Product Description

SALSA® MLPA® Probemix P213-B3 HSP mix-2

To be used with the MLPA General Protocol.

Version B3
Compared to B2 version, three reference probes have been replaced and one probe has a small change in length but no change in sequence detected. For complete product history see page 9.

Catalogue numbers:
- **P213-025R:** SALSA MLPA probemix P213 HSP mix-2, 25 reactions.
- **P213-050R:** SALSA MLPA probemix P213 HSP mix-2, 50 reactions.
- **P213-100R:** SALSA MLPA probemix P213 HSP 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.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 P213 HSP mix-2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplication in *REEP1* and *SPG7* genes, in order to confirm a potential cause for and clinical diagnosis of spastic paraplegia (SPG) type 31 and SPG type 7, 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 P213 HSP 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 the *REEP1* and *SPG7* 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 31 (SPG31) is caused by a pathogenic variant in the receptor expression-enhancing protein 1 (*REEP1*) and accounts for about 5% of all autosomal dominant HSP. The most common type of *REEP1* mutations explaining SPG31 are small frameshift mutations, but nonsense, missense, and microRNA target site alterations have been described (Beetz et al. 2008). 9.5% of pathogenic mutations in *REEP1* are copy number variations (Goizet et al. 2011).

SPG7 is caused by pathogenic variants in *SPG7* and may account for approximately 5% of all autosomal recessive HSP. Of all SPG7 cases, most are caused by *SPG7* point mutations (~98%), while less than 2% can be explained by *SPG7* copy number variations (Klebe et al. 2012, Pfeffer et al. 2015).

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

Gene structure
The *REEP1* gene spans 124 kilobases (kb) on chromosome 2p11.2 and contains 8 exons. The *REEP1* LRG_713 is available at www.lrg-sequence.org and is identical to GenBank NG_013037.1.

The *SPG7* gene spans 67 kilobases (kb) on chromosome 16q24.3 and contains 17 exons.

Transcript variants
For *REEP1*, multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/65055). Transcript variant 1 is the most predominant (NM_001164730.2; 3741 nt; coding sequence 8-634). This sequence is a reference standard in the NCBI RefSeq project.

For *SPG7*, multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/6687). Transcript variant 1 is the most predominant and the longest transcript (NM_003119.4; 3076 nt; coding sequence 16-2403). This sequence is a reference standard in the NCBI RefSeq project.

Exon numbering
The *REEP1* exon numbering used in this P213-B3 HSP mix-2 product description is the exon numbering from the LRG_713 sequence. The *SPG7* exon numbering used in this P213-B3 HSP mix-2 product description is the exon numbering from the RefSeq transcript NM_003119.4. 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 P213-B3 HSP mix-2 contains 42 MLPA probes with amplification products between 128 and 436 nucleotides (nt). This includes 10 probes for the *REEP1* gene and 21 probes for the *SPG7* gene. Additional probes are included for the exons 1 and 3 of *REEP1*, for exon 1 of *SPG7*, and for the last exon of alternative splice variant NM_199367.3 of *SPG7*, which is located within intron 9 of NM_003119.4. 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 HG00246, HG02128 and NA21108 from the Coriell Institute have been tested at MRC-Holland and can be used as positive control samples. HG00246 shows a heterozygous triplication of *REEP1* exons 3 to 8. HG02128 shows a heterozygous deletion of *SPG7* exons 1 to 7 and the *SPG7* upstream probe. NA21108 shows a heterozygous duplication of the complete *SPG7* gene. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration	Altered target genes in P213-B3	Expected copy number alteration
HG00246	Coriell Institute	2p11.2	*REEP1* exons 3-8	Heterozygous triplication
HG02128	Coriell Institute	16q24.3	*SPG7* exons 1-7 + *SPG7* upstream probe	Heterozygous deletion
NA21108	Coriell Institute	16q24.3	Complete *SPG7* gene	Heterozygous duplication

Performance characteristics
The frequency of *REEP1* deletions and duplications in SPG31 cases is ~9.5%. Less than 2% of SPG7 cases can be explained by *SPG7* deletions and duplications. The analytical sensitivity and specificity for the detection of deletions or duplications in the *REEP1* and *SPG7* 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 *REEP1* and *SPG7* 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 or in or near the *REEP1* 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.
- 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 *REEP1* and *SPG7* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P213 HSP mix-2.
- 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/SPG7 and https://databases.lovd.nl/shared/genes/REEP1. 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 *REEP1* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P213-B3 HSP mix-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*REEP1*	*SPG7*
64-105	Control fragments – see table in probemix content section for more information			
128	Reference probe 00797-L00093	5q31		
133	Reference probe 16316-L20697	3q21		
142	**REEP1 probe** 07248-L06819		**Exon 3**	
148	**SPG7 probe** 07265-L06836			**Exon 8**
154	Reference probe 04531-L05030	2q24		
160	**SPG7 probe** 17745-L21891			**Exon 2**
166	**SPG7 probe** 07268-L06839			**Exon 11**
172	**REEP1 probe** 20988-L29219		**Exon 5**	
177 ^	**SPG7 probe** 17746-L21892			**Intron 9**
184 *	Reference probe 19450-L25864	14q31		
190	**SPG7 probe** 07262-L21916			**Exon 5**
196	**SPG7 probe** 07271-L21917			**Exon 14**
202 «	**REEP1 probe** 07246-L06817		**Exon 2**	
208	**SPG7 probe** 07263-L06834			**Exon 6**
214	**SPG7 probe** 07257-L21923			**Exon 1**
220	**REEP1 probe** 07253-L08404		**Exon 7**	
226	**SPG7 probe** 07274-L08405			**Exon 17**
232	**SPG7 probe** 07266-L06837			**Exon 9**
238 «	**REEP1 probe** 17747-L21893		**Exon 1**	
245	**SPG7 probe** 07261-L08406			**Exon 4**
253	Reference probe 06236-L01311	21q11		
260	**REEP1 probe** 07254-L08407		**Exon 8**	
268	**SPG7 probe** 07256-L08408			**Upstream**
276	**SPG7 probe** 17902-L22475			**Exon 13**
283	**SPG7 probe** 17749-L21895			**Exon 3**
292	Reference probe 08936-L09031	11p15		
298 ¥	**SPG7 probe** 21471-L21918			**Exon 7**
310	**REEP1 probe** 07249-L21919		**Exon 3**	
319	**SPG7 probe** 07272-L06843			**Exon 15**
328	**REEP1 probe** 07252-L06823		**Exon 6**	
337	**SPG7 probe** 07267-L06838			**Exon 10**
346	Reference probe 04835-L04219	5p13		
355	Reference probe 05991-L05416	20p12		
363	**SPG7 probe** 07273-L21920			**Exon 16**
373	Reference probe 10693-L11275	6p12		
382 «	**REEP1 probe** 07245-L21921		**Exon 1**	
391 ^	**SPG7 probe** 17750-L21896			**Intron 9**
400	**REEP1 probe** 07250-L06821		**Exon 4**	
408	**SPG7 probe** 17751-L21897			**Exon 12**
417 *	Reference probe 18456-L23632	7q31		
427	**SPG7 probe** 07258-L06829			**Exon 1**
436 *	Reference probe 04279-L23590	12q12		

* New in version B3.
¥ Changed in version B3. Minor alteration, no change in sequence detected.
« 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.
^ These probes detect an alternative exon 10 which is only present in transcript variant 2 NM_199367.2 (see Table 2b).

Table 2. P213-B3 probes arranged according to chromosomal location

Table 2a. *REEP1* gene

Length (nt)	SALSA MLPA probe	REEP1 exon[a]	Ligation site NM_001164730.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*8-10 (exon 1)*		
382 «	07245-L21921	Exon 1	27 nt before exon 1	TCTGTAGAATTT-TAAGTAGGGAGG	0.1 kb
238 «	17747-L21893	Exon 1	42-43	ACAGACTGAAGA-GGTTAGGTCTGG	0.5 kb
202 « ๑	07246-L06817	Exon 2	534 nt after exon 1; reverse	CACCACCAGCCT-GGAGATGATCCA	55.3 kb
142	07248-L06819	Exon 3	100-99; reverse	TTCACAGCCTTG-TAGGAATAATAC	0.2 kb
310	07249-L21919	Exon 3	107 nt after exon 3	GAAGCAGGGAAA-AGGAGTTCCTTC	18.1 kb
400	07250-L06821	Exon 4	200-201	TCACAGACATCT-TCCTTTGTTGGT	9.2 kb
172	20988-L29219	Exon 5	272-273	CTCCCTACACAA-AAGGCTCCAGCC	2.7 kb
328	07252-L06823	Exon 6	11 nt before exon 6	TGCCTCTGTTTT-TCCTTTGACAGG	19.3 kb
220	07253-L08404	Exon 7	458-459	GACAGGGTGCCT-TATCGGAGAGAC	15.9 kb
260	07254-L08407	Exon 8	811-812	TGCAGCCTCACA-AACATGGCCTTT	
		stop codon	*632-634 (exon 8)*		

Table 2b. *SPG7* gene

Length (nt)	SALSA MLPA probe	SPG7 exon[a]	Ligation site NM_003119.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*16-18 (exon 1)*		
268	07256-L08408	Upstream	0.9 kb before exon 1	GACAGACCATCT-AGTCTTTGCTTT	0.9 kb
214	07257-L21923	Exon 1	13-14	GCTTTCAGGCCA-ACATGGCCGTGC	0.5 kb
427	07258-L06829	Exon 1	307 nt after exon 1	GTGGAATCCAGT-AACGGTTTCCGG	1.7 kb
160	17745-L21891	Exon 2	1 nt after exon 2	GCAACTTTTAGG-TATGTATCTGTT	2.4 kb
283	17749-L21895	Exon 3	383-384	GGCGCCTGAAGA-GGACGAAGGTAT	11.1 kb
245	07261-L08406	Exon 4	524-525	CTGGAACGACTT-TGTCCACGAGAT	2.2 kb
190	07262-L21916	Exon 5	686-687	CAAGTTTGAAGA-GAAGCTTCGAGC	3.2 kb
208	07263-L06834	Exon 6	868-869	AAGGTGGATTCA-GTGCTTTTGTAA	1.2 kb
298 ¥	21471-L21918	Exon 7	956-957	CGTGGCAGGAAT-GCACGAAGCCAA	1.1 kb
148	07265-L06836	Exon 8	1 nt before exon 8	TGCTGCCGTCCA-GAGCCCAGAACG	0.6 kb
232	07266-L06837	Exon 9	1259-1258; reverse	TGGAGCGCTTCT-TGCCCACCGCGT	4.5 kb
177 ∧	17746-L21892	Intron 9	4.5 kb after exon 9	CCCTTTATGCAG-AGCCACAGCTGT	0.4 kb
391 ∧	17750-L21896	Intron 9	4.8 kb after exon 9	AAGACGACGGCC-CCTGACGTGCAG	7.2 kb
337	07267-L06838	Exon 10	1409-1410	GGACGGTGCTCT-GATGAGGCCAGG	1.9 kb
166	07268-L06839	Exon 11	1481-1480; reverse	GGTGCTGCTCAA-AAATCTCCCGCC	1.3 kb
408	17751-L21897	Exon 12	1579-1580	GGGCTGACATCG-CCAACATCTGCA	2.5 kb
276	17902-L22475	Exon 13	1690-1691	GGACTGCCAAAA-AGAGCAAGATCC	2.5 kb
196	07271-L21917	Exon 14	12 nt before exon 14	TGGGTCATCTTG-ACCTTGTGCCAG	0.9 kb
319	07272-L06843	Exon 15	2008-2009	TGGTGAAGCAGT-TTGGGATGGCAC	0.7 kb
363	07273-L21920	Exon 16	2161-2162	GACACACCGAGA-AGGTGCTGCAGG	2.5 kb
226	07274-L08405	Exon 17	2372-2373	GCAGCCTCCACT-TGGAGGCGAAGA	
		stop codon	*2401-2403 (exon 17)*		

[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.

« 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.
๑ This probe detects exon 2 which is present in transcript variant 2 NM_022912.3 with ligation site 138-137 (reverse).
¥ Changed in version B3. Minor alteration, no change in sequence detected.

^ These probes detect an alternative exon 10 which is only present in transcript variant 2 NM_199367.3, with ligation sites 1673-1674 (probe 177nt) and 2056-2057 (probe 391nt) in NM_199367.3.

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

Related SALSA MLPA probemixes

- P165 HSP: Contains probes for *ATL1* and *SPAST*.
- P211 HSP region: Contains probes for *SPAST*, *DPY30*, *SLC30A6*, *TUBGCP5*, *NIPA2* and *NIPA1*.
- P306 Spastic Paraplegia 11 (SPG11): Contains probes for *SPG11*.

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P213 product history	
Version	*Modification*
B3	Three reference probes have been replaced. One target probe has a small change in length but no change in sequence detected.
B2	The length of one probe was adjusted.
B1	Two probes for *SPG7* have been added and four replaced, in addition one probe for *REEP1* has been added and two removed and six reference probes have been replaced, 88 and 96 nt control fragments have been replaced (QDX2).
A2	Four extra control fragments at 88-96-100-105 nt.
A1	Several *REEP1* and *SPG7* probes have a small change in length. No changes in sequences detected. Some reference probes have been replaced.
A0	First release.

Implemented changes in the product description

Version B3-02 — 15 January 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- Ligation sites of the probes targeting the *REEP1* and *SPG7* genes updated according to new version of the NM_ reference sequence.

Version B3-01 – 21 February 2019 (04)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version for P213 (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).

Version 16 (55) – 24 June 2016
- Product description adapted to a new product version (version number changed, lot number added, new picture included).
- Changed name of *SPG3a* gene to *ATL1* at page 1, adapting HUGO nomenclature.
- Changed probemix name from “HSP2” to “HSP mix-2”.

Version 15 – 23 February 2016 (55)
- Ligation sites of the *SPG7* gene adjusted according to reference sequence NM_003119.3 and sequence NM_199367.2 in Table 1 and 2b.
- Various minor textual changes throughout the document.

Version 14 (53)
- Exon numbering of the *SPG7* gene has been changed in Table 1 and 2b.

Version 13 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 12 (48)
- Product description adapted to a new product version (version number changed, lot number added, new picture included).
- Various minor textual changes throughout the document.

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