VARIANT ASSESSMENT

- The output sequences are aligned to the human reference genome hg19 (GRCh37). The alignments and variant calling is done using the ION S5 torrent server. Variants are identified and interpreted using Ion Reporter Software. The identified variants are annotated according to HGVS sequence variant nomenclature
- Multiple in-silico predictors, such as SIFT, PolyPhen, MutationTaster, NNSPLICE, and ASSP etc. are used for variant impact on the protein function. Population and literature databases such as dbSNP, Exome Aggregation Consortium (ExAC), genome Aggregation Database (gnomAD), ClinVar, HGMD and PubMed etc are used for variant summary and classification
- Variants are labelled based on the American College of Medical Genetics (ACMG) recommendations for 5-tier variant classification system: **Pathogenic, Likely Pathogenic, Variant of Uncertain Significance (VUS), Likely Benign and Benign**
- Clinically relevant variants identified by NGS are continuously validated in-house by a second independent method (Sanger) for quality aspects; therefore those variants which do not meet our internal QC criteria (based on extensive validation processes) are confirmed by Sanger sequencing

SANGER SEQUENCING RESULT

Since the identified variant was a novel variant, therefore, the variant was confirmed by Sanger sequencing.