

SALSA MLPA KIT P091-B1 CFTR

Lot 0708: a mutation-specific probe detecting the wildtype allele of the d508 mutation has been included. In addition, extra control fragments at 88, 96, 100 and 105 nt have been added.

CYSTIC FIBROSIS and congenital bilateral aplasia of the vas deferens can be caused by mutations in the CFTR gene. Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel and controls the regulation of other transport pathways.

The CFTR gene has 24 exons. The CFTR gene spans 190 Kb of chromosomal sequence and is located on 7q31.2. Most defective CFTR genes are the result of point mutations and small deletions / insertions that can be detected by sequencing, DHPLC and other methods. Several deletions of one or more CFTR exons have been identified. Copy number changes of one or more exons will account for less than 10% of all CFTR mutations in most populations.

The database of genome variants mentions no copy number changes of this genomic region in healthy individuals (see <http://projects.tcag.ca/variation/>).

This P091-B1 CFTR probemix contains probes for each of the 24 exons and a second probe for exon 6, 14, 17 and for exon 24. In addition, the probemix P091-B1 contains a mutation specific that detects the wildtype allele of the d508 mutation.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the CFTR 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).

More information

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Data analysis

The P091-B1 P091 CFTR probemix contains 43 MLPA probes with amplification products between 130 and 472 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 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 copy number changes. 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 A.O.H. Nygren & 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.

SALSA MLPA P091-B1 CFTR probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		reference	CFTR
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 0797-L0463	5q31	
136	GASZ probe 3571-L3264	58 Kb before CFTR	
142	CFTR probe 2956-L13079		Exon 13
148	CFTR probe 3842-L3315		Exon 23
154	CFTR probe 2944-L2376		Exon 1 promoter region
160	CFTR probe 2957-L2389		Exon 14A
166	Reference probe 2881-L2348	19q12	
172	CFTR probe 3578-L2939		Exon 24
178	CFTR probe 2958-L2390		Exon 14B
184	Reference probe 2882-L2349	19q13	
190	CFTR probe 3574-L2400		Exon 24
198	CFTR probe 2946-L13077		Exon 2
204	CFTR probe 2959-L13078		Exon 15
211	Reference probe 0472-L0088	12q14	
220	CFTR probe 2947-L2379		Exon 3
229	CFTR probe 2960-L2392		Exon 16
238	CFTR probe 3839-L3312		Exon 1
247	CFTR probe 2948-L2380		Exon 4
256	CFTR probe 2961-L2393		Exon 17A
265	Reference probe 2318-L1809	19p13	
274	CFTR probe 2949-L2381		Exon 6A
283	CFTR probe 2962-L2394		Exon 17B
292	CFTR probe 3841-L3314		Exon 12
301	CFTR probe 8145-L8069		Exon 6B
310	CFTR probe 3576-L13076		Exon 18
320	Reference probe 1866-L1425	1p34	
328 § *	CFTR probe 3322-L2739 d508 WT		Exon 10
337	CFTR probe 2951-L2383		Exon 7
346	CFTR probe 3840-L3313		Exon 5
353	CFTR probe 3577-L2396		Exon 19
364	CFTR probe 2952-L2384		Exon 8
373	Reference probe 1589-L1161	13q14	
382	CFTR probe 2965-L2397		Exon 20
391	CFTR probe 2953-L2385		Exon 9
400	Reference probe 2598-L2069	5q35	
409	CFTR probe 2966-L2398		Exon 21
418	CFTR probe 2955-L2387		Exon 11
427	Reference probe 0680-L0121	7q35	
436	CFTR probe 2967-L2399		Exon 22
445	CORTBP2 probe 3572-L3267		58 Kb after CFTR
454	Reference probe 0605-L0018	15q26	
463	CFTR probe 2954-L2386		Exon 10
472	Reference probe 2757-L2206	11p13	

* New in version B1 (from lot 0708 onwards)

§ This probe detects the wildtype allele of the DF508 mutation. The presence of the DF508 deletion results in a lower signal. We have no information on the effect of polymorphisms close to the DF508 site.

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 and the complete sequences are available on request: info@mlpa.com.

7q31.2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000492.3	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	<i>133-135</i>		
136	3571-L3264	GASZ Exon 3		TGCTGCTAGT-GTTGCCAATG	58.0 Kb
154	2944-L2376	CFTR exon 1	594-595 in M55106.1	TAGAGCAAAT-TTGGGGCCGG	0.1 Kb
238	3839-L3312	CFTR exon 1	44-45	AAAAAGGGTT-GAGCGGCAGG	24.4 Kb
198	2946-L13077	CFTR exon 2	242-241 Rev.	ATTTGGTATA-TGTCTGACAA	4.7 Kb
220	2947-L2379	CFTR exon 3	325-326	GCTTCAAAGA-AAAATCCTAA	21.8 Kb
247	2948-L2380	CFTR exon 4	449-450	GAAGAATCAT-AGCTTCTAT	3.3 Kb
346	3840-L3313	CFTR exon 5	660-661	TAAAATAAGT-ATTGGACAAC	0.9 Kb
274	2949-L2381	CFTR exon 6A	788-789	GGGAGTTGTT-ACAGGCGTCT	1.2 Kb
301	8145-L8069	CFTR exon 6B	87842-87843 in AC000111.1	GATCAGTGAA-AGACTTGTGA	3.5 Kb
337	2951-L2383	CFTR exon 7	1050-1051	ATACTCAAT-AGCTCAGCCT	2.1 Kb
364	2952-L2384	CFTR exon 8	573-574 in M55113.1	ATTTTCTAGA-TTAAGAAGTA	5.9 Kb
391	2953-L2385	CFTR exon 9	87.868-87867 Rev. in AC000111.1	ACTCCATCAC-ACTGGTAGCA	11.4 Kb
463	2954-L2386	CFTR exon 10	1583-1582 Rev.	CTTCCACTGT-GCTTAATTTT	0.1 Kb
328 §	3322-L2739	CFTR exon 10 d508 WT	1653-1654	AAAATATCAT-CTTTGGTGTT	28.2 kb
418	2955-L2387	CFTR exon 11	1740-1741	TGCAGAGAAA-GACAATATAG	2.8 Kb
292	3841-L3314	CFTR exon 12	451-452	ATTGCATTTT-ACCTCTTGAG	1.9 Kb
142	2956-L13079	CFTR exon 13	2358-2359	ACCAGATTCT-GAGCAGGGAG	2.6 Kb
160	2957-L2389	CFTR exon 14A	2672-2671 Rev.	AGGTATGTGT-TCCATGTAGT	7.9 Kb
178	2958-L2390	CFTR exon 14B	2763-2764	GGCTGCTTCT-TTGGTTGTGC	0.8 Kb
204	2959-L13078	CFTR exon 15	2917-2918	TTGCTTGCTA-TGGGATTCTT	3.3 Kb
229	2960-L2392	CFTR exon 16	638-639 in M55122.1	TAGATGTAAT-AGCTGTCTAC	3.5 Kb
256	2961-L2393	CFTR exon 17A	3179-3178 Rev.	GTTGCAACAA-AGATGTAGGG	1.1 Kb
283	2962-L2394	CFTR exon 17B	3384-3383 Rev.	GGCAGTATGT-AAATTCAGAG	3.0 Kb
310	3576-L13076	CFTR exon 18	3546-3547	AGCCATGAAT-ATCATGAGTA	13.0 Kb
353	3577-L2396	CFTR exon 19	3787-3786 Rev.	CTTCTGTGTA-TTTTGCTGTG	14.9 Kb
382	2965-L2397	CFTR exon 20	3957-3958	GTCTTGGGAT-TCAATAACTT	10.3 Kb
409	2966-L2398	CFTR exon 21	4082-4081 Rev.	TCTGCAACTT-TCCATATTTT	11.8 Kb
436	2967-L2399	CFTR exon 22	4124-4125	TAGAACAGTT-TCCTGGGAAG	0.7 Kb
148	3842-L3315	CFTR exon 23	4350-4349 Rev.	TTCCAGCATT-GCTTCTATCC	1.4 Kb
190	3574-L2400	CFTR exon 24	4418-4419	CCATCCAGAA-ACTGCTGAAC	0.1 Kb
172	3578-L2939	CFTR exon 24	4535-4534 Rev.	GTCTCCTCTT-TCAGAGCAGC	58.0 Kb
		<i>stop codon</i>	<i>4573-4575</i>		
445	3572-L3267	CORTBP2 gene	NM_033427.1 4202-4203	AGAAGCAATA-TTGCAAGAG	

§ Detects the common allele of the d508 polymorphism. The presence of the rare allele will result in a lower signal.

- The **exon 1** probe (154 nt) ligation site is located 234 bp before the ATG startcodon.
- The **exon 4** probe signal (247 nt) might be influenced by the R117X mutation (probably not).
- The **exon 8** probe (364 nt) is located in intron 8 at a distance of 145 bp from the end of exon 8.
- The **exon 9** probe (391 nt) is located in intron 8 at a distance of 490 bp from the exon 9 start. A sequence with high similarity to exon 9 might be present on chromosome 20.
- The **exon 10** probe (463 nt) sequence is outside the DF508 / DF507 mutation sites.
- The **exon 10** probe (328 nt) detects the common allele of the d508 polymorphism and might be influenced by rs332.
- The **exon 11** probe signal (418 nt) is likely to be influenced by the 1717 G>A and G542X mutations.
- The **exon 14A** probe signal (160 nt) is influenced by the W846X mutation (D. Bunyan, Salisbury).
- The **exon 16** probe (229 nt) is located in intron 16 at a distance of 233 bp from the end of exon 16.

SALSA MLPA kit P091-B1 CFTR sample picture

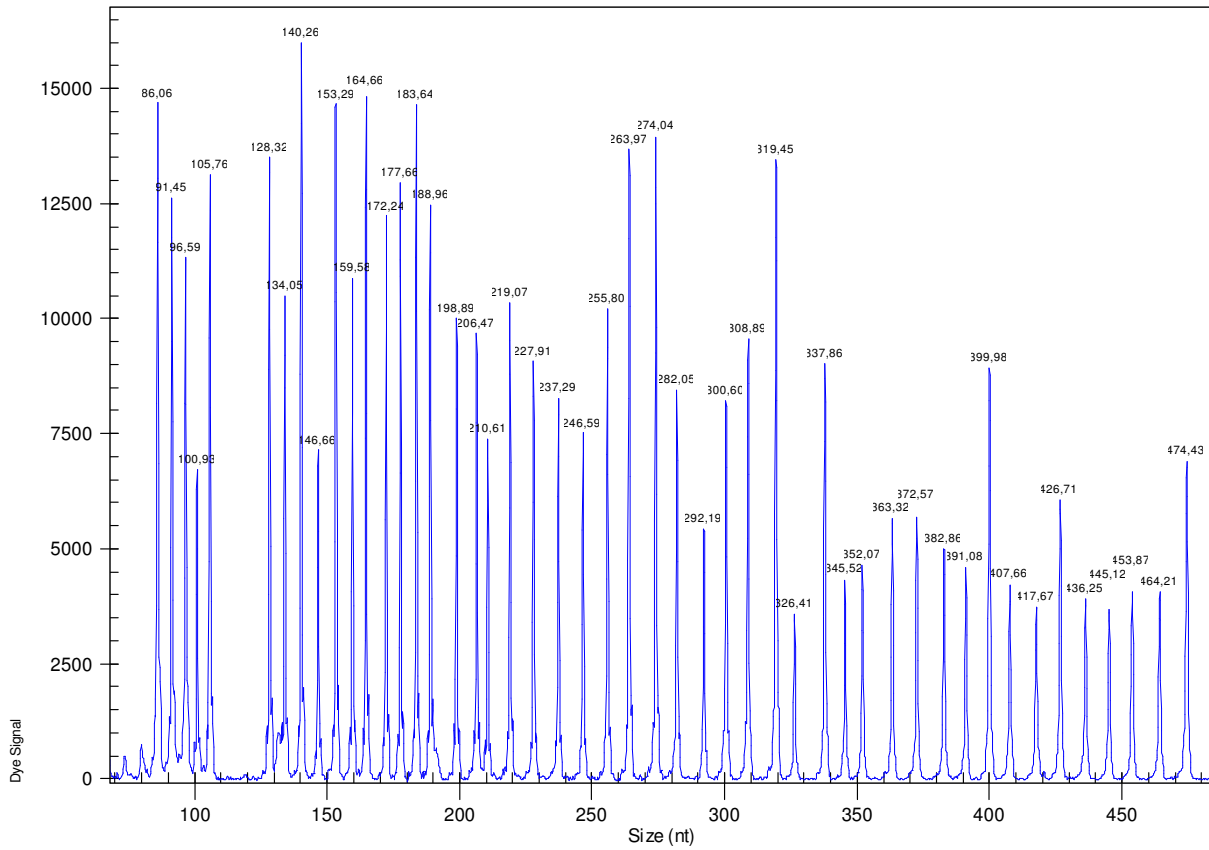


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P091-B1 CFTR (lot 0708).