

SALSA MLPA KIT P062-B LDLR

Lot 0307, 0606

THE LOW DENSITY LIPOPROTEIN RECEPTOR (LDLR) is a cell surface receptor that plays an important role in cholesterol homeostasis. The LDLR gene contains 18 exons and is located on chromosome 19p13.2. The binding of LDL to LDLR results in suppression of cholesterol synthesis by the repression of HMG CoA reductase. Several deletions have been described in the LDLR gene in patients with familial hypercholesterolemia. This is an autosomal dominant disorder characterized by elevation of serum cholesterol bound to low density protein (LDL).

This SALSA MLPA kit contains probes for each of the 18 LDLR exons. Two probes are present for exon 1. In addition, two probes that recognize other genes located just upstream and downstream of LDLR on chromosome 19 have been included. For reference, 16 probes for other genes located on different chromosomes are included.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the LDLR gene. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. 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. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test.

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 SALSA 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).

References of SALSA MLPA kit P062

- Tosi I. et al. (2006). Genetic defects causing familial hypercholesterolaemia: Identification of deletions and duplications in the LDL-receptor gene and summary of all mutations found in patients attending the Hammersmith Hospital Lipid Clinic. *Atherosclerosis*. 2006 Nov 7.
- Damgaard, D. (2005). Detection of large deletions in the LDL receptor gene with quantitative PCR methods. *BMC Med Genet*. 2005 Apr 20;6(1):15.
- Wang J et al. (2005). Multiplex ligation-dependent probe amplification of LDLR enhances molecular diagnosis of familial hypercholesterolemia. *J Lipid Res.*; 46(2):366-72.
- Holla OL et al. (2005). Identification of deletions and duplications in the low density lipoprotein receptor gene by MLPA. *Clin Chim Acta*. 2005 Jun;356(1-2):164-71.

More information

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Data analysis

The P062B LDLR probemix contains 37 MLPA probes with amplification products between 130 and 445 nt. In addition, it contains 5 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) and one synthetic ligation-dependent control fragment at 92 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 intra-normalized by dividing the peak area of each probe's amplification product by the total area of only the reference probes in this probemix (block normalization). Secondly, normalisation can be achieved by dividing this 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.

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 S. Guerrero Caballero & 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 first probemix designer could be made a coauthor.

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

SALSA MLPA P062-B LDLR probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		reference	LDRLR
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	Ligation dependent control fragment at 2q14		
130	Reference probe 0797-L0463	5q31	
136	Reference probe 1819-L1384	16p13	
142	Reference probe 2488-L2236	SMARCA4	
148	LDLR probe 2309-L1800		Exon 1
154	Reference probe 1008-L0577	3p21	
160	LDLR probe 2310-L1801		Exon 2
166	LDLR probe 2311-L1802		Exon 11
175	Reference probe 1114-L0383	5q12	
184	LDLR probe 2312-L1803		Exon 3
193	LDLR probe 2313-L1804		Exon 12
202	Reference probe 1116-L0620	8q21	
211	LDLR probe 2314-L1805		Exon 4
220	LDLR probe 2315-L1806		Exon 14
229	Reference probe 1603-L1185	13q12	
238	LDLR probe 2316-L1807		Exon 5
247	LDLR probe 2317-L1808		Exon 15
256	Reference probe 0518-L0098	2q14	
265	LDLR probe 2318-L1809		Exon 6
274	LDLR probe 2319-L1810		Exon 16
283	Reference probe 1326-L0873	17p13	
292	LDLR probe 2320-L1811		Exon 7
301	LDLR probe 2321-L1812		Exon 17
310	Reference probe 1333-L0876	7q11	
319	LDLR probe 2322-L1813		Exon 8
328	LDLR probe 2323-L1814		Exon 18
337	Reference probe 1919-L1463	1q21	
346	LDLR probe 2324-L1815		Exon 9
355	Reference probe 2325-L1816	KIAA1518	
364	Reference probe 1234-L0781	10p14	
373	Reference probe 1085-L0653	14q32	
382	Reference probe 1581-L1135	22q12	
391	LDLR probe 3003-L2442		Exon 10
400	LDLR probe 3004-L2443		Exon 13
409	Reference probe 0963-L0550	2p14	
418	Reference probe 3065-L2494	4p16	
436	LDLR probe 6281-L5786		Exon 1
445	Reference probe 3988-L3255	3p12	

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 probe and the complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

LDLR probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	LDLR exon	Ligation site NM_000527	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
142	2488-L2236	SMARCA4	4940-4941*	TCTTGCACTC-GGCTTTCACC	26.0 Kb
436	6281-L5786	promotor	150 nt before transcript start	GGAGTGGGAA-TCAGAGCTTC	0.2 Kb
148	2309-L1800	Exon 1	56-57	GCAGGTCGTG-ATCCGGGTCG	10.7 Kb
160	2310-L1801	Exon 2	206-207	AAGACGGGAA-ATGCATCTCC	2.4 Kb
184	2312-L1803	Exon 3	305-306	AAATCCGGGG-ACTTCAGCTG	2.6 Kb
211	2314-L1805	Exon 4	484-485	TGTGACTCAG-ACCGGACTG	1.3 Kb
238	2316-L1807	Exon 5	854-855	GCAGCCGGCA-GTGTGACCGG	0.8 Kb
265	2318-L1809	Exon 6	1003-1004	GACTGCCGGG-ACTGGTCAGA	3.1 Kb
292	2320-L1811	Exon 7	1092-1093	TGACCTTAAG-ATCGGCTACG	0.9 Kb
319	2322-L1813	Exon 8	1216-1217	GAGGGTGGCT-ACAAGTGCCA	1.8 Kb
346	2324-L1815	Exon 9	1336-1337	ATGACGCTGG-ACCGGAGCGA	0.2 Kb
391	3003-L2442	Exon 10	1635-1636	ATTCAGGGAG-AACGGCTCCA	2.6 Kb
166	2311-L1802	Exon 11	1716-1717	TCCCGCCAAG-ATCAAGAAAG	0.6 Kb
193	2313-L1804	Exon 12	1778-1779	ACCTCTCCTT-ATCCACTTGT	3.2 Kb
400	3004-L2443	Exon 13	1990-1991	AGTGCCAACC-GCCTCACAGG	0.2 Kb
220	2315-L1806	Exon 14	2128-2129	GGCTGCCAGT-ATCTGTGCCT	2.8 Kb
247	2317-L1808	Exon 15	2348-2349	CCTCCCGGCT-GCCTGGGGCC	4.7 Kb
274	2319-L1810	Exon 16	2436-2437	AGGAAATGAG-AAGAAGCCCA	1.6 Kb
301	2321-L1812	Exon 17	2563-2564	ATCAACTTTG-ACAACCCCGT	1.7 Kb
328	2323-L1814	Exon 18	2665-2666	CTGGAGGATG-ACGTGGCGTG	9.3 Kb
355	2325-L1816	KIAA1518	1288-1289 §	GTGGCCTTGG-ACGCAGGGCA	

* Genbank NM_003072

§ Genbank XM_170889

SALSA MLPA kit P062-B LDLR sample picture

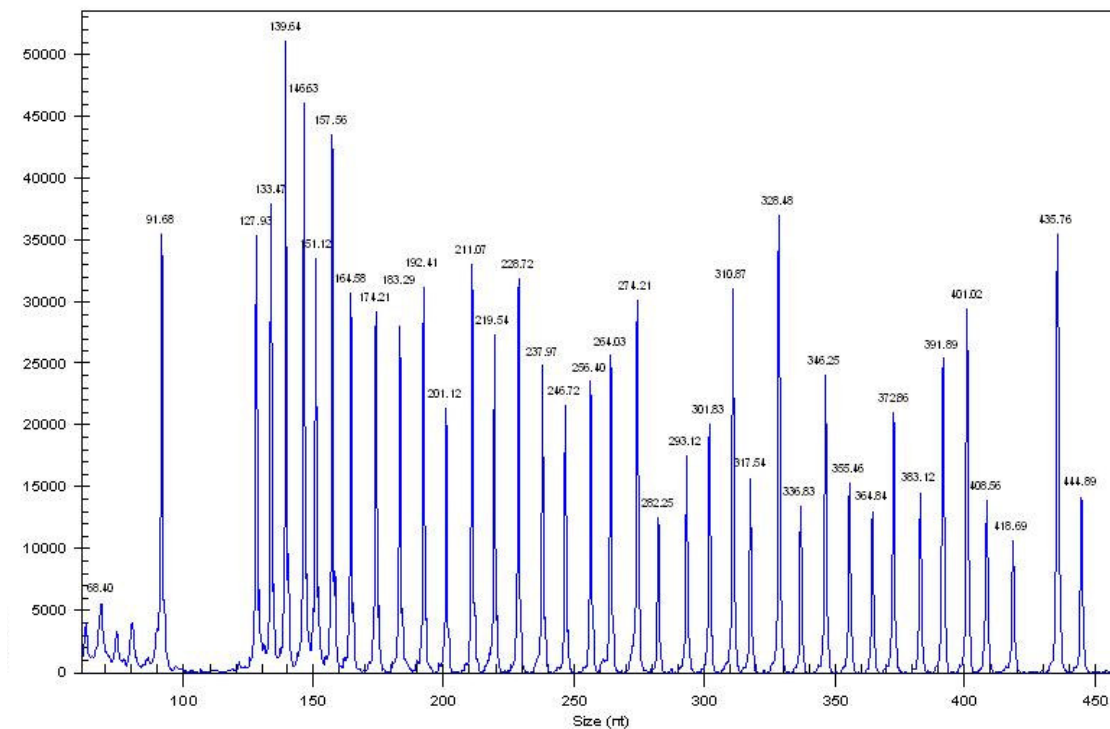


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P062-B LDLR (lot 0307).

