Instructions for Use

SALSA® MLPA® Probemix P051 Parkinson mix 1 and SALSA® MLPA® Probemix P052 Parkinson mix 2

See also the MLPA General Protocol, and the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD067, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P051 Parkinson mix 1 and SALSA® MLPA® Probemix P052 Parkinson mix 2 product pages on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P051 Parkinson mix 1**
Version	**D2**
Catalogue numbers	**P051-025R (25 reactions)** **P051-050R (50 reactions)** **P051-100R (100 reactions)**
Basic UDI-DI:	**872021148P0515M**
Ingredients	**Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA**

Product Name	**SALSA® MLPA® Probemix P052 Parkinson mix 2**
Version	**D2**
Catalogue numbers	**P052-025R (25 reactions)** **P052-050R (50 reactions)** **P052-100R (100 reactions)**
Basic UDI-DI:	**872021148P0525P**
Ingredients	**Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA**

Additional Test Components	**Catalogue numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD067	SD067

Storage and Shelf Life

Recommended conditions	-25°C -15°C	

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. These products should not be exposed to more than 25 freeze-thaw cycles. Do not use the products if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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Any serious incident that has occurred in relation to these products should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in these Product Versions

P051 version D2. As compared to version D1, two probes have a change in length and sequence.

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.

1. Intended Purpose

The SALSA MLPA Probemix P051 Parkinson mix 1 and SALSA MLPA Probemix P052 Parkinson mix 2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assays[2] for the detection of duplications and triplications in the *SNCA* gene (P051), deletions and duplications in the *PARK2* gene, and the presence of one point mutation, G2019S in the *LRRK2* gene (P051 and P052), in genomic DNA isolated from human peripheral whole blood specimens. P051 Parkinson mix 1 and P052 Parkinson mix 2 are intended to confirm a potential cause for early-onset (*PARK2* deletions/duplication, *SNCA* triplications, *LRRK2* G2019S mutation) and late-onset (*SNCA* duplications, *LRRK2* G2019S mutations) Parkinson's disease and for molecular genetic testing of at-risk family members[3].

Copy number variations (CNVs) and the *LRRK2* point mutation, G2019S, detected with P051 Parkinson mix 1 and P052 Parkinson mix 2 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 *SNCA* and *PARK2* are point mutations, which will not be detected by MLPA, with the exception of the aforementioned *LRRK2* G2019S mutation. It is therefore recommended to use this assay in combination with sequence analysis.

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.

These devices are 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 these probemixes are for in vitro diagnostic (IVD) use in the countries specified on page 1 of this product description. In all other countries, the products are 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.

[3] Certain probes included in P051 and P052 targeting other genes may only be used in a research setting. The following table summarises which probes are for IVD or exclusively restricted to RUO use:

	IVD targets	**RUO targets only**
P051	CNVs: *SNCA*; *PARK2* Mutation: *LRRK2* G2019S	CNVs: *PARK7*, *ATP13A2*, *PINK* Mutation: *SNCA* A30P
P052	CNVs: *PARK2* Mutation: *LRRK2* G2019S	CNVs: *UCHL1*, *LRRK2*,*GCH1*, *ATP13A2*, *CAV1/2*

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration (and) treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes except for mutation-specific probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of Parkinson's Disease (PD).	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Binning Sample (Initial Experiment)	• SALSA® Binning DNA SD067, provided by MRC Holland • Required in initial experiment to determine suitable bin set • Should never be used as a reference sample	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemixes product pages on our website.
Validation Samples (Required)	• In the validation experiments of this probemix, the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.	

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemixes	
Length (nt)	**Function**
64-70-76-82	DNA Quantity control fragments
88-96	DNA Denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80-1.20	2	Normal

Typical Results of Probes Targeting Two Copies (e.g. *SNCA*, *PARK2*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to centre around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of Mutation -Specific Probes

Signal Strength	Mutation Status
≥10% median peak height reference probes	Mutation is detected (expected only in positive samples)
<10% median peak height reference probes	Mutation is **not** detected (expected in most samples from healthy individuals)

6. Performance Characteristics

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table can be used. Cut-off values for copy number determination were verified with P051 in 46 samples from healthy individuals with normal copy number and eight samples with known CNVs and with P052 in 43 samples from healthy individuals with normal copy number and five samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Expected values for point mutation detection in normal and affected populations	The mutation-specific probe will only generate a signal when the *LRRK2* sG2019S (196 nt for P051 and 172 nt for P052) 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 relative peak height of the mutation-specific probe in mutation-positive samples to the relative peak height in reference samples. A clear signal (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes. The expected value for mutation-specific probes was verified with P051 and P052 using one positive sample for the *LRRK2* G2019S mutation and 46 and 43 samples from healthy individuals without the mutation, respectively, and the expected mutation status was found in each case.
Limit of detection	A study that evaluated the acceptable minimum and maximum amount of sample DNA revealed that the use of 50-250 ng of human DNA is the recommended input. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P051 on one sample with known CNVs and using P052 on four samples with known CNVs/mutations and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	Impurities in the DNA sample can affect the MLPA reaction. To minimise this effect, see Sample quality section under Precautions and warnings of the MLPA General Protocol. A study using P051 and P052 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA from samples with known CNVs/mutations. For most probes,

expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results* P051	Results* P052
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Ambiguous FR for 4/108 probes Mutation: Expected status for 6/6 probes	Copy number: Expected FR for 50/50 probes Mutation: Expected status for 1/1 probe
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Ambiguous FR for 7/108 probes Mutation: Expected status for 6/6 probes	Copy number: Ambiguous FR for 2/50 probes Mutation: Expected status for 1/1 probe
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Ambiguous FR for 7/108 probes Mutation: Expected status for 6/6 probes	Copy number: Expected FR for 50/50 probes Mutation: Expected status for 1/1 probe
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Ambiguous FR for 7/108 probes Mutation: Expected status for 6/6 probes	Copy number: Ambiguous FR for 1/50 probes Mutation: Expected status for 1/1 probe
Haemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 17/108 probes Mutation: Expected status for 6/6 probes	Copy number: Expected FR for 25/50 probes Mutation: Expected status for 1/1 probe

* Results are summarised for all probes across all six and five samples tested for P051 and P052, respectively.

Exogenous interfering substances (EDTA, heparin, salts (NaCl), and $FeCl_3$) were tested and shown to have a mild effect, leading to, at the most, ambiguous ratios and potential delayed results. Haemoglobin had the largest effect on the FRs, in particular for copy number determination.

Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores.

To minimise variability across samples, 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.

Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. Trueness: previously genotyped samples were tested and found to have the expected results. Precision: results are not affected by operator, day, or laboratory site. For trueness, ten previously genotyped samples were tested using P051, and six using P052, and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutations over multiple days, and by multiple operators. Overall, 99% correct calls (1610/1620 probes) for CNVs and 100% (90/90 probes) correct calls for mutation status were obtained throughout the precision experiments for P051. For P052, 99% correct calls (820/825 probes) for CNVs and 100% correct calls (75/75 probes) for mutation status were obtained throughout the precision experiments.
Clinical validity*	• *SNCA (P051 only)*: less than 1% of Parkinson's disease is caused by duplications and triplications in *SNCA* [1]. • *PARK2*: 2% of early onset and idiopathic, late-onset Parkinson's disease is caused by deletions and duplications in *PARK2* [2]. • *LRRK2*: the frequency of *LRRK2* G2019S mutation varies across populations [3-5]. *(Based on a 2000-2024 literature review)

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

SALSA MLPA Probemix P051-D2 Parkinson mix 1

Chr. position	Target	Exon	Mutation	Distance to next probe	Length (nt)	Probe number	Warnings
1p36.23	*TNFRSF9*			20.6 kb	136	20271-L27994	¬ ∫
1p36.23	*PARK7*	Upstream (Exon 1)		0.3 kb	429	03690-L27587	Ø
1p36.23	*PARK7*	Exon 1		1.1 kb	350	20279-L27649	
1p36.23	*PARK7*	Exon 2		2.6 kb	272	20274-L27644	
1p36.23	*PARK7*	Exon 3		4.0 kb	413	20101-L27586	
1p36.23	*PARK7*	Exon 4		1.6 kb	319	20277-L27647	
1p36.23	*PARK7*	Exon 5		6.8 kb	370	20254-L27588	
1p36.23	*PARK7*	Exon 6		7.4 kb	245	20273-L27643	
1p36.23	*PARK7*	Exon 7		9.2 **M**b	457	02189-L27590	
1p36.13	*ATP13A2*	Exon 9		5.4 kb	178	11715-L27546	
1p36.13	*ATP13A2*	Exon 2		3.6 **M**b	215	11716-L28013	
1p36.12	*PINK1*	Exon 1		4.1 kb	149	21469-L30271	Δ
1p36.12	*PINK1*	Exon 2		2.1 kb	172	03692-L27545	
1p36.12	*PINK1*	Exon 3		4.5 kb	209	12067-L28012	
1p36.12	*PINK1*	Exon 4		1.2 kb	405	20280-L27650	
1p36.12	*PINK1*	Exon 5		2.9 kb	143	20270-L27992	
1p36.12	*PINK1*	Exon 6		0.5 kb	310	20276-L27646	
1p36.12	*PINK1*	Exon 7		2.0 kb	229	03698-L03154	
1p36.12	*PINK1*	Exon 8			469	03697-L27591	
4q22.1	*SNCA*	Exon 6		2.6 kb	184	02169-L28011	
4q22.1	*SNCA*	Exon 5		93.1 kb	486	03689-L27592	
4q22.1	*SNCA*	Exon 4		5.8 kb	166	02168-L27544	
4q22.1	*SNCA*	Exon 3		7.4 kb	253	04616-L27552	
4q22.1	*SNCA*	Exon 2	A30P	0.1 kb	154	02166-L27543	§
4q22.1	*SNCA*	Exon 2		1.3 kb	279	20255-L03103	
4q22.1	*SNCA*	Exon 1			450	04096-L27589	
6q25.3	*LPA*			817.2 kb	265	20224-L27548	¬ †
6q26	*PARK2*	Exon 12		10.4 kb	395	02184-L27585	
6q26	*PARK2*	Exon 11		26.7 kb	377	02183-L27896	
6q26	*PARK2*	Exon 10		162.0 kb	359	02182-L27556	
6q26	*PARK2*	Exon 9		20.5 kb	325	02181-L27555	
6q26	*PARK2*	Exon 8		216.5 kb	302	02180-L27553	
6q26	*PARK2*	Exon 7		187.5 kb	494	20283-L27895	
6q26	*PARK2*	Exon 6		80.8 kb	343	20278-L27648	
6q26	*PARK2*	Exon 5		147.0 kb	423	20281-L27651	
6q26	*PARK2*	Exon 4		61.4 kb	202	20272-SP0951-L27900	Ж
6q26	*PARK2*	Exon 3		180.9 kb	477	20282-L27652	
6q26	*PARK2*	Exon 2		284.3 kb	287	02174-L27554	
6q26	*PARK2*	Exon 1			237	20225-L24881	
12q12	*LRRK2*	Exon 41	G2019S		196	04575-L27549	§
2p	Reference				500	19555-L27674	
2q	Reference				335	18737-L27897	
3p	Reference				294	18776-L27898	
5q	Reference				130	00797-L00463	
6p	Reference				436	10731-L11313	
8q	Reference				222	06746-L27899	
9p	Reference				190	08067-L19457	
11p	Reference				385	18677-L30318	
15q	Reference				160	09787-L10202	
18q	Reference				260	16433-L27655	

SALSA MLPA Probemix P052-D2 Parkinson mix 2

Chr. position	Target	Exon	Mutation	Distance to next probe	Length (nt)	Probe number	Warning
1p36.13	*ATP13A2*	Exon 27		9.4 kb	448	20295-L27934	
1p36.13	*ATP13A2*	Exon 14			230	11717-L27610	
4p13	*UCHL1*	Exon 1		0.2 kb	166	03679-L27600	
4p13	*UCHL1*	Exon 2		0.6 kb	422	21888-L30748	
4p13	*UCHL1*	Exon 3		3.1 kb	294	20290-L27667	
4p13	*UCHL1*	Exon 4		1.0 kb	254	20288-SP0953-L28061	Ж
4p13	*UCHL1*	Exon 5		0.1 kb	177	03681-L03096	
4p13	*UCHL1*	Exon 6		1.3 kb	142	20285-L27662	
4p13	*UCHL1*	Exon 7		0.9 kb	443	20294-L27671	
4p13	*UCHL1*	Exon 8		4.0 kb	238	20287-L27664	
4p13	*UCHL1*	Exon 9			203	02937-L27602	
6q26	*PARK2*	Exon 12		10.5 kb	217	06135-L27603	
6q26	*PARK2*	Exon 11		26.7 kb	395	04614-L27622	
6q26	*PARK2*	Exon 10		162.2 kb	350	03369-L27619	
6q26	*PARK2*	Exon 9		20.4 kb	286	20289-L27933	
6q26	*PARK2*	Exon 8		216.4 kb	196	20286-L27663	
6q26	*PARK2*	Exon 7		187.5 kb	161	03366-L27599	
6q26	*PARK2*	Exon 6		80.8 kb	244	03365-L27611	
6q26	*PARK2*	Exon 5		147.1 kb	148	20257-L27598	
6q26	*PARK2*	Exon 4		61.5 kb	343	19810-L27618	
6q26	*PARK2*	Exon 3		180.6 kb	274	05654-L28095	
6q26	*PARK2*	Exon 2		283.9 kb	303	20291-SP0954-L27668	Ж
6q26	*PARK2*	Intron 1			136	03204-L02565	Ø
7q31.2	*CAV2*	Exon 3		53.0 kb	477	04091-L27626	
7q31.2	*CAV1*	Exon 3			404	21889-L30747	
12q12	*LRRK2*	Exon 1		0.4 kb	379	04278-L27621	
12q12	*LRRK2*	Exon 2		25.9 kb	486	20296-L27936	
12q12	*LRRK2*	Exon 10		23.2 kb	429	04279-L27624	
12q12	*LRRK2*	Exon 15		29.3 kb	466	04280-L28024	
12q12	*LRRK2*	Exon 27		36.3 kb	281	04281-L27614	
12q12	*LRRK2*	Exon 41		0.1 kb	190	20256-L23585	
12q12	*LRRK2*	Exon 41	G2019S	24.8 kb	172	04574-L27601	§
12q12	*LRRK2*	Intron 49 (Exon 49)			334	04283-L27617	Ø
14q22.2	*GCH1*	Exon 6		1.8 kb	388	20292-L27669	
14q22.2	*GCH1*	Exon 5		1.3 kb	328	03685-L27616	
14q22.2	*GCH1*	Exon 4		12.6 kb	369	15131-L27620	+
14q22.2	*GCH1*	Exon 3		5.6 kb	209	04405-L27930	
14q22.3	*GCH1*	Exon 2		37.7 kb	319	03683-L27615	
14q22.3	*GCH1*	Upstream (Exon 1)			261	04618-L28062	Ø
2p	Reference				500	19555-L27674	
2q	Reference				154	14199-L27215	
5q	Reference				130	00797-L19287	
6p	Reference				359	10727-L26803	
8q	Reference				224	06746-L28025	
9q	Reference				415	12747-L27779	
10q	Reference				310	18380-L25673	
11q	Reference				183	09496-L28060	
16q	Reference				268	16225-L18478	
20q	Reference				460	16287-L25505	
21q	Reference				494	19137-L26747	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *PINK1, ATP13A2, PARK7, SNCA, PARK2, LRRK2, UCHL1, CAV2, CAV1,* and *GCH1* exon numbers are derived from the MANE Select transcripts. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region were altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

§	These probes will only generate a signal when the mutation is present.
¬	These probes are flanking probes, included to help determine the extent of a deletion/duplication.
Δ	This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
Ж	These probes consist of three parts and have two ligation sites. A low signal of these probes can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Ø	These probes target sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.

+	The ligation site of this probe is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
∫	Copy number alterations of this probe are suspected to have clinical significance, but the association is not yet fully established.
†	Copy number alterations of only this probe are of unknown clinical significance.

Probemix-specific precautions

1. These products are not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for these products**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by these products should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The clinical significance of the following findings is not yet clear/clearly established: multi-exon deletions in *TNFRSF9* may be associated with PD [6].
2. The mutation-specific probes can only detect the presence of the mutation and should not be used to determine zygosity.
3. Target probes for *PARK7*, *ATP12A2*, *PINK*, *UCHL1*, *GCH1* and *LRRK2* CNVs and the *SNCA* A30P mutation are included to be used for research purposes only and not for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Schulte, C. and T. Gasser, Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. Appl Clin Genet, 2011. 4: p. 67-80.
2. Ambroziak, W., et al., *Genomic instability in the PARK2 locus is associated with Parkinson's disease.* J Appl Genet, 2015. 56(4): p. 451-461.
3. Lesage, S., et al., LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med, 2006. 354(4): p. 422-3.
4. Ozelius, L.J., et al., LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med, 2006. 354(4): p. 424-5.
5. Ross, O.A., et al., Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol, 2011. 10(10): p. 898-908.
6. Güler, S., et al., Early-Onset Parkinson's Disease: A Novel Deletion Comprising the DJ-1 and TNFRSF9 Genes. Mov Disord, 2021. 36(12): p. 2973-2976.

Implemented Changes in the Product Description

Version D2/D2-05 – 24 January 2025 (03S)

- Product description updated to new template.
- Intended purpose was updated; for P051: CNVs for *PARK7*, *ATP13A2*, *PINK* genes and the *SNCA* A30P mutation removed, and for P052: CNVs for *UCHL1*, *LRRK2*, *GCH1, ATP13A2, CAV1/2* genes removed.
- Salt warning has been removed from ATP13A2 probe 11716-L28013.
- SNV rs566749983 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warning for a ligation site >20nt away from the nearest exon added to probe 15131-L27620.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for the target being outside the transcript region added for probes 03690-L27587, 04283-L27617, 04618-L28062, and 03204-L02565.
- Separate warnings for clinical significance added for probes 20271-L27994 and 20224-L27548.
- Probe 03204-L02565 in content table renamed from PACRG exon 1 to PARK2 intron 1 probe.
- Probemixes are now IVDR certified.