Simultaneous detection of copy number variation (CNV) and point mutations with next generation sequencing (NGS) using Agilent HaloPlex custom designs

Christine Haag[1], Susanne Rondot[1], Marvin Schanz[1], Kai Hauschulz[2], Joachim Strub[3], Egbert Schulze[1]

[1] Endocrine Practice, Heidelberg, Germany
[2] Agilent Technologies Sales & Services GmbH & Co. KG, Germany
[3] JSI medical systems GmbH, Kippenheim, Germany

Introduction

Copy number variation (CNV) is a frequent form of structural variation in the human genome. Usually, CNV refers to the duplication or deletion of DNA segments larger than 1 kbp. Copy number variations have been recognized as pathogenic mutations for many years. Alterations in DNA copy number are also a common feature in many cancers and the detection of these changes shows promise for the diagnosis of a disease and also for therapeutic or prognostic purposes.

Methods

We used the Agilent HaloPlex Target Enrichment system (on the Illumina MiSeq platform) for the combined detection of point mutations and copy number variations. Copy number variations are detected by comparing the coverage of regions in the sample of patients with control samples. The copy number variation analysis was performed with the CNV function of the Sequence Pilot software (JSI Medical Systems GmbH, Kippenheim, Germany).

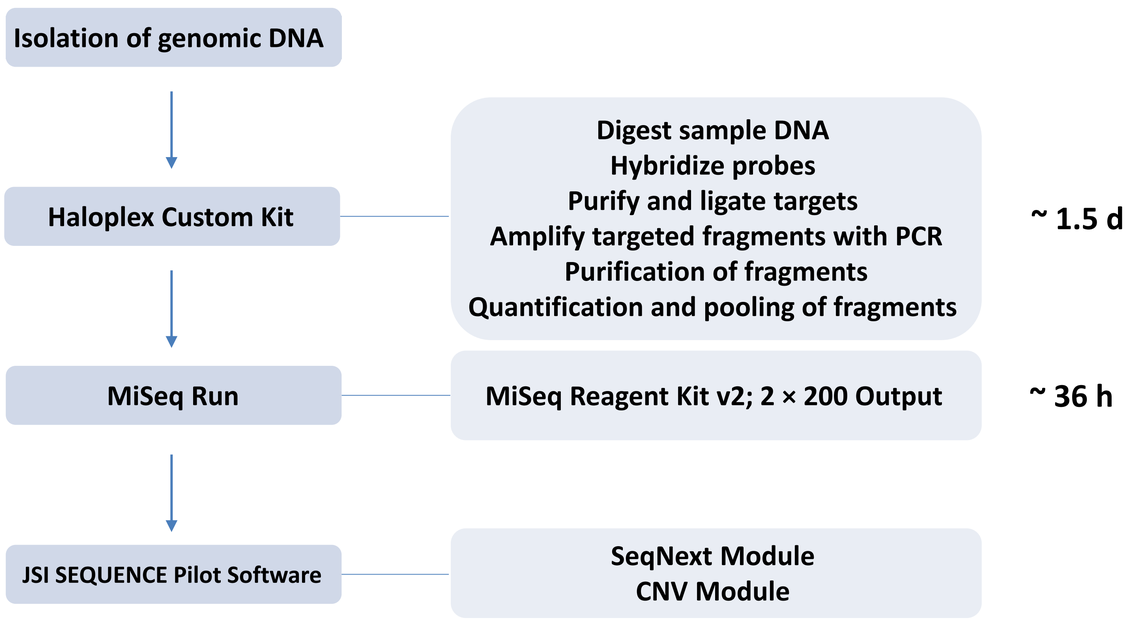
Results

We used three different Haloplex custom designs including 19 genes for hereditary endocrine diseases like pheochromocytoma or multiple endocrine neoplasia for the validation of the Haloplex assay. Altogether, we analyzed 45101 bp covered by 2999 amplicons.
The Haloplex assay was validated using known positive control samples (analyzed by MLPA and Sanger Sequencing) comprising samples with point mutations, exon duplications/deletions as well as indels. All mutations were successfully identified with a high coverage (>1000 fold average exon coverage) and 99.2% of the targeted bases were covered with a least 50 reads. Regions with no coverage account for 0.5%.

Summary

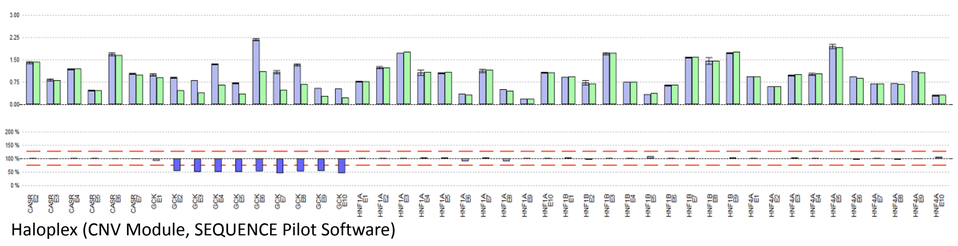
In summary, Haloplex custom designs can be used as a targeted NGS resequencing approach that facilitates the detection of point mutations, indels and duplications/deletions in parallel.

Workflow

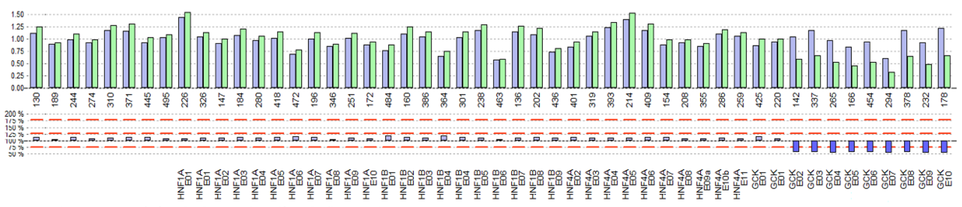


Haloplex Custom Kits	Detected CNV
Kit 1	
MAX	
MEN1	Heterozygous deletion ex 7 Heterozygous deletion ex 6-10
RET	
SDHAF2	
SDHB	Heterozygous deletion ex 1-3
SDHC	
SDHD	Heterozygous deletion ex 1
TMEM127	
VHL	Heterozygous deletion ex 1-3
Kit 2	
CASR	
GCK	Heterozygous deletion ex 2-10
HNF1A	
HNF1B	Heterozygous deletion ex 1-9
HNF4A	
Kit 3	
LDLR	Heterozygous duplication ex 2-8 Heterozygous deletion ex 13-14 Heterozygous deletion ex 16-18 Heterozygous deletion ex 3-6
CDC73	
STAR	
HSD3B2	
PHEX (X-chromosome)	Heterozygous duplication ex 1-22 Heterozygous duplication ex 4-12

example of a deletion of 9 exons (GCK gene)

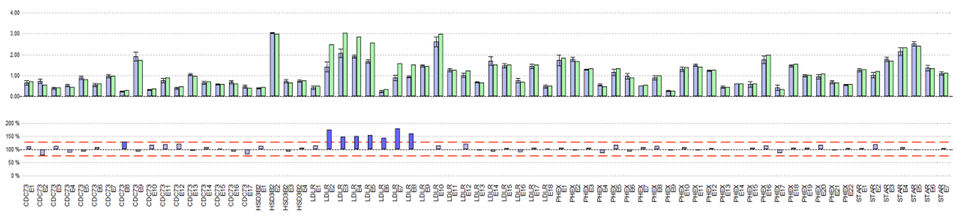


Haloplex (CNV Module, SEQUENCE Pilot Software)

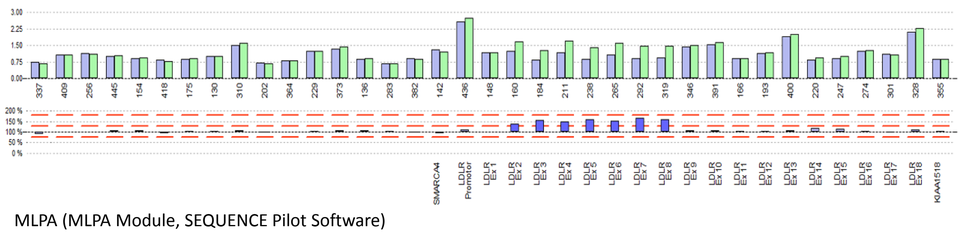


MLPA (MLPA Module, SEQUENCE Pilot Software)

example of a duplication of 7 exons (LDLR gene)



Haloplex (CNV Module, SEQUENCE Pilot Software)



MLPA (MLPA Module, SEQUENCE Pilot Software)