Sanger Validation-Variant

PATIENT	REPORT DATE	BOOKING ID
Sujal Raj Giri	24 Mar 2022	#012202050203

Test Description
The MolQ Sanger Validation test confirms the variants obtained from high through put technology using sanger sequencing.

Clinician
Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Therapy and Cancer Research (IPTCR)
Pathologist: Not Provided

Patient Demographic
Name: Mr Sujal Raj Giri
Sex: Male
Date of Birth/Age: 8 years
Disease: Metastatic Medullary Carcinoma of Thyroid

Specimen
Booking ID: 012202050203
Site: NA
Sample Type: Blood
Date of Collection: 05-02-2022
Date of Booking: 05-02-2022

CLINICAL SYNOPSIS

Mr Sujal Raj Giri, incisional biopsy from left level IV lymph node- metastatic medullary carcinoma thyroid [as per the clinical report dated 10-11-2021 provided along with the Test Requisition Form]. The tumor was identifiable in the FFPE block [2703/21]. The NGS sequencing data analysis of this patient has identified a variant in *RET* gene. The same variation is being validated by Sanger sequencing.

RESULTS

Variant is confirmed to be present by Sanger sequencing.

Analysis For: Variation in *RET* Gene		Gene Name: *RET* (Exon 13)
S.No.	**Variation Detected in NGS**	**Sanger Validation Result**
1.	chr10:g.43613906G>T; c.2370G>T; p.Leu790Phe	Present (Heterozygous)

*The variant analysis in Sanger sequencing is based on the *RET* reference sequence ENST00000355710.3[1]. The exon number and nucleotide numbers will differ based on the reference file chosen and the database used.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

Variant description: A missense variation in exon 13 of the *RET* gene (**chr10:g.43613906G>T; c.2370G>T**) that results in the amino acid substitution of Leucine for Phenylalanine at codon 790 (**p.Leu790Phe**) was detected in Mr Sujal Raj Giri by NGS.

The same variation was detected in heterozygous condition in this patient by Sanger sequencing (Figure 1)

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

APPENDIX 1: TEST METHODOLOGY

METHOD

Targeted gene Sanger sequencing: Exon 13 of the *RET* gene was PCR-amplified and the products were sequenced using Sanger sequencing. In case of mosaicism in leucocytes, the detection limits of Sanger sequencing for presence of variation is ~20%. The sequences were aligned to available reference sequences ENST00000355710.3[1] to detect variations using variant analysis software programs.

DISCLAIMER

About 0.44% of total cases are susceptible to allele dropout/dropin phenomenon, which can lead to misdiagnosis[2].

REFERENCES

1. ENSEMBL: http://www.ensembl.org.
2. Blais, Jonatan *et al.* Risk of Misdiagnosis Due to Allele Dropout and False-Positive PCR Artifacts in Molecular Diagnostics. The Journal of Molecular Diagnostics, Volume 17, Issue 5, 505 – 514.

Figure 1: Sequence chromatogram and alignment to the reference sequence showing the variant in exon 13 of the *RET* gene (chr10:g.43613906G>T; c.2370G>T; p.Leu790Phe) detected in heterozygous condition in Mr Sujal Raj Giri.