Product Description
SALSA® MLPA® Probemix P216-C1 Growth Hormone Deficiency

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 9.

Catalogue numbers:
- **P216-025R:** SALSA MLPA Probemix P216 Growth Hormone Deficiency, 25 reactions.
- **P216-050R:** SALSA MLPA Probemix P216 Growth Hormone Deficiency, 50 reactions.
- **P216-100R:** SALSA MLPA Probemix P216 Growth Hormone Deficiency, 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 P216 Growth Hormone Deficiency is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GH1*, *POU1F1*, *PROP1*, *GHRHR*, *LHX3*, *LHX4*, and *HESX1* genes, which are associated with Growth Hormone Deficiency (GHD).

GHD is characterised by short stature, delayed growth velocity, and delayed skeletal maturation. Idiopathic growth hormone deficiency is the most common cause of GHD in children.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1347/.

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 *GH1*, *POU1F1*, *PROP1*, *GHRHR*, *LHX3*, *LHX4*, and *HESX1* exon numbering used in this P216-C1 Growth Hormone Deficiency product description is the exon numbering from the NM_000515.5, NG_008225.2, NG_015889.2, NG_021416.1, NG_008097.1, NG_008081.1, and NG_008242.2 sequences, respectively. 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 P216-C1 Growth Hormone Deficiency contains 48 MLPA probes with amplification products between 130 and 505 nucleotides (nt). This includes four probes for the *GH1* gene, five

probes for the *POU1F1* gene, three probes for the *PROP1* gene, 12 probes for the *GHRHR* gene, six probes for the *LHX3* gene, six probes for the *LHX4* gene, and finally four probes for the *HESX1* gene. In addition, eight 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 Growth Hormone Deficiency. 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 or in or near the *LHX3* 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 *GH1*, *POU1F1*, *PROP1*, *GHRHR*, *LHX3*, *LHX4*, and *HESX1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P216 Growth Hormone Deficiency.

- 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.

Mutation database

https://databases.lovd.nl/shared/genes. 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 *GHRHR* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P216-C1 Growth Hormone Deficiency

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	Target gene
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 20108-L27306	8p	
137	**GHRHR probe** 07905-L31750		**Exon 2**
142 «	**LHX3 probe** 07230-L06880		**Exon 6**
148	**POU1F1 probe** 07241-L06891		**Exon 6**
154	**GHRHR probe** 07212-L06862		**Exon 9**
163	**PROP1 probe** 07904-L07642		**Exon 1**
170	**LHX4 probe** 07232-L08335		**Exon 2**
175	Reference probe 19291-L31751	4q	
181	**POU1F1 probe** 07240-L08337		**Exon 4**
190	**GH1 probe** 17430-L21430		**Exon 4**
196	**LHX4 probe** 07235-L08508		**Exon 5**
202	**PROP1 probe** 21349-L31752		**Exon 3**
214	**HESX1 probe** 07223-L20027		**Exon 3**
220 *	Reference probe 12427-L13428	22q	
226	**POU1F1 probe** 07238-L21577		**Exon 2**
232 Ж	**GH1 probe** 17015-SP0428-L21431		**Upstream**
240 Ж	**HESX1 probe** 17016-SP0429-L21432		**Exon 4**
247	**POU1F1 probe** 07239-L06889		**Exon 3**
254 ¥ «	**LHX3 probe** 07228-L32956		**Exon 4**
260 « Δ	**LHX3 probe** 15634-L20686		**Exon 1**
265	**GH1 probe** 07218-L06868		**Exon 3**
271 Ж	**GHRHR probe** 17017-SP0430-L20065		**Exon 3**
278	**LHX4 probe** 07233-L20028		**Exon 3**
285 «	**LHX3 probe** 07907-L20029		**Exon 2**
292	Reference probe 08790-L11322	10q	
301 «	**LHX3 probe** 07679-L06879		**Exon 5**
310	**HESX1 probe** 07221-L08511		**Exon 1**
319	Reference probe 17521-L21420	2q	
328	**GHRHR probe** 07214-L06864		**Exon 11**
337	**POU1F1 probe** 07237-L06887		**Exon 1**
347	**GHRHR probe** 07207-L06857		**Exon 4**
355	**LHX4 probe** 07236-L06886		**Exon 6**
364	Reference probe 14946-L16679	6q	
375	**GHRHR probe** 07213-L06863		**Exon 10**
382	**PROP1 probe** 07243-L06893		**Exon 2**
391	**LHX4 probe** 07234-L06884		**Exon 4**
400	**GHRHR probe** 07678-L06854		**Exon 1**
409	**GHRHR probe** 07208-L06858		**Exon 5**
419	**GHRHR probe** 07215-L06865		**Exon 12**
427	**HESX1 probe** 15091-L16864		**Exon 2**
436	**GHRHR probe** 07210-L06860		**Exon 7**
445	**GHRHR probe** 07216-L06866		**Exon 13**
454	**LHX4 probe** 12872-L06881		**Exon 1**
463	Reference probe 14308-L15978	15q	
472	**GH1 probe** 17018-L20066		**Exon 5**
480 «	**LHX3 probe** 17019-L20067		**Exon 3**
490 Ж	**GHRHR probe** 17020-SP0431-L20068		**Exon 6**
505	Reference probe 14883-L27237	14q	

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

* New in version C1.

¥ Changed in version C1. 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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. P216-C1 probes arranged according to chromosomal location

Table 2a. *LHX4*

Length (nt)	SALSA MLPA probe	*LHX4* exon[a]	Ligation site NM_033343.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*267-269 (Exon 1)*		
454	12872-L06881	Exon 1	229-230	GAGAGCGAGAGA-TCTCCGTAGACT	17.8 kb
170	07232-L08335	Exon 2	366-367	GCGCTGGCTGCA-ACCAGCACATCC	18.3 kb
278	07233-L20028	Exon 3	20 nt after exon 3	CATGGCCCCGCA-TGGTCCCCTCTC	4.8 kb
391	07234-L06884	Exon 4	738-739	AGGCTGGAGCTA-AGCGGCCCCGGA	0.5 kb
196	07235-L08508	Exon 5	899-900	AGAAGGGCCAAA-GAGAAACGCCTG	2.4 kb
355	07236-L06886	Exon 6	1092-1093	GGATTTATGGCA-ACGTGGGGGACG	
		stop codon	*1437-1439 (Exon 6)*		

Table 2b. *HESX1*

Length (nt)	SALSA MLPA probe	*HESX1* exon[a]	Ligation site NM_003865.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*61-63 (Exon 1)*		
310	07221-L08511	Exon 1	48-47, reverse	CTCTCGTGGTCT-GCACAGAGCAAC	1.1 kb
427	15091-L16864	Exon 2	276-277	ATTTCATTCCCT-AGCGTGGTGGAT	0.5 kb
214	07223-L20027	Exon 3	474-475	ATCGATATTAGA-GAAGACTTAGCT	0.3 kb
240 Ж	17016-SP0429-L21432	Exon 4	638-639 and 675-676	CTAAACAAGTGA-37 nt spanning oligo-AAATATTAAGTG	
		stop codon	*616-618 (Exon 4)*		

Table 2c. *POU1F1*

Length (nt)	SALSA MLPA probe	*POU1F1* exon[a]	Ligation site NM_000306.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*123-125 (Exon 1)*		
337	07237-L06887	Exon 1	205-206	TCTGATAATGCA-TCACAGTGCTGC	3.0 kb
226	07238-L21577	Exon 2	310-311	TTATGGAAACCA-GCCATCAACCTA	8.9 kb
247	07239-L06889	Exon 3	406-407	TCCTATACACCA-GCCTCTTCTGGC	2.3 kb
181	07240-L08337	Exon 4	615-616	ATGGCTCTGAAT-TCAGTCAAACAA	2.2 kb
148	07241-L06891	Exon 6	861-862	AGATCATGAGGA-TGGCTGAAGAAC	
		stop codon	*996-998 (Exon 6)*		

Table 2d. *PROP1*

Length (nt)	SALSA MLPA probe	*PROP1* exon[a]	Ligation site NM_006261.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*310-312 (Exon 1)*		
163	07904-L07642	Exon 1	266-265, reverse	TCTGACTTGAGA-TTTCTCTGCTTC	1.6 kb
382	07243-L06893	Exon 2	2 nt before exon 2, reverse	AGCACTCGAGTC-TGAGAACGGAGA	1.7 kb
202	21349-L31752	Exon 3	999-1000	TGAGGTCAAACA-AGTACCACCAAG	
		stop codon	*988-990 (Exon 3)*		

Table 2e. *GHRHR*

Length (nt)	SALSA MLPA probe	*GHRHR* exon[a]	Ligation site NM_000823.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*117-119 (Exon 1)*		
400	07678-L06854	Exon 1	166-167	GTTGAGCCCGTT-ACCGACCGTGAG	4.7 kb
137	07905-L31750	Exon 2	29 nt before exon 2, reverse	CAGGATGAGCCA-AGCCATTTGGGT	0.3 kb
271 Ж	17017-SP0430-L20065	Exon 3	304-305, 328-329	GGATGGGCTGCT-24 nt spanning oligo -CGAGTGGGTCAC	0.8 kb
347	07207-L06857	Exon 4	408-409	GGGATTGTACTA-TCACTGGCTGGT	1.3 kb
409	07208-L06858	Exon 5	553-554	AGCCCTCTTCGT-GGCCATCACCAT	0.8 kb
490 Ж	17020-SP0431-L20068	Exon 6	9 nt before exon 6, 598-599	CATTTCTCCCAT-27 nt spanning oligo -GAACTACGTCCA	2.1 kb
436	07210-L06860	Exon 7	777-778	TCAGCTGGCTGT-TGGCAGAAGCCG	1.0 kb
154	07212-L06862	Exon 9	982-983	CAAAGGGCCCAT-TGTCCTCTCGGT	0.8 kb
375	07213-L06863	Exon 10	1058-1059	CTGGAGCCAGCT-CAGGGCAGCCTC	0.6 kb
328	07214-L06864	Exon 11	1128-1129	TGATCCCACTCT-TTGGAATTCACT	0.9 kb
419	07215-L06865	Exon 12	1259-1260	TTCCTCAACCAA-GAGGTGTGTGAT	1.9 kb
445	07216-L06866	Exon 13	1405-1404, reverse	GTGGACTCCAGT-GGCGTGATGAGG	
		stop codon	*1386-1388 (Exon 13)*		

Table 2f. *LHX3*

Length (nt)	SALSA MLPA probe	*LHX3* exon[a]	Ligation site NM_178138.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*117-119 (Exon 1)*		
260 « Δ	15634-L20686	Exon 1	4 nt after exon 1	GACTCGGGGTAA-GCCCCAGCAGGA	4.3 kb
285 «	07907-L20029	Exon 2	273-274	GCCACTGGCACA-GCAAGTGTCTCA	1.0 kb
480 «	17019-L20067	Exon 3	544-543, reverse	TTTCGTAGTCCG-CCTTGCACACGA	0.7 kb
254 «	07228-L32956	Exon 4	633-634	TGGAGACGCTGA-AGAGCGCTTACA	0.3 kb
301 «	07679-L06879	Exon 5	854-855	GACAGCGTTCAG-GAGGGGCAGGAC	1.8 kb
142 «	07230-L06880	Exon 6	1782-1783	TTGGCCTTGCCT-GTCGAGGCAAGA	
		stop codon	*1308-1310 (Exon 6)*		

Table 2g. *GH1*

Length (nt)	SALSA MLPA probe	*GH1* exon[a]	Ligation site NM_000515.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*64-66 (Exon 1)*		
232 Ж #	17015-SP0428-L21431	Upstream	730 nt and 703 nt before exon 1	ATGGGAGGAAGA-27 nt spanning oligo -TTTCTGTTTCTT	1.5 kb
265 #	07218-L06868	Exon 3	300-299, reverse	GACTCTGAGAAA-CAGAGGGAGGTC	0.3 kb
190 #	17430-L21430	Exon 4	459-460	TACGGCGCCTCT-GACAGCAACGTC	0.5 kb
472 #	17018-L20066	Exon 5	773-772, reverse	CTGGAGTGGCAA-CTTCCAGGGCCA	
		stop codon	*715-717 (Exon 5)*		

[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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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

P217 IGF1R	Contains probes for *IGF1R* and *IGFBP3*.
P262 GH1	Contains probes for *IGF1*, *GHR*, *JAK2*, and *STAT5B*.
P026 Sotos	Contains probes for *NSD1* and *NFIX*.
P018 SHOX	Contains probes for *SHOX* and several other probes in the PAR region.
P210 BTK	Contains probes for *BTK*.

References

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- Takagi, M et al. (2017). A novel heterozygous intronic mutation in POU1F1 is associated with combined pituitary hormone deficiency. *Endocr J*, 64(2), 229-234.
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P216 product history	
Version	*Modification*
C1	One reference probe has been replaced, one probe length adjusted and one *LHX3* probe removed.
B2	Four reference probes have been replaced and three probe lengths have been adjusted.
B1	Reference probes are included and several target probes are exchanged.
A1	First release.
B2	Four reference probes have been replaced and three probe lengths have been adjusted.

Implemented changes in the product description

Version C1-01 – 03 August 2023 (04P)
- Product renamed from Growth Hormone Deficiency mix-1 to Growth Hormone Deficiency
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *GH1, POU1F1, GHRHR, LHX3, LHX4*, and *HESX1* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version B2-02 — 01 June 2021 (02P)
- Gene name is corrected in table 2g from *LHX3* to *GH1*.

Version B2-01 — 14 August 2019 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *LHX3* gene updated according to new version of the NM_ reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 13 – 31 August 2015 (55)
- Product description adapted to a new lot (lot number, changes in Table 1 and Table 2, new picture included).
- Exon numbering of the *LHX3* gene has been updated according to NM_178138.4.
- Various minor textual changes.

Version 12 (54) – 26 February 2015
- New sample picture included in product description.
- Data analysis method has been modified.
- Updated link for “Database of Genomic Variants”.

Version 11 (51)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- New references added on page 1.
- Warning added below Table 2 that the NM_ reference sequence has changed.
- Warning added: ๏ The significance of exon 1 deletions is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

- Various minor textual changes on page 1.
- Various minor layout changes.
- Several warnings added in Table 1 and 2.
- Data analysis method has been modified.
- Ligation sites of the probes targeting the *POU1F1* gene updated according to new version of the NM_reference sequence.

Version 10 (48)

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

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