NHS Lothian
South East Scotland Genetic Laboratories

HEREDITARY ATAXIA AND HEREDITARY SPASTIC PARAPLEGIA GENE PANEL - TECHNICAL INFORMATION

Design: The hereditary ataxia (HA) and hereditary spastic paraplegia (HSP) gene panel was designed as part of a custom probe set from Twist Bioscience (TE-98175847) to cover 70 genes associated with HA and/or HSP. This panel design provides coverage of 100% of the target coding regions and flanking intronic sequences (+/- 15bp) for the 70 genes listed below.

Method: Library preparation and target enrichment was performed using the custom designed TE-98175847 probe set (Twist Bioscience) and Nextera Flex for Enrichment (Illumina). Sequencing was performed using a 150bp paired-end sequencing kit on a MiSeq (Illumina). All stages of the workflow were performed according to the manufacturer's instructions.

Coverage criteria: For each sample reported, >95% of the target coding and flanking intronic regions of the 70 genes was covered to a minimum depth of 20 reads (20x). Any regions of the SPAST gene not covered to 20x depth for HSP referrals and any region of the SPG7 gene not covered to 20x depth in cases where one SPG7 pathogenic/likely pathogenic variant was detected were flagged for follow-up Sanger sequencing. Specific details of coverage and depth for individual tests are available from the laboratory on request.

Variant identification and interpretation: Sequence data were mapped and variants identified using GenomeAnalysisToolKit (GATK) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference. Variants identified were subsequently classified according to recent ACGS Best Practice Guidelines for Variant Classification using all available evidence. Any clinically significant variants were confirmed by Sanger sequencing.

Variant reporting: Variants were reported according to HGVS guidelines using the accession numbers listed below. Variants categorised as non-pathogenic or likely non-pathogenic were filtered out at the variant interpretation stage and were not included in the clinical report. Details of these variants are available from the laboratory on request.

Genes included and associated sequence accession numbers:

AAAS (NM_015665.6), ABCB7 (NM_004299.3); ABCD1 (NM_000033.4); ADAR (NM_001111.5); AFG3L2 (NM_006796.1); ALS2 (NM_020919.3); ANO10 (NM_018075.3); APTX (NM_001195248.1); ATL1 (NM_015915.4); ATM (NM_000051.3); ATP1A3 (NM_001256214.1); ATP7B (NM_000053.3); BSCL2 (NM_001122955.3); CACNA1A (NM_000068.3); CACNA1G (NM_018896.4); CAPN1 (NM_001198868.1); COQ8A (NM_020247.4); CYP27A1 (NM_000784.3); CYP7B1 (NM_004820.4); DDHD2 (NM_015214.2); FA2H (NM_024306.4); FGF14 (NM_175929.2); FTL (NM_000146.3); FXN (NM_000144.4); GBA2 (NM_020944.2); GCH1 (NM_000161.2); GRID2 (NM_001510.3); HSPD1 (NM_199440.1); IFIH1 (NM_002168.4); ITPR1 (NM_001168272.1); KCNA1 (NM_000217.2); KCNC3 (NM_004977.2); KCND3 (NM_004980.4); KIF1A

(NM_001244008.1); KIF5A (NM_004984.2); L1CAM (NM_001278116.1); NIPA1 (NM_144599.4); OPA3 (NM_025136.4); PDYN (NM_001190898.2); PLP1 (NM_000533.3); PNPLA6 (NM_001166111.1); POLG (NM_002693.2); PRKCG (NM_002739.3); PRNP (NM_000311.3); PRRT2 (NM_145239.2); REEP1 (NM_001164730.1); RNaseH2B (NM_024570.4); RTN2 (NM_005619.4); SACS (NM_014363.4); SETX (NM_015046.5); SIL1 (NM_022464.4); SLC1A3 (NM_004172.4); SLC2A1 (NM_006516.2); SPART (NM_001142294.1); SPAST (LRG714t1); SPG11 (NM_025137.3); SPG21 (NM_016630.6); SPG7 (NM_003119.2); SPTBN2 (NM_006946.2); STUB1 (NM_005861.3); SYNE1 (NM_182961.3); TGM6 (NM_198994.2); TMEM240 (NM_001114748.1); TTBK2 (NM_173500.3); TTPA (NM_000370.3); TWNK (NM_021830.4); UBAP1 (NM_016525.4); VPS13D (NM_015378.3); WASHC5 (NM_014846.3); ZFYVE26 (NM_015346.3).