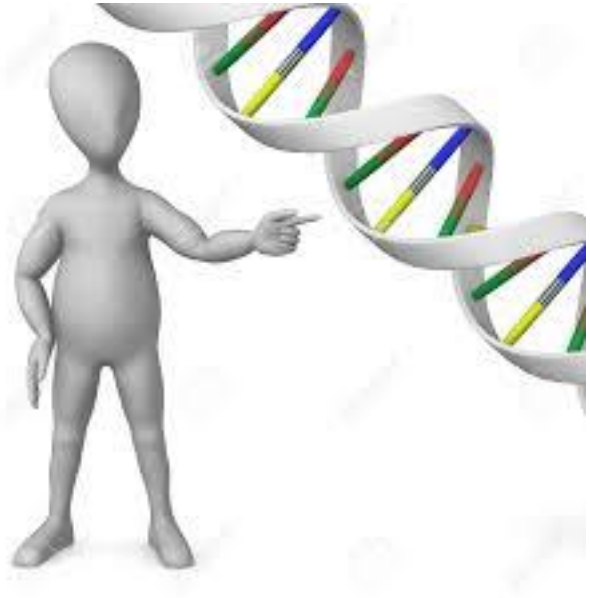
Implementación de análisis de secuenciación masiva automatizada mediante Genexus en tumores sólidos



- *Dr. Javier Hernández Losa*

- *28 Mayo 2021*

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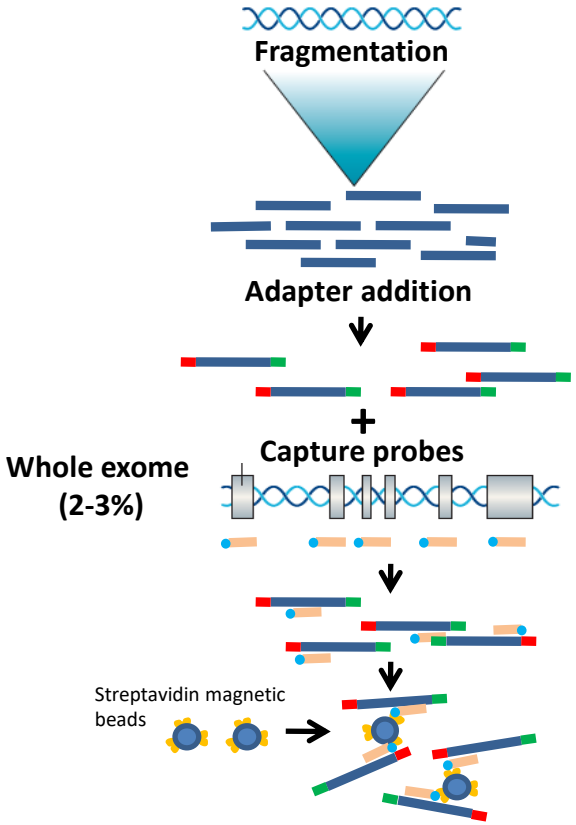
Target enrichment

Hybrid capture

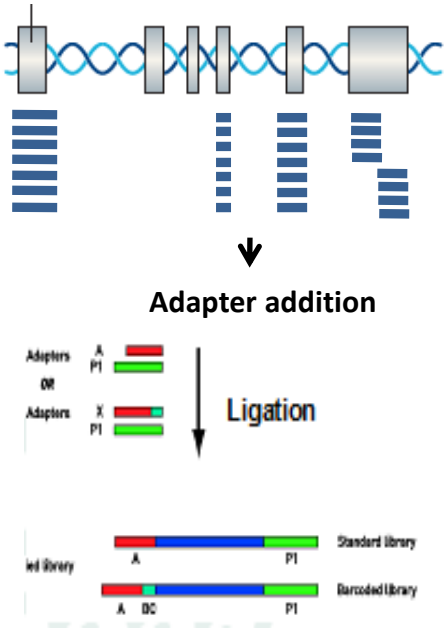


Advantages:
Full exomes to gene panels
Detects tanslocation and CNV
High sensitivity

Disadvantage:
Lower on-target efficiency
large amount of high quality DNA
Turnaround Time (1-2 days)

Predominant applications
. Point mutations
. Copy number variation

PCR based or Ligation based



Advantage:
Compatible with FFPE
Turnaround Time (5-6h)
High on target efficiency
high sensitivity

Disadvantage:
Small target regions
Primer design
Amplification optimization

Predominant applications
. Point mutations
. Copy number variation

Imagen cedida Dr. Pedro Jares

Análisis de Datos secundario

Second stage of NGS analysis. Secondary analysis: read alignment and variant calling.

Ion Reporter

Hi, javier Hernandez 22.7 GB/1 TB Help Sign Out

Home | Samples | Analyses | Workflows | Admin

Overview | Launch | My Variants

Biologia Molecular Hospital Univ Valle Hebron • Ion Reporter 5.10.5

Analysis Results

MyVariants | Download | Visualize | Selected Variants | Send to Report Role | Switch To | Generate Report

Analysis Name: Fusion_24_v2_0a29be42-d49d-430d-a079-3bf19... **Fusion Sample QC**: PASS,[TotalMappedFusionPanelReads>20000;M...
Fusion Overall Call: NOCALL ,[3pGene=ROS1,IsoformsDetected=No... **Total Mapped Fusion Panel Reads**: 133182 **Total Unmapped Reads**: 510

To learn more about reviewing your results, visit the help guide

Summary | Fusions | Functional | Population | Ontologies | Pharmacogenomics | QC

Search | Go | Preferences

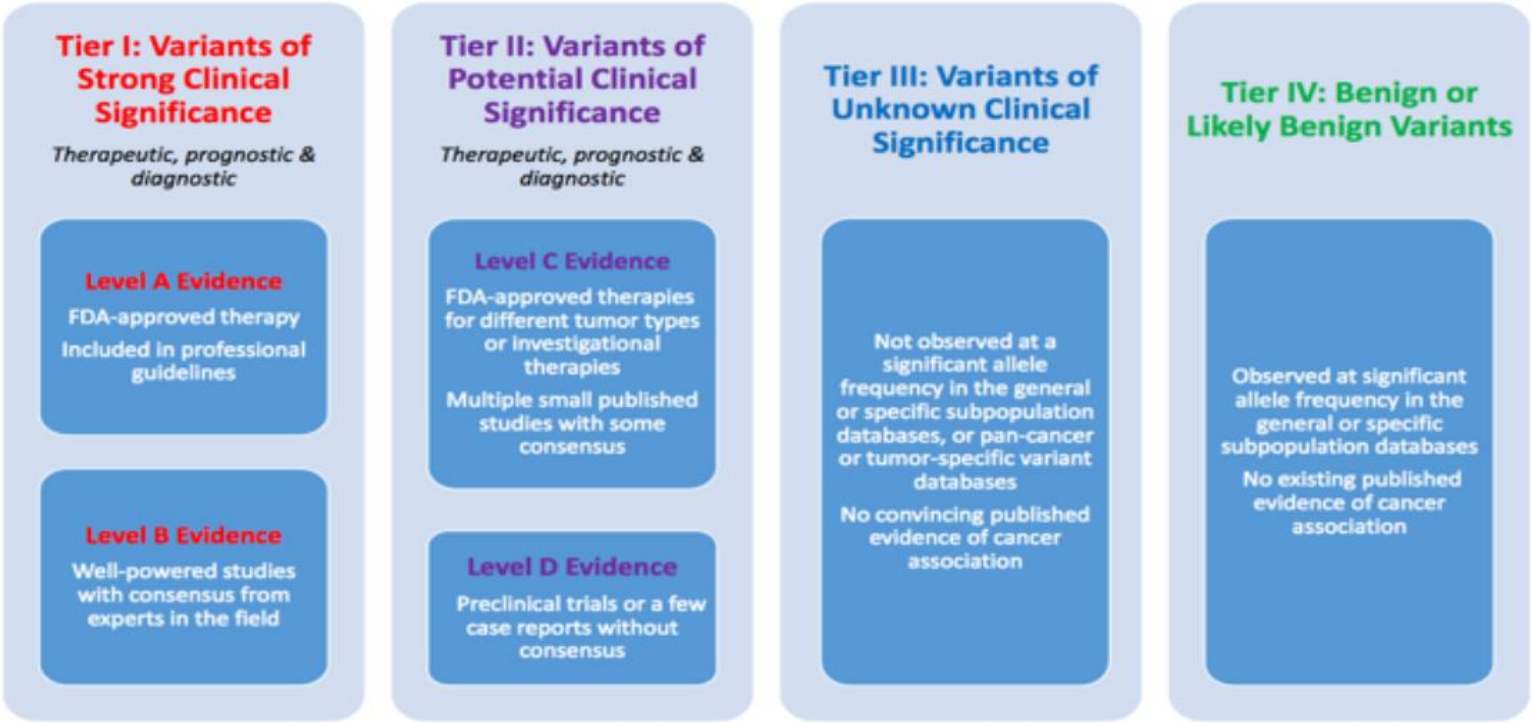
Classification	Locus	Genes	Transcript	Coding	Amino Acid Chan...	Variant Effect	PhyloP	SIFT
Unclassified	chr2:212530084	ERBB4	NM_005235.2	c.1835G>A	p.Arg612Gln	missense	3.84	0
Unclassified	chr10:89685268	PTEN	NM_000314.6, NM_000314.6	c.165-2A>., c.165-2A>.	p.?, p.?	unknown, unknown	8.43	
Unclassified	chr10:89685269	PTEN	NM_000314.6, NM_000314.6	c.165-1GGTTTTTGG c.165-1GGTTTTTGG	p.?, p.?	unknown, unknown	9.16, 9.16, 7.45, 7.45, 5.71, 2.94, 7.45, 5.86, 9.16, 8.67, 4.56, 7.45, 7.24, 3.05, 8.67, 8.67, 2.41, 7.24, 8.67, 7.45, 8.67, 8.67, 8.67, 6.86, 8.67, 3.58, 7.24, 8.67, 5.73, 7.45, 8.67, 1.81, 8.67, 8.67, 6.0, 8.67,	

Table 1 Databases Relevant to Interpretation of Somatic Sequence Variants

Utility/function	Database	Location (web address)
Population databases to exclude polymorphisms	1000 Genomes Project[16]	*http://browser.1000genomes.org*
	Exome Variant Server	*http://evs.gs.washington.edu/EVS*
	dbSNP[17]	*http://www.ncbi.nlm.nih.gov/snp*
	dbVar[18]	*http://www.ncbi.nlm.nih.gov/dbvar*
	ExAC	*http://exac.broadinstitute.org*
Cancer-specific variant databases	Catalog of Somatic Mutations in Cancer[19]	*http://cancer.sanger.ac.uk/cosmic*
	My Cancer Genome	*http://www.mycancergenome.org*
	Personalized cancer therapy, MD Anderson Cancer Center	*https://pct.mdanderson.org*
	cBioPortal, Memorial Sloan Kettering Cancer Center[20]	*http://www.cbioportal.org*
	Intogen[21]	*https://www.intogen.org/search*
	ClinicalTrials.gov	*https://clinicaltrials.gov*
	IARC (WHO) TP53 mutation database[22]	*http://p53.iarc.fr*
	Pediatric Cancer Genome Project (St. Jude Children's Research Hospital—Washington University)	*http://explorepcgp.org*
	International Cancer Genome Consortium[23]	*https://dcc.icgc.org*
Sequence repositories and data hosts	NCBI Genome	*http://www.ncbi.nlm.nih.gov/genome*
	RefSeqGene[24]	*http://www.ncbi.nlm.nih.gov/refseq/rsg*
	Locus Reference Genomic[25]	*http://www.lrg-sequence.org*
	UCSC table browser[26]	*https://genome.ucsc.edu/cgi-bin/hgTables*
	Ensemble BioMart[27]	*http://useast.ensembl.org/biomart/martview*
Other disease/mutation databases useful in the context of variant interpretation for cancer genomics	ClinVar[28]	*http://www.ncbi.nlm.nih.gov/clinvar*
	Human Gene Mutation Database[29]	*http://www.hgmd.org*
	Leiden Open Variation Database[30]	*http://www.lovd.nl*
	dbNSFP (compiled database of precomputed *in silico* prediction scores for nonsynonymous SNVs)[31]	*https://sites.google.com/site/jpopgen/dbNSFP*
	Ensemble Variant Effect Predictor[15]	*http://www.ensembl.org/info/docs/tools/vep/index.html*

Guia de recomendación de informes

AMP Guidelines At a Glance



Li, M. et.al., Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. The Journal of Molecular Diagnostics, Vol19., No. 1, January 2017

J Mol Diagn. 2017 Jan;19(1):4-23

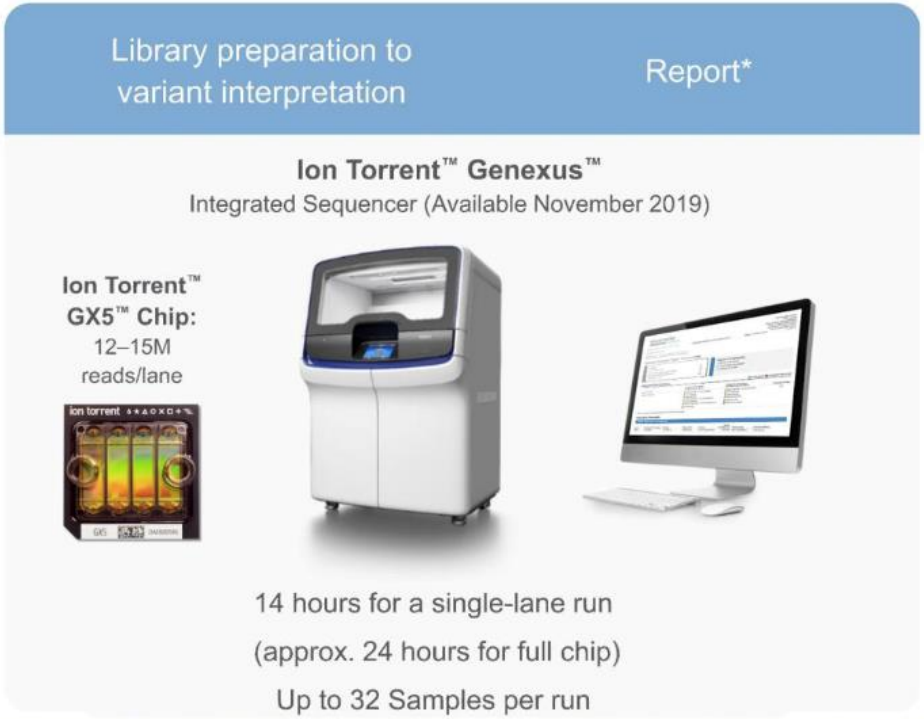
Porque realizar análisis de NGS

- **Caracterización molecular de tumores**
  Sarcomas
  Tumores Pediátricos

- **Análisis de Biomarcadores**
  Cancer de pulmón
  Cancer de colon
  Otros tipos tumorales

- Investigación traslacional

¿Porque Usar Genexus?



For Research Use Only. Not for use in diagnostic procedures.

- ✓ Workflow optimization (n=4 samples/line)
- ✓ Full automation
- ✓ Reduction in TAT
- ✓ Possibility to combine FFPE and Liquid Biopsy analysis

ONCOMINE PRECISION ASSAY (OPA)

Biomarcadores de Cáncer de pulmón (DNA + RNA)

DNA

Genes con cobertura Hotspot:

AKT1	ESR1	MAP2K2
AKT2	FGFR1	MET
AKT3	FGFR2	MTOR
ALK	FGFR3	NRAS
AR	FGFR4	NTRK1
ARAF	FLT3	NTRK2
BRAF	GNA11	NTRK3
CDK4	GNAQ	PDGFRA
CDKN2A	GNAS	PIK3CA
CHEK2	HRAS	PTEN
CTNNB1	IDH1	RAF1
EGFR	IDH2	RET
ERBB2	KIT	ROS1
ERBB3	KRAS	SMO
ERBB4	MAP2K1	TP53

45 genes

CNV (ganancia y pérdida de copias):

ALK
AR
CD274
CDKN2A
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
KRAS
MET
PIK3CA
PTEN

14 genes

RNA

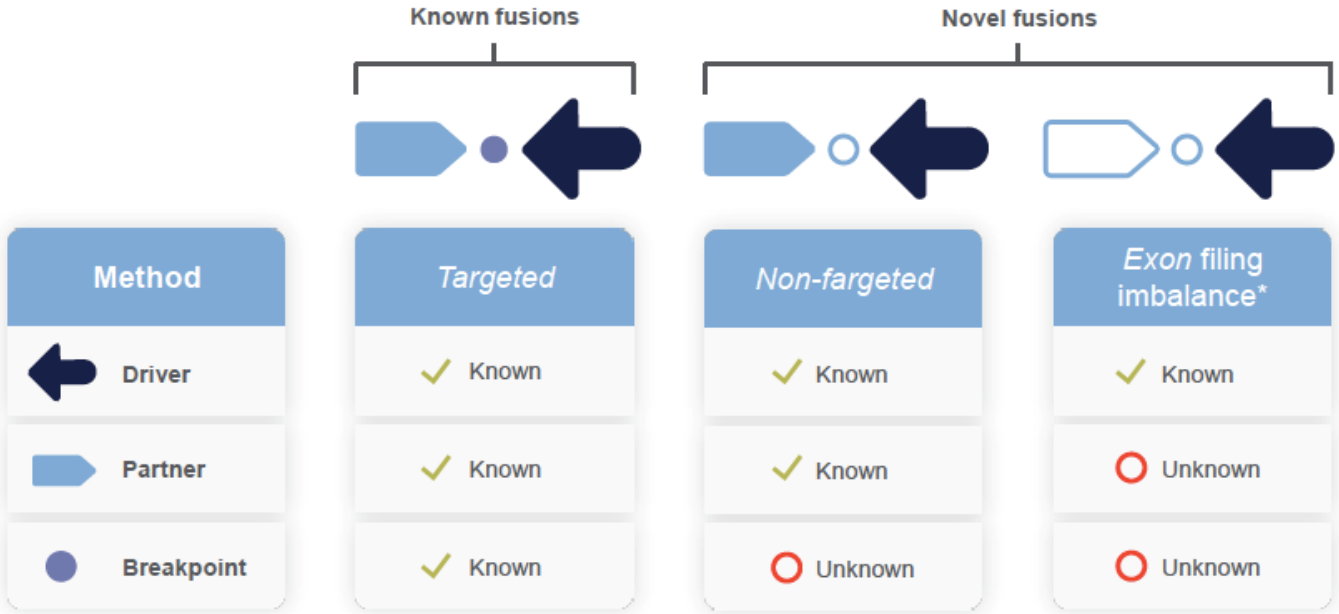
Fusiones Génicas:

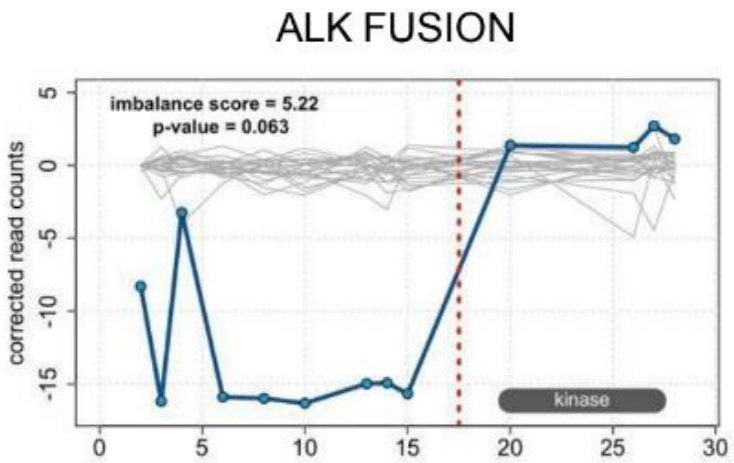
ALK	RET
AR	ROS1
BRAF	RSPO2
EGFR	RSPO3
ESR1	
FGFR1	
FGFR2	
FGFR3	
MET	
NRG1	
NTRK1	
NTRK2	
NTRK3	
NUTM1	

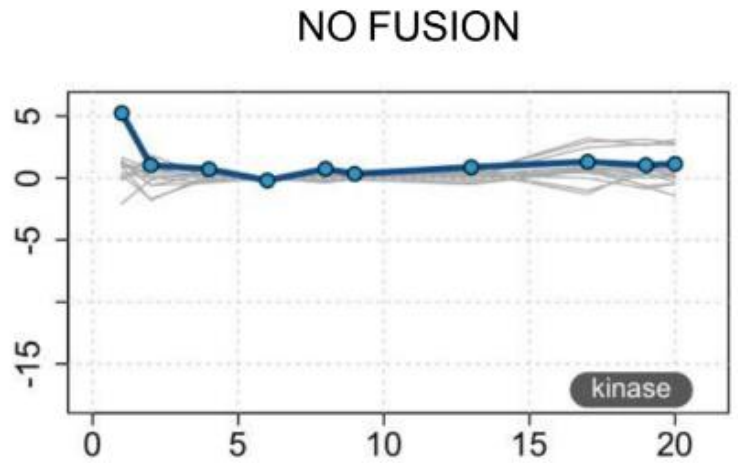
18 variantes

FusionSync Detection Technology

FusionSync Detection Technology is a synchronous approach that combines three methods for sensitive, specific, and broad detection of known and novel fusions





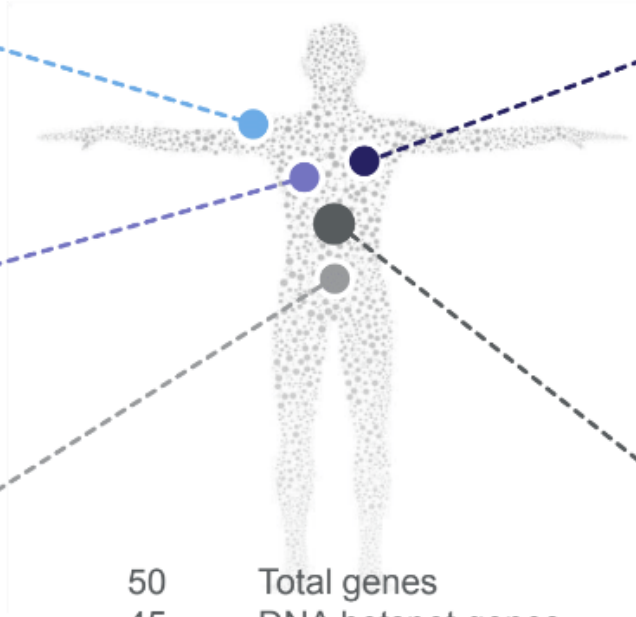


Oncomine Precision Assay Throughput on Genexus

	Assay	1 Lane	2 Lanes	3 Lanes	4 Lanes
Max Number of Samples	Oncomine Precision Assay (for DNA & RNA workflow)	4	8	12	16
	Oncomine Precision Assay (for cfTNA workflow)	1	2	3	4

Workflow	# of samples
Simultaneous DNA- and RNA-based variant analysis	up to 16 FFPE tissue samples (one DNA reaction and one RNA reaction per sample)
DNA-only or RNA-only variant analysis	up to 32 FFPE tissue samples

Posibles aplicaciones de NGS con OPA



Melanoma

- *BRAF* mutations
- *KIT* mutations

Breast, Gastric, Esophageal, Head and Neck, and Endometrial

- *ERBB2 (HER2)* amplification
- *PIK3CA* mutations

Colon

- *BRAF* mutations
- *NRAS* mutations
- *KRAS* mutations

NSCLC

- *EGFR* mutations
- *BRAF* mutations
- *KRAS* mutations
- *ALK* fusions
- *ERBB2 (HER2)* mutations
- *MET* amplification
- *ROS1* fusions
- ***RET fusions****
- MET Exon 14 skip

All solid tumor

- *NTRK1* fusions
- *NTRK2* fusions
- *NTRK3* fusions

50	Total genes
45	DNA hotspot genes
14	CNV genes
16	Inter-genetic fusions
3	Intra-genetic fusions

Evidence generated using Oncomine Reporter

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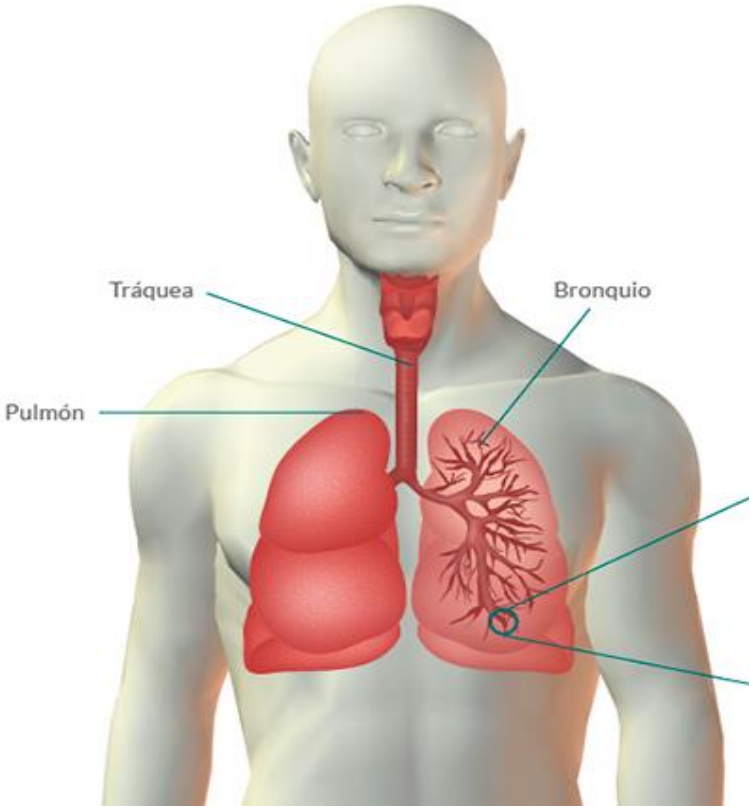
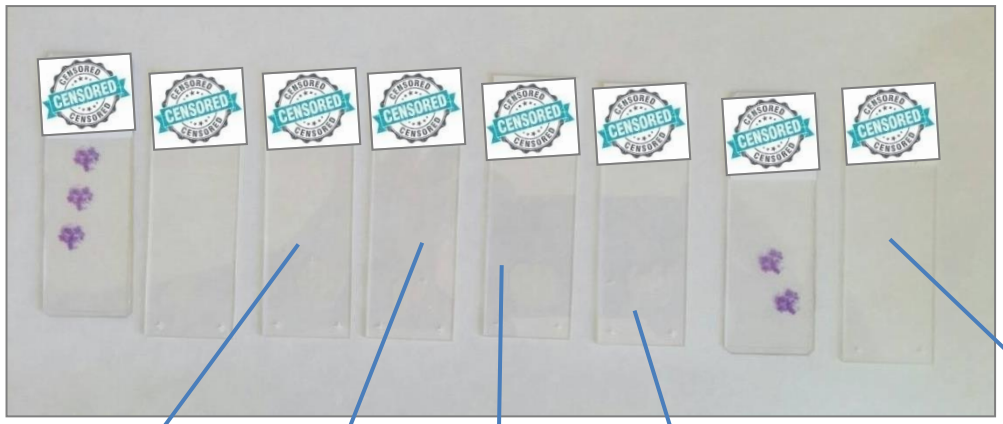
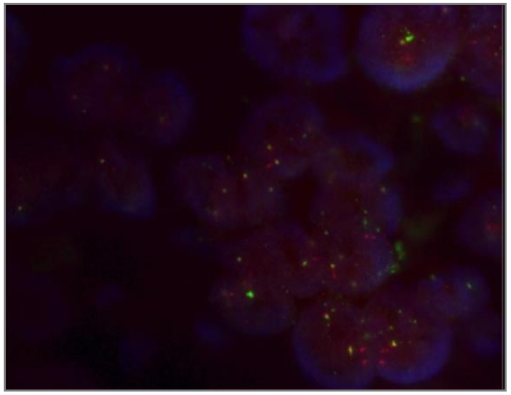
Experiencia en Cancer de pulmon

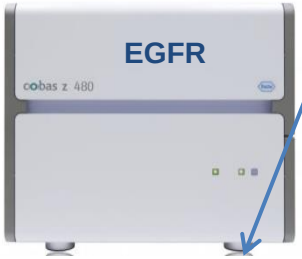


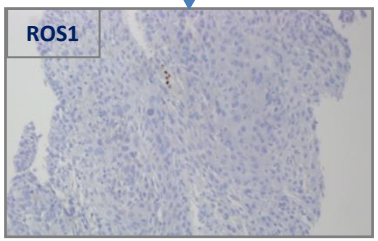
Hospital Universitario Vall d´Hebron

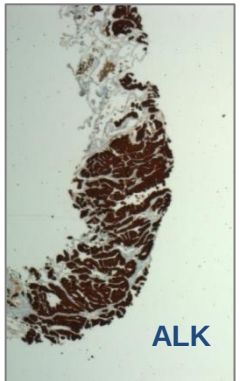


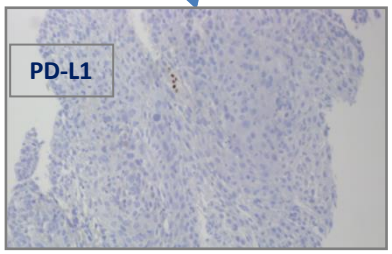
FISH ALK







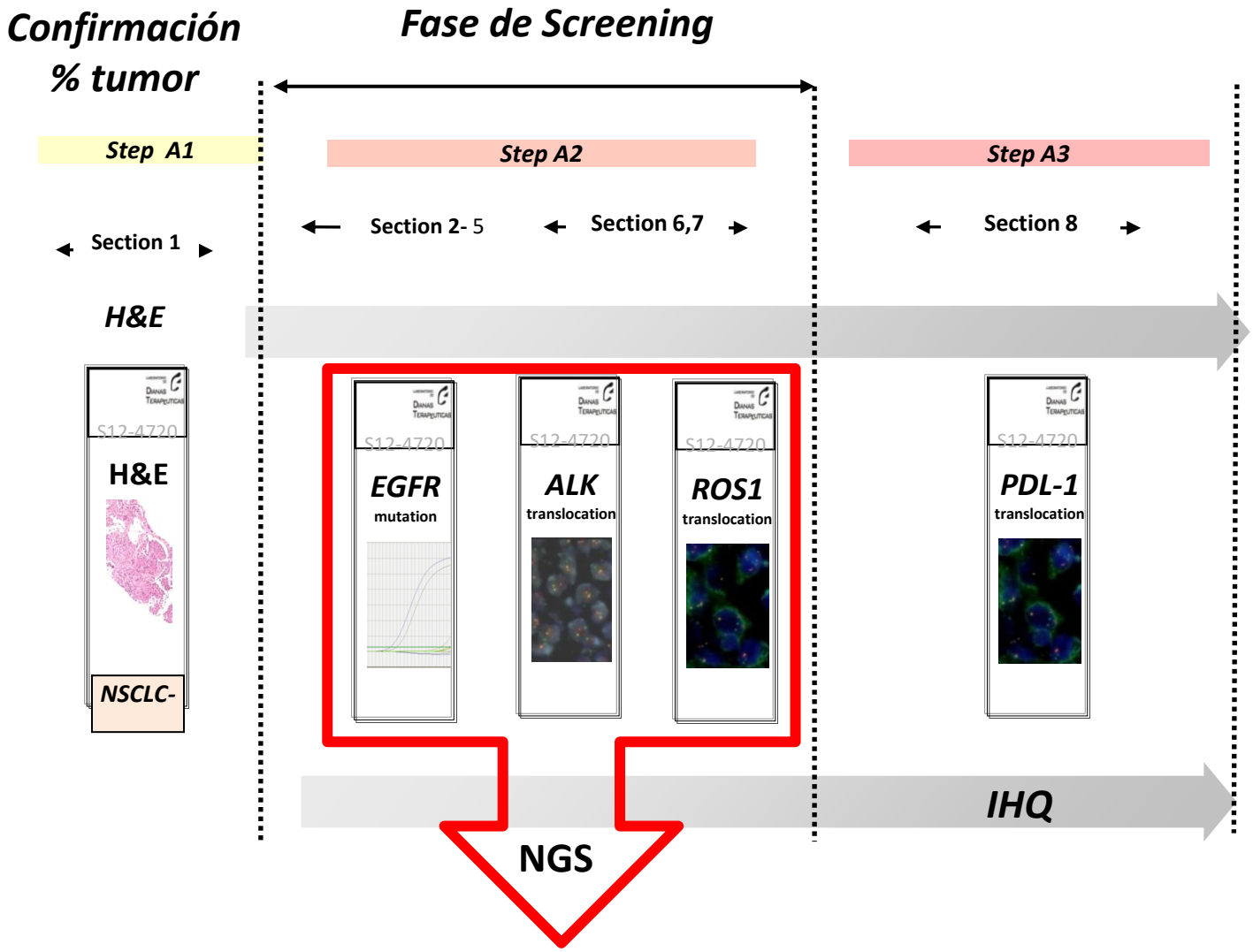




ALK Positive
74% rearranged cells
Deletion variant

Biología Molecular. Vall d´hebron

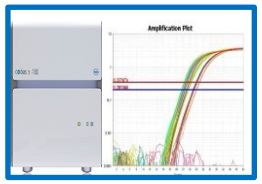
Cambios de flujo de Trabajo

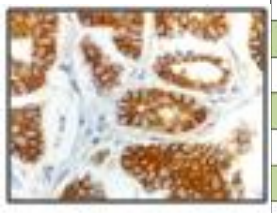


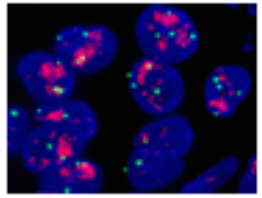
Adaptado Conde et al. Clin Transl Oncol 2013

100% Concordance vs qPCR

Gold Standard







PATIENT	Parafin (FFPE) / Extension (PAAF)	GOLD STANDARD (EGFR)	NGS RESULTS (EGFR)	NGS RESULTS (OTHER VARIANTS IN OTHER GENES)
1	FFPE	p.L861Q	p.Leu861Gln	
2	FFPE	WT	WT	DDR2 p.Met441Ile TP53 p.Val157Phe
3	FFPE	WT	WT	FGFR3 p.Phe384Leu
5	FFPE	p.L747_A750>P	p.L747_A750>P	
6	FFPE	Wt	WT	TP53 p.Gly244Val KRAS p.Gly12Cys
7	FFPE	WT	WT	SMAD4 p.Gln256Leu
8	FFPE	p.L858R	p.Leu858Arg	DDR2 p.Met441Ile
9	FFPE	WT	WT	TP53 p.Tyr236Cys
10	FFPE	WT	WT	TP53 p.Val217fs (frame shift deletor-INDEL)
11	FFPE	p.E746_A750	p.Glu746_Ala750del	TP53 p.His168Arg
12	FFPE	Wt	WT	TP53 p.Pro278Ala
15	FFPE	WT	WT	
16	FFPE	S768I L858R	p.Ser768Ile p.Leu858Arg	
17	FFPE	DEL 19	p.Glu746_Ala750del	FGFR3 p.Phe384Leu MET p.Thr1010Ile
18	FFPE	WT	WT	KRAS p.Gly12Cys
19	FFPE	L858R	p.Leu858Arg	
20	FFPE	DEL 19	p.Leu747_Pro753delinsSe	MET p.Glu168Asp
21	FFPE	L858R	p.Leu858Arg	MET p.Glu168Asp
22	FFPE	DEL 19 T790M	p.Glu746_Ala750del p.Thr790Met	EGFR p.Cys797Ser AKT1 c.47-12G>A TP53 p.Cys242Phe
23	PAAF	NOT DONE	p.Glu746_Ala750de p.Thr790Met	TP53 p.His168Arg
24	PAAF	NOT DONE	WT	

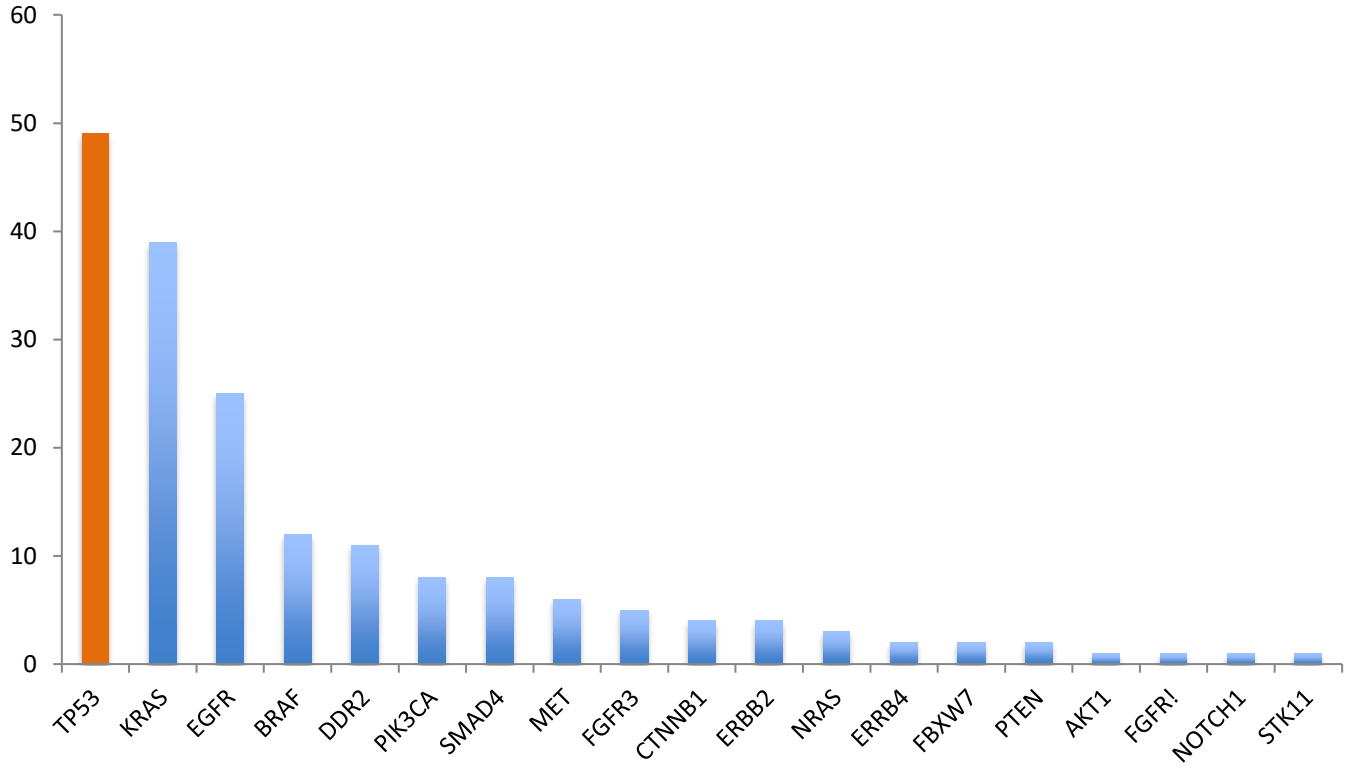
NGS

Biología Molecular. Vall d´hebron

Empleo de S5xl + IonChef

-Análisis de Biomarcadores de Cancer de pulmón (OST)
-Análisis de biomarcadores (Childhood)
-Análisis de Biomarcadores (Myeloid)

Experience in NSCLC



N= 221 NSCLC samples

INCORPORACIÓN DE GENEXUS

Instalación de Genexus 2020

COVID-19
Coronavirus

Jaime Vicioso. Ingeniero Thermo Fisher Scientific

Different handicaps

Hospital Accessibility
Genexus installation
Software installation
Genexus setup
Training ➔ ***Webex***

Live Lens®

Rescue®

OST vs OPA comparative Study in NSCLC samples

OST + OPA		OPA		
AKT1	**FGFR3**	AKT2	FLT3	NTRK1
ALK	**KRAS**	AKT3	GNA11	NTRK2
BRAF	**MAP2K1**	AR	GNAQ	NTRK3
CTNNB1	**MET**	ARAF	GNAS	PDGFRA
EGFR	**NRAS**	CDK4	HRAS	RAF1
ERBB2	**PIK3CA**	CDKN2A	IDH1	RET
ERBB4	**PTEN**	CHEK2	IDH2	
FGFR1	**TP53**	ERBB3	KIT	
FGFR2		ESR1	MAP2K2	
		FGFR4	MTOR	

NSCLC Samples analyzed by OST
n = 15 samples

OST vs OPA comparative Study in NSCLC samples

ID Mostra	GEN	Canvi AA	Canvi nt	Freq Al·lèlica	Profunditat
1	KRAS	p.Gly12Asp	c.35G>A	13,2	2.577
	RET	p.Cys620Arg	c.1858T>C	3,3	849
2	BRAF	p.Gly469Ala	c.1406G>C	32,4	5.023
3	CD274 Gain-of-Function Copy Number 12,05				
4	KIT	p.Val530Ile	c.1588G>A	32	571
	TP53	p.Gly245Val	c.734G>T	37,3	643
	AR LOSS 0,82				
5	EGFR	p.Leu858Arg	c.2573T>G	20,2	4.664
6	KRAS	p.Gly12Cys	c.34G>T	11,7	2.577
7	EGFR	p.E746_A750del	c.2236_2250delGAATTAAGAGAAGCA	30	5.032
8	KRAS	p.Gln61His	c.183A>C	13,7	4.159
9	KRAS	p.Gly12Val	c.35G>T	44,8	6.961
10	EGFR	p.Leu858Arg	c.2573T>G	24,1	1.563
11	Sense Alteracions				
12	CTNNB1	p.Asp32Asn	c.94G>A	39,4	1.205
	KIT	p.Val530Ile	c.1588G>A	6,7	1.179
	TP53	p.Asp281Gly	c.842A>G	46,3	721
13	Sense Alteracions				
14	P53	p.Val157Phe	c.469G>T	22,8	5.001
	KRAS AMPLIFICATION CNV Ratio 3,52				
15	ERBB3	p.Asp297Tyr	c.889G>T	20,6	1.867
	PIK3CA	p.Glu545Asp	c.1635G>C	7,7	3.522

Oncomine precision assay is labeled For Research Use Only. Not for use in diagnostic procedures.
Oncomine Solid Tumour assay is a product for in vitro diagnostic use

Results by OPA

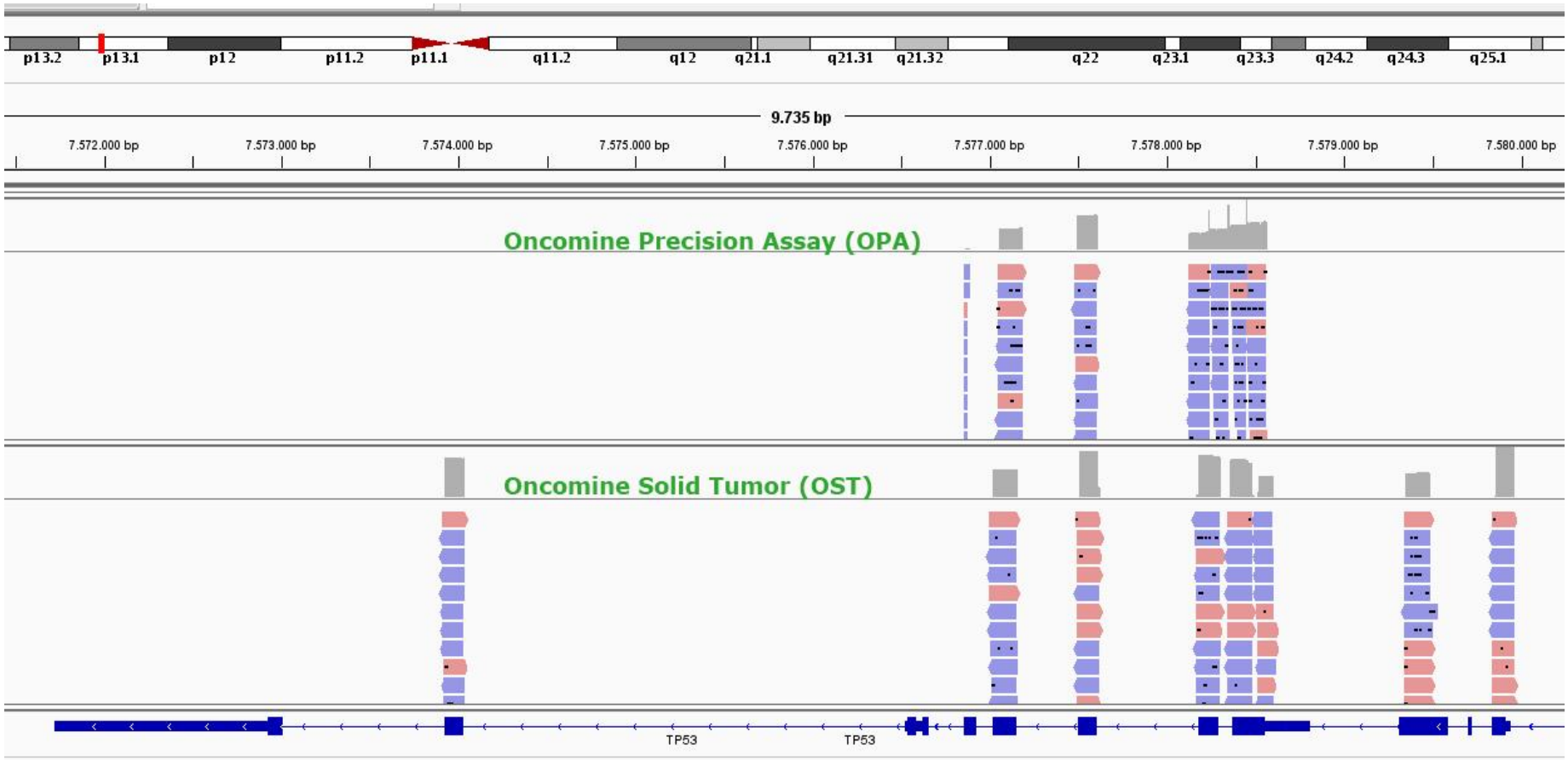
OST vs OPA comparative Study in NSCLC samples

ID Mostra	GEN	Canvi AA	Canvi nt	Freq Al·lèlica	Profunditat
1	KRAS	p.Gly12Asp	c.35G>A	6,32	1.771
2	BRAF	p.Gly469Ala	c.1406G>C	28,96	2.738
3	SMAD4	p.His132Tyr	c.394C>T	34,77	3.103
	TP53	p.Glu349Ter	c.1045G>T	16,65	2.721
4	ERBB4	p.Gln346His	c.1038G>T	22,12	3.996
	TP53	p.Gly245Val	c.734G>T	34,7	3.971
5	EGFR	p.Leu858Arg	c.2573T>G	18,2	3.988
6	KRAS	p.Gly12Cys	c.34G>T	11,96	3.963
	TP53	p.Gln104Ter	c.310C>T	12,68	1.554
7	EGFR	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAGAAGCA	22,85	3.921
8	KRAS	p.Gln61His	c.183A>C	12,91	3.998
9	KRAS	p.Gly12Val	c.35G>T	41,6	3.966
	TP53	p.Arg110Pro	c.329G>C	30,4	1.589
	DDR2	p.Met441Ile	c.1323G>A	21,08	4.000
10	EGFR	p.Leu858Arg	c.2573T>G	25,35	3.988
11	Sense Alteracions				
12	CTNNB1	p.Asp32Asn	c.94G>A	52,89	2.320
	TP53	p.Arg110His	c.329G>A	74,98	2.370
	TP53	p.Asp281Gly	c.842A>G	54,29	2.387
13	TP53	p.Val217fs	c.649delG	30,21	3.969
14	TP53	p.Val157Phe	c.469G>T	35,8	3.109
	DDR2	p.Met441Ile	c.1323G>A	26,46	3.998
15	SMAD4	p.Gln256Leu	c.767A>T	5,15	1.185
	PIK3CA	p.Glu545Asp	c.1635G>C	4,9	3.960

Oncomine precision assay is labeled For Research Use Only. Not for use in diagnostic procedures.
Oncomine Solid Tumour assay is a product for in vitro diagnostic use

Results by OST

TP53 is full covered in OST, not in OPA



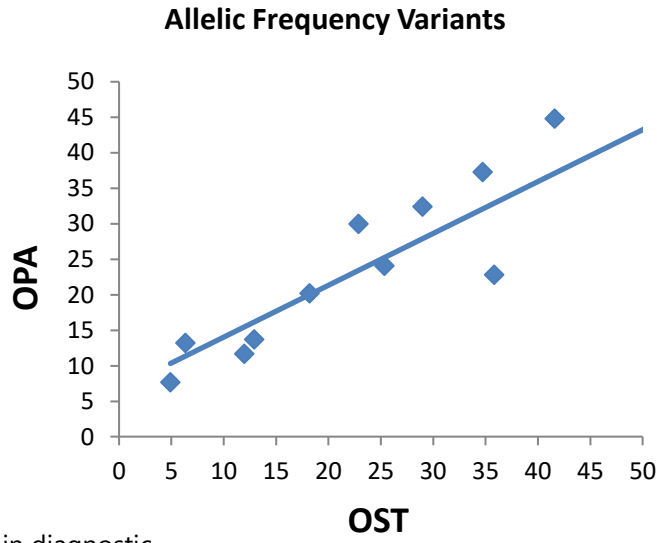
Fijarse bien que contiene cada panel

Oncomine precision assay is labeled For Research Use Only. Not for use in diagnostic procedures.
Oncomine Solid Tumour assay is a product for in vitro diagnostic use

OST vs OPA comparative Study in NSCLC samples

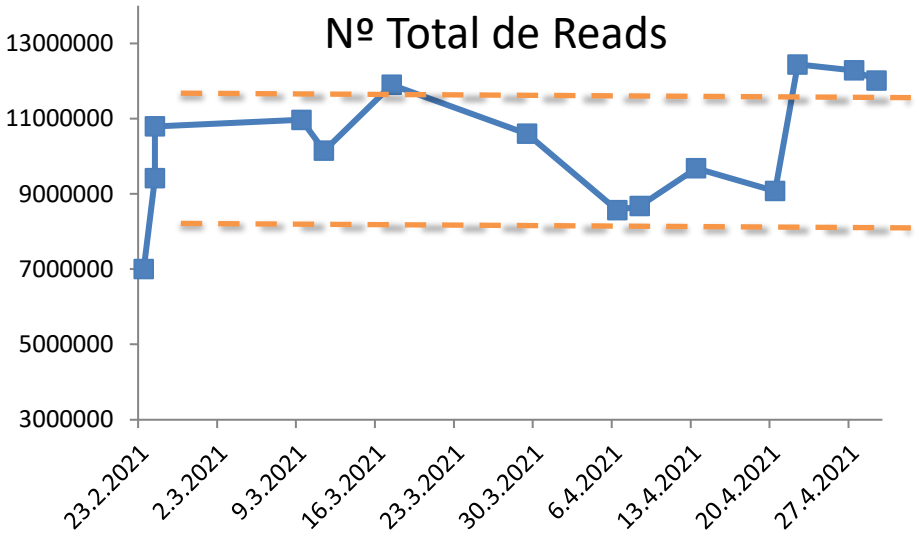
				OST		OPA	
Sample ID	GEN	Aminoacid	Nucleotide	FA	Reads	FA	Reads
1	*KRAS*	p.Gly12Asp	c.35G>A	6,32	1771	13,2	2577
2	*BRAF*	p.Gly469Ala	c.1406G>C	28,96	2738	32,4	5023
4	*TP53*	p.Gly245Val	c.734G>T	34,7	3971	37,3	643
5	*EGFR*	p.Leu858Arg	c.2573T>G	18,2	3988	20,2	4664
6	*KRAS*	p.Gly12Cys	c.34G>T	11,96	3963	11,7	2577
7	*EGFR*	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAGAAGCA	22,85	3921	30	5032
8	*KRAS*	p.Gln61His	c.183A>C	12,91	3998	13,7	4159
9	*KRAS*	p.Gly12Val	c.35G>T	41,6	3966	44,8	6961
10	*EGFR*	p.Leu858Arg	c.2573T>G	25,35	3988	24,1	1563
12	*TP53*	p.Asp281Gly	c.842A>G	54,29	2387	46,3	721
12	*CTNNB1*	p.Asp32Asn	c.94G>A	52,89	2320	39,4	1205
14	*TP53*	p.Val157Phe	c.469G>T	35,8	3109	22,8	5001
15	*PIK3CA*	p.Glu545Asp	c.1635G>C	4,9	3960	7,7	3522

NSCLC Samples analyzed by OST
n = 15 samples



Oncomine precision assay is labeled For Research Use Only. Not for use in diagnostic procedures.
Oncomine Solid Tumour assay is a product for in vitro diagnostic use

Métricas de calidad



Nuevo Software Genexus

Genexus | Ion Torrent

Samples Runs Monitor Results Assays

Samples / Manage Samples

Filter Samples by... | Sample Name | Enter Sample Name

+ Create Sample | Import Samples | Delete | Export | + Prepare Library Batch

Sample Name	Collection Date	Created On	Disease Category	Cancer Type	Sample Type	Gender	Actions
DNA_21_1374_RNA_21_1437	2021-04-13	2021-04-13 12:51	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1492-2_RNA_21_1493	2021-04-13	2021-04-13 12:51	Cancer	Unknown Primary Origin	FFPE	Female	Edit | Audit | Notes
DNA_21_1490-2_RNA_21_1491	2021-04-13	2021-04-13 12:48	Cancer	Non-Small Cell Lung Cancer	FFPE	Female	Edit | Audit | Notes
DNA_21_1539_RNA_21_1540	2021-04-13	2021-04-13 12:47	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1537_RNA_21_1538	2021-04-13	2021-04-13 12:47	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1535_RNA_21_1536	2021-04-13	2021-04-13 12:46	Cancer	Non-Small Cell Lung Cancer	FFPE	Female	Edit | Audit | Notes
DNA_21_1529_RNA_21_1530	2021-04-13	2021-04-13 12:45	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1542_RNA_21_1543	2021-04-13	2021-04-13 12:42	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes

Métricas de calidad

Genexus | Ion Torrent — Samples | Runs | Monitor | Results | Assays

Results / 2021_04_27 / OPA DNA-Fus-w2.6.0 / DNA_21_1784_RMA_21_1785

DNA_21_1784_RMA_21_1785 — Summary | QC ✓ | Variants | Plugins

Run QC —

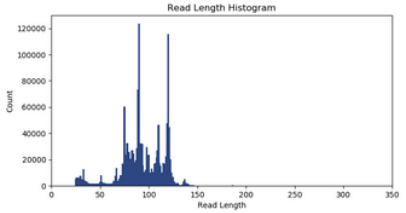
Key Signal	—	57
Not Set		
Percent Loading	—	92%
Not Set		
Raw Read Accuracy	—	98,8
Not Set		

Templating Control QC - CF-1 —

Average Reads Per Lane	—	69.168
Not Set		
Base Call Accuracy	—	96,7%
Not Set		
Mean AQ20 Read Length (bp)	—	73
Not Set		

Sample QC - DNA ✓

MAPD	✓	0,24
[0-0.5]		
Mapped Reads	—	1.523.989
Not Set		
Mean AQ20 Read Length (bp)	—	81
Not Set		
Mean Read Length (bp)	—	93
Not Set		
Uniformity Of Base Coverage	—	96,13
Not Set		



Sample QC - RNA ✓

Mapped Reads	—	440.090
Not Set		
Mean Read Length (bp)	—	90
Not Set		
RNA Expression Ctrls Detected	✓	7
>=5		

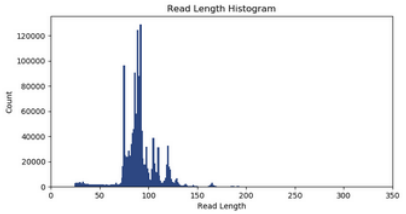
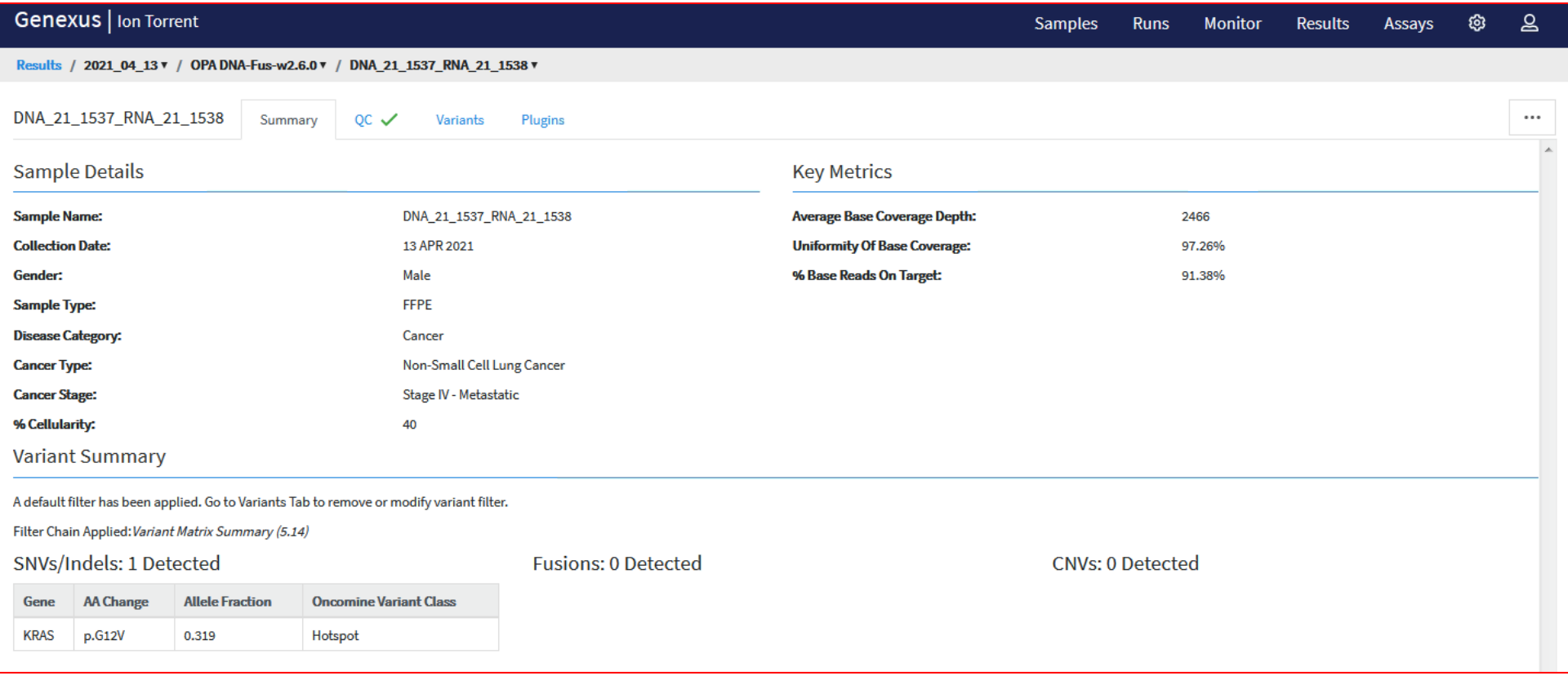
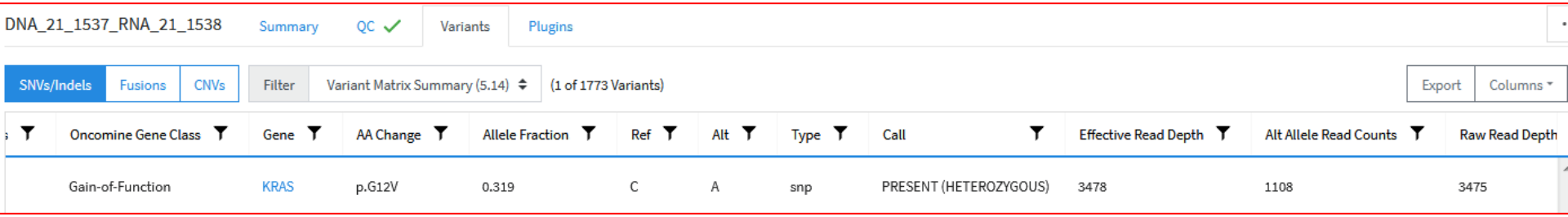


Table 6 Example QC metrics for FFPE DNA+RNA isolated from tumor FFPE samples *(continued)*

QC metric	Value
Sample QC – DNA	
MAPD	0.18–0.24
Mapped Reads	525,000–1,000,000
Mean AQ20 Read Length (bp)	85–95
Mean Read Length (bp)	85–100
Uniformity Of Base Coverage	97–99%
Sample QC – RNA	
Mapped Reads	105,000–650,000
Mean Read Length (bp)	70–100
RNA Expression Ctrls Detected	5–7


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / 2021_04_13 / OPA DNA-Fus-w2.6.0 / DNA_21_1537_RNA_21_1538
DNA_21_1537_RNA_21_1538
Summary
QC
Variants
Plugins
Sample Details
Sample Name: DNA_21_1537_RNA_21_1538
Collection Date: 13 APR 2021
Gender: Male
Sample Type: FFPE
Disease Category: Cancer
Cancer Type: Non-Small Cell Lung Cancer
Cancer Stage: Stage IV - Metastatic
% Cellularity: 40
Key Metrics
Average Base Coverage Depth: 2466
Uniformity Of Base Coverage: 97.26%
% Base Reads On Target: 91.38%
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: 0 Detected
CNVs: 0 Detected
Gene
AA Change
Allele Fraction
Oncomine Variant Class
KRAS
p.G12V
0.319
Hotspot


DNA_21_1537_RNA_21_1538
Summary
QC
Variants
Plugins
SNVs/Indels
Fusions
CNVs
Filter
Variant Matrix Summary (5.14)
(1 of 1773 Variants)
Export
Columns
Oncomine Gene Class
Gene
AA Change
Allele Fraction
Ref
Alt
Type
Call
Effective Read Depth
Alt Allele Read Counts
Raw Read Depth
Gain-of-Function
KRAS
p.G12V
0.319
C
A
snp
PRESENT (HETEROZYGOUS)
3478
1108
3475

BIOLOGIA MOLECULAR

ANÁLISIS MUTACIONAL Y DE FUSIONES GÉNICAS EN TUMOR SÓLIDO

Muestra

Origen de la muestra: Ganglio
Tipo de muestra: PAAF
Referencia DNA: 21/1490, Muestra Valorable
Referencia RNA: 21/1491, Muestra Valorable
Contenido tumoral: 40%

RESULTADO: **PRESENCIA de variantes en los siguientes genes:**
KRAS [c.34G>T; p.Gly12Cys]; VAF 51%; COSM516
IDH1 [c.395G>T; p.Arg132Leu]; VAF 21,4%; COSM28750

Método

Metodología: secuenciación masiva de nueva generación (Next Generation Sequencing, NGS) de las muestras de DNA y RNA para la determinación de mutaciones puntuales, deleciones e inserciones en 50 genes, de CNVs en 14 genes y fusiones génicas en 19 genes relacionados con patologías de cáncer, siguiendo el protocolo del panel "Oncomine Prescision Assay" (ThermoFisher Scientific). Los genes que cubre el panel son:

DNA
Genes con cobertura Hotspot: AKT1, AKT2, AKT3, ALK, AR, ARAF, BRAF, CDK4, CDKN2A, CHEK2, CTNNB1, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, SMO, TP53
CNV (ganancia y pérdida de copias): ALK, AR, CD274, CDKN2A, EGFR, ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, KRAS, MET, PIK3CA, PTEN

RNA
Fusiones Génicas: ALK, AR, BRAF, EGFR, ESR1, FGFR1, FGFR2, FGFR3, MET, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, RET, ROS1, RSPO2, RSPO3

*NOTA: En este informe no se reportan polimorfismos definidos como variantes con una frecuencia en la población general de >1%, variantes sin efecto en la proteína o variantes por debajo del 5% de frecuencia alélica (VAF)

Dra. Marta Sesé
Dr. Javier Hernández

Responsables informe Molecular

Responsable informe: SESE FAUSTINO, MARTA

EQUIP DIAGNÒSTIC

Patòleg : DINARES FERNANDEZ, MARIA CARME
Citotècnic : GONZALEZ MOYA, SARA

Patólogo Responsable del caso

Biología Molecular. Vall d´hebron

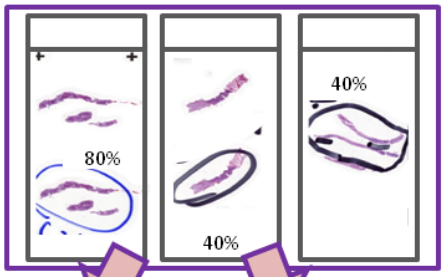
Flujo de trabajo del laboratorio

Lunes	*Martes*	*Miércoles*	*Jueves*	*Viernes*
				
Extracción DNA +RNA 2	Cuantificación 3	Extracción DNA+RNA 2	Comité Molecular	**Análisis** 5
	Carga Genexus 4	**Análisis** 5	Cuantificación 3	**Selección del material**  1
	Selección del material  1		**Carga Genexus** 4	

Biología Molecular. Vall d´hebron

Optimización de la muestra

ALGORITMO MANEJO DE LA MUESTRA PARA NGS EN CÁNCER DE PULMÓN



< 20%T

no hacer NGS

> 20% T

- 3-4 rollitos 10 µM para DNA

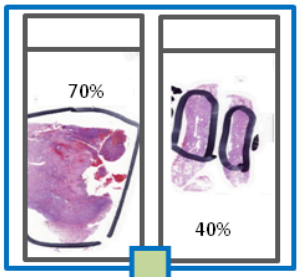
Extracción DNA Manual:

High Pure FFPET DNA Isolation Kit

- 3 rollitos 10 µM para RNA

Extracción RNA Manual:

FormaPure RNA Prep



> 20% T

- 2 rollitos 10 µM para DNA

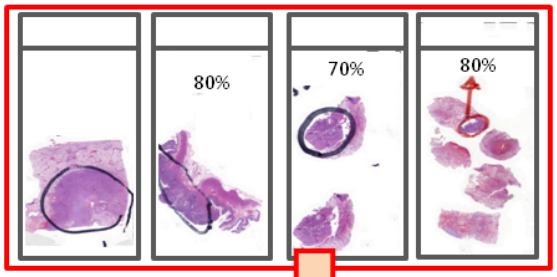
Extracción DNA automática:

Qiasymphony

- 2 rollitos 10 µM para RNA

Extracción RNA Manual:

FormaPure RNA Prep



Macrodisección de la zona tumoral

> 20% T y tamaño zona grande

- 3 rollitos 10 µM para DNA

Extracción DNA automática:

Qiasymphony

- 3 rollitos 10 µM para RNA

Extracción RNA Manual:

FormaPure RNA Prep

> 20% T y tamaño zona pequeña

- 3-4 rollitos 10 µM para DNA

Extracción DNA Manual

High Pure FFPET DNA Isolation Kit

- 3-4 rollitos 10 µM para RNA

Extracción RNA Manual:

FormaPure RNA Prep

CITOLOGÍAS

Diff-Quick:

DNA: **EZ1**

RNA: **FormaPure RNA Prep**

DNA

Si < [5] ng/µL

Concentrar con:

GeneJET FFPE DNA Purification kit

DNA Estandar

RNA

Si < [10] ng/µL

Concentrar con:

GeneJET RNA Purification kit

Protocolo LIBRERÍAS de DNA y RNA

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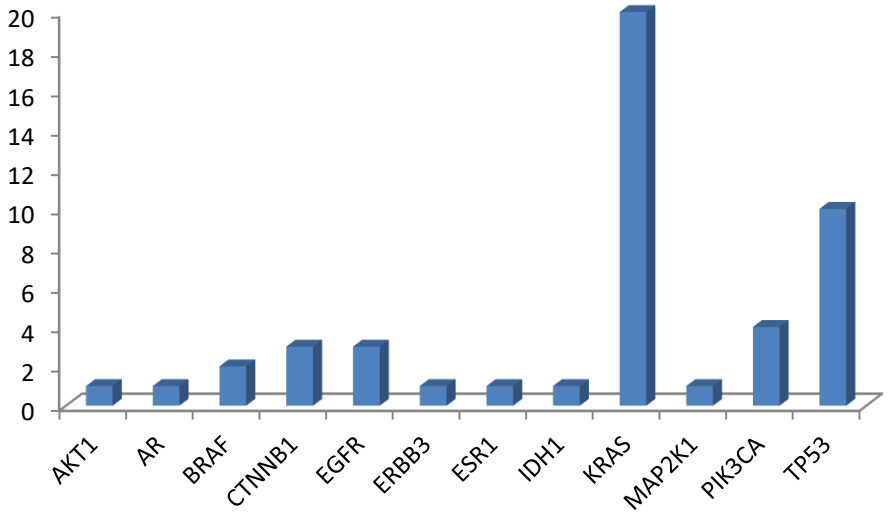
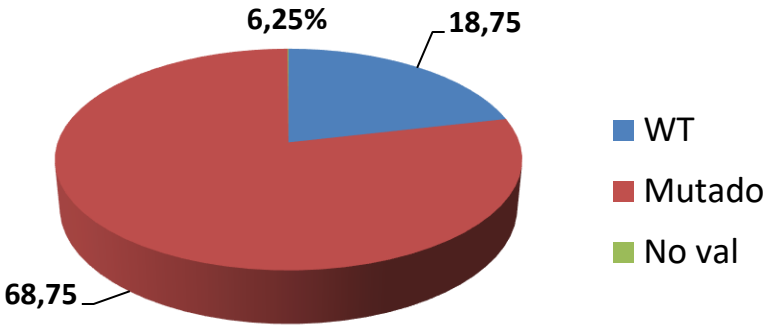
Dentro Video

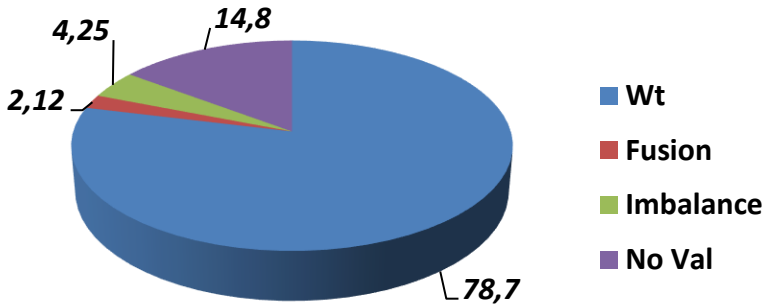
Experiencia Biomarcadores
Cancer Pulmón con el panel OPA



- Inicio análisis 23/02/2021
- 4-8 muestras/ semana
- Puesta punto circuito citologías



Análisis de ADN n=48



Análisis de ARN n=47

Biologia Molecular HUVH

Comite Molecular de Anatomia

- Una vez a la semana (Jueves 8.30) decisión de casos y discusión de resultados
- Priorización de casos para la semana siguiente
- Patólogos y Biólogos integrando los resultados

Experiencia de NGS en diferentes tumores sólidos

- Tumor de Origen Desconocido
- Gliomas
- Sarcomas

Genexus | Ion Torrent — Samples

Samples / Manage Samples

Filter Samples by... | Sample Name | Enter Sample Name | + Create Samp

Sample Name	Collection Date	Created On	Disease Category	Cancer Type	Sample Type
DNA_21_1829_RNA_21_1830	2021-04-29	2021-04-29 10:01	Cancer	Glioblastoma	FFPE
DNA_21_1807_RNA_21_1808	2021-04-29	2021-04-29 10:00	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1805_RNA_21_1806	2021-04-29	2021-04-29 09:59	Cancer	Unknown Primary Origin	FFPE
DNA_21_1624_RNA_21_1625	2021-04-29	2021-04-29 09:58	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1784_RMA_21_1785	2021-04-27	2021-04-27 11:02	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1780_RNA_21_1781	2021-04-27	2021-04-27 11:01	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1766_RNA_21_1767	2021-04-27	2021-04-27 11:00	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1759_RNA_21_1760	2021-04-27	2021-04-27 11:00	Cancer	Melanoma	FFPE

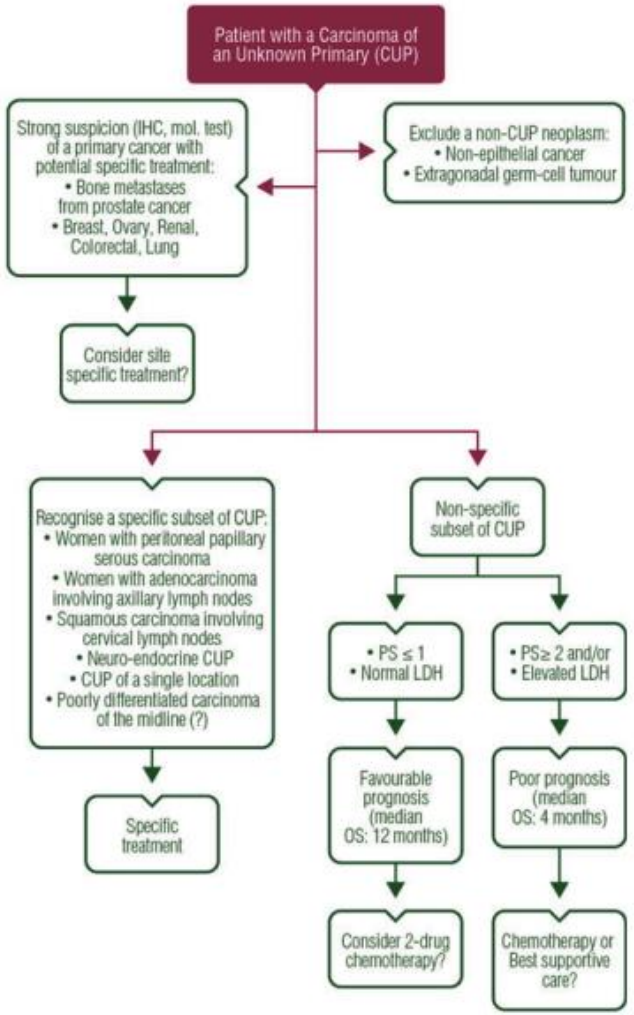
clinical practice guidelines

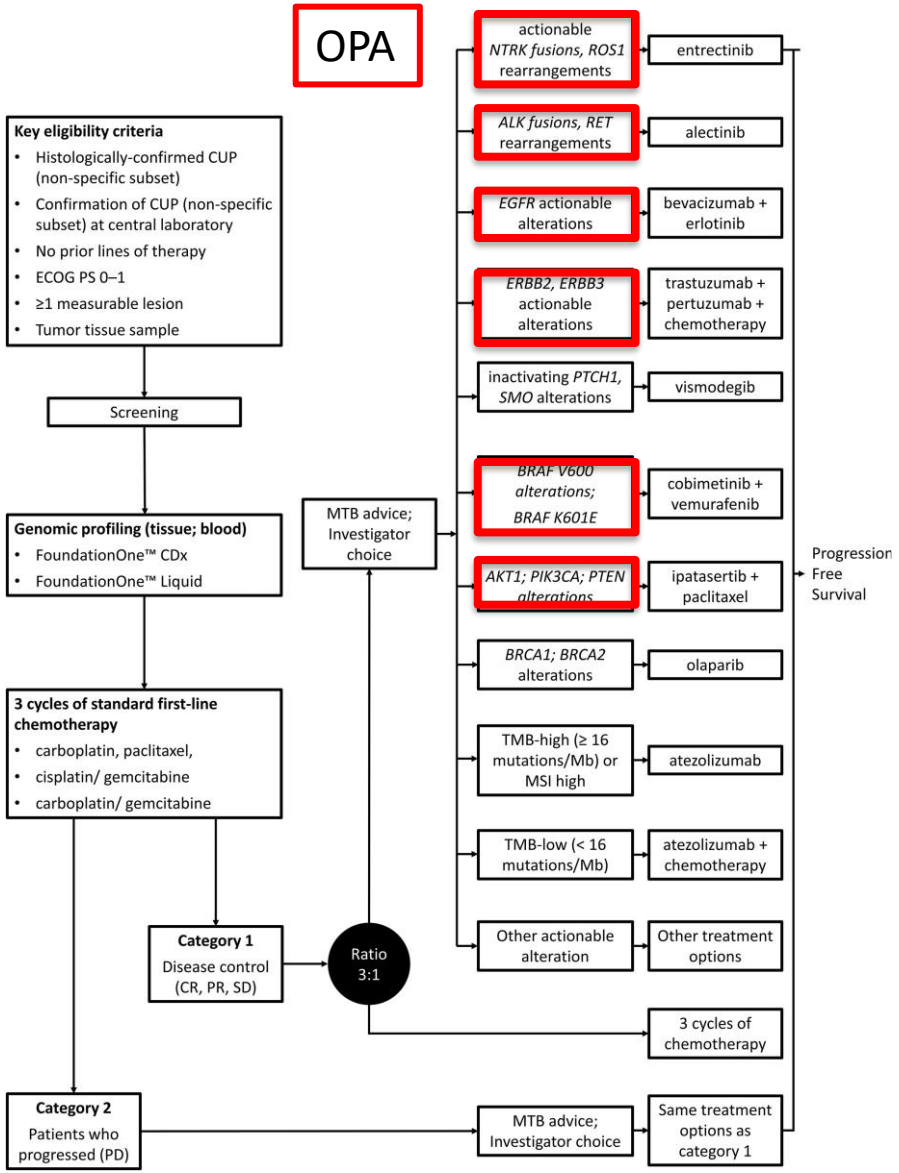
Annals of Oncology 26 (Supplement 5): v133–v138, 2015
doi:10.1093/annonc/mdv305

Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

K. Fizazi[1], F. A. Greco[2], N. Pavlidis[3], G. Daugaard[4], K. Oien[5] & G. Pentheroudakis[3], on behalf of the ESMO Guidelines Committee*

[1]Department of Cancer Medicine, Institut Gustave Roussy, University of Paris Sud, Villejuif, France; [2]Tennessee Oncology, Centennial Medical Center, Nashville, USA; [3]Department of Medical Oncology, University of Ioannina, Ioannina, Greece; [4]Department of Oncology 5073, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [5]University of Glasgow, Institute of Cancer Sciences, Glasgow, UK





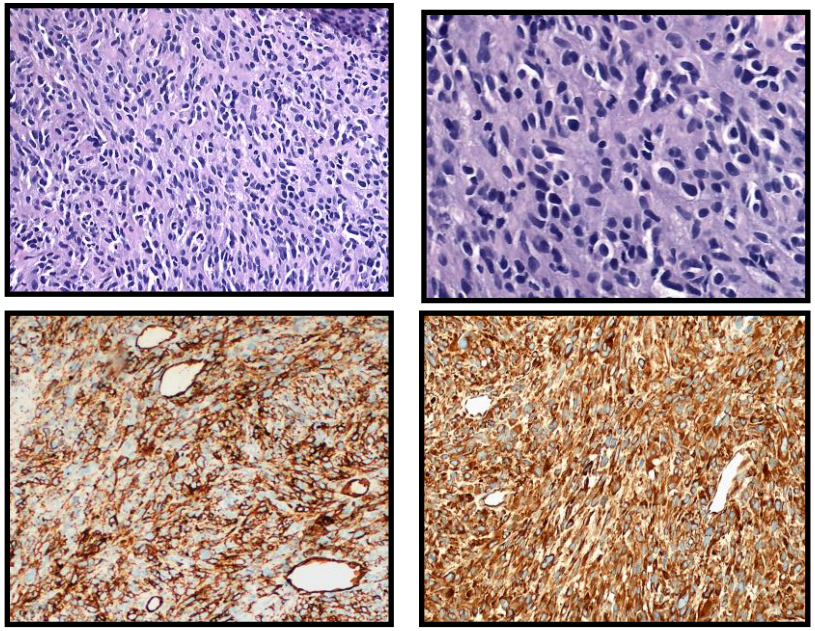
Annals of Oncology 2015 26v133-v138
Pauli C et al. Oncologist 2021

Otros tipos tumorales

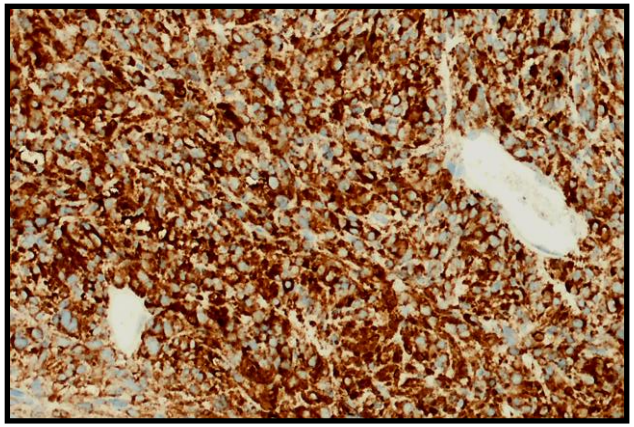
	Sample	Gene	Protein	DNA	Reads	FA	Cosmic
1	Glioma	TP53	p.R273H	c.818G>A	2.430	42	COSM10660
2	CUP	MET	p.R988C	c.2962C>T	5.354	50,9	COSM1666978
3	CUP	KRAS	p.G12C	c.34G>T	5.437	69,5	COSM516
4	Soft Tissue	TP53	p.R248Q	c.743G>A	6.610	42	COSM10662
5	Glioma	KRAS	p.G12V	c.35G>T	3.478	31,9	COSM520
6	Soft Tissue	TP53	p.R213*	c.637C>T	2948	96	COSM10654
7	Glioma	EGFR	p.G598V	c.1793G>T	6230	77,2	COSM21690
7	Glioma	EGFR			CNV 2,61		

	Sample	Results	Reads
1	Glioma	ND	
2	CUP	ND	
3	CUP	ND	
4	Glioma	GTF2I(6) BRAF(10)	1.637
5	CUP	ND	
6	Glioma	KANK1(3) NTRK2(12)	3.433
7	Glioma	ND	
8	Glioma	ND	
9	CUP	ND	
10	Carcinoma	FAIL	
11	Glioma	PTPRZ1(1) MET(2)	3.197
12	Melanoma	TPM3(7) ALK(20)	107
13	Glioma	ND	

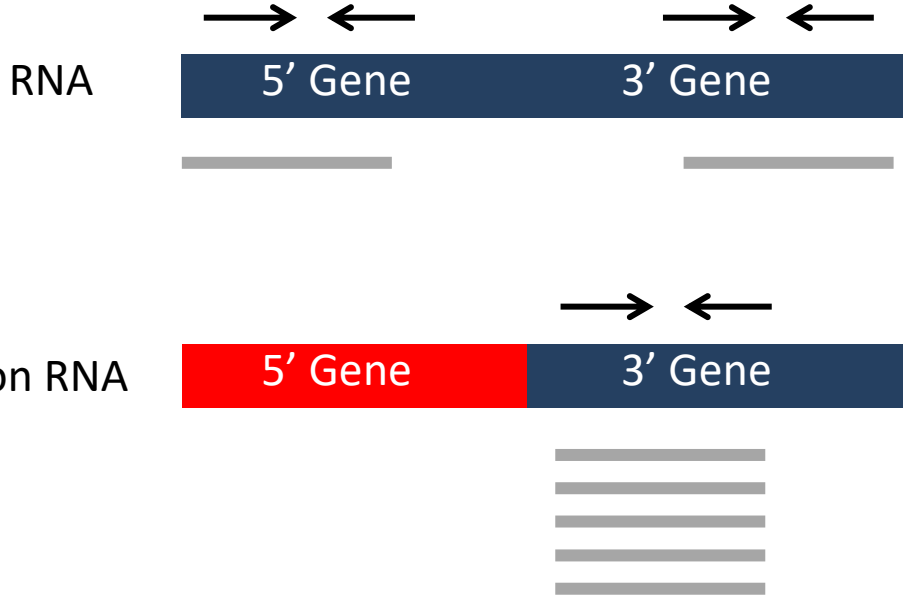
Malignant Mesenquimal Tumour
MNPST??

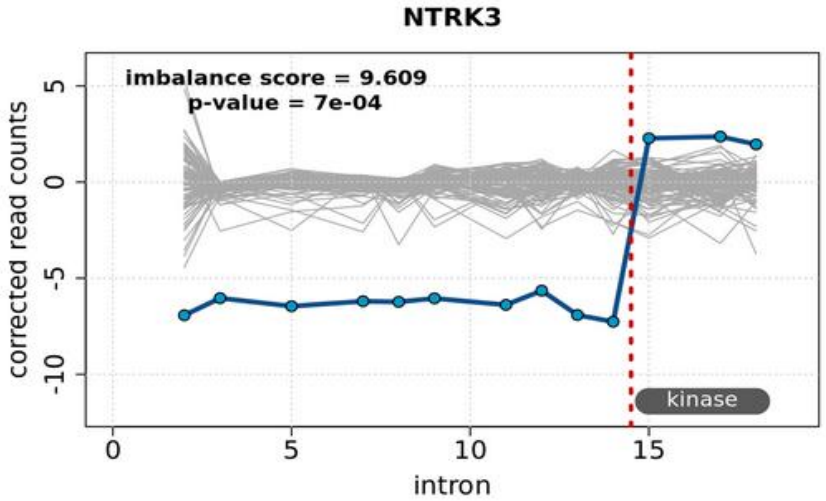


CD34 Vimentin



NTRK





Biología Molecular HUVH

Mensajes Finales

1. *El análisis de tumores sólidos por Genexus posee un flujo óptimo de muestras (múltiplos de 4)*

2. *Los tiempos de respuesta son inferiores a 7 días trás la solicitud*

3. *Los casos solicitados se comentan en el comité semanal de Molecular así como el resultado de los mismos*

4. *El panel de OPA puede aplicarse para el análisis de biomarcadores en diferentes tumores sólidos*

Muchas gracias a todos

Multidisciplinar Team

Molecular Biology. Vall d´hebron