SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region

Lot A1-0909.

The first 3 Mb of the X and Y chromosomes is identical and is called the pseudoautosomal region 1 (PAR1). Among the few genes in this region are SHOX (short stature homeobox gene), CRLF2, CSF2RA and IL3RA. The chromosomal region containing these latter three genes has been linked to schizophrenia and to lymphoid cell transformations in B-cell acute lymphoblastic leukaemia.

This P329 probemix contains 35 MLPA probes in this Xp22.33 PAR1 region and is primarily intended for ALL research. Russell *et al.* and Mullighan et al. recently reported frequent rearrangements in the PAR1 region. These involved a deletion of the IL3RA and CSF2RA genes resulting in the overexpression of the CRLF2 gene in 7% of individuals with B-progenitor ALL and 53% of individuals with ALL associated with Downs syndrome. Please note that we identified a duplication of the complete CRLF2 gene in one of our (not ALL related) reference samples. The occurrence of genomic copy number changes in healthy individuals can be verified in the database of genomic variants (http://projects.tcag.ca/variation).

The IL3RA gene (12 exons) spans ~46 kb of genomic DNA and is located 1.4 Mb from the p-telomere. The CSF2RA gene (13 exons) spans ~41 kb of genomic DNA and is located 1.3 Mb from the p-telomere. The CRLF2 gene (6 exons) spans ~17 kb of genomic DNA and is located 1.3 Mb from the p-telomere. The P329-A1 probemix contains one probe for each exon of the IL3RA gene with the exception of exons 4, 5, 10 and 11; one probe for each exon of the CSF2RA gene and one probe for each exon of the CRLF2 gene with the exception of exon 1. Furthermore, several other probes for the Xp22.33 region are present, including probes for the region between SHOX and IL3RA that is known to contain SHOX enhancer sequences. Finally, 11 reference probes are included in this probemix, detecting 10 different autosomal chromosomal locations.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the above mentioned chromosomal regions in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak area 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 area, 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. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings.

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

The use of this SALSA® MLPA® probemix 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).

Related SALSA® MLPA® probemixes

- P018 SHOX: probes for SHOX gene and several other probes in the PAR region.
- P335 ALL-IKZF1: probes for IKZF1 and various other genes / regions that are often deleted in ALL.

References for CRLF2

- Mullighan *et al.* (2009). Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukaemia. *Nature Genetics 2009 Nov; 41(11) 1243-6*
- Russell, L.J. *et al.* (2009) Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukaemia. *Blood 114:2688-2698.*

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 6, 1057 DN Amsterdam, the Netherlands

Data analysis

The P329-A1 CRLF2-CSF2RA-IL3RA region probemix contains 46 MLPA probes with amplification products between 130 and 490 nt. In addition, it 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.

Data generated by this probemix can be normalised intra-sample by dividing the peak area of each amplification product by the total area of only the reference probes in the 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 targeted by the reference probes. It is strongly recommended to use reference and patient samples of the same sex to minimize variation, as intersex comparison makes analysis more difficult. Sex determination can also be done by visual examination of the electropherogram.

Data normalisation should be performed within one experiment. Always use sample and reference DNA extracted with the same method and derived from the same source of tissue. Confirmation of deletions, duplications 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://projects.tcag.ca/variation. 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.

This probemix was developed by R.J.L. Schuit at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the probemix designer could be made a coauthor.

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

Table 1. SALSA MLPA P329-A1 CRLF2-CSF2RA-IL3RA probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		Reference	IL3RA	CSF2RA	CRLF2	Flanking
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				
136	**CRLF2 probe** 13889-L15427				**Exon 4**	
142	**IL3RA probe** 13597-L15055		**Exon 6**			
148	**CSF2RA probe** 13890-L15428			**Exon 7**		
154 ±	**IL3RA probe** 13891-L16341		**Exon 9**			
160	Reference probe 12741-L13835	21q22				
166	**CSF2RA probe** 13892-L16221			**Exon 10**		
172	**IL3RA probe** 13596-L15054		**Exon 2**			
178	**CSF2RA probe** 13893-L16342			**Exon 6**		
184	**SHOX-AREA probe** 06293-L06219					Xp22.33
191	**CSF2RA probe** 13894-L15432			**Exon 1**		
197	**CSF2RA probe** 13895-L16222			**Exon 13**		
202	**CRLF2 probe** 13896-L15434				**Exon 5**	
208	Reference probe 06047-L05502	4q31				
214	Reference probe 12426-L14685	14q24				
220	**CSF2RA probe** 13897-L15435			**Exon 9**		
226 Ж	**CD99 probe** 14139-SP0141-L15739					Xp22.33
232	**IL3RA probe** 13898-L16224		**Exon 7**			
238	**SHOX-AREA probe** 05650-L16223					Xp22.33
244	**P2RY8 probe** 14140-L15740					Xp22.33
250	**CRLF2 probe** 13899-L16225				**Exon 6**	
256 Ж	**CSF2RA probe** 13900-SP0138-L15438			**Exon 2**		
275	Reference probe 04489-L03878	1p34				
283	**CRLF2 probe** 13902-L15440				**Exon 3**	
292	**CSF2RA probe** 13903-L15441			**Exon 11**		
302	**IL3RA probe** 13904-L15442		**Exon 3**			
310	**ASMT probe** 01153-L00712					Xp22.33
318	**CSF2RA probe** 13905-L15443			**Exon 4**		
328	**IL3RA probe** 13906-L15444		**Exon 8**			
337	Reference probe 01791-L01354	13q14				
346	**IL3RA probe** 13907-L15445		**Exon 1**			
355	**CSF2RA probe** 13908-L15446			**Exon 8**		
364	**ZBED1 probe** 14142-L15742					Xp22.33
373	Reference probe 04537-L03926	2q24				
382	**CSF2RA probe** 13910-L15448			**Exon 14**		
391	**CRLF2 probe** 13911-L15449				**Exon 2**	
400	Reference probe 03266-L02703	3q29				
409	**IL3RA probe** 13912-L16228		**Exon 12**			
418	**CSF2RA probe** 13913-L15451			**Exon 5**		
427	Reference probe 13215-L14404	2p21				
436	**CSF2RA probe** 13915-L15453			**Exon 16**		
445	**P2RY8 probe** 05652-L16229					Xp22.33
463	Reference probe 05950-L05394	2p22				
472	**SHOX-AREA probe** 14700-L16348					Xp22.33
481	**SHOX probe** 03714-L16230					Xp22.33
490 ±	Reference probe 13725-L15206	11p15				

Ж This probe consists of three parts and has two ligation sites.

± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

Note

- **The CSF2RA exon numbering has changed.** From description version 02 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the CSF2RA gene. The exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2.

Table 2. P329 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
481	03714-L16230	SHOX		ACAGCCAACCAC-CTAGACGCCTGC	233.5 kb
184	06293-L06219	SHOX-AREA		TAATTGATGAGA-TGCAGAAGCCAG	128.5 kb
238	05650-L16223	SHOX-AREA		GAAATTCAGTTT-TAATAACACAGA	66.0 kb
472	14700-L16348	SHOX-AREA		CTCTGGTGAGAT-GCCATCTAGAGA	325.2 kb
		CRLF2	NM_022148.2		
		stop codon	*1130-1132*		
250	13899-L16225	Exon 6	697-698	CCTCCCAAACCA-AAGCTGTCCAAA	2.5 kb
202	13896-L15434	Exon 5	631-632	GACTGGTCAGAG-GTGACATGCTGG	3.9 kb
136	13889-L15427	Exon 4	459-460	GGATCTCCTCTA-TGAGGTTCAGTA	4.1 kb
283	13902-L15440	Exon 3	236-237	AGTGCACCAACT-ACCTTCTCCAGG	2.3 kb
391	13911-L15449	Exon 2	165-166	GAATGCCAGCAA-ATACTCCAGGAC	60.1 kb
		Start codon	*17-19*		
		CSF2RA	NM_006140.4		
		start codon	*195-197 (ex 4)*		
191	13894-L15432	Exon 1	24 nt after exon 1	CTTTCCTTCTGT-GGTCTTTGAGCA	5.8 kb
256 Ж	13900-SP0138 -L15438	Exon 2	45nt and 10 nt before exon 2	GTTTCCACTATA- 35 nt spanning oligo -CCTTTCACAGTT	8.0 kb
318	13905-L15443	Exon 4 (3)	236-235 reverse	AATGCTGGGTGT-GGTAACTCACAG	3.1 kb
418	13913-L15451	Exon 5 (4)	316-317	TGTGAGGTTTGA-CTCCAGGACGAT	2.8 kb
178	13893-L16342	Exon 6 (5)	480-481	TCACATTTGAGG-TTCACGTGAATA	0.2 kb
148	13890-L15428	Exon 7 (6)	576-577	TCTCCTGTTTCA-TCTACAATGCGG	1.7 kb
355	13908-L15446	Exon 8 (7)	805-804 reverse	TTGGATGCCAAT-TTCTCGGCTGGT	4.0 kb
220	13897-L15435	Exon 9 (8)	923-924	CCCAGGACCTAT-CAGAAGCTGTCG	1.0 kb
166	13892-L16221	Exon 10 (9)	1003-1002 reverse	TTTCACTTACCA-GTAGGTTTTCCG	5.2 kb
292	13903-L15441	Exon 11 (10)	1124-1125	AGCTCCTGGAGT-GAAGCCATTGAA	3.3 kb
197	13895-L16222	Exon 13 (11)	1156-1155 reverse	CAGAGCCGAGGT-TCCCGTCGTCAG	1.6 kb
382	13910-L15448	Exon 14 (12)	1296-1297	ACAAACTGAATG-ATAACCATGAGG	3.9 kb
436	13915-L15453	Exon 16 (13)	24 nt before exon 13	TGAAGATCTGAC-AGCCTGAACCCT	27.5 kb
		Stop codon	*1395-1397 (ex 16)*		
		IL3RA	NM_002183.2		
		start codon	*350-352 (ex 2)*		
346	13907-L15445	Exon 1	222-223	GGAAGATATCAG-AAACATCCTAGG	5.1 kb
172	13596-L15054	Exon 2	58nt after exon 2	GGTAGACAGACA-CACAATGTCAGC	3.5 kb
302	13904-L15442	Exon 3	480-479 reverse	TCACATTTCTGT-TAAGGTCCCAGG	7.1 kb
142	13597-L15055	Exon 6	943-944	TGCACAGATAAG-TTTGTCGTCTTT	3.8 kb
232	13898-L16224	Exon 7	995-996	ACATGACTGCAA-AGTGTAATAAGA	2.6 kb
328	13906-L15444	Exon 8	3nt after exon 8	ACAGAACAGGTG-AGTGTTCCCTAC	6.3 kb
154	13891-L16341	Exon 9	1148-1149	TCAATCCTGGAA-CGTACACAGTAC	17.3 kb
409	13912-L16228	Exon 12	1469-1470	CTGAAGTACAGG-TCGTGCAGAAAA	83.8 kb
		stop codon	*1484-1486 (ex 12)*		
244	14140-L15740	P2RY8, ex 2	NM_178129.4; 567-568	TTTACGCAAACA-TGTATTCCAGCA	83.1 kb

Length (nt)	SALSA MLPA probe	Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
445	05652-L16229	P2RY8	12.4 kb before exon 1	GAGAAGCCGAGT-GTATTTTGGGGG	83.9 kb
310	01153-L00712	ASMT		GACATCCCAGAA-GTGGTGTGGACG	706.4 kb
364	14142-L15742	ZBED1		TCGTCAAGAGCA-ACACGGAGCAGA	232.9 kb
226 Ж	14139-SP0141 -L15739	CD99		GTCTTGCAGGAA- 30 nt spanning oligo -GGAAAGAAGGGG	

Ж This probe consists of three parts and has two ligation sites.
The NM_006140.4 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering might be different as compared to literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region sample picture

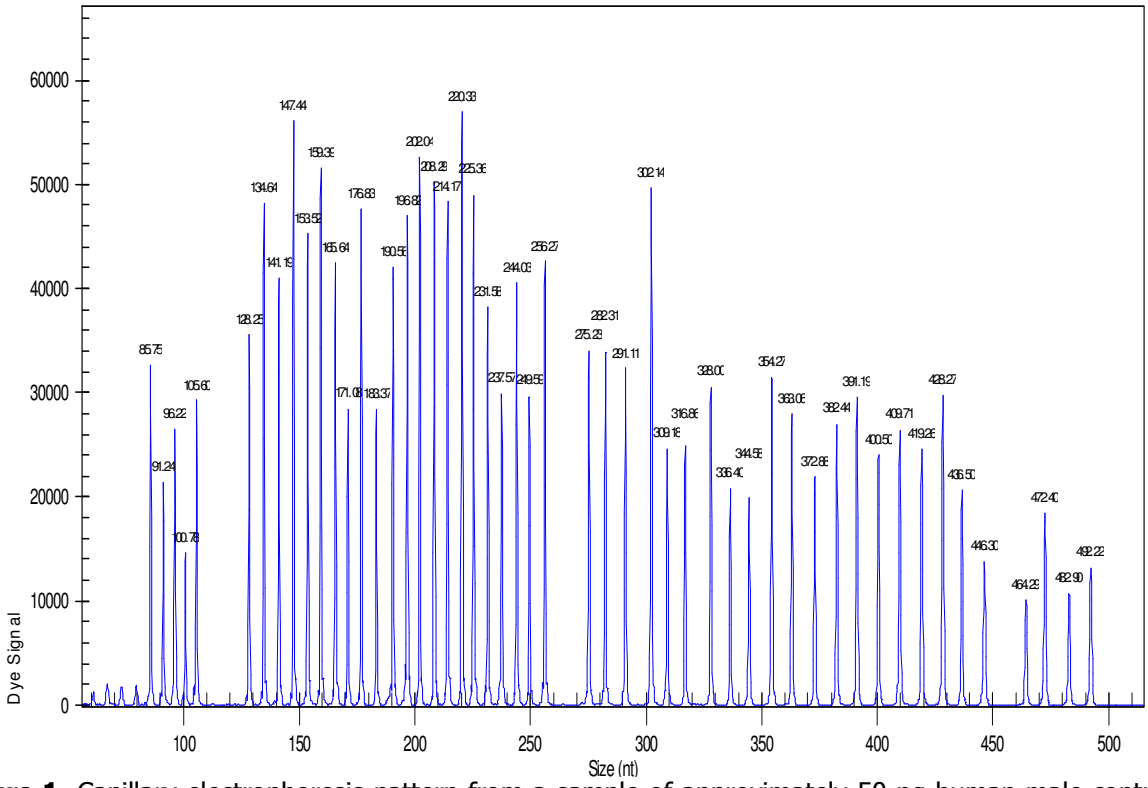

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region (lot A1-0909). The old MLPA buffer (replaced in December 2012) was used. Vials with the old MLPA buffer have a white label.

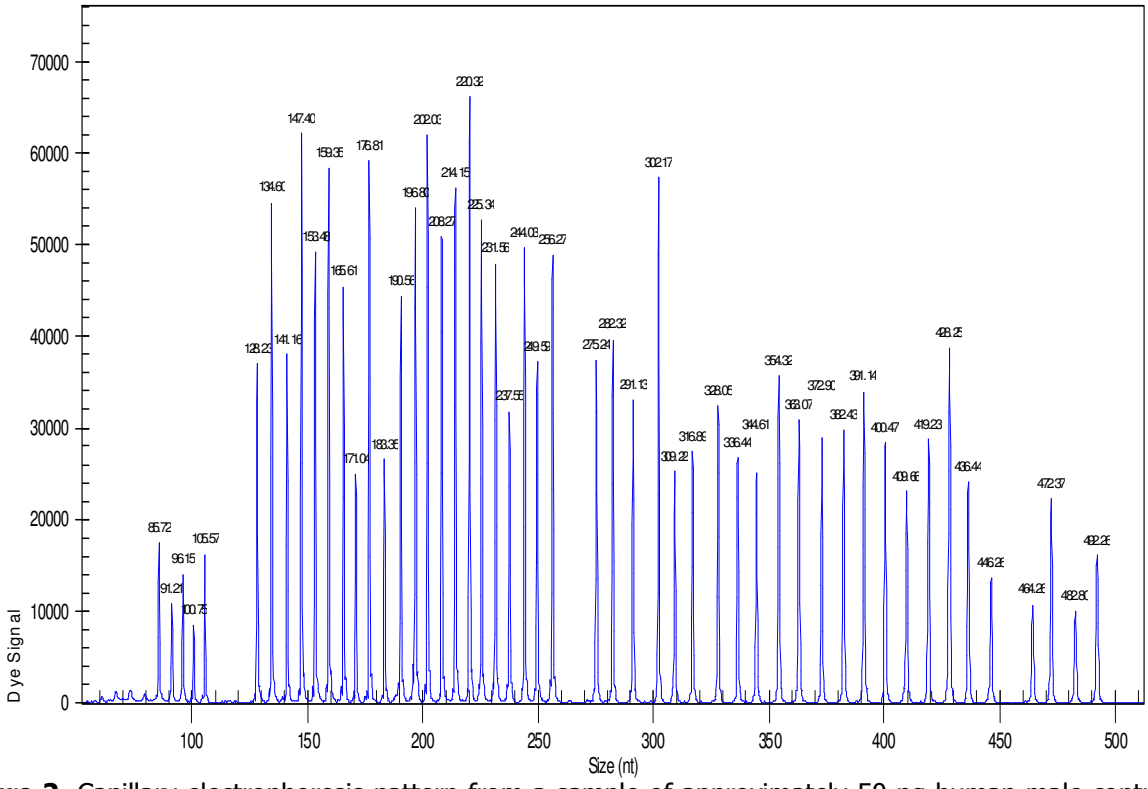

Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region (lot A1-0909). The new MLPA buffer (introduced in December 2012) was used. Vials with the new MLPA buffer have a yellow label.

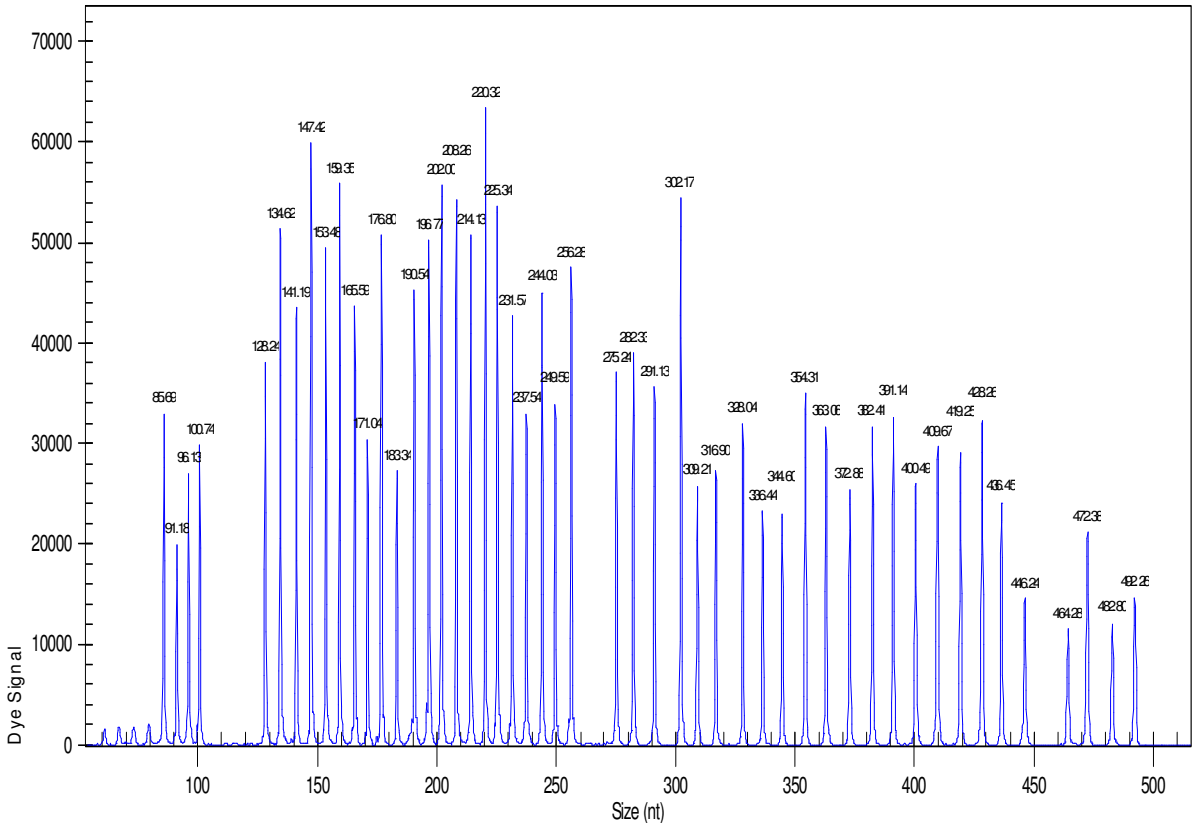

Figure 3. Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analysed with SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region (lot A1-0909). The old MLPA buffer (replaced in December 2012) was used. Vials with the old MLPA buffer have a white label.

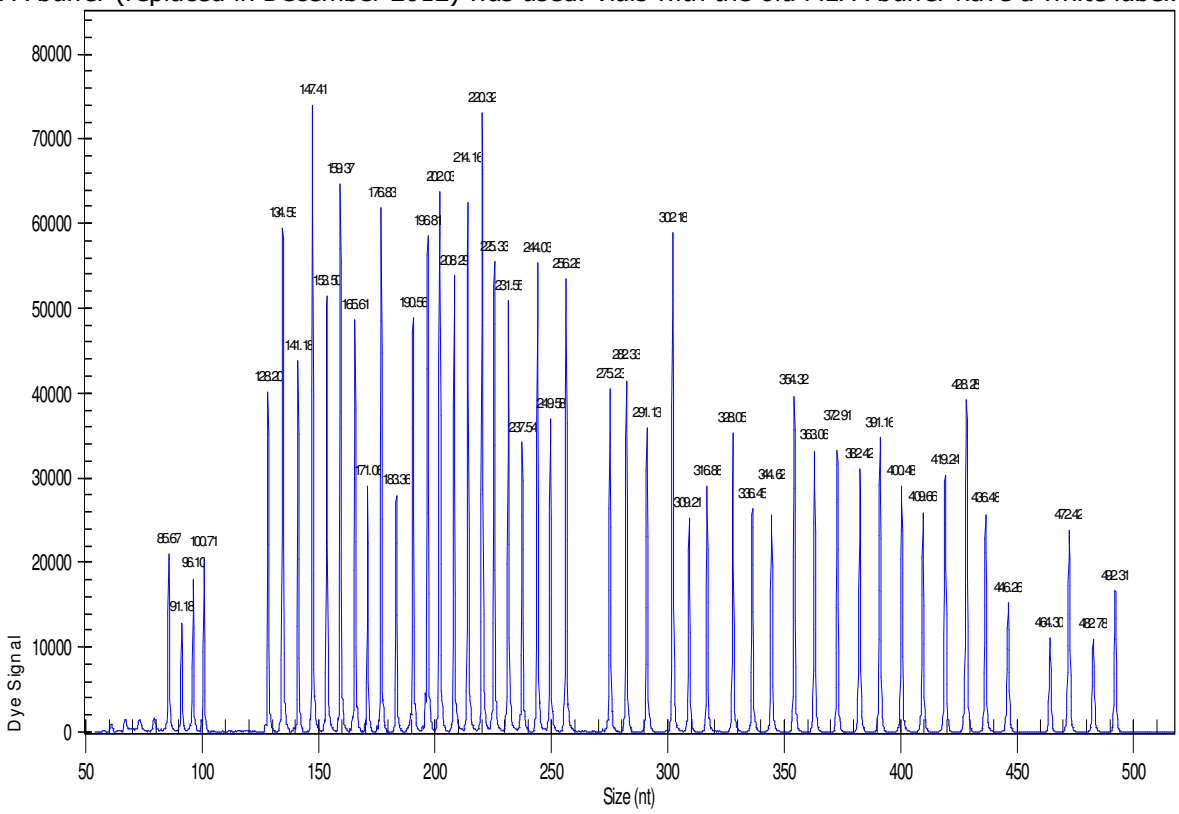

Figure 4. Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analysed with SALSA MLPA probemix P329-A1 CRLF2-CSF2RA-IL3RA region (lot A1-0909). The new MLPA buffer (introduced in December 2012) was used. Vials with the new MLPA buffer have a yellow label.

Implemented Changes – compared to the previous product description version(s).
Version 03 (48) - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 02 (48)* - Remark on RefSeqGene standard and transcript variant added below Table 2. - Exon numbering of the CSF2RA gene has been changed on page 3 and 4. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Data analysis method has been modified. - Various minor textual changes. - Ligation sites of the probes targeting the CSF2RA gene updated according to new version of the NM_reference sequence. *Version 01 (44)* - Not applicable, new document.