

Test Description

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

Patient Demographic

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

Clinician

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

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.

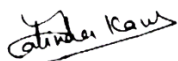
Analysis For: Variation in <i>RET</i> Gene		Gene Name: <i>RET</i> (Exon 13)
S.No.	Variation Detected in NGS	Sanger Validation Result
1.	chr10:g.43613906G>T; c.2370G>T; p.Leu790Phe	Present (Heterozygous)

*The variant analysis in Sanger sequencing is based on the *RET* reference sequence ENST00000355710.3¹. 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)



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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 ENST00000355710.3¹ 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 misdiagnosis².

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.

