NHS Lothian
South East Scotland Genetic Laboratories

DILATED CARDIOMYOPATHY PANEL – TECHNICAL INFORMATION

Design: The dilated cardiomyopathy (DCM) gene panel was designed as part a of custom probe set from Twist Bioscience (ID TE-92106986) to cover 25 genes associated with DCM, including the N2B major cardiac muscle isoform (NM_003319.4) of the Titin (*TTN*) gene. This panel design provides coverage of 100% of the target coding regions and flanking intronic sequences (+/- 15bp) for the 25 genes listed below.

Method: Library preparation and target enrichment was performed using the custom designed 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 25 genes was covered to a minimum depth of 20 reads (20X). Any regions of the *MYBPC3, MYH7, MYL2, TNNI3, TNNT2* and *TPM1* genes not covered at 20X depth 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 and the ClinGen CMP-EP MYH7 recommendations (Kelly et al. Genet Med 2018; 20(3): 351-359) 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, likely non-pathogenic, cold/cool VUS and missense variants in *TTN* 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:
ACTC1 (NM_005159.5); **ACTN2** (NM_001103.4); **BAG3** (NM_004281.4); **CSRP3** (NM_003476.5); **DES** (NM_001927.4); **DMD** (NM_004006.3); **DSP** (NM_0044415.4); **FLNC** (NM_001458.5); **LAMP2** (NM_002294.3); **LMNA** (NM_170707.4; NM_005572.4); **MYBPC3** (NM_000256.3); **MYH7** (NM_000257.4); **MYL2** (NM_000432.4); **MYL3** (NM_000258.3); **NKX2-5** (NM_004387.4); **PLN** (NM_002667.5); **RBM20** (NM_001134363.3); **SCN5A** (NM_000335.5; NM_001099404.2); **TNNC1** (NM_003280.3); **TNNI3** (NM_000363.5); **TNNI3K** (NM_015978.3); **TNNT2** (NM_000364.4; NM_001276345.2); **TPM1** (NM_001018005.2); **TTN** (NM_001267550.2); **VCL** (NM_014000.3).

Please note that, although only the N2B *TTN* transcript (NM_003319.4) is sequenced, the *TTN* transcript variant-IC (NM_001267550.2) is used for reporting in line with HGVS recommendations. This is an inferred, complete transcript, created at the NCBI, that includes all exons for which there is experimental proof.