

SALSA MLPA KIT P250-A1 DiGeorge

Lot 0408

This P250 DiGeorge probemix is a new, extended version of the widely used SALSA MLPA kit P023. The P023 probemix will remain available and should identify most DiGeorge patients. However, we recommend this new P250 kit as it has many more probes in the 22q11 region.

Microdeletions/duplications in the 22q11 region cause a variety of disorders, including DiGeorge syndrome (DGS; MIM 188400), Velocardiofacial syndrome (VCFS; MIM 192430) and Cat Eye syndrome (CES; MIM 115470). DGS and VCFS have a large clinical overlap and are both caused by deletions of a specific 1-3 Mb region on chromosome 22q11. The overall birth prevalence of 22q11 deletions appears to be approximately 1 in 4.000, with 75% of these patients having cardiac abnormalities.

Cat Eye syndrome (CES) has a large phenotypic variability, ranging from near normal to severe malformations. The eyes are predominately affected. CES is caused by the presence of an extra 22q11 copy, usually present as a small extra chromosome, frequently having two centromeres.

Although 90% of cases of DGS appear to be due to a 22q11 deletion, other chromosome defects with features of DiGeorge anomaly have also been described. This P250 MLPA probemix contains probes for several of these regions such as 10p14 (DGS2), 4q35 and 17p13. Please inform us when a deletion of one or more of the probes outside the 22q11 region is detected in a patient with a DiGeorge phenotype.

The high frequency of 22q11 copy number changes is due to the presence of several copies of a repeat sequence (LCR22). Extend of the 22q11 deletions is variable, although 87% extend from the first (LCR22-A) till the fourth (LCR22-D) repeat. This P250 probemix contains 30 different probes in the 22q11 region and can be used to distinguish the most common types of deletion. Five of these 30 probes are in the Cat Eye Syndrome region and 14 are in the most common DiGeorge deletion.

WARNING: The extra chromosome present in Cat Eye Syndrome patients is easily lost during postzygotic divisions, resulting in mosaicism. Detection of CES in mosaic samples might be better by standard karyotype analysis or by FISH. MLPA measures the average copy number of the CES region and may not be able to detect CES in mosaic samples that contain predominantly normal cells.

This SALSA MLPA kit is designed to detect deletions/duplications of the 22q11 region. Deletions of probe recognition sequences will be apparent by a 35-50% reduced relative peak area of the amplification product of those probes. However, mutations/polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Apparent deletions detected by a single probe therefore 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 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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References of SALSA MLPA kit P023 DiGeorge

- Alberti A. et al. (2007). 1.5 Mb de novo 22q11.21 microduplication in a patient with cognitive deficits and dysmorphic facial features. *Clin Genet.* 2007 Feb;71(2):177-82.
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Data analysis

The P250-A1 DiGeorge probemix contains 48 MLPA probes with amplification products between 130 and 481 nt. In addition, it contains 7 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 should be normalized with a more robust method, as changes in the regions targeted by the reference probes more occur. The signals of target-specific probes should be intra-normalized against every single reference probe separately, thereby creating as many ratios as there are reference probes. The median of all produced ratios gives an estimate of the final probe ratio, or ploidy status, of the sample's target-specific probes sequences in an MLPA mix. This way, the signal of each selected reference probe will be used as normalization constant. With the normalization constant, the ratio between reference and patient sample is determined for each target-specific probe.

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 deletions can be done by FISH.

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. Errami, C. McElgunn and 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 P250-A1 DiGeorge probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		Other probes	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		
130	PPIL2 probe 7529-L04870		22q11 D-E
136	Reference (EHMT1) probe 5059-L07380	9q34.3	
142	SLC25A18 probe 5457-L07613		22q11 CES
148*	DGCR8 probe 8475-L08486		22q11 A-B
154	Reference (EHMT1) probe 5058-L07382	9q34.3	
160*‡	HIRA probe 1214-L02328		22q11 A-B
166	SNRPD3 probe 8481-L08492		22q11 G-H
172*	TBX1 probe 5408-L07614		22q11 A-B
178	MICAL3 probe 5458-L04861		22q11 CES
184	Reference (KLKB1) probe 1217-L00694	4q35	
190*	CLTCL1 probe 5462-L05809		22q11 A-B
196*	CLDN5 probe 1218-L06270		22q11 A-B
202*	ZNF74 probe 5927-L07395		22q11 B-C
208*	GP1BB probe 5464-L10114		22q11 A-B
214	GNAZ probe 8478-L08489		22q11 E-F
220	SMARCB1 probe 5928-L07969		22q11 F-G
226	USP18 probe 7528-L04863		22q11 CES
232	Reference (SHANK3) probe 6787-L07383	22q13	
238*	TXNRD2 probe 1223-L05814		22q11 A-B
247	RAB36 probe 8486-L09342		22q11 E-F
254	Reference (RPH3AL) probe 1735-L07385	17p13.3	
259	RTDR1 probe 8484-L09139		22q11 E-F
268	Reference (GATA3) probe 1225-L09140	10p15 DGR2	
274	Reference (GATA4) probe 1226-L03844	8p23.1	
283*	KLHL22 probe 1227-L05815		22q11 B-C
292	TOP3B probe 5930-L04871		22q11 D-E
301*	TBX1 probe 7054-L06663		22q11 A-B
310	HIC2 probe 5931-L04869		22q11 D-E
319*	PCQAP probe 1231-L05816		22q11 B-C
330 †	Reference (FRG1) probe 6766-L08754	4q35	
337	IL17RA probe 1082-L00660		22q11 CES
343	RAB36 probe 5932-L04872		22q11 E-F
349	Reference (DKFZp566) probe 1232-L07388	10p15 DGR2	
355	BID probe 1767-L07389		22q11 CES
364	Reference (AI651963) probe 1234-L00781	10p15 DGR2	
373*	SNAP29 probe 1235-L00773		22q11 C-D
382	Reference (CUGBP2) probe 1522-L00952	10p15 DGR2	
391 †	Reference (CRK) probe 0444-L00007	17p13.3	
400	SMARCB1 probe 5933-L05812		22q11 F-G
409	Reference (GEMIN4) probe 1238-L07390	17p13.3	
418*	LZTR1 probe 1521-L00951		22q11 C-D
427	Reference (MSRA) probe 1240-L00787	8p23.1	
434	Reference (RPH3AL) probe 4081-L03465	17p13.3	
445	Reference (ARSA) probe 1093-L00661	22q13	
454	RTDR1 probe 8479-L08490		22q11 E-F
463*	CDC45L probe 5463-L05808		22q11 A-B
472	Reference (PPP1R3B) probe 1243-L07392	8p23.1	
481	Reference (NEBL) probe 8480-L08491	10p12 DGR2	

* Probes that are expected to have a 35-50% reduced peak height/area in most DiGeorge patients.

*‡ The 160 nt HIRA probe has been reported to be more variable than other 22q probes.

† This probe sequence has been found to contain a polymorphism in some populations.

22q11 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
Cat Eye syndrome region					
337	1082-L00660	IL17RA	GCAGAGTTATCT-GTCCTGCAGCTG	15.959.700	464 Kb
142	5457-L07613	SLC25A18	GCAGTGAGAAGA-GTCGAGTGAAGC	16.423.340	183 Kb
355	1767-L07389	BID	CTACTGGTGTTT-GGCTTCTCCAA	16.606.720	98 Kb
178	5458-L04861	MICAL3	GAAGTACCGCT-GTCCCTGAGGCA	16.704.680	308 Kb
226	7528-L04863	USP18	CTCAGTCCCAC-GTGGAACTCAGC	17.012.950	609 Kb
End of CES region; Start DiGeorge region; LCR22-A					
190	5462-L05809	CLTCL1	TGTTGCCTTGGT-GACCGAGACCGC	17.621.630	77 Kb
160	1214-L02328	HIRA	GGAGCTGCTGAA-GGAGCTGCTACC	17.699.040	148 Kb
463	5463-L05808	CDC45L	ATGTTCTGTGCC-GATTTCCGCAAA	17.847.500	44 Kb
196	1218-L06270	CLDN5	TTCGCCAACATT-GTCGTCCGCGAG	17.891.350	200 Kb
208 †	5464-L10114	GP1BB	CACAACCGAGCT-GGTGCTGACCGG	18.091.550	36 Kb
172 ‡	5408-L07614	TBX1	CCGGGTGAAGCT-TCGCTGGCTGCC	18.127.140	6 Kb
301 ‡	7054-L06663	TBX1	TCATGAGCGCCT-TCGCGCGCTCGC	18.133.450	133 Kb
238	1223-L05814	TXNRD2	GGAGGGTCAGGA-GAGGAGCTGCAG	18.266.250	187 Kb
148	8475-L08486	DGCR8	GGTAATGGACGT-TGGCTCTGGTGG	18.453.640	626 Kb
LCR22-B					
202	5927-L07395	ZNF74	CAGGCAGATTAT-TCCTCGATGCTG	19.079.460	94 Kb
283	1227-L05815	KLHL22	TCTTCGATGTTG-TGCTGGTGGTGG	19.173.330	93 Kb
319	1231-L05816	PCQAP	TGGCATTGGAT-GAAGACACAGGT	19.266.770	305 Kb
LCR22-C					
373	1235-L00773	SNAP29	AGGAGCAAGATG-ACATTCTTGACC	19.572.050	107 Kb
418	1521-L00951	LZTR1	ATGATGAAGGAG-TTCGAGCGCCTC	19.679.220	451 Kb
LCR22-D; End of the commonly-deleted DiGeorge region					
310 ¥	5931-L04869	HIC2	AGGAGGCCGAGG-ACCTGTACAGCAC	20.130.650	249 Kb
130	7529-L04870	PPIL2	GAAGAGCCCTCA-ACCAGTGCCACT	20.379.710	280 Kb
292	5930-L04871	TOP3B	GGTTGCTGAAAA-GCCGTCTTGGC	20.660.040	1074 Kb
LCR22-E					
259	8484-L09139	RTDR1	GGTGTGCATTT-TGACGTCATCCC	21.734.080	61 Kb
214	8478-L08489	GNAZ	TCACCATCTGCT-TTCCCAGTACA	21.795.420	17 Kb
454	8479-L08490	RTDR1	CTCCTTGGAGCT-TCCATTAACAT	21.812.570	5 Kb
343	5932-L04872	RAB36	AGCTGGATGCTT-GGACGCGCCGCT	21.817.590	16 Kb
247	8486-L09342	RAB36	GCAGTCGGTGCT-GCAGGACCTGGA	21.833.130	626 Kb
LCR22-F					
220	5928-L07969	SMARCB1	CTTCGGGCAGAA-GCCCGTGAAGTT	22.459.400	47 Kb
400	5933-L05812	SMARCB1	CATCAGCACACG-GCTCCCACGGAG	22.506.390	777 Kb
LCR22-G					
166	8481-L08492	SNRPD3	CCGGTGAGGTAT-ATCGGGGGAAGC	23.283.730	

† Small change in length from lot 0907 onwards. The detected sequence has not been changed.

‡ The two TBX1 probes are less reliable due to SNPs (172 nt probe) or slow hybridization (301 nt probe). Please disregard apparent deletions of only a single TBX1 probe. We are working on new TBX1 probes.

¥ The 310 nt HIC2 probe is in a region that often shows copy number changes in healthy persons. We recommend disregarding changes in peak height of only this probe.

Ligation site HG18: Distance (nt) to the P-telomere: USHG browser <http://genome.ucsc.edu/cgi-bin/hgBlat>

- FISH probe D22S553 is located around the CLTCL1 gene.
- FISH probe D22S609 is located between CLTCL1 and HIRA.
- FISH probe D22S942 is located between HIRA and CDC45L.
- The TXNRD2 and KLHL22 probes are identical to the KIAA1652 and FLJ14360 probes in P023B.

Most 22q11 DiGeorge deletions (87% according to Shaik, T.H. et al (2000) Human Molecular Genetics 9, 489-501) extend from LCR22-A and LCR22-D. In this P250 DiGeorge probemix, 14 probes are located in this

region. Another 8% extend only from LCR22-A to LCR22-B, while 2% start at LCR22-A and end at LCR22-C. Finally 2% of the cases extended from a site between LCR22-A and LCR22-B and end in LCR22-D. Cat Eye syndrome is usually the result of a DUPLICATION of the 22q11 CES region (small supernumery chromosome that is present in two copies/cell). The SMARCB1 gene is frequently homozygous deleted in rhabdoid tumors. A special P258 SMARCB1 probemix is now available from MRC-Holland.

22q13 probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
445	1093-L00661	ARSA	GGAGGATCAGAT-CTCCGCTCGAGA	49.413.300	94 Kb
232	6787-L07383	SHANK3	ACCAACTGTGAT-CAGTGAGCTCAG	49.507.610	~ 190 Kb to the q-telomere
22q telomere					

Physicians do not always clearly specify whether to investigate 22q11 or 22q13 abnormalities. We have therefore been asked by one DNA diagnostic lab to include these 22q13 telomeric probes.

Deletion / duplication of the 22q13 telomeric region does NOT result in a DiGeorge like phenotype. More probes for 22q13 are present in the P188 22q13 probemix. Distance between these 22q13 probes and the DiGeorge region is more than 25.000 Kb.

10p15 DGR2 probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
268	1225-L09140	GATA3	GAGTGCCTCAAG-TACCAGGTGCC	8.140.590	2447 Kb
349	1232-L07388	DKFZp566	TGTAGACCACAT-GATGGAGATTTG	10.589.000	428 Kb
364	1234-L00781	AI651963	GACATTCCTGT-GGAAATTTGGTG	11.017.050	230 Kb
382	1522-L00952	CUGBP2	TCCCCGGTCAT-GGTCGGAAAAGG	11.247.550	9979 Kb
481	8480-L08491	NEBL	CTGGGATCCTTT-TCTGTTCACTCA	21.226.300	

The GATA3 gene is the center of the HDR syndrome region and CUGBP2 should be in the center of the DGS2 region according to the report of Lichtner et al (2000) J.Med.Genet. 37, 33-37. More GATA3 probes are present in the P234 probemix. We have received one report of a DiGeorge patient with a 10p deletion that was detected with the GATA3 and DKFZp566 probes of the P023 DiGeorge probemix.

The NEBL gene is a long gene (500 Kb) located at almost 10 Mb centromeric of the CUGBP2 gene. The NEBL gene was noticed to be deleted in two patients with a DGS2 deletion by Villanueva et al (2002) Genomics 80:593-600. A patient with a 5.5 Mb deletion on 10p was also found to have a deleted NEBL gene by Yatsenko et al (2004) Clin. Genet. 66:128-136. Please note that the distance between the DGS2 region and NEBL is much more than 5.5 Mb. The significance of NEBL for DiGeorge syndrome is not clear.

8p23 probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
472	1243-L07392	PPP1R3B	ACCGAGCTCCTA- GACAACATTGTG	9.036.270	1067 Kb
427	1240-L00787	MSRA	GCAACAGAACAG- TCGAACCTTTCC	10.102.780	1551 Kb
274	1226-L03844	GATA4	TGGATTTTCTCA- GATGCCTTTACA	11.653.570	

Deletions in the 8p region are associated with congenital heart malformations according to Devriendt et al, (1999) Am. J. Hum. Genet. 64, 1119-1126. More GATA4 probes are present in the P234 probemix.

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.

4q34-qter probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
184	1217-L00694	KLKB1	ATGCCCAATACT- GCCAGATGAGGT	187.390.350	3709 Kb
330	6766-L08754	FRG1	AGGCGGGTTCTA- CAGAGACGTAGG	191.099.040	~ 170 Kb to 4q telomer
4q telomere					

These 4q34.2 probes were included based on the article of Tsai et al, (1999) Am.J.Med.Genet. 82, 336-339, describing a child with 4q34.2 to 4qter deletion and symptoms consistent with VCFS. The FRG1 gene region is complicated and most of our FRG1 probes are sensitive to SNPs. **We strongly recommend disregarding apparent deletions of only the FRG1 probe.**

9q34.3 probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
136	5059-L07380	EHMT1	AAATGCTGCAAA- GCACACTCAGGA	139.731.030	74 Kb
154	5058-L07382	EHMT1	GGACCCCGTTGA- TGAAGCAGCCG	139.805.170	~ 465 Kb to 9q telomer
9q telomere					

Deletions of the 9q telomeric gene EHMT1 are associated with cardiac anomalies, epileptic seizures and mental retardation (OMIM 610253). One customer suggested an overlap of phenotype of 9q telomeric deletions with DiGeorge syndrome and suggested to include EHMT1 probes in this probemix. These EHMT1 probes are different from the EHMT1 probe in the P036 probemix.

17p13.3 probes

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Ligation site HG18	Distance to next probe
Distance from 17p telomere to the first RPH3AL probe is approximately 169 Kb.					
254	1735-L07385	RPH3AL	AGGCGGAATGTG- ATGGGGAACGGC	169.290	14 Kb
434	4081-L03465	RPH3AL	GTAGTGGACACT- TGTACGTGCACT	183.620	413 Kb
409	1238-L07390	GEMIN4	AAACAGTGATAG- ACGTCAGCACAG	596.630	691 Kb
391	0444-L00007	CRK	GACTCCGAATAG- GAGATCAAGAGT	1.287.160	

Deletions of the 17p telomeric region have been suggested to result in a DiGeorge-like phenotype (17p13: Greenberg et al (1988) Am. J. Med. Genet. 31, 1-4). We have indeed received reports of 17p telomeric deletions that were detected while screening patients with suspected DiGeorge syndrome by our P023 DiGeorge probemix.

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.

Suggestions for improvement of this probemix are highly appreciated. The older P023 probemix will remain available.

SALSA MLPA kit P250 DiGeorge sample picture

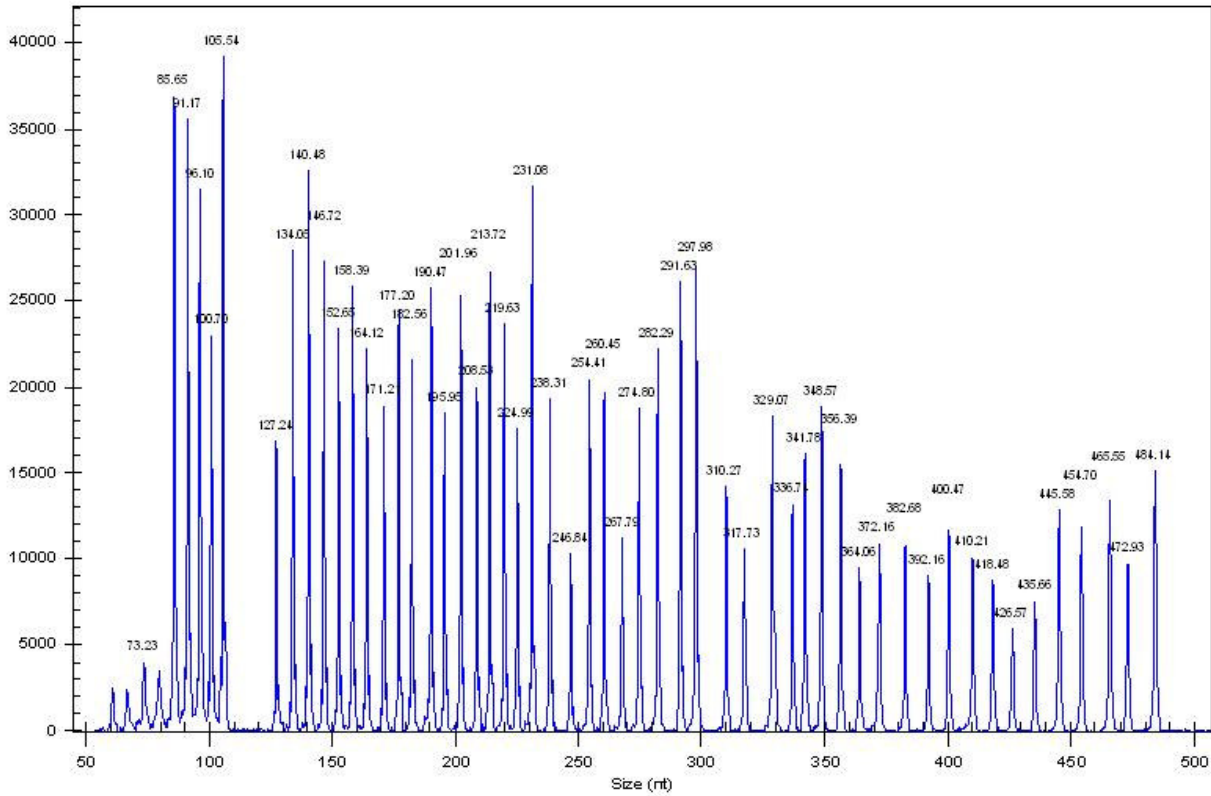


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P250 DiGeorge (lot 0408).