Product Description

SALSA® MLPA® Probemix P165-C3 HSP mix-1

To be used with the MLPA General Protocol.

Version C3
As compared to the previous version C2, one reference probe has been replaced and five probes have had a small change in length but no change in sequence detected. For complete product history see page 9.

Catalogue numbers:

- **P165-025R:** SALSA MLPA Probemix P165 HSP mix-1, 25 reactions.
- **P165-050R:** SALSA MLPA Probemix P165 HSP mix-1, 50 reactions.
- **P165-100R:** SALSA MLPA Probemix P165 HSP mix-1, 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.

Intended purpose
The SALSA MLPA Probemix P165 HSP mix-1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in *ATL1* and *SPAST* genes, in order to confirm a potential cause for and clinical diagnosis of spastic paraplegia (SPG) type 3A and SPG type 4, respectively. This assay is for use with genomic DNA isolated from human peripheral whole blood specimens. This product can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P165 HSP mix-1 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 *ATL1* and *SPAST* genes are point mutations, none of which will be detected by MLPA. 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.

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, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) 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 and Coffalyser.Net analysis software.

Clinical background
Hereditary spastic paraplegias (HSP) are genetically heterogeneous neurodegenerative disorders characterised by progressive spasticity and weakness of the lower limbs due to axonal degeneration in the

pyramidal tract. To date, more than 80 genetic types of HSP have been defined by genetic linkage analysis and identification of HSP-related gene variants.

Spastic paraplegia type 3A (SPG3A) is caused by pathogenic mutations in the *ATL1* gene and accounts for approximately 10% of all autosomal dominant HSP. Currently, there are more than 60 different *ATL1* mutations described for SPG3A patients, which are divided into five broad groups: 91.5% missense mutations, 4% small insertions, 2.8% small deletions, 0.7% splice site mutation, and one (0.7%) whole exon deletion (exon 4) (Sulek et al. 2013).

Mutations in the *SPAST* gene are responsible for both autosomal dominant HSP (40–50%) and sporadic cases (10–15%), causing spastic paraplegia type 4 (SPG4). The most common type of *SPAST* mutations are point mutations (75-80%), and large genomic abnormalities, such as exon deletions, account for up to 20-29% of disease-associated *SPAST* mutations (Beetz et al. 2006; d'Amore et al. 2018; Depienne et al. 2006; Kadnikova et al. 2019).

More information on HSP is available on https://www.ncbi.nlm.nih.gov/books/NBK1509/; https://www.ncbi.nlm.nih.gov/books/NBK1160/; https://www.ncbi.nlm.nih.gov/books/NBK45978/.

Gene structure
The *ATL1* gene spans 100 kilobases (kb) on chromosome 14q22.1 and contains 14 exons. The *ATL1* LRG_360 is available at www.lrg-sequence.org and is identical to GenBank NG_009028.1.

The *SPAST* gene spans 94 kilobases (kb) on chromosome 2p22.3 and contains 17 exons. The *SPAST* LRG_714 is available at www.lrg-sequence.org and is identical to GenBank NG_008730.1.

Transcript variants
For *ATL1,* multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/51062). Transcript variant 3 is the most predominant and is lacking exon 14 and includes exon 15 (NM_001127713.1; 2821 nucleotides (nt); coding sequence 426-2087). This sequence is a reference standard in the NCBI RefSeq project. Transcript variant 3 lacks an alternate in-frame exon and differs in the 5' UTR compared to variant 1.

For *SPAST,* multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/6683). Transcript variant 1 is the most predominant and the longest transcript (NM_014946.4; 5268 nt; coding sequence 277-2127).

Exon numbering
The *ATL1* exon numbering used in this P165-C3 HSP mix-1 product description is the exon numbering from the LRG_360 sequence. The *SPAST* exon numbering used in this P165-C3 HSP mix-1 product description is the exon numbering from the LRG_714 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 LRG 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 P165-C3 HSP mix-1 contains 47 MLPA probes with amplification products between 130 and 481 nt. This includes 16 probes for the *ATL1* gene and 20 probes for the *SPAST* gene. In addition, 11 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 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 different unrelated individuals who are from families without a history of hereditary 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. Sample ID numbers NA10401 and HG00500 from the Coriell Institute have been tested with this P165-C3 probemix at MRC-Holland and can be used as a positive control sample (see the table below).

Sample name	Source	Chromosomal position of copy number alteration	Altered target genes in P165-C3	Expected copy number alteration
NA10401	Coriell Institute	2p22.3	*SPAST*	Heterozygous duplication
HG00500	Coriell Institute	2p22.3	*SPAST* exons 4-17	Heterozygous duplication

Performance characteristics
Less than 1% of SPG type 3A cases can be explained by deletions in *ATL1* gene. Up to 29% of SPG type 4 cases can be explained by deletions or duplications in *SPAST* gene. The analytical sensitivity and specificity for the detection of deletions or duplications in the *ATL1* and *SPAST* genes is very high and can be considered >99% (based on a 2006-2020 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms 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.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 expected results for *ATL1* and *SPAST* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication).

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 *ATL1* and *SPAST* genes are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P165 HSP mix-1.
- 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/SPAST and https://databases.lovd.nl/shared/genes/ATL1. We strongly encourage users to deposit positive results in the LOVD mutation database. 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 *ATL1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P165-C3 HSP mix-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*ATL1*	*SPAST*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q31		
137	**SPAST probe** 05952-L05396			**Exon 13**
142 ¥	**SPAST probe** 22526-L31678			**Exon 1**
149 *	Reference probe 10056-L10480	8q22		
154 ¥	**ATL1 probe** 05277-L26403		**Exon 2b**	
161	**SPAST probe** 11061-L21076			**Exon 2**
166 ¥ ±	**ATL1 probe** 05279-L31680		**Exon 3**	
173	**SPAST probe** 07128-L06737			**Exon 17**
178 ±	**ATL1 probe** 05280-L04661		**Exon 4**	
184	**SPAST probe** 05949-L05391			**Exon 3**
190	Reference probe 16424-L18877	18q21		
196	**SPAST probe** 05268-L04651			**Exon 4**
202	**ATL1 probe** 05281-L04662		**Exon 5**	
208 ¥	**SPAST probe** 05951-L05395			**Exon 11**
214 ¥	**SPAST probe** 22527-L31867			**Exon 5**
220	**ATL1 probe** 05282-L04663		**Exon 6**	
228	Reference probe 08007-L07788	7q21		
235	**SPAST probe** 05265-L04648			**Exon 1**
241	**SPAST probe** 05658-L05111			**Exon 17**
247	**ATL1 probe** 05283-L04664		**Exon 7**	
254	Reference probe 13128-L14348	9q34		
263	**ATL1 probe** 17303-L20798		**Exon 1**	
269	**SPAST probe** 05270-L21080			**Exon 7**
276	**ATL1 probe** 05284-L21081		**Exon 8**	
282	**ATL1 probe** 20719-L28597		**Exon 1**	
290	Reference probe 02338-L21083	12q23		
296	**SPAST probe** 05271-L21084			**Exon 8**
302	**ATL1 probe** 05285-L04666		**Exon 9**	
310	Reference probe 13275-L14608	1p21		
319	**SPAST probe** 05272-L04646			**Exon 9**
327	**ATL1 probe** 05286-L04667		**Exon 10**	
337	Reference probe 07930-L07660	20p13		
346	**SPAST probe** 05273-L04655			**Exon 10**
355	**ATL1 probe** 05287-L05110		**Exon 11**	
364	**SPAST probe** 05953-L05397			**Exon 14**
373	**ATL1 probe** 05288-L04644		**Exon 12**	
382	**ATL1 probe** 05278-L04645		**Exon 2b**	
391	Reference probe 04530-L03919	2q24		
400	**SPAST probe** 05274-L04656			**Exon 12**
409	**ATL1 probe** 05289-L21085		**Exon 13**	
427	**SPAST probe** 05275-L04657			**Exon 15**
436	**ATL1 probe** 05290-L21086		**Exon 15**	
445	**SPAST probe** 20720-L28598			**Exon 16**
454	Reference probe 03856-L03307	17q11		
463	**SPAST probe** 05950-L05394			**Exon 6**
472	**SPAST probe** 07279-L21088			**Exon 1**
481	Reference probe 03328-L02715	3q26		

* New in version C3.
¥ Changed in version C3. Minor alteration, no change in sequence detected.
± SNP rs200452381 could influence the 166 nt probe signal and SNP rs145204580 could influence the 178 nt probe signal. In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.

Table 2. P165-C3 probes arranged according to chromosomal location

Table 2a. *SPAST*

Length (nt)	SALSA MLPA probe	SPAST exon[a]	Ligation site NM_014946.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*277-279 (exon 1)*		
472	07279-L21088	exon 1	123 nt before exon 1	AACTGCACATTG-GGAACTGTAGTT	0.4 kb
142	22526-L31678	exon 1	281-282	GCTGTGAATGAA-TTCTCCGGGTGG	0.1 kb
235	05265-L04648	exon 1	433-434	ACCTGTACTATT-TCTCCTACCCGC	23.5 kb
161	11061-L21076	exon 2	728-729	ATGGTATAAGAA-AGGTATTGAAGA	2.0 kb
184	05949-L05391	exon 3	826-825, reverse	CATAACCAAATT-AGTCATCATTTT	9.3 kb
196	05268-L04651	exon 4	914-915	GGACGTCTATAA-TGACAGTACTAA	15.9 kb
214	22527-L31867	exon 5	1036-1037	AAACAGTTATGA-AAACTGGATCTG	1.0 kb
463	05950-L05394	exon 6	1209-1210	ACTCGTAAGAAA-AAAGACTTGAAG	0.4 kb
269	05270-L21080	exon 7	1335-1336	GCAAAACAAGCA-TTGCAAGAAATT	10.8 kb
296	05271-L21084	exon 8	1418-1419	GCTGTTACTCTT-TGGTCCACCTGG	1.5 kb
319	05272-L04646	exon 9	1494-1495	TTCTTTAATATA-AGTGCTGCAAGT	8.1 kb
346	05273-L04655	exon 10	1557-1556, reverse	CGAGCCACAGCA-AAAAGAGCCCTC	0.3 kb
208	05951-L05395	exon 11	1626-1627	TTGTGTGAAAGA-AGAGAAGGGGAG	0.2 kb
400	05274-L04656	exon 12	1722-1723	AGAGTACTTGTA-ATGGGTGCAACT	4.8 kb
137	05952-L05396	exon 13	1794-1795	CGGGTATATGTG-TCTTTACCAAAT	1.5 kb
364	05953-L05397	exon 14	1878-1877, reverse	GCAAGTTGTGCT-AGTTCTTTTTGG	1.6 kb
427	05275-L04657	exon 15	1916-1917	ATACTCAGGAAG-TGACCTAACAGC	2.3 kb
445	20720-L28598	exon 16	1976-1977	ACTAAAACCAGA-ACAGGTGAAGAA	7.2 kb
241	05658-L05111	exon 17	2086-2087	CTTTAGAAGCGT-ACATACGTTGGA	1.9 kb
173	07128-L06737	exon 17	4016-4017	TAGCCATAAGGT-AAATCATGTCTC	
		stop codon	*2125-2127 (exon 17)*		

Table 2b. ATL1

Length (nt)	SALSA MLPA probe	ATL1 exon[a]	Ligation site NM_001127713.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*426-428 (exon 2b)*		
282	20719-L28597	exon 1	147-148	TATTGCACCTTT-AATGAGACCAGG	0.1 kb
263	17303-L20798	exon 1	256-257	ATTTGGCAACAT-CTCGGTGTCCTT	26.7 kb
154	05277-L26403	exon 2b	72 nt before exon 2b	GTATCTGTGTGA-ACTCGGGCTTGT	0.2 kb
382	05278-L04645	exon 2b	435-436	CCATGGCCAAGA-ACCGCAGGGACA	27.6 kb
166 ±	05279-L31680	exon 3	516-517	AGCCAGTGAAAA-AGGCAGGACCAG	3.1 kb
178 ±	05280-L04661	exon 4	732-733	GGGTTGGAGACT-ACAATGAACCAT	0.6 kb
202	05281-L04662	exon 5	899-898, reverse	GTGGCTGAATCT-CTCAAAGTTGAC	2.3 kb
220	05282-L04663	exon 6	21 nt after exon 6	TTTATTTTCTTT-TTTGTGTATCTG	1.7 kb
247	05283-L04664	exon 7	1026-1027	GCAGACTGGCAA-TGGAGGAAACAT	17.7 kb
276	05284-L21081	exon 8	1100-1101	CCATACGAATTT-TCATATGGAGCC	1.1 kb
302	05285-L04666	exon 9	1185-1184, reverse	GATGTGTTTTCT-GACGTTCTGTAG	6.3 kb
327	05286-L04667	exon 10	1361-1362	AGCCTAGATATT-AAAGAGATCAAT	1.2 kb
355	05287-L05110	exon 11	3 nt after exon 11, reverse	CTCATTAATAAA-TACCTGTAACAT	1.3 kb
373	05288-L04644	exon 12	1494-1495	AAGCTAACAATT-TAGCAGCCGTGG	4.9 kb
409	05289-L21085	exon 13	1594-1595	CTTGCAGACCAA-ACACCTGCAACT	4.2 kb
436	05290-L21086	exon 15	2033-2032, reverse	GGTGTAGGGAAA-GCTTGATGATAC	
		stop codon	*2085-2087 (exon 15)*		

[a] See section Exon numbering on page 2 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.

± SNP rs200452381 could influence the 166 nt probe signal and SNP rs145204580 could influence the 178 nt probe signal. In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.

Note: No probe for *ATL1* exon 14 is present, since this exon is not present in transcript variant 3 (NM_001127713.1).

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P211 HSP region	Contains probes for *SPAST*, *DPY30*, *SLC30A6*, *TUBGCP5*, *NIPA2* and *NIPA1*.
P213 HSP mix-2	Contains probes for *REEP1* and *SPG7*.
P306 Spastic Paraplegia 11 (SPG11)	Contains probes for *SPG11*, *B2M* and *CASC4*.

References

- Beetz C et al. (2006). High frequency of partial SPAST deletions in autosomal dominant hereditary spastic paraplegia. *Neurol*. 67(11):1926-1930.
- D'Amore A et al. (2018). Next generation molecular diagnosis of hereditary spastic paraplegias: an Italian cross-sectional study. *Front. Neurol*. 4.
- Depienne C et al. (2006). Exon deletions of SPG4 are a frequent cause of hereditary spastic paraplegia. *J Med Genet*. 44(4):281-284.
- Kadnikova VA et al. (2019). Mutational spectrum of Spast (Spg4) and Atl1 (Spg3a) genes in Russian patients with hereditary spastic paraplegia. *Scientific Reports* 9: 14412.
- 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.
- Sulek A et al. (2013). Screening for the hereditary spastic paraplaegias SPG4 and SPG3A with the multiplex ligation-dependent probe amplification technique in a large population of affected individuals. *Neurol Sci*. 34(2):239-242.
- 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 P165 mix-1

- Álvarez V et al. (2010). Mutational spectrum of the SPG4 (SPAST) and SPG3A (ATL1) genes in Spanish patients with hereditary spastic paraplegia. *BMC Neurol.* 10: 89.
- Cacheiro P et al. (2017). Evaluating the Calling Performance of a Rare Disease NGS Panel for Single Nucleotide and Copy Number Variants. *Molecular diagnosis & therapy*. 21:303-313.
- de Bot S et al. (2013). ATL1 and REEP1 mutations in hereditary and sporadic upper motor neuron syndromes. *J Neurol.* 260: 869-875.
- Elert-Dobkowska E et al. (2015). Molecular spectrum of the SPAST, ATL1 and REEP1 gene mutations associated with the most common hereditary spastic paraplegias in a group of Polish patients. *J Neurol Sci.* 359: 35-39.
- Erichsen AK et al. (2007). Seven novel mutations and four exon deletions in a collection of Norwegian patients with SPG4 hereditary spastic paraplegia. *Eur J Neurol*. 14(7):809-814.

- Kim TH et al. (2014). Mutation analysis of SPAST, ATL1, and REEP1 in Korean patients with hereditary spastic paraplegia. *J Clin Neurol.* 10: 257-261.
- Lu X et al. (2014). Genetic analysis of SPG4 and SPG3A genes in a cohort of Chinese patients with hereditary spastic paraplegia. *J Neurol Sci*. 347(1-2):368-371.
- Magariello A et al. (2010). Mutation analysis of the SPG4 gene in Italian patients with pure and complicated forms of spastic paraplegia. *J Neurol Sci*. 288(1-2):96-100.
- McCorquodale D et al. (2011). Mutation screening of spastin, atlastin, and REEP1 in hereditary spastic paraplegia. *Clin Genet.* 79: 523-530.
- Meszarosova AU et al. (2016). SPAST mutation spectrum and familial occurrence among Czech patients with pure hereditary spastic paraplegia. *J Hum Genet*. 61(10):845-850.
- Park H et al. (2015). Mutational spectrum of the SPAST and ATL1 genes in Korean patients with hereditary spastic paraplegia. *J Neurol Sci*. 357(1-2):167-172.
- Parodi L et al. (2018). Spastic paraplegia due to SPAST mutations is modified by the underlying mutation and sex. *Brain.* 141:3331-3342.
- Wei QQ et al. (2014). Spastin mutation screening in Chinese patients with pure hereditary spastic paraplegia. *Parkinsonism Relat Disord*. 20(8):845-849.
- Zhu Z et al. (2019). Novel mutations in the SPAST gene cause hereditary spastic paraplegia. *Parkinsonism & related disorders*. 69:125-133.

P165 product history	
Version	*Modification*
C3	One reference probe has been replaced and five probes have had a small change in length but no change in sequence detected.
C2	The length of some probes have been adjusted.
C1	Two probes for exon 1 of the *ATL1* gene have been included. Five reference probes and the 88 and 96 nt control fragments have been replaced.
B1	The 160 nt probe for *SPAST* exon 2 has been replaced.
A1	First release.

Implemented changes in the product description

Version C3-03 — 24-February-2023 (04P)
- Warning added to Table 1 and Table 2b for SNPs that could influence probe signal of 166 nt probe 05279-L31680, and 178 nt probe 05280-L04661.
- 167 nt (05279-L31680) probe length adjusted to 166 nt.
- Selected publications using P165 HSP mix-1 updated.
- Various minor textual and lay-out changes.

Version C3-02 — 15 January 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose is updated.
- Ligation sites of the probes targeting the *SPAST* gene updated according to new version of the NM_ reference sequence.

Version C3-01 — 12 August 2019 (02P)
- Product description rewritten and adapted to a new template.
- P165-C3 is now CE marked.
- Product description adapted to a new product version for P165 (version number changed, small changes in Table 1 and Table 2).
- For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36).

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

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.