

SALSA MLPA KIT P034 / P035 DMD / Becker

Lot 0707, 0906

DUCHENNE MUSCULAR DYSTROPHY and BECKER MUSCULAR DYSTROPHY can be caused by deletions, duplications or point mutations in the DMD gene that encodes dystrophin. Information on DMD and the various deletions/duplications in this gene can be found on www.dmd.nl. In which extend DMD manifests depends on whether the translational reading frame is lost or maintained. Partial gene deletions or duplications in the DMD gene accounts for as much as ~65% of cases of these dystrophies. This extremely high percentage may be due to the nature of the protein and the gene's extreme length (> 2.2 Mb).

This P034/P035 DMD probemix contains probes for each of the exons of the DMD gene on Xp21.2 chromosome. In addition, one probe is present for the alternative exon 1 *DP427c*. These 80 probes have been divided in two probe mixes: P034 and P035. Performing two MLPA reactions is thus sufficient to investigate the copy number of all exons.

Since MLPA offers a more extensive screening than many other methods used in DMD analysis, you may find deletions/duplications that were previously overlooked. For instance, we have been notified by one customer that among 33 samples that were initially found to be normal using the widely used *deletion multiplex test*, 6 samples were actually found to be positive with MLPA (18.1%); 4 of these were duplications and the other 2 had deletions which are not covered by the multiplex PCR test. *Schwartz, M et al (2007; Hum.Mutat. 28, 205)* have reported a completely healthy male with a deletion of exon 16 and part of introns 15 and 16. Their findings suggest that even large gene re-arrangements of the dystrophin gene may not always be disease-causing. Please be caution with the diagnosis of dystrophinopathy in sporadic cases of single exon in-frame deletions.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the aforementioned gene. Deletions of probe recognition sequences in males will be apparent by the absence of the probe amplification product. In female heterozygotes, a 35-50% reduced relative peak area of the amplification product of that probe is expected. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods.

SALSA® MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.

The use of this MLPA kit 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

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References of SALSA MLPA kit P034/P035

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Data analysis

The P034 DMD probemix contains 45 different probes with amplification products between 130 and 490 nt, as well as 6 control fragments generating an amplification product smaller than 130 nt. The control fragments include a 118 nt long chromosome Y specific probe. 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 intra-normalized by dividing the peak area of each amplification product by the total area of only the reference probes in the probemix (block normalization). Secondly, 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. Please note that this type of normalization assumes that no changes occurred in the genomic regions targeted by the reference probes. It is recommended to use reference and patient samples of the same sex to minimize variation, but this is not strictly necessary. Sex determination can also be done by visual examination of the electropherogram.

When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to easily identify exon deletions. Comparison of results should preferably 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 blots or long range PCR.

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

This probemix was developed by J. Coffa & J.P. Schouten 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.

Please note

- We have been informed by one MLPA user that the 490 nt reference probe did not give an amplification product in 1 patient DNA sample (both P034 and P035).
- We have been informed by one MLPA user that the exon 37 probe is sensitive to a (rare?) polymorphism at two nucleotides from the ligation site: 5496G>T, predicted to cause the amino acid change 1763R>L.
- Total length of the DMD gene is 2.217 Kb!! The distance between different exons gives some indication about the likelihood that a single exon deletion/duplication occurs. It is e.g. unlikely that exon 35 is duplicated and exon 36 is not, as these exons are only 0.3 Kb apart. In case you find a deletion/duplication of only one exon, we strongly recommend performing a sequencing analysis of this exon, as mutations / polymorphisms situated close to the probe ligation site can influence the peak area of the amplification product of that probe.

SALSA MLPA P034 DMD probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		Reference	DMD
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation		
118	Y-fragment: Specific for the Y chromosome		
130	Reference probe 1690-L0423	Xq11.2	
138	DMD probe 1353-L1001	Exon 1	
146	DMD probe 1354-L1002	Exon 41	
154	DMD probe 1355-L1615	Exon 21	
162	DMD probe 1356-L1004	Exon 61	
170	DMD probe 1357-L1005	Exon 2	
178	DMD probe 1711-L1279	Exon 42	
186	DMD probe 1359-L1007	Exon 22	
194	DMD probe 1897-L1008	Exon 62	
202	Reference probe 1691-L0465	Xp22	
210	DMD probe 1361-L1009	Exon 3	
218	DMD probe 1362-L1010	Exon 43	
226	DMD probe 1363-L1011	Exon 23	
234	DMD probe 1364-L1012	Exon 63	
242	DMD probe 1365-L1013	Exon 4	
250	DMD probe 1366-L1014	Exon 44	
258	DMD probe 1958-L1518	Exon 24	
266	DMD probe 1368-L1016	Exon 64	
274	Reference probe 1768-L1617	Xq28	
282	DMD probe 1954-L1574	Exon 5	
290	DMD probe 1370-L1287	Exon 45	
298	DMD probe 1371-L1019	Exon 25	
306	DMD probe 1372-L1020	Exon 65	
314	DMD probe 1373-L1021	Exon 6	
322	DMD probe 1374-L1288	Exon 46	
330	DMD probe 1375-L1023	Exon 26	
338	DMD probe 1376-L1024	Exon 66	
354	DMD probe 1713-L1281	Exon 7	
362	DMD probe 1378-L1026	Exon 47	
370	DMD probe 1379-L1616	Exon 27	
378	DMD probe 1960-L1520	Exon 67	
386	DMD probe 1715-L1283	Exon 8	
394	DMD probe 1382-L1030	Exon 48	
402	DMD probe 1716-L1284	Exon 28	
410	DMD probe 2482-L2710	Exon 68	
418 †	Reference probe 3766-L3227	Xq13	
426	DMD probe 1385-L1033	Exon 9	
434	DMD probe 1717-L1285	Exon 49	
442	DMD probe 1387-L1035	Exon 29	
450	DMD probe 1388-L1036	Exon 69	
458	DMD probe 1718-L1286	Exon 10	
466	DMD probe 1390-L1038	Exon 50	
474	DMD probe 1391-L1039	Exon 30	
482	DMD probe 1392-L1040	Exon 70	
490	Reference probe 1692-L1531	Xq28	

† New from lot 0906 onwards.

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

SALSA MLPA P035 DMD probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		Reference	DMD
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	Ligation-dependent control fragment at 2q14		
109	Autosomal fragment at 12q14		
118	Y-fragment: Specific for the Y chromosome		
130	Reference probe 1690-L0423	Xq11.2	
138	DMD probe 1393-L1041		Exon 11
146	DMD probe 1394-L1042		Exon 51
154	DMD probe 1395-L1371		Exon 31
162	DMD probe 1396-L1044		Exon 71
170	DMD probe 1397-L1608		Exon 12
178	DMD probe 2059-L1571		Exon 52
186	DMD probe 1399-L1609		Exon 32
194	DMD probe 1949-L1610		Exon 72
202	Reference probe 1691-L0465	Xp22	
210	DMD probe 1899-L1049		Exon 13
218	DMD probe 1892-L1050		Exon 53
226	DMD probe 1900-L1051		Exon 33
234	DMD probe 1893-L1052		Exon 73
242	DMD probe 1950-L1573		Exon 14
250	DMD probe 1894-L1054		Exon 54
258	DMD probe 1901-L1055		Exon 34
266	DMD probe 1902-L1611		Exon 74
274	Reference probe 1768-L1617	Xq28	
282	DMD probe 1410-L1057		Exon 15
290	DMD probe 1411-L1058		Exon 55
298	DMD probe 1412-L1059		Exon 35
306	DMD probe 1413-L1060		Exon 75
314	DMD probe 2060-L1572		Exon 16
322	DMD probe 1415-L1062		Exon 56
330	DMD probe 1416-L1063		Exon 36
338	DMD probe 1417-L1612		Exon 76
354	DMD probe 1952-L1065		Exon 17
362	DMD probe 1419-L1066		Exon 57
370	DMD probe 3038-L2508		Exon 37
378	DMD probe 1421-L1068		Exon 77
386	DMD probe 1891-L1069		Exon 18
394	DMD probe 1423-L1070		Exon 58
402	DMD probe 1896-L1071		Exon 38
410	DMD probe 1425-L1072		Exon 78
418 ‡	Reference probe 3766-L3227	Xq13	
426	DMD probe 1426-L1073		Exon 19
434	DMD probe 1427-L1074		Exon 59
442	DMD probe 1955-L1613		Exon 39
450	DMD probe 1429-L1076		Exon 79
458	DMD probe 1430-L1077		Exon 20
466	DMD probe 1431-L1614		Exon 60
474	DMD probe 1432-L1079		Exon 40
482	DMD probe 1433-L1080		Exon DP427C
490	Reference probe 1692-L1531	Xq28	

‡ New from lot 0906 onwards.

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.

P034 DMD probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_18533	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
138	1353-L1001	DMD exon 1	26-27	CCCCCTACAG-GACTCAGATC	191.0 Kb
170	1357-L1005	DMD exon 2	266-267	CAAAAGAAAA-CATTCACAAA	170.0 Kb
210	1361-L1009	DMD exon 3	335-336	CTCTTCAGTG-ACCTACAGGA	5.0 Kb
242	1365-L1013	DMD exon 4	435-436	CCCTGAACAA-TGTCAACAAG	21.0 Kb
282	1954-L1574	DMD exon 5	517-518	AGATGGAAAT-CATAAACTGA	7.0 Kb
314	1373-L1021	DMD exon 6	618-619	CCAACAGTGA-AAAGATTCTC	7.0 Kb
354	1713-L1281	DMD exon 7	765-766	GGAATAGTGT-GGTTTGCCAG	110.0 Kb
386	1715-L1283	DMD exon 8	951-952	TTGAAGCCAT-CCAGGAAGTG	1.0 Kb
426	1385-L1033	DMD exon 9	1066-1067	ACAGGGATAT-GAGAGAACTT	53.0 Kb
458	1718-L1286	DMD exon 10	1195-1196	AGACAAGTCA-TTTGGCAGTT	1.0 Kb
154	1355-L1615	DMD exon 21	2916-2917	AAGGACAAGG-ACCCATGTTC	13.0 Kb
186	1359-L1007	DMD exon 22	3051-3052	AGGAGACCAT-GAGTGCCATC	3.0 Kb
226	1363-L1011	DMD exon 23	3200-3201	GGCCTATACT-ATCTCAGCAC	4.0 Kb
258	1958-L1518	DMD exon 24	3441-3442	GGCCTGCCCT-TGGGGATTCA	1.0 Kb
298	1371-L1019	DMD exon 25	3554-3555	CAGAAGATAA-AGAATGAAGC	9.0 Kb
330	1375-L1023	DMD exon 26	3695-3696	GTAAGCCTCC-AGAAAGATCT	6.0 Kb
370	1379-L1616	DMD exon 27	3871-3872	TGAGTCTGTA-AATAGTGTC	7.0 Kb
402	1716-L1284	DMD exon 28	4020-4021	GGCATGAGTT-ATTGTCATAC	3.0 Kb
442	1387-L1035	DMD exon 29	4198-4199	ACAGACCCTA-ACAGATGGCG	26.0 Kb
474	1391-L1039	DMD exon 30	4304-4305	AAGTTGCTTG-AACAGAGCAT	22.0 Kb
146	1354-L1002	DMD exon 41	6019-6020	GGGCTTGCTCT-GAGGATGGG	32.0 Kb
178	1711-L1279	DMD exon 42	6159-6160	CGATGATGGT-GATGACTGAA	22.0 Kb
218	1362-L1010	DMD exon 43	6403-6404	ATTGCAAAGT-GCAACGCCTG	70.0 Kb
250	1366-L1014	DMD exon 44	6589-6590	ACAGTTTCTC-AGAAAGACAC	248.0 Kb
290	1370-L1287	DMD exon 45	6745-6746	AGATGCCAGT-ATTCTACAGG	36.0 Kb
322	1374-L1288	DMD exon 46	6913-6914	CATTGCTAGT-ATCCCCTTG	2.0 Kb
362	1378-L1026	DMD exon 47	7017-7018	TCAAACAATT-AAATGAACT	86.0 Kb
394	1382-L1030	DMD exon 48	7225-7226	GTAAATCAT-CTGCTGCTGT	41.0 Kb
434	1717-L1285	DMD exon 49	7356-7357	AAGAGATTTT-GTCTAAAGGG	17.0 Kb
466	1390-L1038	DMD exon 50	7455-7456	TAAACCGTTT-ACTTCAAGAG	46.0 Kb
162	1356-L1004	DMD exon 61	9325-9326	GCAGCTGCAT-GAAGCCCAC	25.0 Kb
194	1897-L1008	DMD exon 62	9394-9395	TCCCTGGGAG-AGAGCCATCT	63.0 Kb
234	1364-L1012	DMD exon 63	9453-9454	AAACAACCTG-CTGGGACCAT	38.0 Kb
266	1368-L1016	DMD exon 64	9547-9546 Rev.	CTGCAGTCTT-CGGAGTTTCA	13.0 Kb
306	1372-L1020	DMD exon 65	9599-9600	GCTGCATGTG-ATGCCTTGGA	3.0 Kb
338	1376-L1024	DMD exon 66	9806-9807	CTGTCTTTTA-AACTGGCAT	2.0 Kb
378	1960-L1520	DMD exon 67	9987-9986 Rev.	ACACTTGGCT-CAATGTTACT	21.0 Kb
410	2482-L2710	DMD exon 68	10064-10065	GACTGGATGA-GACTGGAACC	2.0 Kb
450	1388-L1036	DMD exon 69	10238-10239	TTTTTTTCTG-GTCGAGTTGC	2.0 Kb
482	1392-L1040	DMD exon 70	10385-10386	CCCCGAATGG-GCTACCTGCC	1.0 Kb

Note: Exon numbering might be different as compared to literature!
Please notify us of any mistakes: info@mlpa.com.

P035 DMD probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM 18533	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
482	1433-L1080	DMD exon DP427c	233-234*	CAGTAATAGA-ATGCTTTTCAG	128.0 Kb
138	1393-L1041	DMD exon 11	1382-1383	TTGACAGCCC-ATCAGGGCCG	30.0 Kb
170	1397-L1608	DMD exon 12	1647-1648	AGCCTCTTGG-ACCTGATCTT	18.0 Kb
210	1899-L1049	DMD exon 13	1762-1763	GGTAGTTGAT-GAATCTAGTG	22.0 Kb
242	1950-L1573	DMD exon 14	1836-1837	GGGCAAACAT-CTGTAGATGG	0.1 Kb
282	1410-L1057	DMD exon 15	1937-1938	TGGCTTTTCAG-AAAAAGAAGA	8.0 Kb
314	2060-L1572	DMD exon 16	2059-2060	GCAATCCATG-GGCAAACGT	20.0 Kb
354	1952-L1065	DMD exon 17	2292-2293	AACAGATCCT-GGTAAAGCAT	27.0 Kb
386	1891-L1069	DMD exon 18	2433-2434	AAGCTGTGTT-GCAGAGTCCT	16.0 Kb
426	1426-L1073	DMD exon 19	2526-2527	AAGCTGAGAA-GTTCAGAAAA	10.0 Kb
458	1430-L1077	DMD exon 20	2650-2651	GTGGATCGAA-TTCTGCCAGT	6.0 Kb
154	1395-L1371	DMD exon 31	4519-4520	GGAGGCTGCC-CAAAGAGTCC	0.4 Kb
186	1399-L1609	DMD exon 32	4674-4675	CTGCATTGGA-AACAAAGAGT	3.0 Kb
226	1900-L1051	DMD exon 33	4766-4767	TCTGAAGTGG-AAATGGTGAT	6.0 Kb
258	1901-L1055	DMD exon 34	4947-4948	GAAAGGAAAT-GAATGTCTTG	15.0 Kb
298	1412-L1059	DMD exon 35	5098-5099	CCTGAAGAGT-ATCACAGAGG	0.3 Kb
330	1416-L1063	DMD exon 36	5288-5289	ATCACAAAGT-GGATCATTCA	2.0 Kb
370	3038-L2508	DMD exon 37	5498-5497 Rev.	TGGCTGCAA-TCGATGGTTG	14.0 Kb
402	1896-L1071	DMD exon 38	5613-5614	CTGAAATTC-ACAGGGGGTG	2.0 Kb
442	1955-L1613	DMD exon 39	5700-5701	TGCAAAGAGG-AGACAACCTTA	3.0 Kb
474	1432-L1079	DMD exon 40	5828-5829	AAGGCTCTAG-AAATTTCTCA	1.0 Kb
146	1394-L1042	DMD exon 51	7630-7631	TCTGGCAGAT-TTCAACCGGG	44.0 Kb
178	2059-L1571	DMD exon 52	7837-7836 Rev.	AGCCTCTTGA-TTGCTGGTCT	50.0 Kb
218	1892-L1050	DMD exon 53	7994-7995	GAGCAGGTCT-TAGGACAGGC	22.0 Kb
250	1894-L1054	DMD exon 54	8116-8117	GCAGACAAAT-GTAGATGTGG	30.0 Kb
290	1411-L1058	DMD exon 55	8276-8277	ACTCATAGAT-TACTGCAACA	120.0 Kb
322	1415-L1062	DMD exon 56	8535-8536	TCCTGTTACA-AAGACGTTTG	10.0 Kb
362	1419-L1066	DMD exon 57	8686-8687	GAAAGATGAT-GAATTAAGCC	18.0 Kb
394	1423-L1070	DMD exon 58	8817-8818	AGACTGTACG-AATATTTCTG	1.0 Kb
434	1427-L1074	DMD exon 59	9005-9006	GATGAGACCC-TTGAAAGACT	33.0 Kb
466	1431-L1614	DMD exon 60	9175-9176	GCCTCTGAAA-GAGAACGTGA	96.0 Kb
162	1396-L1044	DMD exon 71	10450-10451	TCTGATCAAC-TTCTGGCCAG	43.0 Kb
194	1949-L1610	DMD exon 72	10493-10494	CCTCAGCTTT-CACACGATGA	1.0 Kb
234	1893-L1052	DMD exon 73	10571-10570 Rev.	TATCATTTAG-ATAAGATCCA	3.0 Kb
266	1902-L1611	DMD exon 74	10693-10694	CCAGATCTTG-ATTTCTTAG	22.0 Kb
306	1413-L1060	DMD exon 75	10885-10886	TGAGCTCATT-GCTGAGGCCA	1.0 Kb
338	1417-L1612	DMD exon 76	11063-11064	ACCTCTCTAC-AGAGGTCCGA	12.0 Kb
378	1421-L1068	DMD exon 77	11164-11165	CCAGGACACA-AGCACAGGGT	7.0 Kb
410	1425-L1072	DMD exon 78	11245-11246	TGGAAAGCCA-ATGAGAGAGG	5.0 Kb
450	1429-L1076	DMD exon 79	11286-11287	CCACATGGCA-GATGATTTGG	

* Genbank # NM_000109.2

Note: Exon numbering might be different as compared to literature!
Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA kit P034 DMD sample pictures

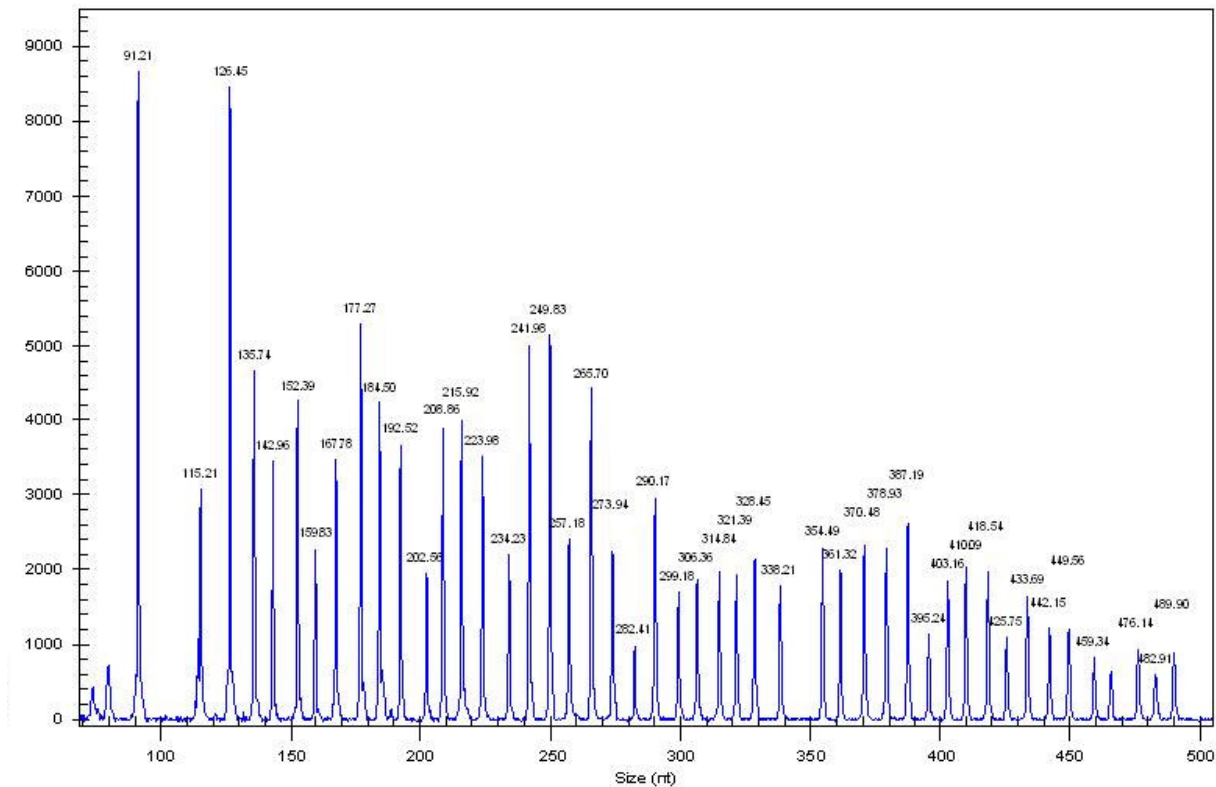


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P034 DMD (lot 0906).

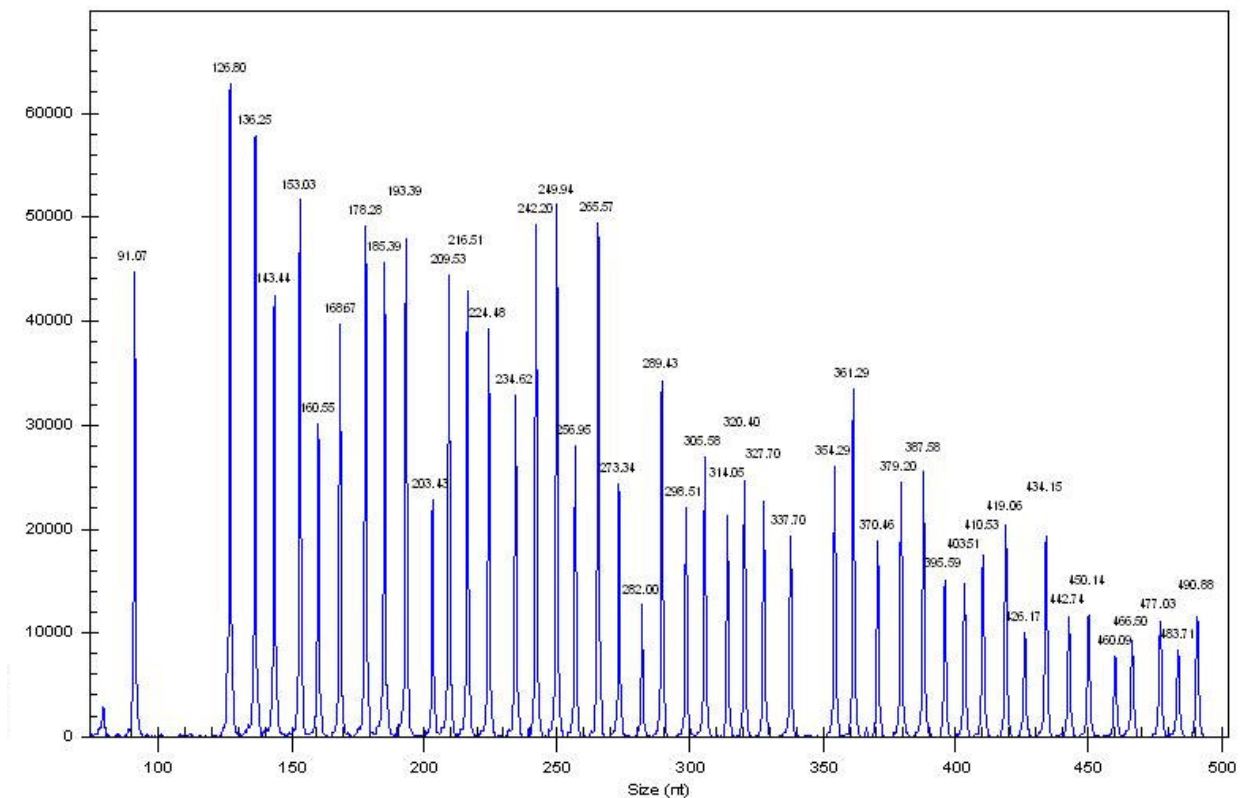


Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analyzed with SALSA MLPA kit P034 DMD (lot 0707).

SALSA MLPA kit P035 DMD sample pictures

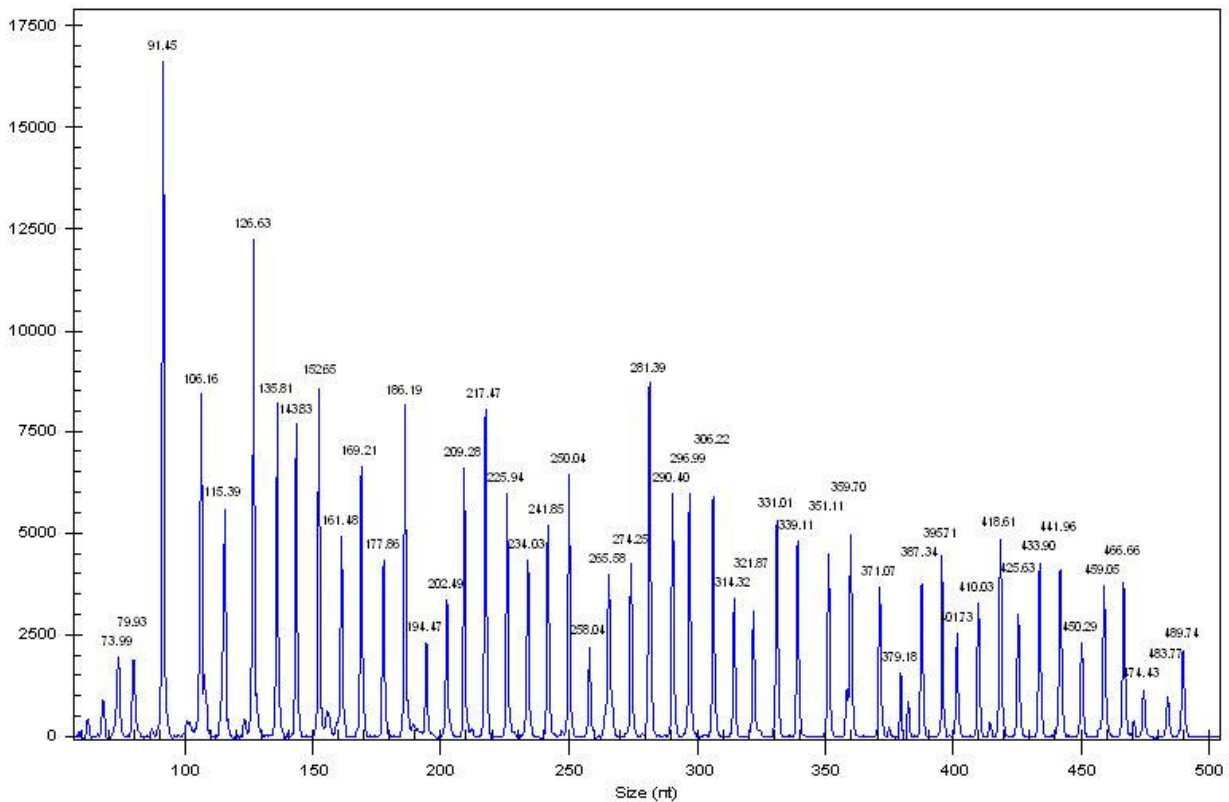


Figure 3. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P035 DMD (lot 0906).

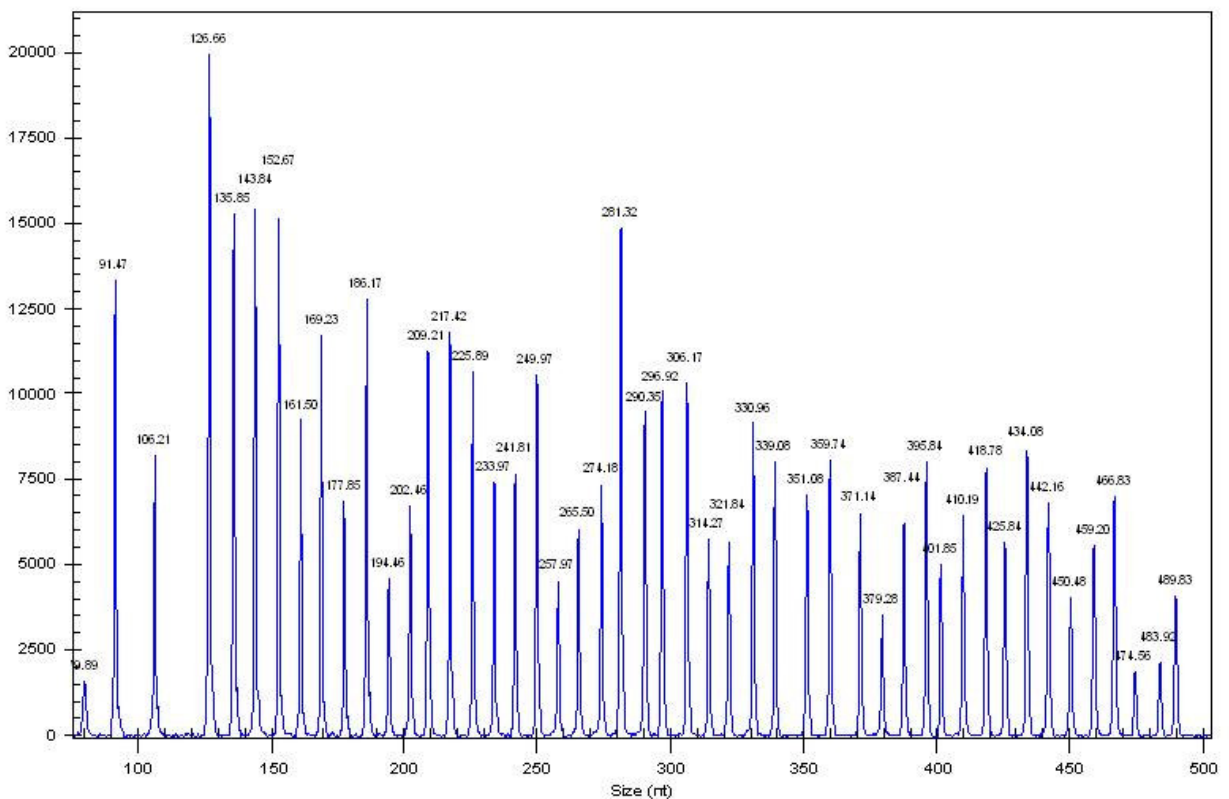


Figure 4. Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analyzed with SALSA MLPA kit P035 DMD (lot 0906).