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 **Sanquin NGS Panel** complement/hemostasis (test code X001 to X006) includes 44 genes and regions of interest. Using this panel, the exones and intron/exon borders of the relevant genes are sequenced. For most proteins, functional/expression level testing is available (see request form no 10: immunodiagnostics and no 4: coagulation).

Table 1: *Genes covered by the complement/hemostasis targeted NGS panel.*

Encoded protein	Context	Gene	Chr	Chr. Positie	OMIM
alpha 2-antiplasmin	Hemostasis/trombosis gene	SERPINF2	17	17p13	613168
Factor IX	Hemostasis/trombosis gene	F9	X	Xq27.1-27.2	300746
Factor V	Hemostasis/trombosis gene	F5	1	1q23	612309
Factor VII	Hemostasis/trombosis gene	F7	13	13q34	613878
Factor VIII	Hemostasis/trombosis gene	F8	X	Xq28	300841
Factor XI	Hemostasis/trombosis gene	F11	4	4q35	264900
Tissue factor	Hemostasis/trombosis gene	F3	1	1p22-21	134390
Von Willebrand factor	Hemostasis/trombosis gene	VWF	12	12p13.3	613160
Factor XIIIa	Hemostasis/trombosis gene	F13A1	6	6p25.3-24.3	134570
Factor XIIIb	Hemostasis/trombosis gene	F13B	1	1q31-32.1	134580
gamma-glutamyl carboxylase	Hemostasis/trombosis gene	GGCX	2	2p12	137167
a Disintergrin and metalloproteinase wih thrombospondin repeats	Hemostasis/trombosis gene	ADAMTS13	9	9q34.2	604134
Antithrombin	Hemostasis/trombosis gene	SERPINC1	1	1q23-25.1	107300
Plasminogen	Hemostasis/trombosis gene	PLG	6	6q26	173350
Protein C	Hemostasis/trombosis gene	PROC	2	2q13-14	612283
protein C receptor, endothelial	Hemostasis/trombosis gene	PROCR	20	20q11.2	600646
Protein S	Hemostasis/trombosis gene	PROS1	3	3q11.2	176880
tissue factor pathway inhibitor -1	Hemostasis/trombosis gene	TFPI	2	2q32	152310
Trombomoduline	Hemostasis/trombosis gene	THBD	20	20p11.21	188040
Methylmalonic aciduria and homocystinuria, cblC type	complement genes	MMACHC	1	1p34.1	609831
Complement component 8 alpha chain	complement genes	C8A	1	1p32.2	120950
Complement component 8 beta chain	complement genes	C8B	1	1p32.2	120960
Complement component 8 gamma chain	complement genes	C8G	9	9q34.3	120930
Complement factor H	complement genes	CFH	1	1q31.3	134370
Complement factor H related 1	complement genes	CFHR1	1	1q31.3	134371
Complement factor H related 2	complement genes	CFHR2	1	1q31.3	600889
Complement factor H related 3	complement genes	CFHR3	1	1q31.3	605336
Complement factor H related 4	complement genes	CFHR4	1	1q31.3	605337
Complement factor H related 5	complement genes	CFHR5	1	1q31.3	608593
Cluster of diffentiation 46	complement genes	CD46	1	1q32.2	120920

Cluster of diffentiation 55	complement genes	CD55	1	1q32.2	125240
Cluster of diffentiation 59	complement genes	CD59	11	11p13	107271
Serine proteinase inhibitor G1 (C1-inhibitor)	complement genes	SERPING1	11	11q12.1	606860
Diacyl glycerol kinase epsilon	complement genes	DGKE	17	17q2	601440
Complement factor D	complement genes	CFD	19	19p13.3	134350
Complement component 3	complement genes	C3	19	19p13.3	120700
Complement factor I	complement genes	CFI	4	4q25	217030
Complement component 9	complement genes	C9	5	5p13.1	120940
Complement component 7	complement genes	C7	5	5p13.1	217070
Complement component 6	complement genes	C6	5	5p13.1	217050
Coagulant factor 12	complement genes	F12	5	5q35.3	610619
Complement factor B	complement genes	CFB	6	6p21.33	138470
Complement component 5	complement genes	C5	9	9q33.2	120900
Complement factor properdin	complement genes	CFP	X	xp11.23	300383

The complement/hemostasis targeted NGS panel is covering 99, 09% of the desired design (exons, flanking intronic regions, untranslated regions and promotor areas). In table 2, the missed regions and their genomic coordinates are listed. In total 9 disease causing variants listed in HGMD (version 2017-4) are not covered in the design.

Table 2: *List of missed bases in the design of complement/hemostasis targeted NGS panel*

Gene	Chr	number missing bases	Position	coverage%	missing HGMD-DM variants
F7	13	6	113,765,157-113,765,163	99,81	
vWF	12	253	6,233,587-6,233,840	98,55	
F13A1	6	105	6,320,819-6,320,924	97,46	
PROS1	3	94	93,647,546-93,647,640	98,05	
TFPI	2	305 121	188,418,927-188,419,232 188,394,090-188,394,211	84,97	
CD59	11	92	33,744,181-33,744,273	94,34	
DGKE	17	163	54,911,945-54,911,622	94,98	
CFI	4	24	110,679,091-110,679,115	99.49	
C6	5	216	41,213,479-41,213,695	95.69	
F12	5	9 13	176,830,994-176,831,003 176,831,810-176,831,823	99,44	1
ADAMTS13	9	67 146	136,288,136-136,288,287 136,291,319-136,291,465	97.11	8
CFP	X	177	47,489,527-47,489,704	96.19	

The percentage of target bases that is covered 20 times (%Base20x), is at least 98.9% for the recommended Mapped Reads of 1.750.000. With this acceptance criteria, 15 amplicons failed to yield >20times coverage over the full amplicon length,listed in Table 3a). 52 published disease making mutations (DM) and 2 likely pathological mutations with"some degree of doubt" (DM?) were missed in these regions (HGMD 2018-2), listed in Table 3b.

Table 3a: *Failed (low coverage) amplicons and their missing genomic locations and published mutations (HGMD, 2018-2)*

Gene	Amplicon	%Base20x amplicon	Locations with base coverage below 20x		missing HGMD
			chromosomal coordinates	description	
VWR	ES7.VWF_60	30%	chr12: 6167087 - 6167247 (160base)	5' exon 14 (124bp) + flanking region (36bp)	8 DM + 2 DM?
GGCX	ES7.GGCX_8	0%	chr2: 85779508 - 85779723 (215base)	volledig exon 10 (chr2: 85779539 - 85779690) + both flanking region	2 DM
F7	ES7.F7_9	24%	chr13: 113771935 - 113772112 (177base)	only intronic region (exon 8 + flanking regions well covered)	none
PROC	ES7.PROC_5	25%	chr2:128180681 - chr2:128180870 (189 base)	3' exon 5 (chr2:128180681-128180747) + 5' exon 6 (chr2:128180850-128180871)	34 DM
PLG	ES7.PLG_21	10%	chr6:161173191 - 161173373 (182 base)	3' exon 18 (chr6:161173191-161173292) + flanking region	1 DM
CFHR3	AMPL7160434353	40%	chr1:196749193 - 196749383 (190 base)	only intronic region (exon 3 + flanking regions well covered)	none
CFP	ES10.CFP_4	13%	chrX:47485795 - 47485956 (161 base)	5' exon 8 (chrX:47485795-47485918) + flanking region	5 DM
F8	ES7.F8_6	0%	chrX:154114288 - 154114495 (208 base)	exon 1 (chrX:154114409-154114433(24 base) + UTR + flanking region	none
CFHR3	AMPL7160434378	59%	chr1:196748218 - 196748346 (129 base)	5' exon 2 (chr1:196748292-196748346(55 base)) + flanking region	none
CFHR3	AMPL7160434330	55%	chr1:196759179 - 196759326 (149 base)	almost complete exon 5 (chr1:196759179 - 196759326)	none
CFHR4	AMPL7160434334	87%	chr1:196882107 - 196882146 (40 base)	only intronic region 3 (exon 3 + flanking regions well covered)	none
CFHR2	AMPL7160434361	63%	chr1:196920144 - 196920250 (107 base)	3' exon 3 (chr1:196920144 - 196920158 (15 base) + flanking region	none
SERPING	AMPL7156549776	42%	chr11:57364916 - 57365109 (194 base)	UTR + 5' exon 1 (chr11:57365027-57365109 (84 base))	2 DM
PLG	ES7.PLG_14	78%	chr6:161152738 - 161152791 (54 base)	5' exon 12: chr6:161152777-chr6:161152791 (14 base) + flanking region	none
VWF	ES7.VWF_24	9%	chr12: 6104972 - 6105179 (208 base)	3' exon 35: chr12:6105168-6105179 (12 base) + flanking region	none

Table 3b: *List of missing published HGMD variants in amplicons with low coverage (HGMD 2018-2)*

GRCh37/hg19	Gene	HGMD Ref	HGVS	Variant class	Phenotype	Reference
chr12:6167208-10	VWF	CD1714155	NM_000552.3: c.1534_1536delCTG	DM	Von Willebrand disease 3	Borràs (2017) Haematologica epub
chr12:6167213	VWF	CS066319	NM_000552.3: c.1534-3C>A	DM	Von Willebrand disease 1	Gallinaro (2006) Thromb Haemost 96: 711
chr12:6167196	VWF	CM1718319	NM_000552.3: c.1548T>A; NP_000543.2: p.Y516*	DM	Von Willebrand disease 1	Lavin (2017) Blood 130: 2344
chr12:6167161	VWF	CM941396	NM_000552.3: c.1583A>G; NP_000543.2: p.N528S	DM	Von Willebrand disease 2c	Haberichter (2010) Blood 115: 4580
chr12:6167137	VWF	CM147756	NM_000552.3: c.1607T>C; NP_000543.2: p.L536P	DM	Von Willebrand disease 2a	Brehm (2014) Thromb Haemost 112: 96
chr12:6167098	VWF	CM1714100	NM_000552.3: c.1646T>C; NP_000543.2: p.F549S	DM	Von Willebrand disease	Borràs (2017) Haematologica epub
chr12:6167096	VWF	CM951301	NM_000552.3: c.1648G>A; NP_000543.2: p.G550R	DM	Von Willebrand disease 2c	Brehm (2014) Thromb Haemost 112: 96
chr12:6167090	VWF	CM1714102	NM_000552.3: c.1654G>A; NP_000543.2: p.A552T	DM	Von Willebrand disease 2n	Borràs (2017) Haematologica epub
chr12:6167173	VWF	CM134325	NM_000552.3: c.1571G>A; NP_000543.2: p.C524Y	DM?	Von Willebrand disease 2a	Flood (2013) Clin Chem 59, 684
chr12:6167119	VWF	CM107439	NM_000552.3: c.1625C>G; NP_000543.2: p.A542G	DM?	Von Willebrand disease	Veyradier (2016) Medicine (Baltimore) 95: e3038
chr2:85779552	GGCX	CM078087	NM_000821.5: c.1426C>T; NP_000812.2: p.R476C	DM	Pseudoxanthoma elasticum	Vanakker (2007) J Invest Dermatol 127: 581
chr2:85779551	GGCX	CM078088	NM_000821.5: c.1427G>A; NP_000812.2: p.R476H	DM	Pseudoxanthoma elasticum	Vanakker (2007) J Invest Dermatol 127: 581
chr2:128180686	PROC	CD104283	NM_000312.3:c.339delC	DM	Protein C deficiency	Pai (2010) ANN HAEMAT 89, 835
chr2:128180687	PROC	CM950991	NM_000312.3:c.340G>C; NP_000303.1:p.G114R	DM	Protein C deficiency	Lind (1995) TH 73, 186
chr2:128180687	PROC	CM950992	NM_000312.3:c.340G>T; NP_000303.1:p.G114C	DM	Protein C deficiency	Reitsma (1995) TH 73, 876
chr2:128180687-701	PROC	CD931043	NM_000312.3:c.340_354delGGCATCGGCAGCTTC	DM	Protein C deficiency	Poort (1993) BCF 4, 273
chr2:128180691-702	PROC	CX005563	NM_000312.3:c.344_355delTCGGCAGCTTCAinsCGT	DM	Protein C deficiency	Alhenc-Gelas (2000) TH 83, 86
chr2:128180693	PROC	CM179761	NM_000312.3:c.346G>A; NP_000303.1:p.G116S	DM	Thrombosis	Chen (2017) TH 117, 1358
chr2:128180695-96	PROC	CI962338	NM_000312.3:c.340_348dupGGCATCGGC	DM	Protein C deficiency	Ireland (1996) TH 76, 867
chr2:128180696-99	PROC	CD123690	NM_000312.3:c.349_352delAGCT	DM	Protein C deficiency	Tang (2012) PLOS ONE 7, e35773
chr2:128180699	PROC	CM910307	NM_000312.3:c.352T>C; NP_000303.1:p.F118L	DM	Protein C deficiency	Reitsma (1991) BLOOD 78, 890
chr2:128180702-19	PROC	CD910546	NM_000312.3: c.355_372delAGCTGCGACTGCCGCAGC	DM	Protein C deficiency	Tsuda (1991) Thromb Haemost 65 647A
chr2:128180705	PROC	CM950993	NM_000312.3:c.358T>G; NP_000303.1:p.C120G	DM	Protein C deficiency	Reitsma (1995) TH 73, 876
chr2:128180707	PROC	CM071944	NM_000312.3:c.360C>A; NP_000303.1:p.C120*	DM	Protein C deficiency	Rovida (2007) HUM MUT 28, 345
chr2:128180707-24	PROC	CD910547	NM_000312.3:c.360_377delCGACTGCCGCAGCGGCTG	DM	Protein C deficiency	Tsuda (1991) TH 65, 647A
chr2:128180719	PROC	CM014374	NM_000312.3:c.372C>G; NP_000303.1:p.S124R	DM	Protein C deficiency	Taliani (2001) GENET TEST 5, 39
chr2:128180720	PROC	CM961152	NM_000312.3:c.373G>C; NP_000303.1:p.G125R	DM	Protein C deficiency	Miyata (1996) TH 76, 302
chr2:128180720	PROC	CM004058	NM_000312.3:c.373G>T; NP_000303.1:p.G125C	DM	Protein C deficiency	Singh (2000) HAEMATOL 85, 891
chr2:128180730	PROC	CM1618220	NM_000312.3:c.383G>A; NP_000303.1:p.G128D	DM	Protein C deficiency	Cheng (2016) BCF 27, 838
chr2:128180733	PROC	CM950994	NM_000312.3:c.386G>A; NP_000303.1:p.R129H	DM	Protein C deficiency	Reitsma (1995) TH 73, 876
chr2:128180735-44	PROC	CD157088	NM_000312.3:c.388_397delTTCTGCCAGC	DM	Protein C deficiency	Boey (2015) BJH epub
chr2:128180736	PROC	CM128563	NM_000312.3:c.389T>G; NP_000303.1:p.F130C	DM	Protein C deficiency	Caspers (2012) TH 108, 247
chr2:128180739	PROC	CM005555	NM_000312.3:c.392G>C; NP_000303.1:p.C131S	DM	Protein C deficiency, type I	Alhenc-Gelas (2000) TH 83, 86
chr2:128180745	PROC	CM005564	NM_000312.3:c.398G>C; NP_000303.1:p.R133P	DM	Protein C deficiency, type I	Alhenc-Gelas (2000) TH 83, 86
chr2:128180746	PROC	CM005565	NM_000312.3:c.399C>T; NP_000303.1:p.R133R	DM	Protein C deficiency, type I	Alhenc-Gelas (2000) TH 83, 86
chr2:128180747	PROC	CM950995	NM_000312.3:c.400G>T; NP_000303.1:p.E134*	DM	Protein C deficiency	Reitsma (1995) TH 73, 876
chr2:128180748	PROC	CS004399	NM_000312.3:c.400+1G>C	DM	Protein C deficiency	Miyata (1998) THROMB RES 92, 181
chr2:128180749	PROC	CS128579	NM_000312.3:c.400+2T>C	DM	Protein C deficiency	Caspers (2012) TH 108, 247
chr2:128180752	PROC	CS910456	NM_000312.3:c.400+5G>A	DM	Protein C deficiency	Reitsma (1991) BLOOD 78, 890
chr2:128180752	PROC	CS910457	NM_000312.3:c.400+5G>C	DM	Protein C deficiency	Reitsma (1991) BLOOD 78, 890
chr2:128180752	PROC	CS910458	NM_000312.3:c.400+5G>T	DM	Protein C deficiency	Reitsma (1991) BLOOD 78, 890
chr2:128180832-47	PROC	CD001911	NM_000312.3:c.401-18_401-3delGCCCTCCCCTGCCCGC	DM	Protein C deficiency	Millar (2000) HUM GENET 106, 646
chr2:128180848	PROC	CS128580	NM_000312.3:c.401-2A>C	DM	Protein C deficiency	Caspers (2012) TH 108, 247
chr2:128180848	PROC	CS930861	NM_000312.3:c.401-2A>G	DM	Protein C deficiency	Soria (1993) HUM GENET 92, 506
chr2:128180867	PROC	CM071947	NM_000312.3:c.418T>C; NP_000303.1:p.C140R	DM	Protein C deficiency	Rovida (2007) HUM MUT 28, 345
chr2:128180871	PROC	CM961153	NM_000312.3:c.422C>A; NP_000303.1:p.S141*	DM	Protein C deficiency	Ireland (1996) TH 76, 867
chr6:161173272	PLG	CM981587	NM_000301.3: c.2251G>A; NP_000292.1: p.G751R	DM	Plasminogen deficiency	Higuchi (1998) Br J Haematol 103, 867
chrX:47485831	CFP	CM972880	NM_002621.2: c.1028A>G; NP_002612.1: p.Q343R	DM	Properdin deficiency	Truedsson (1997) Immunopharmacology 38: 203
chrX:47485823	CFP	CM994439	NM_002621.2: c.1036C>T; NP_002612.1: p.R346C	DM	Properdin deficiency	Fijen (1999) Mol Immunol 36: 863 PubMed: 10698340
chrX:47485898	CFP	CM972879	NM_002621.2: c.961T>G; NP_002612.1: p.W321G	DM	Properdin deficiency	Truedsson (1997) Immunopharmacology 38: 203
chrX:47485897	CFP	CM001765	NM_002621.2: c.962G>C; NP_002612.1: p.W321S	DM	Properdin deficiency	van den Bogaard (2000) Eur J Hum Genet 8: 513
chrX:47485897	CFP	CM171423	NM_002621.2: c.962G>A; NP_002612.1: p.W321*	DM	Immunodeficiency, primary	Stray-Pedersen (2017) J Allergy Clin Immunol 139: 232
chr11:57365057	SERPING1	HR080001	NM_000062.2: c.-161A>G (-687 relative to initiation codon)	DM	Angioneurotic oedema	Uyguner (2008) Hum Genet 124 309
chr11:57365055	SERPING1	CR961722	NM_000062.2: c.-163C>T (-689 relative to initiation codon)	DM	Angioneurotic oedema	Verpy (1996) Am J Hum Genet 59: 308

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