# 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 10.

**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**
From product description **version B1-02 onwards, the exon numbering from the MANE Select transcripts is used for the *MYCN, FBXW7, WT1*, and *AMER1* genes. For the *TP53* gene, the exon numbering from the LRG transcript is used but the MANE exon numbering can be found in square brackets after the LRG exon numbering in Table 2.** 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**
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 following samples from the Coriell Institute have been tested at MRC Holland with the P380-B1 probemix and can be used as positive control samples to detect copy number alterations in the genes listed. 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 P380-B1 | Expected copy number alteration |
|---|---|---|---|---|
| NA22977 | Coriell Institute | 1p36.33 | *TNFRSF18* | Heterozygous deletion |
| NA22976 Ю | Coriell Institute | 1p36.31-p36.33 | *TNFRSF18, CHD5* | Heterozygous deletion |
| NA50276 | Coriell Institute | 1p36.23-p36.31 | *CHD5, MIR34A* | Heterozygous deletion |
| NA00803 | Coriell Institute | 1q23.3 | *MPZ* | Heterozygous deletion |
| NA06473 | Coriell Institute | 1q43 | *SDCCAG8* | Heterozygous deletion |
| NA10401 | Coriell Institute | 2p24.3-q36.1 | *MYCN, DYSF, PAX3* | Heterozygous duplication |
| NA00945 | Coriell Institute | 2p24.3 | *MYCN* | Heterozygous deletion |
| NA10800 | Coriell Institute | 4q22.1 | *PKD2* | Heterozygous deletion |
| NA10313 | Coriell Institute | 4q31.3 | *FBXW7* | Heterozygous duplication |
| NA00501 | Coriell Institute | 4q31.3 | *FBXW7* | Heterozygous duplication |
| NA09709 | Coriell Institute | 11p13 | *WT1* | Heterozygous deletion |
| NA06226 | Coriell Institute | 16p13.11-p13.3 | *CREBBP, ABCC6* | Heterozygous duplication |
| NA08039 | Coriell Institute | 16p13.11-p13.3 | *CREBBP, ABCC6* | Heterozygous duplication |
| NA13685 | Coriell Institute | 16p13.11 | *ABCC6* | Heterozygous deletion |
| NA05875 | Coriell Institute | 16p11.2 | *VKORC1* | Heterozygous deletion |
| NA12074 | Coriell Institute | 16q22.1 | *CDH1* | Heterozygous deletion |
| NA09687 | Coriell Institute | 16q23.2-q24.3 | *MLYCD, FANCA* | Heterozygous duplication |
| NA01416 | Coriell Institute | Xp22.12-q21.2 | *RPS6KA3, AMER1, CHM* | Heterozygous triplication/ homozygous duplication |

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P380-B1 Wilms' tumour probemix.

Ю This cell line has other copy number alterations in reference probes, which are not mentioned in the table above.

**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 | | | 16**q**21 | | 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 | | | 16**q**23.3 | | 16-082,499 |
| 202 | **KISS1 probe** 04251-L03616 | | 1**q**32.1 | | | 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 | | | 16**q**12.1 | | 16-049,733 |
| 226 ¬ | **CHM probe** 13519-L14318 | | | | X**q**21.2 | X-085,101 |
| 232 | **MPZ probe** 04898-L17028 | | 1**q**23.3 | | | 01-159,543 |
| 238 | **FBXW7 probe** 06044-L05499 | | | | 4q31.3 | 04-153,493 |
| 244 † | **AMER1 probe** 12752-L13868 | | | | X**q**11.1 | X-063,330 |
| 250 | **BCL9 probe** 12945-L14100 | | 1**q**21.2 | | | 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 | | | 16**q**22.1 | | 16-067,420 |
| 292 | **IRF6 probe** 12495-L13539 | | 1**q**32.2 | | | 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 | | 1**q**43 | | | 01-241,609 |
| 346 ¬ | **DYSF probe** 08827-L13322 | | | | 2p13.2 | 02-071,694 |
| 355 † | **AMER1 probe** 15331-L29273 | | | | X**q**11.1 | 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 | | | 16**q**24.3 | | 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 | | | 16**q**13 | | 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 | | | | X**q**11.1 | 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**<br>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 | *TNFRSF18* | 1p36.33 | CGGGTTTCTCAC-TGTGTTCCCTGG | 5.0 **M**b | 01-001,129 |
| 492 « | 09114-L09174 | *CHD5* | 1p36.31 | GTTTCTTCTTCT-TAGGAAGGCTCA | 3.0 **M**b | 01-006,151 |
| 280 | 15299-L29217 | *MIR34A* | 1p36.23 | CTGGCCGGTCCA-CGGCATCCGGAG | 2.8 **M**b | 01-009,134 |
| 418 ± | 06136-L05580 | *MFN2* | 1p36.22 | GGTGGCGCTCTC-CTGGATGTAGGC | 10.4 **M**b | 01-011,972 |
| 401 « | 07158-L20119 | *WNT4* | 1p36.12 | AGCTGGAGAAGT-GCGGCTGTGACA | 71.9 **M**b | 01-022,321 |
| 328 ¬ | 20972-L29215 | *ABCA4* | 1p22.1 | CACGAGGAGCAT-GCAGCGAATTCA | 25.9 **M**b | 01-094,231 |
| 148 ¬ | 05252-L04632 | *HMGCS2* | 1p12 | GCATCTCTTTCA-AGGTTTCTGCTG | 25.5 **M**b | 01-120,113 |
| 250 | 12945-L14100 | *BCL9* | 1**q**21.2 | TTATTCCATCTG-AGAAGCCCAGCC | 14.0 **M**b | 01-145,563 |
| 232 | 04898-L17028 | *MPZ* | 1**q**23.3 | CGGGGTCGTTCT-GGGAGCTGTGAT | 42.9 **M**b | 01-159,543 |
| 202 | 04251-L03616 | *KISS1* | 1**q**32.1 | TTGGGGAGCCAT-TAGAAAAGGTGG | 5.6 **M**b | 01-202,429 |
| 292 | 12495-L13539 | *IRF6* | 1**q**32.2 | GCTTTACCAGAC-TCAGTAGTGGAG | 33.6 **M**b | 01-208,042 |
| 341 | 09027-L29503 | *SDCCAG8* | 1**q**43 | 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 *MYCN* 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 | ***MYCN***, ex 2 | 470-471 | TGGAAGAAGTTT-GAGCTGCTGCCC | 3.4 kb | 02-016,000 |
| 363 | 02572-L02036 | ***MYCN***, ex 3 | 1200-1201 | CTGTCACCACAT-TCACCATCACTG | 0.2 kb | 02-016,003 |
| 445 | 03327-L20117 | ***MYCN***, ex 3 | 1351-1352 | TGCACCCCCACA-GAAGAAGATAAA | 55.7 **M**b | 02-016,003 |
| 346 ¬ | 08827-L13322 | *DYSF* | 2p13.3 | CTCATCGACATT-GATGACAAGGAG | 151.2 **M**b | 02-071,694 |
| 307 ¬ | 05994-L29546 | *PAX3* | 2**q**36.1 | 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 *FBXW7* exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets.<br>*FBXW7*, an ubiquitin ligase component that acts as a tumour suppressor, has been shown to be mutated or deleted in ~4% of Wilms' tumour samples. *FBXW7* inactivation is suggested to associate with epithelial type (intermediate risk) tumour histology in Wilms' tumour (Williams et al. 2010). | | | | | | |
| 263 ¬ | 14738-L20398 | *PKD2* | 4**q**22.1 | TGTCACAACTTT-TGATTTCTTCCT | 64.3 **M**b | 04-089,187 |
| 208 | 06047-L05502 | ***FBXW7***, 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 | ***FBXW7***, ex 9 (11) | 2234-2235 | AGTCCATGGAAA-AGTGCATACATC | 21.9 kb | 04-153,471 |
| 238 | 06044-L05499 | ***FBXW7***, 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 *WT1* exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets.<br>*WT1* is altered either by a deletion or mutation in ~20% of sporadic Wilms' tumours (Huff 1998). Mutations or larger deletions in *WT1* 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 | *IGF2* | 11p15.5 | TGCCCAAACACA-CTTGGGTCGGCC | 30.3 **M**b | 11-002,119 |
| 274 | 05360-L04739 | ***WT1***, ex 10 (11) | 2276-2277 | GTCAGCCAGGCT-GCTAACCTGGAA | 4.2 kb | 11-032,367 |
| 167 Δ | 05358-L29218 | ***WT1***, ex 8 (9) | 1463-1464 | CCATACCAGTGT-GACTTCAAGGAC | 24.9 kb | 11-032,371 |
| 178 | 05354-L04733 | ***WT1***, ex 4 (5) | 1098-1099 | CATCCCAGCTTG-AATGCATGACCT | - | 11-032,396 |
| | | | | | | |
| **Chromosome 16**<br>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 | *CREBBP* | 16p13.3 | GGATGAATTCAT-TTAACCCCATGT | 12.4 **M**b | 16-003,765 |
| 268 | 07416-L17062 | *ABCC6* | 16p13.11 | CCCAAACTCTCA-CCTGCTCCCCAA | 14.8 **M**b | 16-016,184 |
| 154 | 10491-L11044 | *VKORC1* | 16p11.2 | TCCTCCAGGTGT-GCACGGGAGTGG | 18.7 **M**b | 16-031,013 |
| 220 # | 05679-L05121 | *SALL1* | 16**q**12.1 | CAACATCTTCTA-GTCCTTCTCAAG | 5.7 **M**b | 16-049,733 |
| 436 | 15528-L17383 | *SLC12A3* | 16**q**13 | GACCATTTCCTA-CCTGGCCATCTC | 9.7 **M**b | 16-055,470 |
| 136 Δ | 11564-L20523 | *TK2* | 16**q**21 | AGCCTGTGTCCA-AGTGGAGAAATG | 2.3 **M**b | 16-065,128 |
| 286 | 02414-L17063 | *CDH1* | 16**q**22.1 | TGCTGTTTCTTC-GGAGGAGAGCGG | 15.1 **M**b | 16-067,420 |
| 197 ± | 06327-L20116 | *MLYCD* | 16**q**23.3 | GAGATGTCACCA-GTCAGTGCCACG | 5.8 **M**b | 16-082,499 |
| 391 | 15632-L17496 | *FANCA* | 16**q**24.3 | AGCTCAGTCTCA-GCCTTGTGTTTG | - | 16-088,332 |
| | | | | | | |
| ***TP53***, at 17p13.1. Indicated ligation sites are according to the MANE Select transcript NM_000546.6. Exon numbering is according to LRG_321; the MANE exon numbering can be found in between brackets (if different).<br>Inactivation of *TP53*, 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 | ***TP53***, ex 10 | 1174-1175 | TTCCGAGAGCTG-AATGAGGCCTTG | 3.1 kb | 17-007,515 |
| 409 | 01586-L01158 | ***TP53***, ex 7 [8] | 981-982 | CTGTCCTGGGAG-AGACCGGCGCAC | 2.3 kb | 17-007,518 |
| 299 | 17420-L21142 | ***TP53***, ex 3 [4] | 451-450 reverse | TAGCTGCCCTGG-TAGGTTTTCTGG | 10.1 **M**b | 17-007,520 |
| 472 ¬ | 11730-L15418 | *RAI1* | 17p11.2 | CCAAGGATCTCA-TCTGGCCACCGC | - | 17-017,568 |
| | | | | | | |
| ***AMER1*** (previously *FAM123B*), at Xq11.1. Indicated ligation sites and exon numbers are according to the NM_152424.4 sequence, which is also the MANE Select transcript.<br>Inactivation of *AMER1*, either by deletion or by point mutation, is a frequent event in Wilms' tumours. Deletions are more common: *AMER1* has been shown to be deleted in 17-20% of Wilms' tumour samples (Rivera et al. 2007; Wegert et al. 2009). | | | | | | |
| 142 ¬ | 07868-L07682 | *RPS6KA3* | Xp22.12 | TCTCCCACATCA-TCCCTTGACCTC | 43.2 **M**b | X-020,123 |
| 463 † | 12760-L13876 | ***AMER1***, ex 2 | 3244-3245 | TGACCATGTCAA-TATCACTATCAG | 2.7 kb | X-063,327 |
| 244 † | 12752-L13868 | ***AMER1***, ex 2 | 517-518 | TCAGCAAGAGCA-AGACCCACGATG | 12.6 kb | X-063,330 |
| 355 † | 15331-L29273 | ***AMER1***, ex 1 | 130-131 | CTAGGAACCTGA-CCGGGCTGGGTA | 21.8 **M**b | X-063,342 |
| 226 ¬ | 13519-L14318 | *CHM* | X**q**21.2 | AGGTTCACTGGA-CTGATTTCTTCT | - | 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 | *GBE1* | 3p12 | GACCTAGAGGGA-CTCATGATCTTT | 03-081,775 |
| 373 | 02560-L02023 | *ATR* | 3q23 | GTTCTTGACATT-GAGCAGCGACTA | 03-143,651 |
| 256 | 05409-L04215 | *NIPBL* | 5p13 | CAAGTGCCTGTT-TTACAACAGAAC | 05-037,012 |
| 127 Δ | 18709-L26552 | *IL4* | 5q31 | ATCGACACCTAT-TAATGGGTCTCA | 05-132,038 |
| 335 | 15117-L29502 | *CDK5RAP2* | 9q33 | TCAGAAGAAACA-GTGTCTCCCACC | 09-122,370 |
| 185 | 18767-L24189 | *NODAL* | 10q22 | AGAGCGGTTTCA-GATGGACCTATT | 10-071,865 |
| 481 | 15738-L11546 | *GABRB3* | 15q12 | GCTCATGGAAAT-ATTCTGTTGACA | 15-024,344 |
| 320 | 05802-L20124 | *CAPN3* | 15q15 | AACCAGCTCTAT-GACATCATTACC | 15-040,490 |
| 427 | 10781-L11396 | *LDLR* | 19p13 | CTGGTTCCCAAA-GTCAGCCACGCA | 19-011,095 |
| 160 | 08771-L29189 | *CRX* | 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 SALSA MLPA probemixes

- **ME030 BWS/RSS:** Contains probes for rapid detection of most causes of BWS/RSS and for screening of Wilms' tumour.
- **P056 TP53:** Contains probes for all exons of the *TP53* gene.
- **P118 WT1:** Contains probes for the *WT1* and *AMER1* genes involved in Wilms' tumour, WAGR, Denys-Drash and Frasier syndrome.
- **P147 1p36:** Contains probes for the 1p36 region.
- **P431 FOXF1:** Contains probes for all exons of *MYCN* gene.

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## Selected publications using SALSA MLPA Probemix P380 Wilms' tumour


- 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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- Pérez-Linares FJ et al. (2020). MicroRNA Profiling in Wilms Tumor: Identification of Potential Biomarkers. *Front Pediatr*. 8:337.
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- Williams RD et al. (2015). Multiple mechanisms of MYCN dysregulation in Wilms tumour. *Oncotarget.* 6: 7232-43.


| **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 B1-02 – 07 February 2024 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Exon numbering of the *FBXW7* and *WT1* genes has been changed according to MANE.<br>- Information about positive sample NA01416 corrected in the table on page 3 for positive control DNA samples.<br>- Ligation sites of the probes targeting the *FBXW7* and *TP53* genes were updated according to the MANE transcript NM_ reference sequence.<br>- DNA denaturation warnings adjusted in Tables 1 and 2 for TNFRSF18 probe at 315 nt, and WNT4 probe at 401 nt.<br>- Warnings for sensitivity for experimental conditions added in Tables 1 and 2 for TK2 probe at 136 nt and WT1 probe at 167 nt.<br>- 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.<br>*Version B1-01 — 12 November 2020 (02P)*<br>- Product description restructured and adapted to a new template.<br>- Various minor textual or layout changes.<br>- Exon numbering of the *FBXW7* gene has been changed according to LRG_1141.<br>- Ligation sites of the probes targeting the *FBXW7*, *MYCN*, *WT1*, and *AMER1* genes updated according to new versions of their NM_ reference sequences.<br>- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).<br>- Warning added to Tables 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.<br>- Two new references added to the Selected publications using SALSA MLPA Probemix P380 Wilms' tumour section.<br>*Version 08 – 19 March 2019 (T08)*<br>- New references to literature added on page 2.<br>- 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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