ERYTHROCYTOSIS GENE PANEL – TECHNICAL INFORMATION

Design: The Erythrocytosis gene panel was designed as a custom probe set and includes relevant genes from the Hereditary Erythrocytosis panel app panel R405 (v1.19; panelapp.genomicsengland.co.uk). This panel design provides coverage of coding regions and flanking intronic sequences (+/-20bp) of the genes listed below.

Gene List:

EGLN1	NM_022051.2
EPAS1	NM_001430.4
EPO	NM_000799.2
EPOR	NM_000121.3
HBA1	NM_000558.4
HBA2	NM_000517.4
HBB	NM_000518.4
VHL	NM_000551.3

Other genes: A number of "amber" genes are included in the design but are not currently analysed or reported. These are genes where there is some evidence to suggest a role in hereditary erythrocytosis and they are included to facilitate future clinical reporting and/or research and development. These would only be unmasked for analysis after sufficient evidence of clinical utility and with confirmation of appropriate consent via the patient's clinical team. Currently this includes the following genes: *BHLHE41, BPGM, GFI1B, HIF1A, HIF1AN, JAK2, KDM6A* and *SH2B3.*

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

Coverage criteria: For each sample reported, >95% of the target regions are 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 are 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 are mapped and variants identified using GenomeAnalysisToolKit (GATK) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference. Any clinically significant variants are confirmed by Sanger sequencing.

Variant reporting: Variant nomenclature follows HGVS guidelines (https://varnomen.hgvs.org/). Variants are classified and reported using ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020 v4.01 (https://www.acgs.uk.com).