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Trusted Answers

HaloPlex^{HS} Publications

HaloPlex^{HS} is a high-sensitivity, amplicon-based targeted sequencing platform that incorporates molecular barcodes tagging every molecule in the DNA library. This approach not only allows for the identification of duplicate reads but also enables correction of errors that arise during PCR and sequencing to achieve high sensitivity and accuracy of variant-calling.

The HaloPlex^{HS} product offers the flexibility to create custom panels up to 5 Mb in size. It features an accelerated workflow of less than 6 hours, starting with as little as 50 ng gDNA, and supports integrated data analysis using the Agilent SureCall software.

The following research publications cite the HaloPlex^{HS} product:

1 **Hirsch P, *et al*. Precision and prognostic value of clone-specific minimal residual disease in acute myeloid leukemia. *Haematologica* (2017) 102:1227-1237. DOI: 10.3324/haematol.2016.159681.**

Application: Minimal residual disease monitoring of AML

Summary: The genetic landscape of acute myeloid leukemia (AML) is highly heterogeneous. Evaluation of minimal residual disease (MRD) by detecting residual mutations in small number of cells during complete remission can have prognosis significance. The authors first determined the clonal architecture of AML cases using cytogentics, FISH and NGS combining a 122-gene HaloPlex panel and exome sequencing. Then HaloPlex^{HS} panels and FISH were used to detect the mutations and chromosomal aberrations in the follow-up samples from these cases that entered complete remission. This clone-specific strategy enabled the detection of lesions down to 0.5 - 0.4 variant cell frequency. The study found that persistence of 2 and more clonal lesions strongly associated with poor prognosis and higher risk of relapse.

2 **de Kock L, *et al*. High-sensitivity sequencing reveals multi-organ somatic mosaicism causing DICER1 syndrome. *J Med. Genet.* (2016) 53:43-52.**

Application: Detection of somatic mosaicism of low allele frequencies

Summary: Mosaic DICER1 mutations are an important cause of the DICER1 syndrome. However very low frequency mutations are difficult to detect. Using a HaloPlex^{HS} panel targeting miRNA-processing-associated genes followed by deep sequencing, somatic mosaic mutations of 0.24 - 31% were detected in constitutional samples of 3 out of 4 patients with DICER syndrome. This study employed multiple technologies for cross-platform validation and demonstrated the utility of HaloPlex^{HS} assays for FFPE-derived samples with the sensitivity required to detect variants of ultra-low allele frequencies.

3 **Xu, L., *et al*. Acquired mutations associated with ibrutinib resistance in Waldenström macroglobulinemia. *Blood* (2017) 129: 2519-2525. https://doi.org/10.1182/blood-2017-01-761726.**

Application: Detection of acquired somatic mutations associated with drug resistance

Summary: Waldenstrom macroglobulinemia (WM) with *MYD88* mutations show high level response to ibrutinib treatment. However, ibrutinib-resistance can develop in cases with WM. Ibrutinib-resistance in chronic lymphocytic leukemia (CLL) has been reported to be associated with acquired mutations in *BTK*. Using targeted deep sequencing by HaloPlex^{HS} interrogating *BTK* and seven other candidate genes, the authors detected multiple *BTK* mutations and two novel mutations with allele frequencies as low as 0.3% in 3 out of 6 cases that progressed on ibrutinib. Further, screening of 38 cases on ibrutinib without progression at the time using this HaloPlex^{HS} panel identified *BTK* mutations in 2 cases that subsequently progressed. These results suggest acquired mutations in *BTK* may contribute to the ibrutinib-resistance in WM cases.

4 **Davey, S, *et al*. Simultaneous human platelet antigen genotyping and detection of novel single nucleotide polymorphisms by targeted next-generation sequencing. *Transfusion* (2017) 57:1497–1504. doi:10.1111/trf.14092.**

Application: Human Platelet Antigens (HPAs) genotyping

Summary: A HaloPlex^{HS} panel was designed to target all exons plus 50 bp flanking sequences of 6 genes known to encode HPAs. Using the single sample analysis workflow of the Agilent SureCall software, the authors obtained full genotypes of 45 historically HPA-typed samples with this targeted NGS panel at 100% concordance. Additional novel mutations were also identified by comparing the HPA sequences between mothers and babies from suspected neonatal alloimmune thrombocytopenia (FNAIT) cases, using the tumor/normal paired analysis in SureCall. This targeted NGS-based approach enables cost-effective and accurate full HPA genotyping and identifications of novel mutations simultaneously.

5 **Ushijima K, *et al*. The Japanese Study Group of Insulin Therapy for Childhood Adolescent Diabetes. Comprehensive screening for monogenic diabetes in 89 Japanese children with insulin-requiring antibody-negative type 1 diabetes. *Pediatr Diabetes* (2017) 0:1–8. https://doi.org/10.1111/pedi.12544.**

Application: Screening for mutations in genes associated with monogenic diabetes

Summary: Using a HaloPlex^{HS} panel targeting 30 genes known to cause monogenic diabetes, this group identified likely pathogenic mutations in 11 out of 89 cases with childhood autoantibody-negative, insulin-requiring type 1 diabetes. The results of this study suggest significant genetic overlap between monogenic diabetes and autoantibody-negative type 1 diabetes.

6 **Niceta, M, *et al*. Biallelic mutations in DYNC2LI1 are a rare cause of Ellis-van Creveld syndrome. *Clin Genet.* (2017) Aug 30. doi:10.1111/cge.13128.**

Application: Scanning of mutations in candidate genes of Ellis-van Creveld syndrome (EvC)

Summary: Exome sequencing of a family with EvC diagnosis, but free of mutations in the previously identified EvC genes, identified DYNC2LI1 as a gene that highly likely underlie the co-segregation of EvC in this family. Subsequently, a HaloPlex^{HS} panel targeting DYNC2LI1 and other 67 candidate EvC genes was used to screen additional 24 subjects with clinical EvC features. Results from targeted sequencing identified novel and existing mutations in DYNC2LI1 overlapping with the findings from the exome sequencing in 3 individuals, suggesting that a specific combination of mutations in DYNC2LI1 may account for a small subset of EvC cases.

7 **Marsh, AP, *et al*. Mutations in DCC cause isolated agenesis of the corpus callosum with incomplete penetrance. *Nat Genet.* (2017) 49:511-514. doi: 10.1038/ng.3794.**

Application: Scanning of mutations in genes associated with brain malformation

Summary: Following linkage analysis and exome sequencing that identified mutations in DCC in families with isolated agenesis of the corpus callosum (ACC), a HaloPlex^{HS} panel of 287 genes, including DCC, associated with brain malformation was used to scan unrelated individuals with ACC. Five out of the 70 scanned individuals were found to have at least one heterozygous DCC mutation altering a conserved amino acid.

8 Kline AD, *et al*. Cornelia de Lange syndrome and molecular implications of the cohesin complex: Abstracts from the 7th biennial scientific and educational symposium 2016. *Am J Med Genet Part A*. (2017) 173A:1172–1185. https://doi.org/10.1002/ajmg.a.38161.

Application: Analysis of mosaicism in CdLS

Summary: Corenelia de Lange Syndrome (CdLS) is a development disorder that causes a range of physical and cognitive disabilities. Not all CdLS cases have been identified with causative mutations. One of the factors could be the insufficient sensitivity of current screening methods to detect mosaic mutations. The authors used a HaloPlex^{HS} panel targeting genes that are or likely are pathogenic for CdLS to assess the contribution of mosaicism to CdLS. Furthermore, the mutation-negative cases defined by this high sensitivity HaloPlex^{HS} assay would be candidates for whole exome sequencing for discovery of new CdLS causative genes.

To learn more about custom and catalog HaloPlex^{HS} products, visit **www.agilent.com/genomics/haloplex**

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