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PANMEP

PROGRAMA ANDALUZ DE FORMACIÓN EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

EXPERTO UNIVERSITARIO EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

DIAGNÓSTICO MOLECULAR

FORMACIÓN
IAVANTE
Fundación
Progreso y Salud

ORGANIZAN:

- **Consejería de Salud y Consumo**, Fundación Progreso y Salud - IAVANTE
- **Universidad Internacional de Andalucía**

Dr. Michele Biscuola

Director Laboratorio Patología Molecular
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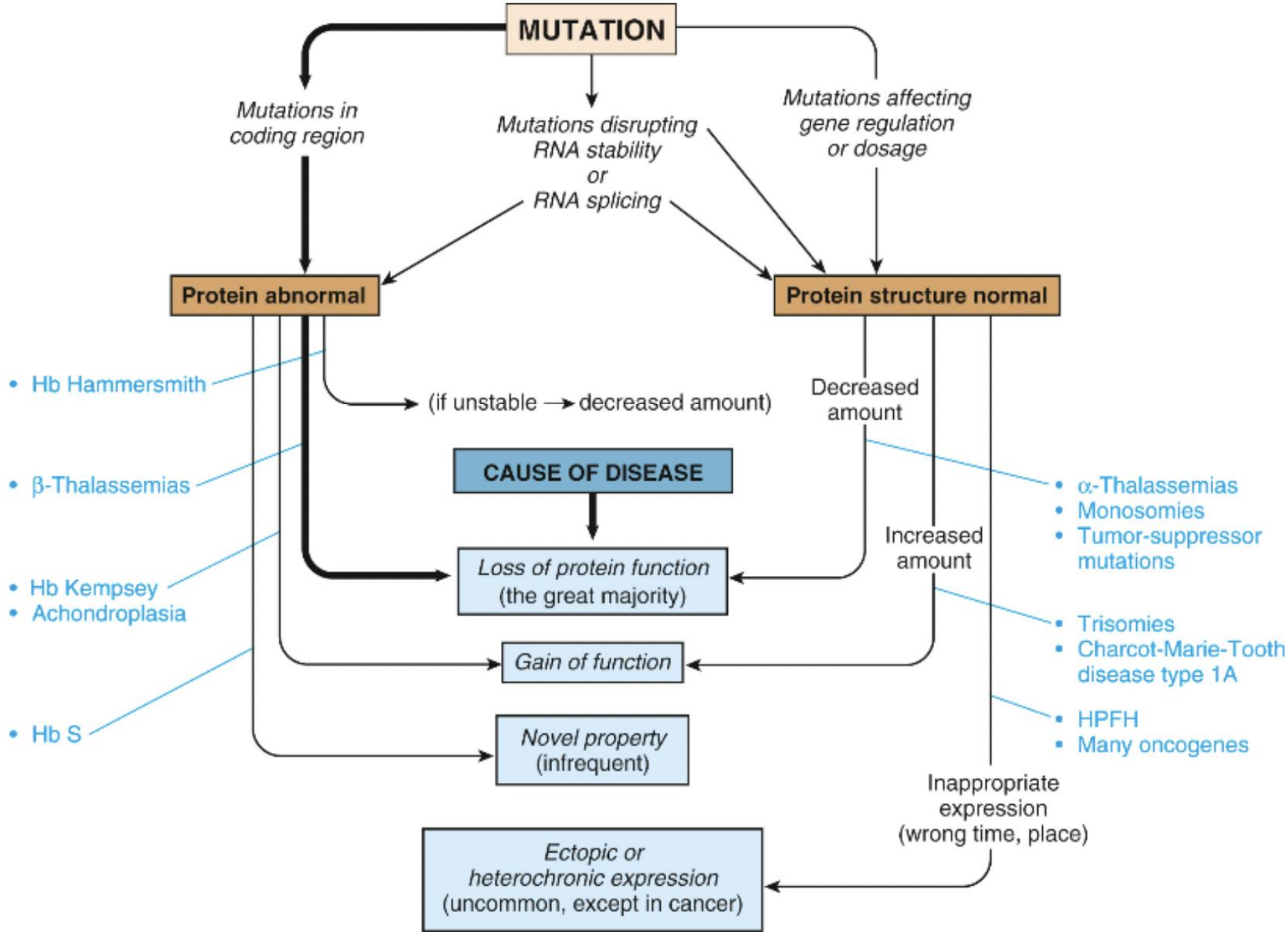
COLABORA
Johnson&Johnson

ORGANIZAN
Universidad Internacional de Andalucía

Junta de Andalucía
Consejería de Salud y Consumo


MUTATION
Mutations in coding region
Mutations disrupting RNA stability or RNA splicing
Mutations affecting gene regulation or dosage
Protein abnormal
Protein structure normal
• Hb Hammersmith
(if unstable → decreased amount)
Decreased amount
CAUSE OF DISEASE
• β-Thalassemias
• α-Thalassemias
• Monosomies
• Tumor-suppressor mutations
Increased amount
• Hb Kempsey
• Achondroplasia
Loss of protein function (the great majority)
Gain of function
• Trisomies
• Charcot-Marie-Tooth disease type 1A
• Hb S
Novel property (infrequent)
• HPFH
• Many oncogenes
Inappropriate expression (wrong time, place)
Ectopic or heterochronic expression (uncommon, except in cancer)

Enfermedades / Patologías

- **Relacionadas con alteraciones genéticas:**
    - Monogénicas
    - Digénicas
    - Oligogénicas
    - Poligénicas
- **Multifactoriales**
- **Otras etiologías**

Enfermedades / Patologías

- **Monogénicas**: Fibrosis quística, Anemia falciforme, Xeroderma pigmentosum, Sd. de Li-Fraumeni, Sd. Cowden, etc.

Examples of notable **Mutations**

Selection of notable mutations, ordered in a standard table of the genetic code of amino acids.

Clinically important missense mutations generally change the properties of the coded amino acid residue between being basic, acidic, polar or nonpolar, while nonsense mutations result in a stop codon.

ΔF508 deletion in cystic fibrosis

1st base \ 3rd base in each row	2nd base: U	2nd base: C	2nd base: A	2nd base: G
U	UUU (Phe/F) Phenylalanine	UCU (Ser/S) Serine	UAU (Tyr/Y) Tyrosine	UGU (Cys/C) Cysteine
	UUC (Phe/F) Phenylalanine	UCC (Ser/S) Serine	UAC (Tyr/Y) Tyrosine	UGC (Cys/C) Cysteine
	UUA (Leu/L) Leucine	UCA (Ser/S) Serine	UAA Ochre (Stop)	UGA Opal (Stop)
	UUG (Leu/L) Leucine	UCG (Ser/S) Serine	UAG Amber (Stop)	UGG (Trp/W) Tryptophan
C	CUU (Leu/L) Leucine	CCU (Pro/P) Proline	CAU (His/H) Histidine	CGU (Arg/R) Arginine
	CUC (Leu/L) Leucine	CCC (Pro/P) Proline	CAC (His/H) Histidine	CGC (Arg/R) Arginine
	CUA (Leu/L) Leucine	CCA (Pro/P) Proline	CAA (Gln/Q) Glutamine	CGA (Arg/R) Arginine
	CUG (Leu/L) Leucine	CCG (Pro/P) Proline	CAG (Gln/Q) Glutamine	CGG (Arg/R) Arginine
A	AUU (Ile/I) Isoleucine	ACU (Thr/T) Threonine	AAU (Asn/N) Asparagine	AGU (Ser/S) Serine
	AUC (Ile/I) Isoleucine	ACC (Thr/T) Threonine	AAC (Asn/N) Asparagine	AGC (Ser/S) Serine
	AUA (Ile/I) Isoleucine	ACA (Thr/T) Threonine	AAA (Lys/K) Lysine	AGA (Arg/R) Arginine
	AUG (Met/M) Methionine	ACG (Thr/T) Threonine	AAG (Lys/K) Lysine	AGG (Arg/R) Arginine
G	GUU (Val/V) Valine	GCU (Ala/A) Alanine	GAU (Asp/D) Aspartic acid	GGU (Gly/G) Glycine
	GUC (Val/V) Valine	GCC (Ala/A) Alanine	GAC (Asp/D) Aspartic acid	GGC (Gly/G) Glycine
	GUA (Val/V) Valine	GCA (Ala/A) Alanine	GAA (Glu/E) Glutamic acid	GGA (Gly/G) Glycine
	GUG (Val/V) Valine	GCG (Ala/A) Alanine	GAG (Glu/E) Glutamic acid	GGG (Gly/G) Glycine

β-Thalassemia

McArdle's disease

β-Thalassemia

- Myotonic dystrophy
- SCA 8

Fragile X Syndrome

Prostate cancer

Colorectal cancer

Sickle-cell disease

Friedreich's ataxia

Amino acids
- Basic
- Acidic
- Polar
- Nonpolar (hydrophobic)

Polyglutamine (PolyQ) Diseases
- Huntington's disease
- Spinocerebellar ataxia (SCA) (most types)
- Spinobulbar muscular atrophy (Kennedy disease)
- Dentatorubral-pallidoluysian atrophy

Mutation type
- = Trinucleotide repeat
- = Deletion
- = Missense
- = Nonsense

Enfermedades / Patologías

- **Monogénicas**: Fibrosis quística, Anemia falciforme, Xeroderma pigmentosum, Sd. de Li-Fraumeni, Sd. Cowden, etc.
- **Digénicas**: Retinitis pigmentosa, Sd. De Bardet–Biedl, etc.
- **Oligogénicas**: Síndrome de Lynch, Sd. QT corto, etc.
- **Poligénicas**: TEA, retrasos mentales no filiados, enfermedades neurológicas, miocardiopatías, etc.

Enfermedades / Patologías

Podemos enfrentarnos a:

- Un gen, una variante patogénica (*HbS*)
- Un gen, distintas variantes en sitios concretos (*KRAS*)
- Un gen, distintas variantes patogénicas en todo el gen (*MLH1*)
- Un gen, distintos tipos (SNV, CNV) de variantes (*BRCA1/2*)
- Distintos genes, una o pocas variantes en cada uno de ellos
- Distintos genes, muchas variantes en todos ellos

Alteración	Herramienta	Observaciones
SNV/InDel	• PCR convencional • Secuenciación • PCR en tiempo real • HRM	• PCR + RFLP • Iª, IIª, IIIª generación • Sondas diferentes • Cribado
CNV	• ISH • MLPA • aCGH • Secuenciación • Cariotipo	• FISH, CISH, SISH • Definir regiones • Resolución • DNA • Interpretación
SV	• ISH • Secuenciación • Cariotipo	• FISH • DNA y RNA • Interpretación

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SV	• ISH • **Secuenciación** • Cariotipo	• FISH • DNA y RNA • Interpretación

Secuenciación de nueva generación (NGS)

- Diferencias entre *Targeted*, WES y WGS
- Protocolo de secuenciación
- Tecnologías
- Diferencias entre capturas de híbridos y amplicones
- Significado de profundidad y cobertura
- Como implementar la NGS en un laboratorio
- Validación y verificación

Secuenciación

Gene-ración	Plataforma	Tecnología	Tamaño máximo de lectura (pb)	Lecturas por corrida (ML)	GB totales	Compañía
1ª	Sanger	Longitud didesoxinucleótidos	~ 1000	-	-	Thermo Fisher Scientific, Waltham, Ma, US
2ª	Roche 454*	Pirosecuenciación	~ 700	1	14	Hoffmann-La Roche Ltd, Basilea, Suiza
	SOLiD	Ligación y codificación por dos bases	2 x 75	100	320	Thermo Fisher Scientific, Waltham, Ma, US
	Illumina/ Solexa	Secuenciación por síntesis	2 x 150	6.000	1800	Illumina Inc., San Diego, Ca, USA
	PGM/ Ion Proton	Tecnología de semiconductores	2.000	80	5	Thermo Fisher Scientific, Waltham, Ma, US
3ª	Helicos*	Secuenciación individual con moléculas fluorescentes	70	20†	20†	Helicos Biosciences, Cambridge, Ma, USA
	PacBio	Secuenciación de una única molécula l en tiempo real	30.000	0,55†	1†	Pacific Biosciencies, Menlo Park, Ca, USA
	Nanopore	Bioporos	200.000	1.250	12.000††	Oxford Nanopore Technologies, Oxford, Reino Unido
	FRET**	Transferencia energética de resonancia de la fluorescencia	-	-	-	Thermo Fisher Scientific, Waltham, Ma, US
	Stratos**	Secuenciación por expansión	-	-	-	Hoffmann-La Roche Ltd, Basilea, Suiza

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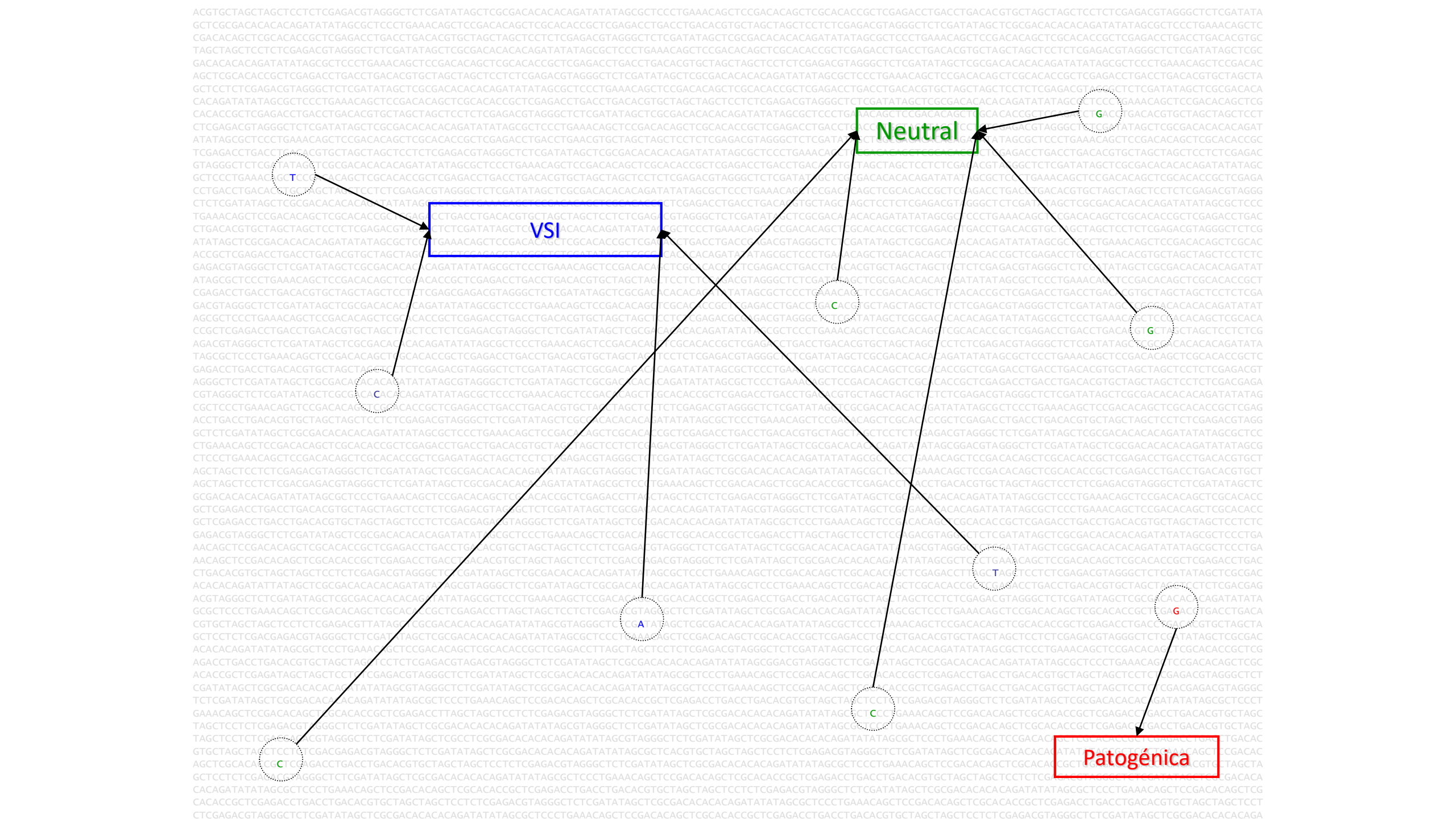
Neutral
VSI
Patogénica
T
C
A
C
G
G
T
G
C
C

NGS	ADN	ARN
WGS		
WES		
RNA-seq		
Targeted		

NGS	ADN	ARN
WGS	• Regiones codificantes • Regiones no codificantes • SNV • CNV • TMB • Reordenamientos estructurales	No aplica
WES		
RNA-seq		
Targeted		

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RNA-seq	No aplica	• Estudio del trascriptoma • Expresión génica *(mRNA, miRNA, snRNA, etc.)* • Eventos de *splicing* alternativo • Regulación génica • Detección de fusiones génicas
Targeted		

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Targeted	• SNV • CNV • TMB* • MSI* • LOH*	• Eventos de *splicing* alternativo • Detección de fusiones génicas

Targeted NGS

A selection of Next Generation Sequencing Platforms and other genetic companion devices.

EClinicalMedicine 25 (2020) 100487

PLATFORM	GENES ASSESSED	FDA APPROVAL	MUTATIONS
FoundationOne CDX (Foundation Medicine)	324	Yes	Copy number alterations, gene fusions, MSI, TMB, PDL-1 (IHC)
MSK IMPACT (Integrated Mutation Profiling Of Actionable Cancer Targets) (Memorial Sloan Kettering)	468	Yes	Somatic single nucleotide variants, insertions, deletions, and microsatellite instability
Oncomine Dx Target Test (Thermofisher)	46	Yes	DNA single-nucleotide variants (SNVs) and deletions in 35 genes, and RNA sequence variantions from 21 genes (Non-small cell lung cancer)
Caris Mollecular Intelligence CDX (Caris Life Sciences)	592	Partial	DNA: copy number alterations, MSI, TMB RNA: gene fusions, mRNA variants
Oncomine Comprehensive Assay (Thermofisher)	161	–	DNA sequencing: copy number alterations, gene fusions
Trusight Oncology 500 (Illumina)	523	–	DNA + RNA assay for assessment of small variants, TMB, MSI, splice variants, and fusions
FoundationOne Liquid	70	–	Plasma: DNA sequencing: copy number alterations, specific gene fusions for lung malignancies, MSI
Guandant360 (Guardant)	76	–	Plasma: DNA sequencing: copy number alterations, 6 gene fusions
GENETIC COMPANION DEVICES			
Praxis Extended RAS Panel (Illumina)	2	Yes	K-ras and N-ras (colorectal cancer)
Therascreen KRAS RGQ PCR Kit (Qiagen)	1	Yes	K-ras (colorectal cancer)
BRACANALYSIS CDX (Myriad Genetic Laboratories)	2	Yes	BRCA1, BRCA2 (Ovarian and Breast cancers)
FoundationFocus CDX BRCA Assay (FoundationOne)	2	Yes	BRCA1, BRCA2 (Ovarian cancer)
Therascreen EGFR RGQ PCR KIT (Qiagen)	1	Yes	EGFR (Non-small cell lung cancer)
COBAS EGFR Mutation Test V2 (Roche Molecular Systems)	1	Yes	EGFR (Non-small cell lung cancer)
THXID BRAF Kit (Biomérieux)	1	Yes	BRAF (Melanoma)
COBAS 4800 BRAF V600 Mutation Test (Roche Molecular Systems)	1	Yes	BRAF (Melanoma)
Therascreen FGFR RGQ RT-PCR Kit (Qiagen)	1	Yes	FGFR (Urothelial cancer)
Therascreen PIK3CA RGQ PCR Kit (Qiagen)	1	Yes	PIK3CA, tissue and plasma (breast cancer)
Myriad MYCHOICE® CDX (Myriad Genetic Laboratories)	Combined asay	Yes	Loss of heterozygosity (LOH), telomeric-allelic imbalance (TAI), large-scale state transitions (LST) (ovarian cancer)

Review

When should we order a next generation sequencing test in a patient with cancer?

Ramon Colomer[a,b,c,d,*], Rebeca Mondejar[a,b,c], Nuria Romero-Laorden[b,d], Arantzazu Alfranca[e], Francisco Sanchez-Madrid[a,c,e], Miguel Quintela-Fandino[a,d,f]

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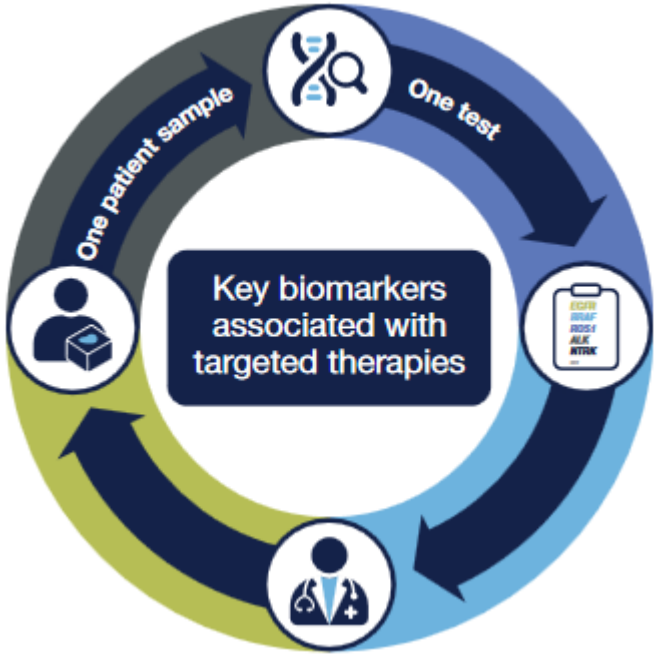
Targeted NGS

Oncomine Dx Target Test



DNA panel, hotspot genes

AKT1	*FGFR2*	*MAP2K1*
ALK	*FGFR3*	*MAP2K2*
AR	*GNA11*	*MET*
BRAF	*GNAQ*	*MTOR*
CDK4	*HRAS*	*NRAS*
CTNNB1	*IDH1*	*PDGFRA*
DDR2	*IDH2*	*PIK3CA*
EGFR	*JAK1*	*RAF1*
ERBB2	*JAK2*	*RET*
ERBB3	*JAK3*	*ROS1*
ERBB4	*KIT*	*SMO*
ESR1	*KRAS*	

RNA panel, fusion drivers

ABL1	*ETV4*	*NTRK2*
ALK	*ETV5*	*NTRK3*
AXL	*FGFR1*	*PDGFRA*
BRAF	*FGFR2*	*PPARG*
ERBB2	*FGFR3*	*RAF1*
ERG	*MET*	*RET*
ETV1	*NTRK1*	*ROS1*

Table 2 Seventy nine genes incorporated in the MammaSeq™ gene panel

ABL1	CDK6	FGFR3	KDR	NOTCH1
AKT1	CDKN1B	FGFR4	KIT	NRAS
AKT3	CDKN2A	FOXA1	KMT2C	PAK1
ALK	CDKN2B	GATA3	KRAS*	PDGFRA
AR	CTCF	GRB7	MAP2K4	PIK3CA
ARID1A	CTNNB1	HIST2H2BE*	MAP3K1	PIK3R1
ATM	DNAH14	HRAS*	MAP3K4	PTCH1
AURKA	EGFR	IDH1*	MDM2	PTEN
AURKB	ERBB2	IGF1R	MDM4	RB1
BRAF	ERBB3	IKBKB	MET	RET
BRCA1	ERBB4	IKBKE	MTOR	RPTOR
BRCA2	ESR1	INPP4B	MYC	RUNX1
CCND1	EZH2*	INSR	NCOA3	SMO
CCNE1	FGF19	JAK2	NCOR1	STK11
CDH1	FGFR1	JAK3	NCOR2	TP53
CDK4	FGFR2	JUN*	NF1	

Targeted NGS

Targeted NGS

Oncomine Comprehensive Assay v3

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	ESR1	KIT	PDGFRB	ARID1A	FBXW7	PTEN	AKT1	FGFR4	AKT2	FGFR2	NUTM1
AKT2	EZH2	KNSTRN	PIK3CB	ATM	MLH1	RAD50	AKT2	FLT3	ALK	FGFR3	PDGFRA
AKT3	FGFR1	KRAS	PIK3CA	ATR	MRE11	RAD51	AKT3	IGF1R	AR	FGR	PDGFRB
ALK	FGFR2	MAGOH	PPP2R1A	ATRX	MSH6	RAD51B	ALK	KIT	AXL	FLT3	PIK3CA
AR	FGFR3	MAP2K1	PTPN11	BAP1	MSH2	RAD51C	AXL	KRAS	BRCA1	JAK2	PRKACA
ARAF	FGFR4	MAP2K2	RAC1	BRCA1	NBN	RAD51D	AR	MDM2	BRCA2	KRAS	PRKACB
AXL	FLT3	MAP2K4	RAF1	BRCA2	NF1	RNF43	BRAF	MDM4	BRAF	MDM4	PTEN
BRAF	FOXL2	MAPK1	RET	CDK12	NF2	RB1	CCND1	MET	CDKN2A	MET	PPARG
BTK	GATA2	MAX	RHEB	CDKN1B	NOTCH1	SETD2	CCND2	MYC	EGFR	MYB	RAD51B
CBL	GNA11	MDM4	RHOA	CDKN2A	NOTCH2	SLX4	CCND3	MYCL	ERBB2	MYBL1	RAF1
CCND1	GNAQ	MED12	ROS1	CDKN2B	NOTCH3	SMARCA4	CCNE1	MYCN	ERBB4	NF1	RB1
CDK4	GNAS	MET	SF3B1	CHEK1	PALB2	SMARCB1	CDK2	NTRK1	ERG	NOTCH1	RELA
CDK6	H3F3A	MTOR	SMAD4	CREBBP	PIK3R1	STK11	CDK4	NTRK2	ESR1	NOTCH4	RET
CHEK2	HIST1H3B	MYC	SMO	FANCA	PMS2	TP53	CDK6	NTRK3	ETV1	NRG1	ROS1
CSF1R	HNF1A	MYCN	SPOP	FANCD2	POLE	TSC1	EGFR	PDGFRA	ETV4	NTRK1	RSPO2
CTNNB1	HRAS	MYD88	SRC	FANCI	PTCH1	TSC2	ERBB2	PDGFRB	ETV5	NTRK2	RSPO3
DDR2	IDH1	NFE2L2	STAT3				ESR1	PIK3CB	FGFR1	NTRK3	TERT
EGFR	IDH2	NRAS	TERT				FGF19	PIK3CA			
ERBB2	JAK1	NTRK1	TOP1				FGF3	PPARG			
ERBB3	JAK2	NTRK2	U2AF1				FGFR1	RICTOR			
ERBB4	JAK3	NTRK3	XPO1				FGFR2	TERT			
ERCC2	KDR	PDGFRA					FGFR3				

DNA GENE LIST: ENTIRE CODING SEQUENCE FOR THE DETECTION OF BASE SUBSTITUTIONS, INSERTION/DELETIONS, AND COPY NUMBER ALTERATIONS

ABL1	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*ALOX12B*	*AMER1 (FAM123B)*	*APC*
AR	*ARAF*	*ARFRP1*	*ARID1A*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*
BCOR	*BCORL1*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*
BTK	*C11orf30 (EMSY)*	*C17orf39 (GID4)*	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	*CCND1*
CCND2	*CCND3*	*CCNE1*	*CD22*	*CD274 (PD-L1)*	*CD70*	*CD79A*	*CD79B*	*CDC73*
CDH1	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*
CDKN2C	*CEBPA*	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CSF1R*	*CSF3R*
CTCF	*CTNNA1*	*CTNNB1*	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*
DDR2	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	*EGFR*	*EP300*	*EPHA3*	*EPHB1*
EPHB4	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*
FAM46C	*FANCA*	*FANCC*	*FANCG*	*FANCL*	*FAS*	*FBXW7*	*FGF10*	*FGF12*
FGF14	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*
FGFR4	*FH*	*FLCN*	*FLT1*	*FLT3*	*FOXL2*	*FUBP1*	*GABRA6*	*GATA3*
GATA4	*GATA6*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3F3A*
HDAC1	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*ID3*	*IDH1*	*IDH2*	*IGF1R*
IKBKE	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	*JAK2*	*JAK3*
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLHL6*
KMT2A (MLL)	*KMT2D (MLL2)*	*KRAS*	*LTK*	*LYN*	*MAF*	*MAP2K1 (MEK1)*	*MAP2K2 (MEK2)*	*MAP2K4*
MAP3K1	*MAP3K13*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*
MERTK	*MET*	*MITF*	*MKNK1*	*MLH1*	*MPL*	*MRE11A*	*MSH2*	*MSH3*
MSH6	*MST1R*	*MTAP*	*MTOR*	*MUTYH*	*MYC*	*MYCL (MYCL1)*	*MYCN*	*MYD88*
NBN	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*
NPM1	*NRAS*	*NSD3 (WHSC1L1)*	*NT5C2*	*NTRK1*	*NTRK2*	*NTRK3*	*P2RY8*	*PALB2*
PARK2	*PARP1*	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1 (PD-1)*	*PDCD1LG2 (PD-L2)*	*PDGFRA*
PDGFRB	*PDK1*	*PIK3C2B*	*PIK3C2G*	*PIK3CA*	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*
POLD1	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PTCH1*
PTEN	*PTPN11*	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*	*RAD51C*
RAD51D	*RAD52*	*RAD54L*	*RAF1*	*RARA*	*RB1*	*RBM10*	*REL*	*RET*
RICTOR	*RNF43*	*ROS1*	*RPTOR*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*
SF3B1	*SGK1*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*
SOX2	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	*STK11*	*SUFU*
SYK	*TBX3*	*TEK*	*TET2*	*TGFBR2*	*TIPARP*	*TNFAIP3*	*TNFRSF14*	*TP53*
TSC1	*TSC2*	*TYRO3*	*U2AF1*	*VEGFA*	*VHL*	*WHSC1*	*WT1*	*XPO1*
XRCC2	*ZNF217*	*ZNF703*						

DNA GENE LIST: FOR THE DETECTION OF SELECT REARRANGEMENTS

ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*CD74*	*EGFR*	*ETV4*
ETV5	*ETV6*	*EWSR1*	*EZR*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*KMT2A (MLL)*
MSH2	*MYB*	*MYC*	*NOTCH2*	*NTRK1*	*NTRK2*	*NUTM1*	*PDGFRA*	*RAF1*
RARA	*RET*	*ROS1*	*RSPO2*	*SDC4*	*SLC34A2*	*TERC**	*TERT***	*TMPRSS2*

*TERC is an NCRNA

**Promoter region of TERT is interrogated

ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER GENOMIC SIGNATURES

Loss of Heterozygosity (LOH) score
Microsatellite (MS) status
Tumor Mutational Burden (TMB)

Targeted NGS

Tabla 3: contenido de ADN incluido en los paneles de TruSight Oncology 500 y TruSight Oncology High-Throughput

ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	

El contenido sombreado de color gris se analiza para la detección de CNV.

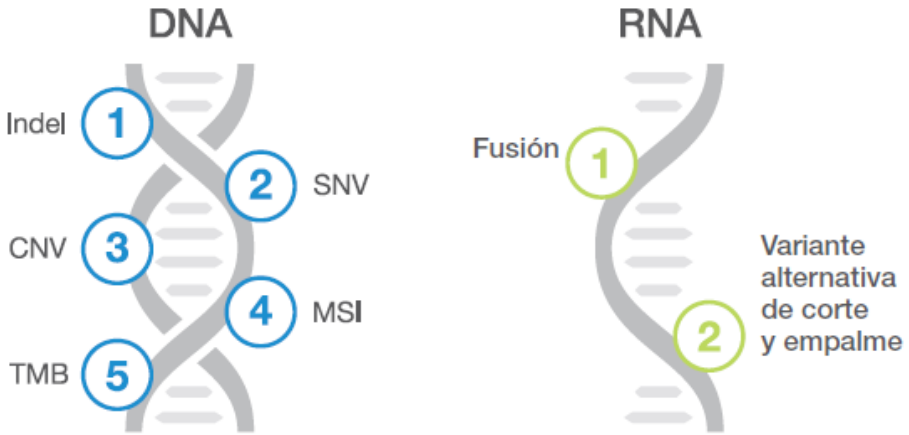
Targeted NGS

Tabla 3: contenido de ADN incluido en los paneles de TruSight Oncology 500 y TruSight Oncology High-Throughput

ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	

El contenido sombreado de color gris se analiza para la detección de CNV.

Targeted NGS



CNMP

Table 3A. List of genomic alterations level I/II/III according to ESCAT in advanced non-squamous non-small-cell lung cancer (NSCLC)

Gene	Alteration	Prevalence	ESCAT	References
EGFR	Common mutations (*Del19, L858R*)	15% (50%–60% Asian)	IA	Midha A, et al. *Am J Cancer Res.* 2015[26]
	Acquired *T790M* exon 20	60% of *EGFR* mutant	IA	Mok T, et al. *J Clin Oncol.* 2018[27]
	Uncommon *EGFR* mutations (*G719X* in exon	NSCLC	IB	Soria J-C, et al. *N Engl J Med.* 2018[28]
	18, *L861Q* in exon 21, *S768I* in exon 20)	10%	IIB	Ramalingam S, et al. *N Engl J Med.* 2020[29]
	Exon 20 insertions	2%		Mok T, et al. *N Engl J Med.* 2017[30]
				Yang JC-H, et al. *Lancet Oncol.* 2015[31]
				Cho J, et al. *J Thorac Oncol.* 2018[32]
				Cardona A, et al. *Lung Cancer.* 2018[33]
				Heymach J, et al. *J Thorac Oncol.* 2018[34]
ALK	Fusions (mutations as mechanism of resistance)	5%	IA	Solomon B, et al. *J Clin Oncol.* 2018[35]
				Soria J-C, et al. *Lancet.* 2017[36]
				Peters S, et al. *N Engl J Med.* 2017[37]
				Zhou C, et al. *Ann Oncol.* 2018[38]
				Camidge D, et al. *N Engl J Med.* 2018[39]
MET	Mutations *ex 14 skipping*	3%	IB	Tong J, et al. *Clin Cancer Res.* 2016[40]
				Drilon A, et al. *Nat Med.* 2020[41]
	Focal amplifications (acquired resistance on EGFR TKI in *EGFR*-mutant tumours)	3%	IIB	Camidge D, et al. *J Clin Oncol.* 2018[52]
BRAF^{V600E}	Mutations	2%	IB	Planchard D, et al. *Lancet Oncol.* 2016[42]
				Planchard D, et al. *Lancet Oncol.* 2017[43]
				Planchard D, et al. *J Clin Oncol.* 2017[44]
ROS1	Fusions (mutations as mechanism of resistance)	1%–2%	IB	Shaw A, et al. *N Engl J Med.* 2014[45]
				Shaw A, et al. *Ann Oncol.* 2019[46]
				Drilon A, et al. *Lancet Oncol.* 2020[47]
NTRK	Fusions	0.23%–3%	IC	Drilon A, et al. *N Engl J Med.* 2018[48]
				Hong D, et al. *Lancet Oncol.* 2020[49]
				Doebele RC, et al. *Lancet Oncol.* 2020[50]
RET	Fusions	1%–2%	IC	Drilon A, et. *J Thorac Oncol.* 2019[51]
KRAS^{G12C}	Mutations	12%	IIB	Barlesi F, et al. *Lancet.* 2016[53]
				Fakih M, et al. *J Clin Oncol.* 2019[54]
ERBB2	Hotspot mutations	2%–5%	IIB	Hyman D, et al. *Nature.* 2018[55]
	Amplifications			Wang Y, et al. *Ann Oncol.* 2018[56]
				Tsurutani J, et al. *J Thorac Oncol.* 2018[57]
BRCA 1/2	Mutations	1.2%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]
PIK3CA	Hotspot mutations	1.2%–7%	IIIA	Cancer Genome Atlas Research Network. *Nature.* 2014[60]
				Vansteenkiste J, et al. *J Thorac Oncol.* 2015[62]
NRG1	Fusions	1.7%	IIIB	Duruisseaux M, et al. *J Clin Oncol.* 2019[59]

REVIEW

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

F. Mosele[1], J. Remon[2], J. Mateo[3], C. B. Westphalen[4], F. Barlesi[5], M. P. Lolkema[6], N. Normanno[7], A. Scarpa[8], M. Robson[9], F. Meric-Bernstam[10], N. Wagle[11], A. Stenzinger[12], J. Bonastre[13,14], A. Bayle[1,15,16], S. Michiels[17,18], I. Bièche[19], E. Rouleau[20], S. Jezdic[21], J-Y. Douillard[22], J. S. Reis-Filho[23], R. Dienstmann[24] & F. André[1,25,26]*

CCR

Table 5. List of genomic alterations level I/II/III according to ESCAT in metastatic colorectal cancer (mCRC)

Gene	Alteration	Prevalence	ESCAT	References
KRAS *NRAS*	Mutations (resistance biomarker)	44% 4%	Not applicable	Van Cutsem E, et al. *J Clin Oncol.* 2015[79] Douillard J-Y, et al. *N Engl J Med.* 2013[80] Sorich M, et al. *Ann Oncol.* 2015[81]
BRAF^{V600E}	Mutations	8.5%	IA	https://doi.org/10.1093/annonc/mdw235 Kopetz S, et al. *N Engl J Med.* 2019[82]
	MSI-H	4%—5%	IA	Overman M, et al. *Lancet Oncol.* 2017[83] Le DT, et al. *J Clin Oncol.* 2020[84]
NTRK1	Fusions	0.5%	IC	Demetri G, et al. *Ann Oncol.* 2018[85] Doebele RC, et al. *Lancet Oncol.* 2020[50]
ERBB2	Amplifications	2%	IIB	Meric-Bernstam F, et al. *Lancet Oncol.* 2019[86] Sartore-Bianchi A, et al. *Lancet Oncol.* 2016[87]
PIK3CA	Hotspot mutations	17%	IIIA	Juric D, et al. *J Clin Oncol.* 2018[90]
ATM	Mutations	5%	IIIA	Wang C, et al. *Transl Oncol.* 2017[92] De Bono J, et al. *N Engl J Med.* 2020[93]
MET	Amplifications	1.7%	IIIA	https://clinicaltrials.gov/ct2/show/NCT03592641[94]
AKT1^{E17K}	Mutations	1%	IIIA	Hyman D, et al. *J Clin Oncol.* 2017[76]
	TMB-high in MSS	1%	IIIA	Fabrizio D, et al. *J Gastrointest Oncol.* 2018[89]
RET	Fusions	0.3%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ALK	Fusions	0.2%	IIIA	Yakirevich E, et al. Clin Cancer Res 2016[88]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

REVIEW

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

F. Mosele[1], J. Remon[2], J. Mateo[3], C. B. Westphalen[4], F. Barlesi[5], M. P. Lolkema[6], N. Normanno[6], A. Scarpa[7], M. Robson[8], F. Meric-Bernstam[9], N. Wagle[10], A. Stenzinger[11], J. Bonastre[12,13], A. Bayle[1,12,13], S. Michiels[12,13], I. Bièche[14], E. Rouleau[15], S. Jezdic[16], J-Y. Douillard[16], J. S. Reis-Filho[17], R. Dienstmann[18] & F. André[1,19,20*]

Páncreas

Table 8. List of genomic alterations level I/II/III according to ESCAT in advanced pancreatic ductal adenocarcinoma (PDAC)

Gene	Alteration	Prevalence	ESCAT	References
BRCA1/2	Germline mutations	1%–4%	IA	The Cancer Genome Atlas Research Network. *Cancer Cell.* 2017[111] Golan T, et al. *N Engl J Med.* 2019[112]
	Somatic mutations	3%	IIIB	Shroff R, et al. *JCO Precis Oncol.* 2018[113]
	MSI-H	1%–3%	IC	Pihlak R, et al. *Cancers.* 2018[115] Marcus L, et al. *Clin Cancer Res.* 2019[97]
NTRK	Fusions	<1%	IC	Cocco E, et al. *Nat Rev Clin Oncol.* 2018[114] Doebele RC, et al. *Lancet Oncol.* 2020[50]
KRAS	Mutations	90%	IIIA	Zeitouni D, et al. *Cancers.* 2016[116]
PIK3CA	Hotspot mutations	3%	IIIA	Heestand G, et al. *Oncotarget.* 2015[117] Payne S, et al. *J Clin Oncol.* 2015[118]
$BRAF^{V600E}$	Mutations	3%	IIIA	Hyman D, et al. *N Engl J Med.* 2015[119]
MDM2	Amplifications	2%	IIIA	Azmi A, et al. *Eur J Cancer.* 2010[120]
ERBB2	Amplifications/ mutations	1%–2%	IIIA	Waddell N, et al. *Nature.* 2015[121] Harder J, et al. *Br J Cancer.* 2012[122] Hyman D, et al. *Nature.* 2018[55]
NRG1	Fusions	1%	IIIA	Jones M, et al. *Clin Cancer Res.* 2019[123]
ALK	Fusions	<1%	IIIA	Singhi A, et al. *J Natl Compr Canc Netw.* 2017[124]
RET	Fusions	<1%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ROS1	Fusions	<1%	IIIA	Pishvaian M, et al. *J Clin Oncol.* 2018[125]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

REVIEW

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

F. Mosele[1], J. Remon[2], J. Mateo[3], C. B. Westphalen[4], F. Barlesi[5], M. P. Lolkema[6], N. Normanno[6], A. Scarpa[7], M. Robson[8], F. Meric-Bernstam[9], N. Wagle[10], A. Stenzinger[11], J. Bonastre[12,13], A. Bayle[1,12,13], S. Michiels[12,13], I. Bièche[14], E. Rouleau[15], S. Jezdic[16], J-Y. Douillard[16], J. S. Reis-Filho[17], R. Dienstmann[18] & F. André[1,19,20*]

Ovario

frontiers | Frontiers in Oncology

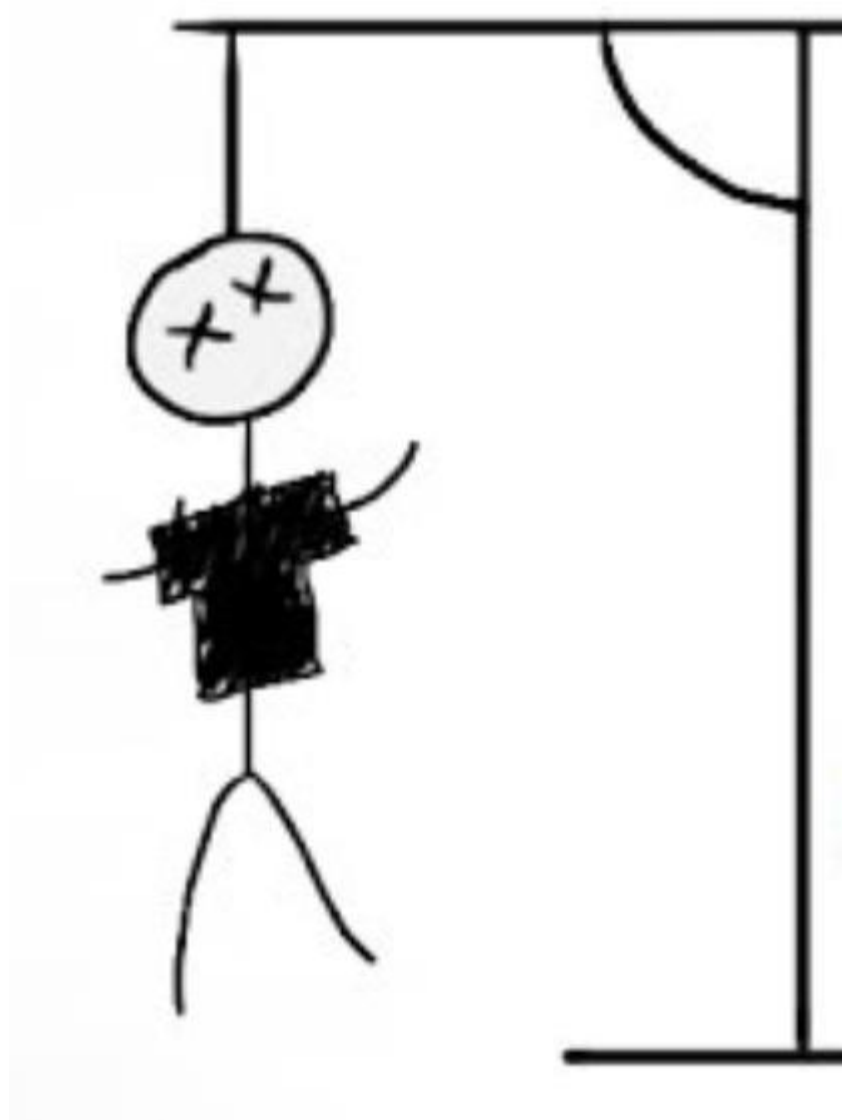
REVIEW
published: 17 June 2022

Homologous Recombination Deficiency in Ovarian, Breast, Colorectal, Pancreatic, Non-Small Cell Lung and Prostate Cancers, and the Mechanisms of Resistance to PARP Inhibitors

Negesse Mekonnen [1,2†], Hobin Yang [1†] and Young Kee Shin [1,3,4,5]*

TABLE 1 | Prevalence of mutation, LOH, and promoter hypermethylation in ovarian cancer.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Promoter Methylation [% (proportion)]
BRCA1	12.2% (31/255) (44); 5% (15/300) (45); 18% (60/333) (46); 15.5% (81/523) (47); 16.5% (26/158) (48)	88% (36/41) (49); 44% (4/9) (50); 67% (6/9) (50); 81.5% (123/151) (51); 60% (60/100) (52); 97% (30/31) (53); 10.13% (16/158) (48)	20% (22/112) (54); 14% (2/14) (50); 14% (5/35) (55); 9.6% (32/332) (56); 14% (38/257) (57); 35% (15/42) (58); 9.34% (45/482) (59); 73.7% (56/76) (60)
BRCA2	9.8% (25/255) (44); 2% (6/300) (45); 3.3% (11/333) (46); 5.5% (29/523) (47); 5.06% (8/158) (48)	58% (24/41) (49); 50% (3/6) (50); 67% (4/6)] (50); 68.9% (104/151) (51); 73% (75/103) (52); 53% (16.5/31) (53); 0.63% (1/158) (48)	21% (3/14) (50); 44% (22/50) (61)
RAD50	7.7% (29/380) (62); 60% (12/20) (63); 2.94% (2/68) (64); 0.63% (1/158) (48)	0.63% (1/158) (48)	–
RAD51	0.3% (1/316) (11)	2% (10/489) (11)	–
RAD51B	2.1% (3/142) (65); 0.06% (2/3.429) (66)	0.8% (4/489) (11)	–
RAD51C	0.7% (1/141) (67); 2.5% (13/523) (47); 0.41% (14/3,429) (66)	97% (30/31) (53); 0.5 (2/429) (68)	1.45% (7/482) (59); 2.7% (9/332) (69); 2.67% (14/524) (70); 3% (9/316) (11)
RAD51D	1.3% (1/77) (67); 2.6% (10/380) (62); 0.35% (12/3429) (66)	0.7% (3/429) (68); 1.2% (6/489) (11)	–
PALB2	3% (9/299) (71); 3.03% (2/66) (72); 0.6% (2/333) (46); 0.63% (12/1915) (73); 2.9% (2/69) (74); 1.1% (6/523) (47); 1.9% (3/158) (48)	0.23% (1/429) (75); 0.7% (3/429) (68); 10.8% (17/158) (48)	3.08% (4/130) (76)
FANCA	4.35% (1/23) (45)	56.45% (17.5/31) (53); 1.16% (1/86) (77); 0.7% (3/429) (75)	–
FANCD2	0.3% (1/316) (11)	32.25% (10/31) (53); 0.23% (1/429) (75)	–
FANCF	0.3% (1/300) (45)	0.2% (1/572) (78)	32.14% (36/112) (79);13.2% (7/53) (80)
FANCI	0.6% (92/300) (45)	1.16% (1/86) (77)	–
FANCM	4.35% (1/23) (45); 2.1% (5/235) (81); 0.96% (5/523) (47)	0.2% (1/489) (11)	–
NBN/ NBS1	1.8% (6/333) (46); 0.28% (9/3236) (82); 0.42% (1/235) (81); 0.38% (2/523) (47)	0.6% (3/489) (11)	–
BARD1	0.12% (4/3,236) (82); 1.6% (4/255) (83); 0.63% (1/158) (48)	0.63% (1/158) (48)	–
ATM	1.78% (7/392) (121); 0.3% (1/333) (46); 16.7% (8/48) (292); 0.82% (3/367 (43); 3.2% (5/158) (48)	29% (9/31) (53); 1.86% (8/429) (75); 1.9% (3/158) (48)	–
ATR	6% (3/50) (293); 69.7% (23/33) (294);; 4.8% (12/141) (295)	29% (9/31) (53) (75);	–
MRE11A	5.92% (17/287) (296); 0.4% (2/523) (47); 0.22% (1/466) (297)		–
BRIP1	7.7% (29/380) (62); 1.47% (1/68) (64); 0.4% (2/523) (47); 1.7% (8/466) (297); 0.52% (1/192) (131); 0.63% (1/158) (48)	0.7% (3/429) (68); 1.3% (2/158) (48)	–
ERCC1	2.6% 10/380) (62); 0.2% (1/523) (78)	0.4% (2/489) (11)	–
CHEK2	20.3% (77/380) (62); 45% (9/20) (63); 1.47% (1/68) (64); 4.2% (12/287) (296); 0.4% (2/523) (47); 1.72% (10/581) (298); 0.43% (2/466) (297); 0.52% (1/192) (131); 0.63% (1/158) (48)	10% (1/10) (298); 7.6% (12/158) (48)	–
EMSY	3.8% (14/380) (62); 8% (25/316) (11); 1.5% (8/523) (78)	0.2% (1/489) (11)	–
TP53	1.47% (1/68) (64); 3.83% (11/287) (296); 0.3% (2/581) (298); 1.04% (2/192) (131); 96% (312/316) (11); 57% (90/158) (48); 71.3% (375/523) (78)	0.63% (1/158) (78)	–
STK11	4.2% (12/287) (296); 1.3% (2/158) (48)	1.6% (8/489) (11)	–
PTEN	5.23% (15/287) (296); 0.43% (2/466) (297); 11.4% (18/158) (48)	6.7% (21/316) (11); 1.9% (3/158) (48); 6.1% (30/489) (11)	16.9% (21/124) (299)
CDH1	7.32% (21/287) (296); 0.52% (1/192) (131)	2.3% (11/489) (11)	–
BLM	0.4% (9/2561) (300); 1.27% (4/316) (11)	0.6% (3/489) (11)	–
RBBP8	1.04% (2/192) (131); 0.32% (1/316) (11); 1.9% (3/158) (48)	0.2% (1/489) (11)	–
CDK12	2.9% (9/316) (11); 4% (21/523) (11)	0.4% (2/489) (11)	–
TP53BP1	1.27% (4/316) (11); 0.8% (4/523) (78)	1.4% (7/489) (11)	–
XRCC1	0.6% (2/316) (11); 0.8% (4/523) (78)	0.4% (2/489) (11)	–
MAD2L2/ REV7	0.3% (1/316) (11)	0.3% (2/572) (78)	–
XRCC5/ Ku80	0.2% (1/523) (78)	–	–
XRCC6/ Ku70	0.3% (1/316) (11); 0.8% (4/523) (78)	0.2% (1/489) (11)	–
SLFN11	0.6% (3/523) (78)	0.8% (4/489) (11)	39% (16/41) (209)



Implementación

- **Región de interés y alteraciones que queremos estudiar**: este punto influye directamente sobre el tamaño de nuestro panel y este aspecto influye directamente sobre el número de muestras que podríamos procesar en paralelo
- Número y tipo de muestras que queremos procesar o, dicho de otra manera, número de librerías por carrera que se podrán cargar juntas para ser secuenciadas en una misma carrera (“multiplexing”)
- Tiempos de respuesta en función de la utilidad clínica (biomarcadores diagnósticos, pronósticos, predictivos)

Implementación

- Estudios de alteraciones somáticas o germinales: este punto condicionará la profundidad de cobertura que necesitamos para las regiones de interés:
    - para las alteraciones somáticas, más profundidad comparado con las alteraciones germinales debido a la presencia de subclones en el tumor en estudio
- Capacidad de secuenciación, entendida como la cantidad de lecturas totales que genera el secuenciador por cada carrera

The Journal of Molecular Diagnostics, Vol. 18, No. 2, March 2016

Clinical Validation and Implementation of a Targeted Next-Generation Sequencing Assay to Detect Somatic Variants in Non-Small Cell Lung, Melanoma, and Gastrointestinal Malignancies

the Journal of Molecular Diagnostics

Pathology - Research and Practice 214 (2018) 713–719

Use of the Ion AmpliSeq Cancer Hotspot Panel in clinical molecular pathology laboratories for analysis of solid tumours: With emphasis on validation with relevant single molecular pathology tests and the Oncomine Focus Assay

Ahwon Lee[a,b], Sung-Hak Lee[a], Chan Kwon Jung[a,b], Gyungsin Park[a], Kyo Young Lee[a], Hyun Joo Choi[c], Ki Ouk Min[d], Tae Jung Kim[e], Eun Jung Lee[e], Youn Soo Lee[a,*]

The Journal of Molecular Diagnostics, Vol. 19, No. 3, May 2017

SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels

the Journal of Molecular Diagnostics
jmd.amjpathol.org

A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

Virchows Arch (2017) 470:5–20
DOI 10.1007/s00428-016-2025-7

Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL

Zandra C Deans[1] · Jose Luis Costa[2] · Ian Cree[3] · Els Dequeker[4] · Anders Edsjö[5] · Shirley Henderson[6] · Michael Hummel[7] · Marjolijn JL Ligtenberg[8] · Marco Loddo[9] ·

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Implementation of NGS in a diagnostic laboratory

Test development and test validation, prior to the implementation and use of any NGS-based diagnostic test, is important. The implementation phase should develop an end-to-end process for testing and documenting the process being validated.

The implementation phase can utilise samples with a range of known mutations and commercially available controls, but should be of the same type as those to be used for diagnostic testing. A range of mutation types must be tested, such as single-nucleotide variants, small indels and copy number variants (if tested for) to provide assurance that they will be detected if present. A range of allelic frequencies must be included so limits of detection for mutation types are established.

Implementación

Paneles “virtuales”

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	ESR1	KIT	PDGFRB	ARID1A	FBXW7	PTEN	AKT1	FGFR4	AKT2	FGFR2	NUTM1
AKT2	EZH2	KNSTRN	PIK3CB	ATM	MLH1	RAD50	AKT2	FLT3	ALK	FGFR3	PDGFRA
AKT3	FGFR1	KRAS	PIK3CA	ATR	MRE11	RAD51	AKT3	IGF1R	AR	FGR	PDGFRB
ALK	FGFR2	MAGOH	PPP2R1A	ATRX	MSH6	RAD51B	ALK	KIT	AXL	FLT3	PIK3CA
AR	FGFR3	MAP2K1	PTPN11	BAP1	MSH2	RAD51C	AXL	KRAS	BRCA1	JAK2	PRKACA
ARAF	FGFR4	MAP2K2	RAC1	BRCA1	NBN	RAD51D	AR	MDM2	BRCA2	KRAS	PRKACB
AXL	FLT3	MAP2K4	RAF1	BRCA2	NF1	RNF43	BRAF	MDM4	BRAF	MDM4	PTEN
BRAF	FOXL2	MAPK1	RET	CDK12	NF2	RB1	CCND1	MET	CDKN2A	MET	PPARG
BTK	GATA2	MAX	RHEB	CDKN1B	NOTCH1	SETD2	CCND2	MYC	EGFR	MYB	RAD51B
CBL	GNA11	MDM4	RHOA	CDKN2A	NOTCH2	SLX4	CCND3	MYCL	ERBB2	MYBL1	RAF1
CCND1	GNAQ	MED12	ROS1	CDKN2B	NOTCH3	SMARCA4	CCNE1	MYCN	ERBB4	NF1	RB1
CDK4	GNAS	MET	SF3B1	CHEK1	PALB2	SMARCB1	CDK2	NTRK1	ERG	NOTCH1	RELA
CDK6	H3F3A	MTOR	SMAD4	CREBBP	PIK3R1	STK11	CDK4	NTRK2	ESR1	NOTCH4	RET
CHEK2	HIST1H3B	MYC	SMO	FANCA	PMS2	TP53	CDK6	NTRK3	ETV1	NRG1	ROS1
CSF1R	HNF1A	MYCN	SPOP	FANCD2	POLE	TSC1	EGFR	PDGFRA	ETV4	NTRK1	RSPO2
CTNNB1	HRAS	MYD88	SRC	FANCI	PTCH1	TSC2	ERBB2	PDGFRB	ETV5	NTRK2	RSPO3
DDR2	IDH1	NFE2L2	STAT3				ESR1	PIK3CB	FGFR1	NTRK3	TERT
EGFR	IDH2	NRAS	TERT				FGF19	PIK3CA			
ERBB2	JAK1	NTRK1	TOP1				FGF3	PPARG			
ERBB3	JAK2	NTRK2	U2AF1				FGFR1	RICTOR			
ERBB4	JAK3	NTRK3	XPO1				FGFR2	TERT			
ERCC2	KDR	PDGFRA					FGFR3				

Paneles “virtuales”

Hotspot genes			
AKT1	ESR1	KIT	PDGFRB
AKT2	EZH2	KNSTRN	PIK3CB
AKT3	FGFR1	KRAS	PIK3CA
ALK	FGFR2	MAGOH	PPP2R1A
AR	FGFR3	MAP2K1	PTPN11
ARAF	FGFR4	MAP2K2	RAC1
AXL	FLT3	MAP2K4	RAF1
BRAF	FOXL2	MAPK1	RET
BTK	GATA2	MAX	RHEB
CBL	GNA11	MDM4	RHOA
CCND1	GNAQ	MED12	ROS1
CDK4	GNAS	MET	SF3B1
CDK6	H3F3A	MTOR	SMAD4
CHEK2	HIST1H3B	MYC	SMO
CSF1R	HNF1A	MYCN	SPOP
CTNNB1	HRAS	MYD88	SRC
DDR2	IDH1	NFE2L2	STAT3
EGFR	IDH2	NRAS	TERT
ERBB2	JAK1	NTRK1	TOP1
ERBB3	JAK2	NTRK2	U2AF1
ERBB4	JAK3	NTRK3	XPO1
ERCC2	KDR	PDGFRA	

Full-length genes		
ARID1A	FBXW7	PTEN
ATM	MLH1	RAD50
ATR	MRE11	RAD51
ATRX	MSH6	RAD51B
BAP1	MSH2	RAD51C
BRCA1	NBN	RAD51D
BRCA2	NF1	RNF43
CDK12	NF2	RB1
CDKN1B	NOTCH1	SETD2
CDKN2A	NOTCH2	SLX4
CDKN2B	NOTCH3	SMARCA4
CHEK1	PALB2	SMARCB1
CREBBP	PIK3R1	STK11
FANCA	PMS2	TP53
FANCD2	POLE	TSC1
FANCI	PTCH1	TSC2

Copy number genes	
AKT1	FGFR4
AKT2	FLT3
AKT3	IGF1R
ALK	KIT
AXL	KRAS
AR	MDM2
BRAF	MDM4
CCND1	MET
CCND2	MYC
CCND3	MYCL
CCNE1	MYCN
CDK2	NTRK1
CDK4	NTRK2
CDK6	NTRK3
EGFR	PDGFRA
ERBB2	PDGFRB
ESR1	PIK3CB
FGF19	PIK3CA
FGF3	PPARG
FGFR1	RICTOR
FGFR2	TERT
FGFR3	

Gene fusions (inter- and intragenic)		
AKT2	FGFR2	NUTM1
ALK	FGFR3	PDGFRA
AR	FGR	PDGFRB
AXL	FLT3	PIK3CA
BRCA1	JAK2	PRKACA
BRCA2	KRAS	PRKACB
BRAF	MDM4	PTEN
CDKN2A	MET	PPARG
EGFR	MYB	RAD51B
ERBB2	MYBL1	RAF1
ERBB4	NF1	RB1
ERG	NOTCH1	RELA
ESR1	NOTCH4	RET
ETV1	NRG1	ROS1
ETV4	NTRK1	RSPO2
ETV5	NTRK2	RSPO3
FGFR1	NTRK3	TERT

New update to the guidelines on testing predictive biomarkers in non-small-cell lung cancer: a National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology

Dolores Isla[1] · Maria D. Lozano[2] · Luis Paz-Ares[3] · Clara Salas[4] · Javier de Castro[5] · Esther Conde[6] · Enriqueta Felip[7] · Javier Gómez-Román[8] · Pilar Garrido[9] · Ana Belén Enguita[10]

Received: 2 November 2022 / Accepted: 7 December 2022

Gene/protein	Predictive alteration	Methodology
EGFR	Mutation	PCR: Sanger sequencing, real-time PCR and NGS
ALK	Rearrangement	IHC, FISH, real-time PCR and NGS
ROS1	Rearrangement	IHC (screening), FISH, real-time PCR and NGS
BRAF V600	Mutation	Real-time PCR and NGS
PD-L1	Overexpression	IHC
NTRK	Rearrangement	IHC (screening), real-time PCR and NGS
RET	Rearrangement	FISH, real-time PCR and NGS
KRAS	Mutation	PCR: Sanger sequencing, real-time PCR and NGS
MET	Mutation Amplification	NGS FISH, real-time PCR and NGS

Table 2 Other biomarkers of interest in NSCLC patients

Gene/protein	Predictive alteration	Methodology
HER2	Mutation Amplification	NGS FISH, real-time PCR, NGS
TMB	Mutations	NGS
STK11	Mutation	NGS
KEAP1	Mutation	NGS
MSI	Pattern of hypermutation	IHC, PCR, NGS

Paneles “virtuales”

DX
PROG./REC.

CNHP

List of gene targets in Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific, Waltham, MA, USA) – 161 gene panel

Hotspot genes	Full-length genes	Copy number genes	Gene fusions (inter- and intragenic)
AKT1	ATM	AKT1	ALK
ALK	BAP1	AR	AXL
AR	BRCA1	CCND1	BRAF
ARAF	BRCA2	CCNE1	EGFR
BRAF	CDKN2A	CDK4	ERBB2
BTK	FBXW7	CDK6	ERG
CBL	MSH2	EGFR	ETV1
CDK4	NF1	ERBB2	ETV4
CHEK2	NF2	FGFR1	ETV5
CSF1R	NOTCH1	FGFR2	FGFR1
CTNNB1	PIK3R1	FGFR3	FGFR2
DDR2	PTCH1	FGFR4	FGFR3
EGFR	PTEN	FLT3	NTRK1
ERBB2	RB1	IGF1R	NTRK3
ERBB3	SMARCB1	KIT	PDGFRA
ERBB4	STK11	KRAS	PPARG
ESR1	TP53	MDM2	RAF1
EZH2	TSC1	MDM4	RET
FGFR1	TSC2	MET	ROS1
FGFR2	ARID1A	MYC	AKT2
FGFR3	ATR	MYCL	AR
FLT3	ATRX	MYCN	BRCA1
FOXL2	CDK12	PDGFRA	BRCA2
GATA2	CDKN1B	PIK3CA	CDKN2A
GNA11	CDKN2B	PPARG	ERBB4
GNAQ	CHEK1	TERT	ESR1
GNAS	CREBBP	AKT2	FGR
HNF1A	FANCA	AKT3	FLT3
HRAS	FANCD2	ALK	JAK2
IDH1	FANCI	AXL	KRAS
IDH2	MLH1	BRAF	MDM4
JAK1	MRE11A	CCND2	MET
JAK2	MSH6	CCND3	MYB
JAK3	NBN	CDK2	MYBL1
KDR	NOTCH2	CDKN2A	NF1
KIT	NOTCH3	CDKN2B	NOTCH1
KNSTRN	PALB2	ESR1	NOTCH4
KRAS	PMS2	FGF19	NRG1
MAGOH	POLE	FGF3	NTRK2
MAP2K1	RAD50	NTRK1	NUTM1
MAP2K2	RAD51	NTRK2	PDGFRB
MAPK1	RAD51B	NTRK3	PIK3CA
MAX	RAD51C	PDGFRB	PRKACA
MED12	RAD51D	PIK3CB	PRKACB
MET	RNF43	RICTOR	PTEN
MTOR	SETD2	TSC1	RAD51B
MYD88	SLX4	TSC2	RB1
NFE2L2	SMARCA4		RELA
NRAS			RSPO2
PDGFRA			RSPO3
PIK3CA			TERT
PPP2R1A			
PTPN11			
RAC1			
RAF1			
RET			
RHEB			
RHOA			
SF3B1			
SMO			
SPOP			
SRC			
STAT3			
U2AF1			
XPO1			
AKT2			
AKT3			
AXL			
CCND1			
CDK6			
ERCC2			
FGFR4			
H3F3A			
HIST1H3B			
MAP2K4			
MDM4			
MYC			
MYCN			
NTRK1			
NTRK2			
PDGFRB			
PIK3CB			
ROS1			
SMAD4			
TERT			
TOP1			

Paneles “virtuales”

CCR

List of gene targets in Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific, Waltham, MA, USA) – 161 gene panel

Hotspot genes	Full-length genes	Copy number genes	Gene fusions (inter- and intragenic)
AKT1	ATM	AKT1	ALK
ALK	BAP1	AR	AXL
AR	BRCA1	CCND1	BRAF
ARAF	BRCA2	CCNE1	EGFR
BRAF	CDKN2A	CDK4	ERBB2
BTK	FBXW7	CDK6	ERG
CBL	MSH2	EGFR	ETV1
CDK4	NF1	ERBB2	ETV4
CHEK2	NF2	FGFR1	ETV5
CSF1R	NOTCH1	FGFR2	FGFR1
CTNNB1	PIK3R1	FGFR3	FGFR2
DDR2	PTCH1	FGFR4	FGFR3
EGFR	PTEN	FLT3	NTRK1
ERBB2	RB1	IGF1R	NTRK3
ERBB3	SMARCB1	KIT	PDGFRA
ERBB4	STK11	KRAS	PPARG
ESR1	TP53	MDM2	RAF1
EZH2	TSC1	MDM4	RET
FGFR1	TSC2	MET	ROS1
FGFR2	ARID1A	MYC	AKT2
FGFR3	ATR	MYCL	AR
FLT3	ATRX	MYCN	BRCA1
FOXL2	CDK12	PDGFRA	BRCA2
GATA2	CDKN1B	PIK3CA	CDKN2A
GNA11	CDKN2B	PPARG	ERBB4
GNAQ	CHEK1	TERT	ESR1
GNAS	CREBBP	AKT2	FGR
HNF1A	FANCA	AKT3	FLT3
HRAS	FANCD2	ALK	JAK2
IDH1	FANCI	AXL	KRAS
IDH2	MLH1	BRAF	MDM4
JAK1	MRE11A	CCND2	MET
JAK2	MSH6	CCND3	MYB
JAK3	NBN	CDK2	MYBL1
KDR	NOTCH2	CDKN2A	NF1
KIT	NOTCH3	CDKN2B	NOTCH1
KNSTRN	PALB2	ESR1	NOTCH4
KRAS	PMS2	FGF19	NRG1
MAGOH	POLE	FGF3	NTRK2
MAP2K1	RAD50	NTRK1	NUTM1
MAP2K2	RAD51	NTRK2	PDGFRB
MAPK1	RAD51B	NTRK3	PIK3CA
MAX	RAD51C	PDGFRB	PRKACA
MED12	RAD51D	PIK3CB	PRKACB
MET	RNF43	RICTOR	PTEN
MTOR	SETD2	TSC1	RAD51B
MYD88	SLX4	TSC2	RB1
NFE2L2	SMARCA4		RELA
NRAS			RSPO2
PDGFRA			RSPO3
PIK3CA			TERT
PPP2R1A			
PTPN11			
RAC1			
RAF1			
RET			
RHEB			
RHOA			
SF3B1			
SMO			
SPOP			
SRC			
STAT3			
U2AF1			
XPO1			
AKT2			
AKT3			
AXL			
CCND1			
CDK6			
ERCC2			
FGFR4			
H3F3A			
HIST1H3B			
MAP3K4			
MDM4			
MYC			
MYCN			
NTRK1			
NTRK2			
PDGFRB			
PIK3CB			
ROS1			
SMAD4			
TERT			
TOP1			

Paneles “virtuales”

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	ESR1	KIT	PDGFRB	ARID1A	FBXW7	PTEN	AKT1	FGFR4	AKT2	FGFR2	NUTM1
AKT2	EZH2	KNSTRN	PIK3CB	ATM	MLH1	RAD50	AKT2	FLT3	ALK	FGFR3	PDGFRA
AKT3	FGFR1	KRAS	PIK3CA	ATR	MRE11	RAD51	AKT3	IGF1R	AR	FGR	PDGFRB
ALK	FGFR2	MAGOH	PPP2R1A	ATRX	MSH6	RAD51B	ALK	KIT	AXL	FLT3	PIK3CA
AR	FGFR3	MAP2K1	PTPN11	BAP1	MSH2	RAD51C	AXL	KRAS	BRCA1	JAK2	PRKACA
ARAF	FGFR4	MAP2K2	RAC1	BRCA1	NBN	RAD51D	AR	MDM2	BRCA2	KRAS	PRKACB
AXL	FLT3	MAP2K4	RAF1	BRCA2	NF1	RNF43	BRAF	MDM4	BRAF	MDM4	PTEN
BRAF	FOXL2	MAPK1	RET	CDK12	NF2	RB1	CCND1	MET	CDKN2A	MET	PPARG
BTK	GATA2	MAX	RHEB	CDKN1B	NOTCH1	SETD2	CCND2	MYC	EGFR	MYB	RAD51B
CBL	GNA11	MDM4	RHOA	CDKN2A	NOTCH2	SLX4	CCND3	MYCL	ERBB2	MYBL1	RAF1
CCND1	GNAQ	MED12	ROS1	CDKN2B	NOTCH3	SMARCA4	CCNE1	MYCN	ERBB4	NF1	RB1
CDK4	GNAS	MET	SF3B1	CHEK1	PALB2	SMARCB1	CDK2	NTRK1	ERG	NOTCH1	RELA
CDK6	H3F3A	MTOR	SMAD4	CREBBP	PIK3R1	STK11	CDK4	NTRK2	ESR1	NOTCH4	RET
CHEK2	HIST1H3B	MYC	SMO	FANCA	PMS2	TP53	CDK6	NTRK3	ETV1	NRG1	ROS1
CSF1R	HNF1A	MYCN	SPOP	FANCD2	POLE	TSC1	EGFR	PDGFRA	ETV4	NTRK1	RSPO2
CTNNB1	HRAS	MYD88	SRC	FANCI	PTCH1	TSC2	ERBB2	PDGFRB	ETV5	NTRK2	RSPO3
DDR2	IDH1	NFE2L2	STAT3				ESR1	PIK3CB	FGFR1	NTRK3	TERT
EGFR	IDH2	NRAS	TERT				FGF19	PIK3CA			
ERBB2	JAK1	NTRK1	TOP1				FGF3	PPARG			
ERBB3	JAK2	NTRK2	U2AF1				FGFR1	RICTOR			
ERBB4	JAK3	NTRK3	XPO1				FGFR2	TERT			
ERCC2	KDR	PDGFRA					FGFR3				

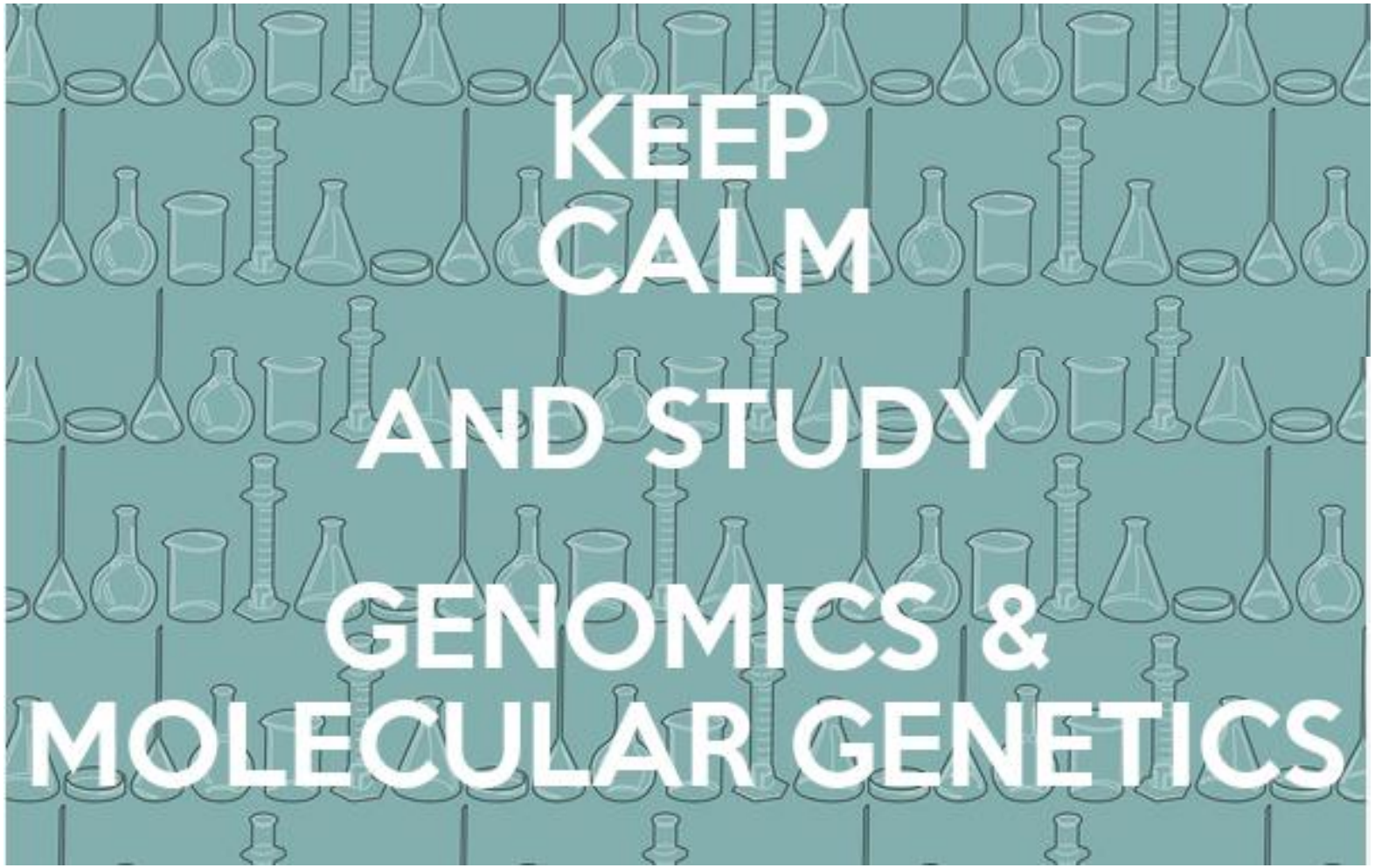
Core Genes - 15

ATM	BARD1	BRCA1	BRCA2	BRIP1	CDK12	CHEK2	FANCD2	MRE11	NBN	PALB2	PPP2R2A	RAD51B	RAD54L	TP53

Core Genes - 28

ATM	BARD1	BRCA1	BRCA2	BRIP1	CDK12	CHEK1	CHEK2	FANCD2	FANCL	KRAS	MRE11	NBN	PALB2	PIK3CA	POLD1	POLE
PPP2R2A	PTEN	RAD50	RAD51	RAD51B	RAD51C	RAD51D	RAD52	RAD54L	TP53	XRCC2						


KEEP
CALM

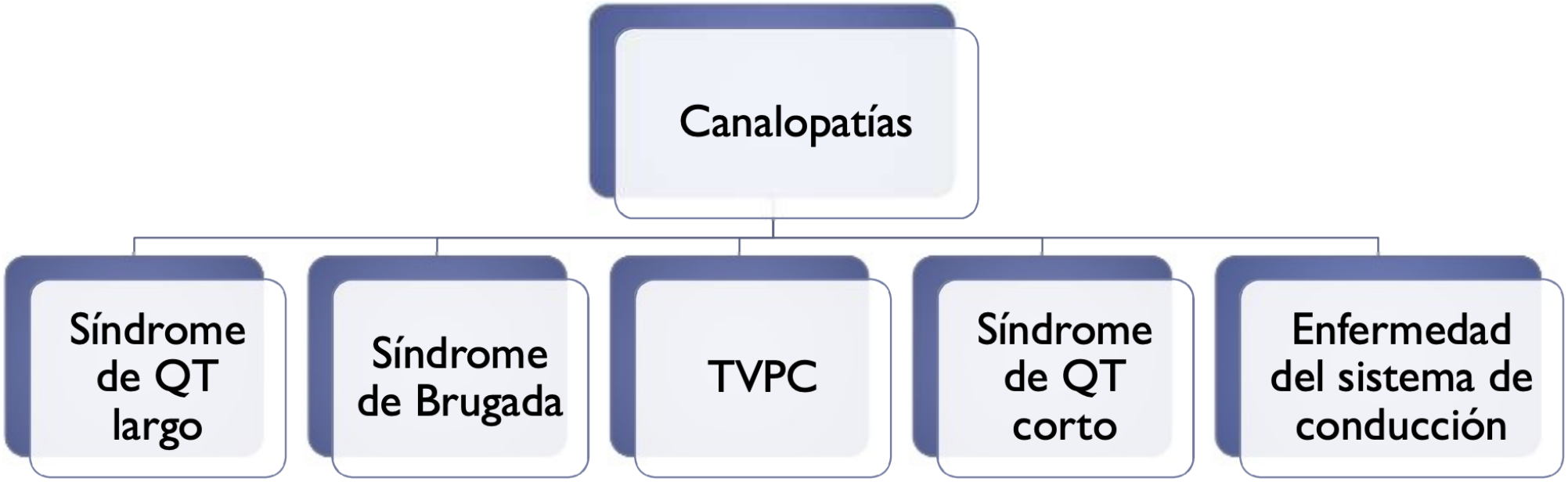

KEEP
CALM
AND STUDY
GENOMICS &
MOLECULAR GENETICS


KEEP
CALM

…y contratad a biólog@s moleculares

Cardiopatías

Cardiopatías



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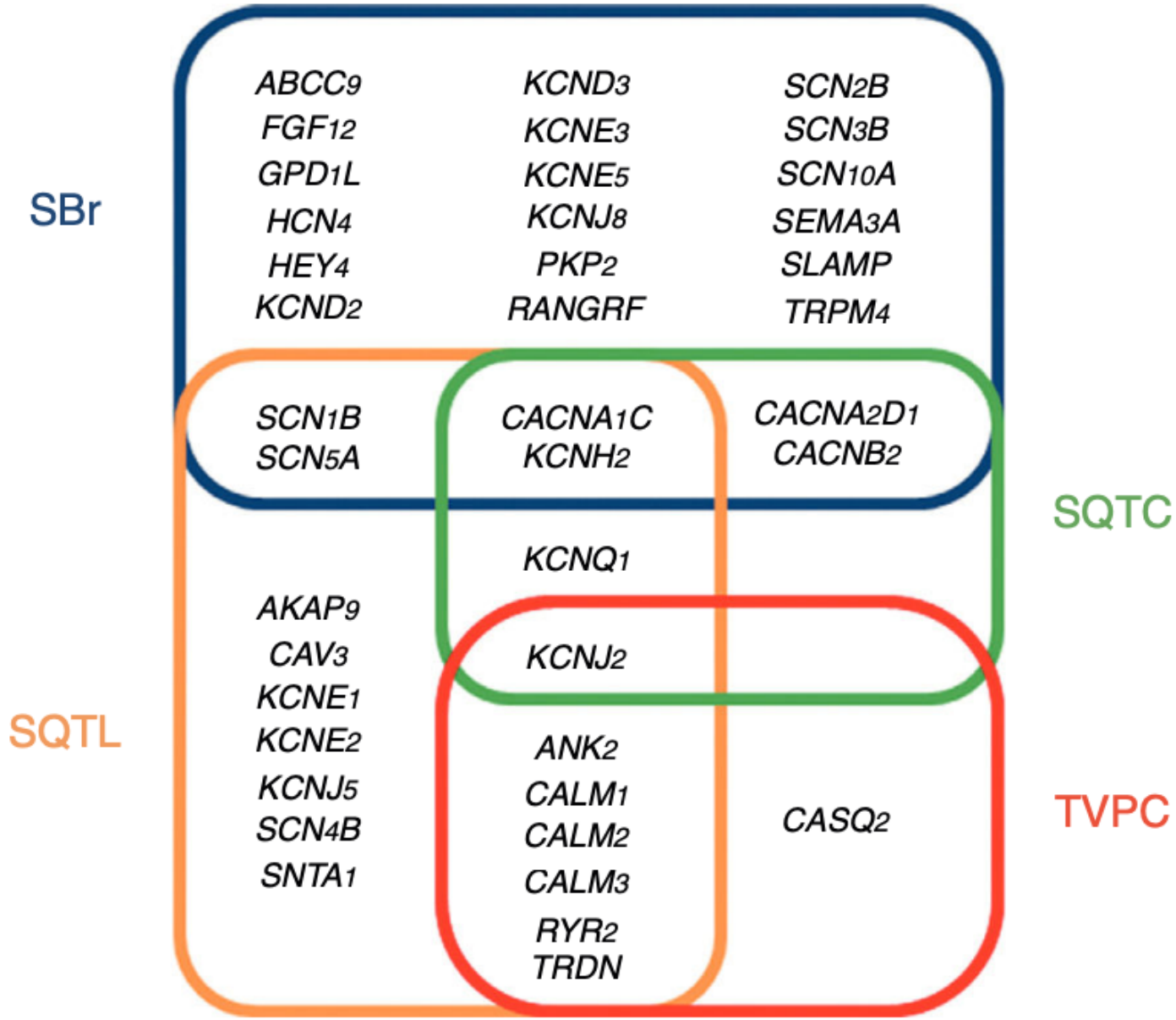


Cardiopatías

SQTL

CACNA1C	***CALM1***	***CALM2***	***CALM3***	***KCNE1***	***KCNE2***	***KCNH2***	***KCNJ2***	***KCNQ1***	***SCN5A***	*AKAP9*	*ANK2*	*CAV3*	*KCNJ5*	*RYR2*	*SCN4B*	*SLC22A5*
SNTA1	*TECRL*	*TRDN*	*CAVIN1**	*FHL2**	*HCN4**	*KCNA5**	*KCND2**	*KCND3**	*KCNE3**	*KCNE5**	*NOS1AP**	*SCN1B**	*SCN3B**	*TRPM4**		

Cardiopatías

SBr

CACNA1C	***CALM1***	***CALM2***	***CALM3***	***KCNE1***	***KCNE2***	***KCNH2***	***KCNJ2***	***KCNQ1***	***SCN5A***	*AKAP9*	*ANK2*	*CAV3*	*KCNJ5*	*RYR2*	*SCN4B*	*SLC22A5*
SNTA1	*TECRL*	*TRDN*	*CAVIN1**	*FHL2**	*HCN4**	*KCNA5**	*KCND2**	*KCND3**	*KCNE3**	*KCNE5**	*NOS1AP**	*SCN1B**	*SCN3B**	*TRPM4**		

Cardiopatías

ESC

DES	***EMD***	***HCN4***	***LAMP2***	***LMNA***	***NKX2-5***	***PRKAG2***	***SCN5A***	***TBX5***	*ACTC1*	*CACNA1D*	*GAA*	*GJA5*	*GLA*	*GNB2*	*KCNJ2*	*MYH6*	*SCN1B*
SLC22A5	*TNNI3K*	*TRPM4*	*TTR*	*CALR**	*CAVIN4**	*DSC2**	*DSG2**	*DSP**	*GATA4**	*HFE**	*IRX3**	*JUP**	*KCNE2**	*KCNH2**	*KCNK17**	*KCNQ1**	
*LDB3**	*MYBPHL**	*MYH7**	*NPPA**	*PITX2**	*PKP2**	*RYR2**	*SCN4B**	*SLMAP**									

Cardiopatías

SQTL

CACNA1C **CALM1** **CALM2** **CALM3** **KCNE1** **KCNE2** **KCNH2** **KCNJ2** **KCNQ1** **SCN5A** AKAP9 ANK2 CAV3 KCNJ5 RYR2 SCN4B SLC22A5

SNTA1 TECRL TRDN CAVIN1* FHL2* HCN4* KCNA5* KCND2* KCND3* KCNE3* KCNE5* NOS1AP* SCN1B* SCN3B* TRPM4*

SBr

CACNA1C **CALM1** **CALM2** **CALM3** **KCNE1** **KCNE2** **KCNH2** **KCNJ2** **KCNQ1** **SCN5A** AKAP9 ANK2 CAV3 KCNJ5 RYR2 SCN4B SLC22A5

SNTA1 TECRL TRDN CAVIN1* FHL2* HCN4* KCNA5* KCND2* KCND3* KCNE3* KCNE5* NOS1AP* SCN1B* SCN3B* TRPM4*

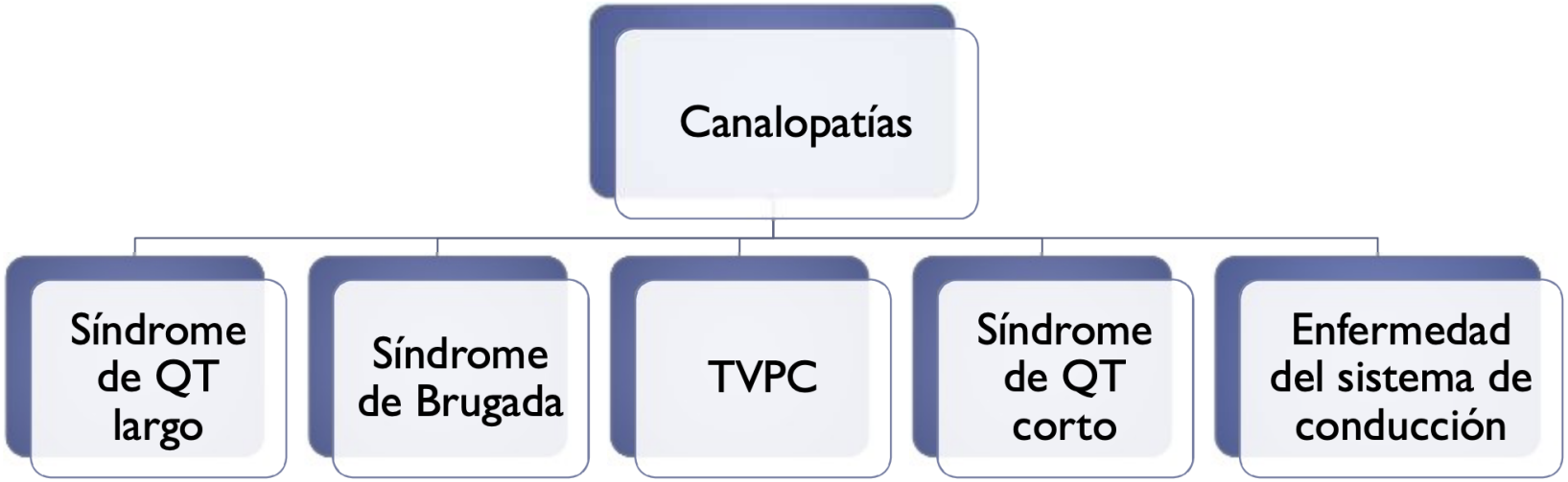
ESC

DES **EMD** **HCN4** **LAMP2** **LMNA** **NKX2-5** **PRKAG2** **SCN5A** **TBX5** ACTC1 CACNA1D GAA GJA5 GLA GNB2 KCNJ2 MYH6 SCN1B

SLC22A5 TNNI3K TRPM4 TTR CALR* CAVIN4* DSC2* DSG2* DSP* GATA4* HFE* IRX3* JUP* KCNE2* KCNH2* KCNK17* KCNQ1*

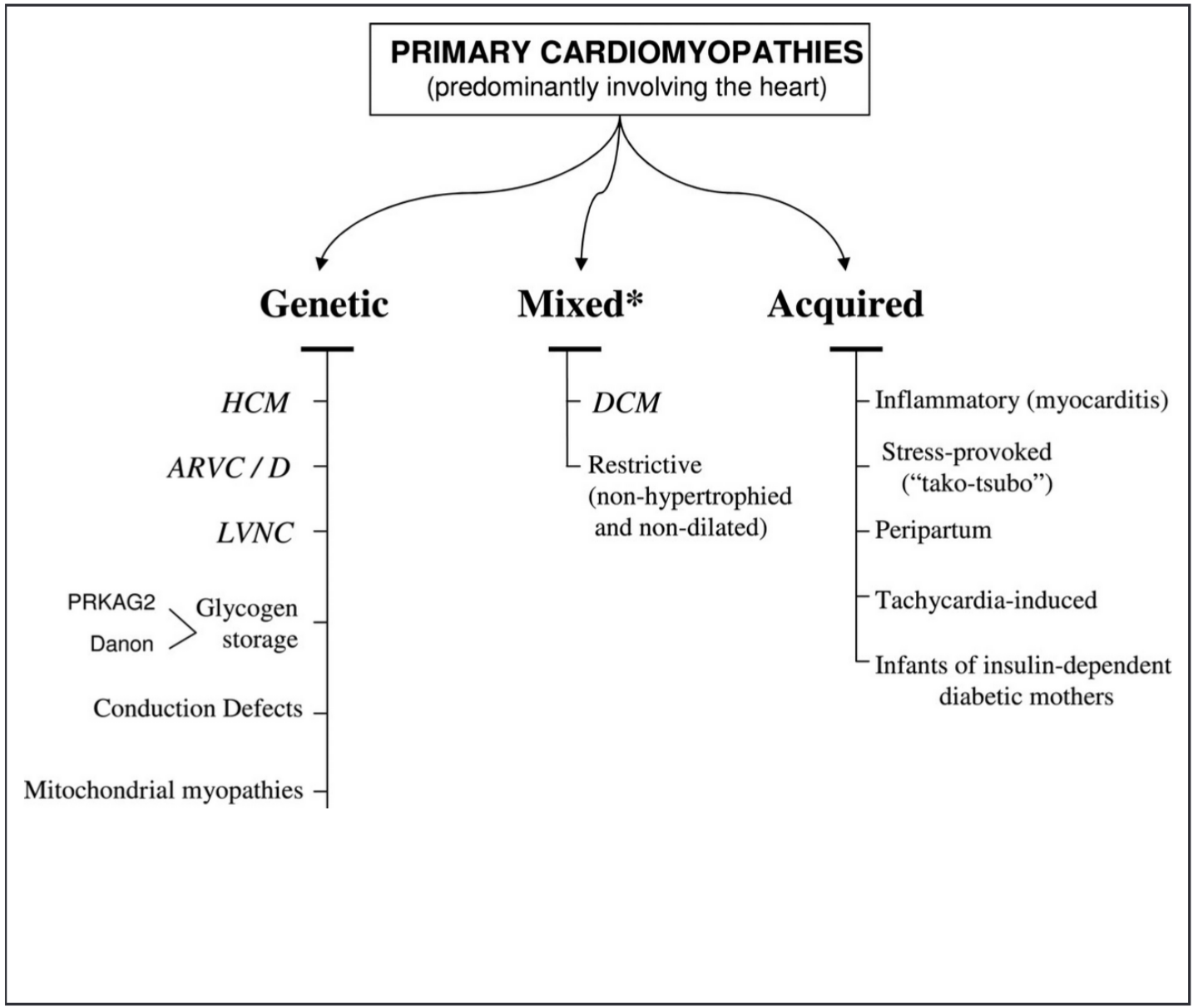
LDB3* MYBPHL* MYH7* NPPA* PITX2* PKP2* RYR2* SCN4B* SLMAP*

Cardiopatías

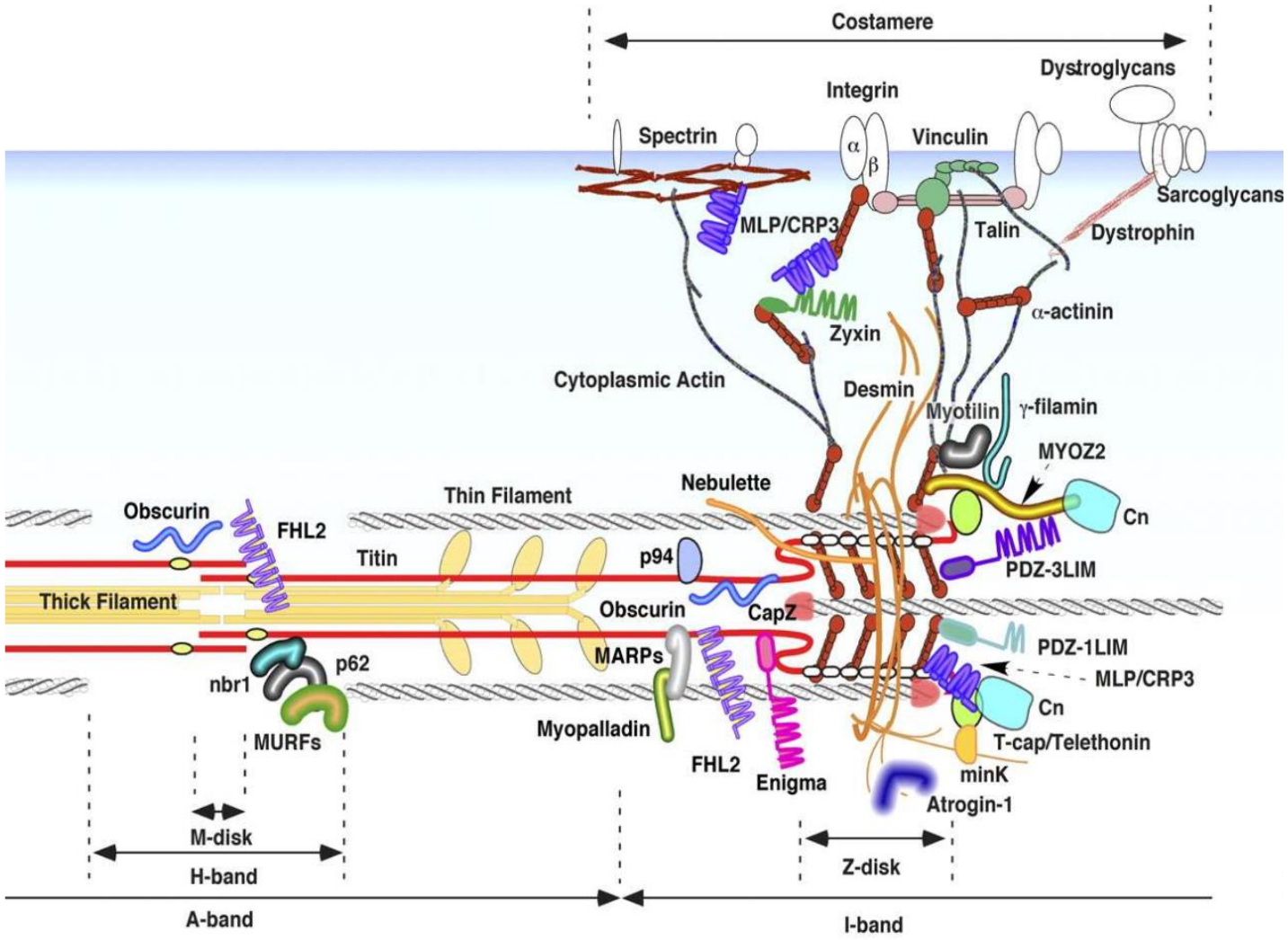


CACNA1C	**CALM1**	**CALM2**	**CALM3**	**CASQ2**	**DES**	**DSC2**	**DSG2**	**DSP**	**EMD**	**FLNC**	**JUP**	**KCNE1**	**KCNE2**	**KCNH2**	**KCNJ2**	**KCNQ1**	**LMNA**
MYH7	**NKX2-5**	**PKP2**	**PLN**	**PRKAG2**	**RYR2**	**SCN5A**	**TNNC1**	**TNNI3**	**TNNT2**	ACTC1	AKAP9	ANK2	CACNA1D	CACNA2D1	CACNB2	CAV3	
FHL2	GAA	GJA5	GLA	GNB2	GPD1L	HCN4	IRX3	KCNA5	KCND3	KCNE3	KCNE5	KCNJ5	KCNJ8	LAMP2	MYH6	PITX2	SCN1B
SCN2B	SCN4B	SLC22A5	SNTA1	TBX5	TECRL	TMEM43	TNNI3K	TRDN	TRPM4	TTR	ABCC9*	ANK3*	CAVIN1*	CAVIN4*	CDH2*		
FGF12*	GATA5*	GJA1*	GREM2*	KCND2*	KCNK17*	KCNK3*	LDB3*	MYBPHL*	NKX2-6*	NOS1AP*	NPPA*	PPA2*	RANGRF*	SCN10A*			
SCN3B*	SLMAP*	SYNE2*	TMEM175*	TPM1*	ZFHX3*												

Miocardiopatías

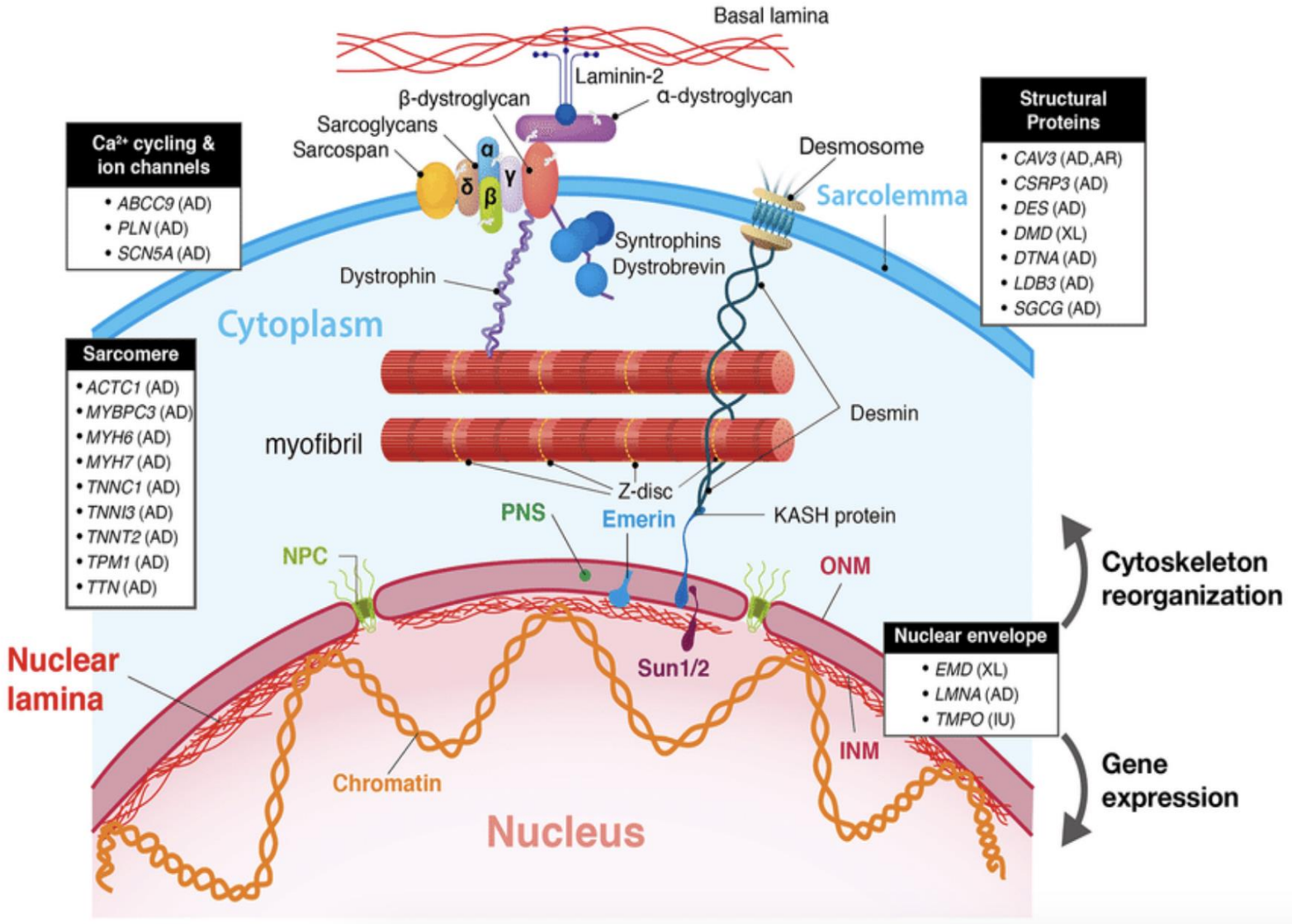


MCH


Costamere
Dystroglycans
Integrin
Spectrin
α
β
Vinculin
Sarcoglycans
MLP/CRP3
Talin
Dystrophin
α-actinin
Zyxin
Cytoplasmic Actin
Desmin
Myotilin
γ-filamin
MYOZ2
Nebulette
Thin Filament
Obscurin
FHL2
Titin
p94
Cn
PDZ-3LIM
Thick Filament
Obscurin
CapZ
PDZ-1LIM
MARPs
MLP/CRP3
p62
nbr1
Cn
T-cap/Telethonin
Myopalladin
FHL2
Enigma
minK
Atrogin-1
MURFs
M-disk
H-band
A-band
Z-disk
I-band

MCD

Genetic Variations Leading to Familial Dilated Cardiomyopathy – Kae Won Cho et al. - 2016



Miocardiopatías

ACTC1 **BAG3** **CACNA1C** **CALM1** **CALM2** **CALM3** **CASQ2** **DES** **DMD** **DSC2** **DSG2** **DSP** **EMD** **FHL1** **FHOD3** **FLNC** **GLA** **JUP**

KCNE1 **KCNE2** **KCNH2** **KCNJ2** **KCNQ1** **LAMP2** **LMNA** **MYBPC3** **MYH7** **MYL2** **MYL3** **NKX2-5** **PKP2** **PLN** **PRKAG2** **PTPN11** **RBM20**

RYR2 **SCN5A** **TNNC1** **TNNI3** **TNNT2** **TPM1** **TRIM63** **TTN** **TTR** AARS2 ACAD9 ACADVL ACTA1 ACTN2 AGK AGL AGPAT2

AKAP9 ALMS1 ALPK3 ANK2 ANO5 ATPAF2 CACNA1D CACNA2D1 CACNB2 CAV3 COA5 COA6 COQ2 COX15 COX6B1 CRYAB

CSRP3 CTNNA3 DLD DNAJC19 DOLK DTNA EYA4 FAH FHL2 FKRP FKTN FOXRED1 GAA GATA4 GATA5 GATA6 GFM1 GJA5

GLB1 GNB2 GNPTAB GUSB GYG1 HCN4 HFE HRAS IRX3 JPH2 KCND3 KCNE3 KCNE5 KCNJ5 KCNJ8 KLHL24 KRAS LAMA2

LDLR LIAS LZTR1 MAP2K1 MAP2K2 MLYCD MRPL3 MRPL44 MRPS22 MTO1 MYBPHL MYOT MYOZ2 MYPN NF1 NRAS PMM2

PPA2 PPCS PRDM16 QRSL1 RAF1 RIT1 SCN1B SCN2B SCO2 SDHA SGCD SGCG SHOC2 SLC22A5 SLC25A3 SNTA1 SOS1 SPEG

SURF1 TAZ TBX20 TBX5 TCAP TECRL TMEM43 TMEM70 TNNI3K TRDN TRPM4 ZBTB17 A2ML1* ABCC9* AKT1* ANK3* ANKRD1*

ATP5F1E* BRAF* BSCL2* C10orf71* CALR* CALR3* CASZ1* CAVIN1* CAVIN4* CBL* CDH2* CHRM2* COL7A1* CTNNA1* CTNNB1*

DNM1L* ELAC2* FBXO32* FGF12* FXN* GATAD1* GJA1* GPD1L* GREM2* GSK3B* IDH2* ILK* ISM2* JARID2* KAT6B* KCNA5*

KCND2* KCNK17* KLF10* LAMA4* LDB3* LMOD2* MAP3K8* MEF2C* MIB1* MYH6* MYLK2* MYOM1* NEBL* NEXN* NKX2-6* NNT*

NONO* NOS1AP* NOTCH1* NPPA* NRAP* OBSCN* OPA3* PDHA1* PDLIM3* PERP* PHKA1* PITX2* PKD2* PKP4* PPP1CB* PPP1R13L*

PSEN1* PSEN2* RANGRF* RASA1* RASA2* RBM24* RRAS* SCN10A* SCN3B* SCN4B* SGCA* SGCB* SLC25A4* SLMAP* SOS2*

SPRED1* SPRY1* SYNE1* SYNE2* SYNGAP1* TGFB3* TMEM175* TMOD1* TOR1AIP1* TRIM54* TSFM* TXNRD2* VCL* WISP1* WT1* XK*

ZFHX3*

Cardiopatías

ACTC1 **BAG3** **CACNA1C** **CALM1** **CALM2** **CALM3** **CASQ2** **DES** **DMD** **DSC2** **DSG2** **DSP** **EMD** **FHL1** **FHOD3** **FLNC** **GLA** **JUP**

KCNE1 **KCNE2** **KCNH2** **KCNJ2** **KCNQ1** **LAMP2** **LMNA** **MYBPC3** **MYH7** **MYL2** **MYL3** **NKX2-5** **PKP2** **PLN** **PRKAG2** **PTPN11** **RBM20**

RYR2 **SCN5A** **TNNC1** **TNNI3** **TNNT2** **TPM1** **TRIM63** **TTN** **TTR** AARS2 ACAD9 ACADVL ACTA1 ACTN2 AGK AGL AGPAT2

AKAP9 ALMS1 ALPK3 ANK2 ANO5 ATPAF2 CACNA1D CACNA2D1 CACNB2 CAV3 COA5 COA6 COQ2 COX15 COX6B1 CRYAB

CSRP3 CTNNA3 DLD DNAJC19 DOLK DTNA EYA4 FAH FHL2 FKRP FKTN FOXRED1 GAA GATA4 GATA5 GATA6 GFM1 GJA5

GLB1 GNB2 GNPTAB GUSB GYG1 HCN4 HFE HRAS IRX3 JPH2 KCND3 KCNE3 KCNE5 KCNJ5 KCNJ8 KLHL24 KRAS LAMA2

LDLR LIAS LZTR1 MAP2K1 MAP2K2 MLYCD MRPL3 MRPL44 MRPS22 MTO1 MYBPHL MYOT MYOZ2 MYPN NF1 NRAS PMM2

PPA2 PPCS PRDM16 QRSL1 RAF1 RIT1 SCN1B SCN2B SCO2 SDHA SGCD SGCG SHOC2 SLC22A5 SLC25A3 SNTA1 SOS1 SPEG

SURF1 TAZ TBX20 TBX5 TCAP TECRL TMEM43 TMEM70 TNNI3K TRDN TRPM4 ZBTB17 A2ML1* ABCC9* AKT1* ANK3* ANKRD1*

ATP5F1E* BRAF* BSCL2* C10orf71* CALR* CALR3* CASZ1* CAVIN1* CAVIN4* CBL* CDH2* CHRM2* COL7A1* CTNNA1* CTNNB1*

DNM1L* ELAC2* FBXO32* FGF12* FXN* GATAD1* GJA1* GPD1L* GREM2* GSK3B* IDH2* ILK* ISM2* JARID2* KAT6B* KCNA5*

KCND2* KCNK17* KLF10* LAMA4* LDB3* LMOD2* MAP3K8* MEF2C* MIB1* MYH6* MYLK2* MYOM1* NEBL* NEXN* NKX2-6* NNT*

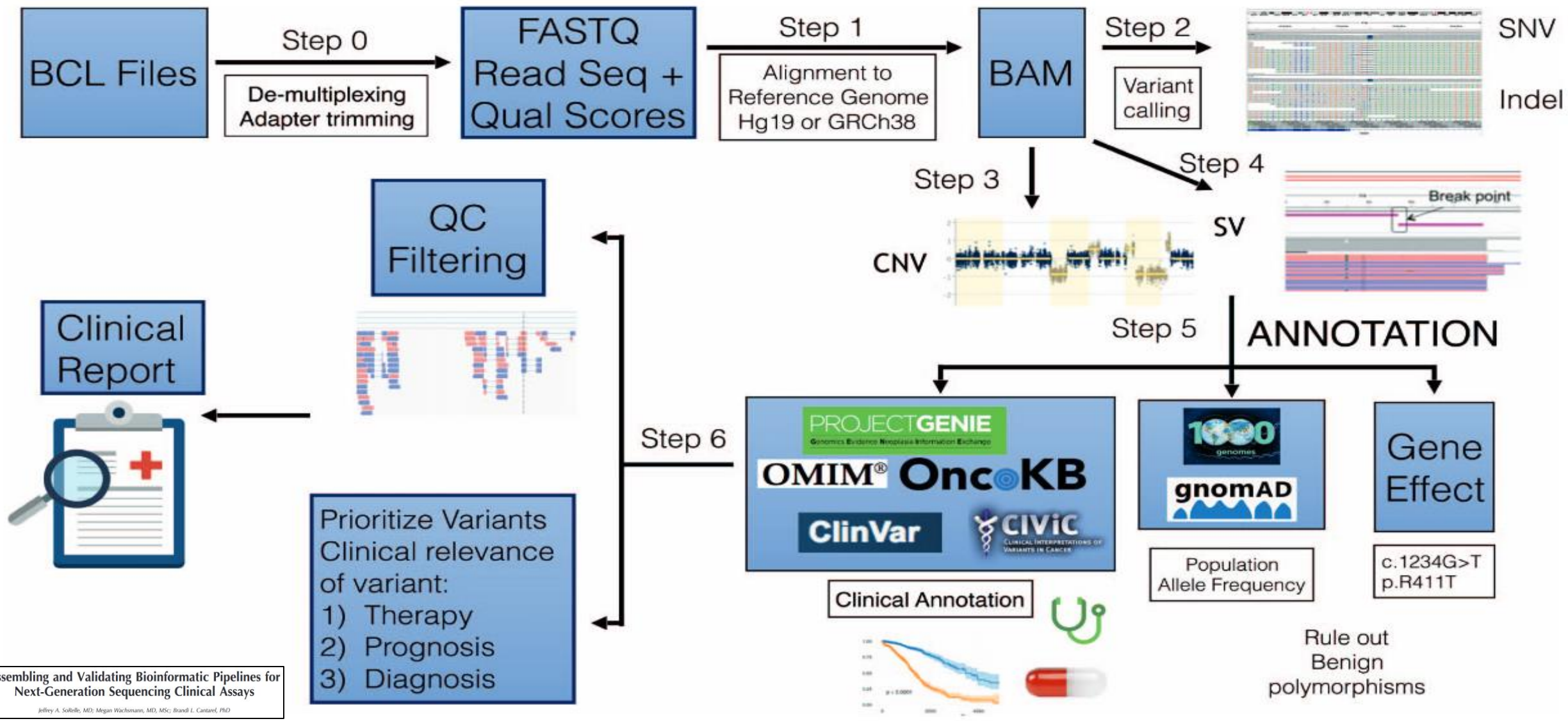
NONO* NOS1AP* NOTCH1* NPPA* NRAP* OBSCN* OPA3* PDHA1* PDLIM3* PERP* PHKA1* PITX2* PKD2* PKP4* PPP1CB* PPP1R13L*

PSEN1* PSEN2* RANGRF* RASA1* RASA2* RBM24* RRAS* SCN10A* SCN3B* SCN4B* SGCA* SGCB* SLC25A4* SLMAP* SOS2*

SPRED1* SPRY1* SYNE1* SYNE2* SYNGAP1* TGFB3* TMEM175* TMOD1* TOR1AIP1* TRIM54* TSFM* TXNRD2* VCL* WISP1* WT1* XK*

ZFHX3*

Flujo de trabajo / Interpretación



Assembling and Validating Bioinformatic Pipelines for Next-Generation Sequencing Clinical Assays

Jeffrey A. Soltelle, MD; Megan Wachsmann, MD, MSc; Brandi L. Cantarel, PhD

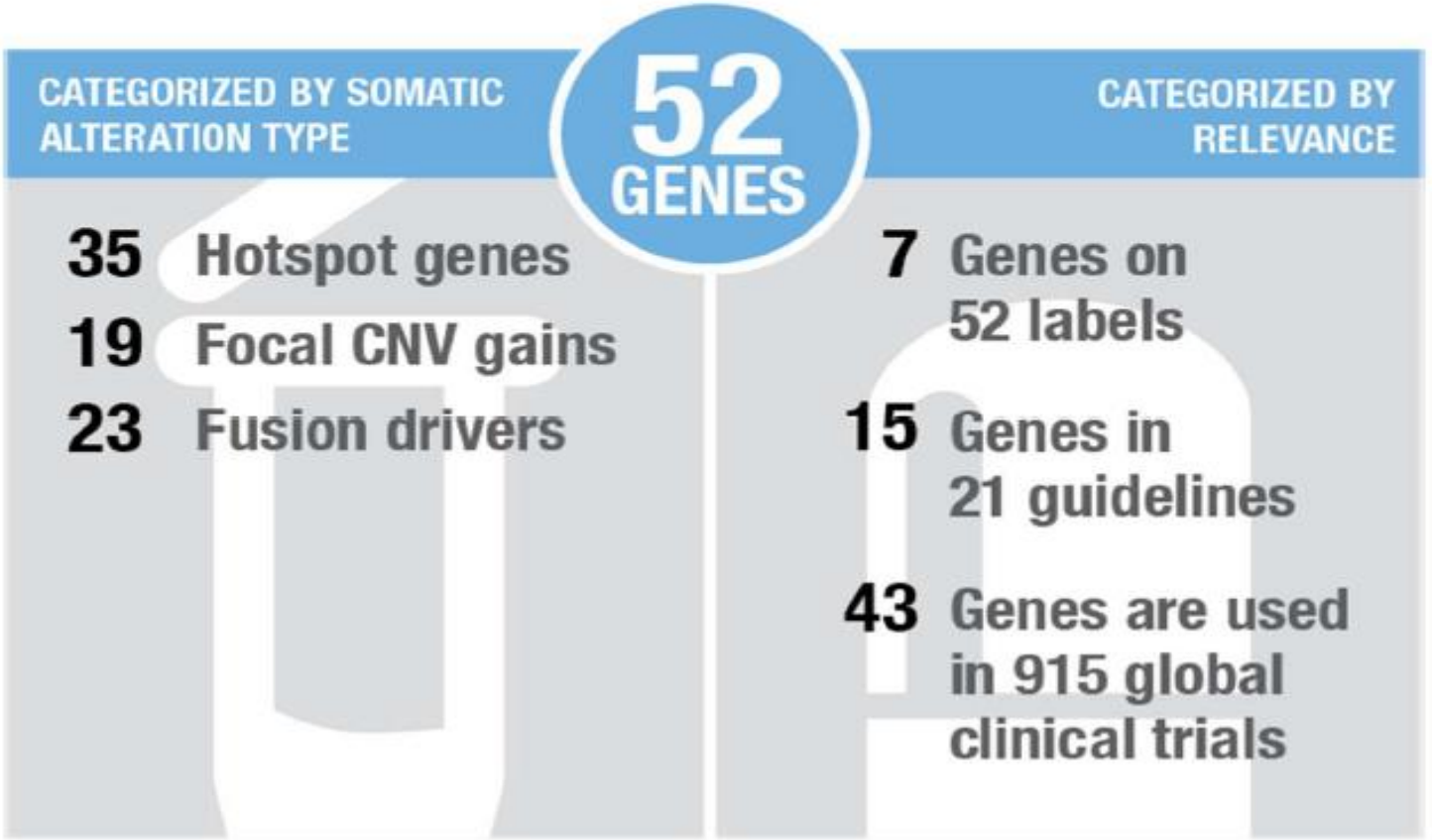
Aspectos importantes

- Regiones cubiertas
- Cobertura/Profundidad
- Número de lecturas
- Pipeline/Filtrado de variantes

Regiones cubiertas

Oncomine Focus Assay* Content and Evidence

Oncomine Reporter
March 2019



Oncomine® Focus Panel 52 Gene List

Hotspot genes, n=35

AKT1
ALK
AR
BRAF
CDK4
CTNNB1
DDR2
EGFR
ERBB2
ERBB3
ERBB4
ESR1
FGFR2
FGFR3
GNA11
GNAQ
HRAS
IDH1
IDH2
JAK1
JAK2
JAK3
KIT
KRAS
MAP2K1
MAP2K2
MET
MTOR
NRAS
PDGFRA
PIK3CA
RAF1
RET
ROS1
SMO

Copy Number Variants, n=19

ALK
AR
BRAF
CCND1
CDK4
CDK6
EGFR
ERBB2
FGFR1
FGFR2
FGFR3
FGFR4
KIT
KRAS
MET
MYC
MYCN
PDGFRA
PIK3CA

DNA Panel

Note: 52 genes total

Fusion drivers, n=23

ABL1
AKT3
ALK
AXL
BRAF
ERG
ETV1
ETV4
ETV5
EGFR
ERBB2
FGFR1
FGFR2
FGFR3
MET
NTRK1
NTRK2
NTRK3
PDGFRA
PPARG
RAF1
RET
ROS1

RNA Panel

Hotspot, **CNV**, **Fusion**, **Hotspot + CNV**, **Hotspot + CNV + Fusion**, **Hotspot + Fusion**, **CNV+ Fusion**

TABLA 1. Genes y regiones estudiados en el análisis de variantes nucleotídicas

GEN	CROMOSOMA	EXONES	CAMBIO DE NUCLEÓTIDO	CAMBIO AMINOACÍDICO	*RefSeq*	BASE DE DATOS	VAF (%)	CATEGORÍA	*TIER*
AKT1	14	3							
ALK	2	11, 21, 22, 23, 24, 25, 27 y 29							
AR	X	6 y 8							
BRAF	7	5, 6, 7, 8, 10, 11, 13, 15 y 18							
CDK4	12	1, 5, 6, 7 y 8							
CTNNB1	3	3							
DDR2	1	5							
EGFR	7	3, 7, 12, 15, 18, 19, 20 y 21							
ERBB2	17	8, 17, 18, 19, 20, 21, 22, 24 y 25							
ERBB3	12	2, 3, 6, 8 y 9							
ERBB4	2	18							
ESR1	6	8							
FGFR2	10	7, 8, 9, 12 y 14							
FGFR3	4	3, 7, 9, 14, 16 y 18							
GNA11	19	4 y 5							
GNAQ	9	4 y 5							
HRAS	11	2 y 3							
IDH1	2	4							
IDH2	15	4							
JAK1	1	14, 15 y 16							
JAK2	9	14							
JAK3	19	11, 12 y 15							
KIT	4	8, 9, 10, 11, 13 y 17							
KRAS	12	2, 3 y 4							
MAP2K1	15	2, 3 y 6							
MAP2K2	19	2							
MET	7	2, 11, 14, 15, 16 y 19							
MTOR	1	30, 39, 40, 43, 47 y 53							
NRAS	1	2, 3 y 4							
PDGFRA	4	7, 12, 14, 18 y 23							
PIK3CA	3	2, 5, 6, 8, 10, 14, 19 y 21							
RAF1	3	7 y 12							
RET	10	10, 11, 13, 15 y 16							
ROS1	6	36 y 38							
SMO	7	4, 6, 8 y 9							

TABLA 1. Genes y regiones estudiados en el análisis de variantes nucleotídicas

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CDK4	12	1, 5, 6, 7 y 8							
CTNNB1	3	3							
DDR2	1	5							
EGFR	7	3, 7, 12, 15, 18, 19, 20 y 21							
ERBB2	17	8, 17, 18, 19, 20, 21, 22, 24 y 25							
ERBB3	12	2, 3, 6, 8 y 9							
ERBB4	2	18							
ESR1	6	8							
FGFR2	10	7, 8, 9, 12 y 14							
FGFR3	4	3, 7, 9, 14, 16 y 18							
GNA11	19	4 y 5							
GNAQ	9	4 y 5							
HRAS	11	2 y 3							
IDH1	2	4							
IDH2	15	4							
JAK1	1	14, 15 y 16							
JAK2	9	14							
JAK3	19	11, 12 y 15							
KIT	4	8, 9, 10, 11, 13 y 17							
KRAS	12	2, 3 y 4							
MAP2K1	15	2, 3 y 6							
MAP2K2	19	2							
MET	7	2, 11, 14, 15, 16 y 19							
MTOR	1	30, 39, 40, 43, 47 y 53							
NRAS	1	2, 3 y 4							
PDGFRA	4	7, 12, 14, 18 y 23							
PIK3CA	3	2, 5, 6, 8, 10, 14, 19 y 21							
RAF1	3	7 y 12							
RET	10	10, 11, 13, 15 y 16							
ROS1	6	36 y 38							
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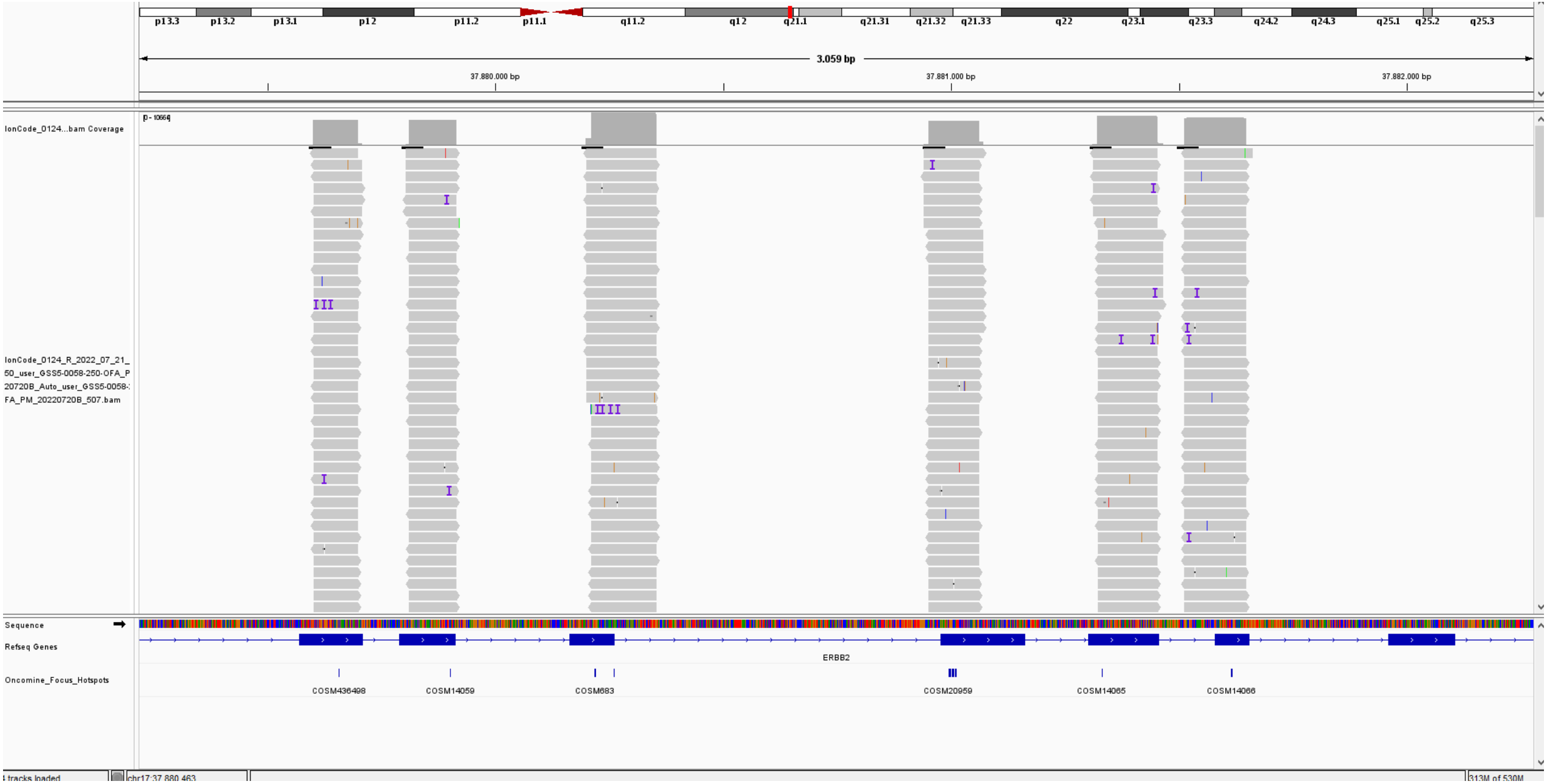
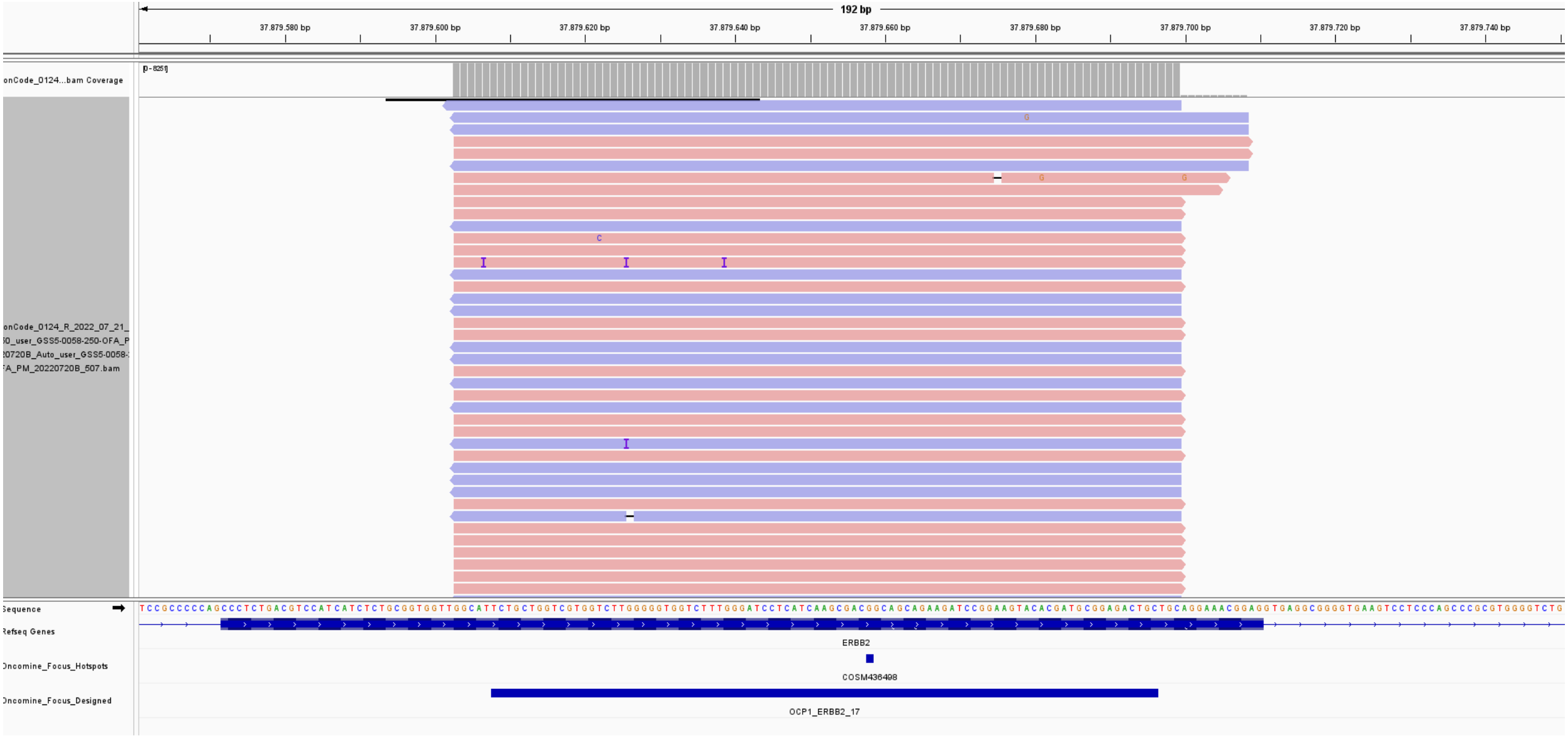
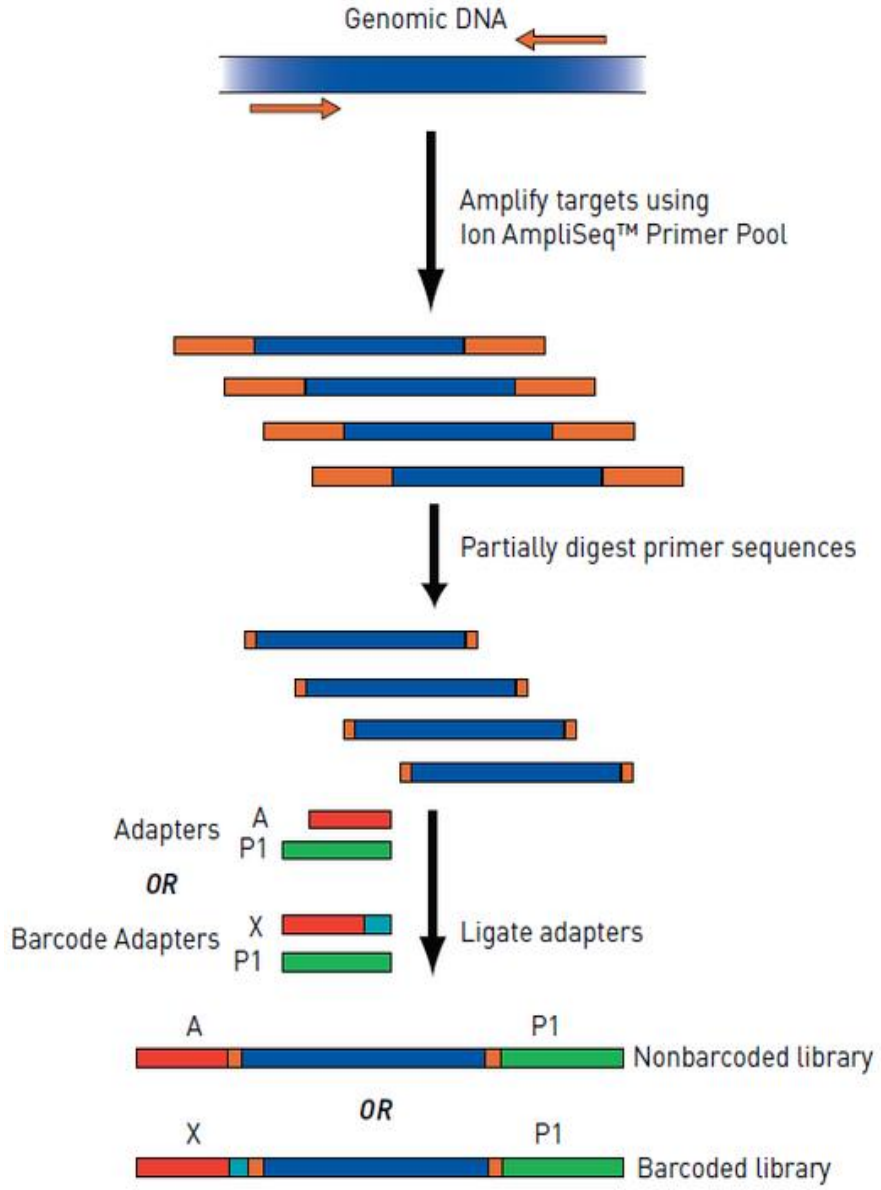
Regiones cubiertas



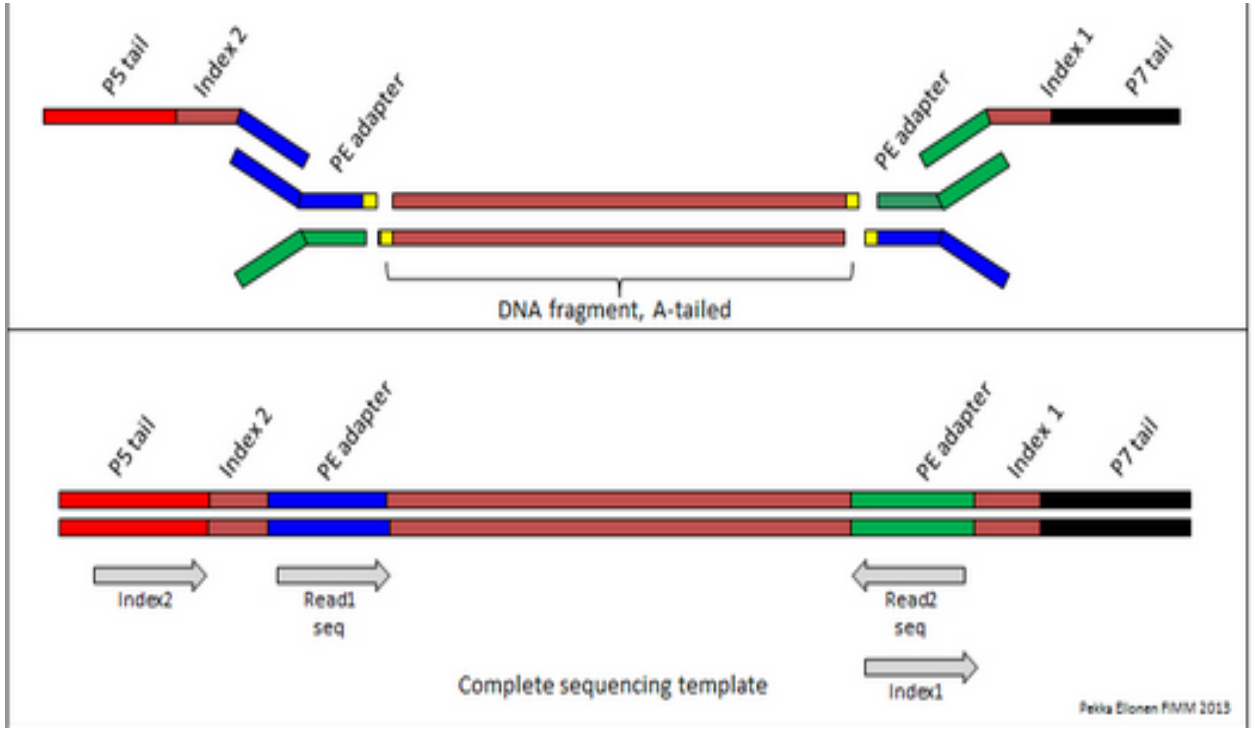
Regiones cubiertas



Librerías

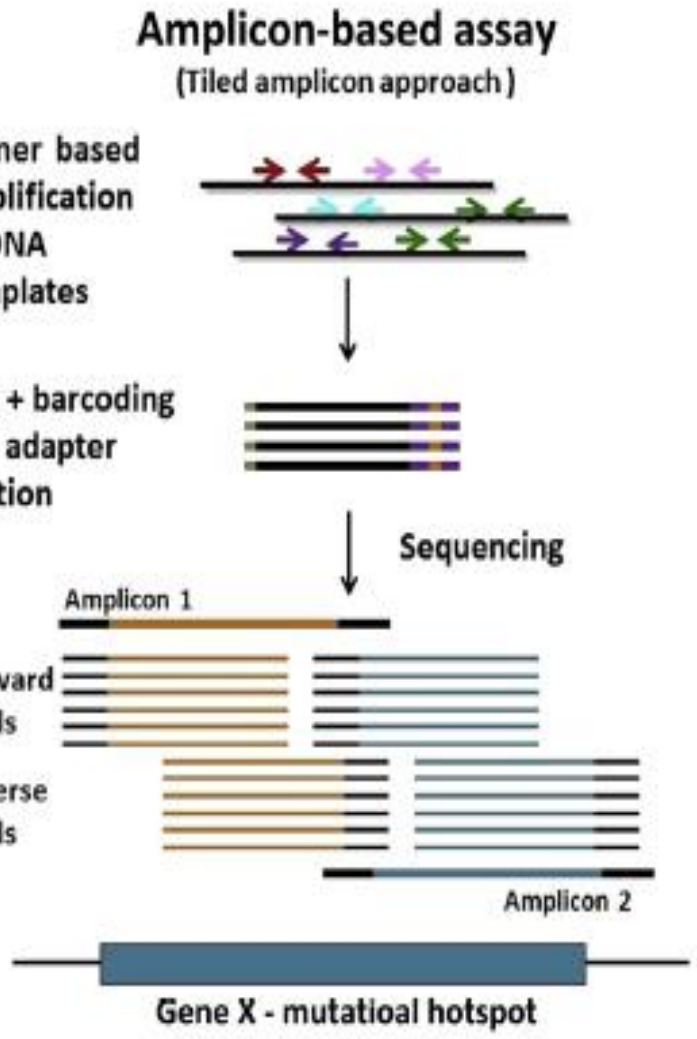


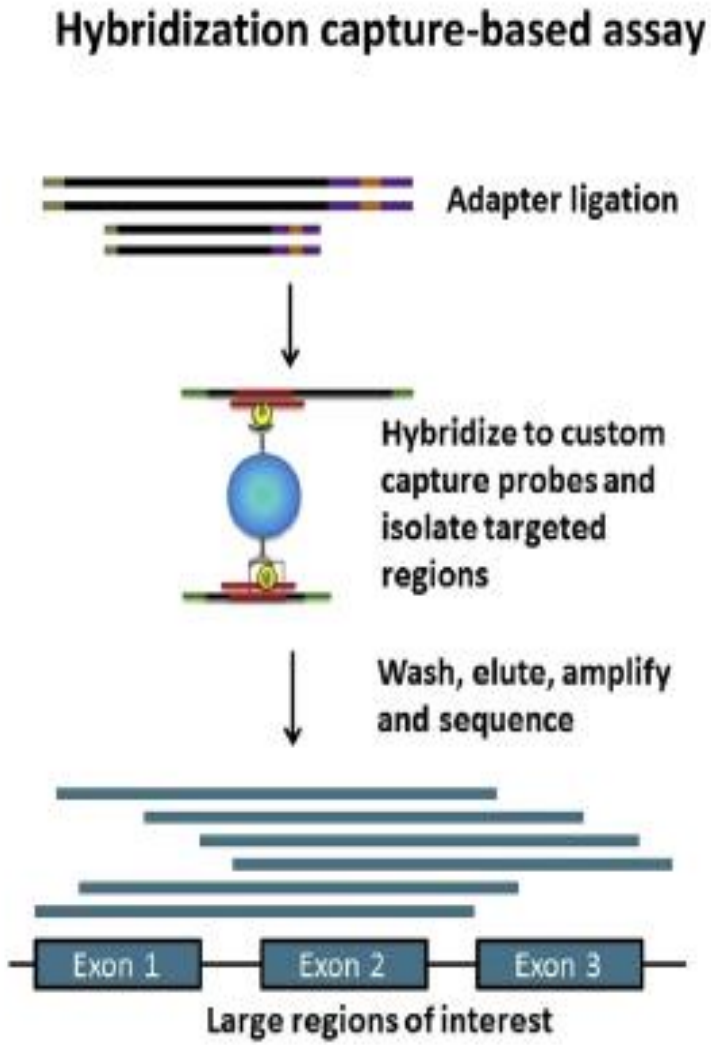
www.thermofisher.com



www.illumina.com

Tecnología





The Journal of Molecular Diagnostics, Vol. 19, No. 3, May 2017

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SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels

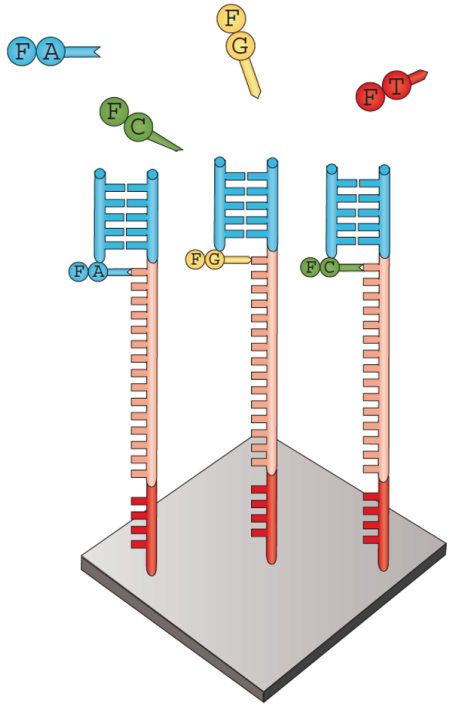
A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

Lawrence J. Jennings,*† Maria E. Arcila,*‡ Christopher Corless,*§ Suzanne Kamel-Reid,*¶‖ Ira M. Lubin,*,** John Pfeifer,*†† Robyn L. Temple-Smolkin,‡‡ Karl V. Voelkerding,*§§¶¶ and Marina N. Nikiforova*‖‖

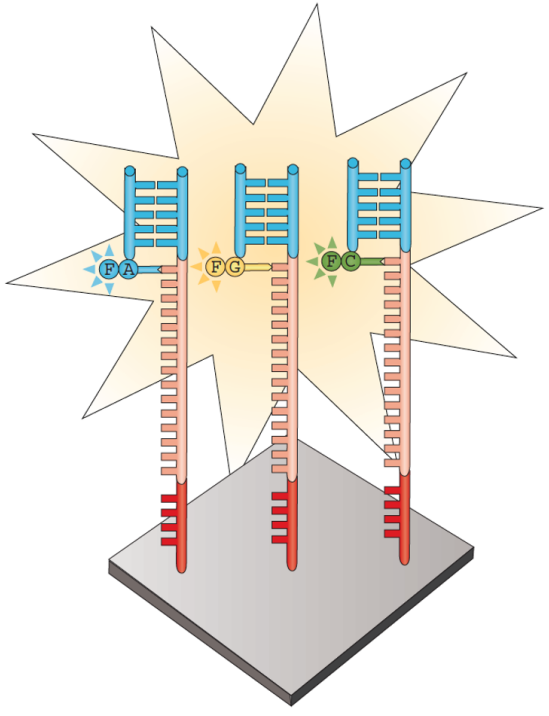
Tecnología

- El método para la preparación y el enriquecimiento de las librerías puede estar basado en captura de híbridos o mediante amplicones
- La tecnología de secuenciación empleada:
  - la secuenciación mediante ligación (Sequencing By Ligation – SBL)
  - la secuenciación mediante síntesis (Sequencing By Synthesis – SBS)
- La secuenciación mediante síntesis a su vez se puede diferenciar en dos tipos:
  - SBS con terminadores fluorescentes reversibles (Cyclic Reversible Termination – CRT)
  - SBS mediante la adición de un único nucleótido (Single-Nucleotide Addition – SNA)

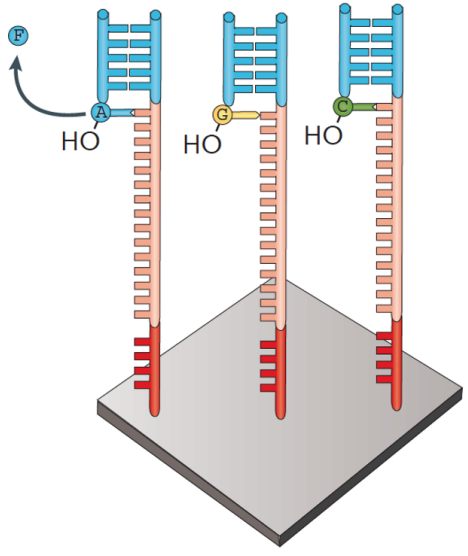
SBS-CRT



Adición de nucleótidos
Los nucleótidos marcados con un fluoróforo y con el extremo 3'-OH bloqueado hibridan con su base complementaria. Cada clúster puede incorporar una base diferente.



Visualización
Cada zona de la *flow cell* se visualiza mediante dos o cuatro láseres. Cada clúster emite un color correspondiente a la base incorporada durante este ciclo.



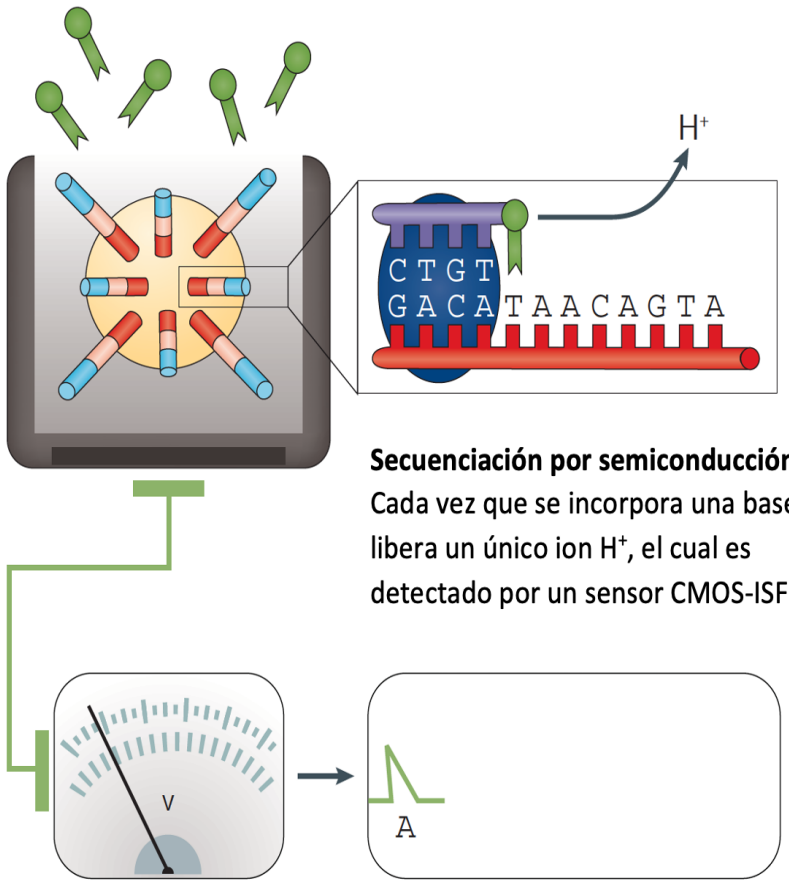
Escisión
Los fluoróforos son escindidos y lavados de la *flow cell* y se regeneran los grupos 3'-OH. A continuación comienza un nuevo ciclo con la adición de nuevos nucleótidos.

NATURE REVIEWS | GENETICS VOLUME 17 | JUNE 2016 | 333

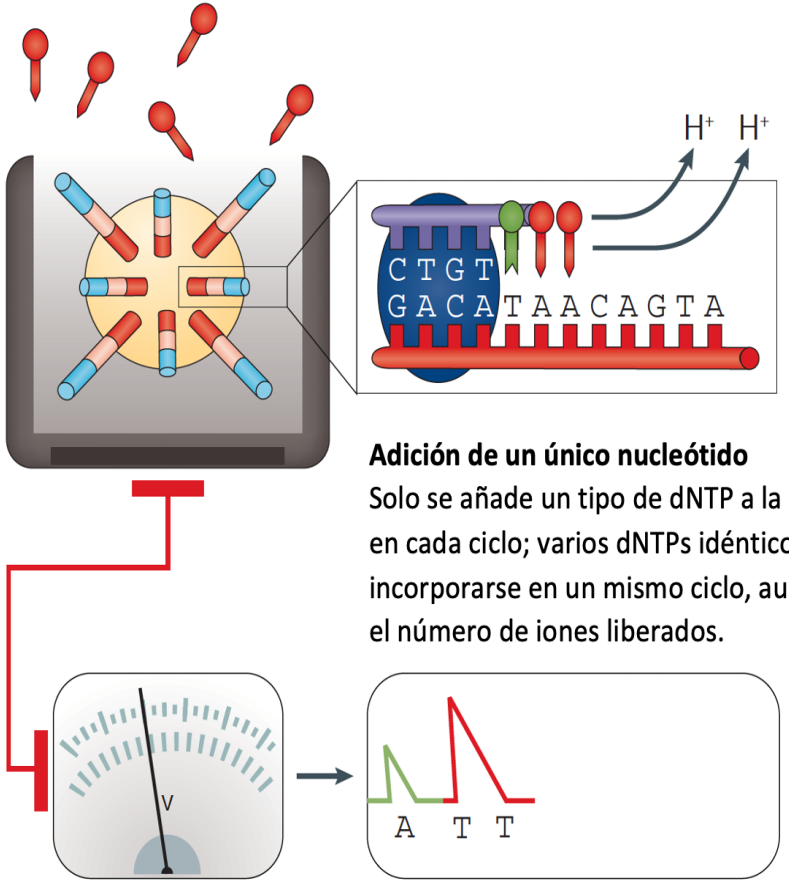
Coming of age: ten years of next-generation sequencing technologies

Sara Goodwin[1], John D. McPherson[2] and W. Richard McCombie[1]

SBS-SNA



Secuenciación por semiconducción
Cada vez que se incorpora una base se libera un único ion H^+, el cual es detectado por un sensor CMOS-ISFET.



Adición de un único nucleótido
Solo se añade un tipo de dNTP a la reacción en cada ciclo; varios dNTPs idénticos pueden incorporarse en un mismo ciclo, aumentando el número de iones liberados.

NATURE REVIEWS | GENETICS VOLUME 17 | JUNE 2016 | 333

Coming of age: ten years of next-generation sequencing technologies

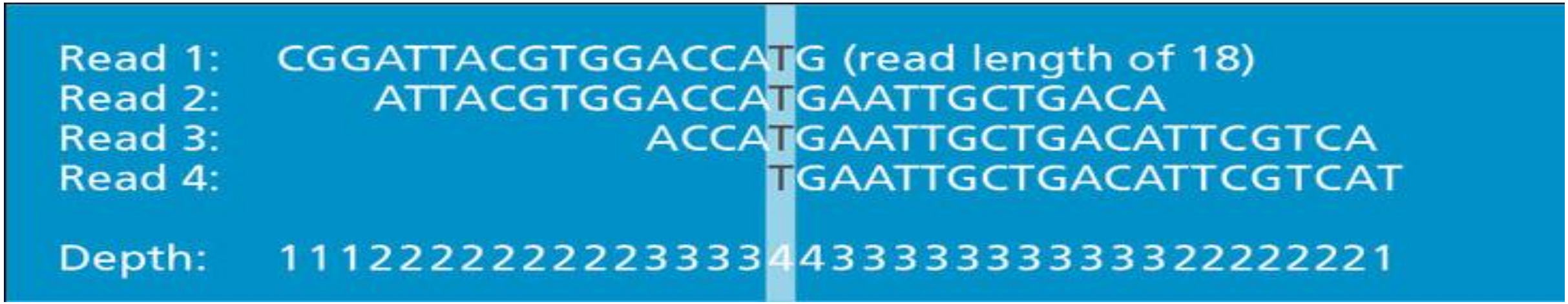
Sara Goodwin[1], John D. McPherson[2] and W. Richard McCombie[1]

Tecnología / Valoración	SBS-CRT	SBS-SNA
Ventajas	• Mayor valor predictivo positivo en la detección de SNVs, indels • Bajo número de falsos positivos • Secuenciación de librerías formadas a partir de captura de híbridos y amplicones • Posibilidad de realizar la secuenciación en un único sentido o en ambos sentidos (*single-end* y *paired-end sequencing*) • Elevado número de lecturas por carrera (400-800 millones)	• Mayor sensibilidad en la detección de SNVs, indels • Duración corta de la carrera de secuenciación (2-4 horas) • Lectura de fragmentos largos (200-400 pb)
Desventajas	• Fragmentos de lectura más cortos (75-150 pb) • Duración de la carrera de secuenciación más larga (11-29 horas)	• Mayor número de falsos positivos, especialmente en homopolímeros • Secuenciación de librerías formadas únicamente a partir de amplicones • Menor número de lecturas por cada carrera (2-80 millones)

Esquema adaptado por Martín M. y Biscuola M.

Profundidad

- Otro aspecto importante en la NGS es el número de veces que cada base de una determinada región está presente en las lecturas (*reads*) de los productos de secuenciación
- Este valor se denomina profundidad de cobertura (*depth of coverage*)
- Es uno de los factores determinantes para evaluar la fiabilidad del nucleótido asignado a esa posición de una determinada región



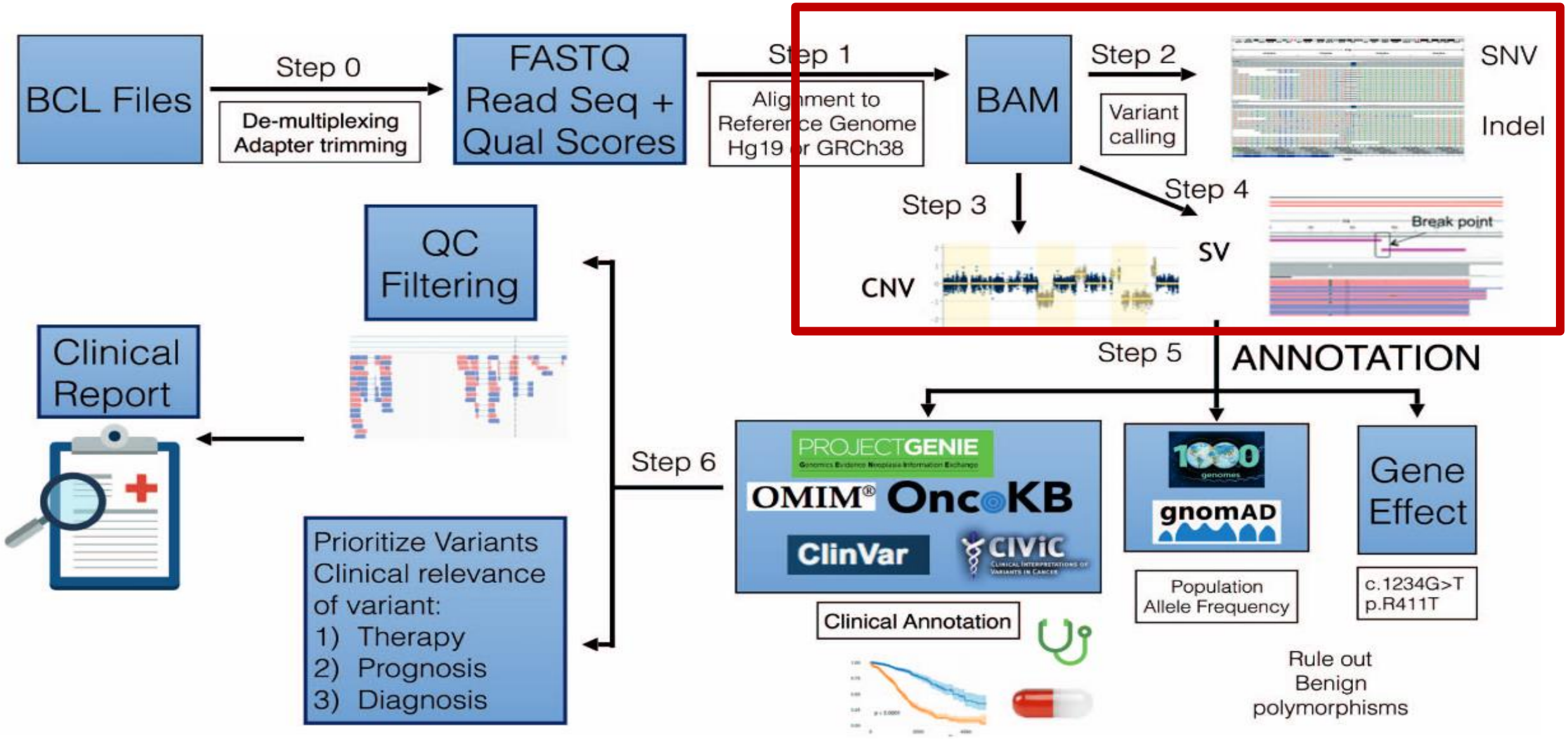
www.google.com/depthofcoverage

Profundidad

Sample	Mapped Reads	On Target	Mean Depth	Uniformity
20PM0133OFAD	2,135,391	98.41%	7,831	99.74%
20PM0134OFAD	1,731,511	98.68%	6,261	98.01%
20PM0135OFAD	2,006,332	98.52%	7,323	100.00%
20PM0136OFAD	386,670	97.92%	1,293	94.38%
20PM0137OFAD	2,513,523	97.70%	9,342	100.00%
20PM0138OFAD	146,706	98.67%	496.7	89.42%
20PM0139OFAD	1,810,707	98.79%	6,602	100.00%
20PM0140OFAD	1,025,386	99.09%	3,610	92.17%

Fuente propia

Pipeline/Filtrado de variantes

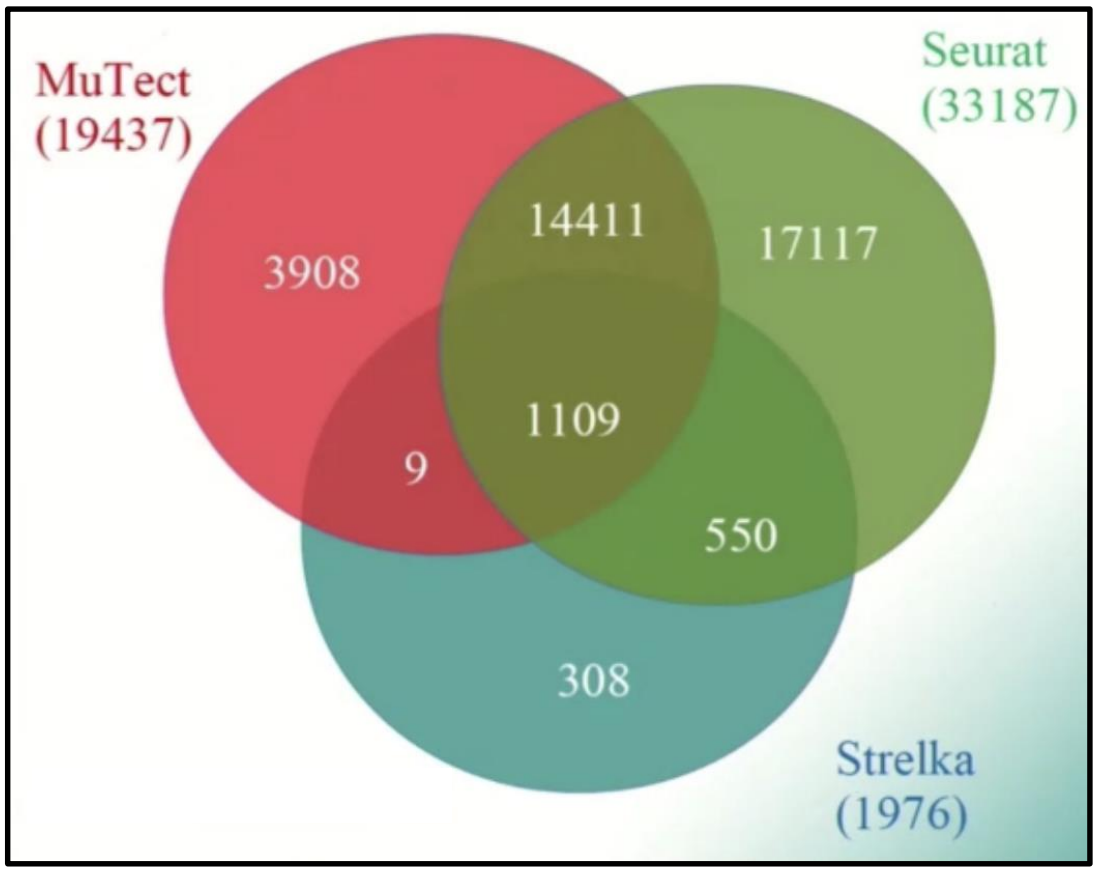


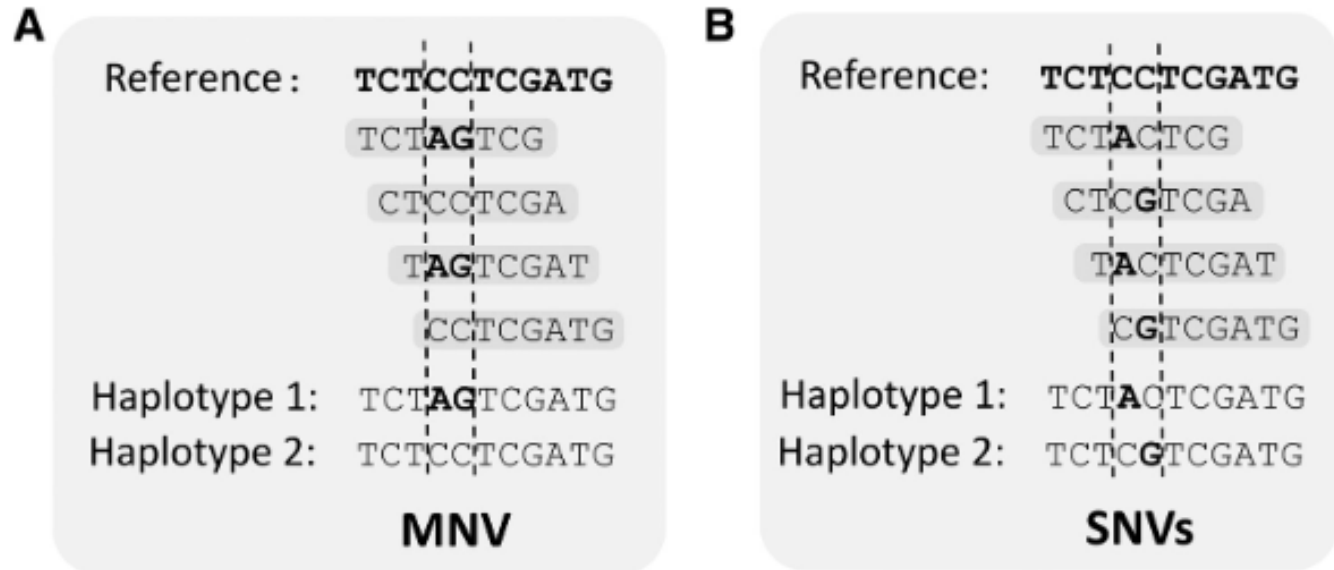
Variant caller	Type of variant	Single-sample mode	Type of core algorithm
BAYSIC [48]	SNV	No	Machine learning (ensemble caller)
CaVEMan [34]	SNV	No	Joint genotype analysis
deepSNV [38]	SNV	No	Allele frequency analysis
EBCall [37]	SNV, indel	No	Allele frequency analysis
FaSD-somatic [31]	SNV	Yes	Joint genotype analysis
FreeBayes [44]	SNV, indel	Yes	Haplotype analysis
HapMuC [42]	SNV, indel	Yes	Haplotype analysis
JointSNVMix2 [30]	SNV	No	Joint genotype analysis
LocHap [43]	SNV, indel	No	Haplotype analysis
LoFreq [36]	SNV, indel	Yes	Allele frequency analysis
LoLoPicker [39]	SNV	No	Allele frequency analysis
MutationSeq [45]	SNV	No	Machine learning
MuSE [40]	SNV	No	Markov chain model
MuTect [35]	SNV	Yes	Allele frequency analysis
SAMtools [8]	SNV, indel	Yes	Joint genotype analysis
Platypus [41]	SNV, indel, SV	Yes	Haplotype analysis
qSNP [24]	SNV	No	Heuristic threshold
RADIA [26]	SNV	No	Heuristic threshold
Seurat [33]	SNV, indel, SV	No	Joint genotype analysis
Shimmer [25]	SNV, indel	No	Heuristic threshold
SNooPer [47]	SNV, indel	Yes	Machine learning
SNVSniffer [32]	SNV, indel	Yes	Joint genotype analysis
SOAPsnv [27]	SNV	No	Heuristic threshold
SomaticSeq [46]	SNV	No	Machine learning (ensemble caller)
SomaticSniper [28]	SNV	No	Joint genotype analysis
Strelka [17]	SNV, indel	No	Allele frequency analysis
TVC [97]	SNV, indel, SV	Yes	Ion Torrent specific
VarDict [18]	SNV, indel, SV	Yes	Heuristic threshold
VarScan2 [9]	SNV, indel	Yes	Heuristic threshold
Virmid [29]	SNV	No	Joint genotype analysis

Computational and Structural Biotechnology Journal 16 (2018) 15–24

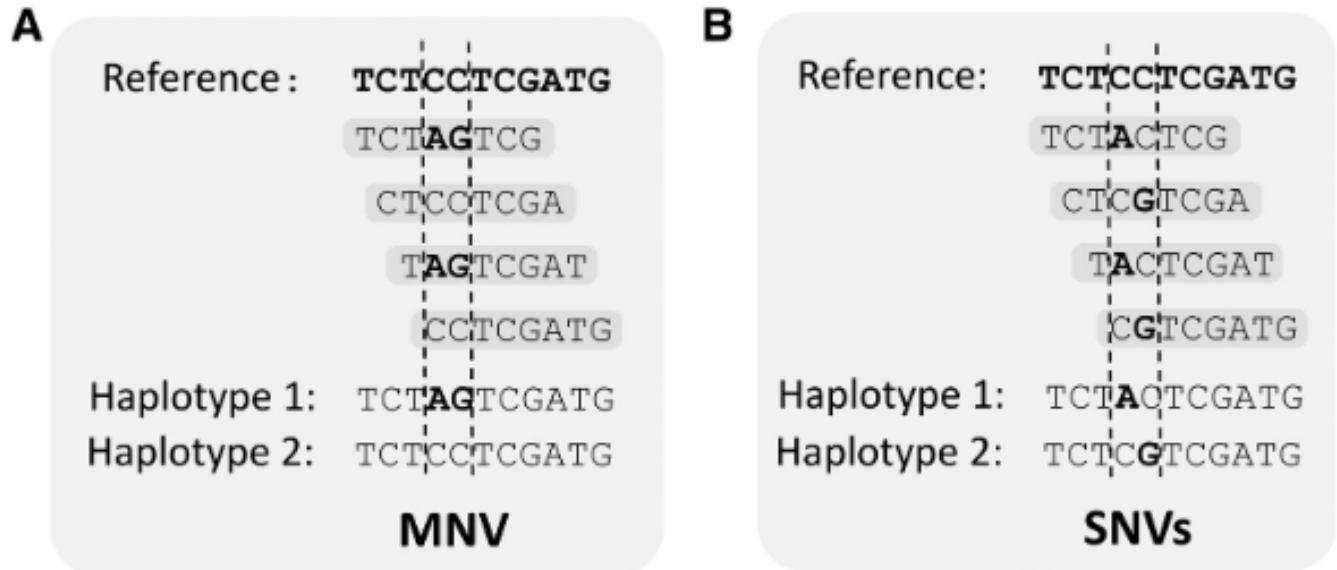
A review of somatic single nucleotide variant calling algorithms for next-generation sequencing data

Chang Xu




A
Reference :
TCTCCTCGATG
TCTAGTCG
CTCCTCGA
TAGTCGAT
CCTCGATG
Haplotype 1:
TCTAGTCGATG
Haplotype 2:
TCTCCTCGATG
MNV
B
Reference:
TCTCCTCGATG
TCTACTCG
CTCGTCGA
TACTCGAT
CGTCGATG
Haplotype 1:
TCTAGTCGATG
Haplotype 2:
TCTCGTCGATG
SNVs

Misannotated MNV in Public Cancer Genomics Datasets



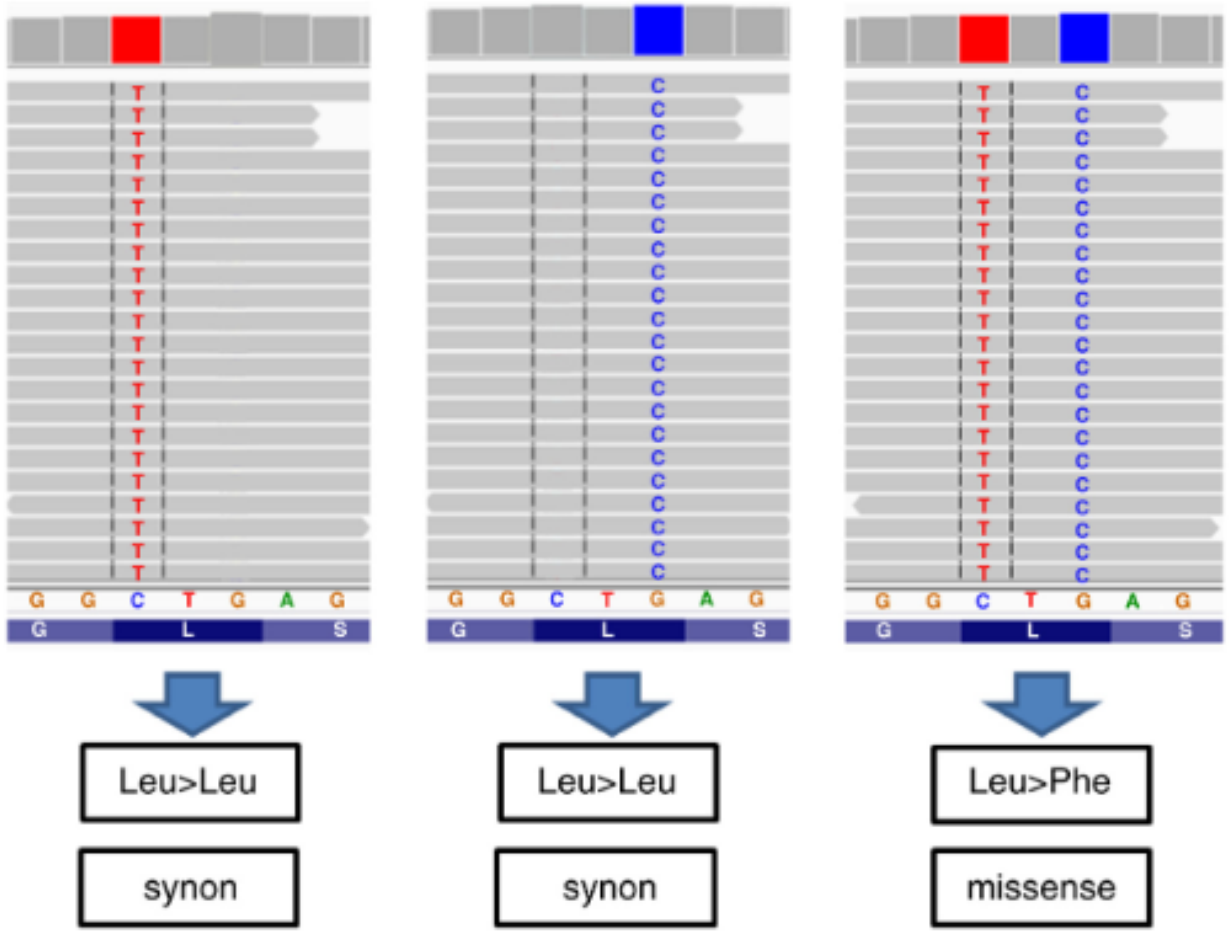
CANCER RESEARCH | GENOME AND EPIGENOME

Misannotated Multi-Nucleotide Variants in Public Cancer Genomics Datasets Lead to Inaccurate Mutation Calls with Significant Implications

Sujaya Srinivasan[1], Natallia Kalinava[1], Rafael Aldana[2], Zhipan Li[2], Sjoerd van Hagen[3], Sander Y.A. Rodenburg[3], Megan Wind-Rotolo[4], Xiaozhong Qian[4,5], Ariella S. Sasson[1], Hao Tang[1], and Stefan Kirov[1]

Misannotation of multiple-nucleotide variants risks misdiagnosis

Matthew N. Wakeling, Formal Analysis, Writing – Review & Editing,[#a,1] Thomas W. Laver, Visualization, Writing – Original Draft Preparation,[#1] Kevin Colclough, Investigation,[2] Andrew Parish, Data Curation,[2] Sian Ellard, Funding Acquisition, Supervision,[1,2] and Emma L. Baple, Conceptualization, Writing – Review & Editing[b,1,3]



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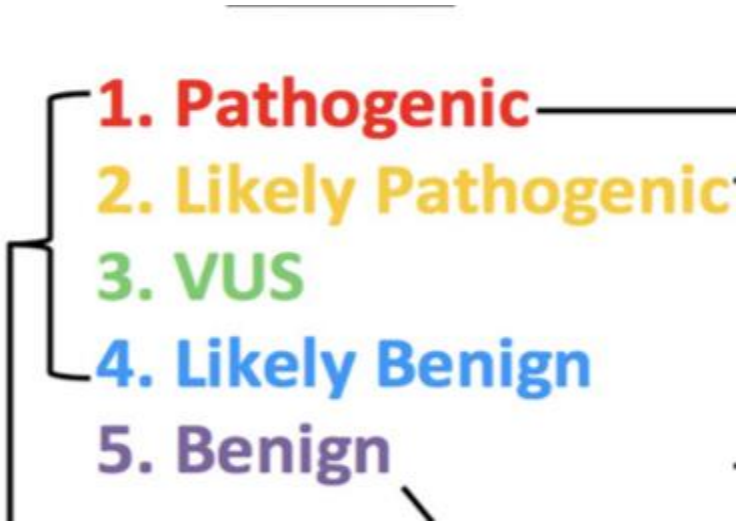
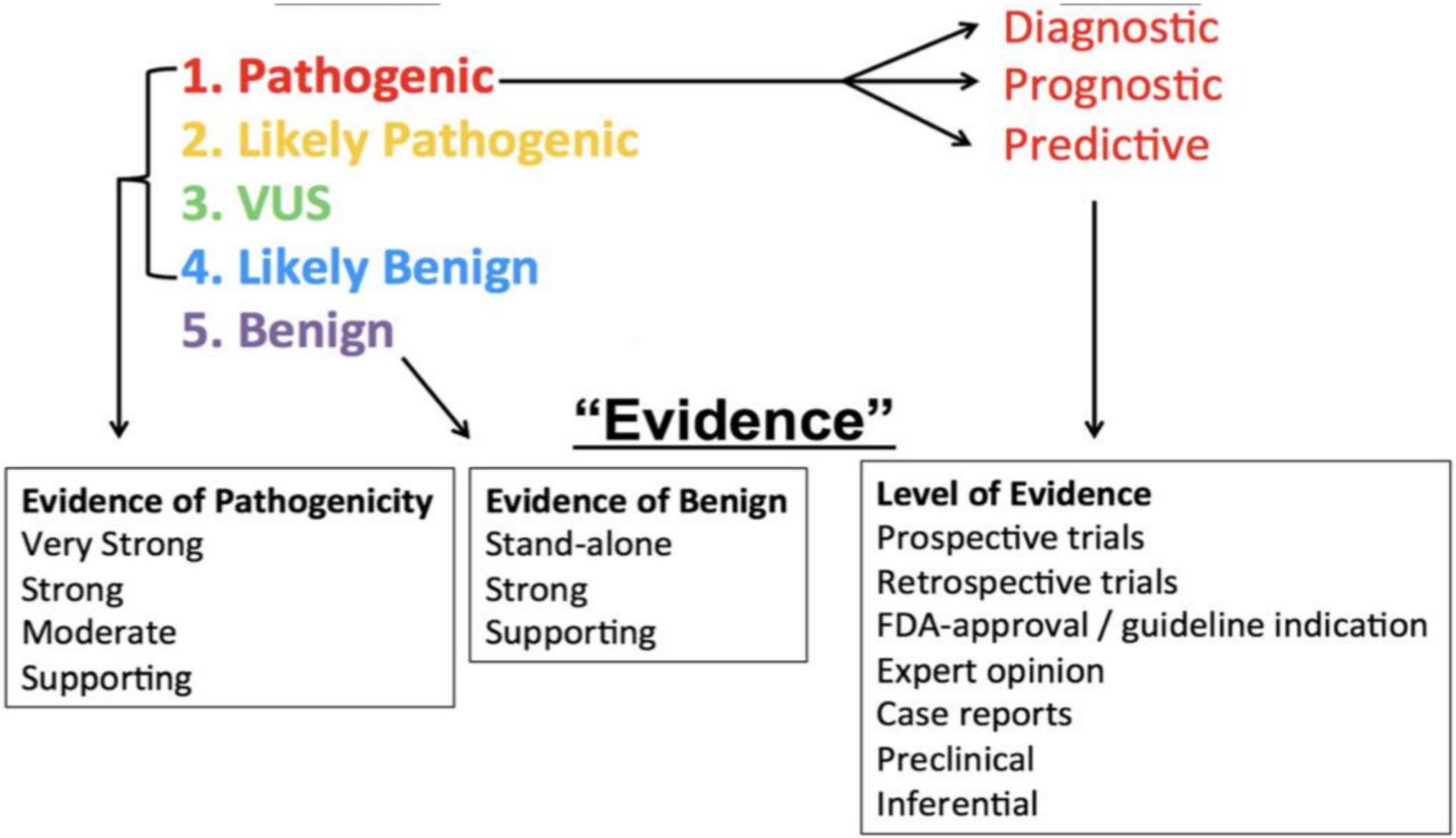
Variantes


1. Pathogenic
2. Likely Pathogenic
3. VUS
4. Likely Benign
5. Benign

Variantes


1. Pathogenic
2. Likely Pathogenic
3. VUS
4. Likely Benign
5. Benign
Diagnostic
Prognostic
Predictive
"Evidence"
Evidence of Pathogenicity
Very Strong
Strong
Moderate
Supporting
Evidence of Benign
Stand-alone
Strong
Supporting
Level of Evidence
Prospective trials
Retrospective trials
FDA-approval / guideline indication
Expert opinion
Case reports
Preclinical
Inferential

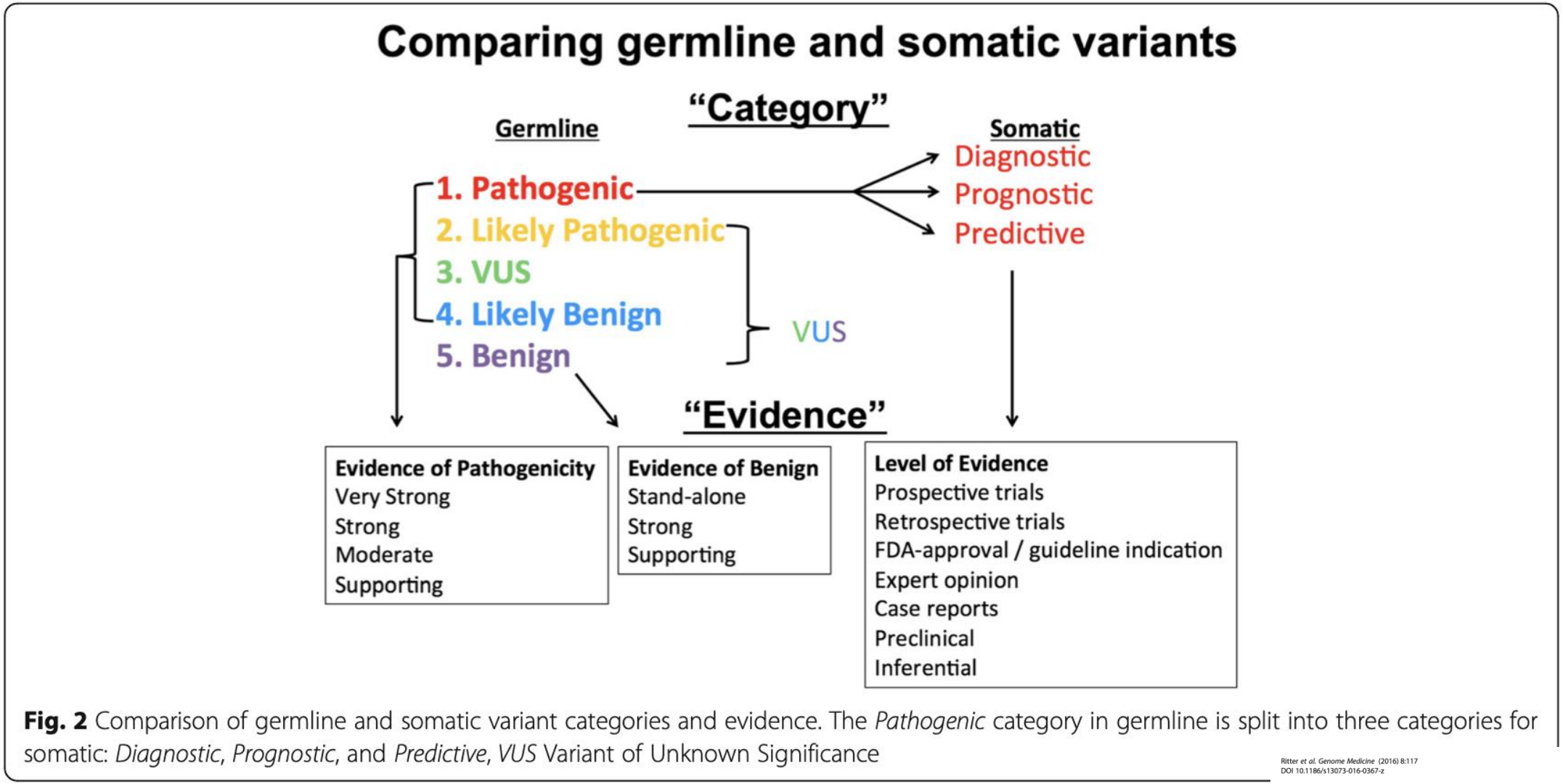


Fig. 2 Comparison of germline and somatic variant categories and evidence. The *Pathogenic* category in germline is split into three categories for somatic: *Diagnostic*, *Prognostic*, and *Predictive*, *VUS* Variant of Unknown Significance

Ritter *et al. Genome Medicine* (2016) 8:117
DOI 10.1186/s13073-016-0367-z

Genome Medicine

RESEARCH Open Access

Somatic cancer variant curation and harmonization through consensus minimum variant level data

Deborah I. Ritter[1†], Sameek Roychowdhury[2†], Angshumoy Roy[1], Shruti Rao[3], Melissa J. Landrum[4], Dmitriy Sonkin[5],

Guias

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ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5],

The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017

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jmd.amjpathol.org

SPECIAL ARTICLE

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

Marilyn M. Li,*† Michael Datto,*‡ Eric J. Duncavage,*§ Shashikant Kulkarni,*¶ Neal I. Lindeman,*‖ Somak Roy,*,** Apostolia M. Tsimberidou,*†† Cindy L. Vnencak-Jones,*‡‡ Daynna J. Wolff,*§§ Anas Younes,*¶¶ and Marina N. Nikiforova*,**

Table 3 Criteria for classifying pathogenic variants

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts
Strong	PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val→Leu caused by either G>C or G>T in the same codon Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level
	PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.
	PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.
	PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance. Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.
Moderate	PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
	PM2 Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.
	PM3 For recessive disorders, detected in *trans* with a pathogenic variant Note: This requires testing of parents (or offspring) to determine phase.
	PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants
	PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.
	PM6 Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease Note: May be used as stronger evidence with increasing segregation data
	PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
	PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.
	PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology
	PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

LOF, loss of function; OR, odds ratio.

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Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5],

Table 4 Criteria for classifying benign variants

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
Strong	BS1 Allele frequency is greater than expected for disorder (see Table 6)
	BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age
	BS3 Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing
	BS4 Lack of segregation in affected members of a family Caveat: The presence of phenocopies for common phenotypes (i.e., cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.
Supporting	BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease
	BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in *cis* with a pathogenic variant in any inheritance pattern
	BP3 In-frame deletions/insertions in a repetitive region without a known function
	BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.
	BP5 Variant found in a case with an alternate molecular basis for disease
	BP6 Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation
	BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

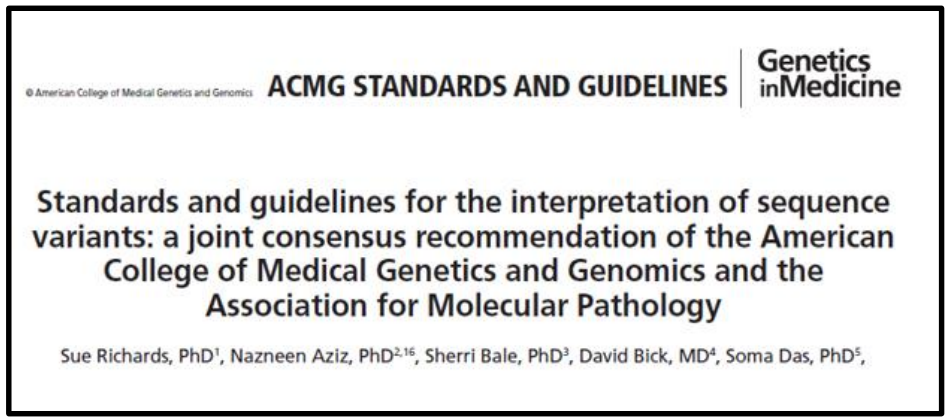

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

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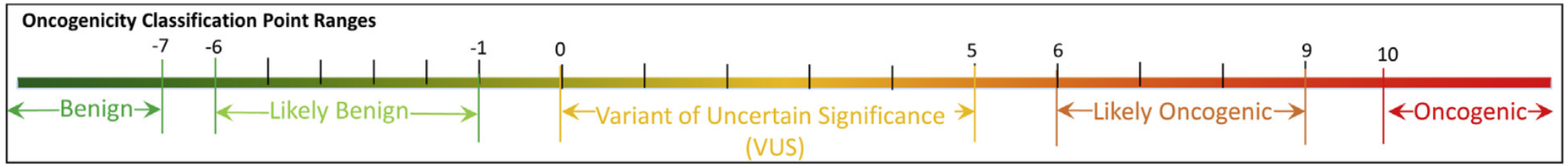
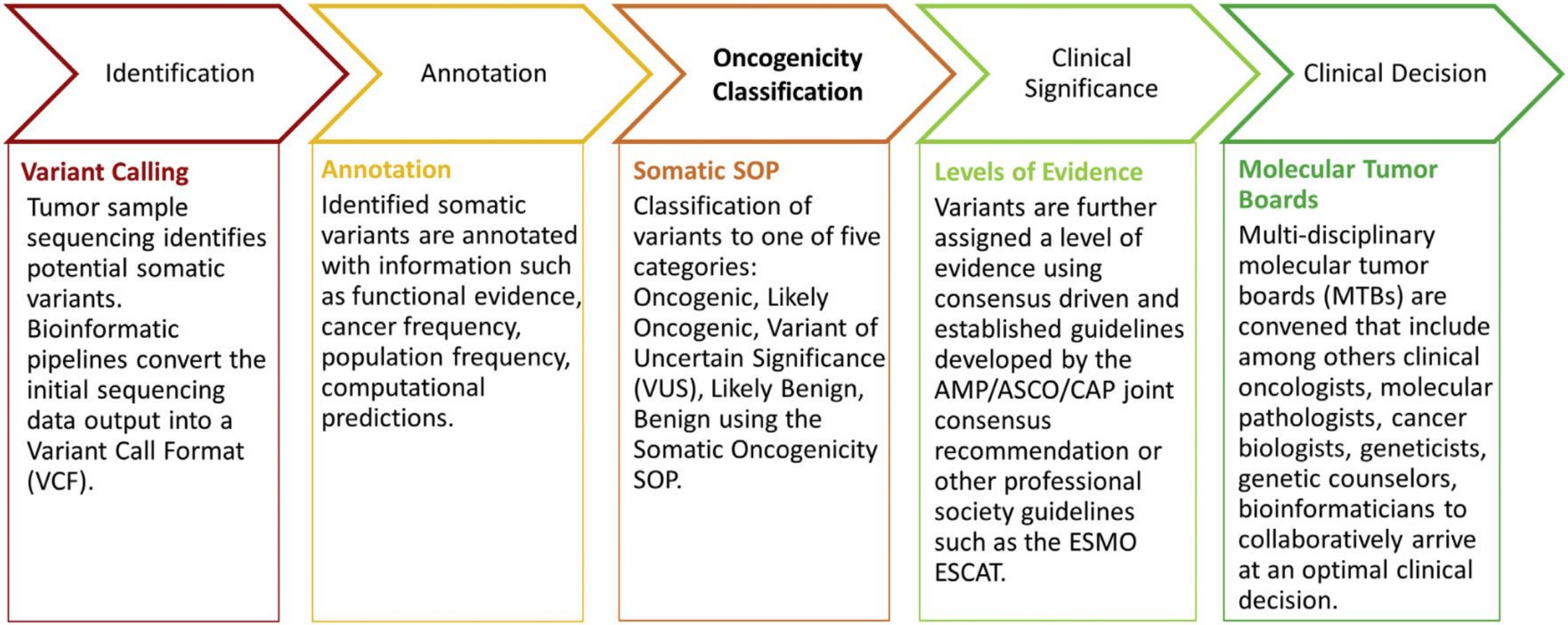
SPECIAL ARTICLE

Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC)



	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			


Identification
Annotation
Oncogenicity Classification
Clinical Significance
Clinical Decision
Variant Calling
Tumor sample sequencing identifies potential somatic variants. Bioinformatic pipelines convert the initial sequencing data output into a Variant Call Format (VCF).
Annotation
Identified somatic variants are annotated with information such as functional evidence, cancer frequency, population frequency, computational predictions.
Somatic SOP
Classification of variants to one of five categories: Oncogenic, Likely Oncogenic, Variant of Uncertain Significance (VUS), Likely Benign, Benign using the Somatic Oncogenicity SOP.
Levels of Evidence
Variants are further assigned a level of evidence using consensus driven and established guidelines developed by the AMP/ASCO/CAP joint consensus recommendation or other professional society guidelines such as the ESMO ESCAT.
Molecular Tumor Boards
Multi-disciplinary molecular tumor boards (MTBs) are convened that include among others clinical oncologists, molecular pathologists, cancer biologists, geneticists, genetic counselors, bioinformaticians to collaboratively arrive at an optimal clinical decision.

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence

FDA-approved therapy

Included in professional guidelines

Level B Evidence

Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence

FDA-approved therapies for different tumor types or investigational therapies

Multiple small published studies with some consensus

Level D Evidence

Preclinical trials or a few case reports without consensus

Tier III: Variants of Unknown Clinical Significance

Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases

No convincing published evidence of cancer association

Tier IV: Benign or Likely Benign Variants

Observed at significant allele frequency in the general or specific subpopulation databases

No existing published evidence of cancer association

Table 2. Three Scales for Classifying Molecular Alterations in Cancer

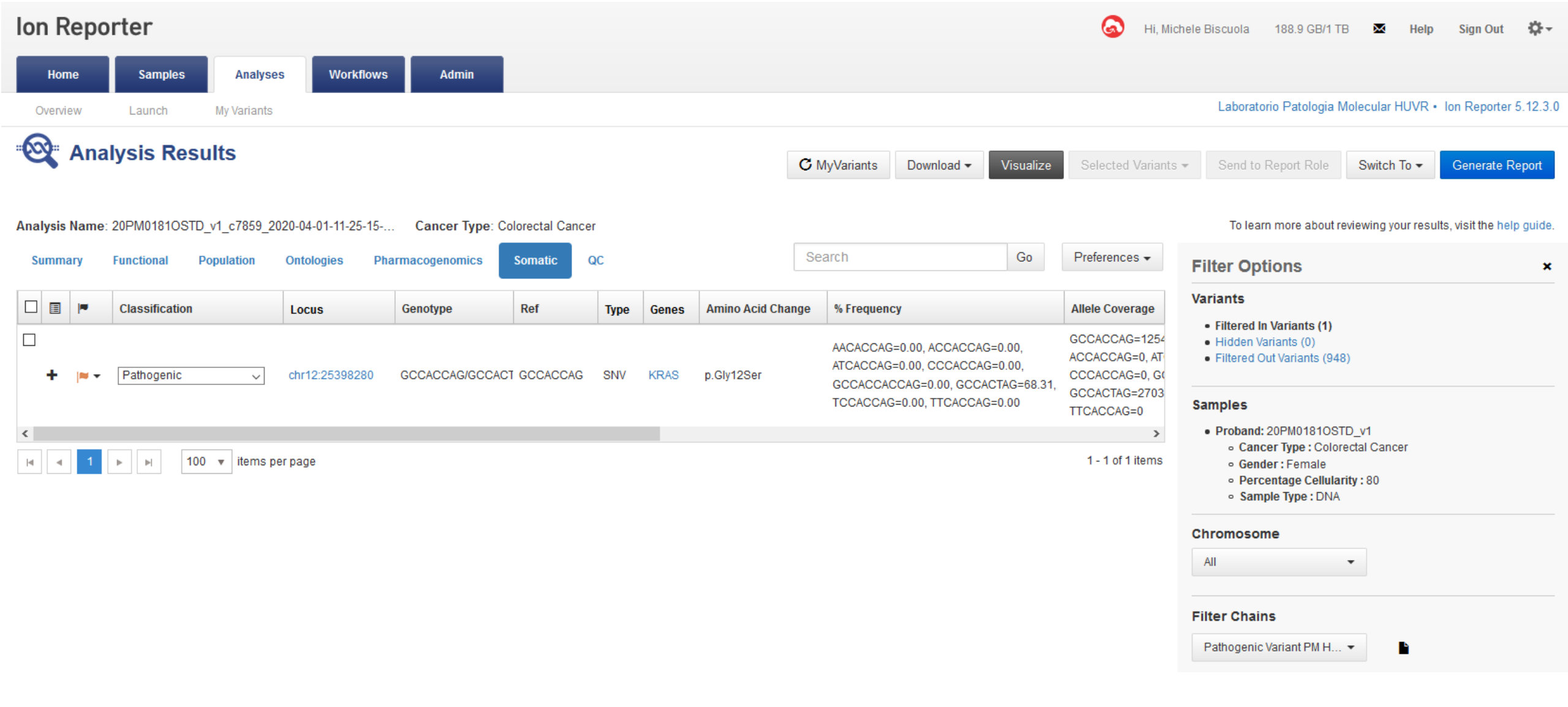
ESCAT		JCR		OncoKB	
ESCAT I	Ready for routine use A: Prospective randomized trials B: Prospective nonrandomized trials C: Basket trials and trials across tumor types	Tier 1	Strong clinical impact A: FDA approved therapy/professional guidelines B: Well-powered studies with expert consensus	Level of actionability	
				Level 1	FDA approval FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication
ESCAT II	Investigational therapeutic options A: Retrospective studies B: Retrospective studies trial; endpoints not currently available	Tier 2	Potential clinical impact C: FDA approval in different tumors/inclusion in clinical trial/multiple small studies with some consensus D: Preclinical trials/case reports	Level 2	Standard care biomarker predictive of response to approved drug[a] A: In this indication B: In another indication
ESCAT III	Hypothetical targets A: As ESCAT I but in other tumor types B: Alteration with predicted impact in same pathways			Level 3	Compelling clinical evidence supports the biomarker as being predictive of response to a drug A: In this indication B: In another indication
ESCAT IV	Preclinical evidences A: *In vivo* or *in vitro* evidences B: *In silico* evidences			Level 4	Compelling biological evidence supports the biomarker as being predictive of response to a drug
ESCAT V	Combination development Objective response but not improved outcomes	Tier 3	Unknown clinical significance		
ESCAT X	Benign variants	Tier 4	Benign variants		
				Level of resistance	
				Level R1	Standard care biomarker predictive of resistance to an FDA-approved drug in this indication
				Level R2	Compelling clinical evidence supports the biomarker as being predictive of resistance to a drug
				Level R3	Compelling biological evidence supports the biomarker as being predictive of resistance to a drug

Molecular Tumor Boards in Clinical Practice

Claudio Luchini,[1,*] Rita T. Lawlor,[2] Michele Milella,[3,*] and Aldo Scarpa[1,2]

[a]Approved drug for OncoKB level 2 is not limited to FDA-approval but also refers to drugs approved by the National Comprehensive Cancer Network (NCCN) or by another expert panel.

SNV


Ion Reporter
Hi, Michele Biscuola
188.9 GB/1 TB
Help
Sign Out
Home
Samples
Analyses
Workflows
Admin
Overview
Launch
My Variants
Laboratorio Patologia Molecular HUVR • Ion Reporter 5.12.3.0
Analysis Results
MyVariants
Download
Visualize
Selected Variants
Send to Report Role
Switch To
Generate Report
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Cancer Type: Colorectal Cancer
To learn more about reviewing your results, visit the help guide.
Summary
Functional
Population
Ontologies
Pharmacogenomics
Somatic
QC
Search
Go
Preferences
Classification
Locus
Genotype
Ref
Type
Genes
Amino Acid Change
% Frequency
Allele Coverage
Pathogenic
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SNV
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GCCACCAG=1254 ACCACCAG=0, AT CCCACCAG=0, G GCCACTAG=2703 TTCACCAG=0
1
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items per page
1 - 1 of 1 items
Filter Options
Variants
Filtered In Variants (1)
Hidden Variants (0)
Filtered Out Variants (948)
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Percentage Cellularity : 80
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All
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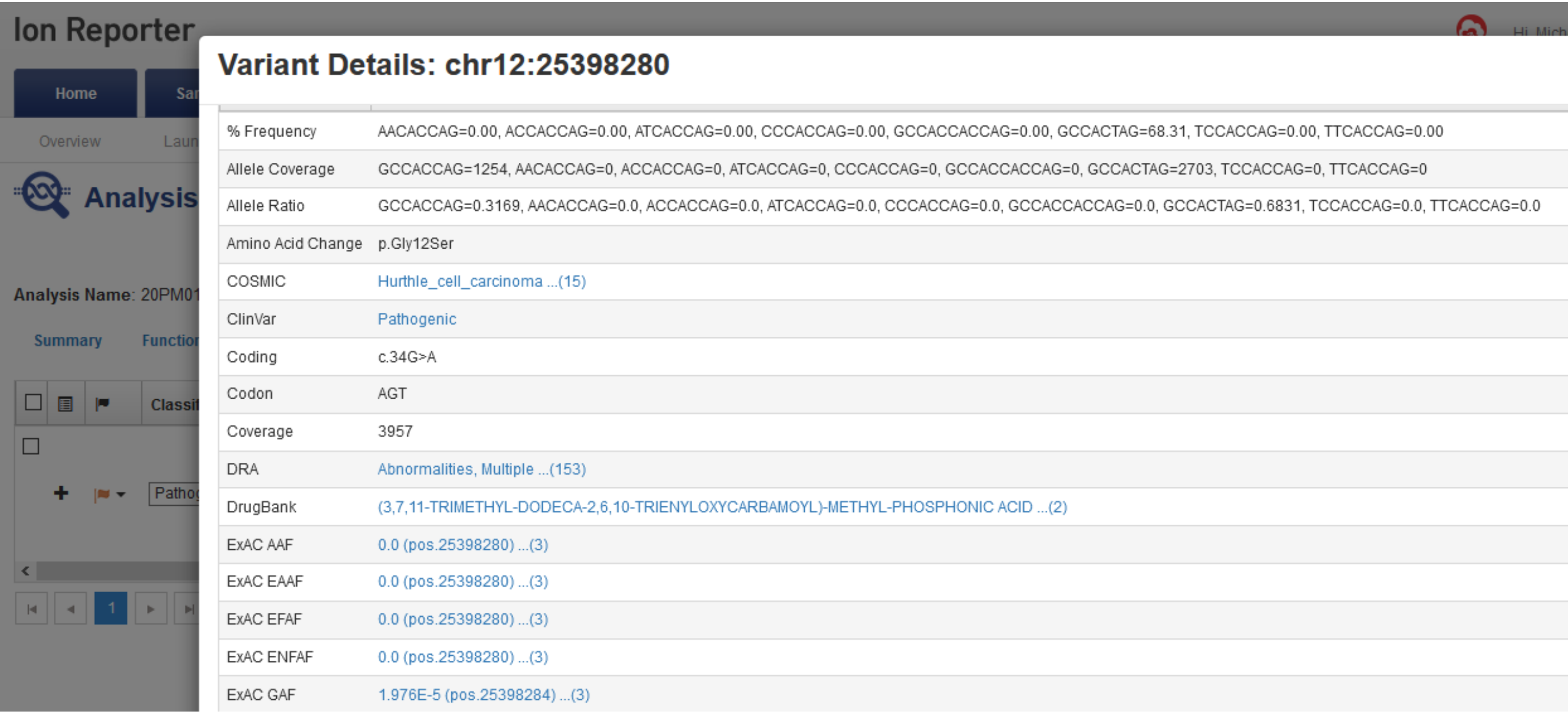
Pathogenic Variant PM H...

SNV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Home
Overview
Analysis
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Amino Acid Change p.Gly12Ser
COSMIC Hurthle_cell_carcinoma ...(15)
ClinVar Pathogenic
Coding c.34G>A
Codon AGT
Coverage 3957
DRA Abnormalities, Multiple ...(153)
DrugBank (3,7,11-TRIMETHYL-DODECA-2,6,10-TRIENYLOXYCARBAMOYL)-METHYL-PHOSPHONIC ACID ...(2)
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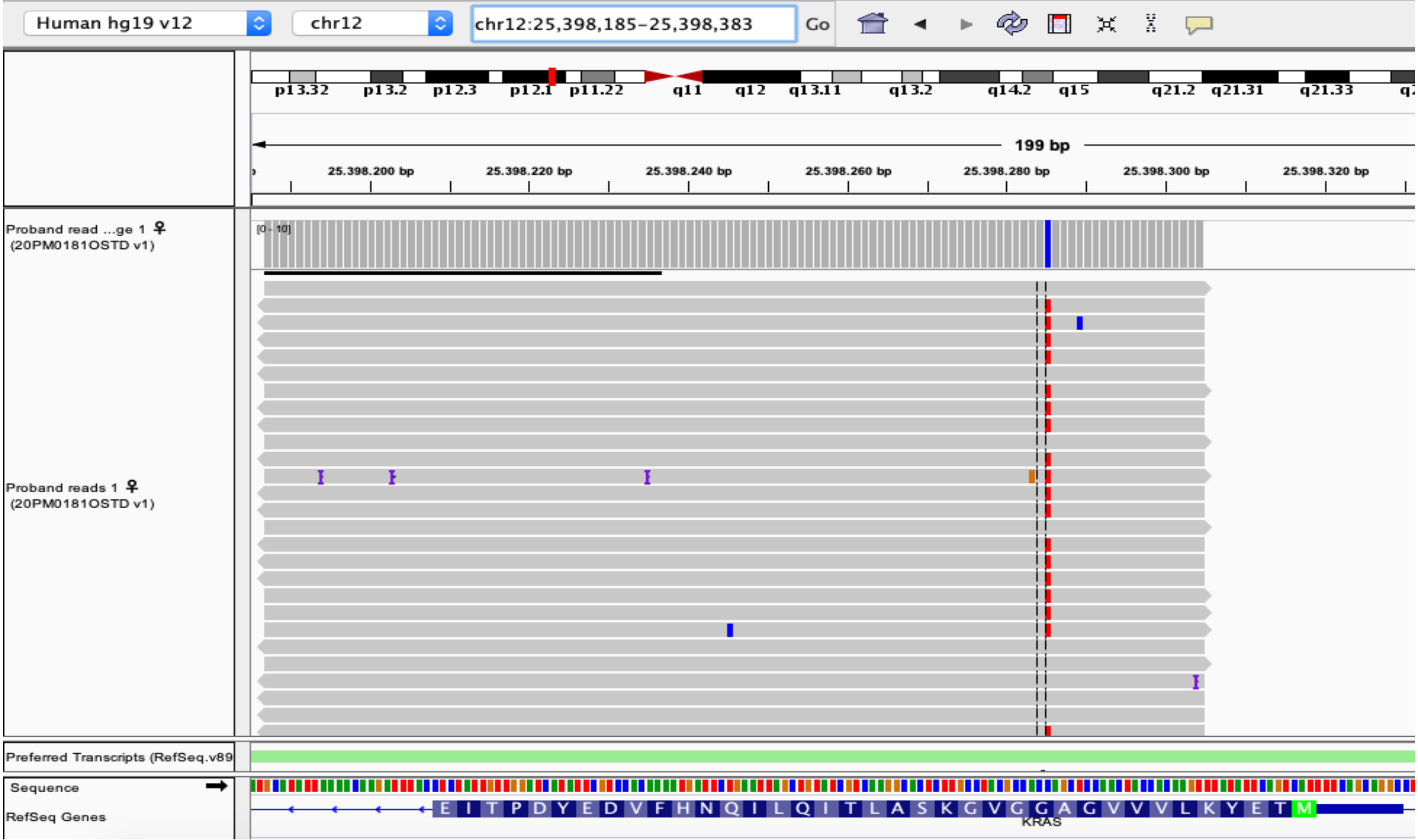
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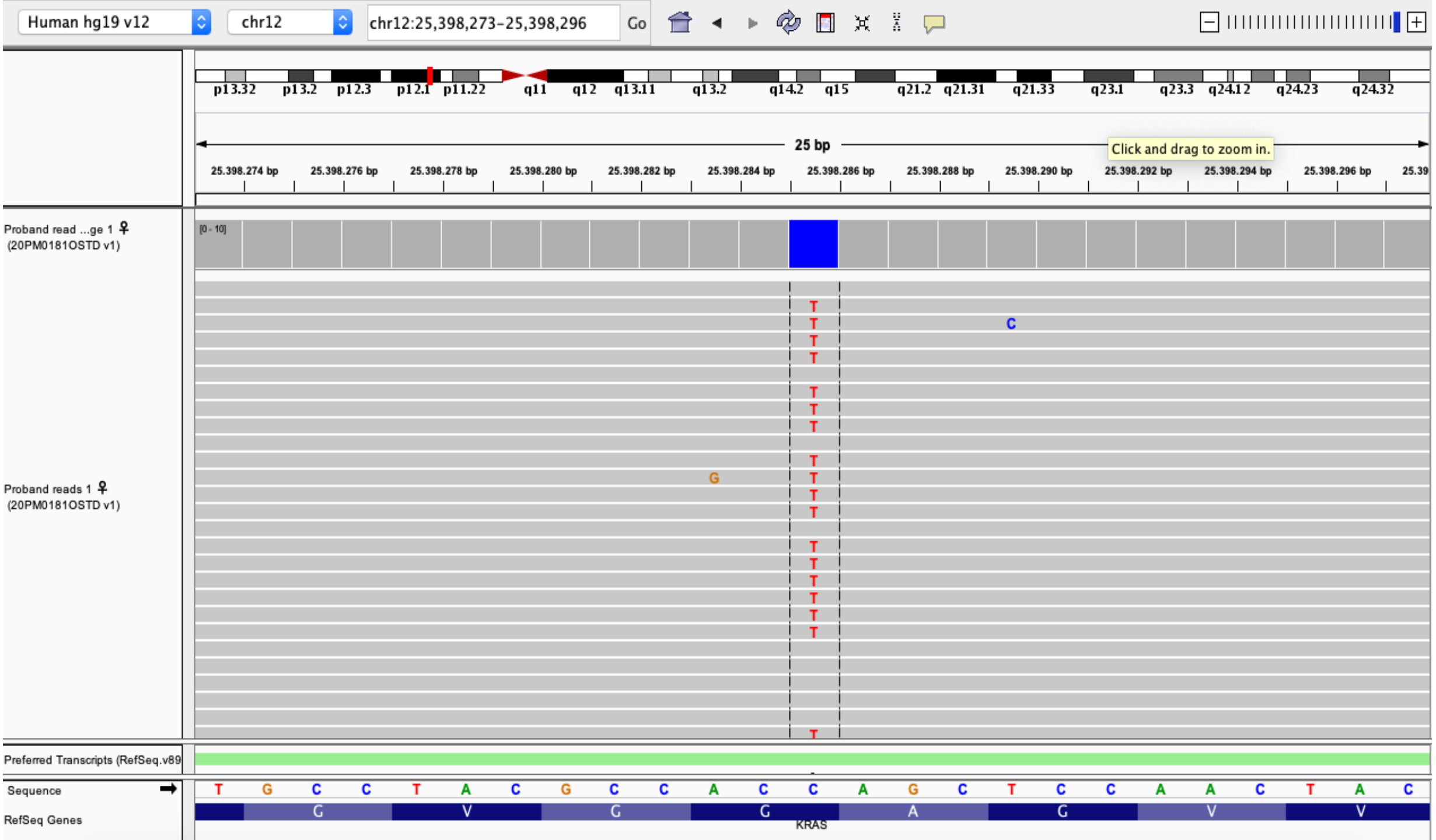
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(20PM0181OSTD v1)
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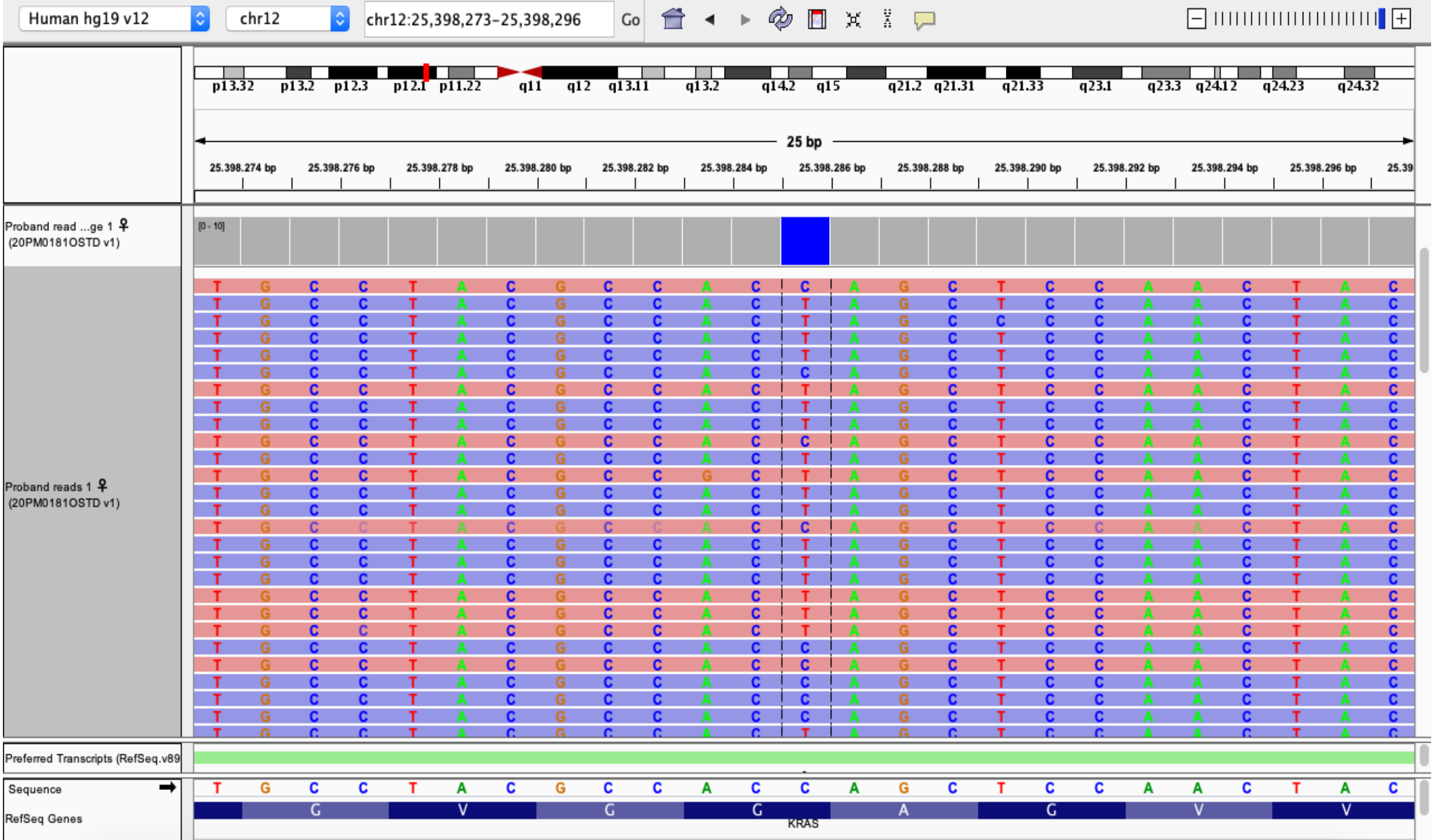
G V G G A G V V
KRAS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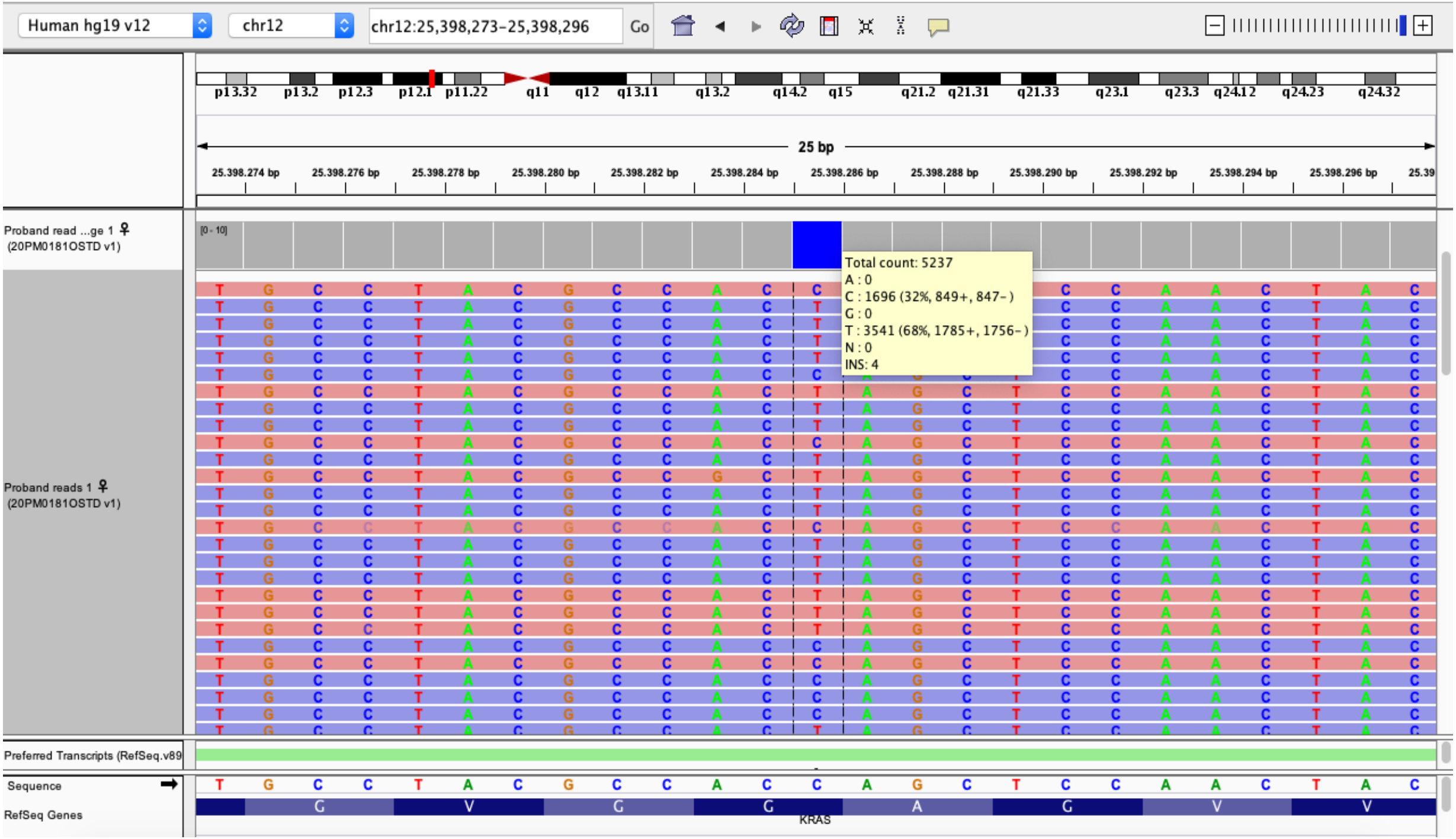
KRAS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Go
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25 bp
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Proband read ...ge 1 ♀
(20PM0181OSTD v1)
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C : 1696 (32%, 849+, 847-)
G : 0
T : 3541 (68%, 1785+, 1756-)
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Preferred Transcripts (RefSeq.v89
Sequence
RefSeq Genes
KRAS

SNV

KRAS G12C

SNV patogénica

Biomarcador predictivo (y diagnóstico)

¿Tiene el mismo significado clínico independientemente de la neoplasia?

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence

FDA-approved therapy

Included in professional guidelines

Level B Evidence

Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence

FDA-approved therapies for different tumor types or investigational therapies

Multiple small published studies with some consensus

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Preclinical trials or a few case reports without consensus

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No convincing published evidence of cancer association

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Observed at significant allele frequency in the general or specific subpopulation databases

No existing published evidence of cancer association

Table 3A. List of genomic alterations level I/II/III according to ESCAT in advanced non-squamous non-small-cell lung cancer (NSCLC)

Gene	Alteration	Prevalence	ESCAT	References
EGFR	Common mutations (*Del19, L858R*)	15% (50%–60% Asian)	IA	Midha A, et al. *Am J Cancer Res.* 2015[26]
	Acquired *T790M* exon 20	60% of *EGFR* mutant	IA	Mok T, et al. *J Clin Oncol.* 2018[27]
	Uncommon *EGFR* mutations (*G719X* in exon	NSCLC	IB	Soria J-C, et al. *N Engl J Med.* 2018[28]
	18, *L861Q* in exon 21, *S768I* in exon 20)	10%	IIB	Ramalingam S, et al. *N Engl J Med.* 2020[29]
	Exon 20 insertions	2%		Mok T, et al. *N Engl J Med.* 2017[30]
				Yang JC-H, et al. *Lancet Oncol.* 2015[31]
				Cho J, et al. *J Thorac Oncol.* 2018[32]
				Cardona A, et al. *Lung Cancer.* 2018[33]
				Heymach J, et al. *J Thorac Oncol.* 2018[34]
ALK	Fusions (mutations as mechanism of resistance)	5%	IA	Solomon B, et al. *J Clin Oncol.* 2018[35]
				Soria J-C, et al. *Lancet.* 2017[36]
				Peters S, et al. *N Engl J Med.* 2017[37]
				Zhou C, et al. *Ann Oncol.* 2018[38]
				Camidge D, et al. *N Engl J Med.* 2018[39]
MET	Mutations *ex 14 skipping*	3%	IB	Tong J, et al. *Clin Cancer Res.* 2016[40]
				Drilon A, et al. *Nat Med.* 2020[41]
	Focal amplifications (acquired resistance on EGFR TKI in *EGFR*-mutant tumours)	3%	IIB	Camidge D, et al. *J Clin Oncol.* 2018[52]
BRAF^{V600E}	Mutations	2%	IB	Planchard D, et al. *Lancet Oncol.* 2016[42]
				Planchard D, et al. *Lancet Oncol.* 2017[43]
				Planchard D, et al. *J Clin Oncol.* 2017[44]
ROS1	Fusions (mutations as mechanism of resistance)	1%–2%	IB	Shaw A, et al. *N Engl J Med.* 2014[45]
				Shaw A, et al. *Ann Oncol.* 2019[46]
				Drilon A, et al. *Lancet Oncol.* 2020[47]
NTRK	Fusions	0.23%–3%	IC	Drilon A, et al. *N Engl J Med.* 2018[48]
				Hong D, et al. *Lancet Oncol.* 2020[49]
				Doebele RC, et al. *Lancet Oncol.* 2020[50]
RET	Fusions	1%–2%	IC	Drilon A, et. *J Thorac Oncol.* 2019[51]
KRAS^{G12C}	Mutations	12%	IIB	Barlesi F, et al. *Lancet.* 2016[53]
				Fakih M, et al. *J Clin Oncol.* 2019[54]
ERBB2	Hotspot mutations	2%–5%	IIB	Hyman D, et al. *Nature.* 2018[55]
	Amplifications			Wang Y, et al. *Ann Oncol.* 2018[56]
				Tsurutani J, et al. *J Thorac Oncol.* 2018[57]
BRCA 1/2	Mutations	1.2%	IIIA	Balasubramaniam S, et al. *Clin Cancer Res.* 2017[63]
PIK3CA	Hotspot mutations	1.2%–7%	IIIA	Cancer Genome Atlas Research Network. *Nature.* 2014[60]
				Vansteenkiste J, et al. *J Thorac Oncol.* 2015[62]
NRG1	Fusions	1.7%	IIIB	Duruisseaux M, et al. *J Clin Oncol.* 2019[59]

REVIEW

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

F. Mosele[1], J. Remon[2], J. Mateo[3], C. B. Westphalen[4], F. Barlesi[5], M. P. Lolkema[5], N. Normanno[6], A. Scarpa[7], M. Robson[8], F. Meric-Bernstam[9], N. Wagle[10], A. Stenzinger[11], J. Bonastre[12,13], A. Bayle[1,12,13], S. Michiels[12,13], I. Bièche[14], E. Rouleau[15], S. Jezdic[16], J-Y. Douillard[16], J. S. Reis-Filho[17], R. Dienstmann[18] & F. André[1,19,20*]

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Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

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Table 5. List of genomic alterations level I/II/III according to ESCAT in metastatic colorectal cancer (mCRC)

Gene	Alteration	Prevalence	ESCAT	References
KRAS *NRAS*	Mutations (resistance biomarker)	44% 4%	Not applicable	Van Cutsem E, et al. *J Clin Oncol.* 2015[79] Douillard J-Y, et al. *N Engl J Med.* 2013[80] Sorich M, et al. *Ann Oncol.* 2015[81]
BRAF^{V600E}	Mutations	8.5%	IA	https://doi.org/10.1093/annonc/mdw235 Kopetz S, et al. *N Engl J Med.* 2019[82]
	MSI-H	4%—5%	IA	Overman M, et al. *Lancet Oncol.* 2017[83] Le DT, et al. *J Clin Oncol.* 2020[84]
NTRK1	Fusions	0.5%	IC	Demetri G, et al. *Ann Oncol.* 2018[85] Doebele RC, et al. *Lancet Oncol.* 2020[50]
ERBB2	Amplifications	2%	IIB	Meric-Bernstam F, et al. *Lancet Oncol.* 2019[86] Sartore-Bianchi A, et al. *Lancet Oncol.* 2016[87]
PIK3CA	Hotspot mutations	17%	IIIA	Juric D, et al. *J Clin Oncol.* 2018[90]
ATM	Mutations	5%	IIIA	Wang C, et al. *Transl Oncol.* 2017[92] De Bono J, et al. *N Engl J Med.* 2020[93]
MET	Amplifications	1.7%	IIIA	https://clinicaltrials.gov/ct2/show/NCT03592641[94]
AKT1^{E17K}	Mutations	1%	IIIA	Hyman D, et al. *J Clin Oncol.* 2017[76]
	TMB-high in MSS	1%	IIIA	Fabrizio D, et al. *J Gastrointest Oncol.* 2018[89]
RET	Fusions	0.3%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ALK	Fusions	0.2%	IIIA	Yakirevich E, et al. Clin Cancer Res 2016[88]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

Table 8. List of genomic alterations level I/II/III according to ESCAT in advanced pancreatic ductal adenocarcinoma (PDAC)

Gene	Alteration	Prevalence	ESCAT	References
BRCA1/2	Germline mutations	1%—4%	IA	The Cancer Genome Atlas Research Network. *Cancer Cell.* 2017[111] Golan T, et al. *N Engl J Med.* 2019[112]
	Somatic mutations	3%	IIIB	Shroff R, et al. *JCO Precis Oncol.* 2018[113]
	MSI-H	1%—3%	IC	Pihlak R, et al. *Cancers.* 2018[115] Marcus L, et al. *Clin Cancer Res.* 2019[97]
NTRK	Fusions	<1%	IC	Cocco E, et al. *Nat Rev Clin Oncol.* 2018[114] Doebele RC, et al. *Lancet* [illegible][50]
KRAS	Mutations	90%	IIIA	Zeitouni D, et al. *Cancers.* 2016[116]
PIK3CA	Hotspot mutations	[illegible]	[illegible]	[illegible] *Oncotarget.* 2015[117] Payne S, et al. *J Clin Oncol.* 2015[118]
BRAF^{V600E}	Mutations	3%	IIIA	Hyman D, et al. *N Engl J Med.* 2015[119]
MDM2	Amplifications	2%	IIIA	Azmi A, et al. *Eur J Cancer.* 2010[120]
ERBB2	Amplifications/ mutations	1%—2%	IIIA	Waddell N, et al. *Nature.* 2015[121] Harder J, et al. *Br J Cancer.* 2012[122] Hyman D, et al. *Nature.* 2018[55]
NRG1	Fusions	1%	IIIA	Jones M, et al. *Clin Cancer Res.* 2019[123]
ALK	Fusions	<1%	IIIA	Singhi A, et al. *J Natl Compr Canc Netw.* 2017[124]
RET	Fusions	<1%	IIIA	Drilon A, et al. *J Clin Oncol.* 2018[91]
ROS1	Fusions	<1%	IIIA	Pishvaian M, et al. *J Clin Oncol.* 2018[125]

ESCAT, European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high.

SNV

Analysis Name: 20PM0184OSTD_v1_c9917_2020-04-01-11-25-15-... **Cancer Type**: Colorectal Cancer

Summary | Functional | Population | Ontologies | Pharmacogenomics | Somatic | QC

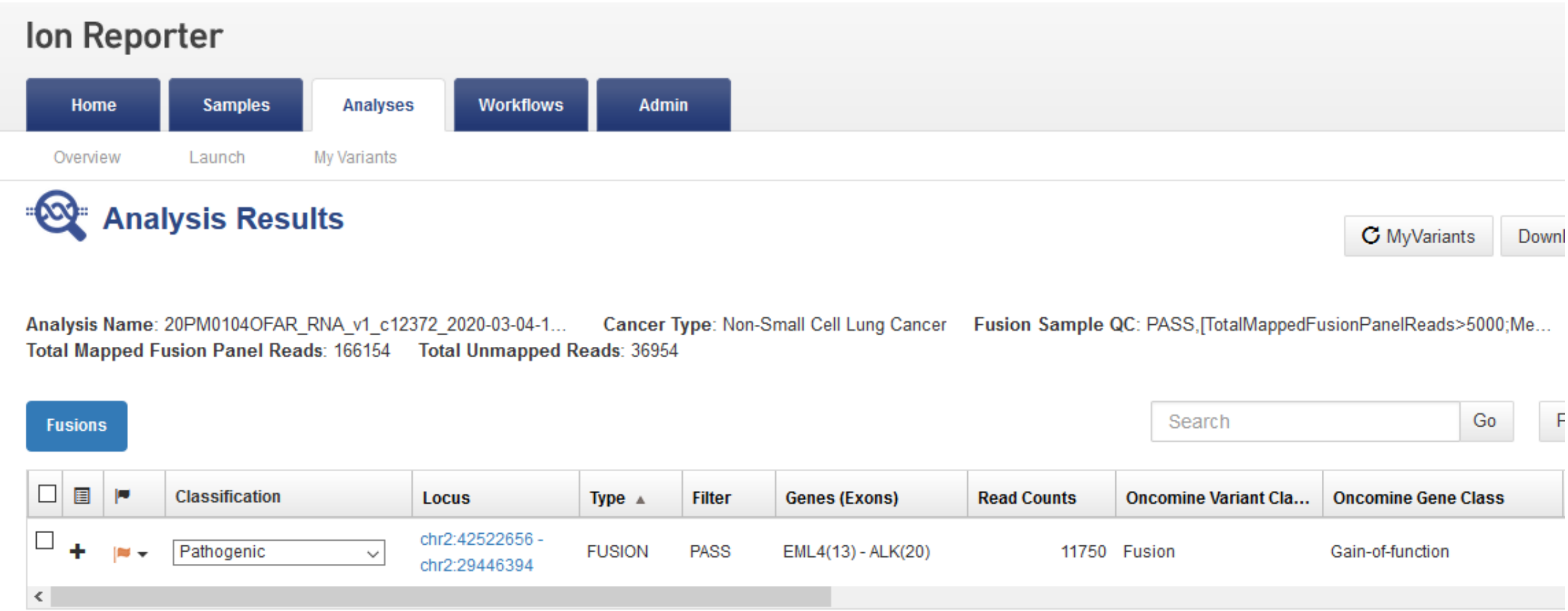
		Classification	Locus	Gen...	Ref	Type	Genes	Amino Acid Change
☐	+	Pathogenic	chr3:178936082	G/A	G	SNV	PIK3CA	p.Glu542Lys
☐	+	Pathogenic	chr17:7578368	CACCAT(	CACCAT(	SNV	TP53	p.His179Tyr

Variant Details: chr3:178936082

Variant Details | Notes

Annotation Sour...	20PM0184OSTD_v1
% Frequency	A=22.06, C=0.00
Allele Coverage	G=3116, A=882, C=0
Allele Ratio	G=0.7794, A=0.2206, C=0.0
Amino Acid Change	p.Glu542Lys
COSMIC	ER-PR-HER-positive_carcinoma ...(89)
ClinVar	Pathogenic/Likely pathogenic
Coding	c.1624G>A
Codon	AAA
Coverage	3998

SV


Ion Reporter
Home
Samples
Analyses
Workflows
Admin
Overview
Launch
My Variants
Analysis Results
MyVariants
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Cancer Type: Non-Small Cell Lung Cancer
Fusion Sample QC: PASS,[TotalMappedFusionPanelReads>5000;Me...
Total Mapped Fusion Panel Reads: 166154
Total Unmapped Reads: 36954
Fusions
Search
Go
Classification
Locus
Type
Filter
Genes (Exons)
Read Counts
Oncomine Variant Cla...
Oncomine Gene Class
Pathogenic
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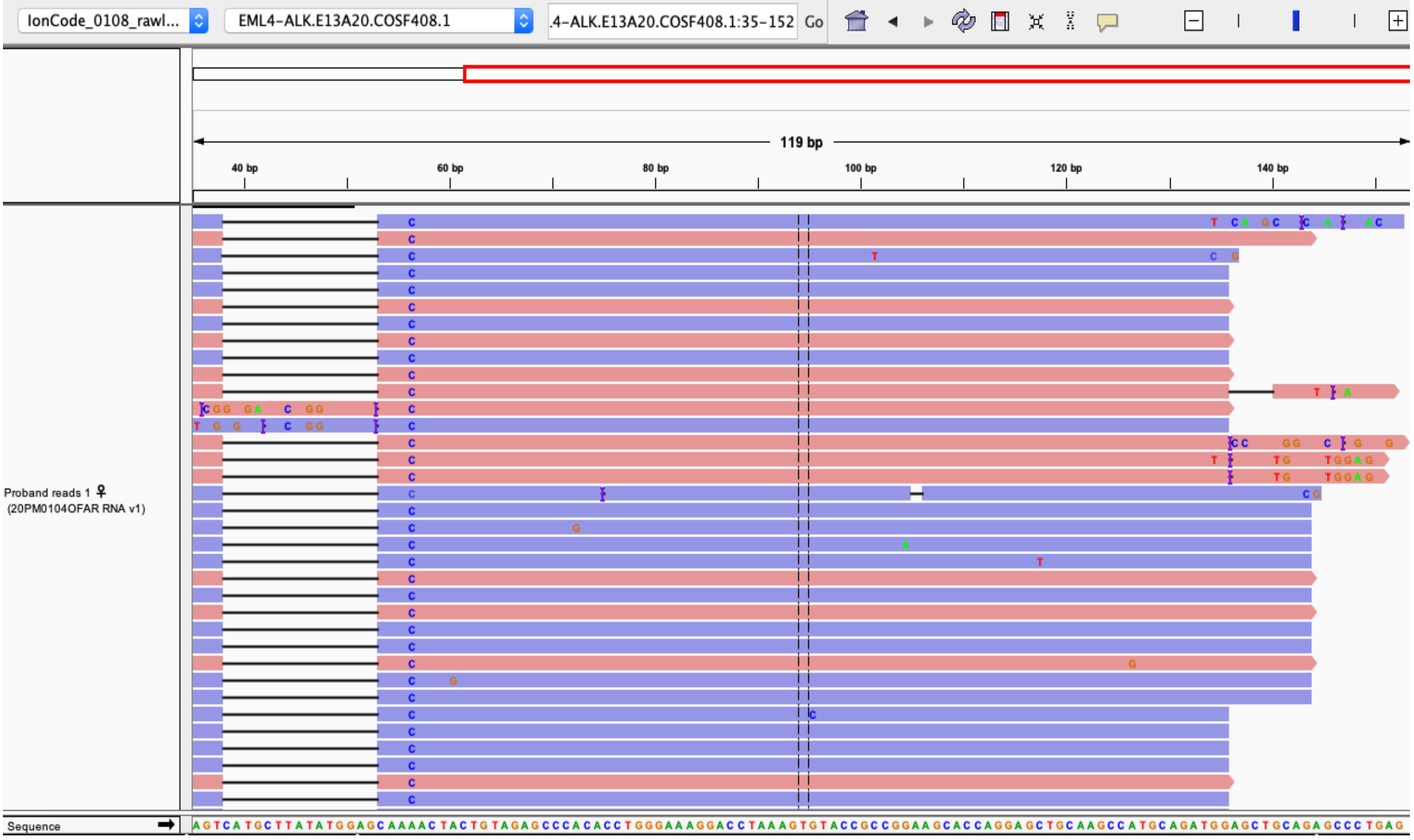
FUSION
PASS
EML4(13) - ALK(20)
11750
Fusion
Gain-of-function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40 bp
60 bp
80 bp
100 bp
120 bp
140 bp
Proband reads 1 ♀
(20PM0104OFAR RNA v1)
Sequence
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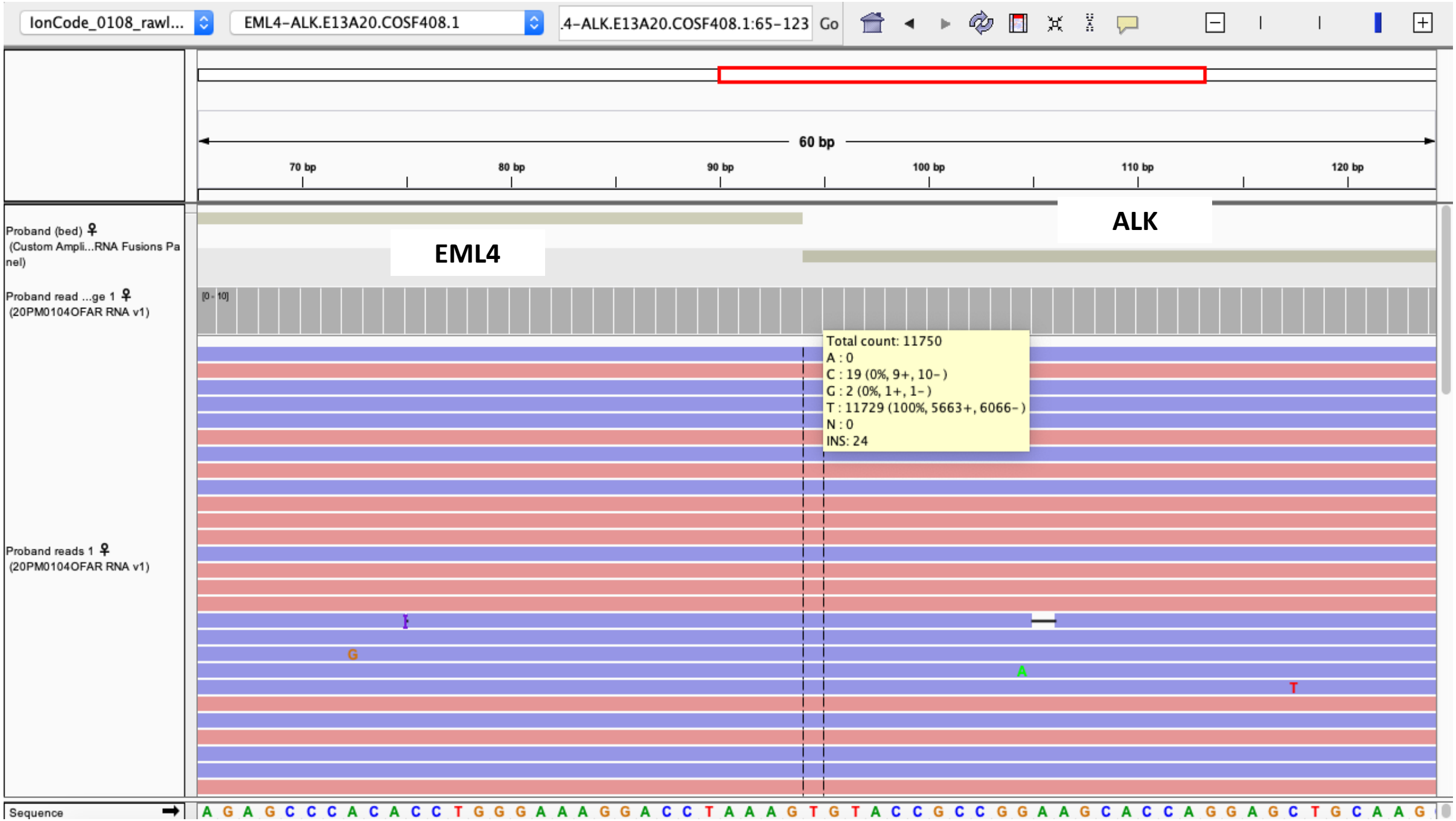
IonCode_0108_rawl...
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.4-ALK.E13A20.COSF408.1:65-123
Go
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70 bp
80 bp
90 bp
100 bp
110 bp
120 bp
Proband (bed) ♀
(Custom Ampli...RNA Fusions Panel)
EML4
ALK
Proband read ...ge 1 ♀
(20PM0104OFAR RNA v1)
[0 - 10]
Total count: 11750
A : 0
C : 19 (0%, 9+, 10-)
G : 2 (0%, 1+, 1-)
T : 11729 (100%, 5663+, 6066-)
N : 0
INS: 24
Proband reads 1 ♀
(20PM0104OFAR RNA v1)
G
A
T
Sequence
A G A G C C C A C A C C T G G G A A A G G A C C T A A A G T G T A C C G C C G G A A G C A C C A G G A G C T G C A A G

Variantes

Analysis Results

MyVariants | Download | Visualize | Selected Variants

Analysis Name: 20PM0136OFAD_v1_c6741_2020-03-17-18-56-07-... **Cancer Type**: Thyroid Cancer **MAPD**: 0.532 **Tumor Cellularity Percentage**: 100% (manual)

Summary | Oncomine | Functional | Population | Ontologies | Pharmacogenomics | Somatic | QC

Search | Go | Preferences

Classification	Locus	Genotype	Ref	Type	Genes	% Frequency	Amino Acid Change	Allele Coverage
Pathogenic	chr1:115252204	C/T	C	SNV	NRAS	3.97	p.Ala146Thr	C=1597, T=66
Likely Pathogenic	chr3:178916891	G/A	G	SNV	PIK3CA	A=3.85, T=0.10	p.Arg93Gln	G=1921, A=77, T=2
Likely Pathogenic	chr3:178922364	G/A	G	SNV	PIK3CA	A=7.18, T=0.00	p.Cys378Tyr	G=789, A=61, T=0
Pathogenic	chr3:178952084	C/T	C	SNV	PIK3CA	4.41	p.His1047Tyr	C=1257, T=58
Pathogenic	chr7:55249071	C/T	C	SNV	EGFR ...(2)	16.62	p.Thr790Met	C=291, T=58
Unknown	chr10:43617397	C/T	C	SNV	RET	19.11	p.Arg912Trp	C=398, T=94
Pathogenic	chr12:25380283	C/T	C	SNV	KRAS	A=0.00, T=5.30	p.Ala59Thr	C=1894, A=0, T=106
VUS	chr14:105246550	T/G	T	SNV	AKT1	6.20	p.Glu17Ala	T=242, G=16

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