Product Description
SALSA® MLPA® Probemixes P011-B4 / P012-B4 VWF

To be used with the MLPA General Protocol.

P011 version B4
For complete product history see page 9.

P012 version B4
For complete product history see page 9.

Catalogue numbers:
- **P011-025R:** SALSA MLPA Probemix P011 VWF mix 1, 25 reactions.
- **P011-050R:** SALSA MLPA Probemix P011 VWF mix 1, 50 reactions.
- **P011-100R:** SALSA MLPA Probemix P011 VWF mix 1, 100 reactions.

- **P012-025R:** SALSA MLPA Probemix P012 VWF mix 2, 25 reactions.
- **P012-050R:** SALSA MLPA Probemix P012 VWF mix 2, 50 reactions.
- **P012-100R:** SALSA MLPA Probemix P012 VWF mix 2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P011/P012 VWF is a **research use only (RUO)** assay for the detection of deletions or duplications in the *VWF* gene, which is associated with von Willebrand Disease (vWD).

vWD is the most common hereditary coagulation abnormality described in humans. vWD is caused by a deficiency of von Willebrand factor (vWF), a blood glycoprotein which mediates the interaction of platelets with damaged endothelial surfaces at sites of vascular injury. vWF also acts as the carrier for factor VIIIC, thus increasing the half-life of VIIIC in the circulation. Furthermore, the vWF protein is involved in a number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and possibly hemolytic-uremic syndrome.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK7014/.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *VWF* exon numbering used in this P011-B4/P012-B4 VWF product description is the exon numbering from the LRG_587 sequence. The exon numbering of the NM_sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequence. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P011-B4 VWF contains 37 MLPA probes with amplification products between 124 and 432 nucleotides (nt). This includes 28 probes targeting 26 out of 52 exons of the *VWF* gene (two probes for exons 2 and 6). In addition, nine reference probes are included that detect autosomal chromosomal locations.
The SALSA MLPA Probemix P012-B4 VWF contains 37 MLPA probes with amplification products between 124 and 433 nucleotides (nt). This includes 28 probes targeting 28 out of 52 exons of the *VWF* gene. In addition, nine reference probes are included that detect autosomal chromosomal locations.
Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

These probemixes contain nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of von Willebrand Disease. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.

- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *VWF* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P011/P012 VWF.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***VWF* mutation database**

https://databases.lovd.nl/shared/genes/VWF. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *VWF* exons 10 and 12 but not exon 11) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P011-B4 VWF mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*VWF*
64-105	Control fragments – see table in probemix content section for more information		
124	Reference probe 19616-L26241	4p	
130	**VWF probe** 11314-L12040		**Exon 5**
136	**VWF probe** 11332-L12057		**Exon 39**
142	Reference probe 21399-L29876	3q	
148	**VWF probe** 11335-L12060		**Exon 45**
154	**VWF probe** 11325-L12050		**Exon 24**
160	**VWF probe** 11330-L12055		**Exon 36**
169	**VWF probe** 11321-L12046		**Exon 14**
178	**VWF probe** 11339-L12064		**Exon 50**
185	**VWF probe** 11328-L12053		**Exon 33**
195	Reference probe 21625-L30241	22q	
202	**VWF probe** 11320-L12045		**Exon 13**
211	**VWF probe** 11331-L29230		**Exon 38**
217	**VWF probe** 11319-L21938		**Exon 9**
226	**VWF probe** 11324-L12049		**Exon 23**
232	**VWF probe** 11338-L12063		**Exon 49**
238	Reference probe 21689-L30520	17q	
244	**VWF probe** 21673-L12051		**Exon 26**
256	**VWF probe** 11334-L14242		**Exon 43**
265	**VWF probe** 13422-L14877		**Exon 2**
274	**VWF probe** 11336-L12061		**Exon 46**
283	**VWF probe** 11329-L12054		**Exon 34**
301	Reference probe 09986-L10445	7q	
310	**VWF probe** 11316-L12042		**Exon 6**
319	**VWF probe** 11333-L12058		**Exon 42**
337	**VWF probe** 13423-L14878		**Exon 2**
350	**VWF probe** 11322-L29229		**Exon 18**
355	**VWF probe** 11318-L12043		**Exon 8**
364 ±	**VWF probe** 20970-L29212		**Exon 20**
373	Reference probe 10718-L11300	6p	
384	**VWF probe** 11327-L21939		**Exon 28**
391	**VWF probe** 13426-L14881		**Exon 47**
400	Reference probe 17960-L22873	18q	
409	Reference probe 10053-L10477	8q	
418	**VWF probe** 11340-L12065		**Exon 52**
427	**VWF probe** 11315-L21940		**Exon 6**
432	Reference probe 10876-L11546	15q	

[a] See section Exon numbering on page 2 for more information.

± SNP rs34510401 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 2. SALSA MLPA Probemix P012-B4 VWF mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*VWF*
64-105	Control fragments – see table in probemix content section for more information		
124	Reference probe S0645-L19362	3p	
130	**VWF probe** 11346-L12071		**Exon 12**
136	**VWF probe** 11362-L12087		**Exon 40**
148	**VWF probe** 11355-L12080		**Exon 28**
154	**VWF probe** 11356-L12081		**Exon 29**
160	**VWF probe** 11359-L12084		**Exon 32**
172	**VWF probe** 11352-L12077		**Exon 22**
178	**VWF probe** 11354-L12079		**Exon 27**
184	**VWF probe** 11350-L12075		**Exon 19**
193	Reference probe 11556-L26606	5q	
202	**VWF probe** 11366-L12091		**Exon 51**
208	**VWF probe** 11363-L12088		**Exon 41**
215	**VWF probe** 11342-L12067		**Exon 4**
223	**VWF probe** 12799-L14243		**Exon 37**
232	**VWF probe** 11341-L12066		**Exon 1**
238	Reference probe 20186-L27463	14q	
247	**VWF probe** 11351-L12076		**Exon 21**
256	**VWF probe** 11349-L12074		**Exon 17**
274	**VWF probe** 11358-L12083		**Exon 31**
283	**VWF probe** 11364-L12089		**Exon 44**
292	**VWF probe** 13425-L14880		**Exon 47**
301	Reference probe 22146-L31174	16p	
310	**VWF probe** 11344-L12069		**Exon 10**
322 +	**VWF probe** 11353-L12078		**Exon 25**
328	**VWF probe** 11348-L21958		**Exon 16**
337	Reference probe 10376-L10928	9q	
346	**VWF probe** 11360-L12085		**Exon 35**
355	**VWF probe** 11365-L12090		**Exon 48**
364	**VWF probe** 11347-L12072		**Exon 15**
373	Reference probe 18296-L25750	8p	
382	**VWF probe** 11343-L12068		**Exon 7**
391	**VWF probe** 11357-L12082		**Exon 30**
400	Reference probe 17960-L22873	18q	
409	Reference probe 21009-L29227	1p	
418	**VWF probe** 11345-L12070		**Exon 11**
427	**VWF probe** 06683-L06261		**Exon 3**
433	Reference probe 16284-L23270	11q	

[a] See section Exon numbering on page 2 for more information.

+ SNP rs4021576 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 3. *VWF* probes arranged according to chromosomal location

Length (nt) P011 P012	SALSA MLPA probe	*VWF* exon[a]	Ligation site NM_000552.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*251-253 (Exon 2)*		
232	11341-L12066	Exon 1	205-206	GCAGCTGAGTTT-CCCAGGGACCTT	1.3 kb
265	13422-L14877	Exon 2	5 nt before exon 2	CTCTTGCTTCTT-TGCAGATGATTC	0.1 kb
337	13423-L14878	Exon 2	22 nt after exon 2	AAGGGCCTCCAT-TTCTCATTCCTG	1.9 kb
427	06683-L06261	Exon 3	375-376	CGGAAGTGACTT-CGTCAACACCTT	10.4 kb
215	11342-L12067	Exon 4	523-524	CTTGGGGAATTT-TTTGACATCCAT	0.4 kb
130	11314-L12040	Exon 5	691-692	GGCAACTTTCAA-GTCCTGCTGTCA	14.9 kb
427	11315-L21940	Exon 6	789-790	CACAGGGACCTT-GACCTCGGACCC	0.1 kb
310	11316-L12042	Exon 6	850-851	GAACAGTGGTGT-GAACGGGCATCT	20.1 kb
382	11343-L12068	Exon 7	1009-1010	TGTGAGAAGACT-TTGTGTGAGTGT	1.7 kb
355	11318-L12043	Exon 8	1154-1155	GTATGGAGTATA-GGCAGTGTGTGT	1.3 kb
217	11319-L21938	Exon 9	1307-1308	CCTGCGTGCATT-CCGGAAAGCGCT	1.1 kb
310	11344-L12069	Exon 10	1383-1384	CAGCCAGTGGAT-CTGCAGCAATGA	6.1 kb
418	11345-L12070	Exon 11	1467-1468	ATACTTCACCTT-CAGTGGGATCTG	0.8 kb
130	11346-L12071	Exon 12	1552-1553	CAGTGTGCTGAT-GACCGCGACGCT	1.4 kb
202	11320-L12045	Exon 13	1759-1760	ATGGACTGGGAT-GGCCGCGGGAGG	4.9 kb
169	11321-L12046	Exon 14	41 nt before exon 14	TATGCCGCTGCT-TTCGCGGCAGCG	1.1 kb
364	11347-L12072	Exon 15	2072-2073	ACCTGCGGAACT-GCCGCTACGACG	4.4 kb
328	11348-L21958	Exon 16	2415-2416	AGAAGACATCTT-CTCAGACCATCA	5.7 kb
256	11349-L12074	Exon 17	13 nt before exon 17	TCGGCTATGATT-TTCTTCTCTGCA	2.4 kb
350	11322-L29229	Exon 18	2536-2537	CTTCGAGGCAAA-AGGAGCCTATCC	8.0 kb
184	11350-L12075	Exon 19	2726-2727	TGGCCCTGGAAA-GGTGTCCCTGCT	1.7 kb
364 ±	20970-L29212	Exon 20	2835-2836	CACAGACCATGT-GTGTGATGCCAC	3.3 kb
247	11351-L12076	Exon 21	2984-2985	TAGTGGGGAATA-AGGGATGCAGCC	2.0 kb
172	11352-L12077	Exon 22	7 nt before exon 22	TTGCTTTGTCTT-CCTCCAGGTGAA	3.6 kb
226 #	11324-L12049	Exon 23	3342-3343	GAGCTCGCAGTG-TGCTGACACCAG	0.4 kb
154 #	11325-L12050	Exon 24	48 nt after exon 24, reverse	TCCATACCACCA-GGCCAAGCCTTG	1.8 kb
322 +#	11353-L12078	Exon 25	3490-3489, reverse	CAGACATCCAGA-TATGGCTCGGGG	0.5 kb
244 #	21673-L12051	Exon 26	365 nt before exon 26	TATAGAATCTTG-CTTCTTTGGACA	1.4 kb
178 #	11354-L12079	Exon 27	12 nt after exon 27	GTAAAACAGATT-CCTGGGTTGTTT	2.2 kb
384 #	11327-L21939	Exon 28	3936-3937	CCACTGTGATGT-TGTCAACCTCAC	0.7 kb
148 #	11355-L12080	Exon 28	4603-4604	CAGCAAAGGGAC-GAGATCGTTAGC	2.3 kb
154 #	11356-L12081	Exon 29	5367-5368	CCCAGCTTCTTA-TTTTGATGAAAT	0.2 kb
391 #	11357-L12082	Exon 30	5449-5450	GTGTCAGTGCTG-CAGTATGGAAGC	0.5 kb
274 #	11358-L12083	Exon 31	5629-5630	CCGGGAGCCTCA-AAGGCGGTGGTC	2.5 kb
160 #	11359-L12084	Exon 32	7 nt before exon 32	GTCTCTTTGCTA-ACTCTAGGAGTG	1.6 kb
185 #	11328-L12053	Exon 33	67 nt after exon 33	TGTTCCCACTGG-TTAATTTTTCCT	0.4 kb
283 #	11329-L12054	Exon 34	6064-6065	AAAGTGGAAGAG-ACCTGTGGCTGC	15.5 kb
346	11360-L12085	Exon 35	6181-6182	TATGTCCTATTT-CAAAACAAGGAG	1.6 kb
160	11330-L12055	Exon 36	6346-6347	GTCTCTGTTCCT-TACGTGGGTGGG	0.4 kb
223	12799-L14243	Exon 37	6532-6533	AACGGAGCCAAT-GACTTCATGCTG	2.3 kb
211	11331-L29230	Exon 38	6973-6974	CCTCCAGATAAA-GTCATGTTGGAA	6.3 kb
136	11332-L12057	Exon 39	7127-7128	TCAACTGCACAA-CGCAGCCCTGCC	0.5 kb
136	11362-L12087	Exon 40	7195-7196	CTCCGCCAGAAT-GCAGACCAGTGC	1.9 kb
208	11363-L12088	Exon 41	7273-7274	CCTCACTGTGAA-CGTGGCCTCCAG	1.3 kb
319	11333-L12058	Exon 42	7426-7427	TGTGATGAGTAT-GAGTGTGCCTGC	5.7 kb
256	11334-L14242	Exon 43	7563-7564	AAGCACCATCTA-CCCTGTGGGCCA	4.6 kb
283	11364-L12089	Exon 44	7716-7717	GCATGAAGGCGA-GTGCTGTGGAAG	2.4 kb
148	11335-L12060	Exon 45	7873-7874	GAGGAGGTCTTT-ATACAACAAAGG	1.2 kb
274	11336-L12061	Exon 46	8005-8006	TGCATGCTCAAT-GGCACTGTCATT	0.6 kb
292	13425-L14880	Exon 47	8049-8050	GATCGATGTGTG-CACGACCTGCCG	0.1 kb

Length (nt) P011 P012	SALSA MLPA probe	*VWF* exon[a]	Ligation site NM_000552.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
391	13426-L14881	Exon 47	8111-8112	TGGAGTGCAGGA-AGACCACCTGCA	14.0 kb
355	11365-L12090	Exon 48	8184-8185	TGGGAGATGTTT-GCCTACGGCTTG	1.1 kb
232	11338-L12063	Exon 49	8287-8288	TGCAAGGTCAAT-GAGAGAGGAGAG	0.6 kb
178	11339-L12064	Exon 50	8405-8406	GCTGTGACACAT-GTGAGTGCGTTA	2.0 kb
202	11366-L12091	Exon 51	8429-8430	AGTGCAACGACA-TCACTGCCAGGC	0.8 kb
418	11340-L12065	Exon 52	8664-8665	CATGGAGTGCAA-ATGCTCCCCCAG	
		stop codon	*8690-8692 (exon 52)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs34510401 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ SNP rs4021576 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P178 F8	Contains probes for the *F8* gene.
P207 F9	Contains probes for the *F7* and *F9* genes and some probes for the *F8* gene.
P440 F10 + F11	Contains probes for *F10* and *F11* genes involved in several bleeding disorders.

References

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- Baronciani L et al. (2021). Genotypes of European and Iranian patients with type 3 von Willebrand disease enrolled in 3WINTERS-IPS. *Blood Adv*, 5(15), 2987-3001.
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- Vangenechten I et al. (2018). A comparative analysis of different automated von Willebrand factor glycoprotein Ib-binding activity assays in well typed von Willebrand disease patients. *J Thromb Haemost*, 16(7), 1268-1277.
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- Yadegari H et al. (2011). Large deletions identified in patients with Von Willebrand Disease by multiple ligation-dependent probe amplification. *J Thromb Haemost*. May;9(5):1083-6.

P011 product history	
Version	*Modification*
B4	Four reference probes have been replaced and one probe length has been adjusted.
B3	Two reference probes have been replaced and several probe lengths have been adjusted.
B2	Four probes have a small change in length. The 88 and 96 nt control fragments have been replaced.
B1	Exon 2 probe replaced by two new ones, exon 47 replaced by new probe.
A1	First release.

P012 product history	
Version	*Modification*
B4	Five reference probes have been replaced.
B3	Three reference probes have been replaced and one has been removed.
B2	One probe has a small change in length. The 88 and 96 nt control fragments have been replaced.
B1	Probes for exon 12 and exon 47 added.
A1	First release.

Implemented changes in the product description

Version B4/B4-02 – 27 July 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *VWF* gene updated according to new version of the NM_ reference sequence.
- New publication added using SALSA MLPA Probemix P011/P012 VWF.

Version B4/B4-01 — 20 March 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *VWF* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 08 – 26 April 2018 (55)
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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