GALEAS™ Hereditary Plus

A clinically validated NGS panel with optimized bioinformatics for analyzing germline mutations associated with hereditary cancers

Highlights

Enhanced clinically relevant content for assessment of hereditary cancer variants.

Expertly curated coverage for the key clinically relevant regions of 146 genes associated with a predisposition for hereditary cancer including breast, prostrate, Lynch syndrome and Wilms tumor.

A single workflow for all clinically relevant variant types, sample types and cancers.

Validate and run one workflow for all hereditary cancers, regardless of sample type (e.g, blood or saliva) and confidently call all variants including a wide range of CNVs in genes such as APC, MHS2, BRCA1 and PMS2 without the need for additional MLPA.

Supported by GALEAS Analysis software.

Optimized for the GALEAS panels, our cloud-based bioinformatics pipelines deliver accurate calling across all variant types associated with inherited cancers.

Introduction

Between 5-10% of all cancers, including cancers of the breast, ovary, uterus, prostate, and gastrointestinal system can be accounted for by hereditary cancers.[1] The identification of individuals who are at an increased risk of developing inherited cancer is dependent upon the ability to accurately identify the genetic variants associated with heritable cancer syndromes. The ability to perform a comprehensive evaluation of the germline variants is key to understanding their association with cancer predisposition. This can provide a cancer risk assessment, and guide the implementation of additional screening and surveillance which may in turn result in an early diagnosis and guide treatment for both themselves and their families.

Next Generation Sequencing (NGS)-based multigene panel for comprehensive profiling of heritable cancers.

The use of targeted NGS multigene panels to provide a comprehensive analysis of cancer susceptible genes has proven to be a clinically viable option for many laboratories. It allows researchers to profile known genetic associations for hereditary cancer regardless of sample type (blood or saliva) or cancer type.

However, many NGS panels struggle to identify key hereditary cancer copy number variants (CNVs), such as single exon BRCA1 or BRCA2 alterations or those CNVs involved in Lynch syndrome, and require additional multiplex ligation dependant probe amplification (MLPA) analysis to detect them.

GALEAS Hereditary Plus panel design

GALEAS Hereditary Plus has been designed to target germline mutations in 146 genes associated with an increased risk of developing hereditary cancer. These genes have been selected to cover not only the common hereditary cancers like breast or prostate, but also the rarer hereditary cancer types like Phaeochromocytoma and paediatric cancers such as Wilms tumor.

Table 1: Genes included in key guidelines associated with risk of developing hereditary cancers and included in the GALEAS Hereditary Plus panel (see appendix for full gene list * Wilms tumor only).

Cancer type	Recommended genes for screening included in GALEAS Hereditary Plus
Breast	ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, STK11, TP53
Colon	TAPC, AXIN2, BMPR1A, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, PMS2, MSH3, MUTYH, NTLH1, POLD1, POLE, PTEN, RNF43, SMAD4, STK11, TP53
Renal	BAP1, FH, FLCN, MET, SDHB, VHL
Ovarian	ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, SKT11, TP53, RAD51C, RAD51D
Prostate	ATM, BRCA1, BRCA2, CHEK2, MLH1, MSH2, MSH6, PALB2
Gastric/GIST	CDH1, KIT, PDGFRA, SDHC, SDHD, SDHA
Brain	APC, ATM, MLH1, MSH2, MSH6, PMS2, TP53
Sarcoma	EXT1, EXT2, MTAP, NF1, RECQL4, SQSTM1, TP53
Paediatric*	CDKN1C, CTR9, REST, TRIM28, WT1

The design has been carefully curated to ensure that all clinically relevant exons are covered, including selected non-coding regions such as BRCA1/2 5′ UTRs and the APC promoter. The GALEAS Hereditary Plus also includes a panel of tracking SNPs for patient identification purposes. When combined with the GALEAS analysis software this panel allows the sensitive and specific detection of SNVs, INDELs and CNVs.

Table 2: GALEAS Hereditary Plus panel specifications (* for gDNA only)

Parameters	Specification
Enrichment method	Hybridization and Capture
Number of genes	146
Capture Panel size	809 Kb
Sequencing platform	Illumina
Targets	Genes associated with hereditary cancer
Variant types	SNVs, CNVs and INDELs
Input DNA requirements*	10-200 ng
Sample type	gDNA from blood or saliva
Multiplexing guidance for sequencing*	1 million reads per sample required to achieve 100x. This equates to 0.2 Gb per sample

GALEAS Hereditary Plus panel performance

Superior precision and recall ensure confident calling of SNV and indel variants.

GALEAS Hereditary Plus was validated across 437 SNPs using commercially available reference control NA24385.

Table 3: GALEAS Hereditary Plus SNV and indel recall across 4 replicates of reference standard NA24385.

	Recall
SNV	99.78%
Indel	100%

Superior precision and recall ensure confident calling of copy number variants

To evaluate the sensitivity of CNV genotyping with GALEAS Hereditary Plus, the panel was run using NIBSC Lynch Syndrome MLPA cell lines. All CNVs were detected with 100% recall and precision when using sex matched control pools (Table 4).

Table 4: Recall and precision statistics for copy number alterations (CNVs) in NIBSC reference controls using the GALEAS Hereditary Plus panel.

CNV	Genotypic sex	CNV type	Detected
Copy normal	Male	Copy neutral	Yes
MSH2 deletion exons 1-6, heterozygous	Male	Multi-exon deletion	Yes
MSH2 deletion exon 7, heterozygous	1 Male	Single exon deletion	Yes
MSH2 deletion exons 1-2, heterozygous	female	Multi-exon deletion	Yes
MSH2 deletion, exon 1, heterozygous	Male	Single exon deletion	Yes
MLH1 exon 13 amplification (3 or more copies)	Female	Multi-exon amplification	Yes

Panel performance specifications

GALEAS Hereditary Plus panel design delivers a high percentage of on-target reads, lower duplication rates and more consistent vertical coverage with 99% of targets covered at 30x or more (Table 5). This exceptional technical performance delivers high recall and precision across more variants associated with hereditary cancer, including CNVs, than a leading competitor without significantly increasing sequencing costs.

Table 5: Sequencing metrics for GALEAS Hereditary Plus. Compared with another commercial alternative, GALEAS Hereditary Plus delivers 100% more content (including CNV probes) for less than 10% more sequencing.

Key Quality Indicator	GALEAS Hereditary Plus	Company I
Number of genes	146	113
Capture panel size (kb)	809 kb	403 kb
GB required for mean 100x coverage	0.2 Gb	0.12 Gb
Percentage coverage >30x	99%	96%
Percentage on or near bait	81%	61.51%
Percent duplication	2.0%	8.99%
SNV recall	99.7%	98.1%
INDELs recall	100%	97.2%

GALEAS Hereditary Plus clinical validation

The clinical utility of GALEAS Hereditary Plus was assessed using research collaborator samples, including gDNA from 64 patient blood samples with orthogonal data.

SNV recall and precision

SNV recall on clinical samples was shown to be 100%, across a wide range of alteration types, including small and large (>10 bp) INDELs.

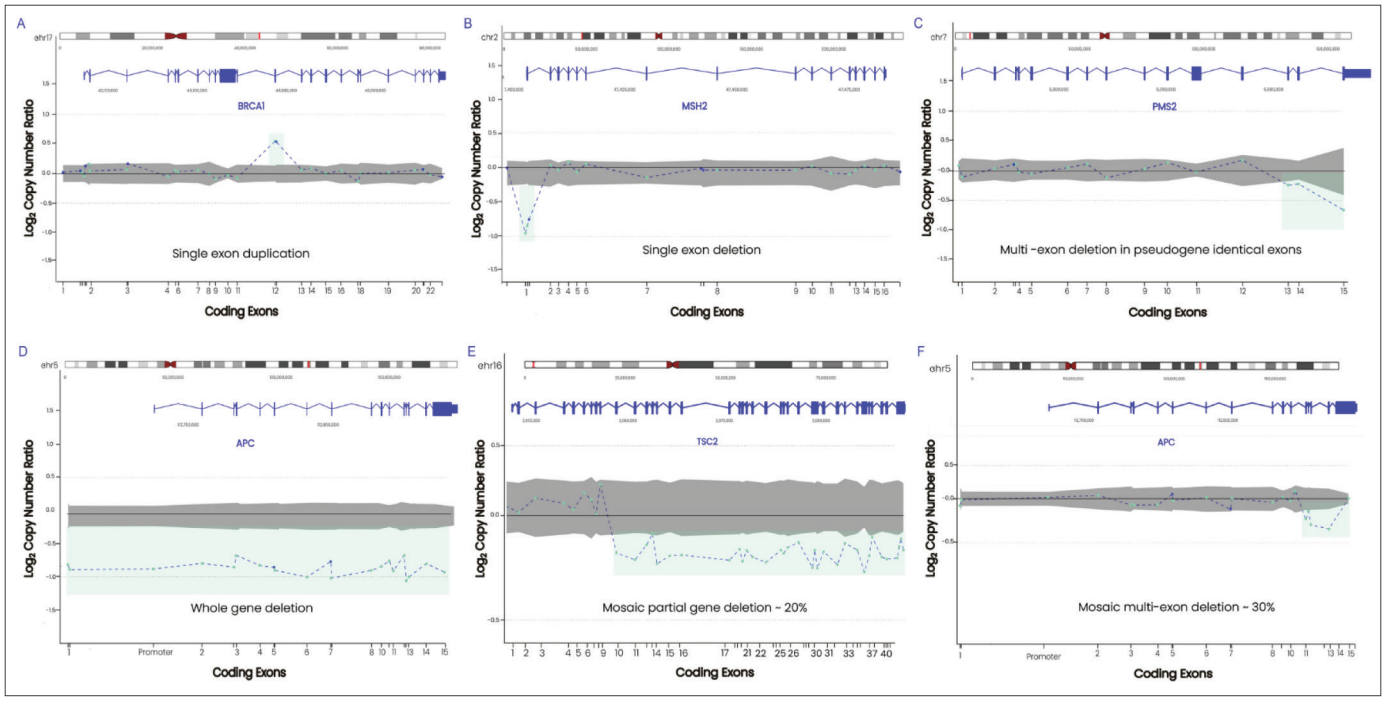
Table 6: SNV recall on clinical samples for GALEAS Hereditary Plus

ID	Gene	HGVS coding	HGVS protein	Genomic position
22	**BRCA1**	c.1175_1214del	p.Leu392fs*5	chr17:43094317
23	**BRCA1**	c.1175_1214del	p.Leu392fs*5	chr17:43094317
64	**MSH2**	c.942+3A>T	P.?	chr2:47414421
65	**PMS2**	c.736_741delins TGTGTGTGAAG	p.(Pro246Cysfs*3)	chr7:5997389
66	**MLH1**	c.1946dupC	p.(Leu650Phefs*14)	chr3:37048561
67	**MSH2**	c.1213_1217dup	p.(Leu407Thrfs*7)	chr2:47429877
68	**MSH6**	c.3562_3563del	1 p.(Ser1188Tyrfs*5)	chr2:47805623

Copy Number Variants (CNVs)

GALEAS Hereditary Plus accurately identifies CNVs from single exons to whole genes in key cancer syndrome susceptibility genes. When combined with the GALEAS Analysis Software, the GALEAS Hereditary Plus panel provides:

- An analytical sensitivity of 100%
- An analytical specificity of 93.5%
- The ability to detect mosaic copy number variations in key genes such as APC and TSC2 (see Figure 1 E and F)
- Capability to deliver CNV detection in PMS2 (see Figure 1C)



GALEAS analysis software

GALEAS Analysis Software is a cloud-based set of optimised bioinformatics pipelines which provides accurate calling of SNVs, INDELs and a wide range of CNVs from single exons to whole genes.

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

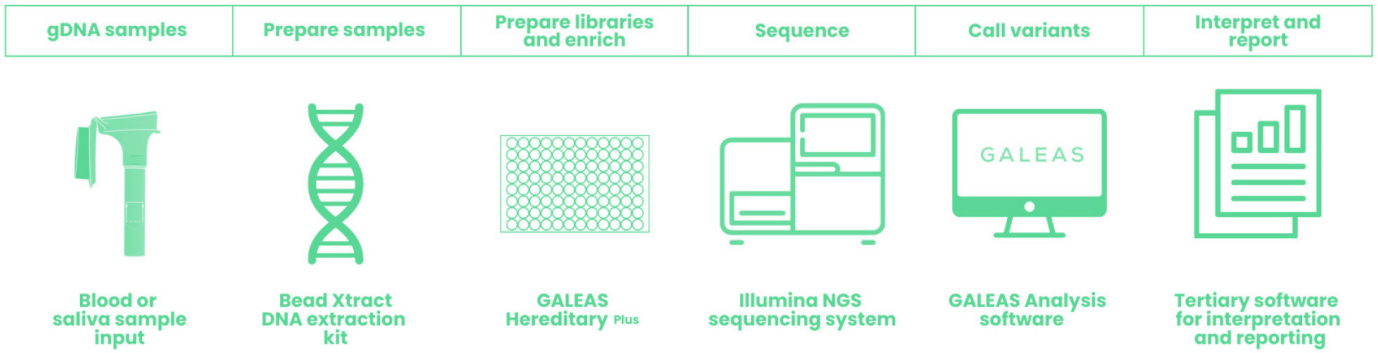
Panel of Normals

To minimize background noise, improve CNV calling and reduce costs, the GALEAS analysis software leverages an in-built 'panel of normals'. Using a large cohort of clinically normal samples, processed with the GALEAS Hereditary Plus library prep and sequenced using an Illumina sequencing workflow, this data set provides a baseline to call CNVs, dramatically improving the accuracy of CNV calling.

Streamlined workflows; quick and easy protocols

GALEAS Hereditary Plus enables laboratories to validate and run a single comprehensive workflow to profile all hereditary cancer types, reducing turnaround time, validation and operating costs. Validate and run one workflow for all hereditary cancers and confidentially call all variants including a wide range of CNVs in genes like APC, MHS2, BRCA1 and PMS2; potentially eliminating the need for MLPA for clinical identification and reporting of CNVs.

The GALEAS Hereditary Plus workflow is simple and easy. Taking less than 10 hours, with less than 2 hours handson 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 run. Indexes are available for up to 384 samples to allow for flexible batch sizes and scalability across all Illumina benchtop sequencers.



Summary

GALEAS Hereditary Plus provides an expertly curated, comprehensive NGS solution for the analysis of genes previously linked with cancer predisposition syndromes. The enhanced probe design, comprehensive coverage, high coverage and uniformity allows the accurate and sensitive detection of SNVs/INDELs and CNVs. Combining this with the GALEAS Analysis software provides a simple and easy sample to analysis workflow. GALEAS Hereditary Plus provides a highly efficient, targeted sequencing and analysis solution to allow the detection of variants associated with cancer predisposition syndromes.

Learn more

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

References

1. Ngeow J, Eng C. Precision medicine in heritable cancer: when somatic tumour testing and germline mutations meet. NPJ Genomic Medicine. 2016;(1):1-3.

Ordering information

Product	Catalog No.
GALEAS Hereditary Plus 16 samples	NGS_GAL_HCP_FR_16
GALEAS Hereditary Plus 96 samples	NGS_GAL_HCP_FR_96

Nonacus Limited
Quinton Business Park
11 Ridgeway
Birmingham
B32 1AF

info@nonacus.com

GALEAS Hereditary^{Plus} gene list

AIP	CDKN1C	EXT2	HNF1A	MSH6	POLE	SDHA	TMEM127
ALK	CDKN2A	EZH2	HOXB13	MTAP	POLH	SDHAF2	TP53
APC	CEBPA	FANCA	HRAS	MUTYH	POT1	SDHB	TRIM28
ATM	CHEK2	FANCB	KIF1B	NBN	PRKAR1A	SDHC	TRIM37
AXIN2	CTNNA1	FANCC	KIT	NF1	PTCH1	SDHD	TRIP13
BAP1	CTR9	FANCD2	KRAS	NF2	PTEN	SEC23B	TSC1
BARD1	DDB2	FANCE	LZTR1	NHP2	PTPN11	SHOC2	TSC2
BLM	DICER1	FANCF	MAP2K1	NRAS	RAD50	SLC25A11	VHL
BMPR1A	DIS3L2	FANCG	MAP2K2	NSD1	RAD51C	SLX4	WRAP53
BRAF	DLST	FANCI	MAX	NTHL1	RAD51D	SMAD4	WRN
BRCA1	EGFR	FANCL	MDH2	PALB2	RAF1	SMARCA4	WT1
BRCA2	ELP1	FANCM	MEN1	PAX5	RB1	SMARCB1	XPA
BRIP1	EPCAM	FH	MET	PAX6	RECQL4	SMARCE1	XPC
BUB1B	ERCC1	FLCN	MITF	PDGFRA	REST	SOS1	XRCC2
CBL	ERCC2	GALNT12	MLH1	PDGFRB	RET	SQSTEM1	
CDC73	ERCC3	GATA2	MRE11	PHOX2B	RHBDF2	STK11	
CDH1	ERCC4	GPC3	ME11A	PMS1	RNF43	SUFU	
CDK4	ERCC5	GPR161	MSH2	PMS2	RTEL1	TERC	
CDKN1B	EXT1	GREM1	MSH3	POLD1	RUNX1	TERT	