Product Description SALSA® MLPA® Probemix P141-A4 NIPBL-1 & P142-A4 NIPBL-2

To be used with the MLPA General Protocol.

P141 Version A4. As compared to version A3, one reference probe has been replaced and one probe has been adjusted in length. For complete product history see page 8.
P142 Version A4. As compared to version A3, one reference probe has been replaced, one reference probe removed, and one probe length has been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P141-025R:** SALSA MLPA Probemix P141 NIPBL-1, 25 reactions.
- **P141-050R:** SALSA MLPA Probemix P141 NIPBL-1, 50 reactions.
- **P141-100R:** SALSA MLPA Probemix P141 NIPBL-1, 100 reactions.

- **P142-025R:** SALSA MLPA Probemix P142 NIPBL-2, 25 reactions.
- **P142-050R:** SALSA MLPA Probemix P142 NIPBL-2, 50 reactions.
- **P142-100R:** SALSA MLPA Probemix P142 NIPBL-2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information: The SALSA MLPA Probemixes P141 NIPBL-1 & P142 NIPBL-2 are **research use only (RUO)** assays for the detection of deletions or duplications in the *NIPBL* gene, which is associated with Cornelia de Lange syndrome (CdLS).

CdLS, also known as Bachmann-de Lange syndrome, is a multiple malformation disorder with characteristic facial features, growth and cognitive retardation, and a variety of other abnormalities affecting a wide range of tissues and organs. Most cases of CdLS are sporadic. Some familial cases have been reported, suggesting autosomal dominant inheritance. The incidence is 1 per 10,000-50,000 live births with no difference based on race or sex.

Among the genes associated with CdLS is the *NIPBL* gene, which is found mutated in 50-60% van CdLS cases. Other genes include the *HDAC8*, *RAD21*, *SMC1A*, and *SMC3* genes, but no probes for these genes are included in these probemixes.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1104/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The *NIPBL* exon numbering used in this P141-A4 P142-A4 product description is the exon numbering from the RefSeq transcript NM_133433.4, which is identical to the NG_006987.2 sequence. This transcript contains 47 exons of which the last two are numbered 46a and 46c. The exon numbering and NM_ sequence used have been retrieved on 07/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P141-A4 NIPBL-1 contains 34 MLPA probes with amplification products between 135 and 418 nucleotides (nt). This includes 24 probes targeting 24 out of the 47 exons of the *NIPBL* gene. In addition, ten reference probes are included that detect autosomal chromosomal locations. The SALSA MLPA Probemix P142-A4 NIPBL-2 contains 34 MLPA probes with amplification products between 135 and 418 nucleotides (nt). This also includes 24 probes targeting 24 out of the 47 exons of the *NIPBL* gene (in total there are two probes for exon 1). In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

These probemixes each contain nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Cornelia de Lange syndrome (CdLS). More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to

inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *NIPBL* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P141 and P142.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***NIPBL* mutation database:** https://databases.lovd.nl/shared/genes/NIPBL. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *NIPBL* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P141-A4 NIPBL-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*NIPBL*
64-105	Control fragments – see table in probemix content section for more information		
135	Reference probe 19551-L26642	2p13	
142	**NIPBL probe** 04822-L04206		**Exon 1**
148	**NIPBL probe** 04839-L04223		**Exon 17**
154	**NIPBL probe** 04855-L04239		**Exon 33**
160	Reference probe 03557-L09329	3p22	
166	**NIPBL probe** 04825-L04209		**Exon 3**
172	**NIPBL probe** 04841-L04225		**Exon 19**
178	**NIPBL probe** 04857-L04241		**Exon 35**
184	Reference probe 03594-L02961	8q12	
190	Reference probe 03915-L03370	15q21	
196	**NIPBL probe** 04827-L04211		**Exon 5**
202	**NIPBL probe** 04843-L04227		**Exon 21**
210	**NIPBL probe** 04859-L04243		**Exon 37**
220	Reference probe 14933-L16666	6q22	
230	**NIPBL probe** 04829-L04213		**Exon 7**
238	**NIPBL probe** 04845-L04229		**Exon 23**
245	**NIPBL probe** 04861-L04245		**Exon 39**
256	**NIPBL probe** 05409-L04215		**Exon 9**
283	**NIPBL probe** 04847-L04231		**Exon 25**
292	**NIPBL probe** 04863-L04247		**Exon 41**
301	Reference probe 16273-L18565	13q14	
310	**NIPBL probe** 04833-L04217		**Exon 11**
318	**NIPBL probe** 04849-L04819		**Exon 27**
328 ¥	**NIPBL probe** 21423-L32290		**Exon 43**
337	Reference probe 01659-L01241	17p13	
346	**NIPBL probe** 04835-L04219		**Exon 13**
355	**NIPBL probe** 04851-L04235		**Exon 29**
364	**NIPBL probe** 04867-L04251		**Exon 45**
373	Reference probe 02560-L02023	3q23	
385	Reference probe 03091-L07348	11p13	
391	**NIPBL probe** 04837-L04221		**Exon 15**
400	**NIPBL probe** 04853-L04820		**Exon 31**
408	**NIPBL probe** 04869-L04253		**Exon 46c**
418 *	Reference probe 20770-L28672	1q24	

a) See above section on exon numbering for more information.

* New in version A4.
¥ Changed in version A4. Minor alteration, no change in sequence detected.

Table 1b. SALSA MLPA Probemix P142-A4 NIPBL-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*NIPBL*
64-105	Control fragments – see table in probemix content section for more information		
135	Reference probe 19551-L26642	2p13	
142	**NIPBL probe** 04824-L05608		**Exon 2**
148	**NIPBL probe** 04840-L04224		**Exon 18**
154	**NIPBL probe** 04856-L04240		**Exon 34**
160	Reference probe 03557-L09329	3p22	
166	**NIPBL probe** 04826-L04840		**Exon 4**
172	**NIPBL probe** 04842-L04226		**Exon 20**
178	**NIPBL probe** 04858-L04242		**Exon 36**
184	Reference probe 03594-L02961	8q12	
190	Reference probe 03915-L03370	15q21	
196	**NIPBL probe** 04828-L04212		**Exon 6**
202	**NIPBL probe** 04844-L04228		**Exon 22**
211	**NIPBL probe** 04860-L04816		**Exon 38**
220	Reference probe 14933-L16666	6q22	
229	**NIPBL probe** 04830-L04817		**Exon 8**
238	**NIPBL probe** 04846-L04230		**Exon 24**
247	**NIPBL probe** 04862-L04246		**Exon 40**
274	**NIPBL probe** 04832-L04216		**Exon 10**
283	**NIPBL probe** 04848-L04232		**Exon 26**
292 ¥	**NIPBL probe** 21389-L32291		**Exon 42**
301	Reference probe 16273-L18565	13q14	
310	**NIPBL probe** 04834-L04218		**Exon 12**
319	**NIPBL probe** 05410-L04234		**Exon 28**
327	**NIPBL probe** 04866-L04250		**Exon 44**
337	Reference probe 01659-L01241	17p13	
346	**NIPBL probe** 04836-L04220		**Exon 14**
355	**NIPBL probe** 04852-L04236		**Exon 30**
363	**NIPBL probe** 04868-L04252		**Exon 46a**
373	Reference probe 02560-L02023	3q23	
385	Reference probe 03091-L07348	11p13	
393	**NIPBL probe** 04838-L04222		**Exon 16**
400	**NIPBL probe** 04854-L04238		**Exon 32**
409	**NIPBL probe** 04823-L04207		**Exon 1**
418 *	Reference probe 20770-L28672	1q24	

a) See above section on exon numbering for more information.

* New in version A4.
¥ Changed in version A4. Minor alteration, no change in sequence detected.

Table 2. *NIPBL* probes arranged according to chromosomal location

Length (nt) P141 P142	SALSA MLPA probe	*NIPBL* exon[a]	Ligation site NM_133433.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*490-492 (Exon 2)*		
142	04822-L04206	Exon 1	242-243	GAGGAGGAGGAA-GAAGAAGCAACG	0.2 kb
409	04823-L04207	Exon 1	396-397	TTGGATTCAGAC-GCCGATTCGCCC	76.6 kb
142	04824-L05608	Exon 2	527-528	CATTACTACTCT-TGCGGGGATTGC	1.9 kb
166	04825-L04209	Exon 3	671-672	GGATGACAATTT-GGTTTCACAGCT	2.5 kb
166	04826-L04840	Exon 4	753-754	AGTGATGACCCA-GAAGGTGACATA	3.4 kb
196	04827-L04211	Exon 5	927-928	CAACAAACCACT-ATCTCACATAGC	0.6 kb
196	04828-L04212	Exon 6	982-983	GCTCTGGGAACA-GATTTATGCCAC	8.8 kb
230	04829-L04213	Exon 7	1149-1150	AACATACATGAT-AATAAAGTTTCT	1.1 kb
229	04830-L04817	Exon 8	1344-1345	GCTGGAAGTGAA-GGAACTCCTAAA	4.2 kb
256	05409-L04215	Exon 9	1797-1798	CAAGTGCCTGTT-TTACAACAGAAC	9.5 kb
274	04832-L04216	Exon 10	3014-3015	TAGGGTTCGAAG-ACCAGAAACATT	10.0 kb
310	04833-L04217	Exon 11	3701-3702	ACGTGTGAAAAT-GAACAAACGCAA	4.8 kb
310	04834-L04218	Exon 12	3899-3900	CTCTCATGAAGG-AAGAAGGAGTTC	0.4 kb
346	04835-L04219	Exon 13	4052-4053	TGAACCAAAACT-AACACCTGAAGG	0.1 kb
346	04836-L04220	Exon 14	4084-4085	ACTCTTCAACTT-TTAAGAGATTCA	1.7 kb
391	04837-L04221	Exon 15	4181-4182	TCCTCAGGAACT-GCTCTTAGGAAA	0.6 kb
393	04838-L04222	Exon 16	4322-4323	GGATGGGTCAAA-GCTTTCCACTTT	3.2 kb
148	04839-L04223	Exon 17	4479-4480	GTGTACATTGAG-GATGTAATTGAA	0.9 kb
148	04840-L04224	Exon 18	4617-4618	GCTAAATGTTCT-ACCCATAAGCAG	0.7 kb
172	04841-L04225	Exon 19	4796-4797	GTGTGCCATTAA-GTTAGTCACTGC	0.6 kb
172	04842-L04226	Exon 20	4867-4868	TTTTTACTTCAC-TTGCAAGATTAC	1.5 kb
202	04843-L04227	Exon 21	4980-4981	GTTTTACAACTT-ATTCAGTGTGTG	4.6 kb
202	04844-L04228	Exon 22	5103-5104	ATGCGAACAGCC-CAAAACTTCCTC	1.4 kb
238	04845-L04229	Exon 23	5245-5246	AACTACTCCTTA-GTTTGTTAGGGA	0.9 kb
238	04846-L04230	Exon 24	5301-5302	ACAGAGATGGCT-TTAAGAGTGGCA	2.3 kb
283	04847-L04231	Exon 25	5478-5479	GATGAAAACACT-GAGACTGATCCT	1.2 kb
283	04848-L04232	Exon 26	5613-5614	CATCATGCAAAG-GAAATTGAGACA	0.2 kb
318	04849-L04819	Exon 27	5754-5755	GATGCTTGCTTG-ATTGTTCGATAC	1.3 kb
319	05410-L04234	Exon 28	5876-5877	CATGAAGTGTTT-GTCTGAGGTTGT	0.2 kb
355	04851-L04235	Exon 29	5955-5956	CGATTGATGGAT-AATTCGACTAGT	2.4 kb
355	04852-L04236	Exon 30	6190-6191	ATGATGAAGAGG-GCATTAAGGTAG	1.6 kb
400	04853-L04820	Exon 31	6266-6267	CAAAGAAGCAAT-GACAAGGAAAAT	1.1 kb
400	04854-L04238	Exon 32	2 nt after exon 32	GCTTCAAAACGT-GAGTGTTCTTTT	9.0 kb
154	04855-L04239	Exon 33	6392-6393	TAAACCTGTGAA-GAAAGCTTGTAC	2.3 kb
154	04856-L04240	Exon 34	6567-6568	CATGCAATGACT-ATGCAACCATAC	5.7 kb
178	04857-L04241	Exon 35	6632-6633	CTGCAATGTTGC-AAAAATCCTAGA	0.3 kb
178	04858-L04242	Exon 36	6776-6777	TGGAGCTGTTGT-AAATAAAGTGAC	0.8 kb
210	04859-L04243	Exon 37	6887-6888	TAACACTTCACT-TCTAACAAACAA	0.7 kb
211	04860-L04816	Exon 38	7065-7066	CAAACAAAAGCT-ATCATTGGTCTA	2.5 kb
245	04861-L04245	Exon 39	7238-7239	ACGTATGCAGCA-GGCAGATAGAGA	0.5 kb
247	04862-L04246	Exon 40	7346-7347	TCTCAAACAGGT-GCTTGAGGCATT	2.6 kb
292	04863-L04247	Exon 41	7506-7507	AACAAGGCTGAT-CAGCAACTTGTG	0.6 kb
291	21389-L32291	Exon 42	7661-7662	CTCTAGCGCTTT-GTGTTCACACCT	4.8 kb
328	21423-L32290	Exon 43	7831-7832	AAGAGCCGTTGT-TTATAATGCATC	1.7 kb
327	04866-L04250	Exon 44	7972-7973	GCGACAGTGAAG-AAGAAGTTTCCA	2.0 kb
364	04867-L04251	Exon 45	8311-8312	CCAAAATCACAG-AAGAGGTGAAAA	3.0 kb
363	04868-L04252	Exon 46a	8518-8519	ATGGGGAGGATA-GAGGAGGAGGCA	0.7 kb
408	04869-L04253	Exon 46c	8688-8689	AAAACCAGCAGT-GGCTTCAGTGTT	
		stop codon	*8902-8904 (Exon 46c)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

References

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Selected publications using SALSA MLPA Probemix P141 NIPBL-1 & P142 NIPBL-2

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- Russo S et al. (2012). Intragenic and large NIPBL rearrangements revealed by MLPA in Cornelia de Lange patients. *Eur J Hum Genet*. 20:734-41.

P141 Product history	
Version	*Modification*
A4	One reference probe has been replaced and one probe has been adjusted in length.
A3	One reference probe has been removed and three reference probes have been replaced.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

P142 Product history	
Version	*Modification*
A4	One reference probe has been replaced, one reference probe removed, and one probe length has been adjusted.
A3	One reference probe has been removed and three reference probes have been replaced.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

Implemented changes in the product description

Version A4-01 — 22 September 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *NIPBL* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 15 – 05 January 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new

picture included).

Version 14 (55) – 20 November 2015

- Product description adapted to a new lot (lot number added, new picture included).
- Various minor textual changes on page 1.
- Ligation sites of the probes targeting the NIPBL gene updated according to new version of the NM_reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 13 (54) – 23 July 2015

- Figures based on the use of old MLPA buffer (replaced in December 2012) removed.
- Various minor textual changes throughout the document.

Version 12 (48)

- Warning added in Table 1, 136 nt probe 05478-L04901.

Version 11 (48)

- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

More information: www.mlpa.com; www.mlpa.eu	
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