# Product Description
## SALSA® MLPA® Probemix P308-B3 MET

To be used with the MLPA General Protocol.

**Version B3**
As compared to the previous B2 lot, the probe length of one target probe has been adjusted, there is no change in the sequence detected. For complete product history see page 9.

**Catalogue numbers:**
- **P308-025R:** SALSA MLPA Probemix P308 MET, 25 reactions.
- **P308-050R:** SALSA MLPA Probemix P308 MET, 50 reactions.
- **P308-100R:** SALSA MLPA Probemix P308 MET, 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 P308 MET is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MET*, *PTEN* and *LRRK2* genes.

The receptor tyrosine kinase MET (also known as hepatocyte growth factor receptor (HGFR) is frequently amplified in human cancers. Amplification results in high cell surface densities and constitutive activation of HGFR even in the absence of growth factor stimulation by its endogenous ligand, hepatocyte growth factor (HGF). *MET* gene is essential for survival and long-distance migration of epithelial and myogenic precursors during embryogenesis. Cancer cells hijack this biological process for invasion and metastasis. Multiple mechanisms can lead to aberrant MET activation, which occurs in many types of cancers.

*MET* gene amplifications leading to constitutive activation of the MET receptor are detected in a wide range of tumour types e.g. brain tumours, gastric cancer and lung tumours (Peters and Adjei 2012). Patients with papillary renal carcinomas carry both germline and somatic point mutations in *MET* (Schmidt et al. 1997; Schmidt et al. 1999). The amplification of leucine-rich repeat kinase-2 (*LRRK2*) is required for oncogenic MET signalling in papillary renal and in thyroid carcinomas and acts downstream of MET activation (Looyenga et al. 2011). Loss of tumour suppressor protein PTEN together with amplification of *MET* can be an alternative mechanism for primary resistance for tyrosine kinase inhibitors (EGFR-TKIs) (Engelman et al. 2007; Sos et al. 2009).

**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/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

**Exon numbering**
The *MET, PTEN* and *LRRK2* exon numbering used in this P308-B3 MET product description is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcripts as indicated in Table 2. 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. Please note that exon numbering for the same genes might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

**Probemix content**
The SALSA MLPA Probemix P308-B3 MET contains 48 MLPA probes with amplification products between 130 and 493 nucleotides (nt). This includes 23 probes for the *MET* gene and three flanking probes for *MET* gene. This probemix also includes four probes for *PTEN* gene and four probes for *LRRK2* gene. In addition, 14 reference probes are included detecting autosomal chromosomal locations, which are relatively copy number stable regions in various cancer types. The identity of the genes detected by the reference probes is available in Table 3. 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 cancer. 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. Samples from the Coriell Institute (see table below) have been tested with this P308 B-version of the probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

| Sample name | Source | Chromosomal position (hg18) of copy number alteration* | Altered target genes in P308-B3 | Expected copy number alteration |
|---|---|---|---|---|
| NA01059 | Coriell Institute | 7q31.2 | *MET* (+flanking genes *CAV2* and *CFTR*) | Heterozygous deletion |
| NA12519 | Coriell Institute | 7q31.2 | *MET* (+flanking genes *CAV2* and *CFTR*) | Homozygous duplication |
| NA20125 | Coriell Institute | 10q23.31 | *PTEN* | Heterozygous 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 P308-B3 MET probemix.

**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 for germline analysis 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/gain | 1.30 < FR < 1.65 |
| Heterozygous triplication/homozygous 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 mosaic or subclonal cases. Analysis of parental samples may be necessary for correct interpretation of complex results in germline level.
- 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.

**P308 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 major cause of genetic defects in the *MET, PTEN* and *LRRK2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P308 MET.
- 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.

**LOVD and COSMIC mutation databases**

LOVD database for *MET* gene: https://databases.lovd.nl/shared/genes/MET; LOVD database for *PTEN* gene: https://databases.lovd.nl/shared/genes/PTEN and LOVD database for *LRRK2* gene: https://databases.lovd.nl/shared/genes/LRRK2; COSMIC database: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in these above mentioned LOVD and COSMIC Databases. 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 *MET* exons 10 and 12 but not exon 11) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P308-B3 MET

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18) / Exon[a] | | | | Location (hg18) in kb |
|---|---|---|---|---|---|---|
| | | Reference | *MET* | *PTEN* | *LRRK2* | |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | | |
| 130 | Reference probe 00797-L21056 | 5q31 | | | | 05-132,038 |
| 139 | Reference probe 10913-L31828 | 2p25 | | | | 02-001,396 |
| 148 | **MET probe** 18448-L23950 | | Exon 21 | | | 07-116,223 |
| 155 | **MET probe** 18449-L23949 | | Exon 5 | | | 07-116,168 |
| 162 | **MET probe** 10327-L24422 | | Exon 17 | | | 07-116,206 |
| 168 | **MET probe** 00453-L23587 | | Exon 2 | | | 07-116,126 |
| 176 | **MET probe** 10317-L23588 | | Exon 7 | | | 07-116,185 |
| 182 | Reference probe 08731-L23593 | 9q21 | | | | 09-078,101 |
| 189 | **MET probe** 22585-L23584 | | Exon 11 | | | 07-116,190 |
| 197 ± | **PTEN probe** 06729-L23164 | | | Exon 2 | | 10-089,644 |
| 203 | **MET probe** 18450-L24423 | | Exon 14 | | | 07-116,199 |
| 211 | Reference probe 04533-L03922 | 2q24 | | | | 02-166,607 |
| 218 | **MET probe** 10320-L23586 | | Exon 10 | | | 07-116,187 |
| 224 | **LRRK2 probe** 04282-L23585 | | | | Exon 41 | 12-039,020 |
| 229 | Reference probe 13569-L23952 | 19p13 | | | | 19-013,425 |
| 236 | **MET probe** 10329-L23575 | | Exon 19 | | | 07-116,211 |
| 241 ¬ | **CFTR probe** 03839-L23953 | | 7q31.2 | | | 07-116,907 |
| 249 | **MET probe** 18451-L23954 | | Exon 18 | | | 07-116,209 |
| 255 | **MET probe** 10323-L23583 | | Exon 13 | | | 07-116,199 |
| 261 | Reference probe 07819-L23623 | 1q31 | | | | 01-194,726 |
| 268 ¬ | **IKZF1 probe** 13873-L31794 | | 7p12.2 | | | 07-050,412 |
| 275 | **MET probe** 18435-L23582 | | Exon 16 | | | 07-116,205 |
| 283 | **MET probe** 18452-L23628 | | Exon 3 | | | 07-116,159 |
| 289 | **MET probe** 22614-L31904 | | Exon 8 | | | 07-116,185 |
| 294 | Reference probe 11900-L23408 | 6p12 | | | | 06-052,028 |
| 303 | **MET probe** 18453-L31792 | | Exon 21 | | | 07-116,225 |
| 310 | **MET probe** 10316-L23581 | | Exon 6 | | | 07-116,183 |
| 318 | Reference probe 16438-L23633 | 18q21 | | | | 18-045,645 |
| 325 | **MET probe** 18454-L23630 | | Exon 4 | | | 07-116,167 |
| 332 | **MET probe** 10325-L24169 | | Exon 15 | | | 07-116,202 |
| 338 | Reference probe 13423-L31795 | 12p13 | | | | 12-006,103 |
| 346 | **MET probe** 18455-L23631 | | Exon 12 | | | 07-116,197 |
| 355 | **PTEN probe** 18694-L24425 | | | Exon 3 | | 10-089,675 |
| 363 | Reference probe 14981-L16717 | 6q22 | | | | 06-129,461 |
| 373 # | **PTEN probe** 03638-L22839 | | | Exon 5 | | 10-089,683 |
| 382 | **LRRK2 probe** 04278-L23577 | | | | Exon 1 | 12-038,905 |
| 393 | **MET probe** 10312-L22033 | | Exon 1 | | | 07-116,100 |
| 400 | Reference probe 09433-L09682 | 11q13 | | | | 11-066,150 |
| 409 | **MET probe** 10330-L23955 | | Exon 20 | | | 07-116,223 |
| 417 | **MET probe** 18456-L23632 | | Exon 2 | | | 07-116,127 |
| 427 ¬ | **CAV2 probe** 04707-L02740 | | 7q31.2 | | | 07-115,928 |
| 436 | **LRRK2 probe** 04279-L23590 | | | | Exon 10 | 12-038,932 |
| 444 | **PTEN probe** 17395-L21062 | | | Exon 9 | | 10-089,715 |
| 454 | Reference probe 12790-L31796 | 2q13 | | | | 02-108,880 |
| 463 | **LRRK2 probe** 04280-L03684 | | | | Exon 15 | 12-038,955 |
| 474 ¥ | **MET probe** 10319-L32155 | | Exon 9 | | | 07-116,186 |
| 483 | Reference probe 06676-L06254 | 11p15 | | | | 11-006,369 |
| 493 | Reference probe 09772-L22978 | 15q21 | | | | 15-042,706 |

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

¥ Changed in version B3. Minor alteration, no change in sequence detected.

± SNV rs146326040 (probe at 197 nt) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¬ 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.

# 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 2. MET, PTEN and LRRK2 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene exon[a] | Location (hg18) / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| ***MET* gene** at 7q31.2. Indicated ligation sites are in NM_000245.4 (MANE Select). | | | | | |
| 268 ¬ | 13873-L31794 | *IKZF1* | 7**p**12.2 | TACGAATGCTTG-ATGCCTCGGGAG | 65.5 **M**b |
| 427 ¬ | 04707-L02740 | *CAV2* | 7q31.2 | CAGCAAATACGT-AATGTACAAGTT | 172.2 kb |
| *Centromeric* | | | | | |
| 393 | 10312-L22033 | ***MET* exon 1** | 366-367 | TTCTCCACTGGT-TCCTGGGCACCG | 26.6 kb |
| | | *start codon* | *397-399 (exon 1)* | | |
| 168 | 00453-L23587 | ***MET* exon 2** | 446-447 | GCTCCTGTTTAC-CTTGGTGCAGAG | 0.5 kb |
| 417 | 18456-L23632 | ***MET* exon 2** | 962-963 | TTCATCTGTAAA-GGACCGGTTCAT | 32.1 kb |
| 283 | 18452-L23628 | ***MET* exon 3** | 1699-1700 | TGGGTCAATTCA-GCGAAGTCCTCT | 8.2 kb |
| 325 | 18454-L23630 | ***MET* exon 4** | 1830-1831 | CCTCATGTGAAT-TTTCTCCTGGAC | 1.0 kb |
| 155 | 18449-L23949 | ***MET* exon 5** | 2040-2039 reverse | AGGCATTCCTCC-GATCGCACACAT | 14.4 kb |
| 310 | 10316-L23581 | ***MET* exon 6** | 2144-2145 | AAGGCTGACCAT-ATGTGGCTGGGA | 2.1 kb |
| 176 | 10317-L23588 | ***MET* exon 7** | 2280-2281 | ACAGTTGGTCCT-GCCATGAATAAG | 0.2 kb |
| 289 | 22614-L31904 | ***MET* exon 8** | 2391-2392 | ATTTCGCCGAAA-TACGGTCCTATG | 0.9 kb |
| 474 | 10319-L32155 | ***MET* exon 9** | 2608-2609 | GCATCTTCAGTT-ACCGTGAAGATC | 0.8 kb |
| 218 | 10320-L23586 | ***MET* exon 10** | 2679-2680 | AGCACAATAACA-GGTGTTGGGAAA | 3.7 kb |
| 189 | 22585-L23584 | ***MET* exon 11** | 2789-2790 | TAATTCAGAGAT-AATCTGTTGTAC | 6.7 kb |
| 346 | 18455-L23631 | ***MET* exon 12** | 3084-3085 | TTATGCACGGTC-CCCAATGACCTG | 1.8 kb |
| 255 | 10323-L23583 | ***MET* exon 13** | 3233-3234 | AACAGCACTGTT-ATTACTACTTGG | 0.3 kb |
| 203 | 18450-L24423 | ***MET* exon 14** | 3387-3388 | GAAATGGTTTCA-AATGAATCTGTA | 3.1 kb |
| 332 | 10325-L24169 | ***MET* exon 15** | 3573-3574 | AGTGCTCTAAAT-CCAGAGCTGGTC | 2.4 kb |
| 275 | 18435-L23582 | ***MET* exon 16** | 3708-3709 | GATGGCAAGAAA-ATTCACTGTGCT | 1.4 kb |
| 162 | 10327-L24422 | ***MET* exon 17** | 3797-3798 | CATGAAAGATTT-TAGTCATCCCAA | 3.3 kb |
| 249 | 18451-L23954 | ***MET* exon 18** | 4019-4020 | CTTGGCTGCAAG-AAACTGTATGTA | 1.3 kb |
| 236 | 10329-L23575 | ***MET* exon 19** | 4149-4150 | AAGTGGATGGCT-TTGGAAAGTCTG | 12.2 kb |
| 409 | 10330-L23955 | ***MET* exon 20** | 6 nt before exon 20 | TGTTTCTTGTTT-TACTAGTGGTCC | 0.5 kb |
| 148 | 18448-L23950 | ***MET* exon 21** | 4559-4560 | CTCCTTCTGGGA-GACATCATAGTG | 1.7 kb |
| | | *stop codon* | *4567-4569 (exon 21)* | | |
| 303 | 18453-L31792 | ***MET* exon 21** | 6242-6243 | TACACCCCACCC-TCATTACATCAT | 682.2 kb |
| *Telomeric* | | | | | |
| 241 ¬ | 03839-L23953 | *CFTR* | 7q31.2 | AGAAAAAGGGTT-GAGCGGCAGGCA | - |
| | | | | | |
| ***PTEN* gene** at 10q23.31. Indicated ligation sites are in NM_000314.8 (MANE Select). | | | | | |
| | | *start codon* | *846-848 (exon 1)* | | |
| 197 ± | 06729-L23164 | ***PTEN* exon 2** | 217 nt after exon 2 reverse | TATCACATAAGT-ACCTGATTATGT | 31.0 kb |
| 355 | 18694-L24425 | ***PTEN* exon 3** | 145 nt before exon 3 | GGGGTATTTGTT-GGATTATTTATT | 7.8 kb |
| 373 # | 03638-L22839 | ***PTEN* exon 5** | 1250-1251 | GGTGTAATGATA-TGTGCATATTTA | 32.1 kb |
| 444 | 17395-L21062 | ***PTEN* exon 9** | 6 nt before exon 9 | TAAATTTTCTTT-CTCTAGGTGAAG | - |
| | | *stop codon* | *2055-2057 (exon 9)* | | |

| ***LRRK2* gene** at 12q12. Indicated ligation sites are in NM_198578.4 (MANE Select). | | | | | |
|---|---|---|---|---|---|
| | | *start codon* | *136-138 (exon 1)* | | |
| 382 | 04278-L23577 | ***LRRK2* exon 1** | 230-231 | AGGAAAACAGAT-AGAAACGCTGGT | 26.3 kb |
| 436 | 04279-L23590 | ***LRRK2* exon 10** | 1286-1285 reverse | TCTTCTCATGTA-AACTGTTTTGGT | 23.2 kb |
| 463 | 04280-L03684 | ***LRRK2* exon 15** | 1904-1905 | GGATTCAGTGCT-TCACACACTGCA | 65.6 kb |
| 224 | 04282-L23585 | ***LRRK2* exon 41** | 6106-6107 | CAGCCATGATTA-TATACCGAGACC | - |
| | | *stop codon* | *7717-7719 (exon 51)* | | |

[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.

± SNV rs146326040 (probe at 197 nt) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¬ 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.

# 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 |
|---|---|---|---|---|---|
| 261 | 07819-L23623 | *KCNT2* | 1q31 | CCTTACCATTCA-TTTCCTTGTGAA | 01-194,726 |
| 139 | 10913-L31828 | *TPO* | 2p25 | GAGTGGCTGTAA-TTTGGGCCATTA | 02-001,396 |
| 454 | 12790-L31796 | *EDAR* | 2q13 | AGAATCAAGGCT-TTTGTGATATGT | 02-108,880 |
| 211 | 04533-L03922 | *SCN1A* | 2q24 | TGAAATGAGAAA-GAGAAGGTCAAG | 02-166,607 |
| 130 | 00797-L21056 | *IL4* | 5q31 | ATCGACACCTAT-TAATGGGTCTCA | 05-132,038 |
| 294 | 11900-L23408 | *PKHD1* | 6p12 | TGCTCTCTGGAT-TCAAGACTGAAA | 06-052,028 |
| 363 | 14981-L16717 | *LAMA2* | 6q22 | TGCCAAAGATGA-TGAGGTCATCTG | 06-129,461 |
| 182 | 08731-L23593 | *PCSK5* | 9q21 | GTCCTGGATTCT-ATGGTGACCAAG | 09-078,101 |
| 483 | 06676-L06254 | *SMPD1* | 11p15 | CTGCTGAAGATA-GCACCACCTGCC | 11-006,369 |
| 400 | 09433-L09682 | *RBM14* | 11q13 | CACCAACTGTCT-TCTTCTCTCGAC | 11-066,150 |
| 338 | 13423-L31795 | *VWF* | 12p13 | AAGGGCCTCCAT-TTCTCATTCCTG | 12-006,103 |
| 493 | 09772-L22978 | *SPG11* | 15q21 | TTTCTTCAGGAT-TGATAGTCATTC | 15-042,706 |
| 318 | 16438-L23633 | *MYO5B* | 18q21 | CGTGGCCGACCA-AGCCACGCAGAA | 18-045,645 |
| 229 | 13569-L23952 | *CACNA1A* | 19p13 | GCTCCTACTTGA-GGAATGGCTGGA | 19-013,425 |

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

## Related SALSA MLPA probemixes

- **P052 Parkinson mix 2:** Contains seven probes for the *LRRK2* gene.
- **P105 Glioma-2:** Contains among other target genes (*PDGFRA, EGFR, CDKN2A, CDK4-MIR26A2-MDM2, NFKBIA* and *TP53*) nine probes for the *PTEN* gene.
- **P225 PTEN:** Contains at least two probes for each of the 9 exons of the *PTEN* gene.
- **P315 EGFR:** Contains 28 probes for the *EGFR* gene, three flanking probes for the *EGFR* gene and two probes specific for the *EGFR* c.2573T>G=p.L858R and c.2369C>T=p.T790M point mutations.

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- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem*. 421:799-801.


## Selected publications using SALSA MLPA Probemix P308 MET


- Sebai M et al. (2022). Novel germline MET pathogenic variants in French patients with papillary renal cell carcinomas type I. *Hum Mutat.* 43:316-27.


| **P308 product history** | |
|---|---|
| *Version* | *Modification* |
| B3 | As compared to the previous B2 lot, the probe length of one target probe has been adjusted, there is no change in the sequence detected. |
| B2 | One flanking probe has been added, five reference probes have been replaced, and several probes have a change in length but no change in the sequence targeted. |
| B1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version B3-01 – 03 October 2023 (04P)<br>- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2) and to a new template.<br>- Various minor textual or layout changes.<br>- Ligation sites of the probes targeting the *MET* gene updated according to new version of the NM_000245.4 MANE Select sequence.<br><br>Version B2-01—05 February 2020 (02P)<br>- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).<br>- Various minor textual or layout changes.<br>- Information about positive control DNA samples added to page 2.<br>-Ligation sites of the probes targeting the MET gene updated according to new version of the NM_001127500.3, PTEN gene according to the NM_000314.8 and LRRK2 gene according to the NM_198578.4 reference sequence. |

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|---|
| - Warning added to Table 1 and 2a for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.<br>- Added probemixes P105, P315 and P458 in the "Related SALSA MLPA probemixes" section on page 7.<br>- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). |

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