

Product Description

SALSA® MLPA® Probemix P380-B1 Wilms' tumour

To be used with the MLPA General Protocol.

Version B1

For complete product history see page 9.

Catalogue numbers:

- **P380-025R:** SALSA MLPA Probemix P380 Wilms' tumour, 25 reactions.
- **P380-050R:** SALSA MLPA Probemix P380 Wilms' tumour, 50 reactions.
- **P380-100R:** SALSA MLPA Probemix P380 Wilms' tumour, 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 P380 Wilms' tumour is a **research use only (RUO)** assay for the detection of deletions or gains on chromosomal arms 1p, 1q, 16p, and 16q. This probemix can further be used to determine the copy number of *MYCN* on 2p24.3, *FBXW7* on 4q31.3, *WT1* on 11p13, *TP53* on 17p13.1, and *AMER1* (previously known as *FAM123B*) on Xq11.1.

Wilms' tumour (WT) is the most common paediatric renal tumour. Most of the cases occur sporadically in otherwise healthy children. However, a minority of cases is associated with other developmental abnormalities such as WARG, Denys-Drash, Beckwith-Wiedemann, Perlman or Frasier syndrome (Maciaszek et al. 2020). While the survival of children with Wilms' tumour has increased to ~90%, the survival of patients with relapse is still low (~50%) (Spreafico et al. 2021). Copy number changes of certain chromosomal regions and genes have been found to be highly significant for prognosis. Tools to classify these high-risk Wilms' tumour patients are needed to intensify the treatment and, in addition, to reduce the treatment burden of the low-risk patients.

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>

Matched Annotation from NCBI and EMBL-EBI (MANE): <https://www.ncbi.nlm.nih.gov/refseq/MANE/> and <http://tark.ensembl.org/>

Exon numbering

The exon numbering from the MANE Select transcripts is used for the *MYCN*, *FBXW7*, *WT1*, *TP53* and *AMER1* genes. Please be aware that the MANE and LRG exon numbering do not always correspond, and MANE exon numbering used here may differ from literature. 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 P380-B1 Wilms' tumour contains 53 MLPA probes with amplification products between 127 and 500 nucleotides (nt). This includes seven probes for chromosomal arm 1p, five probes for 1q, three probes for 16p, six probes for 16q, and three probes for each of the genes *MYCN*, *FBXW7*, *WT1*, *TP53*, and *AMER1*. Additionally, seven flanking probes (for the 2p, 2q, 4q, 11p, 17p, Xp and Xq arms) are included to facilitate the determination of the extent of a deletion or duplication. In addition, 10 reference probes are included that target relatively copy number stable regions in various cancer types including Wilms' tumour. Complete probe sequences 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 Wilms' tumour. It is recommended to use samples of the same sex to facilitate interpretation. 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

See the section Positive samples on the [P380 Wilms' tumour product page](#) on our website.

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 . When this criterion is fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	X chromosome sequences in males	
Normal	Normal	$0.80 < FR < 1.20$
Homozygous deletion	Deletion	$FR = 0$
Heterozygous deletion		$0.40 < FR < 0.65$
Heterozygous duplication/gain		$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication/gain	Duplication/gain	$1.75 < FR < 2.15$
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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- 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 *CHD5*, *WNT4* and *TNFRSF18* 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.

- 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.

P380 specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, the majority of genetic alterations in the genes and chromosomal regions included in this probemix are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P380 Wilms' tumour.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

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.

COSMIC mutation database

<http://cancer.sanger.ac.uk/cosmic>. We strongly encourage users to deposit positive results in the Catalogue Of Somatic Mutations In Cancer (COSMIC). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <http://varnomen.hgvs.org/>.

Please report false positive results due to SNVs and unusual results (e.g., a deletion of *WT1* exons 4 and 10 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P380-B1 Wilms' tumour

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				Location (hg18) in kb
		Reference	Chr 1	Chr 16	Other	
64-105	Control fragments – see table in probemix content section for more information					
127 Δ	Reference probe 18709-L26552	5q31				05-132,038
136 Δ	TK2 probe 11564-L20523			16q21		16-065,128
142 ~	RPS6KA3 probe 07868-L07682				Xp22.12	X-020,123
148 ~	HMGCS2 probe 05252-L04632		1p12			01-120,113
154	VKORC1 probe 10491-L11044			16p11.2		16-031,013
160	Reference probe 08771-L29189	19q13				19-053,030
167 Δ	WT1 probe 05358-L29218				11p13	11-032,371
172	FBXW7 probe 06046-L29190				4q31.3	04-153,471
178	WT1 probe 05354-L04733				11p13	11-032,396
185	Reference probe 18767-L24189	10q22				10-071,865
191	CREBBP probe 09891-L20115			16p13.3		16-003,765
197 ±	MLYCD probe 06327-L20116			16q23.3		16-082,499
202	KISS1 probe 04251-L03616		1q32.1			01-202,429
208	FBXW7 probe 06047-L05502				4q31.3	04-153,464
214	MYCN probe 17046-L20524				2p24.3	02-016,000
220 #	SALL1 probe 05679-L05121			16q12.1		16-049,733
226 ~	CHM probe 13519-L14318				Xq21.2	X-085,101
232	MPZ probe 04898-L17028		1q23.3			01-159,543
238	FBXW7 probe 06044-L05499				4q31.3	04-153,493
244 †	AMER1 probe 12752-L13868				Xq11.1	X-063,330
250	BCL9 probe 12945-L14100		1q21.2			01-145,563
256	Reference probe 05409-L04215	5p13				05-037,012
263 ~	PKD2 probe 14738-L20398				4q22.1	04-089,187
268	ABCC6 probe 07416-L17062			16p13.11		16-016,184
274	WT1 probe 05360-L04739				11p13	11-032,367
280	MIR34A probe 15299-L29217		1p36.23			01-009,134
286	CDH1 probe 02414-L17063			16q22.1		16-067,420
292	IRF6 probe 12495-L13539		1q32.2			01-208,042
299	TP53 probe 17420-L21142				17p13.1	17-007,520
307 ~	PAX3 probe 05994-L29546				2q36.1	02-222,872
315 «	TNFRSF18 probe 02270-L20123		1p36.33			01-001,129
320	Reference probe 05802-L20124	15q15				15-040,490
328 ~	ABCA4 probe 20972-L29215		1p22.1			01-094,231
335	Reference probe 15117-L29502	9q33				09-122,370
341	SDCCAG8 probe 09027-L29503		1q43			01-241,609
346 ~	DYSF probe 08827-L13322				2p13.2	02-071,694
355 †	AMER1 probe 15331-L29273				Xq11.1	X-063,342
363	MYCN probe 02572-L02036				2p24.3	02-016,003
373	Reference probe 02560-L02023	3q23				03-143,651
382 ~ ±	IGF2 probe 15740-L20522				11p15.5	11-002,119
391	FANCA probe 15632-L17496			16q24.3		16-088,332
401 «	WNT4 probe 07158-L20119		1p36.12			01-022,321
409	TP53 probe 01586-L01158				17p13.1	17-007,518
418 ±	MFN2 probe 06136-L05580		1p36.22			01-011,972
427	Reference probe 10781-L11396	19p13				19-011,095
436	SLC12A3 probe 15528-L17383			16q13		16-055,470
445	MYCN probe 03327-L20117				2p24.3	02-016,003
454	TP53 probe 08785-L19640				17p13.1	17-007,515
463 †	AMER1 probe 12760-L13876				Xq11.1	X-063,327
472 ~	RAI1 probe 11730-L15418				17p11.2	17-017,568
481	Reference probe 15738-L11546	15q12				15-024,344

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				Location (hg18) in kb
		Reference	Chr 1	Chr 16	Other	
492 «	CHD5 probe 09114-L09174		1p36.31			01-006,151
500	Reference probe 15203-L20113	3p12				03-081,775

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

« 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 rs77493891 (MLYCD probe at 197 nt), rs187259775 (IGF2 probe at 382 nt), and rs61733200 (MFN2 probe at 418 nt) could influence the signal of these probes. 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.

~ 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.

† The *AMER1* gene was previously called *FAM123B*.

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. P380-B1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon	Location / Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 1						
Loss of heterozygosity at 1p36 (Grundy et al. 2005; Messahel et al. 2009) and gain of 1q (Hing et al. 2001; Chagtai et al. 2016) have been shown to correlate with poor prognosis in Wilms' tumour.						
315 «	02270-L20123	<i>TNFRSF18</i>	1p36.33	CGGGTTTCTCAC-TGTGTTCCCTGG	5.0 Mb	01-001,129
492 «	09114-L09174	<i>CHD5</i>	1p36.31	GTTTCTTCTTCT-TAGGAAGGCTCA	3.0 Mb	01-006,151
280	15299-L29217	<i>MIR34A</i>	1p36.23	CTGGCCGGTCCA-CGGCATCCGGAG	2.8 Mb	01-009,134
418 ±	06136-L05580	<i>MFN2</i>	1p36.22	GGTGGCGCTCTC-CTGGATGTAGGC	10.4 Mb	01-011,972
401 «	07158-L20119	<i>WNT4</i>	1p36.12	AGCTGGAGAAGT-GCGGCTGTGACA	71.9 Mb	01-022,321
328 ~	20972-L29215	<i>ABCA4</i>	1p22.1	CACGAGGAGCAT-GCAGCGAATTCA	25.9 Mb	01-094,231
148 ~	05252-L04632	<i>HMGS2</i>	1p12	GCATCTCTTTCA-AGGTTTCTGCTG	25.5 Mb	01-120,113
250	12945-L14100	<i>BCL9</i>	1q21.2	TTATTCCATCTG-AGAAGCCCAGCC	14.0 Mb	01-145,563
232	04898-L17028	<i>MPZ</i>	1q23.3	CGGGGTCGTTCT-GGGAGCTGTGAT	42.9 Mb	01-159,543
202	04251-L03616	<i>KISS1</i>	1q32.1	TTGGGGAGCCAT-TAGAAAAGGTGG	5.6 Mb	01-202,429
292	12495-L13539	<i>IRF6</i>	1q32.2	GCTTTACCAGAC-TCAGTAGTGGAG	33.6 Mb	01-208,042
341	09027-L29503	<i>SDCCAG8</i>	1q43	GAGACTAACAGA-ACTGCTGGGCGA	-	01-241,609
MYCN , at 2p24.3. Indicated ligation sites and exon numbering are according to the MANE Select transcript NM_005378.6. Gain of <i>MYCN</i> is detected in 9% of Wilms' tumour cases and a significantly higher percentage (32%) is detected in Wilms' tumour of the high risk diffuse anaplastic subtype, thereby making it a putative prognostic marker for Wilms' tumour (Williams et al. 2010).						
214	17046-L20524	<i>MYCN</i> , ex 2	470-471	TGGAAGAAGTTT-GAGCTGCTGCC	3.4 kb	02-016,000
363	02572-L02036	<i>MYCN</i> , ex 3	1200-1201	CTGTCAACACAT-TCACCATCACTG	0.2 kb	02-016,003
445	03327-L20117	<i>MYCN</i> , ex 3	1351-1352	TGCACCCACACA-GAAGAAGATAAA	55.7 Mb	02-016,003
346 ~	08827-L13322	<i>DYSF</i>	2p13.3	CTCATCGACATT-GATGACAAGGAG	151.2 Mb	02-071,694
307 ~	05994-L29546	<i>PAX3</i>	2q36.1	CCGCACTCGCCT-TTCCGTTTCGCC	-	02-222,872
FBXW7 , at 4q31.3. Indicated ligation sites and exon numbering are according to the MANE Select transcript NM_001349798.2 sequence. The <i>FBXW7</i> exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets.						
<i>FBXW7</i> , an ubiquitin ligase component that acts as a tumour suppressor, has been shown to be mutated or deleted in ~4% of Wilms' tumour samples. <i>FBXW7</i> inactivation is suggested to associate with epithelial type (intermediate risk) tumour histology in Wilms' tumour (Williams et al. 2010).						
263 ~	14738-L20398	<i>PKD2</i>	4q22.1	TGTCACAACCTT-TGATTTCTTCCT	64.3 Mb	04-089,187
208	06047-L05502	<i>FBXW7</i> , ex 14 (16)	3206-3207	TGGCGGATCAGA-GCCTCAAACACA	7.8 kb	04-153,464

Length (nt)	SALSA MLPA probe	Gene / Exon	Location / Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
172	06046-L29190	<i>FBXW7</i> , ex 9 (11)	2234-2235	AGTCCATGGAAA-AGTGCATACATC	21.9 kb	04-153,471
238	06044-L05499	<i>FBXW7</i> , intr 4 (6)	2.5 kb before ex 5; NM18315.5: 184-185	CTAATCTTCCTT-TTCTGACGTGCC	-	04-153,493
WT1 , at 11p13. Indicated ligation sites and exon numbering are according to the NM_024426.6 sequence, which is also the MANE Select transcript. The <i>WT1</i> exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets. <i>WT1</i> is altered either by a deletion or mutation in ~20% of sporadic Wilms' tumours (Huff 1998). Mutations or larger deletions in <i>WT1</i> have also been observed in patients with Denys-Drash syndrome, Frasier syndrome and WAGR syndrome which have an increased susceptibility to Wilms' tumours.						
382 ~ ±	15740-L20522	<i>IGF2</i>	11p15.5	TGCCCCAACACA-CTTGGGTCGGCC	30.3 Mb	11-002,119
274	05360-L04739	<i>WT1</i> , ex 10 (11)	2276-2277	GTCAGCCAGGCT-GCTAACCTGGAA	4.2 kb	11-032,367
167 Δ	05358-L29218	<i>WT1</i> , ex 8 (9)	1463-1464	CCATACCAGTGT-GACTTCAAGGAC	24.9 kb	11-032,371
178	05354-L04733	<i>WT1</i> , ex 4 (5)	1098-1099	CATCCAGCTTG-AATGCATGACCT	-	11-032,396
Chromosome 16 Loss of heterozygosity at 16q (Grundy et al. 2005; Messahel et al. 2009) is associated with an increased risk of relapse and death in Wilms' tumour.						
191	09891-L20115	<i>CREBBP</i>	16p13.3	GGATGAATTCAT-TTAACCCCATGT	12.4 Mb	16-003,765
268	07416-L17062	<i>ABCC6</i>	16p13.11	CCCAAACCTCTCA-CCTGCTCCCAA	14.8 Mb	16-016,184
154	10491-L11044	<i>VKORC1</i>	16p11.2	TCCTCCAGGTGT-GCACGGGAGTGG	18.7 Mb	16-031,013
220 #	05679-L05121	<i>SALL1</i>	16q12.1	CAACATCTTCTA-GTCCTTCTCAAG	5.7 Mb	16-049,733
436	15528-L17383	<i>SLC12A3</i>	16q13	GACCATTTCTTA-CCTGGCCATCTC	9.7 Mb	16-055,470
136 Δ	11564-L20523	<i>TK2</i>	16q21	AGCCTGTGTCCA-AGTGGAGAAATG	2.3 Mb	16-065,128
286	02414-L17063	<i>CDH1</i>	16q22.1	TGCTGTTTCTTC-GGAGGAGAGCGG	15.1 Mb	16-067,420
197 ±	06327-L20116	<i>MLYCD</i>	16q23.3	GAGATGTCACCA-GTCAGTGCCACG	5.8 Mb	16-082,499
391	15632-L17496	<i>FANCA</i>	16q24.3	AGCTCAGTCTCA-GCCTTGTGTTTG	-	16-088,332
TP53 , at 17p13.1. Indicated ligation sites and exon numbering are according to the MANE Select transcript NM_000546.6. The exon numbering used in previous versions of this product description can be found in between brackets. Inactivation of <i>TP53</i> , most commonly by point mutation, is seen primarily in the anaplastic Wilms' tumour subtype that is characterised by poor prognosis (Bardeesy et al. 1994).						
454	08785-L19640	<i>TP53</i> , ex 10	1174-1175	TTCCGAGAGCTG-AATGAGGCCTTG	3.1 kb	17-007,515
409	01586-L01158	<i>TP53</i> , ex 8 (7)	981-982	CTGTCTGGGAG-AGACCGGCGCAC	2.3 kb	17-007,518
299	17420-L21142	<i>TP53</i> , ex 4 (3)	451-450 reverse	TAGCTGCCCTGG-TAGGTTTCTGG	10.1 Mb	17-007,520
472 ~	11730-L15418	<i>RAI1</i>	17p11.2	CCAAGGATCTCA-TCTGGCCACCGC	-	17-017,568
AMER1 (previously <i>FAM123B</i>), at Xq11.1. Indicated ligation sites and exon numbers are according to the NM_152424.4 sequence, which is also the MANE Select transcript. Inactivation of <i>AMER1</i> , either by deletion or by point mutation, is a frequent event in Wilms' tumours. Deletions are more common: <i>AMER1</i> has been shown to be deleted in 17-20% of Wilms' tumour samples (Rivera et al. 2007; Wegert et al. 2009).						
142 ~	07868-L07682	<i>RPS6KA3</i>	Xp22.12	TCTCCACATCA-TCCCTTGACCTC	43.2 Mb	X-020,123
463 †	12760-L13876	<i>AMER1</i> , ex 2	3244-3245	TGACCATGTCAA-TATCACTATCAG	2.7 kb	X-063,327
244 †	12752-L13868	<i>AMER1</i> , ex 2	517-518	TCAGCAAGAGCA-AGACCCACGATG	12.6 kb	X-063,330
355 †	15331-L29273	<i>AMER1</i> , ex 1	130-131	CTAGGAACCTGA-CCGGGCTGGGTA	21.8 Mb	X-063,342
226 ~	13519-L14318	<i>CHM</i>	Xq21.2	AGGTTCACTGGA-CTGATTCTTCT	-	X-085,101

^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.

± SNVs rs77493891 (*MLYCD* probe at 197 nt), rs187259775 (*IGF2* probe at 382 nt), and rs61733200 (*MFN2* probe at 418 nt) could influence the signal of these probes. 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.

– 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.

† The *AMER1* gene was previously called *FAM123B*.

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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
500	15203-L20113	<i>GBE1</i>	3p12	GACCTAGAGGGA-CTCATGATCTTT	03-081,775
373	02560-L02023	<i>ATR</i>	3q23	GTTCTTGACATT-GAGCAGCGACTA	03-143,651
256	05409-L04215	<i>NIPBL</i>	5p13	CAAGTGCCTGTT-TTACAACAGAAC	05-037,012
127 Δ	18709-L26552	<i>IL4</i>	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
335	15117-L29502	<i>CDK5RAP2</i>	9q33	TCAGAAGAAACA-GTGTCTCCACACC	09-122,370
185	18767-L24189	<i>NODAL</i>	10q22	AGAGCGGTTTCA-GATGGACCTATT	10-071,865
481	15738-L11546	<i>GABRB3</i>	15q12	GCTCATGGAAAT-ATTCTGTTGACA	15-024,344
320	05802-L20124	<i>CAPN3</i>	15q15	AACCAGCTCTAT-GACATCATTACC	15-040,490
427	10781-L11396	<i>LDLR</i>	19p13	CTGGTTCCCAAA-GTCAGCCACGCA	19-011,095
160	08771-L29189	<i>CRX</i>	19q13	GTGTGGATCTGA-TGCACCAGGCTG	19-053,030

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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

Related products

For related products, see the [product page](#) on our website.

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- Chagtai T et al. (2016). Gain of 1q As a Prognostic Biomarker in Wilms Tumors (WTs) Treated With Preoperative Chemotherapy in the International Society of Paediatric Oncology (SIOP) WT 2001 Trial: A SIOP Renal Tumours Biology Consortium Study. *J Clin Oncol*. 34:3195-203.
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P380 product history	
Version	Modification
B1	One TP53 probe has been replaced, one new reference probe has been added and one reference probe has been replaced, and several probes have small changes in length.
A1	First release.

Implemented changes in the product description**Version B1-03 – 29 July 2025 (04P)**

- Positive control DNA samples section: information moved to product page on website.
- Removed Related SALSA MLPA products section.
- Exon numbering of the *TP53* gene is now reported based on MANE Select Transcript NM_000546.6 in Table 2.

Version B1-02 – 07 February 2024 (04P)

- Product description rewritten and adapted to a new template.
- Exon numbering of the *FBXW7* and *WT1* genes has been changed according to MANE.
- Information about positive sample NA01416 corrected in the table on page 3 for positive control DNA samples.
- Ligation sites of the probes targeting the *FBXW7* and *TP53* genes were updated according to the MANE transcript NM_ reference sequence.
- DNA denaturation warnings adjusted in Tables 1 and 2 for TNFRSF18 probe at 315 nt, and WNT4 probe at 401 nt.
- Warnings for sensitivity for experimental conditions added in Tables 1 and 2 for TK2 probe at 136 nt and WT1 probe at 167 nt.
- One new reference added to the Selected publications using SALSA MLPA Probemix P380 Wilms' tumour section and two new articles added also to the references on page 10.

Version B1-01 – 12 November 2020 (02P)

- Product description restructured and adapted to a new template.
- Various minor textual or layout changes.
- Exon numbering of the *FBXW7* gene has been changed according to LRG_1141.
- Ligation sites of the probes targeting the *FBXW7*, *MYCN*, *WT1*, and *AMER1* genes updated according to new versions of their NM_ reference sequences.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Warning added to Tables 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Two new references added to the Selected publications using SALSA MLPA Probemix P380 Wilms' tumour section.

Version 08 – 19 March 2019 (T08)

- New references to literature added on page 2.
- Length of probe at 185 nt updated in Tables 1 and 2 to better represent the actual peak size.

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

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