Product Description SALSA® MLPA® Probemix P110-C1 & P111-C1 FCGR

To be used with the MLPA General Protocol.

Version P110-C1 & P111-C1. As compared to version P110-B2 & P111-B2, the probemixes have been completely redesigned. New copy number probes, additional HNA1 allele probes and ORF/STOP haplotype probes have been added. Existing target probes have been redesigned, replaced or removed, and all reference probes have been replaced. For complete product history see page 12.

Catalogue numbers:

- **P110-025R:** SALSA MLPA Probemix P110 FCGR mix 1, 25 reactions.
- **P110-050R:** SALSA MLPA Probemix P110 FCGR mix 1, 50 reactions.
- **P110-100R:** SALSA MLPA Probemix P110 FCGR mix 1, 100 reactions.

- **P111-025R:** SALSA MLPA Probemix P111 FCGR mix 2, 25 reactions.
- **P111-050R:** SALSA MLPA Probemix P111 FCGR mix 2, 50 reactions.
- **P111-100R:** SALSA MLPA Probemix P111 FCGR 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.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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.

These SALSA MLPA probemixes are for basic research and intended for experienced users only! The probemixes enable you to detect genomic rearrangements and point mutations in a complex region. Interpretation of results can be complicated. MRC-Holland cannot provide assistance with data interpretation and recommends thoroughly screening any available literature.

Do not deviate from the experimental procedure described in the MLPA General Protocol. Due to the high degree of homology between the regions targeted by the P110-C1 and P111-C1 probemixes, it is of exceptional importance that ligation is performed at the intended temperature to achieve reliable results.

This product requires the identification of suitable reference samples for proper data analysis. For more information, see the sections *Reference samples* and *Interpretation of results*.

General information: The SALSA MLPA Probemixes P110 FCGR mix 1 and P111 FCGR mix 2 are **research use only (RUO)** assays for the detection of genomic rearrangements and point mutations in the 180 kb FCGR2/3 locus at 1q23.3.

Receptors for the Fc portion of IgG play an essential role in the protection of the organism against foreign antigens by removing antigen-antibody complexes from the circulation. Receptors are present on monocytes, macrophages, neutrophils, natural killer (NK) cells and T and B lymphocytes. The receptors participate in diverse functions, such as phagocytosis of immune complexes and modulation of antibody production by B cells. Genes for several low-affinity Fc gamma receptors are clustered on chromosome 1q23.3 Within a 180 kb chromosomal area are genes for the FCGR2A, FCGR2B, FCGR2C, FCGR3A and FCGR3B proteins. In addition, this region contains genes for the HSPA6 and HSPA7 heat shock proteins.

Due to high similarity between these *FCGR* genes and their close proximity, gene rearrangements are frequent in this chromosomal region. Various functionally relevant polymorphisms (SNPs) in these genes, as

well as copy number variation of the *FCGR2C*, *FCGR3A* and *FCGR3B* genes, have been reported. The MLPA probemixes P110/P111 FCGR cover the mentioned *FCGR* genes and are intended to detect both copy number changes of these genes as well as frequent polymorphisms and point mutations, such as *FCGR2B* -386G/C, *FCGR2B* -120A/T, *FCGR3A* p.Val158Phe, *FCGR2B* p.Ile232Thr, *FCGR2A* p.His166Arg, and *FCGR2A* p.Gln62Trp. Probes for *FCGR3B* Human Neutrophil Antigen 1 (HNA1) alleles NA1, NA2 and SH, and *FCGR2C* STOP, classic and non-classic ORF haplotypes are also included.

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 *FCGR2A*, *FCGR2B* and *FCGR2C* exon numbering used in this P110-C1/P111-C1 FCGR product description is adopted from Nagelkerke et al. 2015. *FCGR3A* and *FCGR3B* use the exon numbering from the RefSeq transcripts NM_000569.8 and NM_001271036.1 respectively. The *FCGR3A*/*FCGR3B* exon numbering and NM_ sequences used have been retrieved on 03/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P110-C1 FCGR mix 1 contains a total of 40 MLPA probes with amplification products between 130 and 494 nucleotides (nt) (Table 1a). The SALSA MLPA Probemix P111-C1 FCGR mix 2 contains a total of 38 MLPA probes with amplification products between 130 and 490 nt (Table 1b).

Both P110 FCGR mix 1 and P111 FCGR mix 2 contain 14 probes for determination of copy number changes and genomic rearrangements (Table 2). P110 FCGR also contains 18, and P111 FCGR 16, mutation-specific probes which will only generate a signal when the mutation is present (Table 3). Both probemixes contain eight reference probes targeting copy number stable regions. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

In addition, the 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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment 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.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using DNA samples from 16 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 reference probes over the experiment.

Required specimens: Extracted DNA from 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 unrelated individuals. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Selecting suitable reference samples for the P110/P111 FCGR probemixes is complicated due to the presence of mutation-specific probes in these probemixes. Suitable reference samples have a copy number of two for the target sequences of all reference probes and FCGR copy number probes, and a known copy number for the target sequences of the mutation-specific probes. SALSA Reference Selection DNA SD038 can facilitate the identification of suitable reference DNA samples (see below).

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

SALSA Reference Selection DNA SD038-S02: The selection of suitable reference DNA samples that can be used with P110/P111 FCGR is complicated. To facilitate the selection of suitable reference DNA samples from your own sample collection, a reference selection DNA sample (catalogue number SD038) is provided with these probemixes from MRC-Holland. Reference selection DNA SD038-S02 should only be used for initial experiments on DNA samples from healthy individuals with the intention to identify suitable reference samples. **SD038-S02 should not be used as a reference sample in subsequent experiments.** To aid in determining copy numbers of your own selected reference samples, the copy numbers of Reference Selection DNA SD038-S02 are detailed in the SD038-S02 product description, available online: www.mlpa.com.

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.mlpa.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 reference probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results:

Copy Number status: Probes with one copy in reference samples	Dosage quotient
No copies	DQ = 0
One copy	0.80 < DQ < 1.20
Two copies	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy Number status: Probes with two copies in reference samples	Dosage quotient
No copies	DQ = 0
One copy	0.40 < DQ < 0.65
Two copies	0.80 < DQ < 1.20
Three copies	1.30 < DQ < 1.65
Four copies	1.75 < DQ < 2.15
Ambiguous copy number	All other values

For probes with a copy number of four in the reference samples, the expected normal copy numbers are two, three and four (see Table 3), corresponding to probe ratios of 0.5, 0.75 and 1, respectively. The probe ratios of probes detecting four copies in the reference samples should be interpreted together with the results of surrounding copy number probes.

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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 or a relatively high number of rare mutation-specific 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

P110/P111 FCGR specific information:
We recommend starting the analysis by establishing copy number changes and gene rearrangements with the use of only the copy number probes (Table 2). In this step it is essential to combine copy number results from both P110 and P111 (e.g. in Excel) and then sort these results according to chromosomal location to get a comprehensive view of the genomic arrangement of the loci.

Status of variants detected by mutation-specific probes (Table 3) can subsequently be established. For most mutations, two probes cover the alternative alleles, most often present as a single nucleotide change. These mutation-specific probe pairs also share the same or similar run length in the P110 and P111 probemixes (Table 3). To determine the copy number of specific alleles in separate homologous genes, the first important step is to control that the sum of copies of both alleles matches the sum of the genomic copies in the segments in which the nucleotide changes are present. Collect these copy numbers from the copy number probes (Table 2) designed at genomic locations closest to the mutation-specific probes.

The determination of copy numbers of specific alleles at different loci is best illustrated with an example: The 256 nt probe in P110 detects the -120A allele, while the probe at the same run length in P111 detects the -120T allele, both in *FCGR2B*. In addition, the 256 nt probe in P111 detects the T at the corresponding position in *FCGR2C*. Results show one copy of the A allele and four copies of the T allele, while *FCGR2B* and *FCGR2C* copy numbers in the corresponding gene segments have been determined to three and two, respectively. The total copies of the alleles match the total number of gene copies; both are five. In this example it is furthermore likely that *FCGR2B* harbours one -120A and two -120T alleles, while the additional two T copies belong to *FCGR2C*, as an A allele in the promoter region of *FCGR2C* has not been reported.

Limitations of the procedure:

- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P110-C1 FCGR

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Other	FCGR2A	FCGR3A	FCGR2C	FCGR3B	FCGR2B
64-105	Control fragments – see table in probemix content section for more information						
130 *	Reference probe 19551-L26105	2p13					
137 *	**FCGR3A probe** 21806-L30537			Intron 1			
143 *	Reference probe 10113-L31635	8q22					
148 ¥	**FCGR2A/2C probe** 21814-L30545		**p. Leu273Pro**		Exon 8		
160 *	**FCGR3B probe** 21819-L30550					Intron 3	
166 *	**FCGR3A/3B probe** 21822-L30553			Exon 3		**p.Leu38= NA1**	
172 *	Reference probe 16647-L19180	10q23					
178 *	**HSPA7 probe** 21816-L30547	HSPA7					
184 ¥	**FCGR2B probe** 21824-L30555						**-386C**
190 *	**FCGR2A probe** 21799-L30530		**p. Val204_Gln 205insLeu**				
196 *	**FCGR3A probe** 21803-L30534			Exon 5			
202 *	**FCGR2C/2B probe** 21827-L31274				Exon 5		**p.Ile232**
211	**FCGR2C probe** 03609-L02976				**c.798+1A n.c. ORF1/2**		
220 *	**FCGR2C/2B probe** 21826-L30557				**c.392-20G>C ORF**		Intron 3
238 * Ж	**FCGR2A/2C probe** 21813-SP1007-L30544		Intron 7		**c.799-1C>G ORF; n.c. ORF1**		
247 *	**FCGR2A probe** 21958-L30771		**p.Gln62**				
256 ¥	**FCGR2B probe** 21825-L30556						**-120A**
265 *	**FCGR2A probe** 21958-L30772		**p.Gln62Ter**				
274 *	**FCGR2A probe** 21795-L30526		Exon 2				
283 ¥	**FCGR2C probe** 21810-L30541				**p.Ter57; STOP**		
292 *	Reference probe 18491-L23716	3q12					
301 *	**FCGR3A probe** 21959-L30773			Exon 5			
319 *	**HSPA6 probe** 21802-L30533	HSPA6					
328 *	**FCGR2A probe** 21800-L30531		Intron 4				
337 *	**FCGR2B probe** 21828-L30559						Exon 8
346 *	**FCGR3B probe** 21821-L30552					**p.Asn65 NA1**	
355 ¥	**FCGR2A probe** 21797-L30528		**p.His166**				
364 *	**FCGR3A probe** 21804-L30535			Intron 4			
373 *	Reference probe 04278-L03682	12q12					
382 *	**FCGR3A/3B probe** 21820-L30551			Exon 3		**p.Val106Ile; NA2 and SH**	
392 ¥	**FCGR3A probe** 21866-L31482			**p. Val158Phe**			
400 *	**FCGR2C probe** 21809-L30540				**c.134-45T STOP**		
409 *	Reference probe 16934-L19877	4q12					
418 *	**FCGR2C probe** 21808-L30539				Upstream		
436 *	**FCGR2B probe** 21968-L30786						**p.Asn106del**
444 *	Reference probe 09077-L23425	19p13					
454 *	**FCGR2A probe** 21801-L30532		Intron 7				
463 *	**FCGR3A probe** 21807-L30538			Upstream			
474 *	**FCGR2C probe** 21815-L30546				Downstream		
494 *	Reference probe 19137-L26747	21q22					

* New in version C1.

¥ Changed in version C1. Minor alteration, no change in sequence detected.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

a) See above section on exon numbering for more information.

Table 1b. SALSA MLPA Probemix P111-C1 FCGR

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Other	FCGR2A	FCGR3A	FCGR2C	FCGR3B	FCGR2B
64-105	Control fragments – see table in probemix content section for more information						
130 *	Reference probe 19551-L26105	2p13					
137 *	**FCGR3B probe** 21840-L30581					Exon 1	
142 *	**FCGR2A probe** 21841-L30582		Exon 1				
147 *	**FCGR2A/2C probe** 21842-L30583		Exon 8		**p. Pro280Leu**		
160 *	**FCGR3A probe** 21845-L30586			Intron 3			
166 *	**FCGR3B probe** 21846-L31114					**p.Leu38; NA2 and SH**	
172 *	Reference probe 16647-L19180	10q23					
178 *	**HSPA6 probe** 21847-L30588	HSPA6					
182 *	**FCGR2C/2B probe** 21848-L31275				Upstream		**-386G**
187 *	**FCGR2A probe** 21849-L30590		**c.739+ 871A**				
196 *	**FCGR3B probe** 21803-L30591					Exon 5	
203 *	**FCGR2B probe** 21851-L31575						**p.Ile232Thr**
209 ¥ Ж	**FCGR2A/2C probe** 21852-SP1009-L30594		Intron 7		**c.798+1 A>G**		
219 ¥	**FCGR2C probe** 21853-L30595				**c.392-20G STOP**		
229 *	**FCGR3A probe** 21854-L30596			Downstream			
238 *	**FCGR2C probe** 21855-L30597				**c.799-1C n.c. ORF 2**		
247 *	**FCGR2A probe** 21856-L31576		**p.Gln62Trp**				
256 *	**FCGR2C/2B probe** 21857-L30599				Upstream		**-120T**
265 *	Reference probe 12434-L27286	14q24					
274 *	**FCGR2B probe** 21858-L30600						Exon 7
283 *	**FCGR2C/2B probe** 21859-L30601				**p.Ter57Gln; ORF**		Exon 3
292 *	Reference probe 18491-L23716	3q12					
301 *	**FCGR3B probe** 21960-L30774					Exon 5	
320 *	**HSPA7 probe** 22377-L31573	HSPA7					
337 *	**FCGR3B probe** 21862-L30605					**p.Ala78Asp; SH**	
346 *	**FCGR3A/3B probe** 21863-L30606			Exon 3		**p.Asn65Ser; NA2 and SH**	
355 Δ	**FCGR2A probe** 04814-L10736		**p. His166Arg**				
364 *	**FCGR3B probe** 21864-L30607					Intron 4	
373 *	Reference probe 04278-L03682	12q12					
393 ¥	**FCGR3A/3B probe** 21866-L30609			**p.Val158**		Exon 4	
400 *	**FCGR2C/2B probe** 21867-L30610				**c.134-45 T>C ORF**		Intron 2
409 *	Reference probe 16934-L19877	4q12					
418 *	**FCGR2B probe** 21868-L30611						Upstream
444 *	Reference probe 09077-L23425	19p13					
454 *	**FCGR2C probe** 21870-L30613				Intron 7		
463 *	**FCGR3B probe** 21871-L30614					Upstream	
472 *	**FCGR2A probe** 21872-L30615		Downstream				
490 *	Reference probe 19137-L25693	21q22					

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Δ This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

a) See above section on exon numbering for more information.

Table 2. P110-C1/P111-C1 FCGR copy number probes arranged according to chromosomal location

Length (nt) P110	Length (nt) P111	SALSA MLPA probe	Gene	Exon[a]	Partial sequence[b] (24 nt adjacent to ligation site)	Location ligation site (hg18)	Distance to next probe
	142	21841-L30582	FCGR2A	Exon 1	TCACCCAGCAGC-AGCAAAACTGTC	01-159.741.959	0.5 kb
274		21795-L30526	FCGR2A	Exon 2	ACTCACCAGCTT-GACTGTCTGCAG	01-159.742.416	4.7 kb
328		21800-L30531	FCGR2A	Intron 4	GAATCTTGCATT-GGTGAGTGACTC	01-159.747.105	6.6 kb
454		21801-L30532	FCGR2A	Intron 7	GAGATCTTCAAC-CATTTCTTTTGA	01-159.753.716	2.5 kb
	472	21872-L30615	FCGR2A	Downstream	TGCCTTTCTGAC-AACTTGTGTTCC	01-159.756.252	4.1 kb
319		21802-L30533	HSPA6	Upstream	TCTGGCCATTCA-CTAAGGAACCAG	01-159.760.390	6.2 kb
	178	21847-L30588	HSPA6	Downstream	ACTGCTCCCTGA-TTTCATAGACCA	01-159.766.552	9.8 kb
	229	21854-L30596	FCGR3A	Downstream	GCTCTCTGTGGG-TTCGGGGGTTCC	01-159.776.367	2.0 kb
196		21803-L30534	FCGR3A	Exon 5	TCAAATCCTTCA-TCATGTCAGTTC	01-159.778.363	1.1 kb
301		21959-L30773	FCGR3A	Exon 5	AGACAAACATTC-GAAGCTCAACAA	01-159.779.491	0.3 kb
364		21804-L30535	FCGR3A	Intron 4	CACCAAACACTG-AGCAAAGGCTCC	01-159.779.822	2.1 kb
	160	21845-L30586	FCGR3A	Intron 3	TATTGCTCAGCC-TGGCAATTCGTG	01-159.781.941	4.0 kb
137		21806-L30537	FCGR3A	Intron 1	TGGATTGAGCTC-CTAGGACAAGCC	01-159.785.975	4.5 kb
463		21807-L30538	FCGR3A	Upstream	TAGGAATGAAAA-AGTGTTTAGTCA	01-159.790.443	13.6 kb
418		21808-L30539	FCGR2C	Upstream	CAAGTTAATAAT-AATGACATCTTT	01-159.804.087	31.3 kb
	454	21870-L30613	FCGR2C	Intron 7	GAGATCTTTAAG-CATTTCTTTTGA	01-159.835.371	2.5 kb
474		21815-L30546	FCGR2C	Downstream	GAACACAAGTTC-TCAGAAAGGCAA	01-159.837.903	4.1 kb
	320	22377-L31573	HSPA7	Upstream	TCTGGCCATTCC-TTAAGGAAACAG	01-159.842.039	6.1 kb
178		21816-L30547	HSPA7	Downstream	ACTGCTCCCTGT-TTTCATAGACCA	01-159.848.156	11.7 kb
	196	21803-L30591	FCGR3B	Exon 5	TCAAATCCTTCT-TCATGTCAGTTC	01-159.859.800	1.1 kb
	301	21960-L30774	FCGR3B	Exon 5	TGTTGAGCTTCA-AATGTTTGTCTT	01-159.860.931	0.3 kb
	364	21864-L30607	FCGR3B	Intron 4	GAGCCTTTGCTA-AGTGTTTGGTGA	01-159.861.262	2.1 kb
160		21819-L30550	FCGR3B	Intron 3	TAGTGCTCAGAG-TGGCAATTCGTG	01-159.863.391	3.9 kb
	137	21840-L30581	FCGR3B	Exon 1	TGGATTGAGCTA-CCAGGACAAGCC	01-159.867.333	4.5 kb
	463	21871-L30614	FCGR3B	Upstream	CACTAAACACTA-TTTCATTCCTAC	01-159.871.803	13.8 kb
	418	21868-L30611	FCGR2B	Upstream	GAGCCTTCTGAA-AGTGATGTGTCA	01-159.885.573	28.2 kb
	274	21858-L30600	FCGR2B	Exon 7	TTGTCAGCCTCA-TCAGGATTAGTG	01-159.913.761	0.1 kb
337		21828-L30559	FCGR2B	Exon 8	AATAGGTGATTG-TGTTCTCAGCCT	01-159.913.900	

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 3. P110-C1/P111-C1 FCGR mutation-specific probes

Lenght (nt) P110	Lenght (nt) P111	SALSA MLPA probe	Gene / Variant	Partial sequence (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
FCGR2A-p.Val204_Gln205insLeu enhances binding of FcγRIIa to all subclasses of IgG and was found exclusively among carriers with the FCGR2A 166Arg/Arg genotype (Omar et al. 2012). The **FCGR2A c.739+871A>G** mutation caused retention and expression of exon 6 demonstrating enhanced cellular activation (van der Heijden et al. 2013). Probe detects the normal allele.							
190		21799-L30530	FCGR2A c.612_613insCTT; p.Val204_Gln205insLeu	ATCACTGTCCTT-CAAGGTATGGGG	0-2	01-159.746.485	rs150311303
	187	21849-L30590	FCGR2A c.739+871A	AAACCAGGTGAA-TACAGAGTTGTC	0-2	01-159.748.241	rs72717038
FCGR2A-p.Gln62Trp (formerly known as p.Gln27Trp): The nucleotide polymorphism CA>TG (p.Gln62Trp) was first described in common variable immunodeficiency (CVID) and CVID-like patients (Flinsenberg et al. 2014) and has shown strong linkage disequilibrium (LD) with classic FCGR2C – ORF haplotype in Europeans (Nagelkerke et al. 2019). The FCGR2A c.184C>T polymorphism alone without a change in c.185 to G has not been found before (rs9427397); it would result in a change from glutamine (CAG) to stop (TAG) at position p.62. Such a change will be detected by the 265 nt probe in P110.							

Lenght (nt) P110	P111	SALSA MLPA probe	Gene / Variant	Partial sequence (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
247		21958-L30771	FCGR2A c.184C c.185A; p.Gln62	TCTGACAAGCCA-GGGGGCTCGCAG	0-2	01-159.742.828	rs201218628
	247	21856-L31576	FCGR2A c.184C>T c185A>G; p.Gln62Trp	GCGAGCCCCCCA-GCATGTCAGAGT	0-2	01-159.742.828	rs201218628
265		21958-L30772	FCGR2A c.184C>T; p.Gln62Ter	TCTGACAAGCTA-GGGGGCTCGCAG	0-2	01-159.742.828	rs9427397
FCGR2A-p.His166Arg (formerly known as p.His131Arg): A single nucleotide polymorphism results in a histidine or arginine at position 166. Histidine at this position has higher affinity for IgG1 and IgG2 in comparison with arginine and has been associated with Kawasaki disease (KD) in genome wide association studies (Khor et al. 2011), while an arginine instead has been associated with increased risk of developing systemic lupus erythematosus (SLE, Yuan et al. 2009).							
	355	04814-L10736	FCGR2A c.497A>G; p.His166Arg	TGGGATCCAAAC-GGGAGAATTTCT	0-2	01-159.746.369	rs1801274
355		21797-L30528	FCGR2A c.497A; p.His166	GAAATTCTCCCA-TTTGGATCCCAC	0-2	01-159.746.369	rs1801274
FCGR2A-p.Leu273Pro/FCGR2C-p.Pro280Leu: FCGR2A c.818T and FCGR2C c.839C are homologous positions with one nucleotide difference in FCGR2A and FCGR2C, respectively. The c.818T>C nt polymorhism (p.Leu273Pro) is introduced to FCGR2A and c.839C>T (p.Pro280Leu) to FCGR2C in FCGR2A/2C and FCGR2C/2A chimeric genes, respectively (Nagelkerke et al. 2015).							
	147	21842-L30583	FCGR2A wt / FCGR2C c.839C>T; p.Pro280Leu	TGGTTTCTTCAA-GTTGTCTCTTTC	2-4	01-159.754.429/ 01-159.836.084	FCGR2C: rs867055986
148		21814-L30545	FCGR2A c.818T>C; p.Leu273Pro / FCGR2C wt	AAAGAGACAACC-TGAAGAAACCAA	2-4	01-159.754.429/ 01-159.836.084	FCGR2A: rs382627
FCGR3A-p.Val158Phe: A single nucleotide polymorphism results in a valine or phenylalanine at position 158 (p.Val158Phe). Valine in this position has higher affinity for IgG1 and IgG3 compared to phenylalanine and has been found overrepresented in idiopathic thrombocytopenic purpura patients (Breunis et al. 2008, Carcao et al. 2003). Europeans homozygous for 158Val have a higher risk of developing rheumatoid arthritis (Lee et al. 2008).							
392		21866-L31482	FCGR3A c.526G>T; p.Val158Phe	GCAGGGGGCTTT-TTGGGAGTAAAA	0-2	01-159.781.166	rs396991
	393	21866-L30609	FCGR3A c.526G; p.Val158 / FCGR3B wt	GCAGGGGGCTTG-TTGGGAGTAAAA	2-4	01-159.781.166/ 01-159.862.610	rs396991
FCGR3B HNA1 variants: The NA1, NA2 and SH haplotypes determine the allotypic variants of the Human Neutrophil Antigen 1 (HNA1), involved in allo-immunisation against neutrophilic granulocytes (Matsuo et al. 2000; Steffensen et al. 1999).							
382		21820-L30551	FCGR3A wt / FCGR3B c.316G>A; p.Val106Ile; NA2 and SH	TAGAAGTCCATA-TCGGTGAGTTGA	2-4	01-159.784.838/ 01-159.866.195	FCGR3B: rs2290834
	337	21862-L30605	FCGR3B c.233C>A; p.Ala78Asp; SH	CTTCATTGACGA-TGCCACAGTCAA	0-2	01-159.866.278	rs5030738
346		21821-L30552	FCGR3B c.194A; p.Asn65; NA1	TCACAATGAGAA-CCTCATCTCAAG	0-2	01-159.866.317	rs448740
	346	21863-L30606	FCGR3A wt / FCGR3B c.194A>G; p.Asn65Ser; NA2 and SH	TTGAGATGAGGC-TCTCATTGTGAA	2-4	01-159.784.961/ 01-159.866.317	FCGR3B: rs448740
166		21822-L30553	FCGR3A wt / FCGR3B c.114T>C; p.Leu38=; NA1	TACAGGTTGCTC-GAGAAGGACAGT	2-4	01-159.785.040/ 01-159.866.397	FCGR3B: rs527909462
	166	21846-L31114	FCGR3B c.114T; p.Leu38; NA2 and SH	CTGTCCTTTTCA-AGCACGCTGTAC	0-2	01-159.866.397	rs527909462

Lenght (nt) P110 P111		SALSA MLPA probe	Gene / Variant	Partial sequence (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
FCGR2C STOP/ORF haplotypes: In most people, the FCGR2C gene is not expressed due to a stop codon at c.169 in exon 3 (FCGR2C-p.Ter57). The classic ORF haplotype consists of eight nucleotide changes in FCGR2C in intron 2, including FCGR2C c.134-45T>C, and exon 3, with the most important a FCGR2C c.169T>C polymorphism converting the stop codon into a glutamine and thereby enabling expression of the gene (FCGR2C-p.Ter57Gln) (Metes et al. 1998; Su et al. 2002). The FCGR2C c.392-20G>C alteration in intron 3 has also been shown linked to the ORF haplotype (Nagelkerke et al. 2015). In non-classical (n.c.) ORF1 and 2, presence of an A at the splice donor site c.798+1 result in splicing out of exon 7 and loss of expression due to a frameshift and activation of a novel stop codon in exon 8. In n.c. ORF 2, presence of C at 799-1 activates a cryptic acceptor splice site within intron 7 causing re-introduction of a 62 bp sequence of intron 7 into the transcript (van der Heijden et al. 2012). The expressed ORF haplotype has been associated with susceptibility to KD in Europeans and to idiopathic thrombocytopenic purpura (ITP) (Breunis et al. 2008; Nagelkerke et al. 2019).							
400		21809-L30540	FCGR2C c.134-45T STOP	GAGGAAGCCCAA-GAGCCTGAGAGG	0-2	01-159.825.931	rs549681560
	400	21867-L30610	FCGR2C c.134-45T>C ORF / FCGR2B wt	CTCTCAAGCTCC-TGGGCTTCCTCT	2-4	01-159.825.931/ 01-159.907.762	FCGR2C: rs549681560
283		21810-L30541	FCGR2C c.169T; p.Ter57;STOP	GTTGATCCACTA-GGGCTCGAGTTT	0-2	01-159.826.011	rs759550223
	283	21859-L30601	FCGR2C c.169T>C; p.Ter57Gln;ORF / FCGR2B wt	ACTCGAGCTCCA-GTGGATCAACGT	2-4	01-159.826.011/ 01-159.907.842	FCGR2C: rs759550223
	219	21853-L30595	FCGR2C c.392-20G STOP	CACAGAAAACCC-CAGAGGACCCGG	0-2	01-159.827.538	rs530707246
220		21826-L30557	FCGR2C c.392-20G>C ORF / FCGR2B wt	CGGGTCCTCTGC-GGTTTTTTGTGT	2-4	01-159.827.538/ 01-159.909.370	FCGR2C: rs530707246
	209	21852-SP1009-L30594	FCGR2A wt / FCGR2C c.798+1A>G	GGAGAGAAGGGA-CAAGGCAGGAAGAAA AGGAGATGGCTGGG ATTACTCA**C-** CTCAAATTGGGC	2-4	01-159.750.347/ 01-159.832.005	FCGR2C: rs76277413
211		03609-L02976	FCGR2C c.798+1A n.c. ORF 1 and 2	CCCAATTTGAGA-TGAGTAATCCCA	0-2	01-159.832.005	rs76277413
238		21813-SP1007-L30544	FCGR2A wt / FCGR2C c.799-1C>G ORF; n.c. ORF 1	CGTCCAGGTGG**C**-TGCAGGAAAGCATTT AAAACCCATAGGATA ATTCA-ATACACCGGGGA	2-4	01-159.754.388/ 01-159.836.043	FCGR2C: rs430178
	238	21855-L30597	FCGR2C c.799-1C n.c. ORF 2	GCTTTCCTGCAC-CCACCTGGACGT	0-2	01-159.836.043	rs430178
The **FCGR2B-p.Asn106del** variant abolishes binding of FcγRIIb to IgG1 (Jonsson et al. 2017). dbSNP also reports identical AAT deletions in corresponding sites in the homologous genes FCGR2A (rs760608327) and FCGR2C (rs765184850). In case an AAT deletion is detected, a long-range PCR is needed to identify which gene is affected.							
436		21968-L30786	FCGR2B c.316_318del, p.Asn106del	CTCCCCGCTGTC-GTTGTTGGCCTT	0-2	01-159.907.988	rs755222686
FCGR2B/2C -386G/C and -120T/A variants: Two polymorphic sites in the FCGR2B and FCGR2C promoters form two haplotypes: 2B.1 (-386G/-120T) and 2B.2 (-386C/-120T). A third haplotype, 2B.4, is formed in FCGR2B only (-386C/-120A) (Su et al. 2004; Breunis et al. 2008; Tsang-A-Sjoe et al. 2016). The 2B.4 haplotype has been reported associated with SLE (Su et al. 2004; Blank et al. 2005).							
	182	21848-L31275	FCGR2C wt/ FCGR2B -386G	AAAGGGTGATGC-AGGACAGCGTGC	2-4	01-159.817.466/ 01-159.899.270	FCGR2B: rs3219018
184		21824-L30555	FCGR2B -386C	CACGCTGTCCTC-CATCACCCTTTC	0-2	01-159.899.270	rs3219018

Lenght (nt) P110	P111	SALSA MLPA probe	Gene / Variant	Partial sequence (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
	256	21857-L30599	FCGR2C wt/ FCGR2B -120T	AGTGAAAAAGAA-ATGTTCTGTTTT	2-4	01-159.817.732/ 01-159.899.536	FCGR2B: rs780467580
256		21825-L30556	FCGR2B -120A	AAACAGAACATA-TCTTTTTCACTT	0-2	01-159.899.536	rs780467580
FCGR2B-p.Ile232Thr: A single nucleotide polymorphism results in an isoleucine or threonine at position 232 (p.Ile232Thr). The polymorphism affects downstream signalling and Ile232 provides stronger inhibitory signals compared to Thr232 (Li et al. 2003). Homozygosity for Thr232 protects against malaria, but the same allele is also strongly associated with susceptibility to SLE (Willcocks et al. 2010).							
	203	21851-L31575	FCGR2B c.695T>C; p.Ile232Thr	GGTCACTAGGAC-TGCTGTAGCGGC	0-2	01-159.910.422	rs1050501
202		21827-L31274	FCGR2C wt/ FCGR2B c.695T; p.Ile232	CCGCTACAGCAA-TCCCAGTGACCA	2-4	01-159.828.591/ 01-159.910.422	FCGR2B: rs1050501

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Selected publications using SALSA MLPA Probemixes P110-B2/P111-B2 FCGR

- Breunis WB et al. (2008). Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura *Blood.* 111:1029-1038.
- Nagelkerke SQ et al. (2015). Nonallelic homologous recombination of the FCGR2/3 locus results in copy number variation and novel chimeric FCGR2 genes with aberrant functional expression. *Genes Immun.* 16:422-429.
- Nagelkerke SQ et al. (2019). Extensive ethnic variation and linkage disequilibrium at the FCGR2/3 locus: different genetic associations revealed in Kawasaki disease. *Front Immunol.* 10:185.

P110 Product history	
Version	*Modification*
C1	Probemix has been completely redesigned. New copy number probes, additional HNA allele probes and ORF/STOP haplotype probes have been added. Existing target probes have been redesigned, replaced or removed, and all reference probes have been replaced.
B2	Two reference probes have been replaced, one reference probe has been added, and one reference probe has been changed in length but not in sequence detected. In addition, the control fragments have been replaced (QDX2).
B1	First commercial release

P111 Product history	
Version	*Modification*
C1	Probemix has been completely redesigned. New copy number probes, additional HNA allele probes and ORF/STOP haplotype probes have been added. Existing target probes have been redesigned, replaced or removed, and all reference probes have been replaced.
B2	Two reference probes have been replaced, one reference probe has been added, and one reference probe has been changed in length but not in sequence detected. In addition, the control fragments have been replaced (QDX2).
B1	First commercial release

Implemented changes in the product description

Version C1/C1-01 — 17 June 2020 (02P)
- Product description completely rewritten and adapted to a new template.
- Product description adapted to new product versions (version numbers changed, changes in Tables).
- Both probemixes should be used with the SD038-S02 version.

Version 15 – 05 April 2017 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Minor textual and layout changes.

Version 14 – 02 September 2016 (55)
- Information on the specificity of the 166 nt probe in P111 adjusted.
- Manufacturer's address adjusted.

- Various minor textual changes.

Version 13 (53)

- Information about SD038 Reference DNA added on page 2 and corresponding electropherogram added on page 11.
- Extra reference articles included.

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