Specifications of the NGS panel RBC enzyme deficiency

Targeted sequencing with the Ion Torrent System is able to identify single nucleotide variants, small insertions and small deletions. Variants in repeat sequences, large homopolymers and large insertions/deletions are not or difficult to identify.

The **RBC Enzym AmpliSeq Panel v5** (IAD95230_241) consists of 327 amplicons and is covering 99.48% of desired regions (all coding regions (exons), flanking intronic regions, untranslated regions and promotor areas with known (likely) pathogenic variants) from the 22 genes listed in Table 4.

Table 1: List of submitted genes and the coverage of the designed panel:

Gene	Chromosome	NCBI Transcript	Exons	Coverage	Coverage 5' of initiation site (flanking sequence and 5'UTR)	Amplicons
ABCB7	chrX	NM_004299.4	16	100,00%	-22 relative to initiation codon	24
AK1	chr9	NM_000476.2	6	100,00%	-87 relative to initiation codon	9
ALAS2	chrX	NM_000032.4	11	100,00%	-240 relative to transcription initiation site	19
ALDOA	chr16	NM_001243177.1	10	100,00%	-8 relative to initiation codon	12
CYB5R3	chr22	NM_001171660.1	9	97.25%	-189 relative to initiation codon	12
ENO1	chr1	NM_001428.	12	100,00%	-119 relative to initiation codon	12
G6PD	chrX	NM_000402.4	13	100,00%	-176 relative to initiation codon	19
GAPDH	chr12	NM_002046.5	9	77.48%	-36 relative to initiation codon	8
GCLC	chr6	NM_001498.3	16	100,00%	-713 relative to initiation codon	22
GPI	chr19	NM_000175.3	18	100,00%	-79 relative to initiation codon	22
GSR	chr8	NM_000637.3	13	98.94%	-115 relative to initiation codon	15
GSS	chr20	NM_000178.2	13	100,00%	-151 relative to initiation codon	15
HK1	chr10	NM_033497.2	22	100,00%	-157 relative to initiation codon	27
NT5C3A	chr7	NM_001002010.2	9	100,00%	-60 relative to initiation codon	13
PFKM	chr12	NM_001166686.1	25	100,00%	-129 relative to initiation codon	22
PGD	chr1	NM_002631.2	13	98.93%	-175 relative to initiation codon	13
PGK1	chrX	NM_000291.3	11	100,00%	-75 relative to initiation codon	12
PKLR	chr1	NM_000298.5	11	100,00%	-305 relative to initiation codon	16
PRDX2	chr19	NM_005809.5	6	100,00%	-48 relative to initiation codon	5
UGT1A1	chr2	NM_000463.2	5	100,00%	-124 relative to initiation codon	11
SLC25A38	chr3	NM_017875.2	7	100,00%	-199 relative to initiation codon	10
TPI1	chr12	NM_000365.5	7	100,00%	-65 relative to initiation codon	9

Table 2: List of missed bases in design:

Genes	Coverage	Nr. Missing Bases	GRCh37/hg19 coordinates	Description	Missing HGMD-DM variants
GSR	98.94%	19	chr8:30585140-30585158	located central in exon 1	non
CYB5R3	97.25%	31	chr22:43045295-43045326	complete exon 1 in splice variant with 10 exons	non
PGD	98.93%	17	chr1:10478877-10478894	5' exon 11 (12 base) and flanking region	non
GAPDH	77.48%	245	chr12:6646744-6646989	5' exon 7 (240 base) and flanking region	non

The percentage of target bases that is covered at least 20 times (%Base20x) is at least 98.31% for the recommended Mapped Reads of 750.000. With this acceptance criteria five amplicons failed to yield >20 times coverage over the full amplicon length, listed in Table 3a Two published disease making mutations (DM) and 4 likely pathological mutations with ''some degree of doubt'' (DM?) were missed, listed in Table 3b (Version HGMD 2015.3).

Table 3a: Failed amplicons and their missing bases in exons and flanking intronic regions

Gene	Amplicon	%Base20x amplicon	Locations with base coverage below 20x		missing HGMD
			chromosomal coordinates	description	
GSR	AMPL7155931151	0%	chr8:30585159-30585467	initiation site and first 194 bases of exon 1	none
G6PD	ES7.G6PD_17_18	11%	chrX:153775085-153775261	initiation site and first 2 bp of exon 1	none
TPI1	ES7.TPI1_1	20%	chr12:6976555-6976758	initiation site and first 27 bp of exon 1	1x DM + 3x DM?
G6PD	AMPL7158818825	16%	chrX:153759784-153760063	termination site	1x DM?
GSR	AMPL7155931073	0%	chr8:30546605-30546927	exon 9 and both flanking regions	1x DM

Table 3b: HGMD DM and DM? variants located in failed amplicons

Gene	HGMD reference	GRCh37/hg19	HGVS	Variant class	Phenotype	Reference
TPI1	CR961739	chr12:6976669	c.-62T>G	DM?	Triosephosphate isomerase deficiency	Watanabe (1996) Am J Hum Genet 58: 308 PubMed: 8571957
TPI1	CR993998	chr12:6976685	c.-46T>G	DM?	Triosephosphate isomerase deficiency	Watanabe (1996) Am J Hum Genet 58: 308 PubMed: 8571957
TPI1	CR962730	chr12:6976688	c.-43T>G	DM?	Triosephosphate isomerase deficiency	Watanabe (1996) Am J Hum Genet 58: 308 PubMed: 8571957
TPI1	CM962414	chr12:6976732	c.2T>A	DM	Triosephosphate isomerase deficiency	Schneider (1996) Blood Cells Mol Dis 22: 82 PubMed: 8807088
G6PD	CR133485	chrX:153759858	c.*357A>G	DM?	Glucose-6-phosphate dehydrogenase deficiency	Amini (2013) J Hum Genet 58: 189 PubMed: 23389243
GSR	CM074267	chr8:30546726	c.993G>A	DM	Glutathione reductase deficiency	Kamerbeek (2007) Blood 109: 3560 PubMed: 17185460

Reporting

Only clinical relevant variants will be reported. Variants with classification *Certainly Pathogenic* (class 5) and *Likely Pathogenic* (class 4) are always reported. Variants with category *Unknown significance* (class 3) will only be reported if the variant is expected to be involved in the phenotype of the patient. Category *Certainly Benign* (class 1) and *Likely Benign* (class 2) variants will not be reported. (see: *http://www.acgs.uk.com/quality/best-practice-guidelines/Variant Guidelines*. *Document: Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics*).

Besides reporting the clinical relevant variants we report whether a patients is heterozygous, homozygous, expected compound heterozygous or hemizygous for a mutation and how this may relate to disease phenotype.

All the variants are annotated and reported as designated by the Human Genome Variation Society (HGVS) nomenclature, as described at their website http://varnomen.hgvs.org