HaloPlex^{HS}

Get to Know Your DNA. Every Single Fragment.

High sensitivity rare variant detection using molecular barcodes

Key Benefits

Unparalleled Sensitivity

- Uniquely tag DNA fragments with more than a million 10-nt molecular barcodes
- Confidently detect mutations present at below 1% allele frequency

Superior Accuracy

- Differentiation of true variants from PCR or formalin-fixation artifacts by targeting both DNA strands
- High on-target specificity and deep coverage of target bases so key variants are not missed

Accelerated Solution

- Complete target enrichment in less than 6 hours from only 50 ng of gDNA
- Streamline analysis using SureCall Data Analysis Software

Overview

The detection of somatic variants in heterogenous cell populations is crucial in a wide variety of applications such as evolution, the study of immunological diversity and particularly, in cancer genetics where genetic heterogeneity is believed to be the driver of cancer initiation and progression[1]. Unlike inherited mutations, somatic variants often occur at very low allele frequencies that require sensitive methods for detection. Next generation sequencing (NGS) now allows for parallel high resolution sequencing of billions of nucleotides that has revolutionized the field of genetics. Although theoretically, subclonal DNA populations of any size should be detectable when deep sequencing sufficient numbers of molecules, the identification of rare variants is practically limited by the error rate associated with the sequencing process[2].

HaloPlex^{HS} is a high sensitivity targeted sequencing method based on the HaloPlex next generation PCR technology that incorporates >10^6 unique molecular barcodes in the DNA library, allowing for the identification of duplicate reads hence significantly improving base calling accuracy even at low allelic frequencies compared to conventional NGS methods. Figure 1 shows the detection of allele frequencies down to 0.5% in HapMap mixtures at fixed ratios.

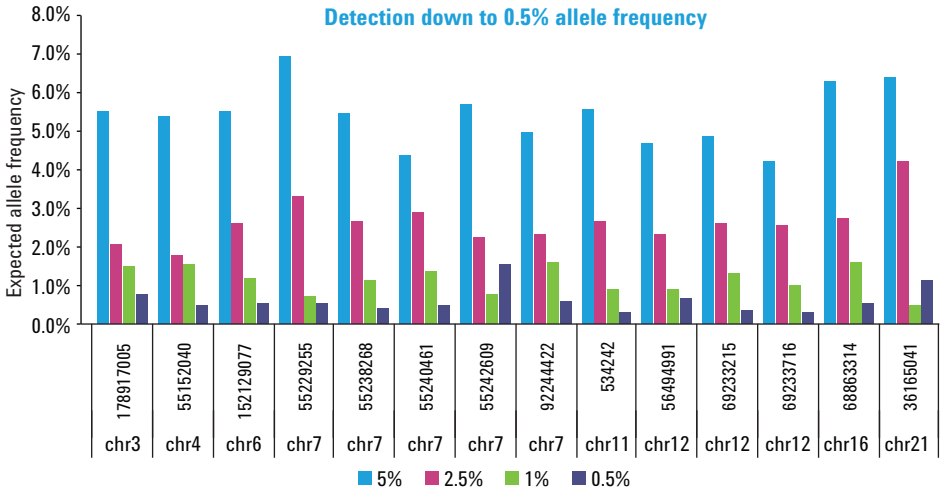

Figure 1. HapMap cell lines, NA18507 and NA10831, were mixed to generate allelic fractions ranging from 0.5% – 5%. The close agreement between expected and observed frequency at various chromosomal positions demonstrates the high sensitivity of HaloPlex^{HS} for low frequency variant detection. Data shown is representative of replicates (sequencing depth = 2000x – 4000x)

1. Kennedy, S.R et al (2014) **Nature Protocols** 9(11) : 2586 – 2606
2. Gundry, M and Vijg, J (2012) **Mutation Research** 729 : 1-15

Excellent Performance and Accuracy

HaloPlex^HS provides high coverage of target bases and on-target specificity plus excellent uniformity in both high quality and FFPE samples, ensuring that variants of interest are detected without having to perform excessive sequencing (Figures 2A and B). In addition to the unique molecular barcoding of each DNA library fragment, superior accuracy is achieved by the targeting of both DNA strands which allows for differentiation of true variants from PCR or formalin-fixation artifacts. Combined with the ability to easily create gene panels of interest through customization using SureDesign and compatibility with leading benchtop sequencing platforms, HaloPlex^HS is a flexible and high sensitivity assay well-suited for clinical research.

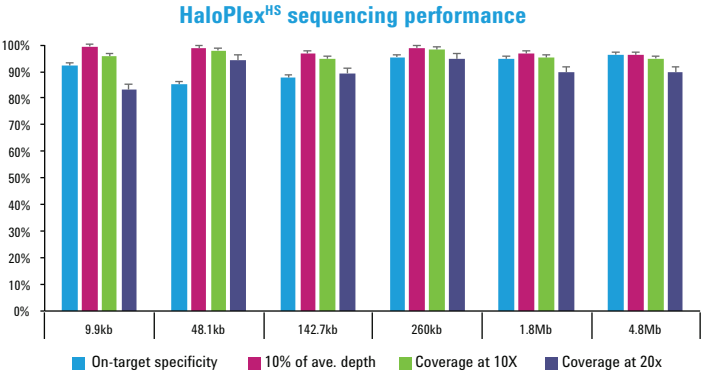

Figure 2A. High on-target specificity (>85%) and uniform coverage of target bases (>95% covered at 10% of average depth) ensure that variants of interest are detected with confidence. Data is representative of duplicates and normalized to 200x average depth.

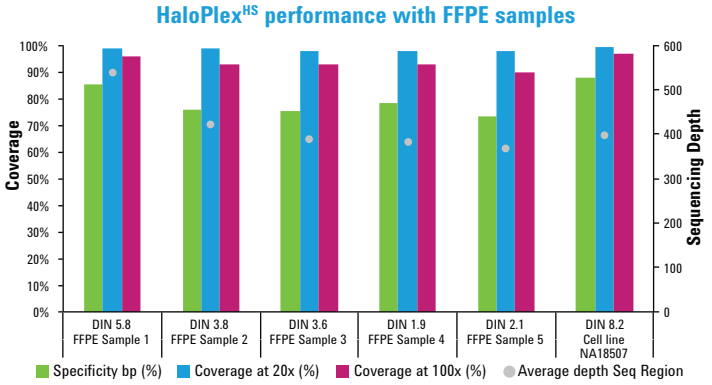

Figure 2B. Excellent coverage of target bases (>90% covered at 100x) even with poor quality FFPE DNA. A custom cancer panel was used to enrich FFPE DNA of varying qualities as indicated by the 2200 Tapestation DNA Integrity Number (DIN), where a DIN of 10 and 1 indicate intact gDNA and completely fragmented gDNA respectively.

Accelerated Solution

SureCall 3.0 software is optimized for the analysis of NGS data generated using HaloPlex^HS. SureCall performs alignment, de-duplication of reads and variant calling using streamlined, guided workflows (Figure 3). When taken together with an efficient HaloPlex^HS workflow which allows for complete target enrichment in less than 6 hours, time to results is reduced.

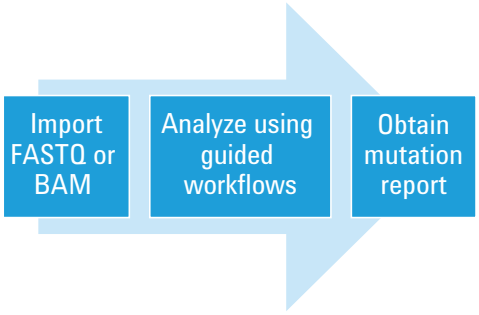

Figure 3. Accelerate time-to-results with a simplified 3-step analysis workflow using SureCall .

Ordering Information:

HaloPlex^HS is available for custom, catalog and made-to-order designs.

Request more information or buy online:
www.agilent.com/genomics/haloplexhs

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PR7000-0011

Printed in USA, December 1, 2015
5991-5551EN