

Specification of the NGS platelet panel

Targeted sequencing with the lon 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

overview genes present in thrombo Amphised 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

Part 1: N		designed regions						
Gene		o 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		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-pro	tchr12	6235179	6235199		85.30	21	No	
VWF-pro	chr12	6235593	6235865		97.31	273	Yes	CR070430
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Table 2b: List of regions missed by coverage in Thrombo AmpliSeq panel.

art 2: Missed by coverage in experiments								
io coordinate start	coordinate end	Exon	% Gene covered	Missed number of bases	HGMD 2017.2	HGMD Accession	#reads	Remarks
64019122	64019146	exon 1	99.37	25	No		2	
36223787	36223989	exon 51	97.85	203	No		2]
44052023	44052162	exon 7	93.24	140	Yes	CM104323 & CI092640	<10]
148847506	148847732	exon 1	92.84	227	No		<30]
71628940	71629010	exon 1	93.25	69	No		<20	1
19748423	19748593	exon 3	86.88	170	No		<10	1
19754123	19754156	exon 9		34	Yes	CM072072	<10	
57430136	57430326	exon 1	94.37	191	No		<12	1
81962158	81962234	exon 24	98.12	77	No		>10 <20]
						CM134325, CM941396, CM147756,		1
						CM107439, CM951301, CM1211909,		
6167087	6167215	exon 14	98.82	129	Yes	C\$066319 & CI941933	<10	
Will differ in ear	ch sample, due to							
repeat. Therefor a	n average has been	exon 2	~97,7	45	Yes	CM169008 & CI117143	<10	
taken as numbe	r of missed bases.							This amplicon is possible to sequence with Sanger.
	ocoordinate start 64019122 36223787 44052023 148447506 77562940 19784423 19754123 57430136 81962138 6167087 Will differ in ear repeat. Therefor a	occordinate start coordinate end 64019122 64019146 36223787 36223989 44052003 44052162 148847506 148847732 7162840 7162910 19748423 1974593 19754123 1974556 57430136 5743026 81962158 81962234 6167087 6167215 Will differ in each sample, due to	ocondinate start condinate end Exon 64019122 64019124 exon 1 36223787 36223989 exon 51 44050203 44052162 exon 7 148847506 148847750 exon 1 71628340 71629010 exon 1 1974423 1974513 exon 3 19754123 19754156 exon 3 1975423 19754126 exon 1 51430136 57430126 exon 1 6167087 6167215 exon 14 Will differ in exon a sample, due to repeat. Therefore an average has been exon 2	ocondinate start coordinate end Xon % Gene covered 64019122 64019146 exon 1 99.37 36223787 36223787 exon 51 97.85 44050203 44052162 exon 7 93.24 148847506 148847750 exon 1 92.28 17162840 716289010 exon 1 92.28 19748473 19748973 exon 3 86.88 19754123 19754156 exon 2 57430136 57430126 exon 1 94.37 51890128 818902234 exon 24 98.12 98.12 6167087 6167215 exon 14 98.82 6167087 6167215 exon 14 98.82	occontinue start coordinate and Sc Gene covered Missed number of bases 64019122 64019124 64019126 exon 1 99.37 25 36223787 36223789 exon 51 97.85 203 44050203 440520162 exon 7 93.24 140 148847506 148847750 exon 1 92.284 227 7162840 71859010 exon 1 93.25 69 19748423 1974593 exon 3 86.88 170 19748423 1974593 exon 3 86.88 170 19748423 19754126 exon 1 94.37 191 57430136 57430126 exon 1 94.37 191 51870158 81896224 exon 24 98.12 77 6167087 6167215 exon 14 98.82 123 Will differ in exch sample, due to repeat. Therefor an average has been exon 2 "97,7 45	occondinate start coordinate end Xon % Gene covered Missed number of bases HGMD 2017.2 64019122 64019124 exon 1 99.37 25 No 36223787 36223789 exon 51 97.85 203 No 44052023 44022162 exon 7 93.24 140 Yes 148547505 148547505 148547705 152.264 227 No 71628940 716289010 exon 1 93.25 69 No 19748423 1974593 exon 3 86.88 170 No 19748423 1974593 exon 1 94.37 191 No 19749423 1974593 exon 1 94.37 191 No 57430136 57430126 exon 14 98.12 77 No 6167087 6167215 exon 14 98.82 129 Yes 6167087 6167215 exon 14 98.82 129 Yes	o coordinate start coordinate cond 64019122 64019122 64019124 64019124 64019124 16000 Accession 64023787 3623789 exon 1 99.37 25 No 26223787 3622389 exon 51 97.75 203 No 440502023 44052162 exon 7 93.24 140 Yes CM104323 & C1092040 440502023 44052162 exon 1 92.24 227 No 148847506 148947732 exon 1 93.25 69 No 1974423 1974593 exon 3 86.88 170 No 19754123 1974593 exon 3 86.88 170 No 19754123 19754156 exon 9 34 Yes CM072072 57430136 57430126 exon 1 94.37 191 No 197657 6167716 exon 14 98.12 77 No CM104323, CM941396, CM147756, CM147756, CM104333, CM941396, CM147756 CM104325, CM941396, CM147756, CM147756, CM104335, CM941396, CM147756, CM104735, CM941396, CM147756, CM104736, CM951012, CM1211909, 616701 616721 exon 14 98.82 129 Yes C5066319 & CI941933 Will differ in exh sample, due to xon 2 "97,7 45 Yes CM169008 & CI117143	io: coordinate stat coordinate stat coordinate stat coordinate stat coordinate stat coordinate stat reads 64018122 64018124 exon 1 99.37 25 No <2

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