

SALSA MLPA KIT P070 HUMAN TELOMERE-5

Lot 0607, 0407, 1006, 0906, 0106, 0805

MENTAL RETARDATION is caused by aberrant copy numbers of subtelomeric regions in up to 8 % of all cases. The SALSA MLPA kit P070 contains one probe for each subtelomeric region from chromosome 1-22 + the two X/Y pseudoautosomal regions. The P069 probemix does not contain probes for the acrocentric chromosome arms 13p, 14p, 15p, 21p and 22p. The 1q, 17p, 20p and Xp probes in P070 detect a different sequence than the corresponding probes in P069. All other P070 probes recognize the same sequence as the corresponding probes in P069.

Most probes are designed for a well-characterised gene near the telomere. Chromosomes 13, 14, 15, 21 and 22 have each more than 10 Mb of repeat sequences at one end, covering most or all of the p arms. For these chromosomes we have used a probe recognition sequence in one of the first genes following this region. The "p" probes for the acrocentric chromosomes are therefore actually located in the q-arm, close to the centromere. Approximately 2500 Kb of DNA sequence at the p telomeric ends of the X and Y chromosomes are identical. This sequence is called the pseudoautosomal region 1, or PAR1. Similarly, a region of approximately 800 Kb DNA at the q telomeric ends of these chromosomes is identical, and is called PAR2. Although encoded by the sex chromosomes X and Y, the genes in the PAR regions have identical copy numbers in most males and females, and behave like autosomal inherited genes.

For independent confirmation of results, the P036C probemix can be used. Except for the X/Yq probe SYBL, all sequences detected by probes in the P069/P070 probemixes are different from the probes in the P036C mix. The P036C MLPA kit is similar to this P070 kit in having one probe for each subtelomeric region in a single probemix.

This MLPA kit is designed to detect deletions/duplications of each subtelomeric region. Deletions of probe recognition sequences will be apparent by a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations/polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. We recommend to test samples with both P036C and P070 (or P069).

On page 3, you can find a complete overview of probemixes which can offer a more thorough analysis of specific subtelomeric locations.

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 P070

- Martinez-Glez V. (2006). Clinical presentation of a variant of Axenfeld-Rieger syndrome associated with subtelomeric 6p deletion. *Eur J Med Genet.* 2006 Oct 28.
- Bruno D.L. (2006). High-throughput analysis of chromosome abnormality in spontaneous miscarriage using an MLPA subtelomere assay with an ancillary FISH test for polyploidy. *Am J Med Genet A.* 2006 Dec 15;140(24):2786-93.
- Northrop, EL et al (2005). Detection of cryptic subtelomeric chromosome abnormalities and identification of anonymous chromatin using a quantitative multiplex ligation-dependent probe amplification (MLPA) assay. *Hum. Mutat.* 26 (5): 477-86.

– Rooms, L. et al. (2006). Multiplex ligation-dependent probe amplification to detect subtelomeric rearrangements in routine diagnostics. *Clin Genet.* 2006 Jan;69(1):58-64.

Please note

On the basis of comments from MLPA users, the content of these probemixes is likely to be adjusted in the future. The development of the perfect telomere MLPA assay is complicated, however. Firstly, genes located very close to the telomere are sometimes polymorphic in copy number, leading to the finding of (harmless) duplications or deletions in healthy persons. Secondly, probes for genes at a larger distance from the telomere might result in false negatives. We are very grateful for user's feedback, so we can continuously strive to improve our probemixes.

Please note: No method will be capable to detect all chromosomal aberrations. The P036C and P069/P070 probemixes are unable to detect telomeric deletions/duplications whose breakpoint is located closer to the telomeres than the MLPA probes in these probemixes, nor will many interstitial deletions / duplications be detected. A website with very useful information on MLPA and FISH probe locations is <http://mlpa.omnilounge.co.uk/>.

The exact chromosomal location of some probes, in particular the distance towards the telomere, might be incorrect. The location of the probes in this mix description is based on the NCBI Mapview build 35 database. Please inform us on results obtained with cytogenetically well characterised DNA samples. If needed, some probes will be replaced in the future. NCBI mapview database: http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search?chr=hum_chr.inf&query. We use this NCBI mapview database and the NCBI blast services to localise each probe recognition sequence on the chromosome maps. MapView build 35 position 03-094.9 means that the recognition site of this probe on chromosome 3 has a distance of 94.9 Mb towards the p-telomere according to the NCBI Mapview build 35 database.

The **P036C and P069 and P070 probes for 1p, 3p, 11p, 20p and 5q** detect sequences that have been found to be duplicated in a normal individual (**1p**: Kathleen Claes, Ghent; **11p**: M. Palomares Bralo, Madrid; **20p**: Valerie Biancalana; Strasbourg ; **5q**: Kathy Mann, London + Liesbeth Rooms & Sara Seneca, Antwerpen ; All personal communications). As deletions of these regions might well have consequences we will not replace these probes. In one family, a duplication of the P070 NDN probe for "**15p**" has been detected in a healthy mother (M. Raynaud, Tours). In this family, the P036C probe for "15p" was normal.

The P036C and P069/P070 probes for 3p (CHL1 gene) were found to be duplicated in healthy parents by Joo Wook Ahn (Guy's London) and Eric Sistermans, Nijmegen. In another family, a deletion of the P036C and P069-P070 probes for 3p was detected in a healthy parent (Joo Wook Ahn, London). We recommend to use the P208 probemix for further analysis of 3p deletions / duplications. According to Dijkhuizen et al (2006; *Am.J.Hum.Genet.* 140A, 2482-87), defects in the more centromeric CNTN4 and CRBN genes might be more important for the 3p syndrome than CHL1 loss.

A **3q29** microdeletion syndrome has been described recently: Willatt, L. et al *A. J. Hum. Genet.* 77: 154-160, 2005. Although this syndrome is due to an interstitial deletion, the commonly deleted region is located very close to the 3q telomere. The sequence detected by the P070 3q probe is located between this interstitial deletion region and the telomere, and will therefore not detect this microdeletion. The probe in P036C however detects a sequence which is within the commonly deleted region.

The P069/P070 probes for 9p (FLJ00026 = DOCK8 gene) were found to be duplicated in a healthy parent in four different families by Joo Wook Ahn (Guy's London). In one other family, a deletion of the this probe was detected in a healthy parent (Joo Wook Ahn, London). We recommend to use the P208 probemix for further analysis of 3p deletions / duplications.

Data analysis

This P070 probemix contains 46 probes with amplification products between 130 and 442 nt, as well as 7 control fragments generating amplification products smaller than 130 nt. Two control fragments recognize the Y chromosome. Length difference between consecutive amplification products is 6 or 8 nt. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blots or long range PCR. The

length of the amplification products does not include the 3' Adenine, which is usually added by a non-proofreading polymerase such as the SALSA polymerase.

MRC-Holland usually defines relative probe signals by dividing each measured peak area by the sum of all peak areas of that sample. The ratio of each peak's relative probe area is then compared to that obtained on a DNA control sample. Note that in case of large deletions, this method of analysis will result in a less than 50% reduction of relative probe signal. An alternative method is to define relative probe signals of probes by comparing them to the combined signals of the control probes. However, for the P036C, P069 and P070 probemixes, it is highly unlikely that more than two probes show a copy number change. We therefore recommend using all probes as control 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, since intra-assay variation is larger. Only samples purified by the same method should be compared.

The 106 and 118 nt chromosome Y specific probes are more variable than the longer probes. Aberrant chromosome Y copy numbers should be treated with caution. These Y probes do not detect subtelomeric sequences. Note that the Coffalyser MLPA-DAT, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Control fragments

Each SALSA MLPA probemix includes four DQ (DNA Quantity) control fragments. The amplification products generated by these DQ fragments are 64, 70, 76 and 82 nt long and thus much shorter than the amplification products generated by the probes. The purpose of these short DQ fragments is to give a warning signal when the amount of sample DNA used was lower than the 20 ng human DNA required for reliable MLPA results. Note that the amplification products of the DQ fragments are only visible when little or no DNA is present, and even when ligation did not take place. Their peak size is inversely correlated with the amount of DNA present in the sample: when more than 100 ng of sample DNA is used, the four 64-82 nt DQ amplification products will be hardly visible.

The SALSA MLPA probemix also contains a 92 nt amplification product that is generated by an MLPA probe consisting of two synthetic probe oligonucleotides detecting a 2q14 DNA sequence. The peak area of this 92 nt fragment should be of similar size as most of the other MLPA amplification products. In contrast to the DQ control fragments, the 92 nt amplification product is sample DNA- and ligation-dependent and is thus only present when sufficient sample has been added and ligation occurred. The ratio between this 92 nt amplification product and the four DQ fragments can provide information over possible errors in the MLPA reaction.

When 50 ng human DNA is used in the MLPA reaction, the DQ fragments will be visible, but with a much smaller peak size than the 92 nt amplification product. However, in case the 64-70-76-82 nt DQ amplification products have peak sizes which are larger than or comparable to those of the 92 nt fragment and the 130-472 nt MLPA probe amplification products, either the ligation reaction has failed or the amount of sample DNA was less than 20 ng. In either case, the results obtained may not be reliable.

This probemix was developed by J. Coffa, C.J. 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 probemix designer could be made a coauthor.

More specific subtelomeric analysis

For more specific subtelomeric analysis, the following MLPA kits are available:

Subtelomeric region	SALSA MLPA kit	Subtelomeric region	SALSA MLPA kit
1p	P147 1p36	9p	P230 telo-7
2p	P208 telo-6	10p	P230 telo-7
3p	P208 telo-6	11p	P230 telo-7
4p	P096 MR2	12p	P230 telo-7
5p	P096 MR2	13p	P163 GJB
6p	P208 telo-6	15p	ME028 PWS/AS
8p	P208 telo-6	22q	P188 22q13

More SALSA MLPA kit for subtelomeric specific analysis are in development.

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

SALSA MLPA P070 Human Telomere-5 probemix

Length (nt)	Chr. position	Gene detected	SALSA MLPA probe	MapView build 35 position
64-70-76-82*		DQ-Control fragments		
92	2q14	Synthetic Control probe		
106	Yq11	UTY	NPK001-L0464 new	Y-014.5
118	Yq11	DBY (DDX3Y)	NPK003-L0313 new	Y-014.0
130	1q	KIAA1720	4084-L3605	01-245.3
137	2q	APG4B	2781-L3168	02-242.9
144	3q	KIAA0226	2690-L2842	03-198.8
151	4q	FRG1	2691-L2843	04-191.6
158	5q	GNB2L1	2790-L2232	05-180.8
165	6q	TBP	2694-L2844	06-170.7
172	7q	VIPR2	2793-L3167	07-158.1
179	8q	RECQL4	2695-L0610	08-145.7
186	9q	EU-HMTase1	2792-L2846	09-135.9
194	10q	ECHS1	2696-L2847	10-134.7
202	11q	KIAA1030	2697-L2848	11-133.3
210	12q	ZNF10	2686-L2849	12-132.0
218	13q	CDC16	2698-L0753	13-112.9
226	14q	MTA1	2699-L2850	14-103.9
234	15q	FLJ22604	2701-L2851	15-099.9
242	16q	GAS11	2702-L0734	16-089.8
250	17q	SECTM1	2703-L3169	17-081.0
258**	18q	CTDP1	2704-L3607	18-075.6
266	19q	BC-2	2705-L2853	19-063.7
274	20q	FLJ20517	2706-L0642	20-063.3
282	21q	S100B	2587-L2854	21-046.9
290	22q	ARSA	2707-L0661	22-049.2
298	Xq (PAR2)	SYBL1	2708-L2855	X-153.6
306	1p	TNFRSF18	2270-L1762	01-001.0
314	2p	ACP1	2709-L2856	02-000.3
322	3p	CHL1	2896-L2363	03-000.3
330	4p	ZNF141	2779-L2221	04-000.3
338	5p	LOC133957	2791-L2233	05-000.3
346	6p	IRF4	4077-L3462	06-000.3
354	7p	UNC84A	2780-L2857	07-000.6
362	8p	FBXO25	2715-L0973	08-000.4
370	9p	FLJ00026	2716-L0688	09-000.4
378***	10p	BS69	5180-L2227	10-000.2
386****	11p	BET1L	2784-L2226	11-000.2
394	12p	RBBP2	2787-L2229	12-000.3
402	"13p"	PSPC1	2717-L3608	13-018.0
410	"14p"	ADPRTL2	2718-L0732	14-018.8
418	"15p"	NDN	4026-L1542	15-021.5
426	16p	DECR2	2720-L0648	16-000.4
434	17p	RPH3AL	4081-L3465	17-000.2
442	18p	THOC1	2789-L2231	18-000.2
450	19p	PPAP2C	3501-L2880	19-000.2
458	20p	FLJ22115	2723-L0641	20-000.3
466	"21p"	STCH	2724-L0334	21-014.7
474	"22p"	IL17R	2725-L0660	22-016.0
482	Xp (PAR1)	SHOX	3714-L0910 new	X-000.5

* Not ligation-dependent; these fragments indicate the amount of DNA used;

** Higher signal than similar probe in previous lots. No change in sequence detected;

*** In previous lots of this P070 probemix, the 378 nt 10p probe sometimes resulted in a "double peak": two peaks differing in length by 1 nucleotide. From lot 0805 onwards, this probe has been replaced. The new probe 5180-L2227 detects exactly the same sequence as the old probe 2785-L2227, but performs better.

SALSA MLPA kit P070 Human Telomere-5 sample picture

Lot 0106

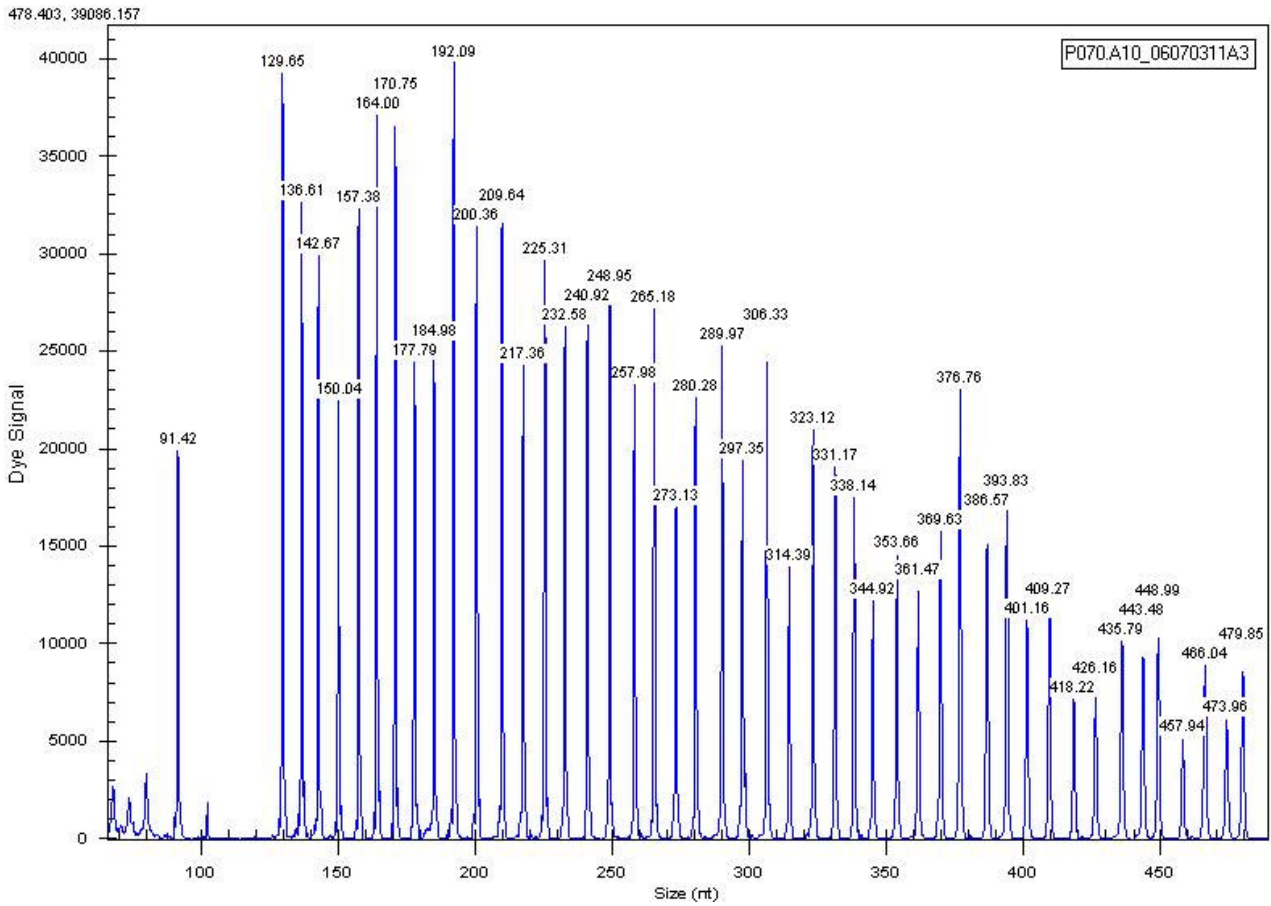


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male DNA analyzed with SALSA MLPA kit P070 Human Telomere-5 (lot 0106).