Product Description
SALSA® MLPA® Probemix
P477-A1 Head and neck carcinoma

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 9.

Catalogue numbers
- **P477-025R:** SALSA® MLPA® Probemix P477 Head and neck carcinoma, 25 reactions
- **P477-050R:** SALSA® MLPA® Probemix P477 Head and neck carcinoma, 50 reactions
- **P477-100R:** SALSA® MLPA® Probemix P477 Head and neck carcinoma, 100 reactions

SALSA® MLPA® Probemix P477 Head and neck carcinoma (hereafter: P477 Head and neck carcinoma) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P477-025R	**P477-050R**	**P477-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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
SALSA® MLPA® Probemix P477 Head and neck carcinoma is a **research use only (RUO)** assay for the detection of copy number alterations (CNAs) in multiple chromosomal regions and genes associated with distinct subgroups in head and neck squamous cell carcinoma (HNSCC): 2q36.2 (*CUL3*), 3q26.32-q26.33 (*PIK3CA*,

SOX2), 4q35.2 (*FAT1*), 5q15 (*KIAA0825*), 7p11.2 (*EGFR*), 8q11.21 (*SNAI2*), 9p22.3 (*NFIB*), 11q13.2-q13.3 (*CCND1*, *FADD*), 18p11.31 (*TGIF1*) and 20q11.21 (*BCL2L1*).

Head and neck squamous cell carcinoma (HNSCC) – the most common form of head and neck cancer, accounting for ~8% of all cancers worldwide with higher frequencies in Southeast Asia and Asia-Pacific regions, and incidence rates are projected to increase. HNSCCs are characterized by clinical, phenotypic and genetic heterogeneity with unstable genomes, exhibiting multiple chromosomal gains and losses, and other structural aberrations. For the most prevalent HNSCCs, oral cavity squamous cell carcinomas (OCSCCs), distinct subgroups were identified (Muijlwijk T et al, 2024): (i) *CNA-quiet* with no or few CNAs, and (ii) *CNA-other*, featuring multiple CNAs in chromosomal regions reported in the Cancer Genome Atlas (TCGA) consortium publication (Cancer Genome Atlas Network, 2015). *CNA-quiet* OCSCCs have a distinct cancer driver gene profile, histological and clinical features, and have more favourable prognosis. P477 Head and neck carcinoma was shown to be a suitable pre-screening method to select *CNA-other* OCSCC (Muijlwijk T et al, 2024).

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering
The exon numbering used for all genes in this P477-A1 Head and neck carcinoma product description is the exon numbering derived from MANE project (release version 1.4) based on MANE Select transcripts for *CUL3*, *PIK3CA*, *SOX2*, *FAT1*, *KIAA0825*, *EGFR*, *SNAI2*, *NFIB*, *CCND1*, *FADD*, *TGIF1* and *BCL2L1* genes. 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
P477-A1 Head and neck carcinoma contains 49 MLPA probes with amplification products between 129 and 500 nucleotides (nt). This includes 36 probes for the *CUL3*, *PIK3CA*, *SOX2*, *FAT1*, *KIAA0825*, *EGFR*, *SNAI2*, *NFIB*, *CCND1*, *FADD*, *TGIF1* and *BCL2L1* genes. In addition, 13 reference probes are included that target relatively copy number stable regions in HNSCC. The identity and partial sequences 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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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 formalin-fixed, paraffin-embedded (FFPE) tissues, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from healthy individuals 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 Leibniz Institute DSMZ listed have been tested with P477-A1 Head and neck carcinoma at MRC Holland and can be used as a positive control samples to detect various CNAs as described in the table below. The quality of cell lines can change; therefore deviations to the indicated CNA findings might occur.

Sample name	Source	Chromosomal position (hg18) of CNA*	Altered target genes in P477-A1 Head and neck carcinoma	Expected copy number alteration
CAL-27 (ACC-446)	DSMZ	4q35.2	*FAT1*	Heterozygous deletion
		7p11.2	*EGFR*	Gain
		11q13.2-q13.3	*CCND1, FADD*	
OAC-M5.1† (ACC-696)	DSMZ	8q11.21	*SNAI2*	Gain
		9p22.3	*NFIB*	Heterozygous deletion
		18p11.31	*TGIF1*	

* 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 P477-A1 Head and neck carcinoma.
† Some of the reference probes are also affected by copy number alterations.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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.

P477 Head and neck carcinoma 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 many tumour samples, genetic alterations e.g. in the *PIK3CA* and *FAT1* genes, are small (point) mutations, none of which will be detected by using P477 Head and neck carcinoma.
- 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 in sequence data 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 (Leiden Open Variation Database) - https://www.lovd.nl/
COSMIC database for somatic mutations in cancer - https://cancer.sanger.ac.uk/cosmic

We strongly encourage users to deposit positive results in the above-listed databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://varnomen.hgvs.org.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *CUL3* exons 2 and 12 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. P477-A1 Head and neck carcinoma

Length (nt)	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			
129	Reference probe 19552-L26106	2p13		02-071,749
137	**FAT1 probe** 21914-L30711		4q35.2	04-187,792
142	**TGIF1 probe** 06368-L05884		18p11.31	18-003,446
152	Reference probe 14199-L25033	2q13		02-108,894
160	**CUL3 probe** 21915-L31517		2q36.2	02-225,071
166	**FADD probe** 17218-L20545		11q13.3	11-069,731
172	Reference probe 12943-L14461	12p11		12-027,724
176 #	**SOX2 probe** 22002-L31142		3q26.33	03-182,914
181	**BCL2L1 probe** 21966-L18065		20q11.21	20-029,774
187	**KIAA0825 probe** 21925-L30722		5q15	05-093,944
193	**CUL3 probe** 21916-L30713		2q36.2	02-225,131
198	**EGFR probe** 15736-L30852		7p11.2	07-055,193
205	**TGIF1 probe** 22003-L31516		18p11.31	18-003,441
212	Reference probe 15470-L17310	1p36		01-011,958
220	**PIK3CA probe** 03826-L30737		3q26.32	03-180,430
226	**NFIB probe** 21917-L30714		9p22.3	09-014,110
232	**FAT1 probe** 12766-L18056		4q35.2	04-187,868
238	**EGFR probe** 06407-L29068		7p11.2	07-055,209
246	Reference probe 05658-L30738	2p22		02-032,233
253	**PIK3CA probe** 16057-L30739		3q26.32	03-180,410
260 #	**SOX2 probe** 07072-L30740		3q26.33	03-182,913
267	**CCND1 probe** 05401-L30741		11q13.2	11-069,167
274	Reference probe 17140-L20332	1p22		01-094,295
281	**NFIB probe** 21918-L31141		9p22.3	09-014,103
288	**BCL2L1 probe** 01928-L30746		20q11.21	20-029,717
298	**SNAI2 probe** 21931-L30939		8q11.21	08-049,996
303	Reference probe 17876-L30742	19q13		19-038,051
311	**CUL3 probe** 21919-L30716		2q36.2	02-225,080
318	**CCND1 probe** 22004-L30847		11q13.2	11-069,176
328	**FAT1 probe** 21920-L30717		4q35.2	04-187,787
337	**KIAA0825 probe** 21921-L30718		5q15	05-093,513
346 #	**TGIF1 probe** 12817-L06800		18p11.31	18-003,448
355	**SNAI2 probe** 14484-L16204		8q11.21	08-049,994
364	Reference probe 13216-L30743	1q22		01-154,373
373	**EGFR probe** 17639-L20672		7p11.2	07-055,178
381	**FADD probe** 22005-L31139		11q13.3	11-069,730
391	**BCL2L1 probe** 05066-L30853		20q11.21	20-029,774
399	**SNAI2 probe** 21930-L18175		8q11.21	08-049,995
406	Reference probe 09720-L30744	12q24		12-116,181
415	Reference probe 09070-L09239	19p13		19-013,259
425	**FADD probe** 22007-L30850		11q13.3	11-069,730
433 Δ	**PIK3CA probe** 03827-L21697		3q26.32	03-180,400
441	**KIAA0825 probe** 21926-L30723		5q15	05-093,831
454	Reference probe 10667-L30745	6p12		06-052,045
463	**CCND1 probe** 05402-L27353		11q13.2	11-069,168
476	**FADD probe** 22008-L30851		11q13.3	11-069,727
485	Reference probe 13413-L22367	6q12		06-065,384
494	**NFIB probe** 21924-L31471		9p22.3	09-014,140
500	Reference probe 14894-L27890	15q15		15-042,665

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 2. Target probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene, exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
2q36.2 *CUL3* exon numbering and ligation site information are according to MANE select transcript NM_003590.5.					
160	21915-L31517	***CUL3***, ex 12	2004-2003, reverse	ATATGATGCTGG-AGTGTGAGCTGT	9.1 kb
311	21919-L30716	***CUL3***, ex 7	1328-1329	GAGGGAGCAAGG-TAAAGCTCTTGT	50.8 kb
193	21916-L30713	***CUL3***, ex 2	559-560	TTTTGCATAAAC-ATGGAGAAAAGC	-
3q26.32-q26.33 *PIK3CA and SOX2* exon numbering and ligation site information are according to MANE select transcript NM_006218.4 and NM_003106.4, respectively.					
433 Δ	03827-L21697	***PIK3CA***, ex 2	650-651	GGCAACCGTGAA-GAAAAGATCCTC	10.5 kb
253	16057-L30739	***PIK3CA***, ex 7	1525-1526	TCGTGCTGCTCG-ACTTTGCCTTTC	20.4 kb
220	03826-L30737	***PIK3CA***, ex 19	3026-3027	ACACGTTCATGT-GCTGGATACTGT	2.5 **M**b
260 #	07072-L30740	***SOX2***, ex 1	760-761	CACCCGGATTAT-AAATACCGGCCC	1.2 kb
176 #	22002-L31142	***SOX2***, ex 1	1986-1987	CTGAAATTTAGG-ACAGTTGCAAAC	-
4q35.2 *FAT1* exon numbering and ligation site information are according to MANE select transcript NM_005245.4.					
328	21920-L30717	***FAT1***, ex 8	4777-4778	AAACTGGATCAT-GAAGCTGTTCAC	5.3 kb
137	21914-L30711	***FAT1***, ex 7	4436-4435, reverse	AATGATGGTTCC-AGTTCCCTTGTC	75.9 kb
232	12766-L18056	***FAT1***, ex 2	316-317	CTGCAGTTTACA-CACCTCGAGTAC	-
5q15 *KIAA0825* exon numbering and ligation site information are according to MANE select transcript NM_001145678.3.					
337	21921-L30718	***KIAA0825***, ex 21	6450-6451	AGAAAGGAAGTA-TAGTAAGGGGAA	318.4 kb
441	21926-L30723	***KIAA0825***, ex 10	2038-2039	TCTACAGTTTCA-GGTTACGAACTA	112.5 kb
187	21925-L30722	***KIAA0825***, ex 2	174-175	AGTTCTTCTGCT-GACAACTCTTCA	-
7p11.2 *EGFR* exon numbering and ligation site information are according to MANE select transcript NM_005228.5.					
373	17639-L20672	***EGFR***, ex 2	440-441	TAACTGTGAGGT-GGTCCTTGGGAA	15,3 kb
198	15736-L30852	***EGFR***, ex 11	1496-1497	GGCTTGGCCTGA-AAACAGGACGGA	16,3 kb
238	06407-L29068	***EGFR***, ex 18	2346-2347	CTTACACCCAGT-GGAGAAGCTCCC	-
8q11.21 *SNAI2* exon numbering and ligation site information are according to MANE select transcript NM_003068.5					
355	14484-L16204	***SNAI2***, ex 3	1001-1002	ACTCGAACAGAA-TGCATTTCTTCA	1.2 kb
399	21930-L18175	***SNAI2***, ex 2	713-714	ACCCACACATTA-CCTTGTGTTTGC	1.3 kb
298	21931-L30939	***SNAI2***, ex 1	200-201	AAGCATTTCAAC-GCCTCCAAAAAG	-
9p22.3 *NFIB* exon numbering and ligation site information is according to MANE select transcript NM_001190737.2					
281	21918-L31141	***NFIB***, ex 10	2090-2091	CTTCCTACATCA-GCAACAGGTGGG	7.5 kb
226	21917-L30714	***NFIB***, ex 8	1809-1810	CCTCCCCACCTG-AATCCTCAGGAT	29.7 kb
494	21924-L31471	***NFIB***, ex 5	1410-1411	GTCAATCTTCAG-AGGTCTCTGTCT	-
11q13.2-q13.3 *CCND1* and *FADD* exon numbering and ligation site information are according to MANE select transcript NM_053056.3 and NM_003824.4, respectively.					

Length (nt)	MLPA probe	Gene, exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
267	05401-L30741	***CCND1***, ex 2	437-438	TCGCTGGAGCCC-GTGAAAAAGAGC	0.7 kb
463	05402-L27353	***CCND1***, ex 3	598-599	CCTGGTGAACAA-GCTCAAGTGGAA	8.7 kb
318	22004-L30847	***CCND1***, ex 5	2292-2293	TAAGTTCCTTTC-CTTTTCTTTAAA	0.6 **M**b
476	22008-L30851	***FADD***, ex 1	20-19, reverse	GCCTGATTCACT-ACAGGAAATCGA	2.9 kb
425	22007-L30850	***FADD***, ex 2	528-527, reverse	CTTGGTGTCTGA-GACTTTGAGCTG	0.4 kb
381	22005-L31139	***FADD***, ex 2	921-920, reverse	TGAGTTCAGAAG-CAGGTGGTCTGT	0.2 kb
166	17218-L20545	***FADD***, ex 2	1122-1123	GTCACACTGTTA-CTCCACAGCGGA	-
18p11.31 *TGIF1* exon numbering and ligation site information are according to MANE select transcript NM_003244.4.					
205	22003-L31516	***TGIF1***, intr 1	995 nt after ex. 1, reverse	GTTTTGAACCAC-TCCACATAAATC	4.9 kb
142	06368-L05884	***TGIF1***, ex 2	377-378	GGATGAGGACAG-CATGGACATTCC	1.5 kb
346 #	12817-L06800	***TGIF1***, ex 3	1109-1110	GGCTGCAGAGAT-GGAGCTTCAGGC	-
20q11.21 *BCL2L1* exon numbering and ligation site information are according to MANE select transcript NM_138578.3.					
288	01928-L30746	***BCL2L1***, ex 3	1083-1084	AGTCGGAAATGA-CCAGACACTGAC	56.2 kb
181	21966-L18065	***BCL2L1***, ex 2	412-413	AGCTGGTGGTTG-ACTTTCTCTCCT	0.1 kb
391	05066-L30853	***BCL2L1***, ex 2	337-338	GTGAGTGAGCAG-GTGTTTTGGACA	-

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

SNVs located in the target sequence of a probe can influence probe hybridisation 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)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
212	15470-L17310	*PLOD1*	1p36	ATGGCTGGGGCT-CTCCGTGGTGTT	01-011,958
274	17140-L20332	*ABCA4*	1p22	ACAGCGACCCAT-TCATCCTCTTCC	01-094,295
364	13216-L30743	*LMNA*	1q22	TGTGTCCACAGA-TCATGGCTATTA	01-154,373
246	05658-L30738	*SPAST*	2p22	CTTTAGAAGCGT-ACATACGTTGGA	02-032,233
129	19552-L26106	*DYSF*	2p13	ACCCCACGGAGA-GCCAGAAGGTGA	02-071,749
152	14199-L25033	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108,894
454	10667-L30745	*PKHD1*	6p12	TCAAGGAGACAA-ATGGGTTACTCC	06-052,045
485	13413-L22367	*EYS*	6q12	GCTTGAGTGCAT-TCCCAACTCATG	06-065,384
172	12943-L14461	*PPFIBP1*	12p11	ATTCACAGAGGA-ACAGTCCCTTCC	12-027,724
406	09720-L30744	*NOS1*	12q24	AGAATATGACAT-TGTGCACCTGGA	12-116,181
500	14894-L27890	*SPG11*	15q15	GGACAATTCGCT-TTGGCCAGGAGG	15-042,665
415	09070-L09239	*CACNA1A*	19p13	ACAACATGAAGA-ACAACAAGCTGG	19-013,259
303	17876-L30742	*SLC7A9*	19q13	CCTAAGACCACC-AGTCTCCAAAAG	19-038,051

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

Related products

For related products, see the product page on our website.

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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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- Muijlwijk T et al. (2024). Hallmarks of a genomically distinct subclass of head and neck cancer. *Nat Commun.* 15:9060.

P477 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 20 December 2024 (04P) - Adapted to new template. - General information and Table 2 rewritten. - New References added. - Positive control DNA samples table added. Version A1-01 — *30 January 2019 (01P)* Not applicable, new document.

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