Cell3™ Target

Cell-Free DNA Target Enrichment System

Highlights

Cell-Free DNA (cfDNA) optimized Target Enrichment System

Developed for, and validated on, ctDNA and cffDNA for both oncology and prenatal applications. Also validated on gDNA, FF and FFPE to enable testing of matched samples and from as little as 1 ng input.

Reduced cost and manufacturing time with NGS validation of all panels

Novel manufacturing method enables rapid delivery, and QC validation by NGS ensures uniformity of sequencing coverage for custom NGS enrichment panels.

Highest confidence calling of ultra-low frequency somatic or mosaic germline variants

Built in molecular identifiers allow accurate and confident calling of ultra-low frequency mutations down to 0.1% VAF. Dual indexing approach provides further confidence through complete removal of mis-assigned index reads caused by index hopping; further reducing false positive mutation calls.

Small, medium or large? Optimized target enrichment ensures highest performance with even the smallest designs

Cell3 Target has been optimized to deliver efficient on target capture regardless of panel size from 1 gene to >1,000 genes to ensure unrivalled on target performance.

Flexible protocol includes enzymatic shearing of FFPE/FF and gDNA or no shearing for cfDNA

Cell3 Target library preparation includes options for enzymatic fragmentation (FFPE/FF and HMW gDNA), no shearing for ctDNA /cffDNA or Covaris shearing for gDNA.

Introduction

The utility of cell-free DNA (cfDNA) in both translational research and diagnostic settings has increased dramatically since its discovery. Areas of research involving cfDNA are diverse and include prenatal, oncology, transplantation and ageing. Often the predominant interest is mutational analysis of selected genomic regions and for the detection of both low <20% VAF and ultra-low <5% VAF mutations and for the study of either somatic cancer or germline mosaic mutations.

While detection of low frequency mutations <20% VAF has been possible with NGS methods for a number of years, accurate detection of ultra-low frequency mutations has been hampered by excessive false positive or artefactual variant calls below 5% VAF arising from cumulative sequencing and PCR errors.

In addition, more recently[1] an Illumina Sequencing chemistry artefact termed index hopping has been identified as causing artefact mis-assigned reads at between 0.1-2% with the potential of false positive calls when looking for ultra-low frequency mutations. Index hopping has more recently been confirmed by Illumina.[2]

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The need to accurately call all variants both above and below 5% VAF has led Nonacus to develop a new target enrichment approach validated on cell-free DNA (ctDNA and cffDNA), FFPE and FF samples to ensure confident calling of all mutations down to 0.1% VAF.

Reduced cost and manufacturing time along with NGS validation of all custom panels

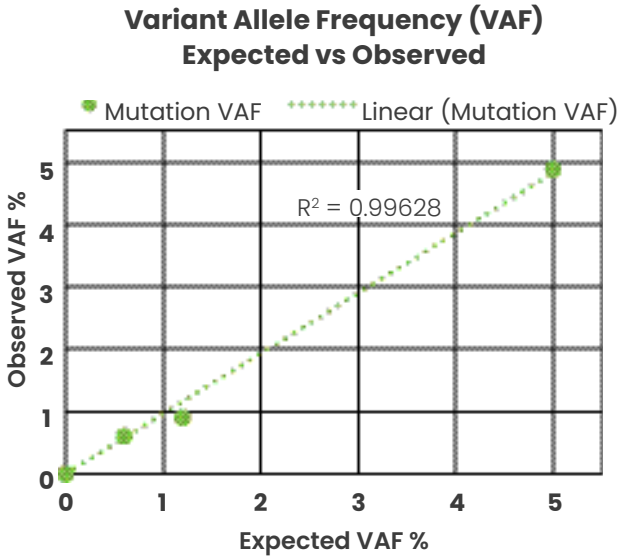
Cell3 Target manufacture process enables customers to receive completely custom target enrichment panel designs within weeks. Minimum orders of 48 sample custom panels enable content to be optimized to meet research or patient needs. By default custom panels are NGS validated to ensure the uniformity of coverage meets our QC requirements.

Validated, quick and convenient workflow

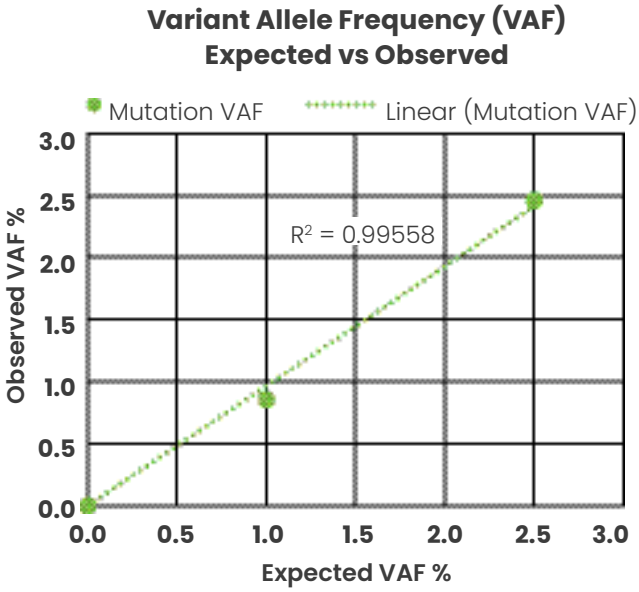
Cell3 Target offers quick, Covaris free, enzymatic shearing for FFPE/FF samples and no requirement to shear for cell-free DNA (ctDNA or cffDNA). The quick and easy workflow taking less than 10 hours, with less than 2 hours hands-on time, enables manual or automated preparation of between 1 – 96 samples in a single batch and with 384 sample indexes available.

Robust, low frequency variant detection

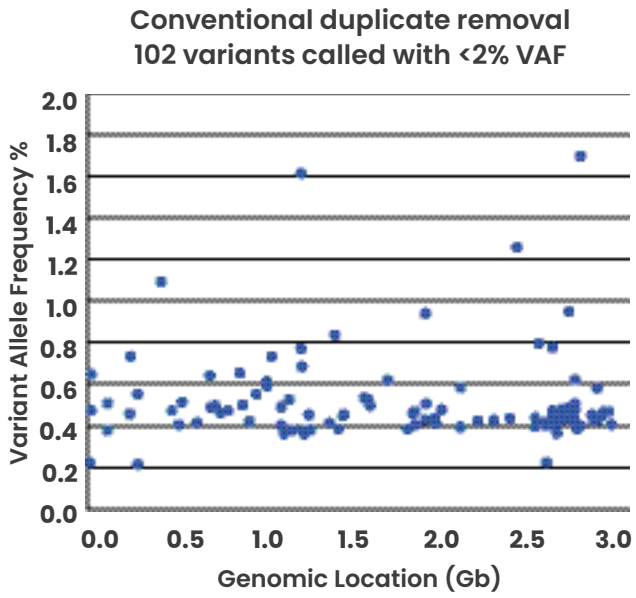
Accuracy and sensitivity of the Cell3 Target technology was tested on commercially available ctDNA reference standards (SeraCare) containing multiple variants at different allele frequencies down to a known VAF of 0.6%. Targeted enrichment was performed using the Cell3 Target Cancer Panel 50, which targets the exonic region of 50 well characterized cancer related genes. The following graph shows the detected vs expected variant frequencies for the SeraCare ctDNA wild-type, 0.6%, 1.2% and 5% VAF reference standards (average sequencing depth of 1,000x consensus reads).

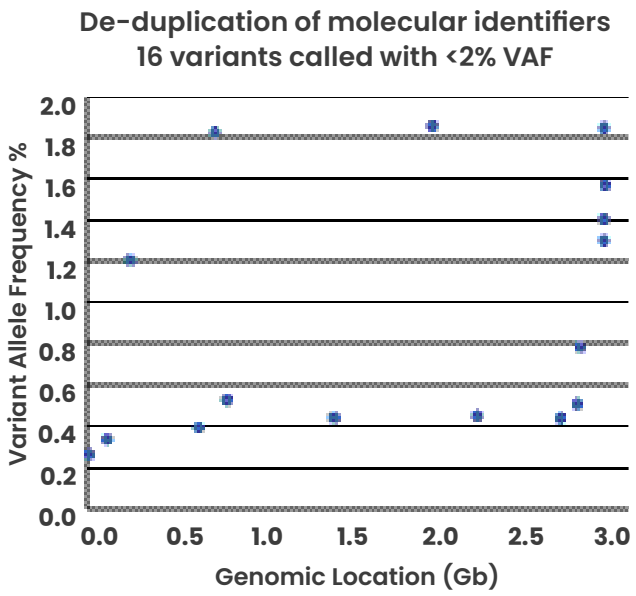


A similar analysis was also conducted after Cell3 Target enrichment using a single gene capture panel (HBB) on artificially prepared genomic DNA samples containing SNVs on the HBB gene at a known VAF of 0%, 1% and 2.5%. The graph below shows a consistent correlation between observed and expected VAF for each sample.



The advantage of using unique molecular identifiers was demonstrated by comparing the amount of variants called after conventional duplicate removal and after de-duplication with molecular identifiers. Cell3 Target enrichment was performed using a 40 kb panel on a cfDNA sample obtained from a pregnant woman. A read family size of three or more reads tagged with the same molecular identifier was used to build a consensus read. The graphs below show an 84% reduction in variants called with <2% VAF when using de-duplication of molecular identifiers during data analysis compared to conventional duplicate removal.



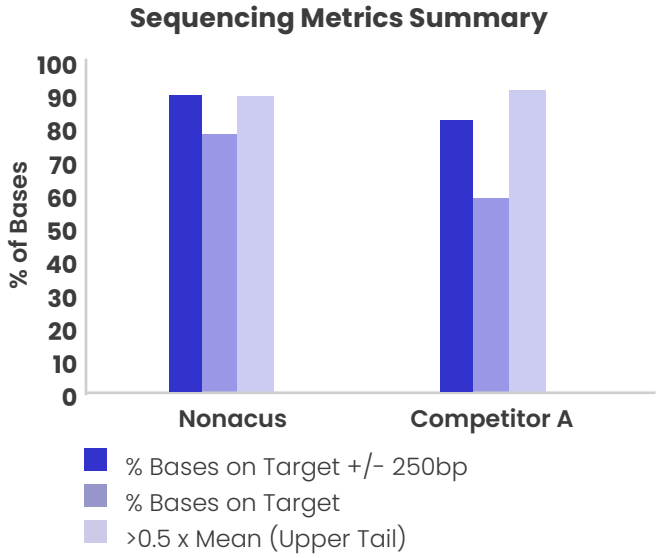


Optimized for small and large panels

Most capture target enrichment technologies suffer from a significant percentage of off target sequencing reads when targeting small regions of the genome. Cell3 Target has been developed so that regardless of whether you are wishing to sequence 1 gene or 1,000 genes you will find lower off target and more uniform coverage when compared with alternative capture technologies.

A 40 Kb panel was designed using both Cell3 Target technology and Company (A) High Sensitivity enrichment kit. Targeted enrichment was performed on genomic DNA samples and sequencing was conducted on the Illumina MiSeq.

The histogram below shows equivalent coverage uniformity and increased on target capture % when compared to Company (A).

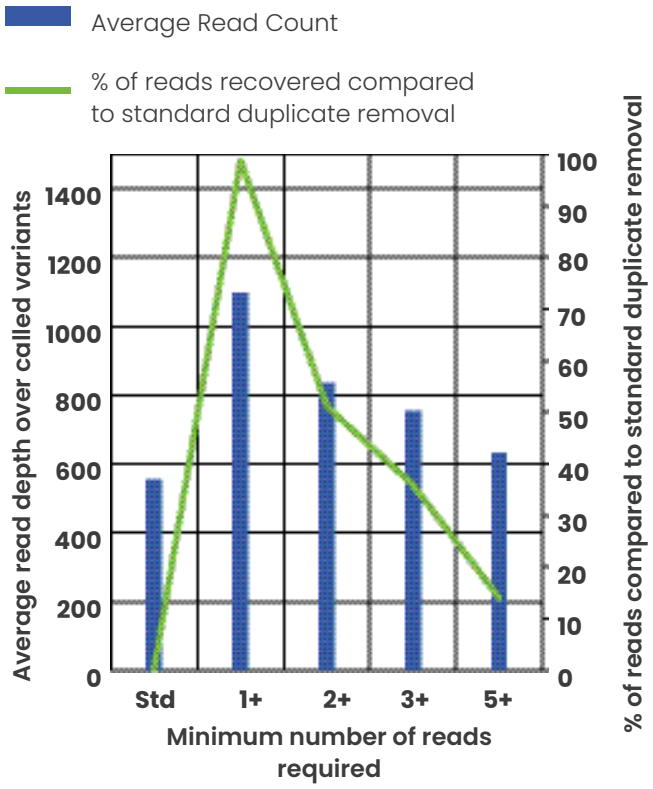


Molecular identifiers can improve coverage of constitutional, somatic or inherited cancer variants

Standard analysis of sequencing data generated by target enrichment methods involves the removal of duplicate reads which have identical start and stop genomic coordinates. Cell3 Target uses molecular identifiers to tag individual molecules prior to PCR; enabling the recovery of unique molecules which have identical start and stop coordinates.

To measure the quantity of reads recovered by using molecular identifiers, sequencing data generated on the Illumina NextSeq (using 25 ng of SeraCare ctDNA reference standard as input and the Cell3 Target Cancer Panel 50 for targeted enrichment) was analyzed using both approaches. The average depth of reads containing called variants is compared in the chart below. The blue columns represent the average unique read count obtained after standard duplicate removal and de-duplication by molecular identifiers with a minimum of 1, 2, 3 and 5 reads required to build a consensus read. The overlaid green line chart represents the % of reads recovered by using molecular identifiers compared to standard duplicate removal. Use of one or more reads to create a consensus read enables the recovery of >98% of reads compared to standard duplicate removal in this case, thus allowing to halve sequenced.

Read count recovery using molecular identifiers



Learn more

To learn more about the Cell3™Target and to download the protocols, application notes, and white papers please visit: www.nonacus.com

References

1: doi: https://doi.org/10.1101/125724 Index Switching Causes 'Spreading-Of-Signal' Among Multiplexed Samples In Illumina HiSeq 4000 DNA Sequencing

2: https://www.illumina.com/content/dam/illumina marketing/documents/products/whitepapers index hopping-white paper-770-2017-004.pdf

Ordering information

Cell3 Target Enrichment System is available as Catalogue Panels, with fixed gene content and immediate availability, or Custom Panels with customer specified gene content and rapid delivery.

All Cell3 Target panels are available with two fragmentation options;
1) non-fragmentation eg (cffDNA/ctDNA),
2) fragmentation eg gDNA or FFPE,
Currently available Cell3 Target products are available at www.nonacus.com. For the most up to date information please visit our website or email: www.nonacus.com or email info@nonacus.com.

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