Specifications of the NGS panel RBC membrane disorders

The **RBC Membrane AmpliSeq Panel v3** (IAD89957_197) is covering 99.05% of submitted areas (all coding regions (exons), flanking intronic regions and (part of) untranslated regions) from the following 10 genes: **SPTA1, SPTB, ANK1, SLC4A1, EPB41, EPB42, RHAG, PIEZO1, SEC23B and UGT1A1** and include all known disease making variants listed in HGMD professional (Version HGMD 2015.3) (see Table 1and 2).

Table 1: List of submitted genes and coverage of the design:

Gene	Chromosome	NCBI Transcript	Exons	Submitted Bases	Coverage	Coverage 5' of initiation site (flanking sequence and 5'UTR)	Amplicons
ANK1	chr8	NM_001142446.1	43	3026	100,00%	204 base before initiation site	57
EPB41	chr1	NM_001166005.1	21	2806	100,00%	149 base before initiation site	20
EPB42	chr15	NM_000119.2	13	2296	100,00%	68 base before initiation site	15
PIEZO1	chr16	NM_001142864.2	51	8076	99,42%	base 34 of exon1	62
UGT1A1	chr2	NM_000463.2	5	1892	100,00%	124 base before initiation site	10
RHAG	chr6	NM_000324.2	10	1330	100,00%	95 base before initiation site	10
SEC23B	chr20	NM_032986.3	20	3455	100,00%	46 base before initiation site	28
SLC4A1	chr17	NM_000342.3	20	2926	100,00%	83 base before initiation site + regions of -3420 and -2865 before init. site	23
SPTA1	chr1	NM_003126.2	52	7780	96,85%	154 base before exon 2	52
SPTB	chr14	NM_001024858.2	35	7406	100,00%	240 base before exon 1 (incl promotor)	50

Table 2: List of missed bases in design:

Genes	Coverage	Number Missing Bases	GRCh37/hg19 coordinates	Description	Missing HGMD-DM variants
ANK1	99,42%	38	chr8:41753965-41754003	flanking region and first 33 bases exon 1	non
PIEZO1	96,85%	74	chr16:88851303-88851377	both flanking regios and Exon 1 (64 bases)	non
		17	chr16:88803041-88803058	flanking region and last 12 bases exon 11	non
		5	chr16:88800153-88800158	flanking site (5base) of 5' exon 18	non
		158	chr16:88782374-88782532	flanking region and first 153 bases exon 50	non

The percentage of target bases that is covered at least 20 times (%Base20x) is at least 99.44% for the recommended Mapped Reads of 750.000. This acceptance criteria will result in a coverage of all published disease causing variants listed in HGMD (downloaded November 2015). Two partial exon regions and their flanking intronic regions are not covered for 20 times. Both regions do not contain disease causing variants listed in HGMD professional (Version HGMD 2015.3).

Table 3: Bases possibly missed in exons and flanking intronic regions

Chr	GRCh37/hg19 coordinates		Amplicon id	Gene	Missing Bases	Exon	Missing HGMD
	Start	End					
chr16	88798299	88798318	AMPL7155929112	PIEZO1	20	22	non
chr16	88786834	88786940	AMPL7155930944	PIEZO1	106	41	non

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