Product Description
SALSA® MLPA® Probemix P236-B1 CFH Region

To be used with the MLPA General Protocol.

Version B1
As compared to version A3, the name of the product has been changed to CFH Region. Four new probes for *CFHR4* and five new probes for *CFH* have been included, and several target probes have been replaced. One additional probe for *CFHR1* and two additional probes for *CFHR5* have been included. Most reference probes have been replaced and the flanking probes have been removed. The probes detecting polymorphic sequences have been removed. For complete product history see page 11.

Catalogue numbers:
- **P236-025R:** SALSA MLPA Probemix P236 CFH Region, 25 reactions.
- **P236-050R:** SALSA MLPA Probemix P236 CFH Region, 50 reactions.
- **P236-100R:** SALSA MLPA Probemix P236 CFH Region, 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.

This product requires the identification of suitable reference samples for proper data analysis. Suitable reference samples have two copies of all target sequences. Reference Selection DNA SD072 can be used to facilitate the selection of suitable reference samples and needs to be ordered separately.

Intended purpose
The SALSA MLPA Probemix P236 CFH Region 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 *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* genes in genomic DNA isolated from human peripheral whole blood specimens. P236 CFH Region is intended to confirm a potential cause for and clinical diagnosis of atypical hemolytic uremic syndrome (aHUS), systemic lupus erythematosus (SLE) or C3 glomerulopathy, and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P236 CFH Region should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require conformation by another method. Most defects in the *CFH* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis. Suitable reference samples should be identified for proper data analysis. Not all exons of the aforementioned genes are covered.

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 use (IVD) 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

Hemolytic uremic syndrome (HUS) is a rare disease characterised by haemolytic anaemia, thrombocytopenia and acute renal failure. Most cases result from infections with Shigatoxin-producing *E. coli* (STEC). Around 10% of HUS cases are not associated with STEC infections (atypical HUS, aHUS). The onset of aHUS ranges from the neonatal period to adulthood. Genetic aHUS accounts for an estimated 60% of all aHUS. Predisposition to aHUS is inherited in an autosomal recessive or autosomal dominant manner with incomplete penetrance.

aHUS is caused by hyperactivation of the alternative complement pathway. This hyperactivation can be due to mutations in complement regulators (CFH, CFI and CFHR proteins). Patients with genetic aHUS predominantly carry pathogenic variants in complement factor H (*CFH*), membrane cofactor protein CD46 (*CD46*) and complement factor I (*CFI*). The latter two genes are covered by probes in SALSA MLPA Probemix P296 aHUS. Genetic aHUS can also be caused by large genomic rearrangements in the *CFH* region (*CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5*) through mechanisms of gene conversion and non-homologous recombination. The *CFH*, *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2* and *CFHR5* genes are arranged in tandem in the 1q31.3 region, approximately 195 Mb from the p-telomere. Most major rearrangements in the *CFH* region result in the deletion of the neighbouring *CFHR1* and *CFHR3* genes and, occasionally, in the generation of *CFH*::*CFHR1* hybrid genes (Szarvas et al. 2016; Valoti et al. 2014; Venables et al. 2006; Zipfel et al. 2007). Gene rearrangements without copy number changes will not be identified by MLPA.

CFHR1-CFHR3 deletions have also been reported to occur in healthy individuals, with ethnic variations in its prevalence, including ~50% of Africans, ~25% in Europeans and less than 10% of Asians (https://www.ncbi.nlm.nih.gov/books/NBK1367/). *CFHR1-CFHR3* deletions are associated with a decreased risk of developing age-related macular degeneration (ARMD). This deletion was found in nearly 20% of the chromosomes of control individuals and in only 8% of the chromosomes of individuals with ARMD (Hughes et al. 2006).

Copy number changes in the *CFH* region are also associated with an increased risk for the polygenic autoimmune disease systemic lupus erythematosus (SLE) (Zhao et al. 2011) and the complement-mediated renal disease C3 glomerulopathy (https://www.ncbi.nlm.nih.gov/books/NBK1425/).

Gene structure

The *CFH* gene spans ~95 kilobases (kb) on chromosome 1q31.3 and contains 22 exons. The *CFH* LRG_47 is available at www.lrg-sequence.org and is identical to GenBank NG_007259.1.

The *CFHR1* gene spans ~12 kb on chromosome 1q31.3 and contains 6 exons. The *CFHR1* LRG_149 is identical to GenBank NG_013060.1.

The *CFHR2* gene spans ~16 kb on chromosome 1q31.3 and contains 5 exons. The *CFHR2* LRG_1216 is identical to GenBank NG_042816.1.

The *CFHR3* gene spans ~21 kb on chromosome 1q31.3 and contains 6 exons. The *CFHR3* LRG_175 is identical to GenBank NG_015993.1.

The *CFHR4* gene spans ~31 kb on chromosome 1q31.3 and contains 10 exons. The *CFHR4* LRG_1224 is identical to GenBank NG_028159.1.

The *CFHR5* gene spans ~32 kb on chromosome 1q31.3 and contains 10 exons. The *CFHR5* LRG_227 is identical to GenBank NG_016365.1.

Transcript variants
For *CFH,* two variants have been described. Transcript variant 1 encodes isoform a (NM_000186.4; 3962 nucleotides (nt) coding sequence 76-3771; https://www.ncbi.nlm.nih.gov/gene/3075). *CFH* transcript variant 1 represents the longer variant.

For *CFHR1,* multiple variants have been described. Transcript variant 1 represents the longer variant (NM_002113.3; 1297 nt; coding sequence 115-1107; https://www.ncbi.nlm.nih.gov/gene/3078).

For *CFHR2,* three variants have been described. Transcript variant 1 encodes isoform 1 (NM_005666.4; 1498 nt; coding sequence 144-956; https://www.ncbi.nlm.nih.gov/gene/3080). *CFHR2* transcript variant 1 represents the longer variant.

For *CFHR3,* two variants have been described. Transcript variant 1 encodes isoform 1 (NM_021023.6; 2934 nt; coding sequence 48-1040; https://www.ncbi.nlm.nih.gov/gene/10878). *CFHR3* transcript variant 1 represents the longer variant.

For *CFHR4,* multiple variants have been described. Transcript variant 1 encodes isoform 1 (NM_001201550.3; 2063 nt; coding sequence 100-1836; https://www.ncbi.nlm.nih.gov/gene/10877). *CFHR4* transcript variant 1 represents the longer variant.

For *CFHR5,* one transcript variant has been described encoding the full length protein (NM_030787.4; 2814 nt; coding sequence 110-1819; https://www.ncbi.nlm.nih.gov/gene/81494).

Exon numbering
The exon numbering used in this P236-B1 CFH Region product description for all genes is the exon numbering from their respective LRG sequences (details can be found in the gene structure section). 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 P236-B1 CFH Region contains 53 MLPA probes with amplification products between 130 and 500 nt. This includes 16 probes for *CFH*, 8 probes for *CFHR3*, 6 probes for *CFHR1*, 4 probes for *CFHR4*, 4 probes for *CFHR2*, and 5 probes for *CFHR5*. In addition, 10 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).

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 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, excluding CFHR1 and CFHR3 probes, over the experiment.

Required specimens
Extracted DNA from human peripheral whole blood specimens, 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. This product requires the identification of suitable reference samples for proper data analysis. Reference samples should be derived from different unrelated individuals who are from families without a history of aHUS, SLE or C3 glomerulopathy. Suitable reference samples have two copies of all target sequences. SD072 can be used as a reference selection DNA. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

SALSA Reference Selection DNA SD072
This product requires the identification of suitable reference samples for proper data analysis. Suitable reference samples have two copies of all target sequences. To facilitate the selection of suitable reference DNA samples from your own sample collection, Reference Selection DNA SD072 can be used (catalogue number SD072) which needs be ordered separately from MRC Holland. Reference Selection DNA SD072 should only be used for initial experiments on DNA samples from healthy individuals with the intention to identify suitable reference samples. **SD072 should not be used as a reference sample in subsequent experiments.** For further details, consult the Reference Selection DNA SD072 product description, available online: 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 NA00214, NA00501, HG01770 and HG01894 from the Coriell Institute have been tested with this P236-B1 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number variation	Altered target genes and expected copy number variation in P236-B1
NA00214	Coriell Institute	1q31.3*	Heterozygous deletion of *CFH, CFHR3, CFHR1, CFHR4, CFHR2* and *CFHR5*
NA00501	Coriell Institute	1q31.3	Heterozygous deletion of *CFHR3* and *CFHR4* and homozygous deletion of *CFHR1*
HG01770	Coriell Institute	1q31.3	Heterozygous deletion of *CFHR3* and *CFHR1*
HG01894	Coriell Institute	1q31.3	Heterozygous deletion of *CFHR3* and *CFHR1*

* Indicated chromosomal band accommodate genes targeted by MLPA probes, however, the whole extent of copy number variation (CNV) present in this cell line cannot be determined by this P236-B1 CFH Region probemix.

Performance characteristics

The expected number of large rearrangements in the *CFH* genomic region that can be detected with this MLPA probemix is 3-26.5% of all mutations in aHUS patients (Dragon-Durey et al. 2009; Szarvas et al. 2016). The expected number of large rearrangements in the *CFH* genomic region that can be detected with this MLPA probemix is less than 1% of all mutations in patients with either systemic lupus erythematosus or C3 glomerulopathy (Deltas et al. 2013; Zhao et al. 2011). The analytical sensitivity and specificity for the detection of deletions and duplications in the *CFH* genomic region is very high and can be considered >99% (based on a 2007-2022 literature review and external analytical performance study).

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 the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion) or 3 (heterozygous duplication).

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.

P236 specific note
- The *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* genes have a high sequence homology, which makes the *CFH* region on 1q31.3 prone to gene conversions and recombination events. In rare cases, an apparent duplication of a single probe might be due to a sequence change in one of the homologous genes. The probes that could be affected are marked with a # symbol in Table 2.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *CFH* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P236 CFH Region.
- 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the Leiden Open Variant 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 *CFH* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P236-B1 CFH Region

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]						
		Reference	*CFH*	*CFHR3*	*CFHR1*	*CFHR4*	*CFHR2*	*CFHR5*
64-105	Control fragments – see table in probemix content section for more information							
130	Reference probe 00797-L00463	5q						
135 ¥	**CFHR3 probe** 22996-L32432			**Upstream**				
139 ¥	**CFH probe** 22043-L08618		**Downstream**					
142	**CFH probe** 07821-L07575		**Exon 2**					
148 *	**CFHR4 probe** 22111-L31098					**Exon 10**		
154 *	**CFHR3 probe** 22069-L31040			**Exon 4**				
157 *	Reference probe 02731-L01824	12q						
164 ±	**CFHR3 probe** 07832-L07588			**Exon 1**				
168 ±	**CFHR3 probe** 08218-L09921			**Exon 6**				
172 *	**CFH probe** 22071-L31042		**Exon 15**					
179	**CFH probe** 07822-L07576		**Exon 3**					
184	**CFHR2 probe** 07844-L07600						**Exon 4**	
190 *	Reference probe 03915-L03370	15q						
196 *	**CFHR1 probe** 22072-L31043				**Exon 4**			
202	**CFH probe** 07820-L07574		**Exon 1**					
208 *	**CFH probe** 22073-L31044		**Exon 18**					
214 *	**CFH probe** 22074-L31045		**Exon 14**					
220 *	Reference probe 08879-L08935	2p						
226 ¥	**CFHR2 probe** 21368-L31327						**Exon 3**	
232	**CFHR5 probe** 07847-L07603							**Exon 3**
238 ¥	**CFHR3 probe** 22997-L32433			**Upstream**				
244 *	**CFHR1 probe** 22076-L31047				**Exon 5**			
253 *	**CFHR5 probe** 22077-L31048							**Exon 8**
258 *	Reference probe 16472-L26940	17q						
265	**CFHR2 probe** 07842-L07598						**Exon 2**	
274	**CFHR3 probe** 07833-L07589			**Exon 2**				
283 *	**CFHR1 probe** 22112-L31100				**Intron 1**			
292 *	**CFH probe** 22079-L31050		**Intron 9**					
301	Reference probe 02767-L02196	9q						
310	**CFH probe** 07828-L07583		**Exon 12**					
317 *	**CFHR4 probe** 22994-L32539					**Exon 5**		
324 ¥	**CFH probe** 22044-L31698		**Exon 22**					
330 ¥	**CFHR5 probe** 07845-L30998							**Exon 1**
337	**CFH probe** 07824-L07578		**Exon 6**					
346	**CFHR1 probe** 07839-L07595				**Intron 3**			
355 *	Reference probe 05991-L05416	20p						
364	**CFHR3 probe** 07835-L07591			**Intron 4**				
373 ±	**CFH probe** 07827-L07582		**Intron 11**					
382 ±	**CFH probe** 07830-L07586		**Exon 17**					
392	**CFHR3 probe** 07834-L07590			**Exon 3**				
400 *	**CFHR4 probe** 22558-L31052					**Exon 6**		
406 *	**CFHR2 probe** 22113-L31101						**Intron 1**	
414 *	Reference probe 12787-L20671	2q						
419	**CFH probe** 07823-L16758		**Exon 4**					
427	**CFHR5 probe** 07846-L16757							**Exon 2**

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]						
		Reference	*CFH*	*CFHR3*	*CFHR1*	*CFHR4*	*CFHR2*	*CFHR5*
436 *	**CFH probe** 22082-L31053		**Exon 21**					
445 *	**CFHR4 probe** 22084-L31055					**Exon 1**		
454 * ◊	**CFHR1 probe** 22995-L32431				**Exon 6**			
463 *	Reference probe 12460-L13461	22q						
472 *	**CFH probe** 22559-L31056		**Exon 19**					
481 *	**CFHR5 probe** 22086-L31057							**Exon 10**
494 *	**CFHR1 probe** 22087-L31058				**Exon 2**			
500 *	Reference probe 09682-L22509	3p						

[a] See section Exon numbering on page 3 for more information.

* New in version B1.

¥ Changed in version B1. Minor alteration, no change in sequence detected.

± SNP rs191734103 could influence the 164 nt CFHR3 probe signal. SNP rs145280059 could influence the 168 nt CFHR3 probe signal. SNP rs16840465 could influence the 373 nt CFH probe signal. SNP rs35292876 could influence the 382 nt CFH probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

◊ Probe can give a background signal in case of heterozygous or homozygous deletion of *CFHR1*.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P236-B1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
***CFH* gene (NM_000186.4)**					
		start codon	*76-78 (Exon 1)*		
202	07820-L07574	Exon 1	120-119 reverse	TCTGCTACACAA-ATAGCCCATAAC	20.9 kb
142	07821-L07575	Exon 2	194-195	CTGGTCTGACCA-AACATATCCAGA	0.9 kb
179	07822-L07576	Exon 3	357-358	ACTCCTTTTGGT-ACTTTTACCCTT	2.1 kb
419	07823-L16758	Exon 4	458-459	TAATTACCGTGA-ATGTGACACAGA	3.7 kb
337	07824-L07578	Exon 6	818-819	TGAAAGAGGAGA-TGCTGTATGCAC	23.4 kb
292 #	22079-L31050	Intron 9	10.6 kb before exon 10 reverse	CAGAGCCACAGA-TAACAAGTCAGC	13.7 kb
373 ±	07827-L07582	Intron 11	1.1 kb after exon 11	CTTGGACACATT-ATGATTGAGTCG	8.4 kb
310	07828-L07583	Exon 12	1893-1894	ATAGTTGGACCT-AATTCCGTTCAG	1.6 kb
214	22074-L31045	Exon 14	2203-2202 reverse	ATCTCCATAGTA-ATAAGGAGGGGA	1.6 kb
172	22071-L31042	Exon 15	2440-2441	AAGGATGGATAC-ACACAGTCTGCA	9.1 kb
382 ±	07830-L07586	Exon 17	2719-2720	ACGGAACCATTA-ATTCATCCAGGT	3.2 kb
208	22073-L31044	Exon 18	2965-2966	TTGAAGGTTTTG-GAATTGATGGGC	1.3 kb
472	22559-L31056	Exon 19	3156-3157	ATGGATGGAGCC-AGTAATGTAACA	3.7 kb
436 #	22082-L31053	Exon 21	86 nt before exon 21 reverse	AACACAGCACTG-TATATAATATCA	2.1 kb
324 #	22044-L31698	Exon 22	283 nt after exon 22	TATCAATACATA-AATGCACCAAAA	8.5 kb
		stop codon	*3769-3771 (Exon 22)*		
139 #	22043-L08618	Downstream	8.8 kb after exon 22	TGCACTTATACA-TGCAATCCGTTG	12.5 kb
***CFHR3* gene (NM_021023.6)**					
135 #	22996-L32432	Upstream	6.1 kb before exon 1	TTAGTCCGAGGT-AGAAAGGGACAT	4.7 kb
238	22997-L32433	Upstream	1.4 kb before exon 1	GGGTGGTAATCT-TGGCTCTCAGTG	1.5 kb
		start codon	*48-50 (Exon 1)*		
164 # ±	07832-L07588	Exon 1	8 nt after exon 1	CAAGGTAAGTTA-AAAGAGATCTAA	4.1 kb
274 #	07833-L07589	Exon 2	100 nt before exon 2 reverse	AACATTTTCTTG-TGGAATTACAGC	1.0 kb

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
392	07834-L07590	Exon 3	61 nt after exon 3	CACGGACGACAG-TCTCAGACTTGT	8.6 kb
154 #	22069-L31040	Exon 4	287 nt after exon 4	ATCAGCAAAATA-TGTTAGTTGCCA	0.7 kb
364 #	07835-L07591	Intron 4	680 nt before exon 5	GGGGGTTATATG-AATTCCTACATT	4.1 kb
168 # ±	08218-L09921	Exon 6	1005-1004 reverse	TATCCCTTCCCG-ACACACTGCTTG	27.2 kb
		stop codon	*1038-1040 (Exon 6)*		
***CFHR1* gene (NM_002113.3)**					
		start codon	*115-117 (Exon 1)*		
283 #	22112-L31100	Intron 1	730 nt after exon 1	TATGTCTGTACA-TGGAGTTTCGAT	5.1 kb
494 #	22087-L31058	Exon 2	12 nt after exon 2 reverse	TTAATGAACAGA-GCATTTACTCAC	1.7 kb
346 #	07839-L07595	Intron 3	396 nt after exon 3 reverse	AGAGAGTTTCAG-GTCCATGTGTAG	0.5 kb
196 #	22072-L31043	Exon 4	194 nt before exon 4 reverse	CAGGTACAAGCT-TTGATGTTTTAA	2.9 kb
244	22076-L31047	Exon 5	64 nt after exon 5	ATTTTGCTGTTG-GTAACAAAATAA	1.3 kb
454 # ◊	22995-L32431	Exon 6	1120-1121	ATCAATCATAAA-ATGCACACCTTT	55.9 kb
		stop codon	*1105-1107 (Exon 6)*		
***CFHR4* gene (NM_001201550.3)**					
		start codon	*100-102 (Exon 1)*		
445	22084-L31055	Exon 1	135 nt before exon 1 reverse	CTGGTATGTACA-TGTACAGCTTTA	19.6 kb
317 #	22994-L32539	Exon 5	849-850	CAGGGTTCTAAA-TATGTAACATGT	2.9 kb
400 #	22558-L31052	Exon 6	938-939	TCAACATGGACA-TCTATATTATGA	8.1 kb
148	22111-L31098	Exon 10	1872-1873	TGTCCAACTTCC-ACTTCTCACTCT	26.2 kb
		stop codon	*1834-1836 (Exon 10)*		
***CFHR2* gene (NM_005666.4)**					
		start codon	*144-146 (Exon 1)*		
406 #	22113-L31101	Intron 1	729 nt after exon 1 reverse	TCGAAACTCCAA-GTACAGACATAG	5.1 kb
265 #	07842-L07598	Exon 2	74 nt after exon 2	CAAGATCATAAA-CACTTGATAATC	1.6 kb
226	21368-L31327	Exon 3	268 nt after exon 3	GTAATACCTGTG-TGTGGTTTATAG	6.7 kb
184 #	07844-L07600	Exon 4	655-656	ATATGCTCCAGG-TTCATCAGTTGA	19.7 kb
		stop codon	*954-956 (Exon 5)*		
***CFHR5* gene (NM_030787.4)**					
		start codon	*110-112 (Exon 1)*		
330	07845-L30998	Exon 1	152-153	CATGGGTATCCA-CTGTTGGGGGAG	5.2 kb
427	07846-L16757	Exon 2	223-224	GATGAAGAAGAT-TATAACCCTTTT	1.1 kb
232 #	07847-L07603	Exon 3	414-415	ATCTTCAGGACT-AATACATCTGGA	18.6 kb
253	22077-L31048	Exon 8	1380-1379 reverse	CTTTTGCTTCTG-GAAGTAGATAGT	6.0 kb
481	22086-L31057	Exon 10	1762-1761 reverse	CGAAATGGTGGT-GATGATATCATC	
		stop codon	*1817-1819 (Exon 10)*		

[a] See section Exon numbering on page 3 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 rs191734103 could influence the 164 nt CFHR3 probe signal. SNP rs145280059 could influence the 168 nt CFHR3 probe signal. SNP rs16840465 could influence the 373 nt CFH probe signal. SNP rs35292876 could influence the 382 nt CFH probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

◊ Probe can give a background signal in case of heterozygous or homozygous deletion of *CFHR1*.

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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- P296 aHUS: Contains probes for the genes *CD46* and *CFI*.

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P236 product history	
Version	*Modification*
B1	Name of the product has been changed from ARMD mix-1 to CFH Region. Four new probes for *CFHR4* and five new probes for *CFH* have been included, and several target probes have been replaced. One additional probe for *CFHR1* and two additional probes for *CFHR5* have been included. Most reference probes have been replaced and the flanking probes have been removed. The probes detecting polymorphic sequences have been removed.
A3	One probe has a change in length. No change in sequence detected. Two control fragments at 88 and 96 nt have been replaced and two new control fragments at 100 and 105 nt have been included (QDX2).
A2	Eight probes have a small change in length. No change in sequence detected.
A1	First release.

Implemented changes in the product description
Version B1-02 – 21 December 2022 (04P) - P236 specific note added about high sequence homology in the *CFH* region. - Ligation site for the 324 nt CFH exon 22 probe updated. - Various minor textual changes. Version B1-01 – 17 May 2021 (04P) - Name of the product is changed from ARMD mix-1 to CFH Region. - P236-B1 is now CE marked.

- 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).
- SD072 can be used as a reference selection DNA.
- Ligation sites of the probes targeting the *CFHR3* and *CFHR1* genes updated according to new version of the NM_ reference sequence.
- Added references to the Reference list and updated the Selected publications list.

Version A3-02 – 17 September 2020 (02P)
- Indicated that suitable reference samples need to have similar copy numbers for the allele-specific probes of *CFH*, *C2*/*CFB* and *ARMS2* in the Reference sample section.
- *CFH* rs1061170 minor allele is changed into *CFH* rs1061170 allele C.
- *CFH* rs1410996 major allele is changed into *CFH* rs1410996 allele A.
- More information added to the warnings for the 148 nt and 282 nt probes in Table 1 and 2a.
- A warning for the 454 nt CFHR1 exon 6 probe which may give a residual signal in some patients with a deletion is added to Table 1 and 2a.
- Flanking genes have been adjusted.
- Positive sample added.
- References added.
- Results of six SNP probes on 50 different DNA samples from healthy individuals are indicated in a table.
- Various minor textual and layout changes.

Version A3-01 – 06 December 2019 (02P)
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Exon numbering: Updated text and comments.
- Warning added to Table 1 and Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Ligation sites of the probes targeting the *CFH*, *CFHR5*, *C2*, *ARMS2* and *KCNT2* genes updated according to new version of the NM_ reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

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