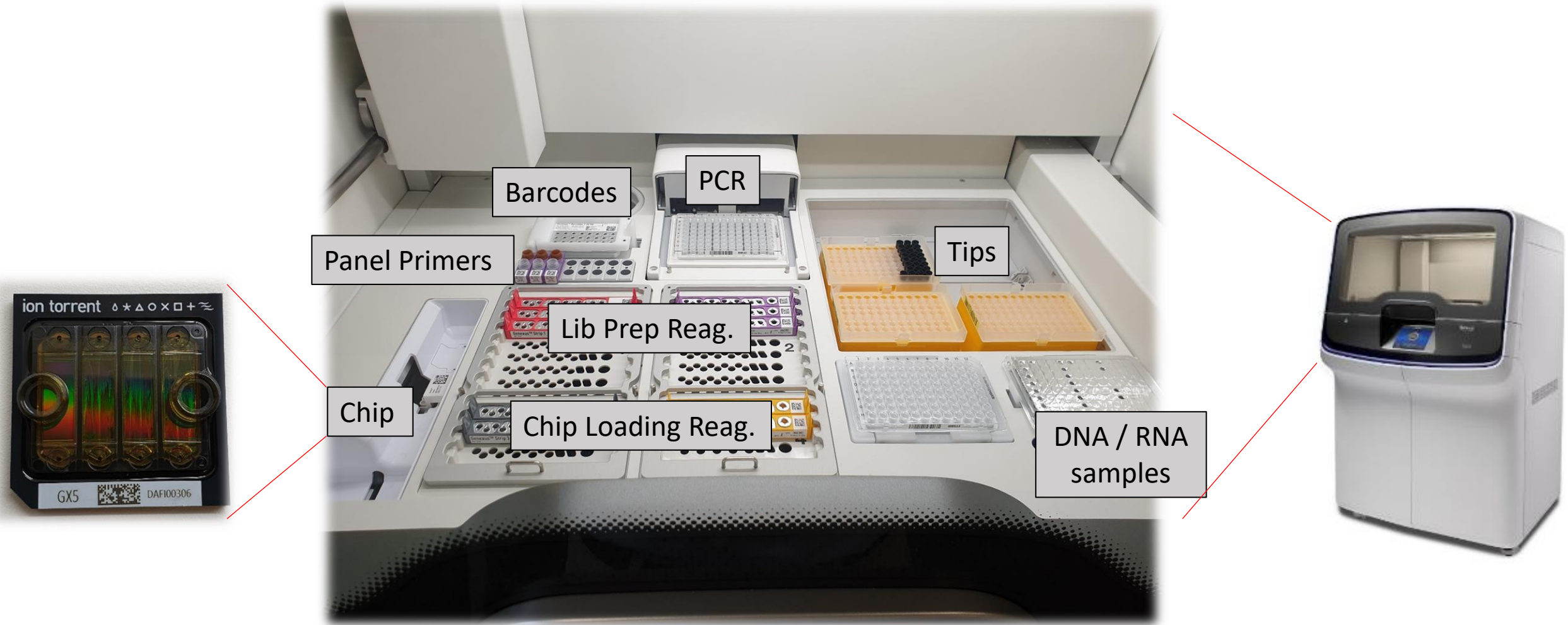


DNA / RNA
Extraction



Library Prep. /
Sequencing / Data
Analysis

The Genexus Sequencer



***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

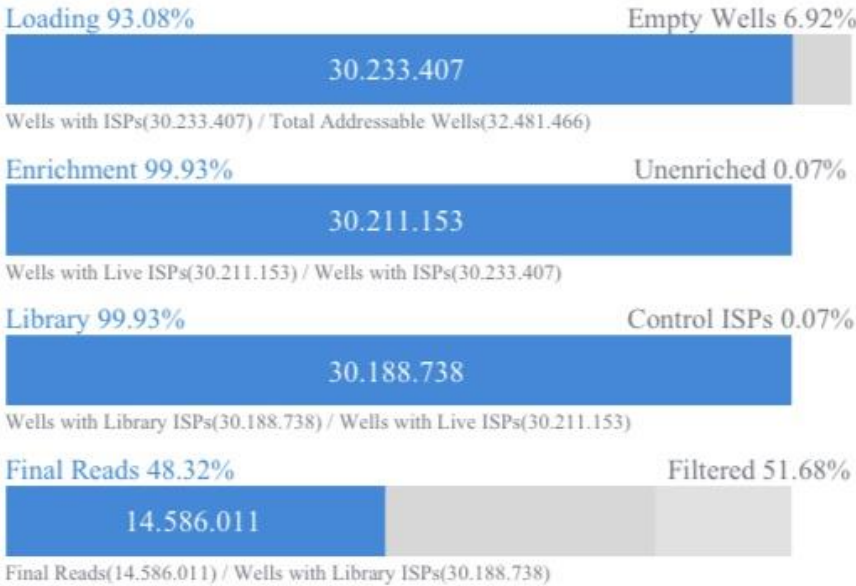
Genexus | Ion Torrent

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 Density

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%	

Genexus Software – DNA sample overview

Genexus Software

gnxs-0293/genexus/results/sample/156/summary

AppGmailYouTubeMapsGenomic Health On...Protocol for Arrivals...INPS - HomeBBtracciaSearch Results/Clini...Istituto Clinico Hum...ZEROCODA

Elenco di lettura

Genexus | Ion Torrent

SamplesRunsMonitorResultsAssays

Results / 20210811 / OPA DNA w2.6.0 / 20-2229

20-2229SummaryQC ✓VariantsPlugins

Sample Details

Sample Name:

20-2229

Collection Date:

11 AUG 2021

Gender:

Unknown

Sample Type:

FFPE

Disease Category:

Cancer

Cancer Type:

Non-Small Cell Lung Cancer

Cancer Stage:

Unknown

% Cellularity:

null

Key Metrics

Average Base Coverage Depth:

4549

Uniformity Of Base Coverage:

99.34%

% Base Reads On Target:

93.18%

Variant Summary

A default filter has been applied. Go to Variants Tab to remove or modify variant filter.

Filter Chain Applied: Variant Matrix Summary (5.14)

SNVs/Indels: 3 Detected

Gene	AA Change	Allele Fraction	Oncomine Variant Class
CTNNB1	p.S45F	0.089	Hotspot
EGFR	p.T790M	0.102	Hotspot
EGFR	p.E746_A750del	0.135	EGFRExon19Deletion

CNVs: 1 Detected

Gene	Gain/Loss	CNV Ratio	Oncomine Variant Class
ERBB3	↑	2.94	Amplification

iontorrent
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EULA | Support

WindowsTaskbarIcons

19:01
12/08/2021

Genexus Software – DNA and RNA sample overview

Genexus | Ion Torrent

SamplesRunsMonitorResultsAssays⚙️👤

Results / VTS2_Run3 ▾ / OPA DNA-Fus-w2.6.0 ▾ / N2604_Z544 ▾

N2604_Z544

SummaryQC ✓VariantsPlugins

⋮

Sample Details

Sample Name:

N2604_Z544

Collection Date:

10 SEP 2020

Gender:

Female

Sample Type:

DNA & RNA

Disease Category:

Cancer

Cancer Type:

Non-Small Cell Lung Cancer

Cancer Stage:

Unknown

% Cellularity:

60

Key Metrics

Average Base Coverage Depth:

5189

Uniformity Of Base Coverage:

98.57%

% Base Reads On Target:

92.16%

Variant Summary

A default filter has been applied. Go to Variants Tab to remove or modify variant filter.

Filter Chain Applied: Variant Matrix Summary (5.14)

SNVs/Indels: 1 Detected

Fusions: 1 Detected

CNVs: 0 Detected

Gene	AA Change	Allele Fraction	Oncomine Variant Class
ERBB2	p.Y772_A775dup	0.554	ERBB2Exon20Insertion

Oncomine Driver Gene	Evidence Level
RET	Targeted Isoforms

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Genexus Software – download files

FILE

HOME

INSERISCI

Taglia

Copia

Copia formato

Appunti

L8

1

Locus

Oncomin

2

chr3:41266137

Hotspot

3

chr7:55249071

Hotspot

4

chr7:55242464

EGFRExor

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Sheet1

Pronto

SAMPLE REPORT

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

My Lab

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

Reported Date: 17 AUG 2021

Draft Report

Sample Details

Sequence Variations: Detected

DNA Sequence Variants

Locus	Oncomin Variant Class	Oncomin 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	PRESEN T (HETER OZYGO US)	2049	COSM516	c.34G>T
chr3:178936094	Hotspot	Gain-of-Function	PIK3CA	p.Q546K	0.247	C	A	snp	PRESEN T (HETER OZYGO US)	682	COSM766	c. 1636C>A
chr17:7577538	Hotspot	Loss-of-Function	TP53	p.R248Q	0.089	C	T	snp	PRESEN T (HETER OZYGO US)	182	COSM10662	c. 743G>A

Copy Number Variations

No CNV sequence variations detected

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DESTRO Annarita ICH

Condividi

Somma automatica

Riempimento

Cancella

Ordina e filtra

Trova e seleziona

Modifica

Change

IC>T

9C>T

15_2249delGGAATTAAGAGAAGC

19:19

12/08/2021

Genexus analysis in FFPE tumor samples

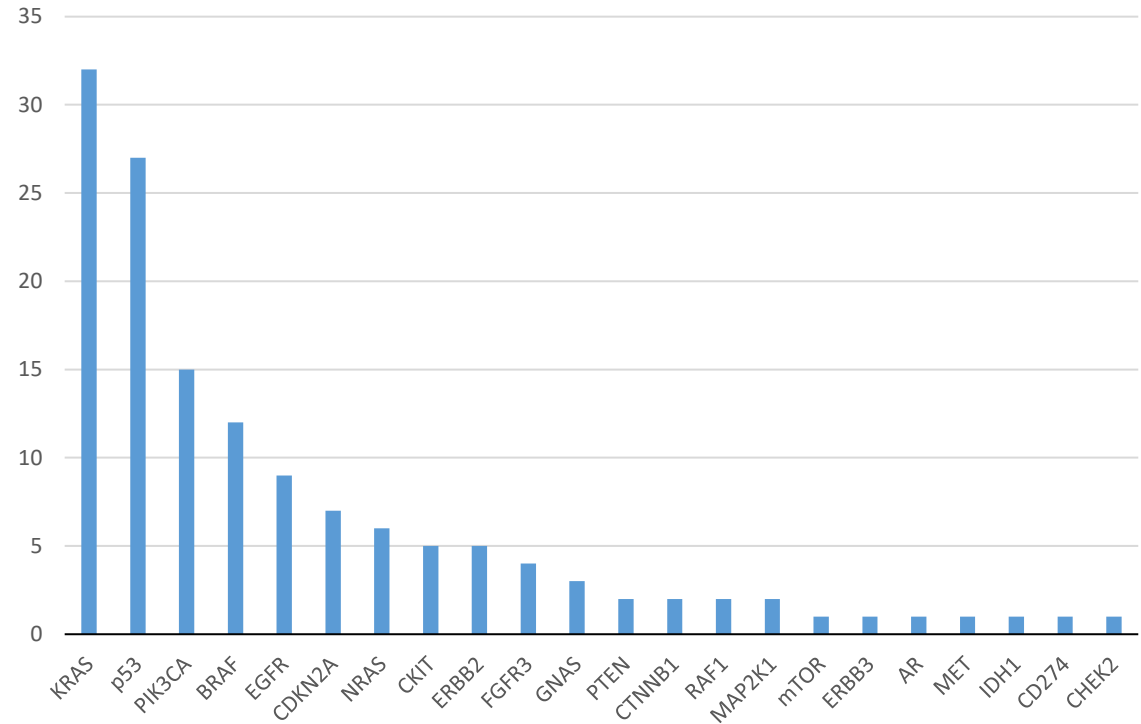
95 tested samples:

- 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

Prevalence of single gene alterations



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 misinterpreted 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 interpreted.
- 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 decalcified-samples. For Research Use Only. Not for use in diagnostic procedures.

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 decalcified bones and melanina-enriched samples
- Good sensitivity in the detection of mutations in tissues with <10% of tumoral cells
- Instrument and Software easily 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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