GALEAS™ Tumor

A clinically validated NGS panel with variant calling software designed in parallel, to support in house comprehensive genomic profiling of solid tumors.

Highlights

Enhanced clinically relevant content

Expertly curated content aligned with UK NHS national genomic test directory, NCCN, FDA and ESMO guidelines. GALEAS Tumor profiles key clinically relevant biomarkers across 519 genes and provides TMB and MSI scores. Content includes 64 pharmacogenomic SNPs, hereditary and pediatric cancer content, HLA profiling for solid tumors, structural variants and enhanced CNV coverage.

Detect key current immuno-oncology biomarkers: microsatellite instability (MSI) and tumor mutational burden (TMB)

GALEAS Tumor has been designed with the analysis of both TMB and MSI in mind, delivering a combined tumor genomic instability measurement that can be used to predict a positive response to immunotherapy treatment.

Optimized target enrichment system

Developed for, and validated on, FFPE to allow genomic analysis and combined TMB/MSI profiling in either primary or metastatic biopsies.

Consolidated workflow for all variant types

Validate and run one streamlined hybridization and capture workflow for all relevant DNA variants implicated in solid tumor cancers.

Supported by GALEAS Analysis Software

Developed in parallel with the panel, the cloud-based GALEAS Tumor pipeline is easily implemented into any laboratory, and provides a rapid and accurate solution for calling SNVs, INDELs, SVs, CNVs, TMB and MSI.

Introduction

Cancer is the second most frequent cause of death worldwide.[1] Numerous types and subtypes of cancer exist, and there is no single pathway responsible for initiating disease onset. Instead, cancers are driven by a myriad of genomic alterations, and their differing combinations impact cancer initiation, development, and response to treatment.[2]

Genomic profiling and use of biomarkers including MSI status or TMB scores can inform scientists and clinicians about tumor genomic profiles and help direct therapeutic strategies.[2] Therefore, it is vital that comprehensive genomic profiling delivers clinically relevant information, in an appropriate time frame to ensure patient access to the most appropriate treatment.

GALEAS Tumor design

GALEAS Tumor is a next generation sequencing (NGS) solution that covers common driver mutations including SNVs, INDELs, CNVs and selected fusions in 519 genes. The solution supports the analysis of immuno-oncology biomarkers including TMB and MSI. Whilst exon focused, the design covers key intronic and promoter regions with the addition of a CNV backbone to support copy number calling across the genome. It is a comprehensive solution that allows the profiling and accurate identification of variants associated with cancer in a single workflow.

The design has been expertly curated by Nonacus to include:

- Common driver mutations including SNVs, CNVs and INDELs in 519 genes
- CNV backbone enabling enhanced CNV calling to a ≤ 1 Mb resolution
- Enhanced coverage of the 1p/19q co-deletion associated with Glioma
- MSI and TMB scoring
- 10 Fusion/Structural rearrangements: ALK, BRAF, EGFR, FGFR2, FGFR3, NTRK1, NTRK2, RET, ROS1, TMPRSS2
- Sample identity tracking SNPs
- 64 Pharmacogenomics (oncology) SNPs
- HLA design relevant for solid tumors

Table 1: GALEAS Tumor technical summary

Parameters	Specification
Enrichment method	Hybridization and Capture
Number of genes	519
Capture panel size	3.74Mb
Sequencing platform	Illumina
Targets	Genes associated with common cancers
Variant types	SNVs, SVs, CNVs and INDELs
Input DNA requirements	10-200ng
Sample types	gDNA from FFPE, frozen tissue or blood
Multiplexing guidance for sequencing	25 million reads (5 Gb) per sample using 2x100 bp PE sequencing to achieve 500x average depth of coverage

GALEAS Tumor validation

The GALEAS Tumor workflow has been validated on reference samples from FFPE and gDNA, assessing SNVs, INDELs, CNVs. Further validation was performed on a clinical cohort consisting of 50 FFPE colorectal cancer (CRC) samples and 50 FFPE healthy donor samples.

Confident calling of SNV and INDEL variants

The efficacy of the GALEAS Tumor workflow was assessed using FFPE reference material containing 23 SNVs and INDELs that had previously been confirmed by ddPCR. A strong correlation between NGS- and ddPCR- determined VAFs were observed with a mean depth of 500x (R^2 = 0.99).

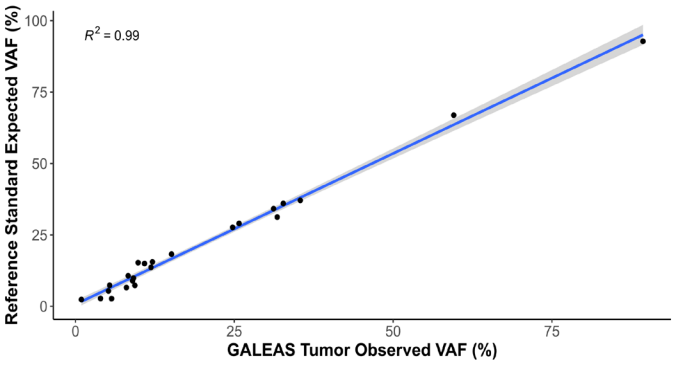


Figure 1: SNV and INDEL recall rate for alterations in reference material from FFPE.

Variant calling on primary tumors

GALEAS Tumor showed 100% recall/precision when comparing somatic variants with orthogonal data* in 50 CRC samples.

*(*orthogonal data available for BRAF, KRAS and NRAS)*

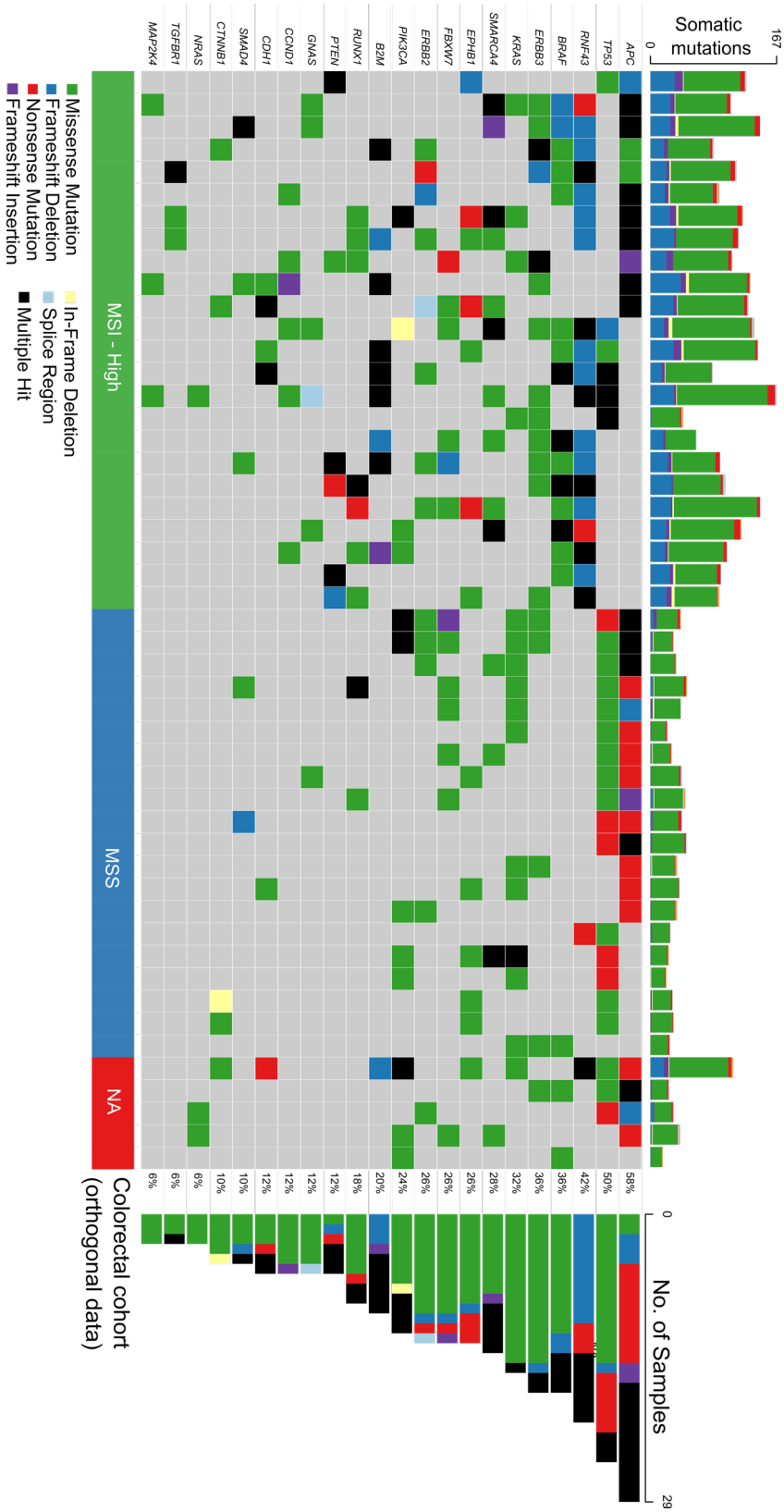


Figure 2: Oncoplot from 50 CRC FFPE cohort highlighting detection of somatic mutations in genes with known cancer hotspots to demonstrate the overall performance of GALEAS Tumor.

Confident calling of copy number variants

GALEAS Tumor has been designed with a copy number backbone enabling enhanced CNV calling to a >1 Mb resolution

Comparison of GALEAS Tumor CNV backbone data with shallow whole genome sequencing (sWGS) demonstrates a strong correlation between the profiles.

To evaluate the sensitivity of CNV genotyping, samples with varying copy numbers were assessed using GALEAS Tumor. The three samples assessed had known copy number variations in EGFR and MET that consist of 3, 6 and 12 copies.

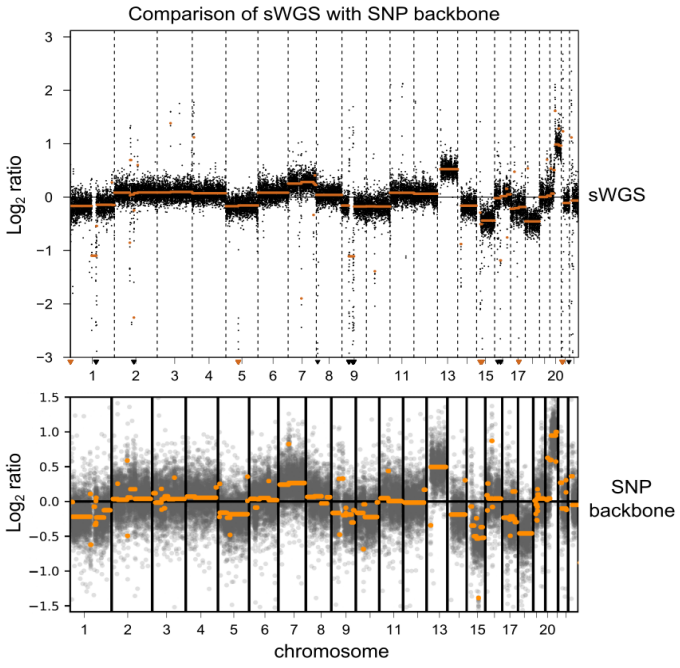


Figure 3: Comparison of GALEAS Tumor SNP backbone data with sWGS. The data shown was obtained from a representative colorectal cancer sample and demonstrates the similarity between the CNV profile obtained from shallow whole genome sequencing (sWGS), with the SNP backbone obtained using GALEAS Tumor.

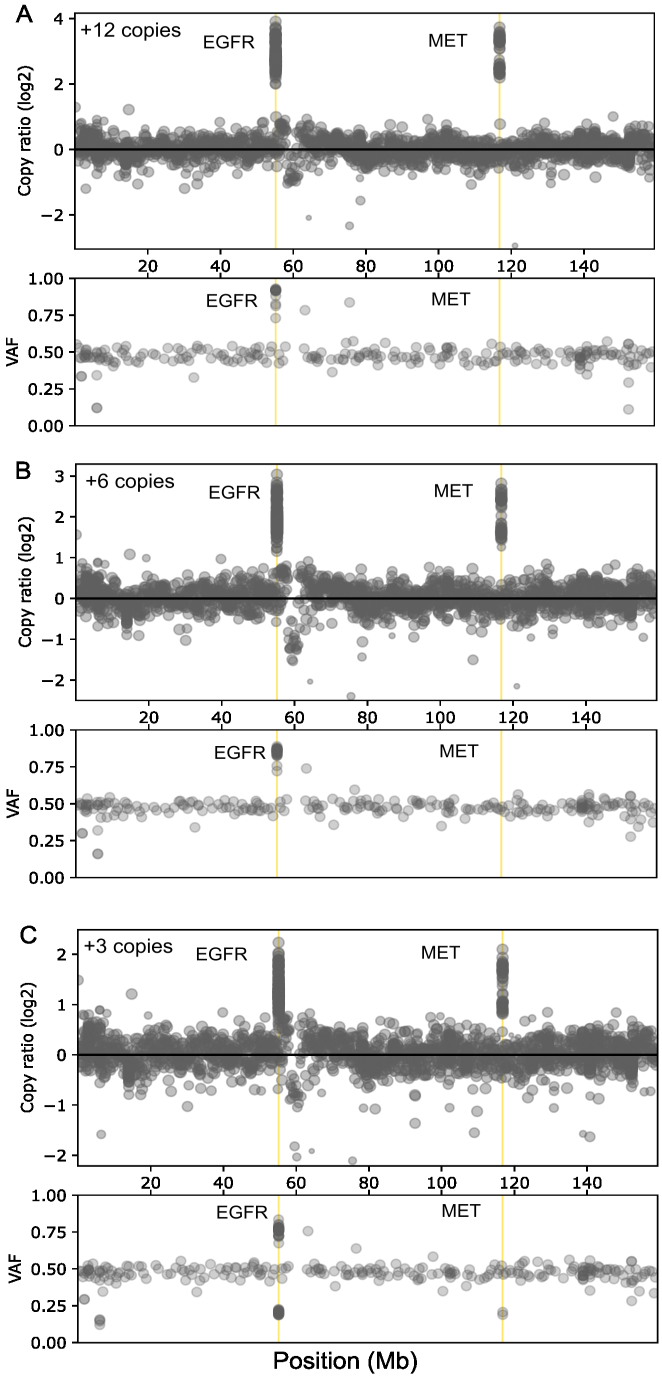


Figure 4: Validating gene level CNV calls with a CNV Lung and Brain Mix reference standard at (A) 12, (B) 6, and (C) 3 copies. Genes highlighted here are EGFR and MET.

Microsatellite instability (MSI) scoring

GALEAS Tumor enables comprehensive detection of MSI. GALEAS MSI scores from control reference material, normal cancer free and colorectal cancer (CRC) FFPE samples were compared to their known MSI status. 100% of MSS CRCs and all normal FFPE samples were confirmed as MSS and normal respectively by the GALEAS analysis software. 23/24 MSI-High CRC FFPE samples were confirmed as MSI-H.

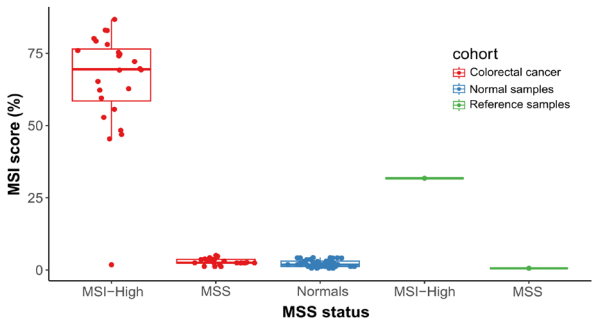


Figure 5.: Comparisons of GALEAS Tumor MSI scores with known MSI status from CRC primary tumor samples (MSS-High), healthy individuals (MSS) and reference standards.

Tumor mutational burden (TMB)

TMB is a key immuno-oncology biomarker across multiple cancer types and has been shown to correlate strongly with MSI status in colorectal cancer.[3, 4] A strong correlation was observed between the GALEAS Tumor derived TMB scores for a CRC cohort (Median TMB 28.24, log2 TMB 1.45) and corresponding sample MSI status (Figure 6).

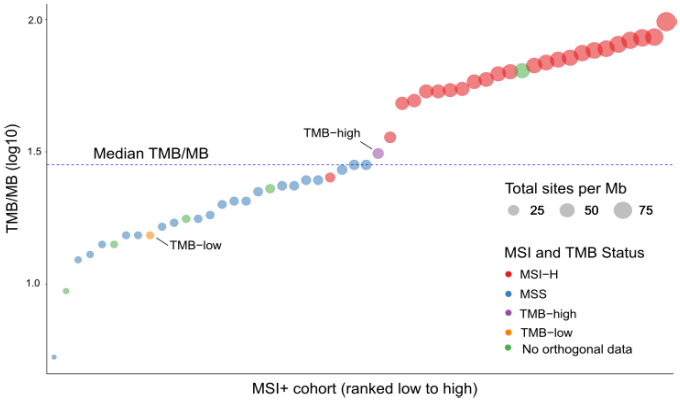


Figure 6: GALEAS Tumor TMB scores compared with MSI status across TMB-low and TMB-high reference standards as well as 50 CRC samples.

High on-target rates and excellent uniformity of coverage delivers more efficient sequencing

The GALEAS Tumor design delivers a high percentage of on-target reads, more uniform coverage and enhanced coverage of key clinically relevant genes. Exceptional technical performance delivers high recall and precision across more variants.

Performance specifications

Table 2: GALEAS Tumor sequencing performance metrics

Key quality indicator	GALEAS Tumor
Number of genes	519
Capture panel size	3.74 Mb
Gb required for mean 500x coverage (2x100 bp PE)	5 Gb
Percentage coverage >250x	98%
Percentage on or near bait	71%
Percentage duplication	9%
SNV recall	100%
INDEL recall	100%

*NOTE: Performance metrics derived from NextSeq 2000 sequencing data

GALEAS analysis software

The GALEAS analysis software delivers on the panel capability. Developed in parallel with the panel, the software is easily integrated into routine laboratory use and provides a robust, intuitive and reliable bioinformatics solution.

Obtaining a precise and reproducible TMB value at low mutation levels can be challenging with smaller panels; GALEAS Tumor combines comprehensive genomic content with validated bioinformatics to provide accurate TMB scoring as well as MSI status indication.

In addition, the GALEAS analysis software provides an easy to use method of uploading batches of FASTQ files and downloading the results in just a few steps.

Streamlined, simple, automatable workflow

The GALEAS Tumor workflow detects all variant types including SNVs, CNVs and INDELs as well as TMB and MSI across 519 genes in a single NGS enrichment. Simplified analysis and reduced costs make this targeted panel an attractive alternative to tumor whole exome sequencing (WES) for routine use. In addition to maximising diagnostic yield, GALEAS Tumor simplifies laboratory workflows helping reduce operating costs.

The workflow is simple and easy, requires as little as 10 ng of DNA and takes less than 10 hours, with less than two hours hands-on time. It is designed with multiple stop points to provide flexibility within laboratory processing.

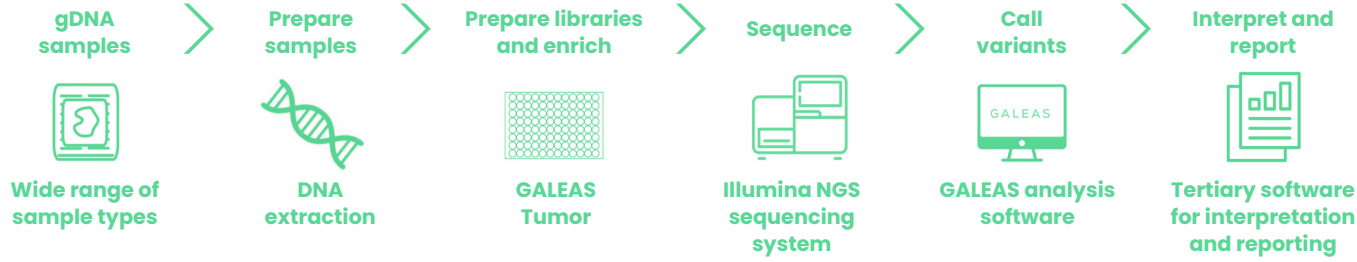
Library preparation can be run manually or automated (up to 96 samples in a single batch). Indexes are available for up to 384 samples to facilitate high throughput laboratories, allow for flexible batch sizes

Summary

GALEAS Tumor provides an expertly curated, clinically validated, comprehensive NGS solution for the analysis of SNVs, CNVs and INDELs as well as TMB and MSI across 519 genes in a single NGS workflow.

The enhanced probe design, comprehensive gene coverage and high uniformity of coverage allows the accurate and sensitive detection of SNVs, INDELs, SVs and CNVs. Combining this with the GALEAS analysis software solution provides a simple and easy sample to analysis workflow. GALEAS Tumor provides a highly efficient, targeted sequencing and analysis solution to allow the detection of clinically relevant DNA variants.

Workflow overview diagram



Learn more

To learn more about GALEAS Tumor and to download the protocols, application notes and white papers please visit: **www.nonacus.com**.

References

1. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. *Nature genetics.* 2013;10):1127-33.

2. The ICGC/TCGA PanCancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. *Nature.* 2020: 82-93.

3. Endris V, Buchhalter I, Allgäuer M, Rempel E, Lier A, Volckmar AL, et al. Measurement of tumor mutational burden (TMB) in routine molecular diagnostics: in silico and real-life analysis of three larger gene panels. *International journal of cancer.* 2019;144(9):2303-12.

4. Schrock AB, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Annals of Oncology.* 2019;30(7):1096-103.

Ordering information

Ordering information	Pack size	Catalog number	Description
GALEAS™ Tumor Frag A (96 samples)	96	NGS_GAL_TCP_FR_96_A	Includes adaptor plate A (1-96 indexes) and complimentary analysis of FASTQ files using GALEAS analysis software for up to 96 samples*
GALEAS™ Tumor Frag B (96 samples)	96	NGS_GAL_TCP_FR_96_B	Includes adaptor plate B (97-192 indexes) and complimentary analysis of FASTQ files using GALEAS analysis software for up to 96 samples*
GALEAS™ Tumor Frag C (96 samples)	96	NGS_GAL_TCP_FR_96_C	Includes adaptor plate C (193-288 indexes) and complimentary analysis of FASTQ files using GALEAS analysis software for up to 96 samples*
GALEAS™ Tumor Frag D (96 samples)	96	NGS_GAL_TCP_FR_96_D	Includes adaptor plate D (289-384 indexes) and complimentary analysis of FASTQ files using GALEAS analysis software for up to 96 samples*
GALEAS™ Tumor Frag (16 samples)	16	NGS_GAL_TCP_FR_16	Includes adaptor plate (1-16 indexes) and complimentary analysis of FASTQ files using GALEAS analysis software for up to 96 samples*

*NOTE: Further charges may apply for reanalysis or reprocessing of FASTQ files, or storage beyond the data retention policy set out in the Terms and Conditions.

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GALEAS Tumor gene list

ABL1	AXL	CCND1	ELOC	FANCD2	FLT3	H1-2	INHBA	LATS1	MLLT3	NPM1	PIK3C2G	PRKN	RICTOR	SMARCD1	TCF7L2	XRCC2
ABL2	B2M	CCND2	EML4	FANCE	FLT4	H2BC5	INPP4A	LATS2	MN1	NR4A3	PIK3C3	PTCH1	RTI1	SMARCE1	TENT5C	YAP1
ABRAXAS1	BAP1	CCND3	EMSY	FANCF	FN1	H3-3A	INPP4B	LIN28B	MPL	NRAS	PIK3CA	PTCH2	RNF43	SMC1A	TERT	YES1
ACVR1	BARD1	CCNE1	EP300	FANCG	FOXA1	H3-3B	INSR	LMO1	MRE11	NRG1	PIK3CB	PTEN	ROS1	SMC3	TET1	YWHAE
ACVR1B	BBC3	CD274	EPCAM	FANCI	FOXL2	H3-5	IRF2	LRP1B	MSH2	NSD1	PIK3CD	PTPN11	RPS6KA4	SMO	TET2	ZBTB2
ADGRA2	BCL10	CD276	EPHA3	FANCL	FOXO1	H3C14	IRF4	LYN	MSH3	NTRK1	PIK3CG	PTPRD	RPS6KB1	SNCAIP	TFE3	ZFHX3
AKT1	BCL2	CD74	EPHA5	FAS	FOXP1	H3C15	IRS1	LZTR1	MSH6	NTRK2	PIK3R1	PTPRS	RPS6KB2	SOCS1	TFEB	ZNF217
AKT2	BCL2L1	CD79A	EPHA7	FAT1	FRS2	H3C2	IRS2	MAGI2	MST1	NTRK3	PIK3R2	PTPRT	RPTOR	SOX10	TFRC	ZNF703
AKT3	BCL2L11	CD79B	EPHB1	FBXW7	FUBP1	H3C3	JAK1	MALT1	MST1R	NUP93	PIK3R3	QKI	RUNX1	SOX17	TGFBR1	ZRSR2
ALK	BCL2L2	CDC73	EPHB2	FGF1	FUS	HGF	JAK2	MAML2	MTOR	NUTM1	PIM1	RAC1	RUNX1T1	SOX2	TGFBR2	
ALOX12B	BCL6	CDH1	ERBB2	FGF10	FYN	HLA-A	JAK3	MAP2K1	MUTYH	PAK1	PIN1	RAD21	RYBP	SOX9	TMEM127	
AMER1	BCOR	CDK12	ERBB3	FGF14	GABRA6	HLA-B	JUN	MAP2K2	MYB	PAK3	PLCG2	RAD50	SDHA	SPEN	TMPRSS2	
ANKRD26	BCORL1	CDK4	ERBB4	FBF19	GATA1	HLA-C	KAT6A	MAP2K4	MYC	PAK5	PLK2	RAD51	SDHAF2	SPOP	TNFAIP3	
APC	BCR	CDK6	ERCC1	FGF2	GATA2	HNF1A	KDM5A	MAP3K1	MYCL	PALB2	PMAIP1	RAD51B	SDHB	SPTA1	TNFRSF14	
AR	BIRC3	CDK8	ERCC2	FGF23	GATA3	HOXB13	KDM5C	MAP3K13	MYCN	PARP1	PMS1	RAD51C	SDHC	SRC	TOP1	
ARAF	BLM	CDKN1A	ERCC3	FGF3	GATA4	HRAS	KDM6A	MAP3K4	MYD88	PAX3	PMS2	RAD51D	SDHD	SRSF2	TOP2A	
ARFRP1	BMPR1A	CDKN1B	ERCC4	FGF4	GATA6	HSD3B1	KDR	MAPK1	MYOD1	PAX5	PNRC1	RAD52	SETBP1	SS18	TP53	
ARID1A	BRAF	CDKN2A	ERCC5	FGF5	GEN1	HSP90AA1	KEAP1	MAX	NBN	PAX7	POLD1	RAD54L	SETD2	STAG2	TP63	
ARID1B	BRCA1	CDKN2B	ERG	FGF6	GID4	ICOSLG	KEL	MCL1	NCOA3	PAX8	POLE	RAF1	SF3B1	STAT3	TRAF2	
ARID2	BRCA2	CDKN2C	ERF11	FGF7	GLI1	ID3	KIAA1549	MDC1	NCOR1	PBRM1	POT1	RANBP2	SGK1	STAT4	TRAF7	
ARID5B	BRD4	CEBPA	ESR1	FGF8	GNA11	IDH1	KIF5B	MDM2	NF1	PDCD1	PPARG	RARA	SH2B3	STAT5A	TSC1	
ASXL1	BRIP1	CHD2	ETS1	FGF9	GNA13	IDH2	KIT	MDM4	NF2	PDCD1LG2	PPM1D	RASA1	SH2D1A	STAT5B	TSC2	
ASXL2	BTG1	CHD4	ETV1	FGFR1	GNAQ	IFNGR1	KLF4	MED12	NFE2L2	PDGFRA	PPP2R1A	RB1	SHQ1	STK11	TSHR	
ATM	BTK	CHEK1	ETV4	FGFR2	GNAS	IGF1	KLHL6	MEF2B	NFKBIA	PDGFRB	PPP2R2A	RBM10	SLIT2	STK40	U2AF1	
ATR	C19MC	CHEK2	ETV5	FGFR3	GPR161	IGF1R	KMT2A	MEN1	NKX2-1	PDK1	PPP6C	RECQL4	SLX4	SUFU	USP6	
ATRX	CALR	CIC	ETV6	FGFR4	GPS2	IGF2	KMT2B	MET	NKX3-1	PDPK1	PRDM1	REL	SMAD2	SUZ12	VEGFA	
AURKA	CARD11	CYP2D6	EWSR1	FH	GREM1	IKBKE	KMT2C	MGA	NOTCH1	PGR	PREX2	RELA	SMAD3	SYK	VHL	
AURKB	CASP8	CREBBP	EZH2	FLCN	GRIN2A	IKZF1	KMT2D	MGMT	NOTCH2	PHF6	PRKAR1A	RET	SMAD4	TAF1	WTF1	
AXIN1	CBFB	CRKL	FANCA	FLI1	GRM3	IL10	KRAS	MITF	NOTCH3	PHOX2B	PRKCI	RHEB	SMARCA4	TBX3	XIAP	
AXIN2	CBL	CRLF2	FANCC	FLT1	GSK3B	IL7R	LAMP1	MLH1	NOTCH4	PIK3C2B	PRKDC	RHOA	SMARCB1	TCF3	XPO1	