Product Description
SALSA® MLPA® Probemix P480-A1 WHS & Achondroplasia

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 8.

Catalogue numbers:
- **P480-025R:** SALSA MLPA Probemix P480 WHS & Achondroplasia, 25 reactions.
- **P480-050R:** SALSA MLPA Probemix P480 WHS & Achondroplasia, 50 reactions.
- **P480-100R:** SALSA MLPA Probemix P480 WHS & Achondroplasia, 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 P480 WHS & Achondroplasia is a **research use only (RUO)** assay for the detection of deletions or duplications in the 4p16 chromosomal region, which is associated with Wolf-Hirschhorn syndrome (WHS). This probemix can also be used to detect the presence of the c.1138G>A (p.Gly380Arg) point mutation, which causes the vast majority of cases of achondroplasia (ACH).

WHS is characterised by severe growth retardation, intellectual disability of variable degrees, microcephaly, “Greek helmet” facies, and closure defects (cleft lip or palate, coloboma of the eye, and cardiac septal defects), among others (Zollino et al. 2008). The prevalence of this syndrome is estimated at about 1 in 50,000 births, although it is suspected to be higher, at about 1 in 20,000 births (Berrocoso et al. 2020). Furthermore, the incidence is twice as high in females than males (Coles et al. 1992). The syndrome is caused by a heterozygous deletion of the WHS critical region on 4p16.3. Most individuals with WHS have *de novo* 4p terminal and interstitial deletions (50-60%), *de novo* microdeletions (25-30%), or an unbalanced translocation (~15%). The remainder have other complex rearrangements leading to 4p16 deletion (South et al. 2008). The deletion extent of the 4p subtelomeric region varies between patients, with smaller deletions resulting in a milder phenotype than larger ones (Zollino et al. 2008).

One of the genes in the 4p16 chromosomal region is the *FGFR3* gene. The c.1138G>A mutation in exon 10 of the *FGFR3* gene causes approximately 98% of cases of ACH (Etlik et al. 2008). ACH is the most common cause of disproportionate short stature. The worldwide prevalence of this genetic disorder is estimated at about 1 in 22,000 births (Foreman et al. 2020).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1183/ (WHS) and https://www.ncbi.nlm.nih.gov/books/NBK1152/ (ACH).

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 *FGFR3* exon numbering used in this P480-A1 WHS & Achondroplasia product description is the exon numbering from the LRG_1021 sequence. The *LETM1, NSD2 (WHSC1) and NELFA* exon numbering used in this P480-A1 WHS & Achondroplasia product description is the exon numbering from the NG_013063.1, the NG_009269.1 and the NG_009232.1 sequence, 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 LRG/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 P480-A1 WHS & Achondroplasia contains 51 MLPA probes with amplification products between 121 and 505 nucleotides (nt). This includes 38 probes for the 4p16 chromosomal region. Furthermore, this probemix also contains one probe specific for the c.1138G>A mutation in *FGFR3* which will only generate a signal when the mutation is present. In addition, 12 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 WHS and ACH. 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 NA00343, NA04126 and NA22601 from the Coriell Institute have been tested with this P480-A1 probemix at MRC Holland and can be used as positive control samples to detect the deletion of the 4p16 chromosomal region covered in this probemix (*FGFR3* c.1138G>A not present). 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 P480-A1	Expected copy number alteration
NA00343	Coriell Institute	4pter-p14	All genes	Heterozygous deletion
NA04126	Coriell Institute	4pter-p15	All genes	Heterozygous deletion
NA22601	Coriell Institute	4pter-p15	All genes	Heterozygous deletion

SALSA Binning DNA SD080

The SD080 Binning DNA provided with this probemix can be used for binning of all probes including the mutation-specific probe (probe 21987-L30825 for the *FGFR3* c.1138G>A mutation). SD080 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD080 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD080 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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. 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

- 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.

WHS and *FGFR3* mutation database
https://databases.lovd.nl/shared/genes/WHSC1 and https://databases.lovd.nl/shared/genes/FGFR3. 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 *LETM1* exons 2 and 8 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P480-A1 WHS & Achondroplasia

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		Location (hg18) in kb
		Reference	WHS region	
64-105	Control fragments – see table in probemix content section for more information			
121	Reference probe S0864-L27364	21q		
130 «	**SPON2 probe** 03100-L03175		***SPON2***	04-001,156
137 «	**CTBP1 probe** 21980-L30818		***CTBP1***	04-001,212
142	**NELFA probe** 21981-L31206		***NELFA*** exon 11	04-001,955
148	Reference probe 20517-L28107	1q		
154	**PIGG probe** 02005-L03588		***PIGG***	04-000,505
160 «	**FGFRL1 probe** 03099-L02502		***FGFRL1***	04-001,010
165	**NELFA probe** 21982-L30820		***NELFA*** exon 6	04-001,958
172	Reference probe 21193-L29851	6p		
178 «	**CTBP1 probe** 03098-L02501		***CTBP1***	04-001,196
184 «	**MSX1 probe** 21983-L30821		***MSX1***	04-004,916
191	**NSD2 (WHSC1) probe** 06057-L06042		***NSD2 (WHSC1)*** upstream	04-001,865
197	**TMEM128 probe** 21984-L30822		***TMEM128***	04-004,299
203 «	**FGFR3 probe** 21985-L31207		***FGFR3*** exon 19	04-001,779
208	**NSD2 (WHSC1) probe** 06059-L13831		***NSD2 (WHSC1)*** exon 16	04-001,929
214	Reference probe 16426-L18879	18q		
220 «	**LETM1 probe** 21986-L31208		***LETM1*** exon 8	04-001,795
226	**NSD2 (WHSC1) probe** 06060-L05515		***NSD2 (WHSC1)*** exon 22	04-001,950
232 «	**LETM1 probe** 04190-L05920		***LETM1*** exon 3	04-001,813
242 § «	**FGFR3 probe** 21987-L30825		***FGFR3*** c.1138G>A	04-001,776
249	Reference probe 06712-L25773	15q		
254 «	**LETM1 probe** 21988-L30826		***LETM1*** exon 14	04-001,786
264	**DOK7 probe** 21989-L30827		***DOK7***	04-003,448
274 «	**GAK probe** 01125-L00683		***GAK***	04-000,835
281	**NELFA probe** 21990-L30828		***NELFA*** exon 1	04-001,980
287 «	**FGFR3 probe** 04183-L31027		***FGFR3*** exon 11	04-001,776
292	Reference probe 18670-L24024	11p		
301 «	**FGFR3 probe** 21991-L30829		***FGFR3*** exon 3	04-001,771
307	Reference probe 17413-L19193	3p		
314 «	**FGFR3 probe** 03094-L31028		***FGFR3*** exon 6	04-001,773
320	**RNF4 probe** 15436-L31029		***RNF4***	04-002,484
328 «	**ZNF141 probe** 14368-L17918		***ZNF141***	04-000,322
335	**PIGG probe** 14440-L31030		***PIGG***	04-000,506
342 «	**TACC3 probe** 03096-L31031		***TACC3***	04-001,695

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		Location (hg18) in kb
		Reference	WHS region	
350	Reference probe 19887-L30323	10q		
357 «	**LETM1 probe** 21992-L30830		***LETM1*** exon 2	04-001,821
365 «	**CPLX1 probe** 21993-L31250		***CPLX1***	04-000,808
373	**STX18 probe** 21994-L30832		***STX18***	04-004,478
389	**ZNF718 probe** 21996-L31247		***ZNF718***	04-000,146
396 «	**SLBP probe** 03097-L31248		***SLBP***	04-001,675
406	Reference probe 09720-L30744	12q		
418	**NSD2 (WHSC1) probe** 03065-L02494		***NSD2 (WHSC1)*** exon 2	04-001,872
427 ±	**ZNF718 probe** 21997-L30835		***ZNF718***	04-000,114
436	Reference probe 12576-L31249	20p		
445	**NSD2 (WHSC1) probe** 06058-L05513		***NSD2 (WHSC1)*** exon 6	04-001,902
453 «	**TACC3 probe** 15440-L21012		***TACC3***	04-001,700
463	**NELFA probe** 03058-L02495		***NELFA*** exon 2	04-001,963
472	Reference probe 21344-L29750	7q		
486 «	**TNIP2 probe** 21998-L30836		***TNIP2***	04-002,717
495	**ADD1 probe** 15435-L17265		***ADD1***	04-002,856
505	Reference probe 14883-L27237	14q		

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

§ Mutation-specific probe. This probe will only generate a signal when the c.1138G>A mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
± SNP rs561407996 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

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

Table 2. P480-A1 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
427 ± #	21997-L30835	*ZNF718*		GCTGCGCGCCTC-ATTCGGCAGTTG	31.6 kb
389 #	21996-L31247	*ZNF718*		TCTTCACATTTC-TAAAATTTTCCT	175.7 kb
328 «	14368-L17918	*ZNF141*		GGTCTGGTAGTA-GCCAGACGCAAA	183.3 kb
154	02005-L03588	*PIGG*		AAAAGCATTCAG-AAGTCCTATTCA	0.8 kb
335	14440-L31030	*PIGG*		GAGTGTGACGTA-GTCCTTCTGCTC	302.6 kb
365 «	21993-L31250	*CPLX1*		ACTGTGGAAGCA-GAGCAATCGCCA	26.5 kb
274 «	01125-L00683	*GAK*		ACGACTTTGAAG-ATCTGTTGTCCA	175.6 kb
160 «	03099-L02502	*FGFRL1*		AGCCCATGGCTA-GTGGCTCATCCC	145.3 kb
130 «	03100-L03175	*SPON2*		CTTCCCCAAGCA-GTACCCCCTGTT	40.1 kb
178 «	03098-L02501	*CTBP1*		TTCGCGTTCCTC-GTTAAGCAGAAG	16.3 kb
137 «	21980-L30818	*CTBP1*		TTCAAAGCCCTC-CGCATCATCGTC	463.1 kb
396 «	03097-L31248	*SLBP*		AACCCCGTTCCA-GATGCTCTGACT	19.9 kb
342 «	03096-L31031	*TACC3*		GGAAGATCGTCT-GTTCTTCGTGTG	5.1 kb
453 «	15440-L21012	*TACC3*		GGACAAAATGGA-TGACCCAAACTT	70.9 kb
		Start codon	*276-278 (exon 2)*		
		FGFR3	**NM_000142.5**		

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
301 «	21991-L30829	Exon 3	652-651 reverse	AGAGCTCACCTG-TCACCCGCACAC	2.1 kb
314 «	03094-L31028	Exon 6	923-924	CTGGTCATGGAA-AGCGTGGTGCCC	2.7 kb
242 § «	21987-L30825	Exon 10	1413-1414; c.1138G>A mutation specific	TCCTCAGCTACA-GGGTGGGCTTCT	0.5 kb
287 «	04183-L31027	Exon 11	1640-1641	GCCAATGTCTCC-GAGCTCGAGCTG	2.3 kb
203 «	21985-L31207	Exon 19	2711-2712	GCCACTGGTCCC-CAACAATGTGAG	7.3 kb
		Stop codon	*2694-2696 (exon 19)*		
		Stop codon	*2424-2426 (exon 14)*		
		LETM1	**NM_012318.3**		
254 «	21988-L30826	Exon 14	2282-2283	GTGTAGGTGATT-GAGCTGGTGGAC	9.1 kb
220 «	21986-L31208	Exon 8	1531-1532	GACCCTCCCAGA-GATTGTGGTACG	18.1 kb
232 «	04190-L05920	Exon 3	395-396	CCTGTGTACACA-TCCTCCAGAGGC	7.4 kb
357 «	21992-L30830	Exon 2	338-339	ACCCTGGGGTTG-AGGAACTGCCTG	44.8 kb
		Start codon	*207-209 (exon 1)*		
		Start codon	*180-182 (exon 2)*		
		NSD2 (WHSC1)	**NM_001042424.3**		
191	06057-L06042	Intron 1	6.7 kb before exon 2 NM_133330.3: 12 nt after exon 3	GTGGGCATTTAT-TTTCCCTTAATG	7.0 kb
418	03065-L02494	Exon 2	481-482	ACTGCGTTTTGA-GTCCCAGGAAAT	29.7 kb
445	06058-L05513	Exon 6	1669-1670	GCTGAGTGAGAA-GCAGAGAGCACG	27.3 kb
208	06059-L13831	Exon 16	3090-3091	CTCGTTTTCGTG-AAATTAAGCTTC	20.7 kb
226	06060-L05515	Exon 22	4032-4033	CTTGGCATCATT-GTGACGTGTGTG	4.8 kb
		Stop codon	*4275-4277 (exon 22)*		
		Stop codon	*1600-1602 (exon 11)*		
		NELFA	**NM_005663.5**		
142	21981-L31206	Exon 11	1455-1456	GTGATCCAGATC-AAGCTGAGCGAG	2.7 kb
165	21982-L30820	Exon 6	786-785 reverse	TCAGAGATGTCC-AGCAGCTGCCAA	5.4 kb
463	03058-L02495	Exon 2	338-339	CTCGCTTAACCT-GGAGCTGGAGGA	17.2 kb
281	21990-L30828	Exon 1	176-177	GGCAGTGAAGCT-CAAGTTGCTACT	503.8 kb
		Start codon	*16-18 (exon 1)*		
320	15436-L31029	*RNF4*		CTACCCATACTC-CCAGAAACGCCA	232.9 kb
486 «	21998-L30836	*TNIP2*		GAGAAGTTGCAG-GAAGAAAATCGA	139.1 kb
495	15435-L17265	*ADD1*		TTACGGTGTAAA-TTGGCAGCGTTT	591.9 kb
264	21989-L30827	*DOK7*		GGAAGCTGTCTG-ACCTCCGGCGCT	850.9 kb
197	21984-L30822	*TMEM128*		TCAACATAATAG-GTCACAACAATG	178.9 kb
373	21994-L30832	*STX18*		GCTTGTTTGATG-AAGTGAGGTATG	438.0 kb
184 «	21983-L30821	*MSX1*		GGGCTACAGCAT-GTACCACCTGAC	

[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.

§ Mutation-specific probe. This probe will only generate a signal when the c.1138G>A mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

± SNP rs561407996 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

« 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.

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.

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

Related SALSA MLPA probemixes

P036 Subtelomeres Mix 1	Detects copy number variations in subtelomeric regions. Contains one probe for each of the 41 subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes.
P070 Subtelomeres Mix 2B	Detects copy number variations in subtelomeric regions. Contains a probe for each of the 41 subtelomeric regions and five probes near the centromeric regions of the five acrocentric chromosomes.
P245 Microdeletion Syndromes-1A	Detects recurrent microdeletions/microduplications. Contains probes for 23 different microdeletion syndromes.
P064 Microdeletion Syndromes-1B	Detects recurrent microdeletions/microduplications. Contains probes for 15 different microdeletion syndromes.
P106 X-linked ID	Contains probes for several genes involved in X-linked intellectual disability.

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P480 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 30 June 2022 (04P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Gene name *WHSC1* changed to *NSD2*, old name can be found in brackets in Table 1 and Table 2. - Ligation sites of the probes targeting the *FGFR3, LETM1, NSD2 (WHSC1)* and *NELFA* genes updated according to new version of the NM_ reference sequence. Version A1-01 – 19 September 2018 (01P) - Not applicable, new document.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200