

Variant Confirmation by Sanger Sequencing

Test Code: SVCNF

Turnaround time: 2 weeks

CPT Codes: 81479 x1

Condition Description

THIS PAGE IS FOR BOTH OF THE FOLLOWING TESTS

VARIANT CONFIRMATION BY SANGER SEQUENCING (SVCNF)

TROUBLESHOOTING FOR VARIANT CONFIRMATION (SVTBL)

Test definition:

This test includes primer design, PCR amplification and Sanger sequencing to confirm variants that fall into the different categories listed below:

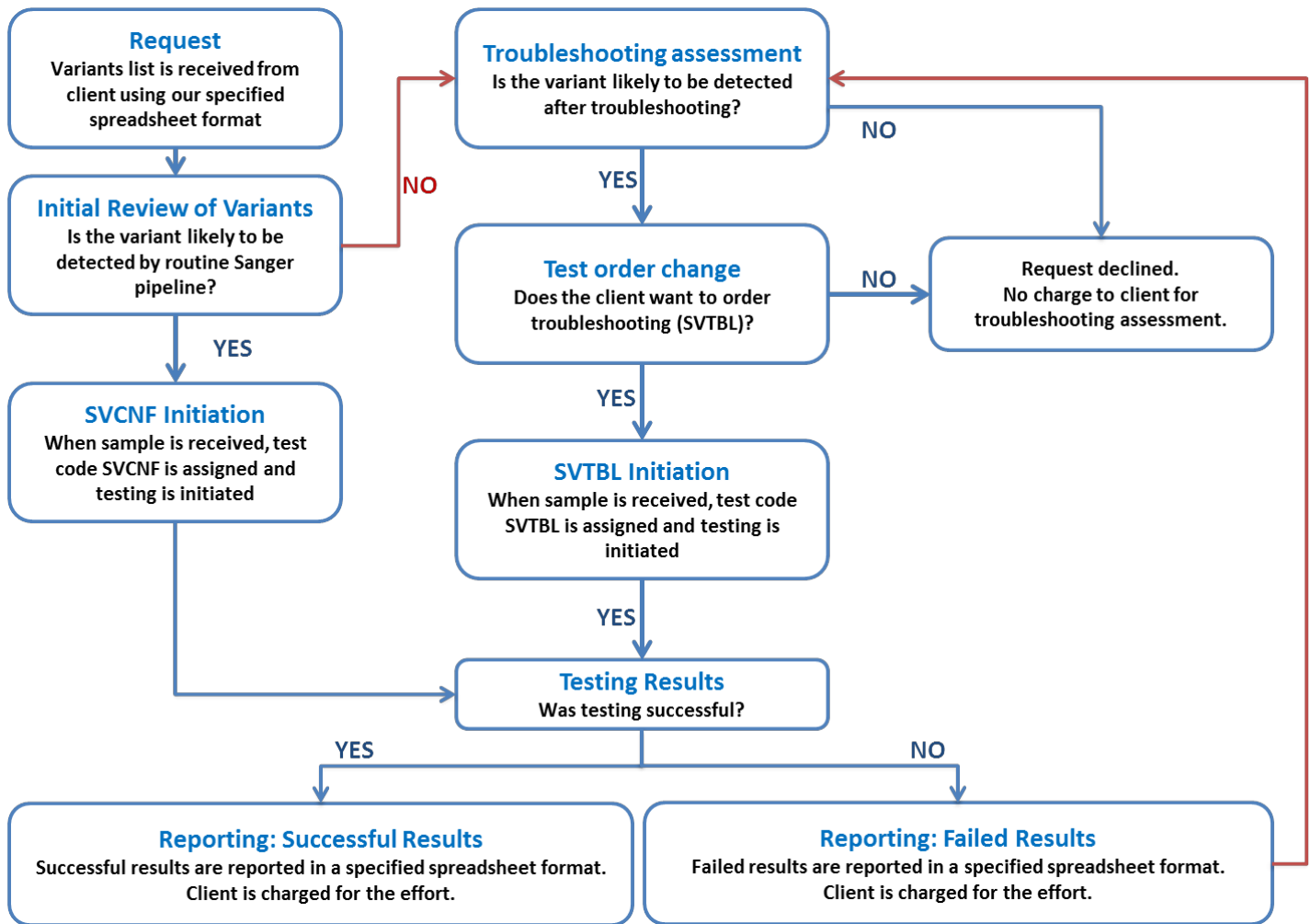
- a. Single nucleotide variants corresponding to a single genomic position.
- b. A genomic region of interest corresponding to a single amplicon.
- c. Small (less than 33 bp) deletion, duplication or indel.

Process:

Variant confirmation by Sanger sequencing (test code SVCNF) includes primer design, PCR amplification, Sanger sequencing, and sequence analysis to detect the presence or absence of a given variant detected by NGS. Most variants can be detected by this routine analysis.

A separate custom test, "troubleshooting for variant confirmation" (test code SVTBL), will confirm variants that are not detected by routine analysis and require additional troubleshooting for accurate identification. These include variants located within DNA segments that are difficult to amplify or sequence due to DNA composition (e.g. highly repetitive sequences, regions where primer design is challenging, etc.). Our team assesses variant requests to determine the likelihood of success and will notify you if the variants cannot be accepted.

Results are reported in a spreadsheet format without interpretation about variant classification. A formal report is not issued with these tests.



Indications

Variant confirmation by Sanger sequencing (SVCNF) and Troubleshooting for variant confirmation (SVTBL) are custom test services. These services are for clinical or research laboratories that require confirmation of variants detected by NGS platforms. Certain variants may not be amenable to confirmation. Prior to initiation of testing, our team assesses each variant request to determine the likelihood of success and to assign the proper SVCNF or SVTBL test code.

Methodology

Suitable primers are designed to amplify the region of the genomic DNA surrounding the variant, or the region of interest, by PCR. Amplicons are sequenced in both the forward and reverse directions.

Detection

Detection rate is determined by the sequence characteristics of the region(s) of interest.

Specimen Requirements

Special Instructions

To order the test, clients are asked to fill out a template spreadsheet. Request a digital copy of this template by emailing eglcsm@egl-eurofins.com

Assessment and test code assignment will be performed by EGL Genetics prior to initiation. Note that results are reported back in a spreadsheet as well.

Spreadsheet Template for Test Order:

| | A | B | C | D | E | F | G | H |
|---|---------------|------------------------|-----|--|--|-------------------|-------------------------------|------------------------------|
| 1 | Sample ID 1 | Sample ID 2 (optional) | Chr | Genomic Start (GRCh37/human genome build 19) | Genomic End (GRCh37/human genome build 19) | Variant | Transcript (e.g. NM_000492.3) | Notes (zygosity, mosaicism?) |
| 2 | C1XXX-KB-XXXX | A-00XXX-D | 10 | 3141495 | 3141495 | c.215C>G (p.S72X) | NM_002627.4 | |
| 3 | C1XXX-xx-XXX5 | SXXXXX1 | 17 | 44103704 | 44103704 | deep intronic | | |