SALSA MLPA probemix P110-B2/P111-B2 FCGR

P110 lot B2-0416, B2-0313 & P111 lot B2-0416, B2-0313. As compared to previous versions B1-0210 and B1-0409, in both P110 and P111 two reference probes have been replaced, and one has been added. In addition, the control fragments have been replaced (QDX2).

These SALSA® MLPA® probemixes are for basic research and intended for experienced MLPA users only! These probemixes enable you to quantify genes in complex regions. Interpretation of results can be complicated. MRC-Holland cannot provide assistance with interpretation of results obtained with this product, and recommends thoroughly screening any available literature.

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 FcG receptors are clustered on chromosome 1q23-24. 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 due to 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 FcG receptor genes and are intended to detect both copy number changes of these genes as well as to detect some frequent polymorphisms and point mutations such as *FCGR2A* R131/H131; *FCGR2B* 232I/232T; *FCGR2B/C* -386 (Alias -343) G/C; *FCGR3A* 158V/158F. Probes specific for *FCGR3B* NA1, NA2 and SH are also included.

Interpretation of results obtained with these P110/P111 FGCR probemixes requires an in-depth knowledge of the function of these genes. For instance, although the FCGR2A and FCGR2B proteins are highly similar, the FCGR2B is an "inhibitory" receptor, while FCGR2A is an activating receptor. Disruption of inhibitory FcG receptors such as FCGR2B may result in autoimmune disorders.

These P110-B2/P111-B2 probemixes are provided with a vial of SD038 reference DNA. More information on SD038 can be found on page 2.

These SALSA® MLPA® probemixes are designed to detect abnormal copy numbers of the *FCGR2A*, *2B*, *2C*, *3A* and *3B* genes and to detect the presence of the aforementioned mentioned polymorphisms and point mutations in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Not all deletions and duplications detected by MLPA will be pathogenic. Not all defects in these genes will be detected by this MLPA test. Users should always verify the latest scientific literature when interpreting their findings.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

More information

Website : www.mlpa.com
E-mail : info@mlpa.com (information & technical questions); order@mlpa.com (for orders)
Mail : MRC-Holland bv; Willem Schoutenstraat 1, 1057 DL Amsterdam, the Netherlands

References for SALSA® MLPA® probemix P110/P111 FCGR

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Data analysis

The P110-B2 FCGR probemix-1 contains 35 MLPA probes with amplification products between 130 and 409 nt. The P111-B2 FCGR probemix-2 contains 30 MLPA probes with amplification products between 130 and 400 nt. This includes several probes specific for mutations or rare variants, which will only generate a signal when that sequence is present in the sample. In addition, each probemix contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

SD038 Reference DNA

Binning: This SD038 DNA contains targets for all probes in SALSA MLPA probemixes P110-B2 FCGR mix 1 and P111-B2 FCGR mix 2 and can therefore be used for binning of the 9 and 7 SNP probes included in the P110-B2 and P111-B2 probemixes, respectively. For details, see the SD product description provided (also available online: www.mlpa.com).
Inclusion of one reaction with 5 µl SD038 DNA in MLPA experiments is recommended. Reference SD should never be used for the quantification of SNP signal(s), as for this purpose true SNP-positive patient samples or cell lines should be used.
Identification of suitable reference DNA samples: The SD038 Reference DNA provided with this probemix can be used to find suitable reference DNA samples. Reference DNA samples for use in MLPA experiments should be derived from the same type of tissue, and be purified by the same method, as the DNA samples to be tested. For certain applications, the selection of suitable reference DNA samples is complicated. Inclusion of one reaction with 5 µl SD038 DNA facilitates the identification of suitable reference DNA samples. We recommend the use of this SD038 Reference DNA only for initial experiments on DNA samples from healthy individuals with the intention to identify suitable reference DNA samples. We do not recommend it for use in all experiments. For further instructions, consult the SD product descriptions provided (also available online: www.mlpa.com).
This product is for research use only (RUO).

Data generated by this probemix can first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes that no changes occurred in the genomic regions recognised by the reference probes.

Data normalisation should be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

Interpretation of results

For most applications, results of both probemix P110 as well P111 will be required. Interpretation of results obtained with these P110/P111 probemixes requires an in-depth knowledge of the function of these genes.

Although the *FCGR2A*, *FCGR2B* and *FCGR2C* genes are more than 92% identical, the FCGR2B protein is an "inhibitory" receptor, while FCGR2A and FCGR2C (and FCGR3A and FCGR3B) are activating receptors. Disruption of inhibitory FCG receptors such as FCGR2B may result in autoimmune disorders and FCGR2B deficient mice are susceptible to induced autoimmune diseases. Certain common variants of FCGR2B have lost their inhibitory activity.

Most individuals lack FCGR2C expression, while some express high levels of FCGR2C capable of triggering cytotoxicity by NK cells. Using these P110/P111 probemixes, Breunis et al. (*Blood 111: 1029-38*, 2008), demonstrated extensive variation in the *FCGR2* and *FCGR3* gene clusters, including copy number variation in *FCGR2C*. They found that 82% of a Caucasian control population had only the *FCGR2C* null allele (STOP codon in exon 3), resulting in the presence of a pseudogene, while the remaining 18% had at least 1 ORF allele and expressed a functionally activating FCGR2C on their immune cells. The *FCGR2C* open reading frame (ORF) allele was significantly overrepresented in patients with immune idiopathic thrombocytopenic purpura (ITP) and Kawasaki disease patients, with 34% of ITP and 28% of Kawasaki patients having at least 1 ORF allele.

The *FCGR3A* and *FCGR3B* genes are highly homologous. The most important difference between the two genes is a C-to-T change at nt 733 of the *FCGR3B* gene, resulting in a stop codon. As a result, *FCGR3A* encodes a protein that has 21 more amino acids than the *FCGR3B* gene product, and FCGR3A is a transmembrane, rather than a GPI-anchored glycoprotein. *FCGR3A* has been reported to be only expressed by NK cells and macrophages, while *FCGR3B* is expressed by neutrophils. The *FCGR3A* and *3B* genes show copy number variation. A low *FCGR3B* copy number or complete *FCGR3B* deficiency may have a key role in the development of specific autoimmunity.

Several variants of *FCGR3B* have been described, most notably the HNA-1a (NA1), HNA-1b (NA2) and HNA-1c (SH) variants. HNA-1a and HNA-1b differ at 5 nucleotides, resulting in 4 amino acids differences. HNA-1b and HNA-1c differ only at one nucleotide/amino acid. Deletions of the *FCGR3B* gene, along with that of the adjacent *FCGR2C* gene, have been described in apparently healthy individuals but may be associated with increased risk of autoimmune disease and susceptibility to bacterial infections.

According to US patent application 2009263795, it can be determined whether a subject is predisposed for developing ITP by determining the amount of the *FCGR2C*-ORF variant as well as by determining the allele frequency of the *FCGR3A* variants 158F/158V and -386G/C.

According to the same patent application, it can be determined whether a subject is predisposed for developing Kawasaki disease by determining the amount of the *FCGR2B* and *FCGR2C*-ORF genes as well as the amount of *FCGR3A*.

We recommend starting the analysis of results by scoring the alleles of the following five variants:

1. The P110 and P111 probes at 355 nt, which are specific for the **FCGR2A 131H** and **131R** variant, respectively.
2. The P110 and P111 probes at 202 nt, which are specific for the **232I** and **232T** variants of the **FCGR2B** and **FCGR2C** genes, respectively.

3. The P110 and P111 probes at 187 nt, which are specific for the C- and G-variant, respectively, at position **-386** in the **FCGR2B** and **FCGR2C** promoter.
4. The P110 and P111 probes at 254 and 255 nt, respectively, which are specific for the **-120A** and **-120T** variants in the **FCGR2C and FCGR2B** promoter. The usual haplotypes are -386G-120T and -386C-120A.
5. The P110 and P111 probes at 391 nt, which are specific for respectively the **158F** and **158V** variants of **FCGR3A**.

For each of these probe pairs, the combined score can be used for copy number analysis of that gene.

Next, the presence of one or more functional FCGR2C copies can be determined by analysis of the following probes:

6. The P110 probe at 400 nt, which is specific for the 2C-stopcodon variant of the FCGR2C exon 3 SNP.
7. The P111 probe at 142 nt, which is specific for FCGR2B + the ORF variant of the FCGR2C exon 3 SNP.
8. The P110 probe at 367 nt, which is specific for the T variant of C259>T, which results in a stop codon.

Next, the following probes can be scored:

9. The P111 probe at 166 nt, which is specific for the **HNA-1b (NA2)** and **HNA-1c (SH)** variants of **FCGR3B**.
10. The P110 probe at 160 nt, which is specific for the **HNA-1a (NA1)** variant of **FCGR3B**. This probe also detects **FCGR3A** exon 3.
11. The P111 probe at 247 nt, which is specific for the **HNA-1c** (**SH**) variant of **FCGR3B**.

Next, the copy number of the various genes can be determined, using the information obtained above as well as the information generated by the following probes:

12. FCGR2A: P110 the 142 and 283 nt probes.
13. FCGR2B: P110 the 180, 274 and 328 nt probes. P111 the 274 and 328 nt probes.
14. FCGR2C: first determine ORF/STOP
15. FCGR3A: P110 the 337 nt probe. P111 the 310 nt probe.
16. FCGR3B: P110 the 310 and 361 nt probes. P111 the 361 nt probe.

The FCGR2A 131H, FCGR3A 158V, FCGR2C-ORF, FCGR3B HNA-1a and FCGR2B 232T variants are associated with a higher susceptibility to autoimmune diseases as they shift the activation-inhibition balance towards activation.

The FCGR2A 131R, FCGR3A 158F, FCGR2C stop, FCGR3B HNA-1b, and FCGR2B 232I variants are associated with less susceptibility as they shift the activation-inhibition balance towards inhibition.

This probemix was developed in a collaboration between Sanquin Amsterdam and MRC-Holland bv.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1a. SALSA MLPA P110-B2 FCGR mix-1 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position						
		other	FCGR2A	FCGR2B	FCGR2C	FCGR3A	FCGR3B	HSPA6/ HSPA7
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA							
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation							
100	X-fragment: Specific for the X chromosome							
105	Y-fragment: Specific for the Y chromosome							
130	Reference probe 00797-L00463	5q31						
138 β	Reference probe 09824-L24582	10q26						
142	**FCGR2A** probe 03605-L02972		Exon 1					
148	**FCGR2C** probe 03610-L02977				Exon 7			
154	Reference probe 02679-L02144	1q31						
160 »	**FCGR3A/3B** probe 03616-L02983					Exon 3a	HNA1a (NA1)	
166	**HSPA7** probe 13416-L14646							Exon 1
172 α	Reference probe 12741-L21983	21q22						
180	**FCGR2B/2C** probe 03611-L02978			Exon 1	Exon 1			
187 § ¥	**FCGR2B/2C** probe 09357-L06620			-386C	-386C			
196	**FCGR2A** probe 03606-L15071		Exon 1					
202 < Θ	**FCGR2B/2C** probe 04812-L04198			232I	232I			
211 □	**FCGR2C** probe 03609-L02976				Exon 6			
220 X	**FCGR2B** probe 03619-L10739			Exon 4				
230	Reference probe 01487-L01095	16q24						
238 α	Reference probe 14970-L16706	6q22						
247 ±	**FCGR2A** probe 04821-L04195		Exon 3					
254 ◊ ¥	**FCGR2B/2C** probe 07014-L10737			120A	120A			
265	Reference probe 01325-L07456	17p13						
274	**FCGR2B** probe 03613-L02980			Exon 7				
283	**FCGR2A** probe 03608-L10738		Exon 5					
292	Reference probe 03162-L02603	11p11						
301	**LMNA** probe 01916-L01460	1q22						
310	**FCGR3B** probe 03618-L02985						Exon 5	
319	**HSPA6** probe 13415-L14874							Exon 1
328	**FCGR2B** probe 03612-L06202			Exon 8				
337	**FCGR3A** probe 03614-L02981					Exon 1		
355 ◎ Θ	**FCGR2A** probe 04813-L04187		131H					
361	**FCGR3B** probe 03615-L12809						Exon 1	
367 #	**FCGR2C** probe 07010-L14886				C259>T (stop)			
373	Reference probe 02560-L02023	3q23						
382	**PAK3** probe 02908-L02302	*Xq23*						
391 ¤ ¥	**FCGR3A** probe 04816-L04190					Exon 4 (158F-variant)		
400 ~	**FCGR2C** probe 04818-L04192				2C-stop			
409 α	Reference probe 14405-L21970	12q13						

α New in version B2 (from lot B2-0313 onwards).
β Changed in version B2 (from lot B2-0313 onwards). Small change in length, no change in sequence detected.
Θ These probes detect the common allele of a polymorphism. The presence of the rare allele will result in a lower signal. No probe signal will be obtained on homozygote rare allele variants.
¥ These probes detect the rare allele of a polymorphism. It will only give a signal, when this rare allele is present.
More information is provided below Table 2.

Notes

- The following five probes detect frequent polymorphisms and should be analysed together with the results obtained with the probe at this length in the P111 probemix: 187 nt, 202 nt, 254 nt, 355 nt, 391 nt. In each case one variant is detected by the P110 and the other by the P111 probemix. The combined copy number should be two except in cases with an unusual copy number of the gene involved.
- Exon numbering used here may differ from literature or databases! We used the same exon numbering as in the previous version of this probemix.

Table 1b. SALSA MLPA P111-B1 FCGR mix-2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position					
		other	FCGR2A	FCGR2B	FCGR2C	FCGR3A	FCGR3B
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA						
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation						
100	X-fragment: Specific for the X chromosome						
105	Y-fragment: Specific for the Y chromosome						
130	Reference probe 00797-L00463	5q31					
138 β	Reference probe 09824-L24582	10q26					
142 ‡	**FCGR2B/2C** probe 04817-L04191			Exon 3a	ORF		
148 ∫	**FCGR2A** probe 03610-L02989		Exon 7				
154	Reference probe 02679-L02144	1q31					
166 *	**FCGR3B** probe 03616-L02990						HNA1b/1c
172 α	Reference probe 12741-L21983	21q22					
180	**FCGR2B/2C** probe 03611-L02978			Exon 1	Exon 1		
187 § Θ	**FCGR2B/2C** probe 07013-L10734			-386G	-386G		
202 < ¥	**FCGR2B** probe 04812-L04186			232T	232T		
211 ∞	**FCGR2A** probe 04815-L04189		Exon 6				
220	**FCGR2C** probe 03619-L02994				Exon 4		
230	Reference probe 01487-L01095	16q24					
238 α	Reference probe 14970-L16706	6q22					
247 ^	**FCGR3B** probe 06639-L06203						HNA1c (SH)
255 ◇ Θ	**FCGR2B/2C** probe 07014-L10735			120T	120T		
265	Reference probe 01325-L07456	17p13					
274	**FCGR2B** probe 03613-L02980			Exon 7			
283	**FCGR2B** probe 07011-L06617			Exon 3a ORF			
292	Reference probe 03162-L02603	11p11					
301	**LMNA** probe 01916-L01460	1q22					
310	**FCGR3A** probe 03618-L02993					Exon 5	
319	Reference probe 06552-L06110	5q13					
328	**FCGR2B** probe 03612-L06202			Exon 8			
355 ◎ ¥	**FCGR2A** probe 04814-L10736		131R				
361	**FCGR3B** probe 03615-L12809						Exon 1
373	Reference probe 02560-L02023	3q23					
382	**PAK3** probe 02908-L02302	Xq23					
391 ¤ Θ	**FCGR3A/3B** probe 04816-L04196					Exon 4 (158V-variant)	Exon 4
400 α	Reference probe 12777-L11598	9q34					

α New in version B2 (from lot B2-0313 onwards).
β Changed in version B2 (from lot B2-0313 onwards). Small change in length, no change in sequence detected.
Θ These probes detect the common allele of a polymorphism. The presence of the rare allele will result in a lower signal. No probe signal will be obtained on homozygote rare allele variants.
¥ These probes detect the rare allele of a polymorphism. It will only give a signal, when this rare allele is present.
More information is provided below Table 2.

Notes

- The following five probes detect frequent polymorphisms and should be analysed together with the results obtained with the probe at this length in the P110 probemix: 187 nt, 202 nt, 255 nt, 355 nt, 391 nt. In each case one variant is detected by the P110 and the other by the P111 probemix. The combined copy number should be two except in cases with an unusual copy number of the gene involved.
- Exon numbering used here may differ from literature or databases! We used the same exon numbering as in the previous version of this probemix!

Table 2. FCGR region probes arranged according to chromosomal location

Please note that some probes are mentioned twice as they detect sequences that are present in one gene as well as a variant of one of the other genes. This table will assist in identifying copy number changes of one or more genes.

Length (nt) P110	Length (nt) P111	SALSA MLPA probe	Gene Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
301	301	01916-L01460	LMNA gene		CTGATAGCTGCT-CAGGCTCGGCTG	5379.3 kb
FCGR2A gene (NM_021642.3)						
			start codon	*54-56 (ex 1)*		
196		03606-L15071	Exon 1	42-41 rev.	CCCAGCACTGTG-CCAACGTCCAGT	0.1 kb
142		03605-L02972	Exon 1	93-94	ATGTATGTCCCA-GAAACCTGTGGC	0.9 kb
247 ±		04821-L04195	Exon 3	238-239	TCTGACATGCCA-GGGGGCTCGCAG	3.5 kb
	355 ◉ ¥	04814-L10736	Exon 4 (131**R** variant; **Arg**)	550-549 rev.	TGGGATCCAAAC-GGGAGAATTTCT	
355 ◉ Θ		04813-L04187	Exon 4 (131**H** variant; **His**)	550-551	GAAATTCTCCCA-TTTGGATCCCAC	0.9 kb
283		03608-L10738	Exon 5	737-738	ACTGCTGTAGCA-GCCATTGTTGCT	3.0 kb
	211 ∞	04815-L04189	Exon 6 (7)	831-830 rev.	GGGATTACTCAC-CTCAAATTGGGC	4.1 kb
	148 ∫	03610-L02989	Exon 7 (8)	871-872	AAAGAGACAACT-TGAAGAAACCAA	6.5 kb
			stop codon	*1002-1004 (ex 7)*		
HSPA6 gene (NM_002155.3; single exon gene)						
			Coding sequence	*414-2345*		
319		13415-L14874	Exon 1	213-214	GCGGGAAGGTGC-GGAAAGGTTCGC	18.6 kb
FCGR3A gene (NM_000569.6)						
			stop codon	*1055-1057 (ex 6)*		
	310	03618-L02993	Exon 5	1025-1026	AGGACCATAAAT-TTAAATGGAGAA	1.7 kb
391 ¤ ¥		04816-L04190	Exon 4 (**F** variant)	818-819	GCAGGGGGCTTT-TTGGGAGTAAAA	
	391 ¤ Θ	04816-L04196	Exon 4 (**V** variant)	818-819	GCAGGGGGCTTG-TTGGGAGTAAAA	3.8 kb
160 »		03616-L02983	FCGR3A/3B exon 3a	406-407	TACAGGGTGCTC-GAGAAGGACAGT	1.3 kb
337		03614-L02981	Exon 1a	95-96	CTTTCCTTCCTG-GTCCTGTTCTAT	31.1 kb
			start codon	*185-187 (ex 2a)*		
FCGR2C gene (NM_201563.5; transcript variant 1)						
			start codon	*100-102 (ex 1)*		
	187 § Θ	07013-L10734	FCGR2B/2C -386**G** variant)	343 nt before ATG start	CACGCTGTCCTG-CATCACCCTTTC	
187 § ¥		09357-L06620	FCGR2B/2C -386**C** variant)	343 nt before ATG start	CACGCTGTCCTC-CATCACCCTTTC	0.2 kb
	255 ◇ Θ	07014-L10735	FCGR2B/2C promoter (120**T** variant)	120 nt before ATG	AAACAGAACATT-TCTTTTTCACTT	
254 ◇ ¥		07014-L10737	FCGR2B/2C promoter (120**A** variant)	120 nt before ATG	AAACAGAACATA-TCTTTTTCACTT	0.2 kb
180	180	03611-L02978	FCGR2B/2C exon 1	214-213 rev.	GCTGTCCACAGA-AGCATATGACCC	8.0 kb
400 ~		04818-L04192	Stop (exon 3)	26 nt before exon 3, forw.	CCTCTTCCTCAC-GCTACCTCCTCT	0.1 kb
	142 ‡	04817-L04191	ORF (exon 3)	26 nt before exon 3, rev.	GAGGAGGTAGCA-TGAAGAAGAGGA	
367 #		07010-L14886	Stop (exon 3)	268-267 rev. C259>**T** specific	GTTGATCCACTA-GGGCTCGAGTTT	1.6 kb
	220	03619-L02994	Exon 4 (3)	20 nt before	CACAGAAAACCC-CAGAGGACCCGG	1.1 kb

Length (nt) P110	Length (nt) P111	SALSA MLPA probe	Gene Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
				exon 4, rev.		
202 <		04812-L04198	FCGR2B/2C exon 5 (232**I** variant; **Ile**)	794-795	GGTCACTGGGAT-TGCTGTAGCGGC	
	202 <	04812-L04186	FCGR2B exon 5 (232**T** variant; **Thr**)	794-795	GGTCACTGGGAC-TGCTGTAGCGGC	3.4 kb
211 □		03609-L02976	Exon 6 (7B)	1 nt after exon 6	CCCAATTTGAGA-TGAGTAATCCCA	4.1 kb
148		03610-L02977	Exon 7 (8)	938-939	AAAGAGACAACC-TGAAGAAACCAA	6.4 kb
			stop codon	*1069-1071 (ex 7)*		
HSPA7 gene (NR_024151.1)						
166		13416-L14646	Exon 1	50-51	AGGTGCGGGAAG-GTTCGCGGCGGC	18.4 kb
FCGR3B gene (NM_000570.3)						
			stop codon	*974-976 (ex 5)*		
310		03618-L02985	Exon 5	1007-1008	AGGACCATAAAC-TTAAATGGAGAA	1.7 kb
	391 ¤ Θ	04816-L04196	Exon 4 (V variant)	800-801	GCAGGGGGCTTG-TTGGGAGTAAAA	3.7 kb
	247 ^	06639-L06203	Exon 4 (3)	507-506 rev. HNA1c variant	CGACTGTGGCAT-CGTCAATGAAGT	0.1 kb
160 »		03616-L02983	FCGR3A/3B exon3	388-389 HNA1a variant	TACAGGGTGCTC-GAGAAGGACAGT	0.1 kb
	166 *	03616-L02990	Exon 3	388-389 HNA1b/1c variant	TACAGCGTGCTT-GAGAAGGACAGT	1.2 kb
361	361	03615-L12809	Exon 1	156-155 rev.	TCTCTGTCACCT-GCCAGTTTCCTT	31.6 kb
			start codon	*275-277 (ex 1)*		
FCGR2B gene (NM_004001.4; transcript variant variant 1)						
			start codon	*128-130 (ex 1)*		
	187 § Θ	07013-L10734	FCGR2B/2C promoter (-386**G** variant)	386 nt before ATG	CACGCTGTCCTG-CATCACCCTTTC	
187 § ¥		09357-L06620	FCGR2B/2C promoter (-386**C** variant)	386 nt before ATG	CACGCTGTCCTC-CATCACCCTTTC	0.2 kb
	255 ◇ Θ	07014-L10735	FCGR2B/2C promoter (D120**T** variant)	120 nt before ATG	AAACAGAACATT-TCTTTTTCACTT	
254 ◇ ¥		07014-L10737	FCGR2B/2C promoter (D120**A** variant)	120 nt before ATG	AAACAGAACATA-TCTTTTTCACTT	0.2 kb
180	180	03611-L02978	FCGR2B/2C exon 1	214-213 rev.	GCTGTCCACAGA-AGCATATGACCC	8.0 kb
	142 ‡	04817-L04191	Exon 3a	25 nt before exon 3, rev.	GAGGAGGTAGCA-TGAAGAAGAGGA	0.1 kb
	283	07011-L06617	Exon 3a	297-298	ACTCGAGCCACA-GTGGATCAACGT	1.5 kb
220 X		03619-L10739	Exon 4	19 nt before exon 4, rev.	CACAAAAAACCG-CAGAGGACCCGG	1.1 kb
202 < Θ		04812-L04198	FCGR2B/2C exon 5 (232**I** variant; **Ile**)	822-823	GGTCACTGGGAT-TGCTGTAGCGGC	
	202 < ¥	04812-L04186	FCGR2B/2C exon 5 (232**T** variant; **Thr**)	822-823	GGTCACTGGGAC-TGCTGTAGCGGC	0.3 kb
274	274	03613-L02980	Exon 7	965-966	CTAATCCTGATG-AGGCTGACAAAG	0.2 Kb
328	328	03612-L06202	Exon 8	1012-1013	TATTCACTTCTC-ATGCACCCGGAT	
			stop codon	*1058-1060 (ex 8)*		

ϴ This probes detect the common allele of a polymorphism. The presence of the rare allele will result in a lower signal. No probe signal will be obtained on homozygote rare allele variants.

¥ These probes detect the rare allele of a polymorphism. They will only give a signal when this rare allele is present.

FCGR2A

± The P110 probe at 247 nt detects the common Q allele of the **Q27W** polymorphism. Its signal will be reduced in case of FCGR2A deletions as well as in the presence of the Q27W (rs9427398) rare W allele (frequency ~ 0.19) and/or the rare allele of the rs9427394 polymorphism (frequency ~ 0.04).

ɷ The P110 and P111 probes at 355 nt are specific for the **FCGR2A 131H** and **131R** variant respectively (rs1801274). The two alleles differ by the presence of arginine or histidine at position 131, resulting in a substantial difference in their ability to ligate human IgG2. The 131H (131A) allele encodes the only Fc-gamma receptor that recognizes IgG2 efficiently and optimal IgG2 handling occurs only in the homozygous state. The 131H allele appears to have a protective effect against systemic lupus erythematosus (SLE) and in particular to lupus nephritis.

∞ The P111 probe at 211 nt detects the most frequent variant of the rs409763 polymorphism (G). The G at this position is in general specific for the FCGR2A gene, while the A is found in FCGR2C null alleles. Please note that when the FCGR2C contains an open reading frame (ORF) in exon 3, the FCGR2C gene will usually also have a G at this position which will increase the probe signal for this probe.

ʃ The 148 nt probe in P111 detects the most frequent variant of the rs382627 polymorphism (T). The T at this position is in general specific for FCGR2A while a C nucleotide is usually present at this position in FCGR2C.

FCGR3A

¤ The P110 and P111 probes at 391 nt are specific for the FCGR3A **158F** and **158V** variants respectively (rs396911). The 158V variant has a higher affinity for IgG1 and IgG3 than the 158F variant. The 158V variant is also present in FCGR3B.

» The 160 nt probe in P110 is specific for the **HNA-1a** (NA1) variant of FCGR3B. This probe also detects FCGR3A exon 3.

FCGR2C

§ The P110 and P111 probes at 187 nt are specific for respectively the C- and G-variant at position **-386** relative to the FCGR2B start of translation (-343 relative to the transcription start site) in the FCGR2B and FCGR2C promoters (rs3219018). The C/C genotype has been reported to be more frequent in SLE patients. In FCGR2B, the -386C allele is almost always accompanied by the -120A allele, and this haplotype showed a 1.8 fold higher transcriptional activity as the -386G-120T haplotype. For FCGR2B, the frequency of the -386G-120T is 91% in Caucasians and that of -386C-120A is 9%. In FCGR2B, the -386C-120T combination is very rare (0.4%) while this combination is more common (12%) in FCGR2C with -386C-120A being very rare (1%) (Su K. et al. 2004, J Immunol. 172:7186-91).

◊ The P110 and P111 probes at 254/255 nt are specific for the **120A** and **120T** variants in the FCGR2C and FCGR2B promoter. This polymorphism has been reported to be linked to transcriptional activity of the FCGR2B gene (see above §).

~ The P110 probe at 400 nt detects the **FCGR2C** exon 3 polymorphism that determines whether a functional open reading frame (**ORF**) containing gln13 is present or a null allele containing a stop codon at position 13. In case the ORF in exon 3 of FCGR2C is present on one allele, the probe signal will be reduced to 0.5. The probe signal is expected to be zero in individuals that are homozygous for the ORF allele. Please note that in rare cases a SNP at +1nt of the ligation point (rs3933769) will also lower the signal to 0.5. In Caucasians, the frequency of homozygous null allele individuals is 82% (full probe signal), while 18% of individuals have at least one functionally activating FCGR2C allele (reduced or absent probe signal). In patients with idiopathic thrombocytopenic purpura, 34% of the individuals had at least 1 ORF allele (Breunis W. et al, 2008).

The P110 probe at 367 nt is specific for the T variant of the C259>T variation in FCGR2C, which generates a stop codon.

□ The P110 probe at 211 nt detects the A allele of rs409763. The presence of a G nucleotide at this position is in general specific for FCGR2A, while an A nucleotide is usually present in FCGR2C. Please note that

when the FCGR2C gene contains an ORF in exon 3, FCGR2C will usually also have a G at this position which will lower the probe signal.

< The P110 and P111 probes at 202 nt are specific for respectively the **232I** and **232T** variants of the FCGR2B and FCGR2C genes (rs1050501). Frequency of the 3 variants in Caucasians has been reported as 81% wildtype (homozygote 232I, Ile), 18% heterozygous and 1% 232Thr/Thr. In 193 Japanese patients with systemic lupus erythematosus (SLE) and 303 healthy controls, Kyogoku et al. (2002) found that homozygosity for an Ile232-toThr polymorphism in the transmembrane region of the FCGR2B gene (I232T) was significantly increased in SLE patients compared with controls. The FCGR2B 232T variant might be unable to inhibit activating receptors.

FCGR3B

FCGR3B is mainly expressed on neutrophils and is found in three polymorphic forms, called human neutrophil antigen **HNA-1a** (formerly NA1), **HNA-1b** (formerly NA2) and **HNA-1c** (formerly SH). HNA-1a and HNA-1b differ by only 5 nucleotides in the FCGR3B coding sequence, resulting in four amino acid alterations. HNA-1c and HNA-1b differ at only one nucleotide. The P110 and P111 probemixes include specific probes for each of the three variants. The frequencies of the three forms are different in different populations. In Caucasians, reported frequencies are approximately 0.35 for HNA-1a, 0.60 for HNA-1b and 0.05 for HNA-1c. In Japanese and Chinese populations, HNA1b is more frequent than HNA-1a. Frequency of HNA-1c has been reported to be as high as 0.35 in African Americans.

¤ The P110 and P111 probes at 391 nt are specific for the FCGR3A **158F** and **158V** variants, respectively (rs396911). The 158V variant has a higher affinity for IgG1 and IgG3 than the 158F variant. The 158V variant is also present in FCGR3B.

» The 160 nt probe in P110 is specific for the **HNA-1a** (NA1) variant of FCGR3B. This probe also detects FCGR3A exon 3.

***** The 166 nt probe in P111 is specific for the **HNA-1b** (NA2) and **HNA-1c** (SH) variants of FCGR3B.

^ The 247 nt probe in P111 is specific for the **HNA-1c** (SH) variant of FCGR3B.

FCGR2B

‡ The P111 probe at 142 nt detects FCGR2B exon 3 and FCGR2C-ORF.

X The P110 probe at 220 nt detects the most frequent C allele of rs4657088. The presence of a C nucleotide at this position is in general specific for FCGR2B, while a G residue is usually present at this position in the FCGR2C gene. When the FCGR2C gene contains an open reading frame (ORF) in exon 3, FCGR2C will often also have a C at that position, which will increase the signal of this probe.

Important Notes:

- Exon numbering and NM_sequences may differ from databases or literature! We here used the same NM_sequences and exon numbering as in the previous version of this probemix!
- Complete probe sequences and the identity of the genes detected by the reference probes is available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P110-B2/P111-B2 FCGR sample pictures

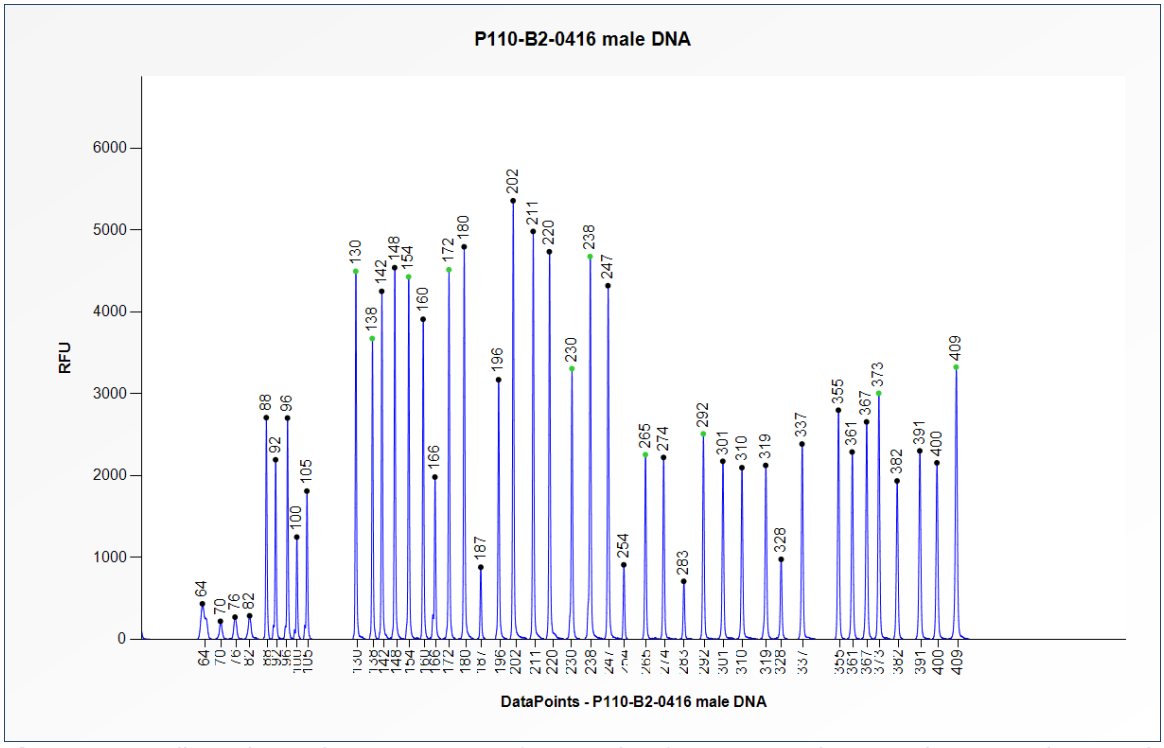

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P110-B2 FCGR (lot B2-0416).

Note: A non-specific peak could appear in the no DNA control at 135 nt. This peak does not correspond to an MLPA probe.

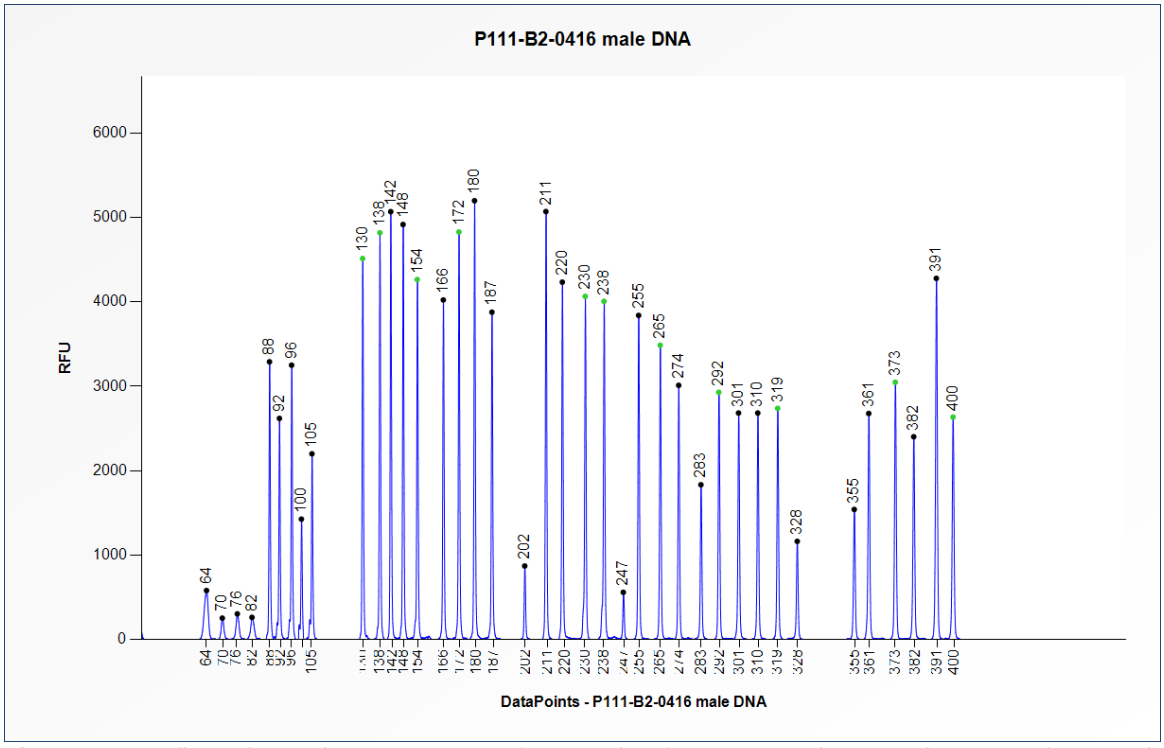

Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P111-B2 FCGR (lot B2-0416).

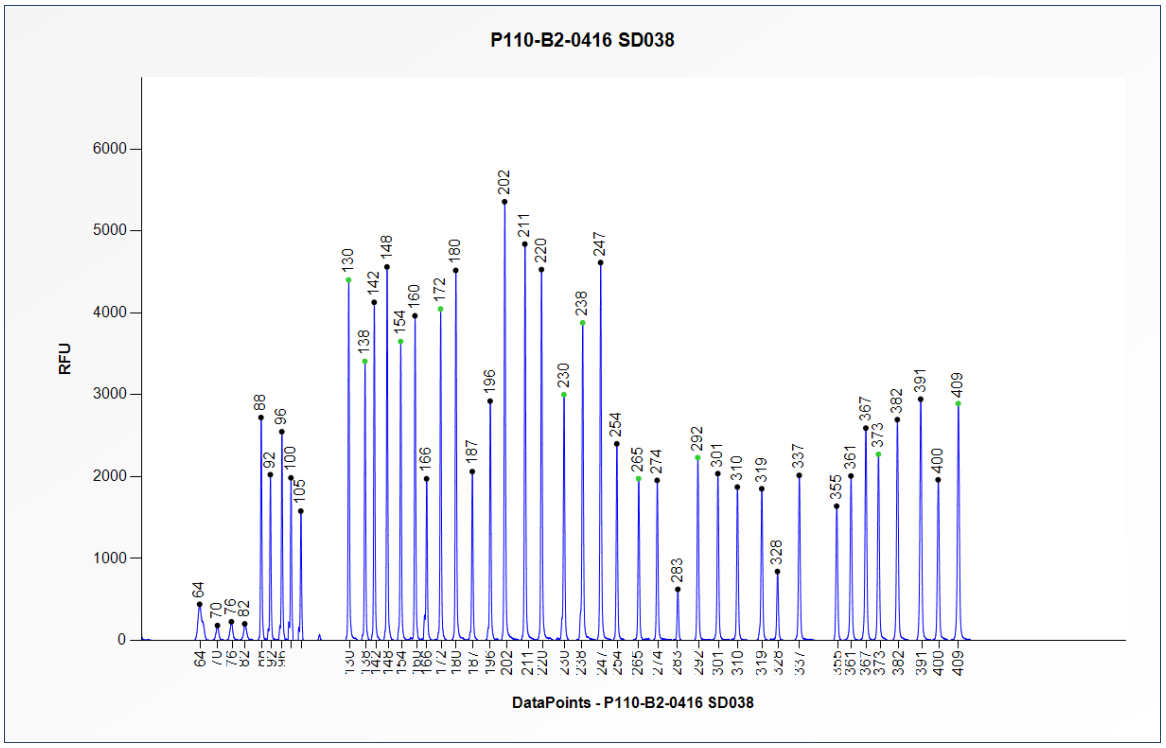

Figure 3. Capillary electrophoresis pattern of SD038 sample DNA (approximately 50 ng) analysed with SALSA MLPA probemix P110-B2 FCGR (lot B2-0416). For more information, see the SD038 Reference DNA product description.

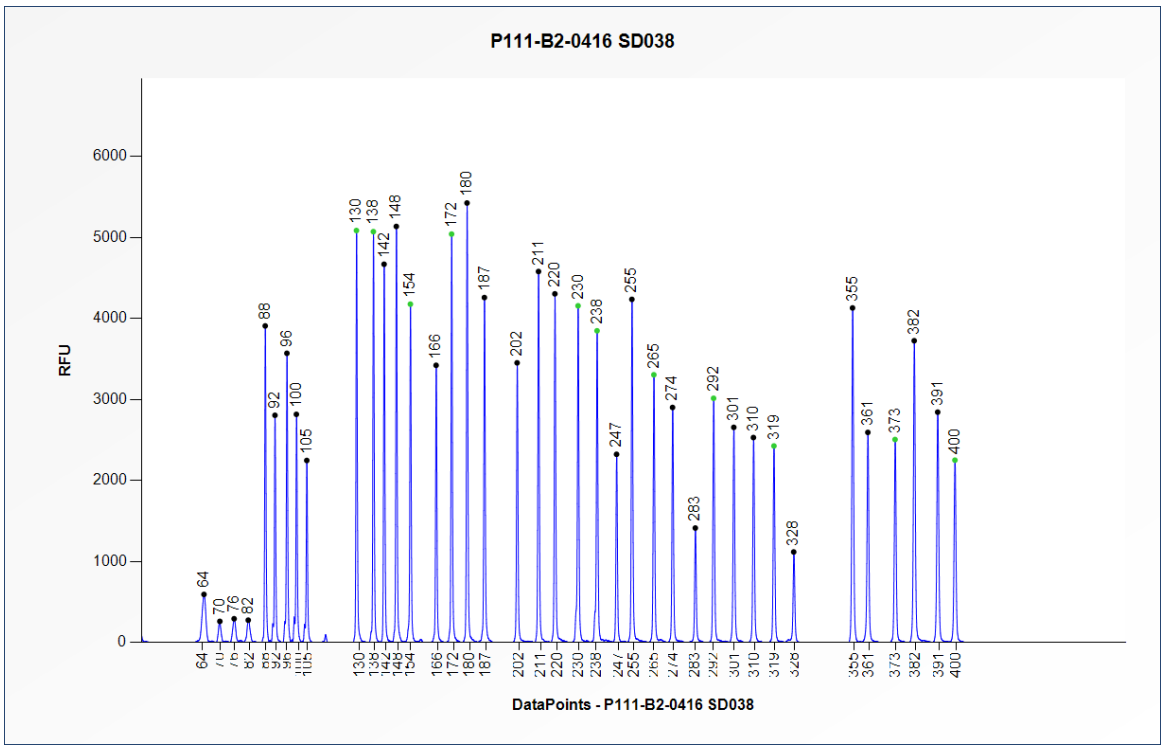

Figure 4. Capillary electrophoresis pattern of SD038 sample DNA (approximately 50 ng) analysed with SALSA MLPA probemix P111-B2 FCGR (lot B2-0416). For more information, see the SD038 Reference DNA product description.

Implemented Changes – compared to the previous product description versions.

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.

Version 12 (49)
- Product description adapted to a new lot (lot number added, changes in Table 1 and Table 2, new picture included).
- Various major textual changes.

Version 11 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 10 (48)
- Various textual changes.
- Exon numbering of the FCGR2C gene has been changed in Table 2.
- Small correction of chromosomal locations in Table 1.

Version 09 (45)
- Various textual changes.
- Exon numbering updated according to the new version of the NM_reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor layout changes.

Version 08 (45)
- Product description adapted to a new lot (lot number added, changes in Table 1 and Table 2, new picture included).
- Various major textual changes.