Product Description
SALSA® MLPA® Probemix P294-C1 Tumour Loss

To be used with the MLPA General Protocol.

Version C1
As compared to version B1, the probemix content is fully revised. For complete product history see page 11.

Catalogue numbers:
- **P294-025R:** SALSA MLPA Probemix P294 Tumour Loss, 25 reactions.
- **P294-050R:** SALSA MLPA Probemix P294 Tumour Loss, 50 reactions.
- **P294-100R:** SALSA MLPA Probemix P294 Tumour Loss, 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 P294 Tumour Loss is a **research use only (RUO)** assay for the detection of copy number aberrations in 15 chromosomal regions that are frequently deleted or mutated in tumour samples.

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
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/ and http://tark.ensembl.org/
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The exon numbering used in this P294-C1 Tumour Loss product description is the exon numbering from the LRG_322 for *VHL*, NG_007551.2 for *FHIT*, LRG_130 for *APC*, LRG_515 for *PTCH1*, LRG_486 for *TSC1*, LRG_311 for *PTEN*, LRG_525 for *WT1*, LRG_293 for *BRCA2*, LRG_517 for *RB1*, LRG_487 for *TSC2*, LRG_321 for *TP53*, LRG_214 for *NF1*, LRG_318 for *SMAD4*, LRG_319 for *STK11*, NM_012181.5 for *FKBP8*, LRG_520 for *SMARCB1*, and LRG_1259 for *AMER1*. For *BRCA1* the traditional exon numbering (exons 1a, 1b, 2, 3 and 5-24), wherein no exon 4 is present is used. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG, NG or NM sequences. **From product description version C1-04 onwards, the exon numbering from the MANE transcripts is used for *CDKN2A*.** Consequently, for *CDKN2A*, the exon numbering has been changed: NM_000077.5 (MANE Select) encoding p16INK4A and NM_058195.4 (MANE Plus Clinical) encoding p14ARF are used. Both NM_000077.5 and NM_058195.4 have distinct first exons (both numbered as exon 1) which contain the translation start codon, and share a common second exon, which is translated in different reading frames (see schematic presentation below). The exon numbering (LRG_11 for *CDKN2A*), used in previous versions of this product description, can be found in between brackets in the 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 P294-C1 Tumour Loss contains 63 MLPA probes with amplification products between 121 and 504 nucleotides (nt). This includes at least 2 probes for the following regions or genes: 1p36, 13q14 (*RB1*), *AMER1*, *APC*, *BRCA1/2*, *CDKN2A/2B*, *FHIT*, *FKBP8*, *NF1*, *PTCH1*, *PTEN*, *SMAD4*, *SMARCB1*, *STK11*, *TP53*, *TSC1/2*, *VHL* and *WT1*. In addition, 12 reference probes are included and detect different autosomal chromosomal locations that are relatively copy number stable in most cancer types. Complete 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 of the same sex 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 healthy individuals without a history of cancer. 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. Sample ID numbers indicated in the table below have been tested with this P294-C1 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 P294-C1	Expected copy number alteration
NA22991	Coriell Institute	1p36.32-p36.33	*TNFRSF4, PRDM16*	Heterozygous deletion
NA50276	Coriell Institute	1p36.22-p36.31	*CHD5, CAMTA1, KIF1B*	Heterozygous deletion
SK-N-MC (ACC-203)◊	DSMZ	1p36.22-p36.33	*TNFRSF4, PRDM16, CHD5, CAMTA1, KIF1B*	Subclonal gain (ratios around 1.3)
		3p14.2-p25.3	*VHL, FHIT*	Heterozygous deletion
		10q23.31	*PTEN*	Heterozygous deletion
		17p13.1	*TP53*	Heterozygous deletion
NA10985	Coriell Institute	3p25.3	*VHL*	Heterozygous deletion
NA03503	Coriell Institute	3p25.3	*VHL*	Heterozygous duplication
NA14234	Coriell Institute	5q22.2	*APC*	Heterozygous deletion
NA02819	Coriell Institute	9p21.3	*CDKN2A, CDKN2B*	Heterozygous duplication
NA09834	Coriell Institute	9q22.32	*PTCH1*	Heterozygous deletion
NA13685	Coriell Institute	9q34.13	*TSC1*	Heterozygous duplication
NA20125	Coriell Institute	10q23.31	*PTEN*	Heterozygous duplication
NA09709	Coriell Institute	11p13	*WT1*	Heterozygous deletion
NA12606	Coriell Institute	13q13.1-q14.2	*BRCA2, RB1, MIR15A, DLEU1*	Heterozygous duplication
NA14164	Coriell Institute	13q14.2-q14.3	*RB1, MIR15A, DLEU1*	Heterozygous deletion
NA02325	Coriell Institute	16p13.3	*TSC2*	Heterozygous duplication
		22q11.23	*SMARCB1*	Heterozygous duplication
NA18949	Coriell Institute	17q21.31	*BRCA1*	Heterozygous deletion of exons 15-16
NA07891	Coriell Institute	18q21.2	*SMAD4*	Heterozygous deletion
NA03623	Coriell Institute	18q21.2	*SMAD4*	Heterozygous duplication
		Xq11.1	*AMER1*	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 P294-C1 Tumour Loss probemix.

◊ In this indicated cell line sample some of the reference probes are affected by CNAs.

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 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		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 or in or near the *TNFRSF4*, *CHD5*, *TSC2* and *STK11* 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.

P294 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 cancer are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P294 Tumour Loss.
- 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 COSMIC mutation database. 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 P294-C1 Tumour Loss

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
121	Reference probe S0864-L25602	21q22		21-037,920
124	Reference probe 19616-L26241	4p13		04-042,278
130 «	**TNFRSF4 probe** 02269-L01761		**1p36.33**	01-001,137
134 +	**AMER1 probe** 19672-L30667		**Xq11.1**	X-063,342
140	**WT1 probe** 14805-L30668		**11p13**	11-032,407
145	**TSC2 probe** 01819-L25996		**16p13.3**	16-002,039
152	**DLEU1 probe** 01062-L21380		**13q14.3**	13-049,577
157	**CDKN2A probe** 16881-L25649		**9p21.3**	09-021,961
162	**KIF1B probe** 04681-L25995		**1p36.22**	01-010,215
167	**MIR15A probe** 04019-L22561		**13q14.3**	13-049,521
172	Reference probe 17970-L22302	14q11		14-022,973
176	**SMARCB1 probe** 08295-L25566		**22q11.23**	22-022,506
182 «	**TSC2 probe** 19344-L25994		**16p13.3**	16-002,077
188	**BRCA2 probe** 01599-L25993		**13q13.1**	13-031,791
193	Reference probe 05703-L02147	3q21		03-123,456
199	**SMAD4 probe** 05147-L30568		**18q21.2**	18-046,840
203	**PTCH1 probe** 03709-L30569		**9q22.32**	09-097,258
209	**APC probe** 01537-L29813		**5q22.2**	05-112,131
214	**BRCA1 probe** 00827-L30570		**17q21.31**	17-038,510
221 #	**PTEN probe** 07686-L30504		**10q23.31**	10-089,716
226 «	**STK11 probe** 03126-L25647		**19p13.3**	19-001,170
232 ±	**FKBP8 probe** 12751-L25986		**19p13.11**	19-018,511
239	**BRCA2 probe** 12302-L30505		**13q13.1**	13-031,843
245	**NF1 probe** 02519-L25985		**17q11.2**	17-026,681
250	Reference probe 06712-L29006	15q24		15-070,436
257	**TSC1 probe** 04108-L13904		**9q34.13**	09-134,810
264 ±	**FKBP8 probe** 12754-L14091		**19p13.11**	19-018,504
270	**TP53 probe** 02376-L26567		**17p13.1**	17-007,519
274	**VHL probe** 01628-L30669		**3p25.3**	03-010,159
281	**TP53 probe** 21581-L25982		**17p13.1**	17-007,532
286	Reference probe 11255-L30670	11q21		11-095,238
292	**FHIT probe** 11728-L30671		**3p14.2**	03-059,972
299 «	**STK11 probe** 03129-L30673		**19p13.3**	19-001,172
304	**TP53 probe** 17420-L30674		**17p13.1**	17-007,520
311	**PTCH1 probe** 17280-L30675		**9q22.32**	09-097,284
317	**SMARCB1 probe** 08280-L25981		**22q11.23**	22-022,459
324	**TSC1 probe** 15301-L25980		**9q34.13**	09-134,768
331	**BRCA1 probe** 02821-L25979		**17q21.31**	17-038,480
341	**FHIT probe** 02290-L22795		**3p14.2**	03-060,783
346	Reference probe 06708-L28235	10p11		10-038,301
355	**PTEN probe** 18694-L24425		**10q23.31**	10-089,675
364 «	**TSC2 probe** 16736-L25977		**16p13.3**	16-002,068
370	**CDKN2A probe** 15242-L30665		**9p21.3**	09-021,958
376 #	**PTEN probe** 03638-L25975		**10q23.31**	10-089,683
382	**CAMTA1 probe** 04695-L25974		**1p36.23**	01-007,728
389	**BRCA1 probe** 18146-L26569		**17q21.31**	17-038,451
396	**VHL probe** 13322-L25972		**3p25.3**	03-010,163
402	**RB1 probe** 19228-L25970		**13q14.2**	13-047,949
409	Reference probe 14423-L30576	12q21		12-084,219
414	**CDKN2B probe** 03814-L25968		**9p21.3**	09-021,999
420	Reference probe 13817-L30577	2q13		02-108,891

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
427 #	**NF1 probe** 04073-L25965		**17q11.2**	17-026,578
433	**SMAD4 probe** 07800-L21445		**18q21.2**	18-046,859
439	**APC probe** 18178-L25963		**5q22.2**	05-112,207
448	Reference probe 17736-L25962	6q21		06-108,321
454	Reference probe 10717-L11299	6p12		06-051,885
463	**TP53 probe** 08785-L30676		**17p13.1**	17-007,515
469 +	**AMER1 probe** 21729-L30508		**Xq11.1**	X-063,327
476	**PRDM16 probe** 04703-L30578		**1p36.32**	01-003,151
481	**WT1 probe** 19585-L26570		**11p13**	11-032,367
490	**RB1 probe** 19180-L25625		**13q14.2**	13-047,815
496 «	**CHD5 probe** 09114-L25620		**1p36.31**	01-006,151
504	Reference probe 09870-L19465	2p15		02-061,126

± SNP rs201904385 could influence the probe signal at 232 nt and SNP rs200681860 could influence the probe signal at 264 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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

+ 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 table above. Single probe aberration(s) must be confirmed by another method.

Table 2. P294-C1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location / Exon[a]	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
1p36 region. Frequently deleted in various tumours. More 1p36 probes in the P147 1p36 probemix.					
130 «	02269-L01761	*TNFRSF4*	1p36.33	GCCGGCCAGCAA-TAGCTCGGACGC	2,0 **M**b
476	04703-L30578	*PRDM16*	1p36.32	ACGGACGTGGAA-GTGTCGCCCCAG	3,0 **M**b
496 «	09114-L25620	*CHD5*	1p36.31	GTTTCTTCTTCT-TAGGAAGGCTCA	1,6 **M**b
382	04695-L25974	*CAMTA1*	1p36.23	AATGAGCTGGCT-GGCCAGTTATCT	2,5 **M**b
162	04681-L25995	*KIF1B*	1p36.22	CTCAGTGAAGGT-GGCTGTCCGGGT	-
VHL gene at 3p25.3. Frequently mutated or deleted in hemangioblastomas, renal carcinomas and pheochromocytomas. More VHL probes in the P016 VHL probemix.					
274	01628-L30669	*VHL*	Exon 1	ACGAGGCCGAGG-TAGGCGCGGAGG	4,7 kb
396	13322-L25972	*VHL*	Exon 2	CGTCAACATTGA-GAGATGGCACAA	49,8 **M**b to *FHIT* gene
FHIT gene at 3p14.2. Frequently mutated or deleted in various tumours. More FHIT probes in the P027 Uveal melanoma probemix.					
292	11728-L30671	*FHIT*	Exon 7	CTCACCTTCACA-GTCTGTCCGGCT	810,6 kb
341	02290-L22795	*FHIT*	Exon 4	CCTGCCTGCTTA-GACCCTCTATAA	-
APC gene at 5q22.2. Frequently mutated or deleted in colorectal tumours. More APC probes in the P043 APC probemix.					
209	01537-L29813	*APC*	Exon 6	CGGGAAGGATCT-GTATCAAGCCGT	76,0 kb
439	18178-L25963	*APC*	Exon 18	GAGCACAGCAAA-CATTCATCATCC	-
CDKN2A/CDKN2B genes at 9p21.3. Frequently mutated, deleted or methylated in various tumours. More CDKN2A-CDKN2B probes in the ME024 9p21 CDKN2A/2B region probemix and in the P419 CDKN2A/2B-CDK4 probemix.					
370	15242-L30665	*CDKN2A*	Exon 3 (4)	TGAAAGAACCAG-AGAGGCTCTGAG	3,0 kb
157	16881-L25649	*CDKN2A*	Exon 2 (3)	TCCTTTCCGTCA-TGCCGGCCCCCA	37,6 kb

Length (nt)	SALSA MLPA probe	Gene	Location / Exon[a]	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
414	03814-L25968	*CDKN2B*	9p21.3	CCTGGAAGCCGG-CGCGGATCCCAA	75,3 **M**b to *PTCH1* gene
PTCH1 gene at 9q22.32. Frequently mutated or deleted in various tumours. More PTCH1 probes in the P067 PTCH1 probemix.					
203	03709-L30569	*PTCH1*	Exon 20	CTGTTCGGCATG-ATGGGCCTCATC	25,8 kb
311	17280-L30675	*PTCH1*	Exon 5	TGTTACAAATCA-GGAGAGCTTATC	37,5 **M**b to *TSC1* gene
TSC1 gene at 9q34.13. Frequently mutated or deleted in tuberous sclerosis associated hamartomas, astrocytomas and renal angiomyolipomas. More TSC1 probes in the P124 TSC1 probemix.					
324	15301-L25980	*TSC1*	Exon 18	CAGCGTGACACT-ATGGTAACCAAG	41,9 kb
257	04108-L13904	*TSC1*	Exon 1	GAGGGACTGTGA-GGTAAACAGCTG	-
PTEN gene at 10q23.31. Frequently mutated or deleted in glioblastomas, prostate tumours and various other tumours. More PTEN probes in the P225 PTEN, P105 Glioma-2 and P158 Juvenile polyposis syndrome (JPS) probemixes.					
355	18694-L24425	*PTEN*	Exon 3	GGGGTATTTGTT-GGATTATTTATT	7,8 kb
376 #	03638-L25975	*PTEN*	Exon 5	GGTGTAATGATA-TGTGCATATTTA	33,3 kb
221 #	07686-L30504	*PTEN*	Exon 9	ACAGCATCTGAA-TTTTAGCACTGG	-
WT1 gene at 11p13. Frequently mutated or deleted in Wilms tumours. More WT1 probes in the P118 WT1 probemix.					
481	19585-L26570	*WT1*	Exon 11	GTCAGCCAGGCT-GCTAACCTGGAA	39,9 kb
140	14805-L30668	*WT1*	Exon 3	GTGGAGTCCTTC-TCCCCTTCTTCC	-
BRCA2 gene at 13q13.1. Frequently mutated or deleted in various tumours. More BRCA2 probes in the P045 BRCA2/CHEK2, P090 BRCA2 and P077 BRCA2 Confirmation probemixes.					
188	01599-L25993	*BRCA2*	Exon 3	AATAATATTCAA-AGAGCAAGGGCT	51,2 kb
239	12302-L30505	*BRCA2*	Exon 19	TTATCATCGCTT-TTCAGTGATGGA	16,0 **M**b to *RB1* gene
13q14 region. ***RB1*** gene at 13q14.2. The *RB1* gene is frequently mutated or deleted in various tumours. The 13q14 region at the *MIR15-MIR16* genes is frequently deleted in CLL. More RB1 probes in the P047 RB1 probemix. More 13q14 probes in the P037 CLL-1 / P038 CLL-2 and P040 CLL probemixes.					
490	19180-L25625	*RB1*	Exon 3	TTTATTGCAGCA-GTTGACCTAGAT	134,1 kb
402	19228-L25970	*RB1*	Exon 25	CCTCCTAAACCA-CTGAAAAAACTA	1,6 **M**b
167	04019-L22561	*MIR15A*	13q14.3	TGGATTTTGAAA-AGGTGCAGGCCA	55,6 kb
152	01062-L21380	*DLEU1*	13q14.3	GAAGAACAGAAC-CTTCAGGAATTG	-
TSC2 gene at 16p13.3. Frequently mutated or deleted in tuberous sclerosis associated hamartomas, astrocytomas and renal angiomyolipomas. More TSC2 probes in the P046 TSC2 and P337 TSC2 Confirmation probemixes.					
145	01819-L25996	*TSC2*	Exon 2	AGCAAAGATTCA-GGCTTGAAGGAG	29,0 kb
364 «	16736-L25977	*TSC2*	Exon 26	TCTGCAGCCGAG-GCCTTCCGGTGC	9,0 kb
182 «	19344-L25994	*TSC2*	Exon 38	GCCCCAGTGCAA-GGCACAGAGGGC	-
TP53 gene at 17p13.1. Frequently mutated or deleted in various tumours. More TP53 probes in the P056 TP53 and P105 Glioma-2 probemixes.					
463	08785-L30676	*TP53*	Exon 10	TTCCGAGAGCTG-AATGAGGCCTTG	4,5 kb
270	02376-L26567	*TP53*	Exon 4b	CAAGATGTTTTG-CCAACTGGCCAA	0,8 kb
304	17420-L30674	*TP53*	Exon 3	TAGCTGCCCTGG-TAGGTTTTCTGG	11,4 kb
281	21581-L25982	*TP53*	Exon 1	GAGAAGCTCAAA-ACTTTTAGCGCC	19,0 **M**b to *NF1* gene
NF1 gene at 17q11.2. Frequently mutated or deleted in neurofibromas. More NF1 probes in the P081 NF1 mix 1, P082 NF1 mix 2 and P122 NF1-area probemixes.					
427 #	04073-L25965	*NF1*	Exon 18	CTTGCCCAACTA-TAACACATTCAT	103,7 kb
245	02519-L25985	*NF1*	Exon 39	CTAGAGACATCA-GGTTTATGTATC	11,8 **M**b to *BRCA1* gene
BRCA1 gene at 17q21.31. Exon numbering is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. Please note that the *BRCA1* exon numbering in LRG_292 is different; this exon numbering is indicated between brackets.					

Length (nt)	SALSA MLPA probe	Gene	Location / Exon[a]	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Frequently mutated or deleted in various tumours. More BRCA1 probes in the P002 BRCA1 and P087 BRCA1 Confirmation probemixes.					
389	18146-L26569	*BRCA1*	Exon 24 (23)	GCTGGAAGCACA-GAGTGGCTTGGC	29,1 kb
331	02821-L25979	*BRCA1*	Exon 15 (14)	CAACAGCTGGAA-GAGTCTGGGCCA	30,5 kb
214	00827-L30570	*BRCA1*	Exon 6 (5)	CGAGATTTAGTC-AACTTGTTGAAG	-
SMAD4 gene at 18q21.2. Frequently mutated or deleted in various tumours. More SMAD4 probes in the P158 Juvenile polyposis syndrome (JPS) probemix.					
199	05147-L30568	*SMAD4*	Exon 8	ATGAGCTTGCAT-TCCAGCCTCCCA	18,5 kb
433	07800-L21445	*SMAD4*	Exon 12	AGCATCAAAGAA-ACACCTTGCTGG	-
STK11 gene at 19p13.3. Frequently mutated or deleted in lung tumours. More STK11 probes in the P101 STK11 probemix.					
226 «	03126-L25647	*STK11*	Exon 3	GCATGCAGGAAA-TGCTGGACAGCG	1,9 kb
299 «	03129-L30673	*STK11*	Exon 6	CTACAAGTTGTT-TGAGAACATCGG	17.3 **M**b to *FKBP8* gene
FKBP8 gene at 19p13.11. Frequently mutated or deleted in tumours. No other FKBP8 probes are in our collection at this moment.					
264 ±	12754-L14091	*FKBP8*	Exon 9	CTGTGGTCATCG-CTGCCAGGAACT	7,2 kb
232 ±	12751-L25986	*FKBP8*	Exon 4	TCAGCCACCCTT-AGGTCTCTGCAG	-
SMARCB1 gene at 22q11.23. Frequently mutated or deleted in rhabdoid tumours. More SMARCB1 probes in the P258 SMARCB1 probemix.					
317	08280-L25981	*SMARCB1*	Exon 1	TGGCGCTGAGCA-AGACCTTCGGGC	47,1 kb
176	08295-L25566	*SMARCB1*	Exon 9	TGGCGCTGGGCT-GTCCCCTCGCCT	-
AMER1 (=*FAM123B*) gene at Xq11.1. Frequently mutated or deleted in Wilms' tumours. More AMER1 probes in the P380 Wilms' tumour probemix.					
469 +	21729-L30508	*AMER1*	Exon 2	TGACCATGTCAA-TATCACTATCAG	15,3 kb
134 +	19672-L30667	*AMER1*	Exon 1	TCCTGGCTGTAC-GCAACGCTTACC	-

[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.

± SNP rs201904385 could influence the probe signal at 232 nt and SNP rs200681860 could influence the probe signal at 264 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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

+ 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
504	09870-L19465	*PEX13*	2p15	TGAGGATGACCA-TGTAGTTGCCAG	02-061,126
420	13817-L30577	*EDAR*	2q13	TGGCCAGGTGAA-CCAGCGACAGCA	02-108,891
193	05703-L02147	*CASR*	3q21	GTGGCTTCCAAA-GACTCAAGGACC	03-123,456
124	19616-L26241	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
454	10717-L11299	*PKHD1*	6p12	TGTCTTAGAGCA-ACTGCCCATGCC	06-051,885

Length (nt)	SALSA MLPA probe	Gene	Chromo-somal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
448	17736-L25962	*SEC63*	6q21	CAGCAGGGTGAA-ACTAACAAGAAC	06-108,321
346	06708-L28235	*ZNF25*	10p11	CAGGTGATTCCT-GGGGCTGCCAGC	10-038,301
286	11255-L30670	*MTMR2*	11q21	AACAAGTTAGCA-GAAATGGAAGAA	11-095,238
409	14423-L30576	*ALX1*	12q21	ATGACACCTTAT-TCTCACTCGCCT	12-084,219
172	17970-L22302	*MYH7*	14q11	AGGCCAAGATCG-TGTCTCGAGAGG	14-022,973
250	06712-L29006	*HEXA*	15q24	GAATGTGTTGGT-TGTCTCTGTAGT	15-070,436
121	S0864-L25602	*KCNJ6*	21q22	AGCTCCTACATC-ACCAGTGAGATC	21-037,920

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

Related SALSA MLPA probemixes

- P175 Tumour Gain: Contains probes for *MDM4*, *MYCN-ALK*, *PDGFRA*, *KIT*, *KDR*, *DHFR*, *EGFR*, *MET*, *SMO*, *BRAF/BRAF* V600E mutation, *FGFR1*, *MYC*, *ABL1*, *RET*, *CCND1/2*, *CDK4*, *MDM2*, *AURKB*, *ERBB2-TOP2A*, *AURKA* and *AR* genes.
- See information in Table 2 for more related probemixes.

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Selected publications using SALSA MLPA Probemix P294 Tumour Loss

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- Stoczynska-Fidelus E et al. (2014). The failure in the stabilization of glioblastoma-derived cell lines: spontaneous in vitro senescence as the main culprit. *PLoS One.* 9:e87136.
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P294 product history	
Version	*Modification*
C1	Ten target probes have been replaced for the 1p36 region, *APC*, *CDKN2A*, *PTEN*, *WT1*, *BRCA2*, *TP53*, *SMAD4* and *STK11* genes. Several probes have a change in length but not in the sequence detected and seven reference probes have been replaced.
B1	18 target probes have been replaced for the *VHL*, *FHIT*, *APC*, *CDKN2A*, *PTCH1*, *TSC1*, *PTEN*, *WT1*, *RB1*, *TSC2*, *TP53*, *NF1*, *BRCA1* and *AMER1* genes. Several probes have a change in length but not in the sequence detected. In addition, 12 reference probes have been added and data analysis method has been modified.
A1	First release.

Implemented changes in the product description

Version C1-04 – 17 January 2023 (04P)
- Exon numbering of the *CDKN2A* gene has been changed according to MANE in Table 2. See also the explanation on page 2.

Version C1-03 – 27 October 2021 (04P)
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Positive samples added to Positive control DNA samples section on page 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added to Table 1 and 2 for SNPs that could influence 232 nt probe 12751-L25986 and 264 nt probe 12754-L14091.
- Warning removed from Table 1 and 2 for low signal caused by salt contamination for 145 nt probe 01819-L25996, 232 nt probe 12751-L25986 and 264 nt probe 12754-L14091.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- New references added in Selected publications section on page 10.

Version C1-02 – 13 April 2018 (01P)
- Warning added to Table 2a for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- New reference added on page 9.
- Minor textual changes.

Version C1-01 – 23 January 2018 (01P)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2a and 2b) and restructured to a new template.
- New references added for P294 probemix on page 9.

Version 10 – 14 September 2017 (T08)

- Warning added in Table 1 and Table 2, 317 nt probe 08280-L25981 and 364 nt probe 16736-L25977. - Minor textual and layout changes.

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