Specification of the NGS platelet panel

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 designed Thrombo AmpliSeq Panel (IAD96657_243) consists of 1468 amplicons and is covering 346,5 Kbase for 73 different genes as listed in Table 1. The panel is covering 98,8% of the desired regions (all coding regions (exons), flanking intronic regions, untranslated regions and promotor areas with known mutations).

Table 1: List of genes in Thrombo AmpliSeq panel, version 1.0

Overview genes present in Thrombo AmpliSeq panel							
ABCG5	BLOC1S4	FLI1	GNB3	HPS6	NBEAL2	PTGS1	VIPAS39
ABCG8	BLOC1S5	FLNA	GP1BA	ITGA2	P2RY1	RASGRP2	VWF
ACTN1	BLOC1S6	GATA1	GP1BB	ITGA2B	P2RY12	RBM8A	WAS
ADRA2A	BLOC1S7 / SNAPIN	GATA2	GP6	ITGB1	PEAR1	RHOA	
ANKRD26	CYCS	GFI1B	GP9	ITGB3	PIK3CG	RUNX1	
ANO6	DTNBP1	GNA12	HOXA11	LYST	PLAU	TBX1	
AP3B1	ETV6	GNA13	HPS1	MASTL	PLCB2	TBXA2R	
BLOC1S1	F2R	GNAI1	HPS3	MPL	PLCB3	TBXAS1	
BLOC1S2	FCGR2A	GNAQ	HPS4	MYH9	PLCG2	THPO	
BLOC1S3	FERMT3	GNAS	HPS5	NBEA	PRKACG	TUBB1	

The percentage of target bases that is covered at least 20 times (%Base20x) is at least 99,1% for the recommended Mapped Reads of 2.800.000. This acceptance criteria will result in a coverage 98,8% of the areas of interest.

For 30 different genes a few bases are missed, either in the design or due to practical coverage, as listed in Table 2. Some pathogenic variants listed in the HGMD database are missed as well, as can be seen in Table 2.

Table 2a: List of regions missed in design of Thrombo AmpliSeq panel.

Part 1: Missed in designed regions

Gene	chromoso	coordinate start	coordinate end	Exon	% Gene covered	Missed number of bases	HGMD 2017.2	HGMD Accession
ADRA2A	chr10	112837927	112837945	Exon1	97.87	19	No	
ADRA2A	chr10	112838691	112838701	Exon1		11	No	
ANKRD26	chr10	27350143	27350176	Exon13	99.29	34	No	
ANKRD26	chr10	27375463	27375467	Intron 5 splicesite		5	No	
BLOC1S4	chr4	6718462	6718495	Exon1	94.88	34	No	
BLOC1S6	chr15	45879893	45879999	Alternative exon 1	82.5	107	No	
BLOC1S6	chr15	45897709	45897717	3' Exon4		9	No	
F2R	chr5	76012128	76012179	5' Exon1	93.02	52	No	
FLNA	chrX	153593277	153593303	Exon 12	99.67	27	No	
GNA12	chr7	2883656	2883800	Exon1, incl startcodon	91.19	145	No	
GNAS	chr20	57466777	57466925	Alternative exon 1	95.11	149	Yes	35 mutations
GNAS	chr20	57474999	57475015	Alternative exon 4		17	No	
GP1BB	chr22	19711756	19711761	Exon 2	99.04	6	Yes	CM1410119 & CM170899
HPS1	chr10	100189217	100189234	3' alternative laatste exon	98.41	18	No	
HPS1	chr10	100193692	100193710	3' of alternative larger exon 5		19	No	
HPS4	chr22	26849129	26849170	3' alternative last exon	98.17	42	No	
HPS5	chr11	18332993	18333003	5' Exon 5	99.69	11	No	
ITGA2B	chr17	42463489	42463504	5' Exon 2	99.53	16	No	
MASTL	chr10	27466725	27466749	Alternative exon 10	98.45	25	No	
MASTL	chr10	27450099	27450116	3' Exon4		18	No	
MYH9	chr22	36698609	36698727	Exon 20	97.79	119	Yes	CM168533 & CM1310514
MYH9	chr22	36685126	36685144	3' Exon 32		19	Yes	CM144001, CM066926 & CM101452
NBEA	chr13	35517156	35517174	Exon1	99.15	19	No	
NBEA	chr13	35615220	35615245	Exon2		26	No	
NBEA	chr13	35692360	35692394	Exon15		35	No	
NBEAL2	chr3	47038022	47038087	3' Exon 16	99.25	66	No	
PEAR1	chr1	156878497	156878511	5' Exon10	98.89	15	No	
PEAR1	chr1	156879538	156879559	5' Exon12		22	No	
PIK3CG	chr7	106508333	106508338	Exon1	99.82	6	No	
PLCB3	chr11	64019147	64019159	Exon1	99.68	13	No	
PRKACG	chr9	71628927	71628939	Exon1	98.78	13	No	
RUNX1	chr21	36164427	36164440	3' last exon incl stopcodon	99.00	14	No	
TBX1	chr22	19748594	19748608	Inside exon 3	99.03	15	No	
TBXAS2R	chr19	3594919	3595145	5' Exon4	32.24	227	No	
VWF-pron	chr12	6235179	6235199		85.30	21	No	
VWF-pron	chr12	6235593	6235865		97.31	273	Yes	CR070430

Table 2b: List of regions missed by coverage in Thrombo AmpliSeq panel.

Part 2: Missed by coverage in experiments

Gene	chromoso	coordinate start	coordinate end	Exon	% Gene covered	Missed number of bases	HGMD 2017.2	HGMD Accession	#reads	Remarks
PLCB3	chr11	64019122	64019146	exon 1	99.37	25	No		<2	
NBEA	chr13	36223787	36223989	exon 51	97.85	203	No		<2	
ABCG5	chr2	44052023	44052162	exon 7	93.24	140	Yes	CM104323 & CI092640	<10	
HPS3	chr3	148847506	148847732	exon 1	92.84	227	No		<30	
PRKACG	chr9	71628940	71629010	exon 1	93.25	69	No		<20	
TBX1	chr22	19748423	19748593	exon 3	86.88	170	No		<10	
TBX1	chr22	19754123	19754156	exon 9		34	Yes	CM072072	<10	
GNAS	chr20	57430136	57430326	exon 1	94.37	191	No		<12	
PLCG2	chr16	81962158	81962234	exon 24	98.12	77	No		>10 <20	
VWF	chr12	6167087	6167215	exon 14	98.82	129	Yes	CM134325, CM941396, CM147756, CM107439, CM951301, CM1211909, C5066319 & CI941933	<10	
GP1BA	chr17	Will differ in each sample, due to repeat. Therefor an average has been taken as number of missed bases.		exon 2	~97,7	45	Yes	CM169008 & CI117143	<10	This amplicon is possible to sequence with Sanger.

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 patient 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