Identification of germline variants for hereditary cancer using a comprehensive NGS Panel

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Introduction

- Understanding the genetic basis of cancer risk is widely accepted.
- Hereditary cancer accounts for approximately 5-10% of all cancers.
- The use of targeted NGS-based multigene panels to provide the comprehensive analysis of cancer susceptible genes has proven to be a clinical viable option.
- 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.
- The Cell3 Target Hereditary Cancer Panel (HCP) allows the comprehensive analysis of SNV and CNVs linked to inherited cancer syndromes, providing a simple streamlined tool for cost-effective cancer risk profiling.

Methods

- 25ng of genomic DNA from 68 anonymised patient and 2 reference DNAs.
- Libraries were prepared using the the Nonacus Cell3 library prep kit. Sequenced on a MiSeq to a mean coverage of 100x .
- Mapping was performed using Sentieon® accelerated bwa-mem with GRCh38 reference Human Genome
- Germline SNV/INDEL calling was performed using Sentieon® HaplotypeCaller
- Copy Number alterations were identified with cn.mops and ExomeDepth. A panel of normal samples was created using 50 samples with no reported familial conditions.

Panel Design

- The Cell3 Target HCP comprehensively covers 129 genes with know associations to hereditary cancers.

Panel Validation

- Cell3 Target HCP was validated on reference DNAs (4 replicates)
- Cell3 Target HCP recall on reference DNA across 437 SNVs
  - SNV – 99.7%
  - INDEL – 100%

CNV	Genotypic Sex	CNV Type	Recall	Position
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 amplification	100%	100%

Table 1. CNV Recall rate for copy number alterations in reference controls

- The Cell3 Target HCP out-performs leading commercial alternative.

Key Quality Indicator	Nonacus HCP	Competitor 1
Number of Genes	129	113
MB required for mean 100x coverage	78.1MB	116.6MB
Percent coverage >30x	98%	96%
Percent on bait	77.6%	37.0%
Percent on or near bait	91.0%	61.5%
Percent Duplication	3.0%	9.0%
SNV Recall	99.7%	98.1%
Indel Recal	100.0%	97.2%

Table 2. Sequencing metrics for the Nonacus Cell3 Target HCP and the leading commercial alternative.

Clinical SNV Validation

- Clinical utility was assessed using 68 patient samples with orthogonal data
- SNV recall on clinical samples 100%, across a wide range of alteration types, including small and large(>10bp) indels.
- MSH2 c.942+3A>T can be genotyped directly using Cell3™ Target HCP, avoiding the need for Sanger sequencing.

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_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 3. Selected SNVs/indels showing the range of variants detectable by the Cell3 Target HCP.

Clinical Copy Number Variation

- Cell3 Target HCP accurately identifies CNVs from single exons to whole genes in key cancer syndrome susceptibility genes.
- CNV detection in 60 clinical samples :
  - Sensitivity 96.6%
  - Specificity 99.6%

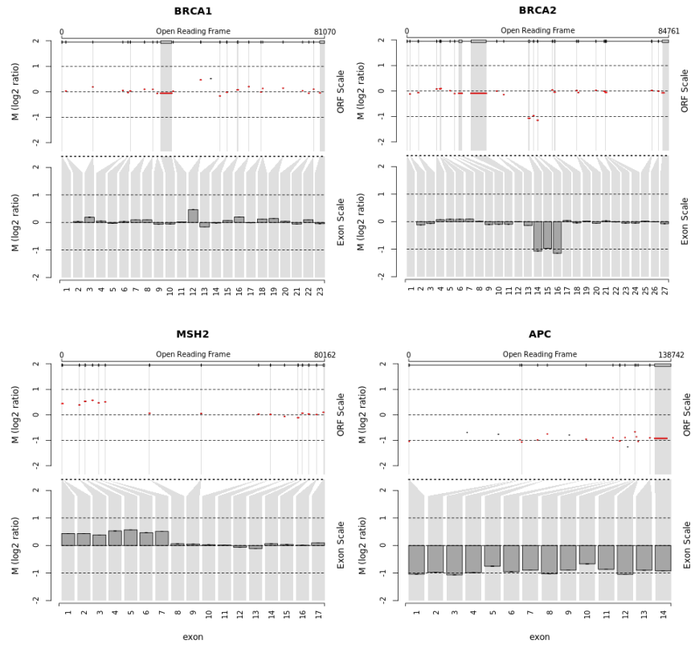

Figure 1. Selected copy number profiles for patients with *BRCA1*, *BRCA2*, *MSH2* and *APC* CNVs

Summary

- The Cell3™ Target HCP shows high concordance with orthogonal sequencing and MLPA data.
- Reduces the need for MLPA for identification of CNVs.
- Provides ~33% reduction in sequencing costs compared with comparable commercial alternatives (32 samples per MiSeq V3).
- Cell3™ Target Hereditary Cancer Panel provides a robust, low cost, automatable workflow for the detection of variants associated with hereditary cancer syndromes.

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