ExomeNext®

SAMPLE REPORT

Ambry Genetics®
A Konica Minolta Company

PATIENT
Name: **Patient , Sample**
Accession #: 00-00000
DOB: 01/01/2009
Sex at birth: Female
MRN: N/A
Indication: Diagnostic

TEST INFORMATION
Portal Order #: 000000
Family #: 0000
Specimen #: N/A
Specimen type: Blood EDTA
Collection date: 01/01/2021
Received date: 01/02/2021
Final Report: 02/20/2021

MEDICAL PROFESSIONAL
Doctor, Sample, MD
Sample Facility

ADDITIONAL RECIPIENTS
GC, Sample, MS, CGC

POSITIVE: Clinically Relevant Alteration(s) Detected

Reportable Findings		
Single gene alterations	**Contiguous gene deletions/duplications**	**Mitochondrial genome**
1(1)*	**None**	**None**

* genes(alterations)

Indication for Testing

Developmental delay, dysmorphic features, relative macrocephaly

Results and Interpretation

Gene (RefSeq ID)	Characterized/ Uncharacterized Gene	Relevant Associated Syndrome	Mode of Inheritance	Genotype	Alteration	Alteration Type	Alteration Classification
TAOK1 (NM_020791)	Characterized	*TAOK1*-related neurodevelopmental disorder	Autosomal dominant	Heterozygous, *de novo*	c.449G>T (p.R150I)	Missense	Pathogenic Mutation

- **Overall, the evidence suggests that the identified *TAOK1* alteration is the cause of the patient's clinical symptoms**. Clinical correlation is recommended.

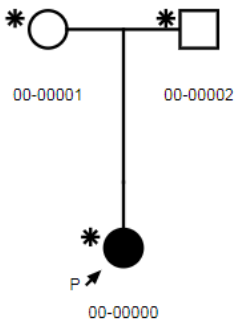
Notes

- Secondary findings were issued in a separate report.
- Genetic counseling is a recommended option for all patients undergoing genetic testing.
- Any tests on hold, previously reported, and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) have not been included in this report. For additional information, please contact Ambry Genetics.

Electronically Signed By Sample Director, on 2/20/2021 at 0:00:00 PM

All content hereafter is supplemental information to the preceding report.

Family Pedigree



✱ Exome Sequencing Performed
Affected
Affected or Partially/Possibly Affected Family Member
Presence of accession number means sample received

Analyses Performed

i) Full exome sequencing, bioinformatics, filtering and manual review based on autosomal and X-linked dominant and recessive and Y-linked inheritance models of the proband, father, and mother was performed. Medical review of characterized genetic etiologies revealed an alteration with likely clinical relevance.

ii) Medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings was not performed at the time of this reclassification. Please contact the laboratory to request reflex to reanalysis of uncharacterized genes, if desired.

iii) Sequencing of mitochondrial DNA (mtDNA) followed by screening and analysis of known pathogenic alterations did not reveal any alterations with likely clinical relevance.

*Uncharacterized genes are not currently established to underlie Mendelian genetic conditions. An uncharacterized gene will be classified as a “candidate” or “suspected candidate” when sufficient evidence, based on Ambry’s comprehensive, rule-based scoring criteria, is available (Farwell Hagman, 2017) (http://www.ambrygen.com/candidate-gene-reporting).

Raw Data

A table with additional variant filtering details can be found with the raw data filtered variant list (if requested). This list includes clinically irrelevant characterized genes and uncharacterized genes which could not be ruled out (if analyzed); these alterations are not systematically confirmed via Sanger sequencing. The filtered variant list can be requested via this form (www.ambrygen.com/file/material/view/1262/Raw_Sequence_Data_Consent_0619_final.pdf).

Metrics and Coverage

The values below represent metrics from the family's exome sequencing. Complete coverage data for this proband can be e-mailed or made available for download through AmbryPort by request.

	Depth of coverage	
Relationship	**% Bases ≥ 10x**	**% Bases ≥ 20x**
Proband	98.64	98.32
Father	98.63	98.21
Mother	98.47	98.13

TAOK1 Gene Details

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
TAOK1	NM_020791	27717943-27878921	160979	20	19	1001 aa

The *TAOK1* gene is located on chromosome 17q11.2 and encodes the serine/threonine-protein kinase TAO1 protein (OMIM_610266), which plays a role in the regulation of mitotic progression (Draviam, 2007). Pathogenic alterations in this gene have been associated with *TAOK1*-related neurodevelopmental disorder, which is an autosomal dominant condition that generally occurs *de novo*.

TAOK1-related neurodevelopmental disorder is characterized by developmental delay with language and motor delays, hypotonia, and intellectual disability. Dysmorphic features in *TAOK1*-related neurodevelopmental disorder include downslanting palpebral fissures, long and narrow face, wide spaced teeth, ptosis, depressed nasal bridge, mild dolichocephaly, macrocephaly, frontal bossing, palate abnormalities, and sparse scalp hair. Affected individuals have also been reported with seizures, behavioral problems, learning disabilities, hearing impairment, visual impairment, feeding difficulties, and joint hypermobility (McLaughlin, 2017; Dulovic-Mahlow, 2019). Loss of function alterations have been reported as the mechanism of disease for *TAOK1*-related neurodevelopmental disorder (Dulovic-Mahlow, 2019).

TAOK1 c.449G>T (p.R150I)

Alteration description:
The c.449G>T (p.R150I) alteration is located in exon 6 (coding exon 5) of the *TAOK1* gene. This alteration results from a G to T substitution at nucleotide position 449. This change occurs in the last base pair of coding exon 5, which makes it likely to have some effect on normal mRNA splicing. In addition to potential splicing impact, this alteration causes the arginine (R) at amino acid position 150 to be replaced by an isoleucine (I).

Population frequency:
Based on data from the Genome Aggregation Database (gnomAD), the *TAOK1* c.449G>T alteration was not observed, with coverage at this position.

Conservation:
The p.R150 amino acid is conserved in available vertebrate species.

***In silico*:**
The p.R150I alteration is predicted to be deleterious by *in silico* analysis.

Structural data:
The p.R150 amino acid is located in the conserved kinase domain, also known as catalytic domain, of the TAOK1 protein. The catalytic domains of protein kinases contain 12 conserved subdomains that fold into a common catalytic core structure. R150 is part of the catalytic loop of subdomain VIB and adjacent to residue D151, which is likely important for the catalytic activity of that region (Hanks, 1995).

Family inheritance:

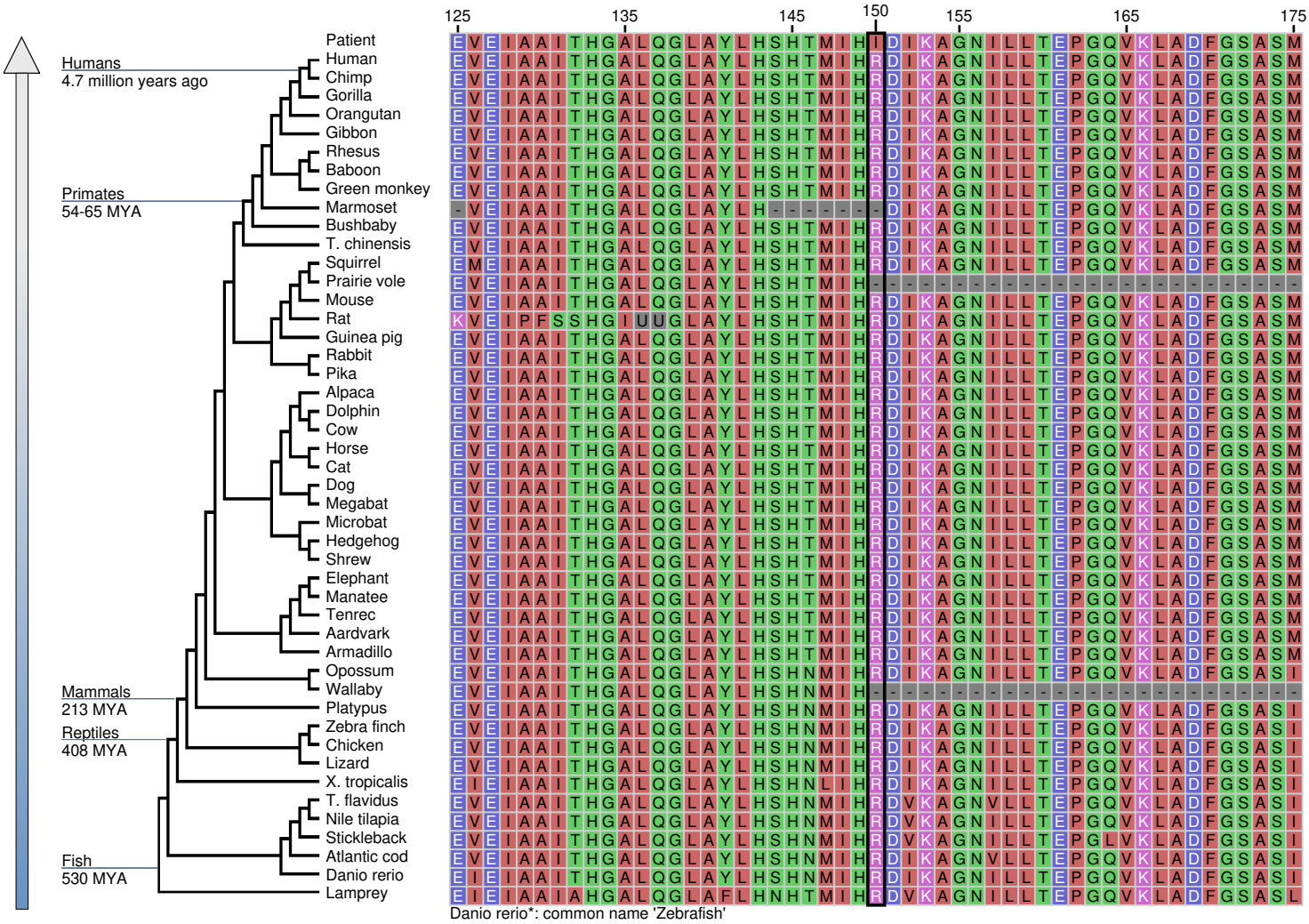
Gene (RefSeq ID)	Alteration	Exon	Proband (19-00000)	Father (19-00001)	Mother (19-00002)	Inheritance
TAOK1^ (NM_020791)	c.449G>T (p.R150I)	CDS 5	Heterozygous	Negative	Negative	*De novo*♦

^Alteration(s) confirmed by automated fluorescence dideoxy sequencing (aka "Sanger") sequencing.

♦Note that the possibility of germline mosaicism cannot be ruled out.

Based on the available evidence, the *TAOK1* c.449G>T (p.R150I) alteration is classified as pathogenic.

TAOK1 c.449G>T (p.R150I)



Trait	Arg (R)	Ile (I)
Amino Acid Name	Arginine	Isoleucine
Polarity/Charge	positively charged	non-polar
pH	basic	neutral
Residue Weight	156	113
Hydrophobicity Score	-4.5	4.5
Hydrophilicity Score	3	-1.8
Secondary Structure Propensity	α indifferent / β indifferent	α former / strong β former

Report References

- Draviam VM, *et al.* (2007) *Nat. Cell Biol.* **9**(5):556-64. **PMID:**17417629
- Dulovic-Mahlow M, *et al.* (2019) *Am. J. Hum. Genet.* **105**(1):213-220. **PMID:**31230721
- Hanks SK, *et al.* (1995) *FASEB J.* **9**(8):576-96. **PMID:**7768349
- McLaughlin HM, et al. (2017) Abstract PgmNr 1040 Novel *de novo TAOK1* variants associated with a neurodevelopmental phenotype, macrocephaly, and joint hypermobility. Presented at the 67th Annual Meeting of the American Society of Human Genetics. Oct 19, 2017, Orlando, Florida (available at https://ep70.eventpilotadmin.com/web/planner.php?id=ASHG17)

Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

- 1000 Genomes [Internet]: 1000 Genomes Project Consortium (2010) *Nature* **467**(7319):1061-1073. Available from: http://www.1000genomes.org.
- Ambry Clinical Validity Assessment: Smith ED, *et al.* (2017) *Hum Mutat.* **38**(5):600-608.
- Berkeley Drosophila Genome Project [Internet]: Reese MG, *et al.* (1997) *J Comp Biol* **4**(3), 311-23. http://www.fruitfly.org/seq_tools/splice.html.
- ClinGen Clinical Validity Classifications [Internet]: https://www.clinicalgenome.org/knowledge-curation/gene-curation/clinical-validity-classifications; Rehm HL, *et al.* (2015) *N Engl J Med* **372**(23):2235-2242.
- Clinical Genomic Database [Internet]: Solomon BD, *et al.* (2013) *Proc Natl Acad Sci U S A.* **110**(24):9851-5. Available from: http://research.nhgri.nih.gov/CGD.
- Combined Annotation Dependent Depletion (CADD) [Internet]: Kircher M, *et al.* (2014) *Nat Genet.* **46**(3):310-5. Available from: http://cadd.gs.washington.edu.
- Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]: Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 135). Available from: http://www.ncbi.nlm.nih.gov/projects/SNP.
- DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. Firth, H.V. *et al* (2009). *Am J Hum Genet* **84:**524-533.
- ESEfinder [Internet]: Smith PJ, *et al.* (2006) *Hum Mol Genet* **15**(16):2490-2508 and Cartegni L, *et al.* (2003) *Nucleic Acid Res* **31**(13):3568-3571. Available from: http://cb.utdallas.edu/tools/ESE
- Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA (URL: http://exac.broadinstitute.org). (Lek M, *et al* 2016: see below)
- Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet]: Seattle, WA. Available from: http://evs.gs.washington.edu/EVS.
- Expression Atlas: Differential and Baseline Expression [Internet]: Petryszak, R. *et al.* (2013) *Nucleic Acids Res* **10**.1093/nar/gkt1270. Available from: http://www.ebi.ac.uk/gxa/home.
- Farwell Hagman KD, *et al.* (2016) *Genet Med* doi:10.1038/gim.2016.95.
- GeneMANIA [Internet]: Warde-Farley D, *et al.* (2010) *Nucleic Acids Res* **38**(Web Server issue):W214-20. doi: 10.1093/nar/gkq537. Available from: http://genemania.org.
- GeneReviews [Internet]: Pagon RA, *et al.* editors. (1993-) Seattle, WA: University of Washington, Seattle. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1116.
- Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org/ (Lek M, *et al* 2016: see below)
- Grantham prediction: Grantham R. (1974) *Science* **185**(4151):862-864.
- Green RC, *et* al. (2013) *Genet Med* **15**(7):565-74.
- HGMD® [Internet]: Stenson PD, *et al.* (2014) *Hum Genet.* **133**(1):1-9. Available from: http://www.hgmd.cf.ac.uk.
- Integrative Genomics Viewer (IGV): Thorvaldsdóttir H, *et al.* (2012) *Brief Bioinform* **14**(2):178-192.
- Kalia SS, *et* al. (2016) *Genet Med* doi:10.1038/gim.2016.190.
- Kyoto Encyclopedia of Genes and Genomes (KEGG) [Internet]: Kanehisa M, *et al.* (2014) *Nucleic Acids Res* **42**. http://www.genome.jp/kegg.
- Lek M, *et al* (2016) Nature 536(7616):285-91.
- Mouse Gene Expression Database (GXD): Finger JH, *et al.* (2011): *Nucleic Acids Res* **39**(suppl 1):D835-D841. Available from: http://www.informatics.jax.org.
- Mouse Genome Database (MGD) [Internet]: Eppig JT, *et al.* (2012) *Nucleic Acids Res* **40**(1):D881-86 Available from: http://www.informatics.jax.org.
- Mutation Assessor (functional impact of protein mutations) [Internet]: Reva BA *et al.* (2011) *Nucleic Acids Res* **39**(17):e118. Available from: http://mutationassessor.org.
- NeXtProt [Internet]: Lane L, *et al.* (2012) neXtProt: a knowledge platform for human proteins. *Nucleic Acids Res* **40**(D1): D76-D83. Available from: http://www.nextprot.org.
- Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol* 2004 **5**(2):89-99.
- OMIM (Online Inheritance in Man) [Internet]: Copyright© 1966-2012 Johns Hopkins University. Available from: http://www.omim.org.
- PolyPhen [Internet]: Adzhubei IA, *et al.* (2010) *Nat Methods* **7**(4):248-249. Available from: http://genetics.bwh.harvard.edu/pph2.
- RefSeq: The NCBI handbook [Internet]: Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project. Available from: http://www.ncbi.nlm.nih.gov/refseq.
- Richards, *et al.* On behalf of the ACMG Laboratory Quality Assurance Committee (2015) Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, 17(5), 405–424. http://doi.org/10.1038/gim.2015.30.
- SIFT [Internet]: Kumar P *et al.* (2009) *Nat Protoc.* **4**(7):1073-81. http://sift.jcvi.org.

ExomeNext™ Assay Information

General Information: Ambry's ExomeNext™ is a cost-effective, comprehensive, integrated exome sequencing assay designed to increase the diagnostic yield for genetic disorders that have eluded definitive delineation using traditional diagnostic approaches. The exome represents virtually all the exons, which are the regions in the human genome that are translated into proteins. It is estimated that the protein-coding regions of the human genome contain about 85% of the disease-causing mutations. Whole-exome sequencing has been successfully applied to identify both inherited and *de novo* mutations in a diverse variety of autosomal dominant, recessive, and X-linked disorders.

Result Reports: A primary clinical report will only be generated for the proband regardless of number of family members submitted. However, it may be possible to infer information about family member's results based on the proband's report. Pathogenic mutation(s) likely to factor into the patient's current clinical condition are always reported. Since new scientific information becomes available on a regular basis, this could alter the interpretation of previously reported results. In the event of a change in interpretation, an unsolicited re-classification/amended report may be issued to the ordering clinician.

Expected (Normal) Value: Diagnostic: 0, 1, or more mutation(s) detected.

Test Limitations: This test was developed and its performance characteristics were determined by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or physician skilled in evaluating the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The following types of mutations are detectable: nucleotide substitutions, small deletions, small insertions and small indels. Exome sequencing is not intended to analyze the following types of mutations: gross deletions/duplications, gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, trinucleotide repeat sequences, mutations involved in tri-allelic inheritance, certain mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, and X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutations in an undetectable region. Ambry's ExomeNext detection rate is 30% for positive or likely positive relevant findings identified in established disease-gene associations, and an additional 7% for candidate gene findings (Farwell K, *et al.*, Genet Med., 2014 and Farwell Hagman K, *et al.*, Genet Med., 2017). Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that may interfere with analysis, or from other sources.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT xGen Exome Research Panel V1.0 (IDT). Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with the exome baits. Captured DNA is eluted and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina NovaSeq, NextSeq or HiSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools using genome assembly GRCh 37/hg19. Data is annotated with the Ambry Variant Analyzer tool (AVA), including: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. Data analysis is focused on small insertions and deletions, canonical splice site alterations, and non-synonymous alterations. Gross deletion/duplication analysis is assessed for proband only for all genes within the targeted exome using a custom pipeline based on coverage and/or breakpoint analysis from NGS data and is followed by a confirmatory orthogonal method, as needed. The following sites are used to search for previously described gene mutations and polymorphisms: the Human Gene Mutation Database (HGMD), the Single Nucleotide Polymorphism database (dbSNP), ExAC, ESP, 1000 genomes, and online search engines (e.g., PubMed). Variants are then filtered further based on applicable inheritance models. Cosegregation studies are performed if family members are available with the exception of gross deletions/duplications, which are confirmed in the proband only.. All relevant findings undergo manual review by molecular geneticists using integrated genomics software (IGV) and/or undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via trio exome sequencing with coverage and alternate read ratios above established confidence thresholds. Additionally, gross deletions/duplications are confirmed by SNP Microarray (Affymetrix® CytoScan™ HD Array), in-house targeted array, MLPA, or Sanger sequencing. Co-segregation results may be confounded by many factors which cannot be completely ruled out including haploinsufficiency, reduced penetrance, age-of-onset, and/or variable expressivity. Relevant findings are evaluated from among the genes in Ambry's internal, dynamic gene database which classifies genes as characterized or uncharacterized Mendelian disease genes based on clinical validity (Smith E, et al., Hum Mutat, 2017). Characterized genes are those currently known to underlie at least one Mendelian genetic condition. Uncharacterized genes are those not currently known or with insufficient evidence to be associated with a Mendelian genetic condition. Characterized genes are analyzed first. If no positive findings are identified, reflex analysis of uncharacterzied genes occurs for potential identification of a candidate gene finding. The analysis of candidate gene findings is only performed when an informative trio is received for testing and focuses on de novo, autosomal recessive, or X-linked inherited alterations. Each alteration remaining after inheritance model filtering is analyzed and/or reviewed by board certified molecular geneticists to identify the most likely causative alteration(s). Interpretation is based on the clinical, family, and test information provided by the referring provider and the current knowledge of genes and alterations at the time of reporting. Screening and analysis of known mtDNA mutations related to the proband's clinical phenotype is included if ordered. Amplification of the entire mitochondrial genome is carried out by long distance PCR and sequencing of mitochondrial DNA (mtDNA) is performed separately on Illumina MiSeq.

Analysis of Alterations: The following lines of evidence are used to assess the pathogenic nature of an alteration: presence in affected and healthy populations, co-segregation information, functional studies, alteration type, conservation, *in silico* predictions, and presence in a functional protein domain. The absence of a particular line of evidence implies that no information was found or it does not apply for that alteration type (eg. *in silico* for truncating alterations).

Analytical range: Approximately 75% of the bases are expected to have quality scores of Q30 or higher, which translates to an expected base-calling error rate of 1:1000, or an expected base-calling accuracy of 99.9%. Additionally, 90% and 95% of the exome will be covered at ≥20x and ≥10x respectively under current run conditions, generally sufficient for high quality heterozygous and homozygous variant calling for germline variants. For any given individual ~ 10% of the targeted exome is not sequenced well enough to make a confident call. Each individual may have slightly different coverage yield distributions within the exome. Exons plus at least 6 bases into the 5' and 3' ends of all the introns are analyzed and reported. The pipeline detects deletions and duplications >5 exons in size in sequences with sufficient resolution. The mean depth of coverage for targeted mitochondrial bases is greater than 1,000X.