Medical College of Wisconsin | Genomic Sciences & Precision Medicine Center

Custom Clinical Sanger Sequencing (CCS)

Turn Around Time:	**30 Days**
CPT Codes:	**Contact Lab**
Test Includes:	✓ **Custom PCR Primer Design** ✓ **DNA Extraction** ✓ **Region of Interest PCR Amplification** ✓ **Dideoxy DNA Sequencing** ✓ **Data Analysis** ✓ **Clinical Report**

Expedited CCS testing is available. Contact the lab for more information.

Test Description

Custom Clinical Sequencing (CCS) is used to confirm a previously suspected genetic variant detected by next generation sequencing or research sequencing. This test may also be used to test the affected status of family members of an individual with a confirmed variant, when the affected family member's blood or DNA is provided as a control.

Sample Requirements

Whole Blood: 2-4mL (4mL preferred) of whole blood in EDTA (purple top tube). For infants, we require a minimum of 1mL of blood. Ship blood tubes overnight at room temperature in an insulated container within 5 days of collection.

gDNA: 5µg of purified gDNA with a minimum concentration of 75ng/µL in a screw cap tube and a 260/280 purity ratio of 1.75-2.0. The tube must be labeled with at least two patient identifiers (patient name/submitter ID number and date of birth). Ship gDNA overnight at room temperature. We do not accept products of genome amplification or other amplification reactions.

Buccal Swab and Saliva: Collection in DNA Genotek kit. Oragene•Dx OGD-575 U Oragene•Dx OGD 500 kits accepted. Contact lab for kids if necessary.

Test Methodology

Region specific PCR primers are designed and optimized to amplify the variant of interest. Extracted gDNA is quantified and PCR amplified. The purified PCR product is then sequenced in the forward and reverse direction using automated fluorescent dideoxy (Sanger) sequencing.

Limitations

The test methodology is limited in its ability to detect duplications, deletions, insertions and mosaicism. The test will not detect variants in the genome that fall outside of the regions analyzed. This technology is limited in its ability to accurately identify variants occurring in regions with high sequence identity to other regions of the genome (e.g. paralogous genes and pseudogenes). The test will not detect variants where the sequencing technique is unable to provide accurate data such as genomic regions that are GC rich and those with repetitive regions such as mono, di- and trinucleotide repeats. This test may not detect polyploidy or balanced rearrangements such as inversion and balanced translocations.

Contact & Submission

GSPMCBusinessOffice@mcw.edu | 414-955-2550

Whole Genome Sequencing (WGS) | References

1. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/