Product Description
SALSA® MLPA® Probemix P178-B4 F8

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, one reference probe has been replaced. For complete product history see page 9.

Catalogue numbers:
- **P178-025R:** SALSA MLPA Probemix P178 F8, 25 reactions.
- **P178-050R:** SALSA MLPA Probemix P178 F8, 50 reactions.
- **P178-100R:** SALSA MLPA Probemix P178 F8, 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.

Intended purpose
The SALSA MLPA Probemix P178 F8 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *F8* gene in genomic DNA isolated from human peripheral whole blood specimens. P178 F8 is intended to confirm a potential cause for and clinical diagnosis of hemophilia A, and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P178 F8 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *F8* gene are intron 22 and intron 1 inversions and point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with targeted intron 22 and intron 1 inversion analysis and sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Hemophilia A, one of the most common coagulation disorders, is caused by complete or partial deficiency in factor VIII (FVIII) clotting activity. Depending on the remaining level of FVIII clotting activity, three types of hemophilia A can be discriminated: (1) severe hemophilia A with <1% FVIII activity, (2) moderate hemophilia A with 1-5% FVIII activity, and (3) mild hemophilia A with 6-40% FVIII activity. Patients with hemophilia A experience prolonged bleeding or oozing following an injury, tooth extraction or surgery, and delayed or

recurrent bleeding prior to complete wound healing. The major cause of disability from bleeding is chronic joint disease, and the leading cause of death related to bleeding is intracranial hemorrhage.

Hemophilia A is an X-linked recessive disease with a prevalence of ~1:5,000 live male births that is caused by mutations in the *F8* gene encoding FVIII. The most common genetic defects in the *F8* gene are intron 22 and intron 1 inversions, which occur in 43-45% and 2-5% of severe hemophilia A cases, respectively. The remaining types of pathogenic variants span the entire mutation spectrum, i.e. missense mutations, small deletions or insertions, nonsense mutations, splice site mutations, deletions and duplications. Deletions and duplications are found in ~5% of hemophilia A patients (http://f8-db.eahad.org/; Kim et al. 2012; Lannoy et al. 2012; Miller et al. 2012; Vencesla et al. 2012), and include single- and multi-exon deletions and duplications throughout the *F8* gene, as well as whole gene deletions. More information about hemophilia A is available at https://www.ncbi.nlm.nih.gov/books/NBK1404/.

Gene structure
The *F8* gene spans ~187 kilobases (kb) on chromosome Xq28 and contains 26 exons. The *F8* LRG_555 is available at www.lrg-sequence.org and is identical to GenBank NG_011403.2.

Transcript variants
For *F8*, two transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/2157). Transcript variant 1 encodes the full-length protein (NM_000132.4; 9032 nucleotides (nt); coding sequence 172-7227). Transcript variant 2 is a shorter variant that contains a unique 5' exon located within intron 22 of transcript variant 1 that is spliced to exons 23-26 (NM_019863.3; 2616 nt; coding sequence 161-811).

Exon numbering
The *F8* exon numbering used in this P178-B4 F8 product description is the exon numbering from the LRG_555 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 sequences. 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 P178-B4 F8 contains 43 MLPA probes with amplification products between 136 and 477 nt. This includes 33 probes for the *F8* gene. Each exon is covered by at least one probe. Two probes are present for exon 1, 3, 7, 12 and 26, and three probes are present for exon 14. In addition, ten reference probes are included that detect locations on the X chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains 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 of the same sex 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 from human peripheral blood, 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 hemophilia A. As all probes in the P178 probemix target the X chromosome, the gender of the reference samples used in an experiment is not important. Target probes are first normalised to reference probes within a sample. Therefore, a ratio of 1 corresponds to 1 copy in male samples as they have one X chromosome. 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. Sample ID numbers NA07503 and NA02325 from the Coriell Institute have been tested with this P178-B4 probemix at MRC Holland and can be used as positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Sample ID number	Source	Gender	Expected CNV*
NA07503	Coriell Institute	female	Heterozygous whole gene deletion of *F8*
NA02325	Coriell Institute	female	Heterozygous duplication of *F8* exon 1-22

* The whole extent of the CNV present in these samples cannot be determined by this P178-B4 F8 probemix.

Performance characteristics
Large deletions and duplications in the *F8* gene are found in ~5% of all hemophilia A patients (*F8* variant database; http://f8-db.eahad.org/; Kim et al. 2012; Lannoy et al. 2012; Miller et al. 2012; Vencesla et al. 2012). The exact percentage varies, however, depending on disease phenotype. For severe, mild and moderate hemophilia A, the percentage of large deletions and duplications is estimated at ~9.7%, ~1.4% and ~0.9%, respectively (*F8* variant database; http://f8-db.eahad.org/). The analytical sensitivity and specificity for the detection of deletions and duplications in the *F8* gene is very high and can be considered >99% (based on a 2008-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for *F8*-specific probes in male samples are allele copy numbers of 1 (normal), 0 (deletion) or 2 (duplication). For female samples, copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication) can be expected. In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) may be obtained. 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:

Copy Number status: Male samples	Final ratio
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
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 or in or near the *F8* gene. 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 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.

P178 specific note:

- Duplication of *F8* exon 1-22 is not expected to result in a risk of hemophilia A, as a normal copy of the *F8* gene is maintained (Lannoy et al. 2013; Lannoy and Hermans 2018).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *F8* gene are intron 22 inversions, intron 1 inversions and small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P178 F8.
- 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.
- DNA samples with gross deletions or duplications of the X chromosome, including the sequences detected by the reference probes, are not suitable for analysis with SALSA MLPA Probemix P178 F8.

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.

Factor VIII gene (*F8*) variant database

http://f8-db.eahad.org/. We strongly encourage users to deposit positive results in the Factor VIII Gene (*F8*) Variant Database. 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 *F8* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P178-B4 F8

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*F8*
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 07103-L14743	Xp22	
142	**F8 probe** 05610-L05060		**Exon 1**
148	**F8 probe** 05618-L05068		**Exon 7**
154	**F8 probe** 05627-L06055		**Exon 14**
160 «	**F8 probe** 05633-L05083		**Exon 20**
166 *	Reference probe 04423-L05579	Xp22	
172	**F8 probe** 05612-L05062		**Exon 2**
179	**F8 probe** 05619-L14947		**Exon 8**
186	**F8 probe** 06290-L17545		**Exon 6**
191	**F8 probe** 05626-L17546		**Exon 14**
197 «	**F8 probe** 05634-L17547		**Exon 21**
202	Reference probe 13203-L14524	Xq13	
209	**F8 probe** 05611-L17548		**Exon 1**
215	**F8 probe** 05620-L17549		**Exon 9**
221	**F8 probe** 06288-L05892		**Exon 5**
229	**F8 probe** 05641-L05091		**Exon 26**
238	Reference probe 06910-L06490	Xq22	
247	**F8 probe** 05617-L05067		**Exon 7**
256	**F8 probe** 05621-L05071		**Exon 10**
265	**F8 probe** 05628-L05078		**Exon 15**
274	**F8 probe** 07044-L05899		**Exon 12**
292	Reference probe 02920-L02314	Xp21	
298	**F8 probe** 05614-L05891		**Exon 4**
305	**F8 probe** 06287-L06058		**Exon 11**
313	**F8 probe** 06506-L06340		**Exon 3**
319	**F8 probe** 05629-L05079		**Exon 16**
328	**F8 probe** 05637-L05087		**Exon 23**
346	Reference probe 05125-L04515	Xq26	
355	**F8 probe** 05623-L05893		**Exon 12**
364	**F8 probe** 05630-L05080		**Exon 17**
372	**F8 probe** 14164-L15766		**Exon 24**
382	Reference probe 02908-L02302	Xq22	
391 «	**F8 probe** 07045-L05898		**Exon 22**
402	**F8 probe** 05624-L05074		**Exon 13**
409	**F8 probe** 05631-L05081		**Exon 18**
418	**F8 probe** 13707-L15181		**Exon 25**
426	Reference probe 13207-L14528	Xp11	
434	Reference probe 13118-L15558	Xq21	
444	**F8 probe** 05613-L14948		**Exon 3**
452	**F8 probe** 05625-L14949		**Exon 14**
459 «	**F8 probe** 05632-L14950		**Exon 19**
468	**F8 probe** 13708-L15182		**Exon 26**
477	Reference probe 01391-L01039	Xp21	

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

* New in version B4.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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 2. *F8* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*F8* exon[a]	Ligation site NM_000132.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*172-174 (Exon 1)*		
142	05610-L05060	Exon 1	24-25	GCACATCCAGTG-GGTAAAGTTCCT	0.3 kb
209	05611-L17548	Exon 1	294-295	AGTGATCTCGGT-GAGCTGCCTGTG	22.9 kb
172	05612-L05062	Exon 2	346-347	AATCTTTTCCAT-TCAACACCTCAG	2.5 kb
444	05613-L14948	Exon 3	462-463	ACCATCCAGGCT-GAGGTTTATGAT	0.1 kb
313	06506-L06340	Exon 3	538-537 reverse	TTTCCAGTAGGA-TACACCAACAGC	4.0 kb
298	05614-L05891	Exon 4	689-690	CCCACTGTGCCT-TACCTACTCATA	5.7 kb
221	06288-L05892	Exon 5	779-780	TTCAGGGAGTCT-GGCCAAGGAAAA	2.5 kb
186	06290-L17545	Exon 6	891-892	CAGGATAGGGAT-GCTGCATCTGCT	15.2 kb
247	05617-L05067	Exon 7	984-985	CACAGGAAATCA-GTCTATTGGCAT	0.2 kb
148	05618-L05068	Exon 7	1132-1133	CACTCTTGATGG-ACCTTGGACAGT	2.7 kb
179	05619-L14947	Exon 8	1213-1214	AAGTAGACAGCT-GTCCAGAGGAAC	0.6 kb
215	05620-L17549	Exon 9	1566-1567	CAGCATGAATCA-GGAATCTTGGGA	4.9 kb
256	05621-L05071	Exon 10	1659-1660	AACATCTACCCT-CACGGAATCACT	4.1 kb
305	06287-L06058	Exon 11	1830-1831	CGCTATTACTCT-AGTTTCGTTAAT	3.1 kb
355	05623-L05893	Exon 12	1975-1976	TTGATGAGAACC-GAAGCTGGTACC	0.1 kb
274	07044-L05899	Exon 12	2063-2062 reverse	TGTGCATGATGT-TGGAGGCTTGGA	6.1 kb
402	05624-L05074	Exon 13	2163-2164	ATTGGAGCACAG-ACTGACTTCCTT	16.5 kb
452	05625-L14949	Exon 14	2655-2656	TCTGATCTCCAA-GAAGCCAAATAT	0.9 kb
191	05626-L17546	Exon 14	3577-3578	ATGGAAAGAACT-CTCTGAACTCTG	1.5 kb
154	05627-L06055	Exon 14	5111-5112	CACCTGGGCAAA-GCAAGGTAGGAC	22.3 kb
265	05628-L05078	Exon 15	5431-5432	AGAAAGTTGTTT-TCCAGGAATTTA	1.6 kb
319	05629-L05079	Exon 16	5587-5588	ATTCCTTCTATT-CTAGCCTTATTT	0.5 kb
364	05630-L05080	Exon 17	5818-5819	CTAACACACTGA-ACCCTGCTCATG	0.5 kb
409	05631-L05081	Exon 18	6073-6074	TCAGCATGGGCA-GCAATGAAAACA	1.9 kb
459 «	05632-L14950	Exon 19	6226-6227	GGCGGGTGGAAT-GCCTTATTGGCG	0.7 kb
160 «	05633-L05083	Exon 20	6308-6309	TCCCCTGGGAAT-GGCTTCTGGACA	1.5 kb
197 «	05634-L17547	Exon 21	6405-6406	TCCGGATCAATC-AATGCCTGGAGC	3.8 kb
391 «	07045-L05898	Exon 22	6572-6571 reverse	AATTTCCTCGAT-AAGTCTGCCACT	32.9 kb
328	05637-L05087	Exon 23	6637-6638	CTGGGATAAAAC-ACAATATTTTTA	1.4 kb
372	14164-L15766	Exon 24	6782-6783	GAGTAAAGCAAT-ATCAGATGCACA	1.3 kb
418	13707-L15181	Exon 25	7007-7008	TGTGAAGGAGTT-CCTCATCTCCAG	22.8 kb
468	13708-L15182	Exon 26	7190-7191	TGCCCTGAGGAT-GGAGGTTCTGGG	1.5 kb
229	05641-L05091	Exon 26	8714-8715	TTTGGAAACTAT-AACATAGCTGTC	
		stop codon	*7225-7227 (Exon 26)*		

[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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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

P011 VWF mix 1	Probes for the *VWF* gene involved in von Willebrand disease.
P012 VWF mix 2	Probes for the *VWF* gene involved in von Willebrand disease.
P207 F9	Probes for the *F7* and *F9* genes involved in factor VII deficiency and haemophilia B.
P440 F10 + F11	Probes for the *F10* and *F11* genes involved in factor X and factor XI deficiency.
P469 F5	Probes for the *F5* gene involved in factor V deficiency.

References

- Kim HJ et al. (2012). Mutation spectrum and inhibitor risk in 100 Korean patients with severe haemophilia A. *Haemophilia*. 18:1008-1013.
- Lannoy N et al. (2012). Computational and molecular approaches for predicting unreported causal missense mutations in Belgian patients with haemophilia A. *Haemophilia*. 18:e331-339.
- Lannoy N et al. (2013). Intron 22 homologous regions are implicated in exons 1-22 duplications of the F8 gene. *Eur J Hum Genet*. 21:970-976.
- Lannoy N and Hermans C. (2018). Review of molecular mechanisms at distal Xq28 leading to balanced or unbalanced genomic rearrangements and their phenotypic impacts on hemophilia. *Haemophilia*. 24:711-719.
- Miller CH et al. (2012). F8 and F9 mutations in US haemophilia patients: correlation with history of inhibitor and race/ethnicity. *Haemophilia*. 18:375-382.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.
- Vencesla A et al. (2012). F8 gene dosage defects in atypical patients with severe haemophilia A. *Haemophilia*. 18:708-713.

Selected publications using SALSA MLPA Probemix P178 F8

- Acquila M et al. (2008). MLPA assay in F8 gene mutation screening. *Haemophilia*. 14:625-627.
- Belvini D et al. (2017). Multiplex ligation-dependent probe amplification as first mutation screening for large deletions and duplications in haemophilia. *Haemophilia*. 23:e124-e132.
- Guo Z et al. (2018). Spectrum of molecular defects in 216 Chinese families with hemophilia A: identification of noninversion mutation hot spots and 42 novel mutations. *Clin Appl Thromb Hemost*. 24:70-78.
- Johnsen JM et al. (2017). Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative. *Blood Adv*. 1:824-834.
- Lannoy N et al. (2009). Identification of de novo deletion in the factor VIII gene by MLPA technique in two girls with isolated factor VIII deficiency. *Haemophilia*. 15:797-801.
- Payne AB et al. (2012). Utility of multiplex ligation-dependent probe amplification (MLPA) for hemophilia mutation screening. *J Thromb Haemost*. 10:1951-1954.
- Rafati M et al. (2011). Identification of ten large deletions and one duplication in the F8 gene of eleven unrelated Iranian severe haemophilia A families using the multiplex ligation-dependent probe amplification technique. *Haemophilia*. 17:705-707.
- Riccardi F et al. (2010). Spectrum of F8 gene mutations in haemophilia A patients from a region of Italy: identification of 23 new mutations. *Haemophilia*. 16:791-800.
- Rost S et al. (2008). Detection of large duplications within the factor VIII gene by MLPA. *J Thromb Haemost*. 6:1996-1999.
- Santacroce R et al. (2009). Detection of new deletions in a group of Italian patients with Hemophilia A by multiplex ligation-dependent probe amplification. *Genet Test Mol Biomarkers*. 13:573-576.
- Shinozawa K et al. (2021). Spectrum of F8 Genotype and Genetic Impact on Inhibitor Development in Patients with Hemophilia A from Multicenter Cohort Studies (J-HIS) in Japan. *Thromb Haemost.* 121:603-615.
- Silva Pinto C et al. (2012). Molecular diagnosis of haemophilia A at Centro Hospitalar de Coimbra in Portugal: study of 103 families - 15 new mutations. *Haemophilia*. 18:129-138.
- Theophilus BD et al. (2011). Deletions and duplications in the factor VIII gene identified using multiplex ligation-dependent probe amplification. *J Thromb Haemost*. 9:605-607.

P178 product history	
Version	*Modification*
B4	One reference probe has been replaced.
B3	Two reference probes have been removed.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Two F8 and five reference probes have been replaced, two reference probes have been added, one GDI flanking probe has been removed and several probes have been changed in length.
A1	First release.

Implemented changes in the product description

Version B4-02 – 01 February 2022 (04P)
- Product description completely rewritten and adapted to a new template.
- P178-B4 is now CE marked.
- Sample NA07503 added to the section on positive control DNA samples.
- Ligation sites of the probes targeting the *F8* gene updated according to new version of the NM_ reference sequence.

Version B4-01 — 13 December 2019 (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).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Probe remark regarding SNPs affecting the 247 nt probe 05617-L05067 and the 418 nt probe 13707-L15181 removed.
- SALSA MLPA probemix P469 F5 added to the related products.
- List of publications using SALSA MLPA probemix P178 F8 updated.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.