Sanger Validation-Variant

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 Cancer Therapy and Research
Pathologist: Not Provided

Patient Demographic
Name: Mr Nursingha Charan Mahapatra
Sex: Male
Date of Birth/Age: 69 years
Disease: Adenocarcinoma Stomach

Specimen
Booking ID: 012306010202
Site: NA
Sample Type: Blood
Date of Collection: 01-06-2023
Date of Booking: 01-06-2023

CLINICAL SYNOPSIS

The index patient, Mr. Nursingha Charan Mahapatra, is a case of Adenocarcinoma of stomach. He was found to harbor a variant of uncertain significance, c.8395_8404del in the ***ATM*** gene. The same variant is being validated by Sanger sequencing.

RESULTS

Variant is confirmed to be present by Sanger sequencing.

Analysis For: Variation in Gene		Gene Name: *ATM* (Exon 57)
S.No.	**Variation Detected in NGS**	**Sanger Validation Result**
1.	chr11:108214065; c.8395_8404del (p.Phe2799LysfsTer4)	Present (Heterozygous)

*The variant analysis in Sanger sequencing is based on the *ATM* gene reference sequence NM_000051.3 (GRCh37)[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 variant of uncertain significance in exon 57 of the ***ATM*** gene [Chr11:108214065; c.8395_8404del; (p.Phe2799LysfsTer4)] was detected in Nursingha Charan Mahapatra 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 57 of the *ATM* gene was PCR-amplified and the product was sequenced using Sanger sequencing. In case of mosaicism in leucocytes, the detection limits of Sanger sequencing for presence of variants are ~20%. The sequences were aligned to available reference sequences NM_000051.3[1] to detect variants using variant analysis software programs.

DISCLAIMER

1. This is a laboratory developed test and the development and the performance characteristics of this test was determined by the reference laboratory.
2. Please note that the tests are performed only after approval of referring/ ordering clinician/physician. Above recommendations /results should not be viewed as only source of information on which treatment or other clinical decisions are made. Clinical correlation is highly recommended.
3. The classification of variants of unknown significance can change over time and MolQ Laboratory cannot be held responsible for this. Please contact MolQ Laboratory later to inquire about any changes.
4. This sanger custom test is not a clinically validated assay for each and every primer set. No form of test can guarantee 100% accuracy. This assay is no such exception, and it has some Inherent pitfalls.
5. The PCR based assay will not amplify genic regions outside primer binding site, may not detect the exact size for the deletion or duplication which is more than 50bp.
6. Pseudogenes can present challenges during sequencing and analysis, which may impact the accuracy and interpretation of the results.
7. Though PCR is a highly sensitive and specific technique, performance may vary based on several factors including allelic dropout and preferential amplification (Chance phenomenon) causing a potential source of misdiagnosis for both dominant and recessive diseases. About 0.44% of total cases are susceptible to allele dropout phenomenon, which can lead to misdiagnosis[2].
8. Testing of affected/carrier index/proband samples parallelly with test samples is highly recommended to rule out false negative/positive results.
9. The accuracy of the results is based on the assumption that samples received were correctly identified, family relationships are true and clinical diagnosis of relatives is correct.
10. In a very few cases genetic tests may not show the correct results leading to false positives and negatives; e.g. because of the quality of the sample provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reason that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading, or even wrong result of testing if such could not be recognized by MolQ Laboratory in advance.
11. Negative results do not negate the absence of mutations that are not covered by the test.
12. The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
13. If results obtained do not match the clinical findings, additional testing should be considered as per the referring clinician's recommendations.
14. MolQ Laboratory hereby recommends the patients and/ or guardians of the patients, as the cause may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report (s).

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 57 of the *ATM* gene [chr11:108214065;c.8395_8404del; (p.Phe2799LysfsTer4)] detected in heterozygous condition in Mr. Nursingha Charan Mahapatra.