Product Description
SALSA® MLPA® Probemix P306-B2 SPG11

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 7.

Catalogue numbers:
- **P306-025R:** SALSA MLPA Probemix P306 SPG11, 25 reactions.
- **P306-050R:** SALSA MLPA Probemix P306 SPG11, 50 reactions.
- **P306-100R:** SALSA MLPA Probemix P306 SPG11, 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.mrcholland.com).

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 Probemix P306 SPG11 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SPG11* gene, which is associated with Spastic Paraplegia 11 (SPG11).

Spastic Paraplegia 11 (SPG11) is a neurological disorder characterised by progressive spasticity and weakness of the lower limbs. Other features associated with the disorder are, amongst others, mild intellectual disability, peripheral neuropathy and increased reflexes in the upper limbs. *SPG11* is inherited in an autosomal recessive manner. The majority of associated mutations in the *SPG11* gene are single nucleotide variants, but large deletions have been described.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1210/.

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 *SPG11* exon numbering used in this P306-B2 SPG11 product description is the exon numbering from the NG_008885.1 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P306-B2 SPG11 contains 51 MLPA probes with amplification products between 124 and 500 nucleotides (nt). The P306-B2 SPG11 probemix contains probes for each of the 40 *SPG11* exons.

In addition, one probe is included for *B2M* located 50 kilobases (kb) upstream, and one probe for *CASC4* located 150 kb downstream of *SPG11*. Furthermore, nine 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.mrcholland.com).

This probemix contains 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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 different unrelated individuals who are from families without a history of Spastic Paraplegia. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples 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.mrcholland.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 final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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.
- 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: a lower injection voltage or a shorter injection time, 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 *SPG11* gene are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P306 SPG11.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

***SPG11* mutation database**

https://databases.lovd.nl/shared/genes/SPG11. 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 SNVs and unusual results (e.g., a duplication of *SPG11* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P306-B2 SPG11

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SPG11*
64-105	Control fragments – see table in probemix content section for more information		
124	Reference probe 03100-L19610	4p	
130	Reference probe 00797-L00463	5q	
136	**SPG11** probe 09799-L10214		**Exon 38**
142	**SPG11** probe 09789-L19085		**Exon 28**
148	**SPG11** probe 21590-L30263		**Exon 24**
154	**SPG11** probe 09769-L10184		**Exon 8**
160	**SPG11** probe 09787-L10202		**Exon 26**
166	**SPG11** probe 09795-L10210		**Exon 34**
172	**SPG11** probe 09771-L10186		**Exon 10**
178 ¬	**CASC4** probe 09802-L12595		**Downstream**
184	Reference probe 19450-L25864	14q	
190	**SPG11** probe 09780-L10195		**Exon 19**
196	**SPG11** probe 09763-L10178		**Exon 2**
202	**SPG11** probe 09800-L10215		**Exon 39**
208	**SPG11** probe 09786-L10201		**Exon 25**
214	**SPG11** probe 09796-L30468		**Exon 35**
220	**SPG11** probe 21647-L30469		**Exon 4**
226	**SPG11** probe 09778-L10193		**Exon 17**
232	Reference probe 10410-L12202	9q	
238	Reference probe 19807-L14372	5p	
247	**SPG11** probe 09774-L10189		**Exon 13**
253	**SPG11** probe 09790-L12916		**Exon 29**
260	**SPG11** probe 09794-L12917		**Exon 33**
265	**SPG11** probe 09782-L30264		**Exon 21**
274	**SPG11** probe 09792-L10207		**Exon 31**
278	Reference probe 18594-L28268	2q	
285	**SPG11** probe 09762-L30262		**Exon 1**
292	**SPG11** probe 09801-L10216		**Exon 40**
301	**SPG11** probe 09781-L10196		**Exon 20**
310	**SPG11** probe 09791-L10206		**Exon 30**
319	**SPG11** probe 09767-L10182		**Exon 6**
329	**SPG11** probe 09776-L10191		**Exon 15**
337	**SPG11** probe 09784-L10199		**Exon 23**
346	**SPG11** probe 09768-L10183		**Exon 7**
355	**SPG11** probe 09783-L10198		**Exon 22**
364	**SPG11** probe 09775-L10190		**Exon 14**
373	**SPG11** probe 09779-L10194		**Exon 18**
382	**SPG11** probe 09798-L10213		**Exon 37**
391	**SPG11** probe 21649-L30467		**Exon 9**
404	Reference probe 09016-L29686	1q	
412	**SPG11** probe 09793-L12593		**Exon 32**
420	**SPG11** probe 09777-L19608		**Exon 16**
427	**SPG11** probe 09764-L19607		**Exon 3**
436	**SPG11** probe 16538-L30466		**Exon 27**
445	**SPG11** probe 21648-L30266		**Exon 5**
454 ¬	**B2M** probe 16539-L19029		**Upstream**
463	**SPG11** probe 09773-L10188		**Exon 12**
472	**SPG11** probe 11803-L12598		**Exon 36**
481	**SPG11** probe 09772-L10187		**Exon 11**
490	Reference probe 10218-L10698	7q	
500	Reference probe 21347-L29753	3p	

[a] See section Exon numbering on page 1 for more information.

¬ 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *SPG11* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SPG11* exon[a]	Ligation site NM_025137.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
454 ¬	16539-L19029	*B2M*		AACATCAACATC-TTGGTCAGATTT	54.3 kb
		Start Codon	*16-18 (exon 1)*		
285	09762-L30262	Exon 1	111-112	CTGTTGGTGCCA-GTCCCCGCCGAG	3.1 kb
196	09763-L10178	Exon 2	407-408	AACCATTTTGTA-TAGCTGTAGTAG	1.3 kb
427	09764-L19607	Exon 3	603-604	CTTCCCTTGCCT-GCACAGGCAGTG	2.0 kb
220	21647-L30469	Exon 4	770-771	GCAGCAACAGGA-GCCAGCCAAGAT	5.0 kb
445	21648-L30266	Exon 5	954-955	CCTAAGGGCGTA-GATGAAGATGAT	0.4 kb
319	09767-L10182	Exon 6	1161-1162	ACAAGTGTGCAG-AGCTGGGCCTTC	2.9 kb
346	09768-L10183	Exon 7	1549-1550	ATGGAAGTGCCA-GCACTGTGGACA	15.3 kb
154	09769-L10184	Exon 8	1626-1627	CAGGCCGGGATA-GAAAATCGTCAG	4.3 kb
391	21649-L30467	Exon 9	1770-1771	GAGCTGATACCA-GCATTGGATTTA	0.6 kb
172	09771-L10186	Exon 10	1961-1962	CTACATTAATGA-ACTTCGAACCTT	2.3 kb
481	09772-L10187	Exon 11	2144-2145	TTTCTTCAGGAT-TGATAGTCATTC	3.7 kb
463	09773-L10188	Exon 12	14 nt after exon 12	AGGTAAAGGTGA-GACTACATAGTA	0.4 kb
247	09774-L10189	Exon 13	2415-2416	GAGAAGCTTTAT-TTGGGACATTTC	0.4 kb
364	09775-L10190	Exon 14	2577-2578	TTAAATTGGGCT-CTGTGGTGGGAT	1.5 kb
329	09776-L10191	Exon 15	2762-2763	TCAGCAGAACAA-ATGGCCCCTTCT	4.8 kb
420	09777-L19608	Exon 16	2918-2919	GAGCCGTATTGG-AGGTGTAATACA	2.0 kb
226	09778-L10193	Exon 17	3116-3117	ACACCCTTGGTT-TGAATTTTTAGT	2.6 kb
373	09779-L10194	Exon 18	3235-3236	CCAGTGTAAGCA-GTATGCTATTGG	2.4 kb
190	09780-L10195	Exon 19	3389-3390	TCCTTACCCCAA-GCTAAAAACTGC	2.5 kb
301	09781-L10196	Exon 20	3531-3532	ACACTAGCTATA-GGAGGTAAGTCA	5.4 kb
265	09782-L30264	Exon 21	3544-3545	TAGATGCATGGA-GTCATCTCCCAC	1.8 kb
355	09783-L10198	Exon 22	3722-3723	AGTAGGCAATGA-AGCCTATGTTAT	0.6 kb
337	09784-L10199	Exon 23	6 nt after exon 23	AAAGAGGTTTGT-GAGTTGCAGTCT	1.4 kb
148	21590-L30263	Exon 24	4124-4125	CAAAGCAAATGA-TTGGCTGCAGTT	0.7 kb
208	09786-L10201	Exon 25	4355-4356	AATTCTGCTCCA-ATGCTCAGAGGA	0.8 kb
160	09787-L10202	Exon 26	4535-4536	GGGACACATTCA-GGACTCAACAGA	2.9 kb
436	16538-L30466	Exon 27	4655-4656	GTCAAAGGATTC-CCCGTTACTACT	3.1 kb
142	09789-L19085	Exon 28	4830-4831	GTGTGTTTCCTT-TTGAAGCTTATG	3.6 kb
253	09790-L12916	Exon 29	5076-5077	GGACAATTCGCT-TTGGCCAGGAGG	1.3 kb
310	09791-L10206	Exon 30	5323-5324	GGAGCAGCATGG-AGGAGCGCCATC	9.4 kb
274	09792-L10207	Exon 31	5963-5964	GCTGACAAGCAA-ATGCCTCCATGG	1.3 kb
412	09793-L12593	Exon 32	6148-6149	CACAGGGCCTTA-AGCCAGATACTG	0.9 kb
260	09794-L12917	Exon 33	6298-6299	AAGACCGCACAT-TGGTAGGCATGA	2.1 kb
166	09795-L10210	Exon 34	6391-6392	CCCATCATTGCT-TCACCCTGACGT	1.1 kb
214	09796-L30468	Exon 35	6494-6495	CACTTCACAGGT-ACGGCTCCTCAC	2.0 kb
472	11803-L12598	Exon 36	6695-6696	GTGCTTCAGCAT-GTGCCGGGAGAT	1.2 kb
382	09798-L10213	Exon 37	6812-6813	ACAACTGCTGCT-GAAGGCCCTGAC	0.3 kb
136	09799-L10214	Exon 38	6887-6888	GGCCCAGCACTG-TCAGCGGCTCAC	1.3 kb
202	09800-L10215	Exon 39	17 nt before exon 39	TGGTGTCCTTCT-TCACCTCTCCTT	1.7 kb
292	09801-L10216	Exon 40	7412-7413	GATGAGAAGAAG-AATGCATTGGAG	148.8 kb
		Stop Codon	*7345-7347 (exon 40)*		
178 ¬	09802-L12595	*CASC4*		AGGGAAACTGGA-AGCCAAACACTG	

[a] See section Exon numbering on page 1 for more information.

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

¬ 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- P165: HSP: Includes probes for *ATL1* (*SPG3A*) and *SPAST*.
- P211: HSP region: Includes probes for the *SPAST* region (15q11 region; *LOC84661*, *SLC30A6*, *TUBGCP5*, *NIPA2* and *NIPA1*).
- P213: HSP mix-2: Includes probes for *REEP1* and *SPG7*.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P306 SPG11

- Meszarosova AU et al. (2020). Two types of recessive hereditary spastic paraplegia in Roma patients in compound heterozygous state; no ethnically prevalent variant found. *Neuroscience Letters*. 721-134800.
- Yoon G et al. (2013). Autosomal recessive hereditary spastic paraplegia—clinical and genetic characteristics of a well-defined cohort. *Neurogen*. 14: 181-188.

P306 product history	
Version	*Modification*
B2	One reference probe has been removed, and four replaced. Also, the length of several probes have been adjusted.
B1	One SPG11, one flanking probe (B2M) and three reference probes have been replaced. Three extra reference probes have been included. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description
Version B2-01 – 24 November 2021 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *SPG11* gene updated according to new version of the NM_ reference sequence.
Version 10 (55) – 01 December 2017 - Product description adapted to a new product lot (lot number added, new picture included). - Small changes in Table 1 and Table 2. - Various minor textual changes.
Version 09 (55) – 09 October 2015 - Product description adapted to a new product lot (lot number added, new picture included). - Various textual changes.
Version 08 (48) – 06 August 2015 - Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

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