Product Description SALSA® MLPA® Probemix P133-C3 Kallmann-2

To be used with the MLPA General Protocol.

Version C3. As compared to version C2, three reference probes have been replaced and one reference probe has been removed. For complete product history see page 8.

Catalogue numbers:
- **P133-025R:** SALSA MLPA Probemix P133 Kallmann-2, 25 reactions.
- **P133-050R:** SALSA MLPA Probemix P133 Kallmann-2, 50 reactions.
- **P133-100R:** SALSA MLPA Probemix P133 Kallmann-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.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P133 Kallmann-2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FGFR1*, *GNRHR*, *KISS1R*, *GNRH1*, *NELF*, *PROK2* and *PROKR2* genes, which are associated with Kallmann syndrome.

Kallmann syndrome is a disease of idiopathic hypogonadotropic hypogonadism with anosmia or hyposmia. Defects in several genes can result in a Kallmann syndrome phenotype.
Loss-of-function mutations in ***FGFR1*** underlie Kallmann syndrome 2 (KAL2), whereas a gain-of-function mutation in *FGFR1* has been shown to cause a form of craniosynostosis. The *FGFR1* gene (18 exons) spans ~58 kb of genomic DNA and is located on chromosome 8p12, 38 Mb from the p-telomere. This P133-C3 Kallmann-2 probemix contains 11 probes for 10 of the 18 exons of the *FGFR1* gene.
Defects in the ***KISS1R*** gene are a possible cause of hypogonadotropic hypogonadism. The *KISS1R* gene (5 exons) spans ~4 kb of genomic DNA and is located on chromosome 19p13.3, 0.9 Mb from the p-telomere. This probemix contains probes for 3 of the 5 exons of the *KISS1R* gene.
A candidate gene for the autosomal form of Kallmann syndrome is the ***NELF*** *(NSMF)* gene. The *NELF* gene (16 exons) spans ~12 kb and is located on chromosome 9q34.3, 137 Mb from the p-telomere. This P133-C3 Kallmann-2 probemix contains probes for 3 of the 16 exons of the *NELF* gene.
An autosomal recessive form of Kallmann syndrome can be caused by defects in the ***GNRH1*** gene. The *GNRH1* gene comprises 3 exons and spans ~5 kb on chromosome 8p21.2, 25 Mb from the p-telomere. Probes for each exon of the *GNRH1* gene are included in this probemix.
Defects in the ***GNRHR*** gene are a cause of hypogonadotropic hypogonadism without anosmia. The *GNRHR* gene (3 exons) spans ~19 kb and is located on chromosome 4q13.2, 68 Mb from the p-telomere. Probes for each exon of the *GNRHR* gene are included in this probemix.
The ***PROK2*** and ***PROKR2*** genes have been implicated in autosomal recessive Kallmann syndrome (Abreu et al. 2008). The *PROK2* gene (4 exons) spans 14 kb on chromosome 3p13, 72 Mb from the p-telomere. The *PROKR2* gene (3 exons), spans 12 kb and is located on chromosome 20p12.3, 5 Mb from the p-telomere. Probes for each of the four *PROK2* exons and for two *PROKR2* exons have been included in this P133-C3 probemix.

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 *FGFR1*, *GNRHR*, *KISS1R*, *GNRH1*, *NELF*, *PROK2* and *PROKR2* exon numbering used in this P133-C3 Kallmann-2 product description is the exon numbering from the RefSeq transcripts NM_023110.2, NM_000406.3, NM_032551.5, NM_000825.3, NM_001130969.1, NM_001126128.2, and NM_144773.3, which are identical to the LRG_993, NG_009293.1, NG_008277.1, NM_000825.3, NG_021362.1, NG_008275.1, and NG_008132.2 sequences, respectively. The exon numbering and NM_ sequence used have been retrieved on 07/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P133-C3 Kallmann-2 contains 40 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 31 probes for the different genes associated with Kallmann syndrome. In addition, 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.mlpa.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.mlpa.com.

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

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *NELF, KISS1R* and *PROK2* genes. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *FGFR1*, *GNRHR*, *KISS1R*, *GNRH1*, *NELF*, *PROK2,* and *PROKR2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P133 Kallmann-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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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. 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 SNPs and unusual results (e.g., a duplication of *FGFR1* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P133-C3 Kallmann-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		reference	other	*GNRHR*	*PROK2*	*KISS1R*	*FGFR1*
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 00797-L13645	5q31					
142 Δ	**FGFR1 probe** 11950-L12769						**Exon 1**
148	**GNRHR probe** 04445-L03831			**Exon 1**			
154 «	**PROK2 probe** 11953-L12773				**Exon 3**		
160 «	**NELF probe** 04453-L03839		**Exon 6**				
165 Δ	**FGFR1 probe** 04432-L03818						**Exon 1**
172	Reference probe 13229-L14562	1p21					
178 Δ	**FGFR1 probe** 04184-L03583						**Exon 5**
184	**GNRHR probe** 04446-L04844			**Exon 1**			
190	**PROKR2 probe** 11954-L12774		**Exon 2**				
196 «	**KISS1R probe** 11955-L12775					**Exon 5**	
202 « Δ	**KISS1R probe** 04449-L03835					**Exon 1**	
210	**FGFR1 probe** 04434-L03820						**Exon 3**
214 « ±	**PROK2 probe** 21880-L30474				**Exon 1**		
220 « ¬ Δ	**STK11 probe** 03126-L03339					**Exon 3**	
229	**GNRH1 probe** 04442-L03828		**Exon 1**				
237	Reference probe 02334-L17301	12q23					
244 «	**PROK2 probe** 11956-L12776				**Exon 4**		
256 «	**NELF probe** 21565-L04184		**Exon 13**				
265	**FGFR1 probe** 04436-L03822						**Exon 6**
274	Reference probe 05960-L05377	7p11					
283	**GNRHR probe** 04447-L05586			**Exon 2**			
292	**FGFR1 probe** 04437-L03823						**Exon 8**
301 *	Reference probe 02471-L01915	15q21					
309 *	Reference probe 15069-L16827	2q37					
319	**FGFR1 probe** 04438-L03824						**Exon 10**
328 « Δ	**KISS1R probe** 04450-L18833					**Exon 4**	
337	**GNRH1 probe** 04443-L03829		**Exon 2**				
346	**FGFR1 probe** 04439-L03825						**Exon 13**
355	Reference probe 03092-L02492	11p13					
363 «	**NELF probe** 04455-L03841		**Exon 16**				
375	**FGFR1 probe** 01046-L00624						**Exon 2**
382 «	**PROK2 probe** 11957-L12777				**Exon 2**		
391	**PROKR2 probe** 11958-L12778		**Exon 3**				
400	**FGFR1 probe** 04440-L03826						**Exon 14**
409	Reference probe 07455-L07103	17q21					
424	**FGFR1 probe** 04441-L21311						**Exon 18**
430	**GNRH1 probe** 04444-L21310		**Exon 3**				
445	**GNRHR probe** 04448-L03834			**Exon 3**			
454 *	Reference probe 09182-L08064	10p13					

a) See above section on exon numbering for more information.

* New in version C3.
± SNP rs372623686 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
¬ 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.

Table 2. P133-C3 probes arranged according to chromosomal location

Table 2a. *FGFR1* gene

Length (nt)	SALSA MLPA probe	***FGFR1*** **exon**[a]	Ligation site NM_023110.3	**Partial sequence**[b] **(24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*744-746 (Exon 2)*		
165 Δ	04432-L03818	Exon 1	154 nt before exon 1	GCTGTAGGAAGA-GAAGGGCAGAGC	0.5 kb
142 Δ	11950-L12769	Exon 1	347-348	GTCTGCAAGGAA-AGTGAGGCGCCG	10.8 kb
375	01046-L00624	Exon 2	732-733	CAACCTCTAACT-GCAGAACTGGGA	27.6 kb
210	04434-L03820	Exon 3	937-938	GCAGAGCATCAA-CTGGCTGCGGGA	1.9 kb
178 Δ	04184-L03583	Exon 5	1282-1283	CAAATGCCCTTC-CAGTGGGACCCC	1.8 kb
265	04436-L03822	Exon 6	1391-1392	TGGAGCATCATA-ATGGACTCTGTG	4.3 kb
292	04437-L03823	Exon 8	1741-1742	AAATGTCTCCTT-TGAGGACGCAGG	3.5 kb
319	04438-L03824	Exon 10	2060-2061	TCCATGAACTCT-GGGGTTCTTCTG	2.4 kb
346	04439-L03825	Exon 13	2511-2512	ACCCCAGCCACA-ACCCAGAGGAGC	1.1 kb
400	04440-L03826	Exon 14	2609-2610	TGCATACACCGA-GACCTGGCAGCC	1.6 kb
424	04441-L21311	Exon 18	3530-3531	AGCCAATGAACA-GGCATGCAAGTG	
		stop codon	*3210-3212 (Exon 18)*		

Table 2b. *GNRH1* gene

Length (nt)	SALSA MLPA probe	***GNRH1*** **exon**	Ligation site NM_000825.3	**Partial sequence (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*1699-1701 (Exon 1)*		
229	04442-L03828	Exon 1	965-966	TAATGATGTCCT-GTCCTTCACTGT	2.5 kb
337	04443-L03829	Exon 2	1917-1918	CACCAGCCACGT-TCTCCCCTCCGA	2.2 kb
430	04444-L21310	Exon 3	1990-1991	AGAAGATTTAAA-TCCATTGGGCCA	
		stop codon	*1987-1989 (Exon 3)*		

Table 2c. *GNRHR* gene

Length (nt)	SALSA MLPA probe	***GNRHR*** **exon**	Ligation site NM_000406.3	**Partial sequence (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*54-56 (Exon 1)*		
148	04445-L03831	Exon 1	1272 nt before exon 1	GGACTGGTCTAA-GCTGCTCAAGAT	1.6 kb
184	04446-L04844	Exon 1	365-366	TGGAACATTACA-GTCCAATGGTAT	9.4 kb
283	04447-L05586	Exon 2	717-718	GCCTCTTCATCA-TCCCTCTTTTCA	4.0 kb
445	04448-L03834	Exon 3	859-860	TCTAAAAATGAC-GGTTGCATTTGC	
		stop codon	*1038-1040 (Exon 3)*		

Table 2d. *NELF* gene

Length (nt)	SALSA MLPA probe	***NELF*** **exon**	Ligation site NM_001130969.1	**Partial sequence (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*233-235 (Exon 1)*		
160 «	04453-L03839	Exon 6	992-993	GGGAGAATGATT-CCGCGTCTGTAA	5.0 kb
256 «	21565-L04184	Exon 13	1499-1500	ATCTCCTCAAGA-ACAAGGTGGCCA	2.0 kb
363 «	04455-L03841	Exon 16	2956-2957	ACGATGTACGAT-ACCCTCATAGTG	
		stop codon	*1823-1825 (Exon 16)*		

Table 2e. *KISS1R* gene

Length (nt)	SALSA MLPA probe	***KISS1R*** **exon**	Ligation site NM_032551.5	**Partial sequence (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*171-173 (Exon 1)*		
202 « Δ	04449-L03835	Exon 1	315-316	TGCCGCTCTTCT-TCGCGGCGCTGA	2.3 kb
328 « Δ	04450-L18833	Exon 4	748-749	CTACTGCAGTGA-GGCCTTCCCCAG	0.6 kb
196 «	11955-L12775	Exon 5	1114-1115	GTCCTACAGCAA-CTCCGCGCTGAA	2.2 kb
		stop codon	*1365-1367 (Exon 5)*		
220 « ¬ Δ	03126-L03339	*STK11* exon 3		GCATGCAGGAAA-TGCTGGACAGCG	

Table 2f. *PROK2* gene

Length (nt)	SALSA MLPA probe	*PROK2* exon	Ligation site NM_001126128.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*97-99 (Exon 1)*		
214 « ±	21880-L30474	Exon 1	4 nt after exon 1	TCACCGGGGTAA-GCGGCCCGGGCG	3.4 kb
382 «	11957-L12777	Exon 2	259-260	GGGTCAAGAGCA-TAAGGATTTGCA	7.1 kb
154 «	11953-L12773	Exon 3	48 nt after exon 3	TGCCCAGAAGAA-TTAGCCTTTACA	1.6 kb
244 «	11956-L12776	Exon 4	420-421	CACACTTGCCCA-TGTCTGCCAGGC	
		stop codon	*484-486 (Exon 4)*		

Table 2g. *PROKR2* gene

Length (nt)	SALSA MLPA probe	*PROKR2* exon	Ligation site NM_144773.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*248-250 (Exon 2)*		
190	11954-L12774	Exon 2	430-431	ATTGCACTGGCA-GGCATCATGCTG	12.0 kb
391	11958-L12778	Exon 3	1262-1263	CGGTCAAGAACA-ACACCATGAAGT	
		stop codon	*1400-1402 (Exon 3)*		

a) See above section on exon numbering for more information.

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

± SNP rs372623686 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
¬ 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.

Related SALSA MLPA probemixes

P132 Kallmann-1 Contains probes for most exons of the *ANOS1* (*KAL1*) gene causing Kallmann syndrome.
P201 Charge Contains probes for the *CHD7* gene, involved in CHARGE syndrome.

References

- Abreu AP et al. (2008). Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome. *J Clin Endocrinol Metab*. 93:4113-4118.
- 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 P133 Kallmann-2

- Başaran Y et al. (2013). Analysis of KAL1, FGFR1, GPR54, and NELF copy number variations by multiplex ligation dependent probe amplification in male patients with idiopathic hypogonadotropic hypogonadism. *Turk J Med Sci.* 43:726-32.
- Francou B et al. (2016). Prevalence of KISS1 Receptor mutations in a series of 603 patients with normosmic congenital hypogonadotrophic hypogonadism and characterization of novel mutations: a single-centre study. *Hum Reprod*, 31(6), 1363-1374.
- Fukami M et al. (2013). Submicroscopic deletion involving the fibroblast growth factor receptor 1gene in a patient with combined pituitary hormone deficiency. *Endocr J* 60(8):1013-20.

- Laitinen EM et al. (2011). Incidence, phenotypic features and molecular genetics of Kallmann syndrome in Finland. *Orphanet J Rare Dis*, 6(1), 41.
- Ohtaka K et al. (2017). FGFR1 Analyses in Four Patients with Hypogonadotropic Hypogonadism with Split-Hand/Foot Malformation: Implications for the Promoter Region. *Hum mut.* 38(5): 503-506.
- Richters RJ et al. (2020). Oculoectodermal Syndrome–Encephalocraniocutaneous Lipomatosis Associated with NRAS Mutation. *Acta Derm Venereol*, 100(6-7), 1-2.
- Takagi M et al. (2016). Novel heterozygous mutation in the extracellular domain of FGFR1 associated with Hartsfield syndrome. *Hum genome var.* 3.

P133 Product history	
Version	*Modification*
C3	Three reference probes have been replaced and one reference probe has been removed.
C2	One reference probe has been replaced and two probe lengths have been adjusted.
C1	Eight reference probes and the 88 and 96 nt control fragments have been replaced (QDX2).
B1	Four probes for the *PROK2* gene, two probes for the *PROKR2* gene, and one additional *KISS1R* probe have been added. One *FGFR1* probe has been replaced. Finally, four extra control fragments at 88-96-100 and 105 nt have been added.
A1	First release.

Implemented changes in the product description

Version C3-01 — 08 September 2020 (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 *FGFR1*, *GNRHR*, *KISS1R*, and *PROK2* genes updated according to new version of the NM_ reference sequence.

Version C2-02 – 21 March 2019 (01P)
- *FGFR1* gene location adjusted to correct hg18 location (8p12).

Version C2-01 – 26 April 2018 (01P)
- Product description restructured and adapted to a new template.
- Exon numbering of the *PROKR2* gene has been changed.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).

Version 11 – 04 December 2015 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and 2, new picture included).
- Various textual changes on page 1 and 2 and minor textual changes throughout the document.
- Exon numbering of the FGFR1, GNRH1, GNRHR and NELF genes has been updated according to the new version of the NM_ reference sequences in Table 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New references added.

Version 10 – 22 July 2015 (54)
- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.
- Various minor textual changes throughout the document.

Version 09 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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