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, MSI and HRD 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.

Simplified workflow for all variant types
Carefully designed to allow analysis of all relevant markers for solid tumors including HRD in one workflow, , GALEAS tumor allows laboratories to streamline processes improving through put and efficiency.

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, MSI and HRD.

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 HRD and 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 in oncology including SNVs, INDELs, CNVs and selected fusions in 519 genes. The solution supports the analysis of immuno-oncology biomarkers including TMB and MSI and calculation of homologous recombination deficiency (HRD). 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
- HRD status.
- 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

Parameter	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	10-200ng
Input DNA requirements	Under 3 hours
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 (R2 = 0.99).

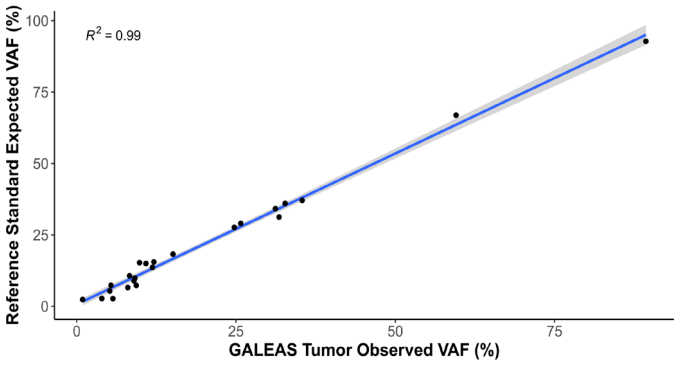


Figure 1. SNV and INDEL recall rate for alterations in reference material from FFPE.

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)*

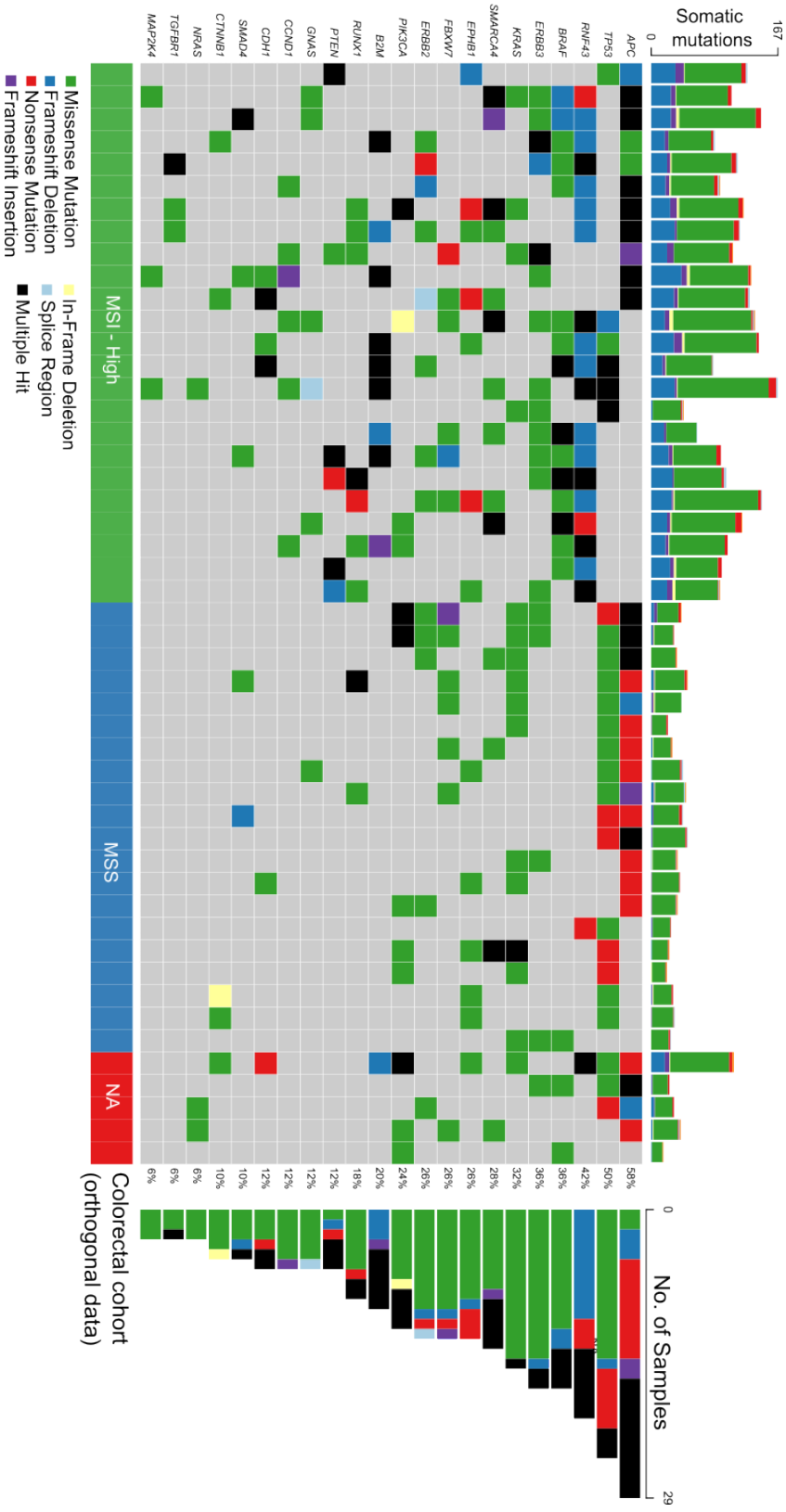


Figure 2. Oncoplot from 50 CRC FFPE cohort highlighting detection of somatic mutations in genes with known cancer hotspots to demonstrate the overall performance of GALEAS Tumor.

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.

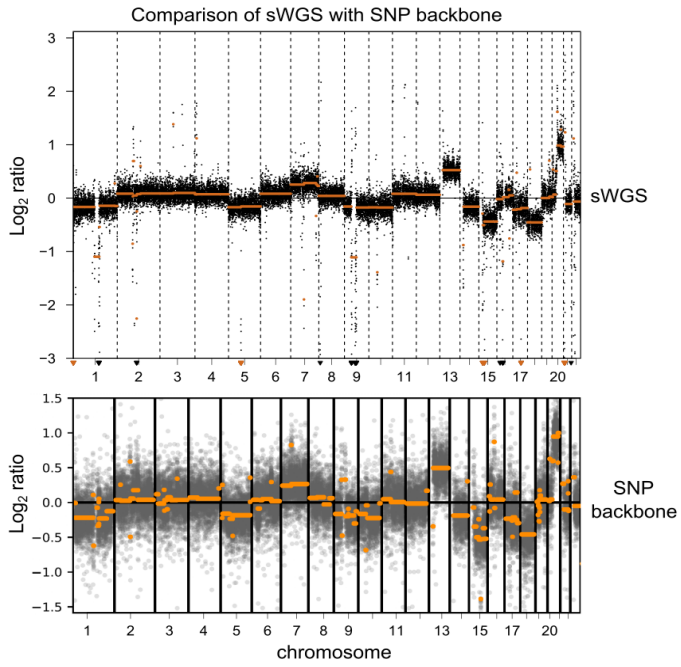


Figure 3. Comparison of GALEAS Tumor SNP backbone data with sWGS. The data shown was obtained from a representative colorectal cancer sample and demonstrates the similarity between the CNV profile obtained from shallow whole genome sequencing (sWGS), with the SNP backbone obtained using GALEAS Tumor.

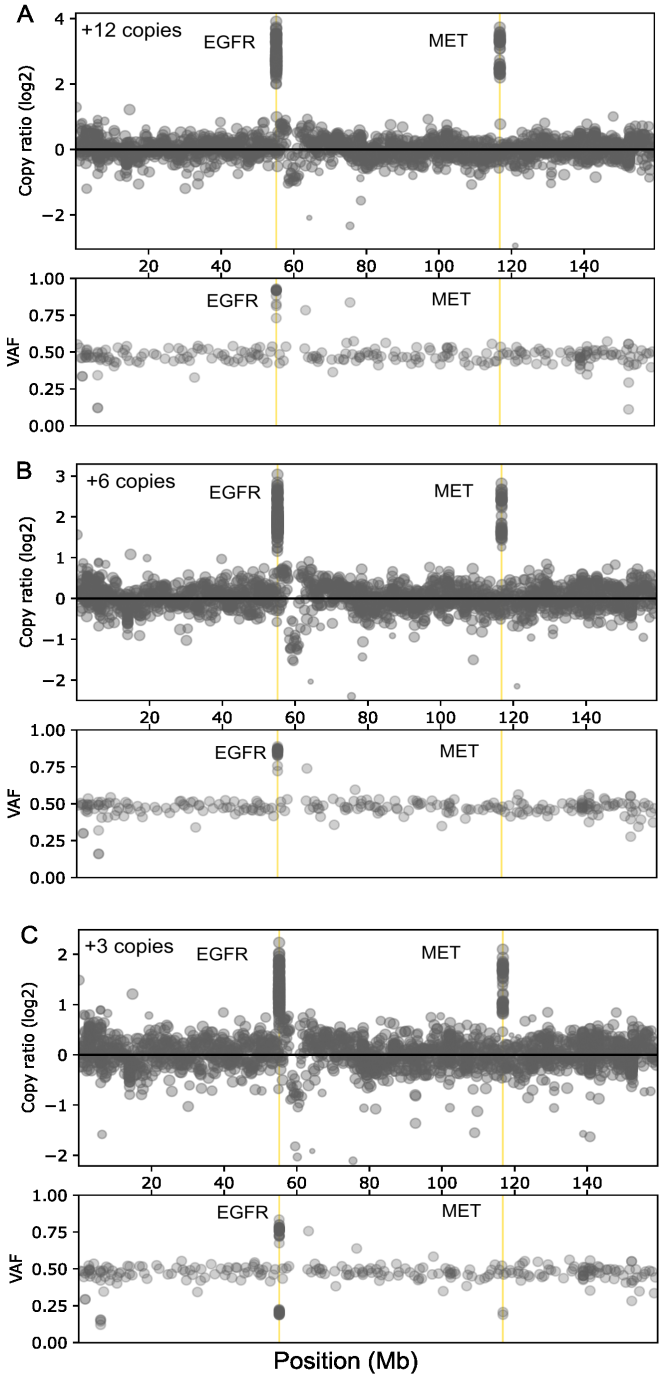


Figure 4. Validating gene level CNV calls with a CNV Lung and Brain Mix reference standard at (A) 12, (B) 6, and (C) 3 copies. Genes highlighted here are EGFR and MET.

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.

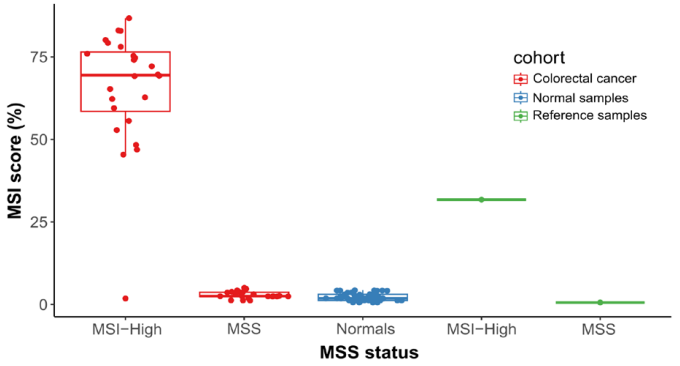


Figure 5. Comparisons of GALEAS Tumor MSI scores with known MSI status from CRC primary tumor samples (MSS-High), healthy individuals (MSS) and reference standards.

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).

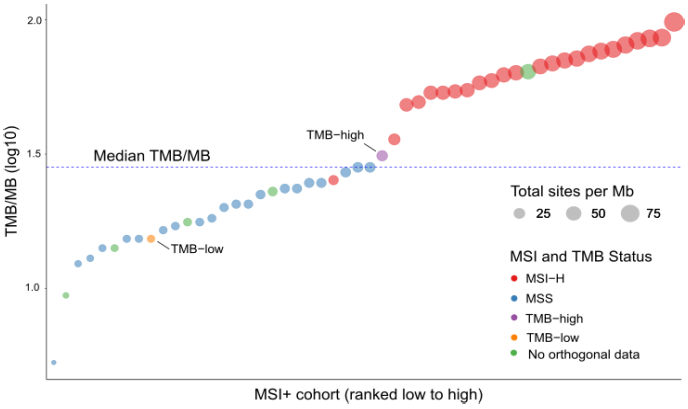


Figure 6. GALEAS Tumor TMB scores compared with MSI status across TMB-low and TMB-high reference standards as well as 50 CRC samples.

Homologous Recombination Deficiency (HRD)

The design of the GALEAS Tumor panel enables an assessment of HRD in a single workflow, removing the need for separate BRCA and genomic instability testing to obtain an HRD score. Shown to deliver high concordance with orthogonal data, GALEAS Tumor offers laboratories a streamlined and efficient way of incorporating HRD scoring into their comprehensive profiling workflow. To test the performance of GALEAS Tumor HRD, 265 samples, 3 Ovarian Cancer Cohorts (OV1 (19 samples) OV2 (50 samples) and OV3 (93 samples) with orthogonal HRD status, HRD high, low and HRP reference standards (3), NOCANCERL FFPE tissue (50) and a cohort of colorectal cancer samples (50) (CRC) were run. Orthogonal HRD status is unknown in the CRC and NOCANCER cohort, but the prevalence is expected to be low. The inclusion of, these samples is to highlight the power of GALEAS Tumor HRD is accurately identifying HRD events caused by Genomic Instability in tumor cohorts. The concordance between the GALEAS Tumor HRD status and the orthogonal HRD status is shown in Figure 7

Orthogonal HRD status is defined by a Genomic Instability Score >=42 or a pathogenic BRCA mutation (BRCAm). For GALEAS Tumor HRD a GALEAS Scar Score (GSS) value of ≥2.6 classified samples as HRD, or a pathogenic BRCA 1/2 mutation (BRCAm) identified using the Varsome5 germline and somatic clinical classification model. Samples with GSS values <2.6 were categorized as HRP (homologous recombination proficient).
A high concordance was observed between the GALEAS Tumor HRD based predictions in combination with BRCAm and the orthogonal HRD status. The overall accuracy was 93.21%, with a sensitivity of 93.82% and specificity of 91.95%, signifying a robust ability to correctly identify both HRD and HRP cases. Precision and F1 scores, of 94.9% and 0.96 respectively,) further underscored the strong discriminative performance of the GALEAS Tumor HRD threshold. The confusion matrix revealed that 80 HRD and 167 HRP samples were correctly classified, with only 18 misclassified instances, indicating high reliability in detecting HRD events. Collectively, these results confirm that a GALEAS Tumor HRD GSS threshold of ≥2.6 and BRCAm detection is a valid and effective metric for distinguishing HRD from HRP in ovarian cancer and in cohorts known to be highly HRP such as CRC.

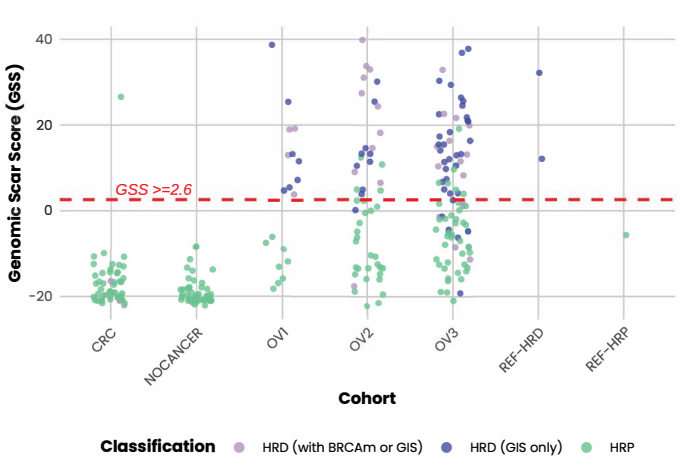


Figure 7. GALEAS Tumor HRD status shows high concordance with orthogonal HD classification.

GALEAS analysis software

The GALEAS analysis software was developed in parallel with the panel, delivering an optimized solution for ultra-sensitive SNV, INDEL and CNV detection and reports for HRD, MSI and TMB.

The software is easily integrated into routine laboratory use and provides a robust, intuitive and reliable bioinformatics solution for variant calling. The output, which consists of variant call format (VCF) and binary alignment map (BAM) files and QC metrics, can be uploaded into any decision support software for data interpretation.

Performance specifications

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.

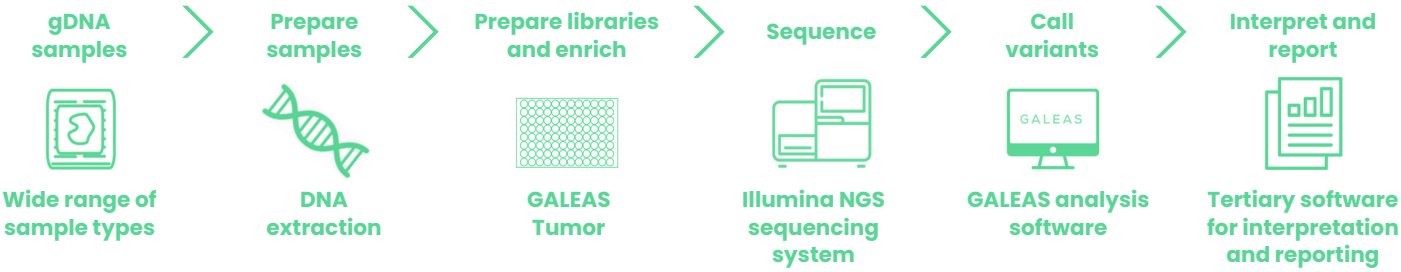
Table 2: GALEAS Tumor sequencing performance metrics

Key quality indicator	GALEAS Tumor
Number of genes	519
Capture panel size	3.74Mb
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%

Streamlined, simple, automatable workflow

The workflow for GALEAS Tumor 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 and to allow for flexible batch sizes.

Workflow overview diagram



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. 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.

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. *Na-ture.* 2020: 82-93.

3. Endris V, Buchhalter I, Allgäuer M, Rempel E, Lier A, Vol- ckmar AL, et al. Measurement of tumor mutational bur- den (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.

5. Kopanos, C., et al. (2019). VarSome: The human genomic variant search engine. *Bioinformatics, 35*(11), 1978–1980. Last accessed 21/01/2025 https://varsome.com/

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.

Nonacus Limited
Quinton Business Park
11 Ridgeway
Birmingham
B32 1AF

info@nonacus.com

© 2024 Nonacus Limited. For research use only (RUO).
Not for use in diagnostic procedures.

GALEAS Tumor gene list

ABL1	B2M	CCND3	CTLA4	EPCAM	FANCL	FOXP1	H3C2	JAK1	MAML2	MUTYH	PAK3	PLK2	RAD51B	SDHC	SRSF2	TP53
ABL2	BAP1	CCNE1	CTNNA1	EPHA3	FAS	FRS2	H3C3	JAK2	MAP2K1	MYB	PAK5	PMAIP1	RAD51C	SDHD	SS18	TP63
ABRAXAS1	BARD1	CD274	CTNNB1	EPHA5	FAT1	FUBP1	HGF	JAK3	MAP2K2	MYC	PALB2	PMS1	RAD51D	SETBP1	STAG2	TRAF2
ACVR1	BBC3	CD276	CUL3	EPHA7	FBXW7	FUS	HLA-A	JUN	MAP2K4	MYCL	PARP1	PMS2	RAD52	SETD2	STAT3	TRAF7
ACVR1B	BCL10	CD74	CUX1	EPHB1	FGF1	FYN	HLA-B	KAT6A	MAP3K1	MYCN	PAX3	PNRC1	RAD54L	SF3B1	STAT4	TSC1
ADGRA2	BCL2	CD79A	CXCR4	EPHB2	FGF10	GABRA6	HLA-C	KDM5A	MAP3K13	MYD88	PAX5	POLD1	RAF1	SGK1	STAT5A	TSC2
AKT1	BCL2L1	CD79B	CYLD	ERBB2	FGF14	GATA1	HNF1A	KDM5C	MAP3K4	MYOD1	PAX7	POLE	RANBP2	SH2B3	STAT5B	TSHR
AKT2	BCL2L11	CDC73	DAXX	ERBB3	FGF19	GATA2	HOXB13	KDM6A	MAPK1	NBN	PAX8	POT1	RARA	SH2D1A	STK11	U2AF1
AKT3	BCL2L2	CDH1	DCUN1D1	ERBB4	FGF2	GATA3	HRAS	KDR	MAX	NCOA3	PBRM1	PPARG	RASA1	SHQ1	STK40	USP6
ALK	BCL6	CDK12	DDIT3	ERCC1	FGF23	GATA4	HSD3B1	KEAP1	MCL1	NCOR1	PDCD1	PPM1D	RB1	SLIT2	SUFU	VEGFA
ALOX12B	BCOR	CDK4	DDR2	ERCC2	FGF3	GATA6	HSP90AA1	KEL	MDC1	NF1	PDCD1LG2	PPP2R1A	RBM10	SLX4	SUZ12	VHL
AMER1	BCORL1	CDK6	DDX3X	ERCC3	FGF4	GEN1	ICOSLG	KIAA1549	MDM2	NF2	PDGFRA	PPP2R2A	RECQL4	SMAD2	SYK	WT1
ANKRD26	BCR	CDK8	DDX41	ERCC4	FGF5	GID4	ID3	KIF5B	MDM4	NFE2L2	PDGFRB	PPP6C	REL	SMAD3	TAF1	XIAP
APC	BIRC3	CDKN1A	DICER1	ERCC5	FGF6	GLI1	IDH1	KIT	MED12	NFKBIA	PDK1	PRDM1	RELA	SMAD4	TBX3	XPO1
AR	BLM	CDKN1B	DIS3	ERG	FGF7	GNA11	IDH2	KLF4	MEF2B	NKX2-1	PDPK1	PREX2	RET	SMARCA4	TCF3	XRCC2
ARAF	BMPR1A	CDKN2A	DNMT1	ERRFI1	FGF8	GNA13	IFNGR1	KLHL6	MEN1	NKX3-1	PGR	PRKAR1A	RHEB	SMARCB1	TCF7L2	YAP1
ARFRP1	BRAF	CDKN2B	DNMT3A	ESR1	FGF9	GNAQ	IGF1	KMT2A	MET	NOTCH1	PHF6	PRKCI	RHOA	SMARCD1	TENT5C	YES1
ARID1A	BRCA1	CDKN2C	DNMT3B	ETS1	FGFR1	GNAS	IGF1R	KMT2B	MGA	NOTCH2	PHOX2B	PRKDC	RICTOR	SMARCE1	TERT	YWHAE
ARID1B	BRCA2	CEBPA	DOT1L	ETV1	FGFR2	GPR161	IGF2	KMT2C	MGMT	NOTCH3	PIK3C2B	PRKN	RIT1	SMC1A	TET1	ZBTB2
ARID2	BRD4	CHD2	DPYD	ETV4	FGFR3	GPS2	IKBKE	KMT2D	MITF	NOTCH4	PIK3C2G	PTCH1	RNF43	SMC3	TET2	ZFHX3
ARID5B	BRIP1	CHD4	DROSHA	ETV5	FGFR4	GREM1	IKZF1	KRAS	MLH1	NPM1	PIK3C3	PTCH2	ROS1	SMO	TFE3	ZNF217
ASXL1	BTG1	CHEK1	E2F3	ETV6	FH	GRIN2A	IL10	LAMP1	MLLT3	NR4A3	PIK3CA	PTEN	RPS6KA4	SNCAIP	TFEB	ZNF703
ASXL2	BTK	CHEK2	EED	EWSR1	FLCN	GRM3	IL7R	LATS1	MN1	NRAS	PIK3CB	PTPN11	RPS6KB1	SOCS1	TFRC	ZRSR2
ATM	C19MC	CIC	EGFL7	EZH2	FLI1	GSK3B	INHBA	LATS2	MPL	NRG1	PIK3CD	PTPRD	RPS6KB2	SOX10	TGFBR1	
ATR	CALR	CYP2D6	EGFR	FANCA	FLT1	H1-2	INPP4A	LIN28B	MRE11	NSD1	PIK3CG	PTPRS	RPTOR	SOX17	TGFBR2	
ATRX	CARD11	CREBBP	EIF1AX	FANCC	FLT3	H2BC5	INPP4B	LMO1	MSH2	NTRK1	PIK3R1	PTPRT	RUNX1	SOX2	TMEM127	
AURKA	CASP8	CRKL	EIF4A2	FANCD2	FLT4	H3-3A	INSR	LRP1B	MSH3	NTRK2	PIK3R2	QKI	RUNX1T1	SOX9	TMPRSS2	
AURKB	CBFB	CRLF2	ELOC	FANCE	FN1	H3-3B	IRF2	LYN	MSH6	NTRK3	PIK3R3	RAC1	RYBP	SPEN	TNFAIP3	
AXIN1	CBL	CSF1R	EML4	FANCF	FOXA1	H3-5	IRF4	LZTR1	MST1	NUP93	PIM1	RAD21	SDHA	SPOP	TNFRSF14	
AXIN2	CCND1	CSF3R	EMSY	FANCG	FOXL2	H3C14	IRS1	MAGI2	MST1R	NUTM1	PIN1	RAD50	SDHAF2	SPTA1	TOP1	
AXL	CCND2	CTCF	EP300	FANCI	FOXO1	H3C15	IRS2	MALT1	MTOR	PAK1	PLCG2	RAD51	SDHB	SRC	TOP2A	