Product Description SALSA® MLPA® Probemix P051-D2 & P052-D2 Parkinson

To be used with the MLPA General Protocol.

P051 version D2. As compared to version D1, two probes have a change in length and sequence. For complete product history see page 14.
P052 version D2. As compared to version D1, one probe has a change in length and sequence and one probe has a change in sequence. For complete product history see page 14.

Catalogue numbers:
- **P051-025R:** SALSA® MLPA® probemix P051 Parkinson mix 1, 25 reactions.
- **P051-050R:** SALSA® MLPA® probemix P051 Parkinson mix 1, 50 reactions.
- **P051-100R:** SALSA® MLPA® probemix P051 Parkinson mix 1, 100 reactions.

- **P052-025R:** SALSA® MLPA® probemix P052 Parkinson mix 2, 25 reactions.
- **P052-050R:** SALSA® MLPA® probemix P052 Parkinson mix 2, 50 reactions.
- **P052-100R:** SALSA® MLPA® probemix P052 Parkinson mix 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.

Intended use: The SALSA MLPA Probemixes P051 and P052 Parkinson are an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplication in *SNCA, PARK2, UCHL1, PINK1, PARK7, ATP13A2, LRRK2, GCH1* genes, and the presence of two point mutations, A30P in the *SNCA* gene and G2019S in the *LRRK2* gene, in genomic DNA isolated from human peripheral whole blood specimens. P051 and P052 Parkinson are intended to confirm a potential cause for and clinical diagnosis of Parkinson's disease and for molecular genetic testing of at-risk family members. Additionally, deletions or duplication in *GCH1* gene, covered by P052 Parkinson mix 2, can be used to confirm a potential cause for and clinical diagnosis of GTP cyclohydrolase 1-deficient dopa-responsive dystonia and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P051 and P052 Parkinson should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *SNCA, PARK2, UCHL1, PINK1, PARK7, ATP13A2, LRRK2, GCH1* genes are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis. Not all exons of *ATP13A2* and *LRRK2* genes are covered.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit, Coffalyser.Net analysis software, and SALSA Binning DNA SD067.

Clinical background: Parkinson's disease is the second most common neurodegenerative disorder and is characterized by the degeneration of dopaminergic neurons of the midbrain. Resting tremor, bradykinesia, rigidity, and postural instability are the main clinical manifestations of the disease. Parkinson's disease occurs in approximately 13 per 100,000 people. The age of onset in patients with Parkinson's disease varies over a wide range and can be defined as either early onset (≤50 years) or late onset (>50 years). The majority of Parkinson's disease cases are sporadic and a family history is reported in approximately 10-20% of patients.

Mutations in multiple genes are associated with autosomal dominant (*SNCA*, *LRRK2*, *GCH1*, *UCHL1*) or autosomal recessive (*PARK2* (also known as *PRKN*), *PINK1*, *PARK7*, *ATP13A2*) Parkinson's disease. Mutations in these genes range from point mutations to larger exonic rearrangements including deletions and duplications. The presence of multiple copies of *SNCA* is known to be associated with Parkinson's disease and the severity of symptoms increases with the number of copies of the gene (Keyser et al. 2010, Matsumoto et al. 2010). The *LRRK2* G2019S mutation (p.Gly2019Ser, c.6055G>A) is the most common Parkinson-associated mutation known today and has been reported in 41% of sporadic and 37% of familial Parkinson patients from the North African Arab population and in 18.3% of Ashkenazi Jewish Parkinson patients (Lesage et al. 2006, Ozelius et al. 2006), while the mutation has been found in only 0.58% Parkinson patients of European and Asian origin (Ross et al. 2011).
Mutations in *PARK2* and *PINK1* are the most common causes of early onset Parkinson's disease (EOPD), however the frequencies vary widely across studies. It has been reported that up to 50% of familial and 18% of sporadic EOPD cases had pathogenic *PARK2* mutations, whereas more recent studies have reported a pathogenic mutation frequency as low as 1.6%. Frequency estimates for *PINK1* mutations tend to fall within a similarly broad range as for *PARK2*, whereas *PARK7* mutations are generally very rare, being estimated in a UK-based study in 0.4% (Kilarski et al. 2012). Parkinson-related mutations in *ATP13A2*, *GCH1* and *UCHL1* are very rare.

GTP cyclohydrolase 1-deficient dopa-responsive dystonia (GTPCH1-deficient DRD), also known as autosomal dominant Segawa syndrome (OMIM #128230) is characterised by a childhood-onset dystonia, postural and motor disturbances showing marked diurnal fluctuation, and late development of parkinsonism (Segawa et al. 1976). All individuals with this disorder, are treated with relatively low doses of levodopa and show complete or near-complete reversal of symptoms. The disorder is caused by mutations in the *GCH1* gene encoding GTP cyclohydrolase 1.

More information is available on https://www.ncbi.nlm.nih.gov/books/NBK1223/; https://www.ncbi.nlm.nih.gov/books/NBK1478/ and https://www.ncbi.nlm.nih.gov/books/NBK1508/.

Gene structure, transcript variants and exon numbering

Gene	Location	Genomic length	# exons	NM_sequence used for exon numbering	LRG	NM-sequence length	Coding sequence
PARK7	1p36.23	24 kb	7	NM_007262.5	NA	1127 nt	107-676
ATP13A2	1p36.13	26 kb	29	NM_022089.4	834 (pending approval)	3996 nt	191-3733
PINK1	1p36.12	18 kb	8	NM_032409.3	NA	2657 nt	92-1837
UCHL1	4p13	12 kb	9	NM_004181.5	NA	1103 nt	50-721
SNCA (*PARK1*)	4q22.1	114 kb	6	NM_000345.4	NA	3177 nt	226-648
PARK2 (*PRKN*)	6q26	1380 kb	12	NM_004562.3	NA	4178 nt	99-1496
LRRK2	12q12	144 kb	51	NM_198578.4	NA	9239 nt	136-7719
GCH1	14q22.2	61 kb	6	NM_000161.3	NA	2916 nt	164-916

NA: not applicable.

The exon numbering used in this P051-D2/P052-D2 Parkinson product description is the exon numbering from the RefSeq transcript NM_sequences as described in the table above. LRG information is available on www.lrg-sequence.org. Transcript variant details are available on https://www.ncbi.nlm.nih.gov/gene. The exon numbering and NM_ sequence used have been retrieved on 09/2020. 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 Probemixes P051-D2/P052-D2 Parkinson both contain 50 MLPA probes with amplification products between 130 and 500 nucleotides (nt). The P051-D2 probemix includes 38 probes for the *PARK7*, *ATP13A2*, *PINK1*, *SNCA* and *PARK2* genes. The P051-D2 probemix also contains two probes specific for the *SNCA* A30P and *LRRK2* G2019S mutations which will only generate a signal when the mutation is present. The P052-D2 probemix includes 36 probes for the *ATP13A2*, *UCHL1, PARK2, LRRK2* and *GCH1* genes. The P052-D2 probemix also contains one probe specific for the *LRRK2* G2019S mutation which will only generate a signal when the mutation is present. *PARK2* maps close to *FRA6E*, one of the most active fragile sites in the human genome. Therefore, the P052-D2 probemix also contains two probes that are located at fragile sites detecting the *CAV1* and *CAV2* genes, which are not related to Parkinson's disease. The content of the P051 and P052 probemixes per gene can be found in Table 2 a-i.
In addition, ten and eleven reference probes are included in the P051-D2 and P052-D2 Parkinson probemixes respectively, 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).

Each of these probemixes 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 from peripheral blood, 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 Parkinson disease or GTPCH1-deficient DRD. 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 diverse collections of biological resources which

may be used as a positive control DNA samples in your MLPA experiments. Sample ID numbers NA21698, ND35201, NA06802, NA07994, NA19750, NA00782, NA10800, ND00196, NA50276, NA10074, NA10947, NA01059, NA12519 from the Coriell Institute have been tested with the P051-D2 and/or P052-D2 probemixes at MRC-Holland and can be used as a positive control samples (see tables below). The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Altered target genes in P051-D2	Expected aberration
NA21698	Coriell Institute	*PARK2*	Heterozygous deletion of *PARK2* exon 1
ND35201	Coriell Institute	*PARK2*	Homozygous deletion of *PARK2* exon 3 and 4
NA06802	Coriell Institute	*PARK2, LPA*	Heterozygous deletion
NA07994	Coriell Institute	*PARK2, LPA*	Heterozygous duplication
NA19750	Coriell Institute	*LRRK2*	*LRRK2* G2019S
NA00782	Coriell Institute	*SNCA*	Heterozygous duplication
NA10800	Coriell Institute	*SNCA*	Heterozygous deletion
ND00196	Coriell Institute	*SNCA*	Triplication
NA50276	Coriell Institute	*PARK7, TNFRSF9*	Heterozygous deletion

Sample name	Source	Altered target genes in P052-D2	Expected aberration
NA21698	Coriell Institute	*PACRG*	Heterozygous deletion of *PACRG* exon 1
ND35201	Coriell Institute	*PARK2*	Homozygous deletion of *PARK2* exon 3 and 4
NA06802	Coriell Institute	*PARK2, PACRG*	Heterozygous deletion
NA07994	Coriell Institute	*PARK2, PACRG*	Heterozygous duplication
NA19750	Coriell Institute	*LRRK2*	*LRRK2* G2019S
NA10074	Coriell Institute	*GCH1*	Heterozygous duplication
NA10947	Coriell Institute	*UCHL1*	Heterozygous deletion
NA01059	Coriell Institute	*CAV1, CAV2*	Heterozygous deletion
NA12519	Coriell Institute	*CAV1, CAV2*	Heterozygous triplication/Homozygous duplication

SALSA Binning DNA SD067: The SD067 Binning DNA provided with this probemix can be used for binning of *SNCA* A30P (c.88G>C = p.A30P) and *LRRK2* G2019S (c.6055G>A = p.G2019S) mutation-specific probes (SNCA probe 02166-L27543, LRRK2 probe 04575-L27549 (P051-D2) and LRRK2 probe 04574-L27601 (P052-D2)). SD067 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD067 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD067 Binning DNA product description, available online: www.mlpa.com.

Performance characteristics: The frequency of *SNCA* deletions or duplications in Parkinson's disease is less than 1%. Mutations in *PARK2* and *PINK1* are the most common causes of EOPD, however the frequencies vary widely across studies. It has been reported that up to 50% of familial and 18% of sporadic EOPD cases had pathogenic *PARK2* mutations, whereas more recent studies have reported a pathogenic mutation frequency as low as 1.6%. Frequency estimates for *PINK1* mutations tend to fall within a similarly broad range as for *PARK2*, whereas *PARK7* mutations are generally very rare, being estimated in a UK-based study in 0.4% (Kilarski et al. 2012). The frequency of *LRRK2* G2019S mutation is 0.5% in sporadic Parkinson's disease and 2%-6% in familial Parkinson's disease in the US and Europe, while in North Africa the frequency is ~30% in sporadic Parkinson's disease and up to 41% in familial Parkinson's disease (Lesage et al. 2006, Ozelius et al. 2006, Ross et al. 2011). Parkinson-related mutations in *ATP13A2*, *GCH1* and *UCHL1* are very rare (https://www.ncbi.nlm.nih.gov/books/NBK1208/). The frequency of *GCH1* deletions or duplications in GTPCH1-deficient DRD is ~13% (https://www.ncbi.nlm.nih.gov/books/NBK1508/).
The analytical sensitivity and specificity for the use of P051 and P052 is very high and can be considered >99% (based on a 2000-2020 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). In rare cases, the expected results for *SNCA* region specific MLPA probes are allele copy numbers of 4 (heterozygous triplication/homozygous duplication) and for *PARK2*, *PINK1*, *ATP13A2* and *PARK7* region specific MLPA probes are allele copy numbers of 0 (homozygous deletion). 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 or in or near the *PINK1*, *ATP13A2*, *PARK7*, *UCHL1* gene. 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.
- Please note that background signals of the mutation-specific probes can be expected above the threshold in some cases. Users should always compare the peak height of the mutation-specific probes in mutation-positive samples to the peak height in reference samples. When the peak height of the mutation-specific probes in sample DNA is comparable to the peak height in reference samples, then it is a false positive result.
- 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 *LRRK2*, *ATP13A2*, *UCHL1*, *GCH1*, *PINK1*, *PARK7* and *PARK2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P051/P052 Parkinson.
- 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.

Mutation databases: https://grenada.lumc.nl/LOVD2/TPI/home.php and https://databases.lovd.nl/shared/genes/GCH1. 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 *PARK7* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA P051-D2 Parkinson probemix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]						
		reference	PARK7	ATP13A2	PINK1	SNCA	PARK2	LRRK2
64-105	Control fragments – see table in probemix content section for more information							
130	Reference probe 00797-L00463	5q						
136 ¬	**TNFRSF9** probe 20271-L27994		Upstream					
143	**PINK1** probe 20270-L27992				**Exon 5**			
149 ¥ Δ	**PINK1** probe 21469-L30271				**Exon 1**			
154 §	**SNCA** probe 02166-L27543					A30P Mut		
160	Reference probe 09787-L10202	15q						
166	**SNCA** probe 02168-L27544					**Exon 4**		
172	**PINK1** probe 03692-L27545				**Exon 2**			
178	**ATP13A2** probe 11715-L27546			**Exon 9**				
184	**SNCA** probe 02169-L28011					**Exon 6**		
190	Reference probe 08067-L19457	9p						
196 §	**LRRK2** probe 04575-L27549							G2019S Mut
202 Ж	**PARK2** probe 20272-SP0951-L27900						**Exon 4**	
209	**PINK1** probe 12067-L28012				**Exon 3**			
215 «	**ATP13A2** probe 11716-L28013			**Exon 2**				
222	Reference probe 06746-L27899	8q						
229	**PINK1** probe 03698-L03154				**Exon 7**			
237	**PARK2** probe 20225-L24881						**Exon 1**	
245	**PARK7** probe 20273-L27643		**Exon 6**					
253	**SNCA** probe 04616-L27552					**Exon 3**		
260	Reference probe 16433-L27655	18q						
265 ¬	**LPA** probe 20224-L27548						Downstream	
272	**PARK7** probe 20274-L27644		**Exon 2**					
279	**SNCA** probe 20255-L03103					**Exon 2**		
287	**PARK2** probe 02174-L27554						**Exon 2**	
294	Reference probe 18776-L27898	3p						
302	**PARK2** probe 02180-L27553						**Exon 8**	
310	**PINK1** probe 20276-L27646				**Exon 6**			
319	**PARK7** probe 20277-L27647		**Exon 4**					
325	**PARK2** probe 02181-L27555						**Exon 9**	
335	Reference probe 18737-L27897	2q						
343	**PARK2** probe 20278-L27648						**Exon 6**	
350 ±	**PARK7** probe 20279-L27649		**Exon 1**					
359	**PARK2** probe 02182-L27556						**Exon 10**	
370	**PARK7** probe 20254-L27588		**Exon 5**					
377	**PARK2** probe 02183-L27896						**Exon 11**	
385 ¥	Reference probe 18677-L30318	11p						
395	**PARK2** probe 02184-L27585						**Exon 12**	
405	**PINK1** probe 20280-L27650				**Exon 4**			
413	**PARK7** probe 20101-L27586		**Exon 3**					
423	**PARK2** probe 20281-L27651						**Exon 5**	
429	**PARK7** probe 03690-L27587		**Exon 1**					
436	Reference probe 10731-L11313	6p						
450	**SNCA** probe 04096-L27589					**Exon 1**		
457	**PARK7** probe 02189-L27590		**Exon 7**					
469	**PINK1** probe 03697-L27591				**Exon 8**			
477	**PARK2** probe 20282-L27652						**Exon 3**	
486	**SNCA** probe 03689-L27592					**Exon 5**		
494	**PARK2** probe 20283-L27895						**Exon 7**	
500	Reference probe 19555-L27674	2p						

a) See table in the gene structure, transcript variants, and exon numbering section.

¥ Changed in version D2 (from lot D2-0618 onwards). Change in length and in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *SNCA* A30P (154 nt) or *LRRK2* G2019S (196 nt) mutation is present. Please note that background signals of the mutation-specific probes

can be expected above the threshold in some cases. Users should always compare the peak height of the mutation-specific probes in mutation-positive samples to the peak height in reference samples.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Δ More variable. This probe is sensitive to certain experimental variations, which increases the quadruplex structure. Deletion of only this probe should be treated with caution and reported to MRC.
± SNP rs566749983 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 1b. SALSA MLPA P052-D2 Parkinson probemix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]						
		reference	ATP13A2	UCHL1	PARK2	CAV1/2	LRRK2	GCH1
64-105	Control fragments – see table in probemix content section for more information							
130	Reference probe 00797-L19287	5q						
136 Ø	**PACRG** probe 03204-L02565				**Intron 1**			
142	**UCHL1** probe 20285-L27662			**Exon 6**				
148	**PARK2** probe 20257-L27598				**Exon 5**			
154	Reference probe 14199-L27215	2q						
161	**PARK2** probe 03366-L27599				**Exon 7**			
166	**UCHL1** probe 03679-L27600			**Exon 1**				
172 §	**LRRK2** probe 04574-L27601						G2019S	
177	**UCHL1** probe 03681-L03096			**Exon 5**				
183	Reference probe 09496-L28060	11q						
190	**LRRK2** probe 20256-L23585						**Exon 41**	
196	**PARK2** probe 20286-L27663				**Exon 8**			
203	**UCHL1** probe 02937-L27602			**Exon 9**				
209	**GCH1** probe 04405-L27930							**Exon 3**
217	**PARK2** probe 06135-L27603				**Exon 12**			
224	Reference probe 06746-L28025	8q						
230	**ATP13A2** probe 11717-L27610		**Exon 14**					
238	**UCHL1** probe 20287-L27664			**Exon 8**				
244	**PARK2** probe 03365-L27611				**Exon 6**			
254 Ж	**UCHL1** probe 20288-SP0953-L28061			**Exon 4**				
261	**GCH1** probe 04618-L28062							**Exon 1**
268	Reference probe 16225-L18478	16q						
274	**PARK2** probe 05654-L28095				**Exon 3**			
281	**LRRK2** probe 04281-L27614						**Exon 27**	
286	**PARK2** probe 20289-L27933				**Exon 9**			
294	**UCHL1** probe 20290-L27667			**Exon 3**				
303 Ж	**PARK2** probe 20291-SP0954-L27668				**Exon 2**			
310	Reference probe 18380-L25673	10q						
319	**GCH1** probe 03683-L27615							**Exon 2**
328	**GCH1** probe 03685-L27616							**Exon 5**
334	**LRRK2** probe 04283-L27617						**Exon 49**	
343	**PARK2** probe 19810-L27618				**Exon 4**			
350	**PARK2** probe 03369-L27619				**Exon 10**			
359	Reference probe 10727-L26803	6p						
369	**GCH1** probe 15131-L27620							**Exon 4**
379	**LRRK2** probe 04278-L27621						**Exon 1**	
388	**GCH1** probe 20292-L27669							**Exon 6**
395	**PARK2** probe 04614-L27622				**Exon 11**			
404 ¥	**CAV1** probe 21889-L30747					**Exon 3**		
415	Reference probe 12747-L27779	9q						
422 ≠	**UCHL1** probe 21888-L30748			**Exon 2**				
429	**LRRK2** probe 04279-L27624						**Exon 10**	
443	**UCHL1** probe 20294-L27671			**Exon 7**				
448	**ATP13A2** probe 20295-L27934		**Exon 27**					
460	Reference probe 16287-L25505	20q						
466	**LRRK2** probe 04280-L28024						**Exon 15**	
477	**CAV2** probe 04091-L27626					**Exon 3**		
486	**LRRK2** probe 20296-L27936						**Exon 2**	
494	Reference probe 19137-L26747	21q						
500	Reference probe 19555-L27674	2p						

a) See table in the gene structure, transcript variants and exon numbering section for more information.

¥ Changed in version D2 (from lot D2-0618 onwards). Change in length and in sequence detected.
≠ Changed in version D2 (from lot D2-0618 onwards). Change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *LRRK2* G2019S (172 nt) mutation is present. Please note that background signals of the mutation-specific probes can be expected above the threshold in some cases. Users should always compare the peak height of the mutation-specific probes in mutation-positive samples to the peak height in reference samples.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of a single intron probe are unlikely to be related to the condition tested.

Table 2. P051 and P052 probes arranged according to chromosomal location

Table 2a. *PARK7*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	PARK7 exon[a]	Ligation site NM_007262.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
136		20271-L27994	TNFRSF9		GAAGACCAAGGA-GTGGAAAGTTCT	20.6 kb
			start codon	*107-109 (exon 2)*		
429		03690-L27587	Exon 1	304 nt before exon 1	GAGCGCGAACTA-AGGAACCCCTCT	0.3 kb
350 ±		20279-L27649	Exon 1	5 nt before exon 1	CGCGTGCGTGCT-GGCGTGCGTTCA	1.1 kb
272		20274-L27644	Exon 2	125-126	CCAAAAGAGCTC-TGGTCATCCTGG	2.6 kb
413		20101-L27586	Exon 3	256-255 *reverse*	GGACAAATGACC-ACATCACGGCTA	4.0 kb
319		20277-L27647	Exon 4	310-309 *reverse*	AGAACCACCACA-TCATATGGTCCC	1.6 kb
370		20254-L27588	Exon 5	409-408 *reverse*	ATGGCGGCTATC-AGGCCCTTCCGG	6.8 kb
245		20273-L27643	Exon 6	487-488	ACAACACACCCT-CTTGCTAAAGAC	7.4 kb
457		02189-L27590	Exon 7	686-687	AGAGCAGCGAAC-TGCGACGATCAC	9.2 **M**b
			stop codon	*674-676 (exon 7)*		to ATP13A2 gene

Table 2b. *ATP13A2*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	ATP13A2 exon[a]	Ligation site NM_022089.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*191-193 (exon 1)*		
215 «		11716-L28013	Exon 2	203-204	TGTCCACAGACA-GCAGCCCTCTCG	5.4 kb
178		11715-L27546	Exon 9	1001-1002	CCTCCATCTCCA-TCTGCCTGTCGC	4.1 kb
	230	11717-L27610	Exon 14	1520-1521	CCATCTACAGCA-TCTTCATCCTCT	9.4 kb
	448	20295-L27934	Exon 27	3311-3312	CCGCACCAGACA-ACCTGCCCAACT	3.6 **M**b
			stop codon	*3731-3733 (exon 29)*		to PINK1 gene

Table 2c. *PINK1*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	PINK1 exon[a]	Ligation site NM_032409.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*92-94 (exon 1)*		
149 ¥Δ		21469-L30271	Exon 1	344-345	TGCAGCGGCAGT-TCGTGGTGCGGG	4.1 kb
172		03692-L27545	Exon 2	564-565	GGAGTATCTGAT-AGGGCAGTCCAT	2.1 kb
209		12067-L28012	Exon 3	864-865	AGCAGTCACTTA-CAGGTAAGTGCC	4.5 kb
405		20280-L27650	Exon 4	884-885	CCAAGAGAGGTC-CCAAGCAACTAG	1.2 kb
143		20270-L27992	Exon 5	1195-1196	TCCGACAACATC-CTTGTGGAGCTG	2.9 kb
310		20276-L27646	Exon 6	1341-1342	GATGGCCCCAGA-GGTGAGTCCCGA	0.5 kb
229		03698-L03154	Exon 7	1430-1431	CCTATGAAATCT-TCGGGCTTGTCA	2.0 kb
469		03697-L27591	Exon 8	2208-2209	GCATGTCTGATT-TGCCACCTGGAT	
			stop codon	*1835-1837 (exon 8)*		

Table 2d. *UCHL1*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	UCHL1 exon[a]	Ligation site NM_004181.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*50-52 (exon 1)*		
	166	03679-L27600	Exon 1	9-10	GCTAGCTGTTTT-TCGTCTTCCCTA	0.2 kb
	422 ≠	21888-L30748	Exon 2	84-85	TGCCTTTCAGAT-GCTGAACAAAGT	0.6 kb
	294	20290-L27667	Exon 3	135-136	GTGGCGCTTCGT-GGACGTGCTGGG	3.1 kb
	254 Ж	20288-SP0953-L28061	Exon 4	15 nt after exon 4 and 355-354; *reverse*	ATGGCTGGCCTC-35 nt spanning oligo-TTATTGGCCACT	1.0 kb
	177	03681-L03096	Exon 5	412-413	CTTTCTGAAACA-GAGAAAATGTCC	0.1 kb
	142	20285-L27662	Exon 6	471-472	GGCCATACAGGC-AGCCCATGATGC	1.3 kb
	443	20294-L27671	Exon 7	520-521	GTAGATGACAAG-GTGAATTTCCAT	0.9 kb
	238	20287-L27664	Exon 8	584-585	CAGATGGACGAA-TGCCTTTTCCGG	4.0 kb
	203	02937-L27602	Exon 9	722-723	AGGCAGCCTAAT-GCTCTGTGGGAG	50 **M**b to SNCA gene
			stop codon	*719-721 (exon 9)*		

Table 2e. *SNCA*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	SNCA exon[a]	Ligation site NM_000345.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*226-228 (exon 2)*		
450		04096-L27589	Exon 1	184-185	AGAACTGGGAGT-GGCCATTCGACG	1.3 kb
279		20255-L03103	Exon 2	234-235	GCCATGGATGTA-TTCATGAAAGGA	0.1 kb
154 §		02166-L27543	Exon 2 88G>C=A30P	313-312 *reverse*	TGTCTTTCCTG**G**-TGCTTCTGCCAC	7.4 kb
253		04616-L27552	Exon 3	6 nt before exon 3	TTGAATTTGTTT-TTGTAGGCTCCA	5.8 kb
166		02168-L27544	Exon 4	406-407	AGAAGACCAAAG-AGCAAGTGACAA	93.1 kb
486		03689-L27592	Exon 5	560-561	ACAGGAAGGAAT-TCTGGAAGATAT	2.6 kb
184		02169-L28011	Exon 6	662-663	AATATCTTTGCT-CCCAGTTTCTTG	
			stop codon	*646-648 (exon 6)*		

Table 2f. *PARK2 (PRKN)*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	PARK2 exon[a]	Ligation site NM_004562.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*99-101 (exon 1)*		
237		20225-L24881	Exon 1	104-105	GTGACCATGATA-GGTACGTGGGTA	0.5 kb
	136	03204-L02565	PACRG; Exon 1	NM_152410.2; 66-65 *reverse*	GCAGCAAAGCAT-TAAACTCTAATG	0.28 **M**b
287		02174-L27554	Exon 2	169-170	CACCAGCATCTT-CCAGCTCAAGGA	0.1 kb
	303 Ж	20291-SP0954-L27668	Exon 2	217-218 and 254-255	TCCGGCTGACCA-37 nt spanning oligo-GACTGGACTGTG	0.18 **M**b
	274	05654-L28095	Exon 3	296-295 *reverse*	ACAATGTGAACA-ATGCTCTGCTGA	0.2 kb
477		20282-L27652	Exon 3	491-490 *reverse*	CCAGCTGGTGGT-GAGTCCTTCCTG	61.3 kb
	343	19810-L27618	Exon 4	537-538	ACAACAGCTTTT-ATGTGTATTGCA	0.1 kb
202 Ж		20272-SP0951-L27900	Exon 4	606-607 and 12 nt after exon 4	GCAGCACCTGCA-38 nt spanning oligo-AGTGTCAGCATG	0.15 **M**b
423		20281-L27651	Exon 5	681-682	GGATGAGTGGTG-AATGCCAATCCC	0.04 kb
	148	20257-L27598	Exon 5	710-711	TGCCCTGGGACT-AGTGCAGTAAGT	80.7 kb
343		20278-L27648	Exon 6	783-784	CTTTGCACCTGA-TCGCAACAAATA	0.1 kb
	244	03365-L27611	Exon 6	13 nt after exon 6	TAAGGATCTAAA-AATAGTGTCACT	0.19 **M**b
494		20283-L27895	Exon 7	873-872 *reverse*	GTCTAAGCAAAT-CACGTGGCGGGA	0.1 kb
	161	03366-L27599	Exon 7	954-955	AACTTGGCTACT-CCCTGCCTTGTG	0.22 **M**b
302		02180-L27553	Exon 8	993-994	ACTCCTTGATTA-AAGAGCTCCATC	0.02 kb
	196	20286-L27663	Exon 8	1009-1008 *reverse*	CCAGAATCCTGA-AGTGATGGAGCT	20.4 kb
	286	20289-L27933	Exon 9	1067-1068	GCAGAGGAGTGT-GTCCTGCAGATG	0.1 kb
325		02181-L27555	Exon 9	1144-1145	TGACCAGAGGAA-AGTCACCTGCGA	0.16 **M**b
359		02182-L27556	Exon 10	1200-1201	TCTGCCGGGAAT-GTAAAGAAGCGT	0.1 kb
	350	03369-L27619	Exon 10	1262-1261 *reverse*	TTCTGTACCTGA-GTAGTTGTTCCT	26.7 kb
377		02183-L27896	Exon 11	1333-1334	AGAAACCATCAA-GAAAACCACCAA	0.04 kb
	395	04614-L27622	Exon 11	1367-1368	CGCTGCCATGTA-CCAGTGGAAAAA	10.3 kb

395		02184-L27585	Exon 12	1841-1842	CAATCCATGTAT-CTGTATGGGTCA	0.1 kb
	217	06135-L27603	Exon 12	1967-1968	TGACAAGGTGTT-TCTCTGTAAAAT	0.82 **M**b
			stop codon	*1494-1496 (exon 12)*		
265		20224-L27548	LPA		AAATATACTTAT-AGTGATTGCACA	

Table 2g. *CAV1/2*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	Gene / Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
	404 ¥	21889-L30747	CAV1; Exon 3	NM_001753.5; 382-383	GGGGCATTTACT-TCGCCATTCTCT	53.0 kb
	477	04091-L27626	CAV2; Exon 3	NM_001233.5; 503-504	CGATGCTTCTCT-TCTGTCAGCCTG	

Table 2h. *LRRK2*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	LRRK2 exon[a]	Ligation site NM_198578.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*136-138 (exon 1)*		
	379	04278-L27621	Exon 1	230-231	AGGAAAACAGAT-AGAAACGCTGGT	0.4 kb
	486	20296-L27936	Exon 2	352-351 *reverse*	ACTCGCGACTCT-CATATAGGAGTC	25.9 kb
	429	04279-L27624	Exon 10	1286-1285 *reverse*	TCTTCTCATGTA-AACTGTTTTGGT	23.2 kb
	466	04280-L28024	Exon 15	1904-1905	GGATTCAGTGCT-TCACACACTGCA	29.3 kb
	281	04281-L27614	Exon 27	3761-3762	CAATGATATTCA-GTACCTACCAGG	36.3 kb
	190	20256-L23585	Exon 41	6106-6107	CAGCCATGATTA-TATACCGAGACC	0.1 kb
196 §		04575-L27549	Exon 41 6055G>A=G2019S	6190-6191	TTGCTGACTAC**A**-GCATTGCTCAGT	-
	172 §	04574-L27601	Exon 41 6055G>A=G2019S	6190-6191	TTGCTGACTAC**A**-GCATTGCTCAGT	24.8 kb
	334	04283-L27617	Exon 49	181 nt after exon 49	TGGAGGGAGTAA-ATGCTCTCAGTC	
			stop codon	*7717-7719 (exon 51)*		

Table 2i. *GCH1*

Length (nt) P051	Length (nt) P052	SALSA MLPA probe	GCH1 exon[a]	Ligation site NM_000161.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*164-166 (exon 1)*		
	261	04618-L28062	Exon 1	186 nt before exon 1	CGCCCTTTTCCT-TCCCTCCCTGCG	37.7 kb
	319	03683-L27615	Exon 2	577-578	ATAGACATGTTT-TCCATGTGTGAG	5.6 kb
	209	04405-L27930	Exon 3	630-631	CCATATTGGTTA-TCTTCCTAACAA	12.6 kb
	369	15131-L27620	Exon 4	61 nt before exon 4	GTTTTATGAGGA-AGGCTTATCAAT	1.3 kb
	328	03685-L27616	Exon 5	729-730	TACAAAACAAAT-TGCTGTAGCAAT	1.8 kb
	388	20292-L27669	Exon 6	861-862	AATGTTGGGTGT-GTTCCGGGAGGA	
			stop codon	*914-916 (exon 6)*		

a) See table in the gene structure, transcript variants and exon numbering section 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.

¥ Changed in version D2 (from lot D2-0618 onwards). Change in length and in sequence detected.
≠ Changed in version D2 (from lot D2-0618 onwards). Change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *SNCA* A30P (154 nt) or the *LRRK2* G2019S (196 nt and 172 nt) mutation is present. Please note that background signals of the mutation-specific probes can be expected above the threshold in some cases. Users should always compare the peak height of the mutation-specific probes in mutation-positive samples to the peak height in reference samples.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
± SNP rs566749983 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe is sensitive to certain experimental variations, which increases the quadruplex structure. Deletion of only this probe should be treated with caution and reported to MRC.

Related SALSA MLPA probemixes

P099 GCH1-TH-SGCE Contains one probe for each exon of *GCH1*, involved in autosomal dominant dopa responsive dystonia.

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P051 Product history	
Version	*Modification*
D2	A change in length and sequence of two probes.
D1	The lacking exons of *PARK7* and *SNCA* were included and multiple target and reference probes have been replaced.
C3	Several probes have a change in length / peak height, but no change in sequence detected.
C2	The 88 and 96 nt control fragments have been replaced (QDX2).
C1	One PINK1 probe has been replaced and several reference probes have been replaced or added.
B1	One PINK1 probe has been replaced, one PARK7 has been removed and two ATP13A2 probes have been added. In addition, new control fragments are included.
A1	First release.

P052 Product history	
Version	*Modification*
D2	One probe has a change in length and sequence and one probe has a change in sequence.
D1	Probes for missing exons of *UCHL1* and *GCH1* were added, several target probes and reference probes were replaced or added.
C2	One reference probe has been replaced and the control fragments have been adjusted (QDX2).
C1	Two new ATP13A2 probes and four extra control fragments have been added.
B1	Two reference probes have been changed. In addition, new control fragments are included.
A1	First release.

Implemented changes in the product description

Version D2/D2-03 — 09 December 2020 (02P)

- Probe length of probe (02189-L27590) was adjusted in Table 1a and Table 2a from 459 to 457, of probe (04096-L27589) was adjusted in Table 1a and Table 2e from 452 to 450, of reference probe (10731-L11313) was adjusted in Table 1a from 437 to 436, and of reference probe (16225-L18478) was adjusted in Table 1b from 265 to 268.
- Information on sub-bands of reference probes was removed from Table 1a and Table 1b. Chromosomal position of probe (03204-L02565) updated in Table 1b.
- Information added in Positive control DNA samples section, positive sample NA19750 was added to table of positive samples tested with P051, and information regarding samples NA21698, NA01059 and NA12519 was updated in table of positive samples tested with P052.

Version D2/D2-02 — 13 October 2020 (02P)

- Product description rewritten and adapted to a new template.

- Intended use updated to a new template; GTPCH1-deficient DRD added to the intended use.
- Clinical background information updated, information regarding GTPCH1-deficient DRD added, and references updated.
- Performance characteristics section and mutation database section updated.
- Israel added as countries with IVD status.
- Ligation sites of the probes targeting the *PARK7, PINK1, UCHL1, SNCA, PARK2, CAV1/2, LRRK2, GCH1* genes updated according to new version of the NM_ reference sequence.
- In the table present in the Gene structure and transcript variant section NM_00456.2 was corrected and updated to NM_004562.3.
- Extra positive control samples tested by MRC Holland were added to the section of Positive control DNA samples.
- Warning added to Table 1a and Table 2a for SNP rs566749983 which could influence the probe (20279-L27649) signal.
- Warning on Table 1a and 2c of salt sensitive probe (21469-L30271) replaced by a warning of variable probe due to experimental conditions.
- Warning of salt sensitive probe added to Table 1a and Table 2b for probe 11716-L28013.
- List of selected publications updated.

Version D2/D2-01 – 03 August 2018 (04)

- Product description restructured and adapted to a new template.
- Product description adapted to new product versions for P051 and P052 (version number changed, changes in Table 1 and Table 2).

Version 28 – 07 December 2018 (54)

- Warning adjusted below Table 1a/b and Table 2 about the mutation specific probes which will only generate a signal when the SNCA A30P (154 nt) or LRRK2 G2019S (196 nt) mutation is present. Please note that background signals of the mutation-specific probes can be expected above the threshold in some cases.

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