BLEEDING & PLATELET DISORDERS PANEL – TECHNICAL INFORMATION

Design: The bleeding and platelet gene panel was designed as a custom probe set. This integrates genes from the ISTH approved gene list (isth.org/page/GinTh_GeneLists) and includes relevant genes from panel app panels in use by other UKHCDO laboratories including Bleeding & Platelet (panel app ref R90) and Thrombophilia (panel app ref R97) (panelapp.genomicsengland.co.uk). This panel design provides coverage of coding regions, flanking intronic sequences (+/-20bp) and 5' and 3' untranslated regions (UTR) for the genes listed below, with the exception of *ANKRD26* where only the 5'UTR is included.

Subpanels	Genes included and associated sequence accession numbers		
Coagulation & Fibrinolysis	***ACVRL1*** (NM_000020.3)	***F10*** (NM_000504.3)	***KLKB1*** (NM_000892.5)
	CHST14 (NM_130468.4)	***F11*** (NM_000128.3)	***KNG1*** (NM_000893.4)
	COL3A1 (NM_000090.3)	***F12*** (NM_000505.3)	***LMAN1*** (NM_005570.3)
	ENG (NM_000118.3)	***F13A1*** (NM_000129.3)	***MCFD2*** (NM_139279.5)
	F2 (NM_000506.4)	***F13B*** (NM_001994.2)	***SERPINE1*** (NM_000602.4)
	F5 (NM_000130.4)	***FGA*** (NM_000508.3)	***SERPINF2*** (NM_000934.3)
	F7 (NM_000131.4)	***FGB*** (NM_005141.4)	***THBD*** (NM_000361.3)
	F8 (NM_000132.3)	***FGG*** (NM_021870.2)	***VKORC1*** (NM_024006.5)
	F9 (NM_000133.3)	***GGCX*** (NM_000821.6)	***VWF*** (NM_000552.3)
Platelet disorders	***ABCG5*** (NM_022436.2)	***GBA*** (NM_000157)	***NBEAL2*** (NM_015175.2)
	ABCG8 (NM_022437.2)	***GFI1B*** (NM_004188.5)	***P2RY12*** (NM_022788.4)
	ACTB (NM_001101.3)	***GNE*** (NM_005476.6)	***PLA2G4A*** (NM_024420.2)
	ACTN1 (NM_001130004.1)	***GP1BA*** (NM_000173.5)	***PLAU*** (NM_002658.3)
	ADAMTS13 (NM_139025.4)	***GP1BB*** (NM_000407.4)	***PTGS1*** (NM_000962.4)
	ANKRD26 (NM_014915.2)	***GP6*** (NM_016363.5)	***RASGRP2*** (NM_153819.1)
	ANO6 (NM_001025356.2)	***GP9*** (NM_000174.4)	***RBM8A*** (NM_005105.4)
	AP3B1 (NM_003664.4)	***HOXA11*** (NM_005523.5)	***RNU4ATAC*** (NR_023343.1)
	AP3D1 (NM_001261826.3)	***HPS1*** (NM_000195.4)	***RUNX1*** (NM_001754.4)
	ARPC1B (NM_005720.4)	***HPS3*** (NM_032383.4)	***SLFN14*** (NM_001129820.1)
	BLOC1S5 (NM_201280.3)	***HPS4*** (NM_022081.5)	***SRC*** (NM_198291.2)
	BLOC1S3 (NM_212550.4)	***HPS5*** (NM_181507.1)	***STIM1*** (NM_003156.3)
	BLOC1S6 (NM_012388.3)	***HPS6*** (NM_024747.5)	***STXBP2*** (NM_006949.2)
	CDC42 (NM_001791.4)	***IKZF5*** (NM_001372123.1)	***TBXA2R*** (NM_001060.5)
	CYCS (NM_018947.5)	***ITGA2B*** (NM_000419.3)	***TBXAS1*** (NM_030984.3)
	DIAPH1 (NM_001079812.2)	***ITGB3*** (NM_000212.2)	***THPO*** (NM_000460.4)
	DTNBP1 (NM_032122.4)	***KDSR*** (NM_002035.4)	***TPM4*** (NM_003290.3)
	ETV6 (NM_001987.4)	***LYST*** (NM_000081.3)	***TUBB1*** (NM_030773.3)
	FERMT3 (NM_178443.2)	***MECOM*** (NM_004991.3)	***VIPAS39*** (NM_001193315.1)
	FLI1 (NM_002017.4)	***MPIG6B*** (NM_025260.3)	***VPS33B*** (NM_018668.4)
	FLNA (NM_001110556.2)	***MPL*** (NM_005373.2)	***VWF*** (NM_000552)
	FYB1 (NM_001465.6)	***MYH9*** (NM_002473.5)	***WAS*** (NM_000377.2)
	GATA1 (NM_002049.3)	***NBEA*** (NM_015678.4)	

Thrombosis	***ADAMTS13*** (NM_139025.4)	***FGG*** (NM_021870.2)	***PROS1*** (NM_000313.3)
	F2 (NM_000506.4)	***HRG*** (NM_000412.4)	***SERPINC1*** (NM_000488.3)
	F5 (NM_000130.4)	***PIGA*** (NM_002641.3)	***SERPIND1*** (NM_000185.3)
	FGA (NM_000508.3)	***PLG*** (NM_000301.3)	***SERPINE1*** (NM_000602.4)
	FGB (NM_005141.4)	***PROC*** (NM_000312.3)	***THBD*** (NM_000361.2)

Other genes: A number of "amber" genes are included in the design but are not currently analysed or reported. These are genes where some evidence is available to suggest a role in genetic platelet disorders and they are included to facilitate future clinical reporting and/or research and development. These will only be unmasked for analysis after sufficient evidence of clinical utility and with confirmation of appropriate consent via the patient's clinical team. These include the following platelet disorder genes: *ABCC4, EPHB2, PRKACG, PTPRJ, TRPM7.*

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 regions were covered to a minimum depth of 20 reads (20X). Any regions of the genes most relevant to the clinical presentation 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 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 above. This analysis cannot reliably detect all deletions/duplications/insertions, structural or copy number variants, and may not detect variants within homopolymeric regions or regions with coverage <20X. Variants were classified and reported using ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2024 v1.2 (https://www.acgs.uk.com). Details of all variants detected in a patient sample are available from the laboratory on request.