Product Description

SALSA® MLPA® Probemix P187-B4 Holoprosencephaly (HPE)

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, one reference probe has been added and two reference probes have been replaced. For complete product history see page 8.

Catalogue numbers:

- **P187-025R:** SALSA MLPA Probemix P187 Holoprosencephaly (HPE), 25 reactions.
- **P187-050R:** SALSA MLPA Probemix P187 Holoprosencephaly (HPE), 50 reactions.
- **P187-100R:** SALSA MLPA Probemix P187 Holoprosencephaly (HPE), 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 P187 Holoprosencephaly (HPE) is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FBXW11*, *PTCH1*, *SHH*, *SIX3*, *TGIF1*, *TRAPPC10* and *ZIC2* genes, which are associated with holoprosencephaly (HPE). Deletions and duplications of the *SIX2* gene, which is adjacent to *SIX3*, and *GLI2*, a former HPE candidate, can also be detected with this probemix.

HPE is a complex brain malformation resulting from incomplete cleavage of the forebrain (prosencephalon) during gastrulation, leading to both intracranial and craniofacial midline defects (GeneReviews: NBK1530). Clinical manifestation is variable and can include, among others, a wide spectrum of dysmorphism of the brain and face, neurologic impairment and seizures.

In 25%-50% of cases, HPE is caused by numeric or structural chromosomal abnormalities, most commonly trisomy 13, trisomy 18 and triploidy. Structural chromosome abnormalities have been reported for almost all chromosomes. Moreover, monogenic syndromic (18-25%) as well as monogenic nonsyndromic HPE has been described. Defects in the genes *SHH* (5.4-5.9%, 7q36.3), *ZIC2* (4.8-5.2%, 13q32.3), *SIX3* (3%, 2p21) and *TGIF1* (<1%, 18p11.31) are among the most common causes of nonsyndromic HPE. Moreover, a 1.24 Mb duplication that included the *FBXW11* gene (5q35.1) has been associated with HPE (Koolen et al., 2006). Other HPE candidate genes include *PTCH1* (9q22.32) (Richieri-Costa et al., 2016), and *TRAPPC10* (21q22.3, previously *TMEM1*) (Yamakawa et al., 1996). While *GLI2* (2q14.2) was postulated to be another HPE candidate, it has become clear that pathogenic variants of *GLI2* cause a phenotype with features overlapping with HPE as well as distinct symptoms (Bear et al., 2014).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1530/

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 exon numbering used in this P187-B4 Holoprosencephaly (HPE) product description is the exon numbering from the RefSeq sequences:

- NG_016222.1 sequence for *SIX3*
- NG_009360.1 sequence for *SIX2*
- NG_009030.1 sequence for *GLI2*
- NG_009275.2 sequence for *FBXW11*
- NG_007504.2 sequence for *SHH*
- LRG_515 sequence for *PTCH1*
- LRG_1157 sequence for *ZIC2*
- NG_007447.2 sequence for *TGIF1*
- NG_008658.1 sequence for *TRAPPC10*

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 or 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 P187-B4 Holoprosencephaly (HPE) contains 50 MLPA probes with amplification products between 124 and 487 nucleotides (nt). Forty of the probes detect sequences in the regions described above. This includes three probes for the *FBXW11* gene, 17 probes for *GLI2*, one probe for *PTCH1*, four probes for *SHH*, two probes for *SIX2*, three probes for *SIX3*, five probes for *TGIF1*, two probes for *TRAPPC10*, and three probes for *ZIC2*. In addition, ten 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 HPE. 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 genes *SIX3*, *SIX2*, *GLI2*, *SHH* and *ZIC2*. 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

- The majority of genetic defects in the targeted genes/regions are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P187 Holoprosencephaly (HPE).
- 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. 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 *GLI2* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P187-B4 Holoprosencephaly (HPE)

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*SIX3/SIX2*	*GLI2*	*FBXW11*	*SHH*	Other
64-105	Control fragments – see table in probemix content section for more information						
124	Reference probe 19616-L26275	4p					
130 «	**ZIC2 probe** 08300-L08262						**ZIC2 exon 3**
136 «	**SHH probe** 06358-L05874					**Exon 2**	
142	**TGIF1 probe** 06368-L05884						**TGIF1 exon 2**
148	**PTCH1 probe** 03707-L03161						**PTCH1 exon 17**
154	**GLI2 probe** 10275-L10787			Upstream			
160 «	**GLI2 probe** 20967-L30330			**Exon 12**			
166	**GLI2 probe** 10277-L10789			**Exon 2**			
172 *	Reference probe 06556-L19388	1q					
178 «	**GLI2 probe** 10278-L10790			**Exon 13**			
184 «	**SHH probe** 06801-L06396					**Exon 1**	
190	Reference probe 10484-L11037	11q					
195	**GLI2 probe** 10279-L10791			Upstream			
202	**TRAPPC10 probe** 07694-L07418						**TRAPPC10 exon 7**
208	**TGIF1 probe** 06369-L05885						**TGIF1 exon 3**
214 «	**SIX3 probe** 06363-L05879		**SIX3 exon 1**				
220 «	**GLI2 probe** 10280-L12843			**Exon 8**			
227	Reference probe 10721-L11303	6p					
232 «	**GLI2 probe** 10282-L30331			**Exon 5**			
238 «	**SHH probe** 06357-L30332					**Exon 1**	
244	Reference probe 15975-L18130	8q					
249	**TRAPPC10 probe** 07695-L30333						**TRAPPC10 exon 21**
256	**FBXW11 probe** 07624-L07308				**Exon 7**		
265	**GLI2 probe** 10283-L10795			**Exon 1**			
272 «	**GLI2 probe** 20968-L30334			**Exon 9**			
283 «	**GLI2 probe** 10285-L10797			**Exon 6**			
288	Reference probe 06499-L06039	3p					
296	**TGIF1 probe** 06366-L30335						**TGIF1** upstream
301	**FBXW11 probe** 07625-L07309				**Exon 13**		
310 *	Reference probe 15455-L17288	12q					
318 «	**ZIC2 probe** 06370-L05886						**ZIC2 exon 1**
328 « Ж	**SIX2 probe** 21290-SP0036-L08636		**SIX2 exon 2**				
337 «	**GLI2 probe** 10291-L10803			**Exon 8**			
346	**TGIF1 probe** 12817-L06800						**TGIF1 exon 3**
355	**GLI2 probe** 10293-L10805			**Exon 1**			
364 «	**GLI2 probe** 10294-L10806			**Exon 7**			
374 «	**SIX2 probe** 12818-L06803		**SIX2 exon 1**				
382 «	**GLI2 probe** 10295-L10807			**Exon 10**			
390	**GLI2 probe** 20969-L12842			**Exon 3**			
396 *	Reference probe 19791-L32741	1q					
400 «	**ZIC2 probe** 06372-L30337						**ZIC2 exon 3**
409	**FBXW11 probe** 07623-L07307				**Exon 2**		
418	**GLI2 probe** 10296-L10808			**Exon 4**			
427 «	**GLI2 probe** 10297-L10809			**Exon 11**			
436 «	**SIX3 probe** 06362-L05878		**SIX3 exon 1**				
445	**TGIF1 probe** 06367-L05883						**TGIF1 exon 1**
454	Reference probe 17392-L21057	10p					
463 «	**SIX3 probe** 06364-L05880		**SIX3 exon 2**				
478 «	**SHH probe** 12816-L21224					**Exon 3**	

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*SIX3/SIX2*	*GLI2*	*FBXW11*	*SHH*	Other
487	Reference probe 18578-L23910	20q					

[a] See section Exon numbering on page 2 for more information.

* New in version B4.

« 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.

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. P187-B4 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 2p21					
		SIX3	**NM_005413.4**		
		start codon	*404-406 (Exon 1)*		
436 «	06362-L05878	Exon 1	455-456	TCTTGTTGCCAA-ACTTCGCCGATT	0.2 kb
214 «	06363-L05879	Exon 1	662-663	CCACCCTCAACT-TCTCGCCGGAGC	2.4 kb
463 «	06364-L05880	Exon 2	1367-1368	CCTCCATCCTCT-CGGTAACCTCCA	61.7 kb
		stop codon	*1400-1402 (Exon 2)*		
		SIX2	**NM_016932.5**		
		stop codon	*1216-1218 (Exon 2)*		
328 « Ж	21290-SP0036-L08636	Exon 2	924-925; 952-953	GAGAACTCCAAT- 28 nt spanning oligo -GCGGCAAGTCGG	2.4 kb
374 «	12818-L06803	Exon 1	621-622	GCACACTACATC-GAGGCGGAGAAG	
		start codon	*343-345 (Exon 1)*		
Chromosome 2q14.2					
		GLI2	**NM_005270.4**		
		start codon	*31-33 (exon 1)*		
195	10279-L10791	Upstream	~5 kb before exon 1	TACAATAATGCT-ATAAATGCATAG	0.1 kb
154	10275-L10787	Upstream	~5 kb before exon 1	AAAGAGCAAGGT-CAGCCGTGGGGG	4.9 kb
265	10283-L10795	Exon 1	3-4	CTCTTTTAGGAT-TGCCACCCAGGA	0.2 kb
355	10293-L10805	Exon 1	6 nt after exon 1	AAGGAGGTACTT-TCTGTTTCGCAC	130.0 kb
166	10277-L10789	Exon 2	247-248	AGGAAGGAAGGT-ACCATTACGAGC	24.0 kb
390	20969-L12842	Exon 3	427-428	ACCTCCGTTCTG-TGCACAGCAGCC	3.9 kb
418	10296-L10808	Exon 4	505-506	AGGAGCACCTTA-AGGAGAGGGGAC	13.4 kb
232 «	10282-L30331	Exon 5	9 nt before exon 5	CTTGTGTTGACT-ATCCCACAGTGT	1.9 kb
283 «	10285-L10797	Exon 6	1071-1072	AGCAGCAACTGT-CTGAGTGACACC	1.4 kb
364 «	10294-L10806	Exon 7	1162-1163	GCAGCAAGGTCA-AGACCGAGCCTG	2.9 kb
337 «	10291-L10803	Exon 8	5 nt before exon 8	CAGCGACCTCTT-TTCAGGGCCAGG	0.1 kb
220 «	10280-L12843	Exon 8	1292-1293	CAAGGAAGATCT-GGACAGGGATGA	3.5 kb
272 «	20968-L30334	Exon 9	1457-1458	CCGCTGGCAGGC-CTGCACGCGGGA	4.4 kb
382 «	10295-L10807	Exon 10	2 nt after exon 10	CTCCAACGAGGT-ACCTCTGCGGGG	1.7 kb
427 «	10297-L10809	Exon 11	1788-1789	AAGCATGTGAAA-ACGGTCCACGGC	2.0 kb
160 «	20967-L30330	Exon 12	2256-2257	TCACTCAAGGAT-TCCTGCTCATGG	1.7 kb
178 «	10278-L10790	Exon 13	2330-2331	CGCAGGCTCCAT-CCTGGAAAACTT	
		stop codon	*4789-4791 (Exon 13)*		

Chromosome 5q35.1					
		FBXW11	**NM_012300.3**		
		stop codon	*1763-1765 (Exon 12)*		
301	07625-L07309	Exon 13	4269-4270	AGTGCACCTAAT-CTGGCTTCATGC	16.5 kb
256	07624-L07308	Exon 7	967-968	AAAGTGTTAACA-GGACACACAGGC	79.6 kb
409	07623-L07307	Exon 2	203-204	CAAGGTCTTTGT-GGCTAGGCTGCG	
		start codon	*137-139 (Exon 1)*		
Chromosome 7q36.3					
		SHH	**NM_000193.4**		
		stop codon	*1728-1730 (Exon 3)*		
478 «	12816-L21224	Exon 3	1063-1064	CTTCCTCACTTT-CCTGGACCGCGA	2.7 kb
136 «	06358-L05874	Exon 2	883-884	CAAGGCACATAT-CCACTGCTCGGT	5.6 kb
238 «	06357-L30332	Exon 1	562-563	CGAGCGATTTAA-GGAACTCACCCC	0.2 kb
184 «	06801-L06396	Exon 1	325-326	CTCGGAAGTCAT-CAGTTCCATGGG	
		start codon	*342-344 (Exon 1)*		
Chromosome 9q22.32					
		PTCH1	**NM_000264.5**		
		start codon	*906-908 (Exon 1)*		
148	03707-L03161	Exon 17	3516-3517	AAATCATGCCAA-ACAATTACAAGA	
		stop codon	*5247-5249 (Exon 23)*		
Chromosome 13q32.3					
		ZIC2	**NM_007129.5**		
		start codon	*282-284 (Exon 1)*		
318 «	06370-L05886	Exon 1	919-920	ACTCCACAACCA-GTACGGCCCCAT	2.6 kb
400 «	06372-L30337	Exon 3	1526-1527	TTGCAGGTCCAT-GAGTCCTCCCCG	1.0 kb
130 «	08300-L08262	Exon 3	2539-2540	TCCCTTCTGTTT-CTTCAGCACATT	
		stop codon	*1878-1880 (Exon 3)*		
Chromosome 18p11.31					
		TGIF1	**NM_170695.5**		
		start codon	*835-837 (Exon 2)*		
296	06366-L30335	upstream	220-221 (NM_173207.4)	CGGGCAAAAGTT-GTGCATTGGCCC	4.0 kb
445	06367-L05883	Exon 1	147-148	GAGAGTGAAATT-AAACTTGAAACT	4.7 kb
142	06368-L05884	Exon 2	833-834	GGATGAGGACAG-CATGGACATTCC	1.4 kb
208	06369-L05885	Exon 3	1452-1453	GGACCAAGTACG-AATACACAGAGT	0.1 kb
346	12817-L06800	Exon 3	1565-1566	GGCTGCAGAGAT-GGAGCTTCAGGC	
		stop codon	*1591-1593 (Exon 3)*		
Chromosome 21q22.3					
		TRAPPC10	**NM_003274.5**		
		start codon	*186-188 (Exon 1)*		
202	07694-L07418	Exon 7	1015-1016	CCAGCCAGTGAA-GAGCTGGAACGG	34.9 kb
249	07695-L30333	Exon 21	3497-3498	CTGACTGAATCT-GATGAGCATTTT	
		stop codon	*3963-3965 (Exon 23)*		

[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.

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.

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

P067 PTCH1 Contains probes for most *PTCH1* exons.

References

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P187 product history	
Version	*Modification*
B4	One reference probe has been added and two reference probes have been replaced.
B3	Three reference probes have been added and five have been replaced. Several probe lengths have been adjusted.
B2	Four probes have been replaced, two target probes have been removed. The 88 and 96 nt control fragments have been replaced (QDX2).
B1	PTCH probes have been replaced by probes for GLI2. New reference probes and control fragments have been added.
A1	First release.

Implemented changes in the product description
Version B4-01 – 11 January 2021 (04P) - 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 *SIX3*, *SIX2*, *FBXW11*, *SHH*, *PTCH1*, *ZIC2*, *TGIF1*, *TRAPPC10* updated according to new version of the NM_ reference sequences. Version 07 – 14 November 2017 (55) - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - New references added on page 2. - Ligation sites of the probes targeting the ZIC2 gene updated according to new version of the NM_reference sequence. - Various minor textual changes on pages 1 and 2.

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