IRON REGULATION DISORDERS GENE PANEL – TECHNICAL INFORMATION

Design: The Iron regulation gene panel was designed as a custom probe set and includes relevant genes from the Iron metabolism panel app panel R96 (v1.2; panelapp.genomicsengland.co.uk). This panel design provides coverage of coding regions and flanking intronic sequences (+/-20bp) of the genes listed below.

Where relevant to the referral, the following tests are also included:

- Sequencing of the 5' untranslated region of the *FTL* gene, which contains the iron responsive element (IRE) associated with hyperferritinaemia cataract syndrome
- Dosage analysis using the P347 Hereditary Haemochromatosis multiplex ligation dependant probe amplification (MLPA) kit is used to measure the copy number of the coding exons of the *HFE, SLC40A1* and *HAMP* genes and selected exons of the *TFR2* and *HFE2* genes (for full details see www.mrcholland.com).

Gene List:

ABCB7	(NM_004299.4)	*HAMP*	(NM_021175.3)
ALAS2	(NM_000032.4)	*HFE*	(NM_000410.3)
ATP7B	(NM_000053.2)	*HFE2*	(NM_213653.3)
BMP6	(NM_001718.6)	*SLC11A2*	(NM_001174125.1)
CP	(NM_000096.3)	*SLC25A38*	(NM_017875.2)
CYBRD1	(NM_02483.3)	*SLC40A1*	(NM_014585.5)
FTL	(NM_000146.3)	*TF*	(NM_001063.3)
GBA	(NM_000157.3)	*TFR2*	(NM_003227.3)
GLRX5	(NM_016417.2)	*TMPRSS6*	(NM_153609.3)

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 genetic iron regulation disorders 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: *FECH, FTH1, STEAP3.*

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).