# GALEAS® Hereditary Plus

## A comprehensive NGS panel for hereditary cancer profiling

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## Introduction

Hereditary cancer accounts for approximately 5-10% of all cancers, and understanding the genetic basis of cancer risk is widely accepted.

The use of targeted NGS-based multigene panels to provide the comprehensive analysis of cancer susceptible genes has potential to be a clinical viable option to reduce costly and time consuming MLPA.

However, many panels struggle to identify key hereditary cancer copy number variants (CNVs), such as single exon BRCA1/2 alterations or those CNVs involved in Lynch syndrome.

GALEAS® Hereditary Plus provides a comprehensive workflow for the analysis of SNVs and CNVs linked to inherited cancer syndromes, providing a simple streamlined tool for cost-effective cancer risk profiling.

## Panel Design

GALEAS® Hereditary Plus profiles 147 highly curated genes with known associations to hereditary cancers.

Includes enhanced content to allow improved CNV calling of key cancer susceptibility loci.

24 SNPs for identity tracking.

**ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, NBN, PALB2, PIK3CA, RAD50, RAD51C, RAD51D, TP53, XRCC2**

**BMPR1A, EPCAM, MKH1, MSH2, MSH6, PM52, PM52CL, MUTYH, PTEN, STK11, APC, NTHL1, RNF43, SMAD4**

## Methods

100ng of genomic DNA from:

- 64 anonymised patient samples
- 50 normal samples
- NA24385 (HG002) and seven NIBSC reference controls gDNA

Libraries prepared using the GALEAS® Hereditary Plus Hybridization and Capture Enrichment kit.

Sequenced to a mean depth of 250x, with 0.3Gb of sequencing, giving 98% coverage at 100x.

Data analysis performed using the GALEAS® Software. A cloud based solution for the analysis of the GALEAS® Hereditary Plus NGS Panel.

## Panel Validation

- GALEAS® Hereditary Plus was validated on reference control NA24385
- GALEAS® Hereditary Plus recall on on NA24385 DNA across 437 SNVs
  - SNV – 99.7%
  - INDEL – 100%

| CNV | Genotypic sex | CNV Type | Recall | Precision |
|---|---|---|---|---|
| Copy normal | male | copy neutral | 100% | 100% |
| MSH2 deletion exons 1-6, heterozygous | male | multi-exon deletion | 100% | 100% |
| MSH2 deletion exon 7, heterozygous | male | single-exon deletion | 100% | 100% |
| MSH2 deletion exons 1-2, heterozygous | female | multi-exon deletion | 100% | 100% |
| MSH2 deletion exon 1, heterozygous | male | single-exon deletion | 100% | 100% |
| MLH1 exon 13 amplification (3+ copies) | female | multi-exon deletion | 100% | 100% |

Table 1: CNV Recall rate for copy number alterations across 7 NIBSC reference control DNA samples

## Clinical SNV Validation

Clinical utility was determined using 64 patient samples with orthogonal data. SNV recall on clinical samples was 100%, across a wide range of alteration types, including small and large (>10bp) INDELs. MSH2 c.942+3A>T can be genotyped directly using GALEAS® Hereditary Plus, avoiding the need for Sanger sequencing.

| ID | Gene | HGVS coding | HGVS protein | Genomic Position (GRCh38) |
|---|---|---|---|---|
| 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_741delinsTGTGTGTGAAG | 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 | p.(Ser1188Tyrfs*5) | chr2:47805623 |

Table 2: Selected SNVs/INDELs showing the range of variants detectable by GALEAS® Hereditary Plus

## Clinical Validation of Copy Number Variation

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 single exon duplications (Fig. 1A), deletions (Fig. 1B)
- Detect whole gene deletions (Fig. 1D)
- The ability to detect mosaics in key genes such as APC and TSC2 (see Figure 1, E and F)
- Capability to accurately distinguish between PMS2 and PMS2CL pseudogene (see Figure 1, C)

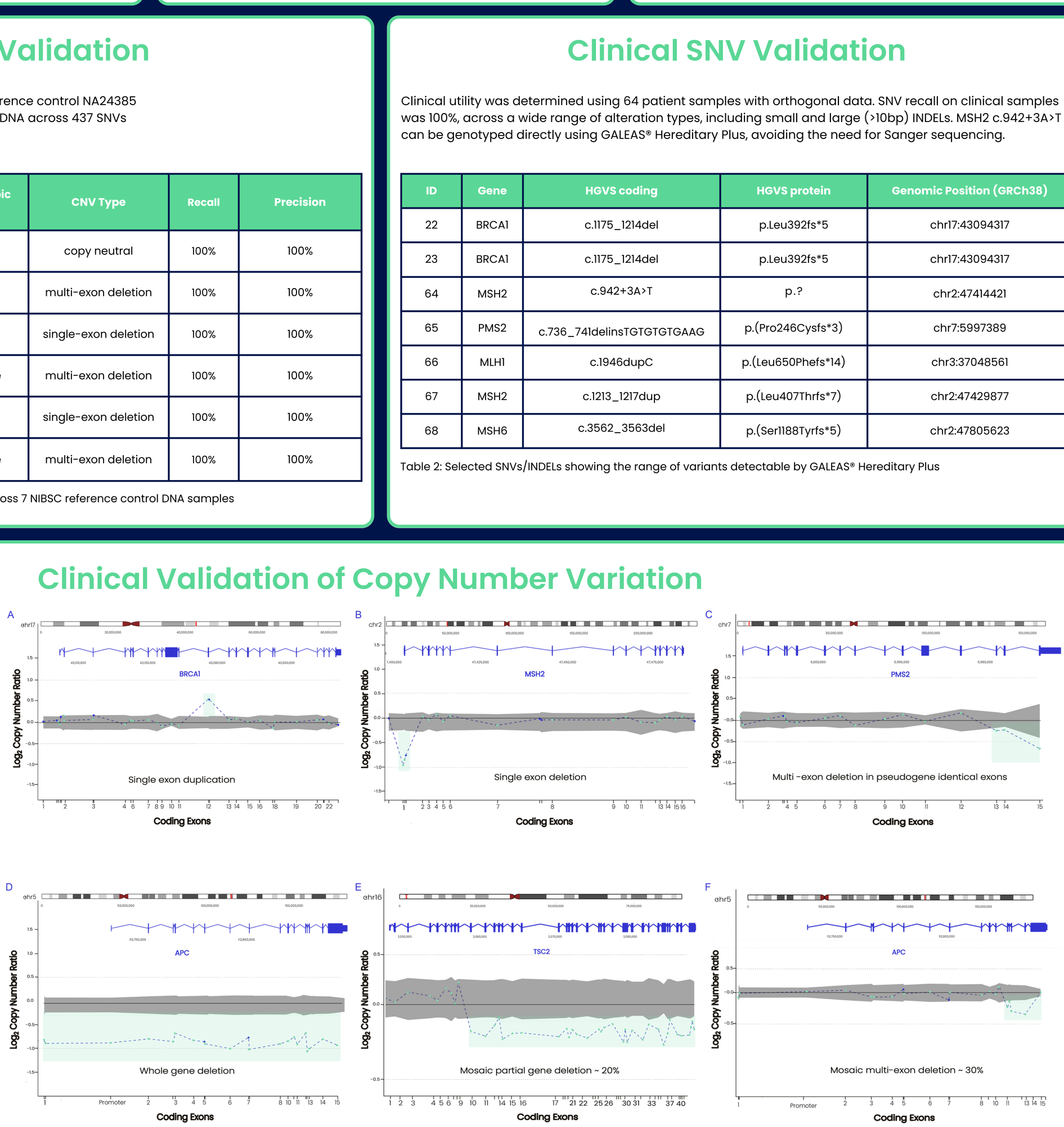


Figure 1: Selected CNV profiles for
A) BRCA1 single exon duplication
B) MSH2 single exon deletion
C) PMS2 multiple exon deletion in pseudogene
D) APC whole gene deletion
E) TSC2 mosaic partial gene CNV ~ 20%
F) APC mosaic partial gene CNV ~ 30%

## Summary

- GALEAS® Hereditary Plus shows high concordance with orthogonal sequencing and MLPA data.
- Accurate detection of SNVs and INDELs and accurate calling across a wide range of CNVs, from single exons to whole genes.
- Efficient differentiation of PMS2 and PM2CL pseudogenes across CNVs and SNVs.
- Potentially removes the need for MLPA for the clinical identification and reporting of CNVs.

## Key Points

- GALEAS® Hereditary Plus provides a cost-effective tool for the profiling and analysis of heritable cancer syndromes and cancer risk profiling.
- Highly curated design to improve CNV detection.
- Bespoke software tool allows the sensitive and accurate detection of even the most challenging SNVs and CNVs.
- Reduces the need for costly and time consuming MLPA.