SALSA MLPA probemix P372-A1 Microdeletion Syndromes 6

Lot A1-0509

The purpose of the P372 probemix is to further investigate results found with the P245 Microdeletion probemix. The P245 probemix provides a possibility to screen samples for 21 different microdeletion syndromes in a single reaction. For confirmation of results obtained with this P245 probemix, four different probemixes are now available with additional probes in these 21 regions: P371, P372, P373 and P374 Microdeletion Syndromes.

This P372 probemix contains probes for the Sotos syndrome region on 5q35 (7 probes), the DiGeorge region on 22q11 (11 probes), the Rubinstein-Taybi CREBBP gene on 16p13 (7 probes), the DiGeorge 2 region on 10p15 (7 probes) and the NF1 gene region on 17q (11 probes).

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned 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 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).

Related SALSA® MLPA® probemixes

- P245 Microdeletion syndromes 1: probes for 21 different microdeletion syndromes.
- P297 Microdeletion syndromes 2: probes for several recently described microdeletion syndromes (1q21.1; 1q21-TAR; 15q13; 16p11; 17q12).
- P371 Microdeletion syndromes 5 (follow-up P245): 2p16, Langer-Giedion region 8q24, 9q22, WAGR syndrome 11p13, 15q24, 17q21.
- P373 Microdeletion syndromes 7 (confirmation P245): 1p36, 3q29, Cri-du-chat, Wolf-Hirschhorn region, 22q13 Phelan-McDermid.
- P374 Microdeletion syndromes 8 (confirmation P245): Williams, Prader-Willi/Angelman, Miller-Dieker, Smith-Magenis, Xq28-MECP2-RETT syndrome.
- P064 MR-1: 1p36, Sotos, Williams, Prader-Willi/Angelman, Miller-Dieker, Smith-Magenis, Alagille, DiGeorge.
- P096 MR-2: Cri-du-chat, Wolf-Hirschhorn, Langer-Giedion, WAGR, Rubinstein-Taybi.

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 P372-A1 Microdeletion-6 probemix contains 43 MLPA probes with amplification products between 142 and 474 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 normalized intra-sample by dividing the peak area of each amplification product by the combined peak area of all peaks in that sample (global normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference samples.

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://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 at MRC-Holland.

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

Table 1. SALSA MLPA P372-A1 Microdeletion-6 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		5q35	10p15	16p13	17q11	22q11
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					
142±	**NSD1 probe** 02589-L11228	5q35				
148	**DGCR8 probe** 08475-L08486					22q11
154	**TCEB1P3 probe** 13355-L14785		10p14			
159	**NSD1 probe** 02597-L02068	5q35				
166	**GATA3 probe** 07635-L14382		10p15			
172	**PROP1 probe** 07244-L06894	5q35				
178	**TCEB1P3 probe** 13353-L14783		10p14			
185	**SUZ12 probe** 03786-L14384				17q11	
191	**CLTCL1 probe** 05462-L05809					22q11
197	**NF1 probe** 12019-L14389				17q11	
202	**ZNF74 probe** 05927-L07395					22q11
208	**NSD1 probe** 03225-L14390	5q35				
214	**GATA3 probe** 07634-L14385		10p15			
220‡	**RNF135 probe** 03783-L03292				17q11	
227	**NSD1 probe** 01304-L00854	5q35				
238	**TXNRD2 probe** 01223-L05814					22q11
244	**COMT probe** 07490-L14391					22q11
252	**ATAD5 probe** 03781-L14392				17q11	
258	**UTP6 probe** 03785-L14394				17q11	
265	**GATA3 probe** 01225-L00776		10p15			
274	**CREBBP probe** 09897-L14386			16p13		
283	**KLHL22 probe** 01227-L05815					22q11
292 *	**ADAP2 probe** 03782-L03291				17q11	
301	**GATA3 probe** 07636-L07321		10p15			
310	**NF1 probe** 04076-L14387				17q11	
317	**CREBBP probe** 09906-L10319			16p13		
328	**GNB1L probe** 07487-L14393					22q11
337	**NF1 probe** 02507-L01938				17q11	
346	**CREBBP probe** 09896-L14388			16p13		
355	**FGFR4 probe** 01311-L00859	5q35				
364	**CREBBP probe** 09884-L10297			16p13		
374	**COMT probe** 07489-L10761					22q11
382	**DGCR8 probe** 08476-L10765					22q11
391	**NF1 probe** 02530-L01961				17q11	
400	**NSD1 probe** 02598-L02069	5q35				
409	**CREBBP probe** 09893-L10306			16p13		
418	**LZTR1 probe** 01521-L00951					22q11
427 ‡	**CREBBP probe** 09880-L10293			16p13		
436	**TCEB1P3 probe** 13364-L14794		10p14			
445	**NF1 probe** 04074-L03710				17q11	
454	**NF1 probe** 03856-L03307				17q11	
465	**CDC45 probe** 05463-L05808					22q11
474	**CREBBP probe** 09888-L10301			16p13		

* The name of this gene has changed. Previous name can be found between brackets in Table 2.

± SNP at 20 nt from ligation site could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

‡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, even when the 88nt and 96nt D-fragments (QDX1, present in all lots until lot 1210) don't indicate denaturation problems.

Note: Exon numbering might be different as compared to literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. P372 probes arranged according to chromosomal location

Table 2a. Sotos syndrome region

Length (nt)	SALSA MLPA probe	Gene / exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
355	01311-L00859	FGFR4 exon 18 (2)	05-176.457194	GCGATTCTGTCT-TCAGCCACGACC	106.6 kb
142 ±	02589-L11228	NSD1 exon 5 (4)	05-176.563758	AGATCCTTCTGA-GAGAGCCTGGGT	42.5 kb
208	03225-L14390	NSD1 exon 11 (10)	05-176.606291	GCCAAGGAAGCG-AAAACGACAGAG	13.3 kb
P245	*02595-L08077*	*NSD1 exon 14 (13)*	*05-176.616671*	*3 kb from 01304-L00854*	
227	01304-L00854	NSD1 exon 15 (14)	05-176.619619	TACCACGCCAAT-GACTTTTGCCTG	9.7 kb
159	02597-L02068	NSD1 exon 17 (16)	05-176.629353	GAGCAGCAAGGA-TAAGATGGGCAA	11.0 kb
400	02598-L02069	NSD1 exon 19 (18)	05-176.640336	GTGCTTTTCCAA-GCGCCAATATCC	711.9 kb
P245	*02600-L02071*	*NSD1 exon 22 (21)*	*05-176.648451*	*8 kb from 02598-L02069*	
172	07244-L06894	PROP1 exon 3	05-177.352269	TGAGGTCAAACA-AGTACCACCAAG	
			Distance to 5q telomere: ~ 3506 kb		

± SNP at 20 nt from ligation site could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

- **The exon numbering of the FGFR4 and NSD1 genes has changed**. From description version 03 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for these genes. This 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.
- More probes for the NSD1 gene are present in the P026 Sotos probemix. Deletion of the complete NSD1 gene is a frequent cause of Sotos syndrome and may result in a more severe phenotype.
- Frequency of complete gene deletions has been reported as 10% (United Kingdom) to 45% (Japan) of all NSD1 mutations detected. More info on Sotos syndrome is in OMIM 117550.
- Distance from the NSD1 gene to the 5q telomeric probes in P036 and P069-70 is approximately 3950 kb.
- Most common cause of Sotos syndrome are point mutations in the NSD1 gene that will not be detected by these MLPA probes.

Table 2b. DiGeorge region II, 10p15 region

Length (nt)	SALSA MLPA probe	Gene / exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
P245	*07632-L07317*	*GATA3*	*10-008.136773*	*4 kb from 01225-L00776*	
265	01225-L00776	GATA3 exon 3	10-008.140560	GAGTGCCTCAAG-TACCAGGTGCCC	5.4 kb
214	07634-L14385	GATA3 exon 4	10-008.145969	GGGGCAACCTCG-ACCCCACTGTGG	5.6 kb
166	07635-L14382	GATA3 exon 5	10-008.151531	TACTACAAGCTT-CACAATGTAAGT	4.3 kb
301	07636-L07321	GATA3 exon 6	10-008.155803	AACAGCTCGTTT-AACCCGGCCGCC	1934.1 kb
178	13353-L14783	TCEB1P3 area	10-010.089853	GTCTTGATTCCA-TTCTGACACTGC	449.3 kb
436	13364-L14794	TCEB1P3 area	10-010.539170	TGCCAGTTCAGA-CCAGTATTGACA	53.5 kb
P245	*01232-L07388*	*Hs.538604*	*10-010.588971*	*4 kb from 13355-L14785*	
154	13355-L14785	TCEB1P3 area	10-010.592708	TCATCCAGAAGA-GTCCATCAACTG	

- More probes for the 10p15 DiGeorge region 2 are in the P250 DiGeorge probemix.
- More info in OMIM 601362.
- Besides this DGS2 region, also deletion of the 17p terminal region can cause a DiGeorge-like phenotype. These 17p deletions should be detected by the P036 and P069/P070 Human Telomere probemixes. The great majority of DiGeorge syndrome patients have a 22q11 deletion.

Table 2c. Rubinstein-Taybi syndrome, CREBBP gene

Length (nt)	SALSA MLPA probe	Gene / exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
317	09906-L10319	CREBBP exon 27	16-003.726660	AAAAAGATGCTG-GACAAGGCGTTT	21.2 kb
274	09897-L14386	CREBBP exon 18	16-003.747897	CTGGCTCATGTT-CAACAATGCCTG	1.0 kb
346	09896-L14388	CREBBP exon 17	16-003.748864	CCCAGAGTCATT-ACCTTTCCGGCA	11.9 kb
409	09893-L10306	CREBBP exon 14	16-003.760750	ACTCAGCCATCA-ACTCCTGTGTCG	8.0 kb
474	09888-L10301	CREBBP exon 9	16-003.768705	AGCCTATGCTAA-GAAAGTGGAAGG	13.3 kb
364	09884-L10297	CREBBP exon 5	16-003.782018	CAAATCATCTCT-CATTGGAAGAAC	87.8 kb
427 ‡	09880-L10293	CREBBP exon 1	16-003.869824	GCTCGCCCGGTT-TCTCGGCGAATG	
P245 ‡	*03087-L02487*	*CREBBP exon 1*	*16-003.869877*	*0.1 kb from 09880-L10293*	

‡ These probes are located within, or close to, a very strong CpG island. A low signal of these probes can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA, even when the 88nt and 96nt D-fragments (QDX1, present in all lots until lot 1210) don't indicate denaturation problems.

- More probes for the CREBBP gene are present in the P313 CREBBP probemix. Only a minority of Rubinstein-Taybi patients can be detected with the use of these probes; most patients have point mutations in CREBBP or EP300.
- More info in OMIM 180849.
- The 16p13.3 deletion syndrome (OMIM 610543) is caused by larger deletions that include the CREBBP gene.

Table 2d. NF1 region / 17q11.2

Length (nt)	SALSA MLPA probe	Gene / exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
252	03781-L14392	ATAD5	17-026.186154	GCAGGTACGCTT-TAAGACAGTTAC	91.8 kb
292 *	03782-L03291	ADAP2 (CENTA2)	17-026.277986	TGAAGGCCAAGT-TCGAAGCCAGAG	57.8 kb
220 ‡	03783-L03292	RNF135	17-026.335798	GGAACATCTTGT-AGACATTGTCAG	111.3 kb
197	12019-L14389	NF1 exon 1	17-026.447083	TCGTCTCATCCT-GCCCCGAGAGCT	125.6 kb
P245	*03778-L11180*	*NF1 exon 12 (10)*	*17-026.557430*	*15 kb from 04076-L14387*	
310	04076-L14387	NF1 exon 15 (11)	17-026.572706	TAACTGGCATGT-ACATATAAAGCT	3.6 kb
337	02507-L01938	NF1 exon 17 (12)	17-026.576312	GGATCATGAAGA-ATTACTACGTAC	5.1 kb
P245	*02508-L02620*	*NF1 exon 20 (15)*	*17-026.578679*	*2 kb from 02507-L01938*	
445	04074-L03710	NF1 exon 23 (18)	17-026.581419	ACTTCAACTAAT-TGACACAGTTTC	107.8 kb
454	03856-L03307	NF1 exon 45 (36)	17-026.689192	TCTTGTTGTCTT-TGGGTGTATTAG	36.0 kb
391	02530-L01961	NF1 exon 58 (49)	17-026.725181	GCCACTGTAACA-GTGGACGAACTC	501.3 kb
258	03785-L14394	UTP6	17-027.226448	TCCCAGAGTCTC-TAAACAATTCAG	113.1 kb
185	03786-L14384	SUZ12	17-027.339502	CAATGATAAATC-TACGGCTCCTAT	

‡ 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.
* The name of this gene has changed. Previous name can be found between brackets in Table 2.

Notes

- **The exon numbering of the NF1 gene has changed.** From description version 03 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for this gene. This 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.
- More probes for the NF1 gene are present in the P081 & P082 NF1 probemixes. More probes for other genes in this area are present in the P122 NF1 area probemix.
- Approximately 5-20% of all NF1 patients carry a heterozygous deletion of approximately 1.5 Mb that includes the NF1 gene. This NF1 microdeletion results in a more severe phenotype that often includes mental retardation, facial dysmorphism and developmental delay.
- More info in OMIM 162200.

Table 2e. DiGeorge region / 22q11

Length (nt)	SALSA MLPA probe	Gene	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
191	05462-L05809	CLTCL1 *(AB-region)*	22-017.621597	TGTTGCCTTGGT-GACCGAGACCGC	225.9 kb
465	05463-L05808	CDC45 *(AB-region)*	22-017.847478	ATGTTCGTGTCC-GATTTCCGCAAA	308.9 kb
P245	*01218-L06270*	*CLDN5 (AB-region)*	*22-017.891318*	*44 kb from 05463-L05808*	
P245	*05464-L10114*	*GP1BB (AB-region)*	*22-018.091521*	*65 kb from 07487-L10114*	
328	07487-L14393	GNB1L *(AB-region)*	22-018.156405	CGGGATCGCCGA-GGTCACGATCCG	109.8 kb
238	01223-L05814	TXNRD2 *(AB-region)*	22-018.266225	GGAGGGTCAGGA-GAGGAGCTGCAG	64.0 kb
374	07489-L10761	COMT *(AB-region)*	22-018.330270	TTGACACCTACT-GCGAGCAGAAGG	5.8 kb
244	07490-L14391	COMT *(AB-region)*	22-018.336105	GTGCGCCAGACT-TCCTAGCACACG	117.5 kb
148	08475-L08486	DGCR8 *(AB-region)*	22-018.453612	GGTAATGGACGT-TGGCTCTGGTGG	24.2 kb
382	08476-L10765	DGCR8 *(AB-region)*	22-018.477850	GACTCAGCGACT-GCACCAGTGGCA	651.6 kb
202	05927-L07395	ZNF74 *(BC-region)*	22-019.079428	CAGGCAGATTAT-TCCTCGATGCTG	93.9 kb
283	01227-L05815	KLHL22 *(BC-region)*	22-019.173307	TCTTCGATGTTG-TGCTGGTGGTGG	505.9 kb
P245	*01235-L00773*	*SNAP29 (CD region)*	*22-019.572014*	*107 kb from 01521-L00951*	
418	01521-L00951	LZTR1 *(CD-region)*	22-019.679191	ATGATGAAGGAG-TTCGAGCGCCTC	

- More probes in the 22q11 DiGeorge region are present in the P250 DiGeorge probemix.
- Deletions in 22q11 are the most frequent cause of DiGeorge syndrome. These 22q11 deletions can be variable in size. The majority (~88%) include the AB, BC and CD regions, though some deletions are smaller (AB only) or larger.
- More info in OMIM 188400.

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 P372-A1 Microdeletion-6 sample picture

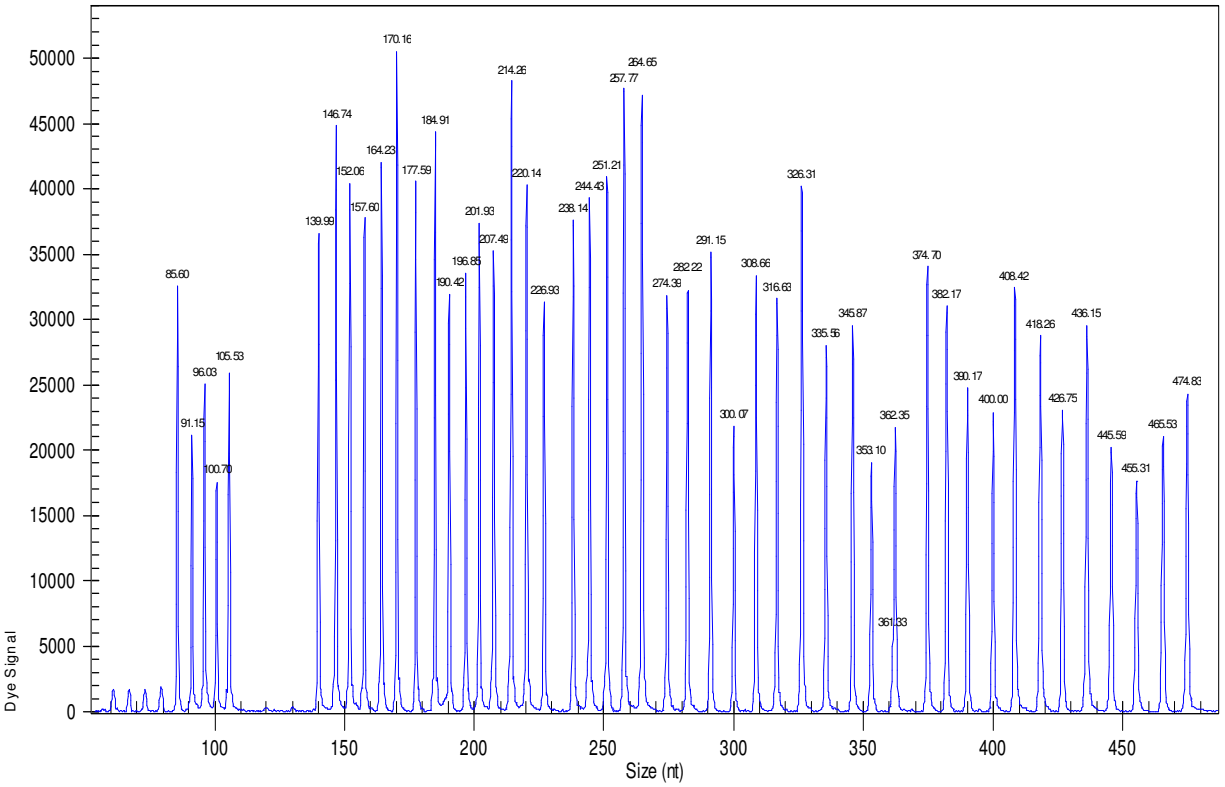

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA® MLPA® probemix P372-A1 Microdeletion-6 (lot A1-0509). The old MLPA buffer (replaced in December 2012) was used. Vials with the old MLPA buffer have a white label.

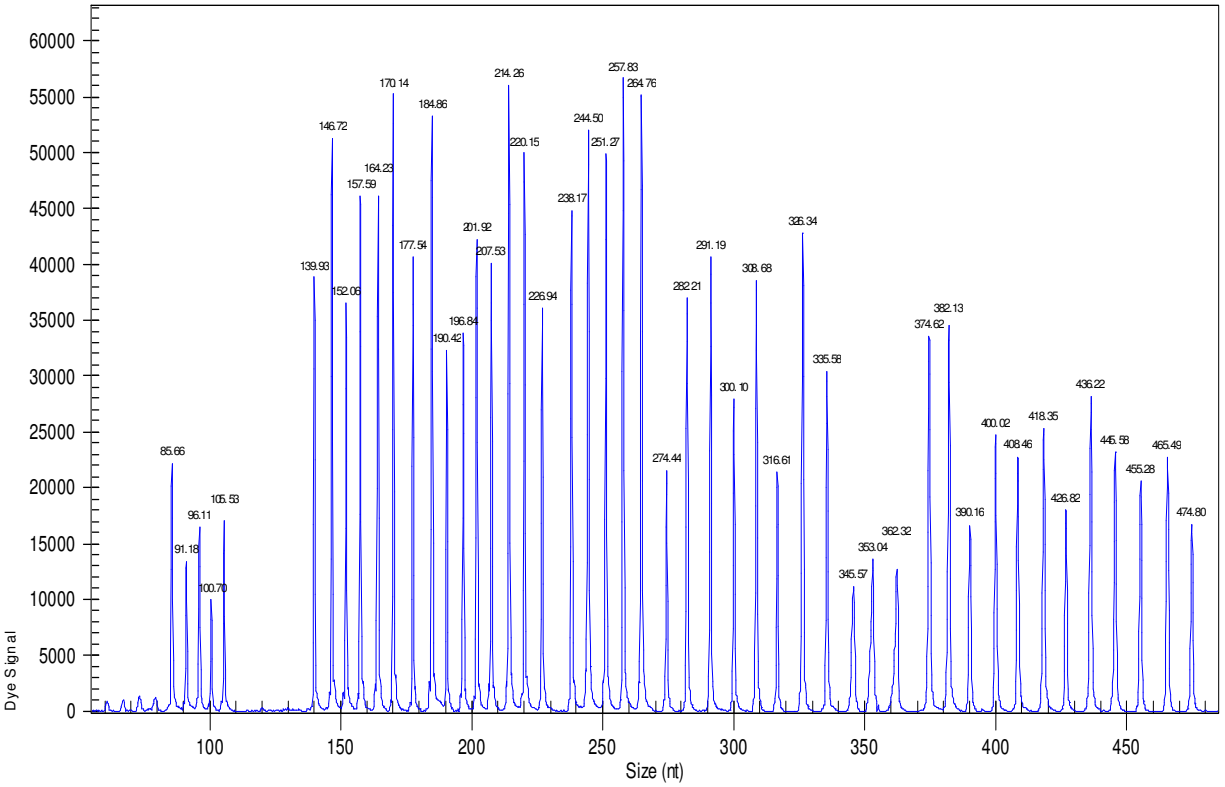

Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA® MLPA® probemix P372-A1 Microdeletion-6 (lot A1-0509). The new MLPA buffer (introduced in December 2012) was used. Vials with the new MLPA buffer have a yellow label.

Implemented Changes – compared to the previous product description versions

Version 06 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 05 (48)
- Warning about salt sensitivity of 427 nt probe 09880-L10293 added to tables.

Version 04 (48)
- Various minor textual changes on pages 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 03 (46)
- Warning added in Table 1 and 2, 142 nt probe 02589-L11228 and 220 nt probe 03783-L03292.
- Exon numbering of the FGFR4, NSD1 and NF1 genes has been changed in Table 2.
- Data analysis method has been modified.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual changes on page 1.
- Various minor layout changes.