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CGT Laboratory

OncoPed

NGS genetic test developed by Cogentech's CGT Laboratory

Introduzione

OncoPed OncoPed is the innovative molecular test proposed by Cogentech, based on an advanced technological process called Next Generation Sequencing (**NGS**), which enables the detection of variations at the level of the patient's genome. These variations include both point mutations (substitutions, small deletions/insertions, or SNVs) and large rearrangements (CNVs) due to deletion/duplication of one or more exons of the genes under investigation. The test is offered at the **germline level** and includes analysis of genes involved in **predisposition** to pediatric cancers, although the technology can also detect variants at the somatic level. The panel allows for molecular diagnostic insights into the predisposition genes for medulloblastoma, Wilms' tumor, some rare ovarian cancers (Sertoly-Leydig and SCCOTH), retinoblastoma, uveal melanoma/melanoma, renal tumors, some lymphomas, rhabdoid tumors, and tumors that develop in Gorlin, DICER1 and Carney syndromes.

The genes included in the current version of the panel are listed below:

SUFU, PTCH1, DICER1, RB1, BAP1, POT1, PRKAR1A, SMARCB1, SMARCA4, TP53, APC, MUTYH, CDKN2A, CDK4, MITF, PTEN, VHL, WT1, FLCN, MET, FH, FBXW7, BRCA1, BRCA2, PALB2, CDH1, MLH1, MSH2, MSH6 and *PMS2*.

How the test is carried out

The test is performed on genomic DNA extracted from a patient's blood sample (**germ sample**) subsequently enriched for the genes of interest by means of the technique termed 'capture,' in which probes designed through a criterion are used to specifically select only well-defined regions. In detail, the probes were created by exploiting Agilent Sure Select technology, including the nucleotide regions of the exons of the selected genes and at least 20 bases of the adjacent intronic regions.

The set of genomic fragments 'captured' by the probes represents the enriched regions of the genes of interest (library), which are subsequently sequenced through a process employing NGS techniques and the use of Illumina's **MiSeq Dx** or **NextSeq 550 Dx** instruments.

Variants found are confirmed by direct Sanger sequencing or by Multiplex Ligation-dependent Probe Amplification (MLPA), on second aliquot of blood, if they are VUS (variants of uncertain significance) or pathogenic variants. In the case of SNV variants in PMS2, Long Range PCR is expected to be used to select the gene against pseudogenes. The ABI3500 Dx Genetic Analyzer platform (Applied Biosystems) is used for both Sanger method sequence identification and MLPA fragments. Computer processing of these data is done with software is Mutation Surveyor and Gene Marker, respectively, both sold by the company SoftGenetics

Bioinformatics analysis

At the end of the NGS sequencing phase, the data undergo a series of advanced **bioinformatic analysis** that include:

- The *primary analysis*, that is, the generation of sequences (*reads*) and the evaluation of their quality;
- the *secondary analysis*, which consists of pairing the data obtained with the corresponding regions of the genome chosen as reference (GRCh37-hg19): the presence of any SNV or CNV variants is defined;
- *tertiary analysis*, which allows the interpretation of variants, which is eventually followed by the issuance of a molecular diagnostic report.

While the primary analysis is performed by the NGS sequencer, the others are performed by an automated procedure (*pipeline*) developed in collaboration with an expert firm in the field (enGenome). The *pipeline* was created so as to:

- Highlight any regions of interest with low sequencing coverage: these areas will be reanalyzed by sequencing with the Sanger method to always ensure 100% coverage of the region of interest;
- Generate results related only to the required genes.

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Panel verification

The robustness of the **OncoPed** panel was verified by comparing the data obtained with this panel with those obtained from the 'analysis of the same sample examined with the OncoPan® panel or with a different NGS approach (WES, Whole Exon Sequencing). *Specificity* and *sensitivity* values were assigned, investigating some genes common to all approaches, relative to coding exons and adjacent intronic sequences (-21 or +7 base pairs from splicing junctions, for SNVs).

	SNV	CNV
Sensitivity	99.9%	99.9%
Specificity	100%	99.9%

Lists the outcomes of this analysis

Panel limitations

This analysis is unable to detect deep intronic variants, genomic rearrangements, triplet expansions, and germline mosaicisms below the 10 percent frequency of the least represented allele.

Conclusions

The **OncoPed** panel proved reliable within the limits of the reported values. To increasingly improve the accuracy of the CNV analysis, additional data will be added to harden the '*baseline*,' i.e., the set of controls necessary for the process to assess the variation in the **read depth** of the regions investigated, with particular attention to those genes that have corresponding pseudogenes (PMS2) in the human genome. The optimal average read depth (calculated with respect to all regions in each sample) was estimated to be around 200x, with a minimum of 30X.

Finally, each region sequenced by NGS technique is visualized using the Broad Institute's Integrative Genomics Viewer (IGV) program to further reduce the possibility of false negatives.