Product Description
SALSA® MLPA® Probemix P451-B1 Chromosome 16

To be used with the MLPA General Protocol.

Version B1
First unrestricted release. For complete product history see page 8.

Catalogue numbers:
- **P451-025R:** SALSA MLPA Probemix P451 Chromosome 16, 25 reactions.
- **P451-050R:** SALSA MLPA Probemix P451 Chromosome 16, 50 reactions.
- **P451-100R:** SALSA MLPA Probemix P451 Chromosome 16, 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 P451 Chromosome 16 is a **research use only (RUO)** assay for the detection of deletions or duplications on chromosome 16, which are associated with several tumour types, including breast cancer, Wilms' tumour and multiple myeloma.

Low-grade breast neoplasias are molecularly characterized by a loss of chromosome arm 16q (De Boer et al. 2018; Rakha et al. 2006). Deletions of 16q arm are amongst the most frequent genetic events in breast cancer, especially in low grade ductal carcinoma, and loss of 16q seems to be associated with better prognosis. Since a loss of chromosome 16q has also been detected in premalignant lesions, this loss is likely to encompass an early step in carcinogenesis (De Boer et al. 2018). Loss of 16q is associated with poor prognosis in multiple myeloma (Jenner et al. 2006) and loss of heterozygosity for 16q is a prognostic factor identifying a high risk of relapse and death in Wilms' tumour (Grundy et al. 2005).

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
PhenoGram Plot: http://visualization.ritchielab.org/phenograms/plot

Probemix content
The SALSA MLPA Probemix P451-B1 Chromosome 16 contains 50 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 36 probe(s) for chromosome 16, targeting 35 genes. In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types, including breast cancer. Probe sequences and the identity of the genes detected by the reference probes are available in table 3 and 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, derived from germline blood samples or from tumour tissue and corresponding healthy tissue, 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 formalin-fixed paraffin-embedded 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 healthy individuals. 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 NA06226, NA08039, NA05875 and NA12074 from the Coriell Institute have been tested with this P451-B1 Chromosome 16 probemix at MRC-Holland and can be used as positive control samples to detect the copy number alterations mentioned in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Coriell sample	Chromosomal position of copy number alteration (hg18)	Affected target gene(s) in P451-B1	Expected copy number alteration
NA06226	16p12.1-p13.3	*TSC2*, *CREBBP*, *ABAT*, *ABCC1*, *UQCRC2*	Heterozygous duplication
NA08039	16p12.1-p13.3	*CREBBP*, *ABAT*, *ABCC1*, *UQCRC2*, *PALB2*	Heterozygous duplication
NA05875	16p11.2	*VKORC1*	Heterozygous deletion
NA12074	16q22.1-q23.1	*CDH1*, *TXNL4B*, *DHX38*, *ZFHX3*, *BCAR1*	Heterozygous deletion

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 final ratio (FR) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

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. 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 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.

P451 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 P451 Chromosome 16.
- 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 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P451-B1 Chromosome 16

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			Location (hg18) in kb
		Reference	16p	16q	
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 20879-L29296	12q24			12-116,137
136	Reference probe 19551-L30449	2p13			02-071,750
142	**CDH8 probe** 20362-L28029			**16q21**	16-060,381
148	**ABAT probe** 13864-L15382		**16p13.2**		16-008,737
154	**ADGRG1 probe** 10195-L10655			**16q13**	16-056,247
160	**ZFHX3 probe** 20053-L27397			**16q22.3**	16-071,391
165	Reference probe 13816-L28133	2q13			02-108,906
172	**SALL1 probe** 20054-L27172			**16q12.1**	16-049,732
183	**ABCC12 probe** 20055-L28030			**16q12.1**	16-046,733
193	Reference probe 12422-L28134	14q24			14-076,842
204	**BCAR1 probe** 21158-L29445			**16q23.1**	16-073,826
211 «	**FOXF1 probe** 21582-L25816			**16q24.1**	16-085,105
218	**ADAMTS18 probe** 20363-L27760			**16q23.1**	16-075,885
226	Reference probe 13554-L08748	9q21			09-078,133
232	**CMTM3 probe** 20366-L29446			**16q21**	16-065,200
240	**FANCA probe** 01487-L29447			**16q24.3**	16-088,379
246	Reference probe 19985-L27453	4p16			04-005,671
256	**GAS8 probe** 02702-L29865			**16q24.3**	16-088,638
264	**CYLD probe** 16225-L27394			**16q12.1**	16-049,386
269	**ABCC1 probe** 20368-L28032		**16p13.11**		16-016,085
277	Reference probe 13393-L28135	6q12			06-064,546
283	**CDH1 probe** 21583-L30451			**16q22.1**	16-067,420
293	**IRF8 probe** 20772-L28737			**16q24.1**	16-084,494
300	**TXNL4B probe** 20370-L27767			**16q22.3**	16-070,682
306	**TK2 probe** 11589-L30694			**16q21**	16-065,120
313	Reference probe 11548-L30695	1p31			01-068,687
319	**DHX38 probe** 20372-L27769			**16q22.3**	16-070,697
325	**CREBBP probe** 09906-L27184		**16p13.3**		16-003,727
333	**CDH11 probe** 20176-L27448			**16q21**	16-063,590
338	**MMP2 probe** 04766-L28160			**16q12.2**	16-054,097
346	**VKORC1 probe** 10487-L11040		**16p11.2**		16-031,014
355	Reference probe 06015-L27179	19q13			19-059,318
364	**CDH1 probe** 12656-L13730			**16q22.1**	16-067,400
371	**VPS35 probe** 05770-L21374			**16q11.2**	16-045,260
379	**PALB2 probe** 07497-L30936		**16p12.1**		16-023,548
386	Reference probe 04278-L30937	12q12			12-038,905
397	**UQCRC2 probe** 20597-L29864		**16p12.1**		16-021,893
402	**SLC12A3 probe** 15527-L29444			**16q13**	16-055,478
409	Reference probe 10681-L11263	6p12			06-051,933
417	**WWOX probe** 03346-L30450			**16q23.1**	16-076,706
427 «	**SPG7 probe** 07258-L06829			**16q24.3**	16-088,103
433	**TSC2 probe** 13549-L30696		**16p13.3**		16-002,060
439	**CDH13 probe** 07946-L30697			**16q23.3**	16-081,218
447	**FBXO31 probe** 20058-L30698			**16q24.2**	16-085,935
454	**MLYCD probe** 20059-L30699			**16q23.3**	16-082,498
461	**RBL2 probe** 20060-L27178			**16q12.2**	16-052,046
471	Reference probe 00979-L21316	10p14			10-012,019
484	**CNTNAP4 probe** 20374-L30693			**16q23.1**	16-075,071
492	Reference probe 17001-L30500	20q11			20-034,954
500	Reference probe 13438-L30452	5q31			05-131,756

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. Chromosome 16 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal position (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
433	13549-L30696	*TSC2*	16p13.3	GCGCCGTGGTGA-GCTGCGTCCTCT	1.7 Mb	16-002,060
325	09906-L27184	*CREBBP*	16p13.3	AAAAAGATGCTG-GACAAGGCGTTT	5.0 Mb	16-003,727
148	13864-L15382	*ABAT*	16p13.2	CCCTGTCCCTCA-AGGGGTCATGGC	7.3 Mb	16-008,737
269	20368-L28032	*ABCC1*	16p13.11	TCACGGACCTGT-AATATGGTTCCT	5.8 Mb	16-016,085
397	20597-L29864	*UQCRC2*	16p12.1	GTATAAATCCCA-AAGAGTCCAGAA	1.7 Mb	16-021,893
379	07497-L30936	*PALB2*	16p12.1	CTTCCTGCTTCT-GATAGCATAAAC	7.5 Mb	16-023,548
346	10487-L11040	*VKORC1*	16p11.2	TTCTTCGCTGTT-TTCCTAACTCGC	14.2 Mb	16-031,014
371	05770-L21374	*VPS35*	16q11.2	CCTTTGGTATTT-GCAGCTTACCAG	1.5 Mb	16-045,260
183	20055-L28030	*ABCC12*	16q12.1	TCCTGAGAAGGC-CTCTCTGAGCCA	2.7 Mb	16-046,733
264	16225-L27394	*CYLD*	16q12.1	AGGCTGAATCAT-AAATATAACCCA	0.3 Mb	16-049,386
172	20054-L27172	*SALL1*	16q12.1	ATCTGGATATGA-GGGTATTTCTCT	2.3 Mb	16-049,732
461	20060-L27178	*RBL2*	16q12.2	TCATCTAAATTC-CCAACAGATAAA	2.1 Mb	16-052,046
338	04766-L28160	*MMP2*	16q12.2	AAGGGTGCCTAT-TACCTGAAGCTG	1.4 Mb	16-054,097
402	15527-L29444	*SLC12A3*	16q13	ACAAGAGGAAGA-TCAAGGCCTTCT	0.8 Mb	16-055,478
154	10195-L10655	*ADGRG1*	16q13	GATTGTGGTACA-GAACACCAAAGT	4.1 Mb	16-056,247
142	20362-L28029	*CDH8*	16q21	CCTGGAGAGGCA-GTTCAACATTAA	3.2 Mb	16-060,381
333	20176-L27448	*CDH11*	16q21	AATTCCGACGGT-GGCTCCAGTGGC	1.5 Mb	16-063,590
306	11589-L30694	*TK2*	16q21	GAGAGGTCGATT-CACAGCGCAAGA	79.4 kb	16-065,120
232	20366-L29446	*CMTM3*	16q21	TCCTCTTTGCTG-ATGCCATGCAGC	2.2 Mb	16-065,200
364	12656-L13730	*CDH1*	16q22.1	TCAGAAGACAGA-AGAGAGACTGGG	19.7 kb	16-067,400
283	21583-L30451	*CDH1*	16q22.1	TGCTGTTTCTTC-GGAGGAGAGCGG	3.3 Mb	16-067,420
300	20370-L27767	*TXNL4B*	16q22.3	TGGGTTATTTCA-AGATGAGCTTCC	15.3 kb	16-070,682
319	20372-L27769	*DHX38*	16q22.3	GGATGCTCTGCA-GATCTATCCCAT	0.7 Mb	16-070,697
160	20053-L27397	*ZFHX3*	16q22.3	GCAGCATGTTCC-TCCCAGCAGCTG	2.4 Mb	16-071,391
204	21158-L29445	*BCAR1*	16q23.1	CGAGTCCTGGGA-GGTGAACTTAGG	1.2 Mb	16-073,826
484	20374-L30693	*CNTNAP4*	16q23.1	TGGTCCCCTGGA-ACCATTTCTTCT	0.8 Mb	16-075,071
218	20363-L27760	*ADAMTS18*	16q23.1	CCTGCAGCTCAG-GTCTGGGGAGAC	0.8 Mb	16-075,885
417	03346-L30450	*WWOX*	16q23.1	GGCGTTTACTGT-GGATGATAATCC	4.5 Mb	16-076,706
439	07946-L30697	*CDH13*	16q23.3	CTCCTGTCCCAG-GTAGGGAAGAGG	1.3 Mb	16-081,218
454	20059-L30699	*MLYCD*	16q23.3	TTCACACGGTGA-ATGCCAGGTAAC	2.0 Mb	16-082,498
293	20772-L28737	*IRF8*	16q24.1	CTCTTCTCCTCA-TTCTCCCAAATC	0.6 Mb	16-084,494
211 «	21582-L25816	*FOXF1*	16q24.1	AGCCGTCTTTTG-CAGGGAGCGGGA	0.8 Mb	16-085,105
447	20058-L30698	*FBXO31*	16q24.2	CCTCCCCATGAC-CCCCACGTCGAT	2.2 Mb	16-085,935
427 «	07258-L06829	*SPG7*	16q24.3	GTGGAATCCAGT-AACGGTTTCCGG	0.3 Mb	16-088,103
240	01487-L29447	*FANCA*	16q24.3	CCTGGTCTTCCT-GTTTACGTTCTT	0.3 Mb	16-088,379
256	02702-L29865	*GAS8*	16q24.3	CTGGCACTAACT-TCATTGACACCT	-	16-088,638

[a] 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 located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 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
313	11548-L30695	*RPE65*	1p31	CAGGGTTGAGCA-TCCTGCTGGTGG	01-068,687
136	19551-L30449	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071,750
165	13816-L28133	*EDAR*	2q13	CTCACATTCCTT-GGTGTTGGGGGG	02-108,906
246	19985-L27453	*EVC2*	4p16	TCAGTCTTCTCC-CTGTCTGAAGAG	04-005,671
500	13438-L30452	*SLC22A5*	5q31	GACTTGTATTAT-TTGGCTACAGTC	05-131,756
409	10681-L11263	*PKHD1*	6p12	TCACAGCTGGTT-TCCTGAAAGGCT	06-051,933
277	13393-L28135	*EYS*	6q12	ATAGAGAGTGGA-ACTAGTGTTTAG	06-064,546
226	13554-L08748	*PCSK5*	9q21	AGAAAGGCCTGA-TCATGAACCCTC	09-078,133
471	00979-L21316	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
386	04278-L30937	*LRRK2*	12q12	AGGAAAACAGAT-AGAAACGCTGGT	12-038,905
130	20879-L29296	*NOS1*	12q24	ACTGCTGAACCT-TTCCTCTGGGAC	12-116,137
193	12422-L28134	*POMT2*	14q24	TCTTGCTGGCTA-CCTGAGTGGATA	14-076,842
355	06015-L27179	*PRPF31*	19q13	AATGCACTGGAT-TACATCCGCACG	19-059,318
492	17001-L30500	*SAMHD1*	20q11	CCCTGTCACCTC-AAGTTTGAGGAT	20-034,954

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

Related SALSA MLPA probemixes

- **P380 Wilms' tumour**: Contains probes for 16p and 16q, as well as two other chromosomal regions and five genes relevant for Wilms' tumour.
- **P425 Multiple Myeloma**: Contains probes for 16q12 and 16q23, as well as nine other chromosomal regions relevant for multiple myeloma.
- **P078 Breast tumour**: Contains probes for *CDH1*, as well as eight other chromosomal regions relevant for breast cancer.

References

- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- De Boer M et al. (2018). Role of columnar cell lesions in breast carcinogenesis: analysis of chromosome 16 copy number changes by multiplex ligation-dependent probe amplification. *Mod Pathol* 31:1816-33.
- Grundy PE et a. (2005). Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National Wilms Tumor Study Group. *J Clin Oncol.* 23:7312-21.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Jenner MW et al. (2006). Abnormalities of 16q in Multiple Myeloma Are Associated with Poor Prognosis: 500K Gene Mapping and Expression Correlations Identify Two Potential Tumor Suppressor Genes, WWOX and CYLD. *Blood.* 108:110.
- Rakha EA et al. (2006). Chromosome 16 tumor-suppressor genes in breast cancer. *Genes Chromosomes Cancer* 45:527-35.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- 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 P451 Chromosome 16

- De Boer M et al. (2018). Role of columnar cell lesions in breast carcinogenesis: analysis of chromosome 16 copy number changes by multiplex ligation-dependent probe amplification. *Mod Pathol.* 31:1816-33.
- Lacle MM et al. (2013). Analysis of copy number changes on chromosome 16q in male breast cancer by multiplex ligation-dependent probe amplification. *Mod Pathol.* 26:1461-7.

P451 product history	
Version	*Modification*
B1	First unrestricted release
A1	Restricted test version

Implemented changes in the product description
Version B1-03 – 30 June 2022 (04P) - Product description rewritten and adapted to a new template. - Various minor textual and layout changes. Version B1-02 – 09 September 2020 (01P) - Name of *GPR56* gene is updated in Tables 1 & 2a to *ADGRG1* according to the new HUGO nomenclature. Version B1-01 - 07 August 2018 (01P) - Not applicable, new document.

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