Product Description SALSA® MLPA® Probemix P378-D1 MUTYH

To be used with the MLPA General Protocol.

Version D1. As compared to version C1, one new reference probe has been added and 11 have been replaced; *MUTYH* exon 8, 11, 12, and exon 16 probes have been replaced, *MUTYH* exon 8 probe has been added, and *MUTYH* exon 7 probe has been removed; *SCG5* exon 5 probe has been replaced and *SCG5* downstream probe (enhancer probe upstream *GREM1*) has been added; and several probes have a small change in length. For complete product history see page 10.

Catalogue numbers:
- **P378-025R:** SALSA® MLPA® probemix P378 MUTYH, 25 reactions.
- **P378-050R:** SALSA® MLPA® probemix P378 MUTYH, 50 reactions.
- **P378-100R:** SALSA® MLPA® probemix P378 MUTYH, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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.

Intended use: The SALSA MLPA probemix P378 MUTYH is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *MUTYH* and *GREM1* genes and in specific regions of the human *SCG5* gene. It further contains two probes that can detect the presence of the p.Y179C and p.G396D point mutations in the *MUTYH* gene. The product is intended for confirming a potential cause and clinical diagnosis for MUTYH-Associated Polyposis (MAP) and Hereditary Mixed Polyposis Syndrome (HMPS1). This product can be used for molecular genetic testing of at-risk family members/individuals.

This assay is for use with human DNA extracted from peripheral blood. Deletions or duplications detected with the P378 MUTYH probemix must be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the *MUTYH* gene are point mutations, which will not all be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the *MUTYH* gene. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must be interpreted by a clinical molecular geneticist or equivalent.
This probemix can be used on tumour material to detect deletions and duplications in a research setting.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: The *MUTYH* (*mutY* homolog, *E. coli*) gene encodes a DNA glycosylase involved in oxidative DNA damage repair. Mutations in this gene result in heritable predisposition to colon cancer, more specifically *MUTYH* associated polyposis (MAP). MAP is an autosomal recessive trait. A single defective copy of the *MUTYH* gene may result in no, or only a small increase in risk for colorectal cancer. In case of bi-allelic *MUTYH* defects, the risk for colorectal cancer is 43% to almost 100% in the absence of timely surveillance according to the NCBI GeneReview for MAP: http://www.ncbi.nlm.nih.gov/books/NBK107219/.

The two most common *MUTYH* mutations associated with hereditary colorectal cancer are p.Y179C (c.536A>G) in exon 7 and p.G396D (c.1187G>A) in exon 13. These two mutations cover approximately 80% of germline alterations found in patients of European origin. In Eastern Asian populations these hotspot mutations are not prevalent. Patients with homozygous p.G396D or heterozygous p.G396D/p.Y179C mutations show a milder phenotype and later age of onset when compared to homozygous carriers of the p.Y179C mutation (NCBI GeneReview for MAP). A third common mutation is the p.E410Gfs*43 (c.1227_1228dupGG) mutation (Guarinos et al. 2014, Morak et al. 2014). Bi-allelic *MUTYH*-related colorectal cancer presents without polyps in one-third of the cases.
More information is available in the Clinical Utility Gene Card for *MUTYH* (http://www.nature.com/ejhg/journal/v21/n1/full/ejhg2012163a.html).

A duplication of 40 kb upstream of the *GREM1* gene and of the 3' end of *SCG5* is linked to an increased risk of developing colorectal cancer. This syndrome is known as hereditary mixed polyposis syndrome (HMPS1). This duplication leads to increased allele-specific *GREM1* expression in the epithelium of the large bowel. Increased *GREM1* expression is predicted to cause reduced bone morphogenetic protein (BMP) pathway activity, a mechanism that also underlies tumorigenesis in juvenile polyposis of the large bowel (Jaeger at al. 2012). Another duplication of ~16 kb in this region has been described more recently in members of a family presenting with atypical FAP (Rohlin et al. 2016). Seven probes are included in the P378 probemix to detect the 40 kb duplication (see Table 1 and Table 2b for more information). Copy number alterations detected by only a single probe specific for the *SCG5* gene, are unlikely to have a relation to the condition tested for.
More information on HMPS1 is available at http://omim.org/entry/601228.

Gene structure:
The *MUTYH* gene, located on chromosome 1p34 spans ~11 kb of genomic DNA and contains 16 exons. The LRG sequence is LRG_220 sequence and is identical to the NCBI NG_008189.1 sequence.
The *SCG5* gene, located on chromosome 15q13 spans ~55 kb of genomic DNA and contains six exons. The NCBI NG sequence for the *SCG5* gene is NG_051230.1. No LRG sequence is available.
The *GREM1* gene, also located on chromosome 15q13 spans ~17 kb of genomic DNA and comprises two exons. The NCBI NG sequence for the *GREM1* gene is NG_033791.1. No LRG sequence is available.

Transcript variants:
The NM_001128425.1 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_001128425.1, represents transcript variant alpha5 of the *MUTYH* gene. This sequence is the reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (217-219) and the stop codon is located in exon 16 (1864-1866).
The NM_001048172.1 transcript (https://www.ncbi.nlm.nih.gov/nuccore/NM_001048172.1) represents variant gamma2 of the *MUTYH* gene, with an alternative start codon as compared to transcript variant alpha5, located in intron 1 of transcript variant alpha5. The ATG translation start site is positioned at 147-149 in NM_001048172.1.
The NM_001048174.1 transcript (https://www.ncbi.nlm.nih.gov/nuccore/NM_001048174.1) represents variant beta3 of the *MUTYH* gene and also has an alternative start codon in intron 1 of transcript variant alpha5. The ATG translation start site is positioned at 66-68 in NM_001048174.1.
The NM_001144757.2 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_001144757.2, represents transcript variant 1 of the *SCG5* gene. This sequence is the reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 2 (174-176) and the stop codon is located in exon 6 (810-812).
The NM_013372.6 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_013372.6, represents transcript variant 1 of the *GREM1* gene. This sequence is the reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 2 (160-162) and the stop codon is located in exon 2 (712-714).

Exon numbering:
The exon numbering used in this P378-D1 product description and in the Coffalyser.Net analysis sheet is identical to the exon numbering found in the aforementioned LRG sequences and NCBI NG sequences. The exon numbering and NM sequence used is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description.

The *MUTYH* exon numbering has changed. From June 2014 onwards, we have adopted the NCBI exon numbering that is present in the NG_008189.1 sequence for this gene, which is similar to the LRG exon numbering (LRG_220). This exon numbering may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2a. The exon numbering is from 01/2018, but can be changed (e.g. by NCBI) after the release of this product description.

Probemix content: The P378-D1 MUTYH probemix contains 47 MLPA probes with amplification products between 116 and 471 nt. It contains probes for the *MUTYH*, *SCG5* and *GREM1* genes. For the *MUTYH* gene, the probemix contains 18 copy number probes and two mutation-specific probes, for the *SCG5* and *GREM1* genes both six copy number probes are included. In addition, the probemix contains 15 reference probes. The identity of the genes detected by the reference probes is available online (www.mlpa.com) and in Table 2c.

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.mpla.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment 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.mlpa.com).

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 from purified blood, 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.

Reference samples: 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 healthy individuals. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number(s) HG00097, HG01095, HG01500, HG01685, NA19789, and NA20522 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample(s) to detect the *MUTYH* heterozygous p.G396D mutation. The quality of cell lines can change, therefore samples should be validated before use. For determining mutation burden of the p.Y179C and p.G396D mutations, a positive sample of the same sample quality should be used.

SALSA Binning DNA SD022: The SD022 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of two mutation-specific probes (MUTYH probe 18416-SP0654-L23441 for the p.Y179C mutation and MUTYH probe 18417-SP0655-L23442 for the p.G396D mutation). SD022 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD022 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s), as for this purpose true mutation positive patient samples or cell lines should be used. It is strongly advised to use DNA sample and reference DNA samples extracted with the same method and derived from the same source of tissue. For further details, please consult the SD022 Binning DNA product description provided. **This product is for research use only (RUO), except when used in combination with a probemix for in vitro diagnostic (IVD) purpose, as specified at the end of this product description.**

Performance characteristics: The Clinical Utility Gene Card for *MUTYH* notes that deletions or duplications for *MUTYH* are rare events, the two common *MUTYH* mutations however account for around 80% of all reported mutant alleles in individuals of North-Western European origin. Penetrance in biallelic mutation carriers is up to 100%. Analytical sensitivity and specificity for MAP is almost 100% if sequencing of all 16 exons is applied in combination with MLPA.

Contrary to MAP, HMPS1 is inherited in a dominant manner. It has been shown that HMPS1 is caused by duplications of the 3' end of the *SCG5* gene and the upstream region of the *GREM1* locus (Jaeger et al. 2012, Rohlin et al. 2015). This probemix contains seven probes targeting this region, see Table 1 and Table 2b for more information.

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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.mlpa.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 expected results for the *MUTYH, SCG5* and *GREM1* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). Copy numbers of 4 (heterozygous triplication/homozygous duplication) or 0 (homozygous deletion) may occur, but are extremely rare.

The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *MUTYH*, *SCG5* and *GREM1* 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 (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 in some cases 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.
- Determining mutation burden: as a reference for the p.Y179C and p.G396D mutations, use positive samples of the same sample quality.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *MUTYH* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P378 MUTYH.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.
- When used on tumour samples (for research use only): 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 through the above mentioned dosage quotients if the percentage of tumour cells is low and if possible subclonality is present.** Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample. The diagnostic use of P378 with DNA extracted from tumour tissue has not been validated.

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 one or 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.

***MUTYH* mutation database:** We strongly encourage users to deposit positive results in MUTYH LOVD (www.lovd.org/mutyh). 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 SNPs and unusual results (e.g., a duplication of *MUTYH* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P378-D1 MUTYH

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]			
		Reference	MUTYH	GREM1	SCG5
64-105	Control fragments – see table in probemix content section for more information				
116 *	Reference probe S0472-L30486	6p12			
123 *	Reference probe 21531-L30487	6p22			
130 *	Reference probe 16316-L18705	3q21			
136 « Δ ◍	**GREM1 probe** 18483-L23305			**Exon 1**	
148 ¥	**MUTYH probe** 15777-L29704		**Exon 3**		
152 ¥	Reference probe 14199-L25033	2q13			
157 ¥ ∫	**SCG5 probe** 18309-L30392				**Exon 6**
161 « Δ ◍	**GREM1 probe** 18350-L23692			**Exon 1**	
166	**MUTYH probe** 15780-L17837		**Exon 6**		
172	**MUTYH probe** 15781-L17838		**Exon 4**		
184 §	**MUTYH probe** 18416-SP0654-L23441		**Y179C**=c.536A>G		
190	**MUTYH probe** 15783-L18347		**Exon 14**		
196 *	**MUTYH probe** 21351-L29755		**Exon 11**		
202 ∫	**SCG5 probe** 18310-L14109				**Exon 3**
208 *	Reference probe 21495-L16542	10q26			
214	**MUTYH probe** 20514-L28229		**Exon 10**		
220 ∫	**SCG5 probe** 18352-L23306				**Exon 4**
226 * ∫	**SCG5 probe** 21353-L29757				**Downstream**
232	**MUTYH probe** 15788-L17845		**Exon 5**		
238 Ø	**MUTYH probe** 15789-L17846		**Intron 1** (Alt. first exon in NM_001048172.1)		
244	**MUTYH probe** 15787-L18348		**Exon 15**		
250	**SCG5 probe** 18353-L23307				**Exon 2**
258 ∞	**MUTYH probe** 18417-SP0655-L23442		**G396D**=c.1187G>A		
267 *	Reference probe 21354-L29758	2p13			
274 ¥	**MUTYH probe** 15791-L30765		**Exon 1**		
283	**MUTYH probe** 15792-L17849		**Exon 2**		
292 *	**MUTYH probe** 21355-L29759		**Exon 12**		
301	Reference probe 02266-L01752	3p25			
310 « ∫	**GREM1 probe** 18354-L23308			**Upstream**	
318 *	**MUTYH probe** 21356-L29760		**Exon 8**		
328	**MUTYH probe** 18355-L23309		**Exon 13**		
337 *	Reference probe 07367-L07014	2q24			
345 « ∫	**GREM1 probe** 18356-L23310			**Upstream**	
351 *	Reference probe 16520-L23853	11p12			
363	**GREM1 probe** 18358-L23312			**Exon 2**	
372 *	Reference probe 05953-L28763	2p22			
382	**GREM1 probe** 18360-L23314			**Exon 2**	
391 * ∫	**SCG5 probe** 21357-L29761				**Exon 5**
400	**MUTYH probe** 18420-L23445		**Exon 9**		
409 *	Reference probe 17462-L21218	12p13			
418 *	**MUTYH probe** 21358-L30391		**Exon 16**		
427 Ø	**MUTYH probe** 18422-L23447		**Intron 1** (Alt. first exon in NM_001048174.1)		
432 *	**MUTYH probe** 21359-L29763		**Exon 8**		
445 *	Reference probe 16571-L19062	11q13			
452 *	Reference probe 19636-L26295	10p11			
463 *	Reference probe 14955-L16688	6q22			
471 *	Reference probe 21532-L27372	11p15			

(a) The exon numbering used in this P378-D1 product description and in the Coffalyser.Net analysis sheet is identical to the exon numbering found in the aforementioned LRG sequences and NCBI NG sequences.

* New in version D1 (from lot D1-1217 onwards).
¥ Changed in version D1 (from lot D1-1217 onwards). Small change in length, no change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the p.Y179C mutation is present. This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
∞ Mutation-specific probe. This probe will only generate a signal when the p.G396D mutation is present. Note that this probe may not generate a signal if the p.G396D mutation is present on the same allele as the pathogenic p.E410Gfs*43 mutation. The signal of this probe may also be reduced in the presence of depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
« 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.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
Ø Intron probe. This probe targets an alternative transcript variant using an alternative start codon. The significance of deletions or duplications for these probe targets are not clear.
ᵒ The significance of only exon 1 deletions is not clear as this exon is non-coding.
ʃ Probes within 40 kb duplication of 3' end of *SCG5* and the upstream region of *GREM1* locus, which can cause HMPS1 (Jaeger et al. 2012).

Note: The exon numbering for the *MUTYH* gene has changed. From June 2014 onwards, we have adopted the NCBI exon numbering that is present in the NG_008189.1 sequence in the product description, which is similar to the LRG exon numbering (LRG_220). This exon numbering may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2a. The exon numbering is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description.

Table 2. P378 probes arranged according to chromosomal location

Table 2a. *MUTYH*

Length (nt)	SALSA MLPA probe	MUTYH exon[a] / mutation	Ligation site[b] NM_001128425.1	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*217-219 (ex 1)*		
274	15791-L30765	Exon 1	50-51	CTCGTGGCTAGT-TCAGGCGGAAGG	0.4 kb
238 Ø	15789-L17846	Intron 1 (ex 2)	NM_001048172.1 alt. ex1; 48-49	GCTAATTGCCTA-TTGGCCTGTGCT	0.1 kb
427 Ø	18422-L23447	Intron 1 (ex 3)	NM_001048174.1 alt. ex 1; 30-31	GGGCCTCCGTGT-TCTGCTGTCTTC	5.5 kb
283	15792-L17849	Exon 2 (4)	352-353	ACAACAGTCAGG-CCAAGCCTTCTG	0.9 kb
148	15777-L29704	Exon 3 (5d)	487-488	TCAGAGACGTAG-CTGAAGTCACAG	0.2 kb
172	15781-L17838	Exon 4 (6)	9 nt after exon 4	CTGGTCAGTACA-TCTCCTGAGAGC	0.1 kb
232	15788-L17845	Exon 5 (7)	635-636	GCTGCAGCAGAC-CCAGGTTGCCAC	0.2 kb
166	15780-L17837	Exon 6 (8)	3 nt before exon 6	TGCCTGTGGCTA-TAGAAGTGGCCT	0.2 kb
184 §	18416-SP0654-L23441	**p.Y179C** =c.536A>G	752-751 and 724-723 reverse	CACGAGAATAG**C**-28 nt spanning oligo-CTCCTGTGGGTA	0.1 kb
318	21356-L29760	Exon 8 (10)	37nt before exon 8 reverse	TATAAGACACCC-AAGACTCCTGGG	0.1 kb
432	21359-L29763	Exon 8 (10)	836-837	TACAGCAGAGAC-CCTGCAGCAGCT	0.2 kb
400	18420-L23445	Exon 9 (11)	971-972	CATTGGTGCTGA-TCCCAGCAGCAC	0.2 kb
214	20514-L28229	Exon 10 (12)	1069-1070	CAGCCATGGAGC-TAGGGGCCACAG	0.2 kb
196	21351-L29755	Exon 11 (13)	1158-1157 reverse	AAGAGCTGTTCC-TGCTCCACCTGA	0.3 kb
292	21355-L29759	Exon 12 (14)	1283-1282 reverse	TGGGGAAGTTGA-CCACTCCCAGGG	0.3 kb
258 ∞	18417-SP0655-L23442	**p.G396D** =c.1187G>A	1403-1404 and 1438-1439	CTCCCTCTCAG**A**-35 nt spanning oligo-CCTGGGAGCCCT	0.1 kb
328	18355-L23309	Exon 13 (15)	1535-1534 reverse	CACTTACCTCCC-CAAGGTGCCGGA	0.1 kb
190	15783-L18347	Exon 14 (16)	1569-1570	CACATCAAGCTG-ACATATCAAGTA	0.7 kb
244	15787-L18348	Exon 15 (17)	Intron 14-1693	CTTCTTGTCTAG-GTTTTCCGTGTG	1.3 kb
418	21358-L30391	Exon 16 (18)	1874-1873 reverse	ATGGGGGCTTTC-AGAGGTGTCACT	
		stop codon	*1864-1866 (ex 16)*		

(a) The exon numbering used in this P378-D1 product description and in the Coffalyser.Net analysis sheets is identical to the exon numbering found in the LRG_220 sequence and the NCBI NG_008189.1 sequence.

(b) Ligation sites of the P378 MUTYH MLPA probes are indicated according to Refseq sequence NM_001128425.1, containing 16 exons. Only the intron probes of 238 and 427 nt are indicated according to sequence NM_001048172.1 and sequence NM_001048174.1, respectively. The NM sequences used are from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

§ Mutation-specific probe. This probe will only generate a signal when the p.Y179C mutation is present. This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
∞ Mutation-specific probe. This probe will only generate a signal when the p.G396D mutation is present. Note that this probe may not generate a signal if the p.G396D mutation is present on the same allele as the pathogenic p.E410Gfs*43 mutation. The signal of this probe may also be reduced in the presence of depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
Ø Intron probe. This probe targets an alternative transcript variant using an alternative start codon. The significance of deletions or duplications for these probe targets are not clear.

Note: The exon numbering for the MUTYH gene has changed. From June 2014 onwards, we have adopted the NCBI exon numbering that is present in the NG_008189.1 sequence in the product description, which is similar to the LRG exon numbering (LRG_220). This exon numbering may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2a. The exon numbering is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description.

Table 2b. *SCG5* and *GREM1*

Length (nt)	SALSA MLPA probe	SCG5 exon[a]	Ligation site[b] NM_001144757.2	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*174-176 (ex 2)*		
250	18353-L23307	Exon 2	194-195	AGGATGGTCTCT-ACCATGCTATCT	36.2 kb
202 ∫	18310-L14109	Exon 3	477-478	TGACTGGAGACA-ACATTCCTAAGG	4.7 kb
220 ∫	18352-L23306	Exon 4	581-582	AACACCCCTGAC-ACTGCAGAGTTC	7.2 kb
391 ∫	21357-L29761	Exon 5	715-716	ACGAAAGCGGAG-GGTAACACGTGC	4.9 kb
157 ∫	18309-L30392	Exon 6	865-866	TCAGCATGGCTT-ATGTGCACGTGT	4.3 kb
226 ∫	21353-L29757	Downstream	3918 nt after exon 6 reverse	AGGTAATTCCAC-CTTTCCCTCTGT	8.6 kb
		stop codon	*810-812 (ex 6)*		

Length (nt)	SALSA MLPA probe	GREM1 exon[a]	Ligation site[b] NM_013372.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*160-162 (ex 2)*		
345 « ∫	18356-L23310	Upstream	8423 nt before exon 1	AGAAACAAACAC-TGCAGGCAAGGT	2.9 kb
310 « ∫	18354-L23308	Upstream	5570 nt before exon 1	ACAGGTTACCCT-GTCTGCAGACAA	5.6 kb
136 «Δø	18483-L23305	Exon 1	4-5	TGCCTGGCACTC-GGTGCGCCTTCC	0.1 kb
161 «Δø	18350-L23692	Exon 1	153-154	ACCCGCCGCACT-GACAGGTGAGCG	12.7 kb
363	18358-L23312	Exon 2	292-293	ACAATGACTCAG-AGCAGACTCAGT	0.3 kb
382	18360-L23314	Exon 2	592-593	GCTCCTTCTGCA-AGCCCAAGAAAT	
		stop codon	*712-714 (ex 2)*		

(a) The exon numbering used in this P378-D1 product description and in the Coffalyser.Net analysis sheet is identical to the exon numbering found in the NCBI NG_051230.1 sequence for *SCG5* and in the NCBI NG_033791.1 sequence for *GREM1*.

(b) Ligation sites of the P378 MUTYH MLPA probes are indicated according to Refseq sequence NM_001144757.2 for *SCG5*, containing 6 exons, and NM_013372.6 for *GREM1*, containing 2 exons. The NM_001144757.2 sequence used is from 01/2018 and the NM_013372.6 sequence used is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.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.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
° The significance of only exon 1 deletions is not clear as this exon is non-coding.
∫ Probes within 40 kb duplication of 3' end of *SCG5* and the upstream region of *GREM1* locus, which can cause HMPS1 (Jaeger et al. 2012)

Table 2c. Reference probes

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Location (hg18)
372	05953-L28763	SPAST	2p22	GCAAGTTGTGCT-AGTTCTTTTTGG	02-032.222 kb
267	21354-L29758	DYSF	2p13	GAACCAAAGTCA-TCAAGAACAGCG	02-071.562 kb
152	14199-L25033	EDAR	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108.894 kb
337	07367-L07014	SCN1A	2q24	GCAACAGGAGGC-AGCTCAGGTAAA	02-166.611 kb
301	02266-L01752	GHRL	3p25	GGCTTTTCGCTT-GCTTCTGCAGCA	03-010.302 kb
130	16316-L18705	RAB7A	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130.000 kb
123	21531-L30487	KIAA0319	6p22	GAGGAGGAACAA-GTGGGACGGCGA	06-024.754 kb
116	S0472-L30486	PKHD1	6p12	GTAACCATCTCA-GGTCTCTGATGA	06-052.018 kb
463	14955-L16688	LAMA2	6q22	CATGTCAATGTA-ATGGACACAGCA	06-129.691 kb
452	19636-L26295	PARD3	10p11	CCTGCAGCAAAT-AAAGAGCAGTAT	10-034.712 kb
208	21495-L16542	UROS	10q26	AGTGTATGTGGT-TGGAAATGCTAC	10-127.491 kb
471	21532-L27372	SMPD1	11p15	CTGCTGAAGATA-GCACCACCTGCC	11-006.369 kb
351	16520-L23853	RAG2	11p12	GTTTAGCGGCAA-AGATTCAGAGAG	11-036.576 kb
445	16571-L19062	SHANK2	11q13	TCGAGGTACGAT-GCGAAGGCAGAA	11-070.014 kb
409	17462-L21218	GRIN2B	12p13	CTGTTCTGGCAA-GCCTGGCATGGT	12-013.611 kb

Colorectal cancer (CRC) related SALSA MLPA probemixes

	Condition		Gene	Probemix
COLORECTAL CANCER (CRC) PROBEMIXES	Lynch Syndrome (HNPCC)		MLH1	P003, ME011, P248 (Confirmation of P003), ME042
			MSH2	P003, ME011, P248 (Confirmation of P003)
			MSH6	P072, ME011
			PMS2	P008, ME011
			EPCAM	P003, P072
	Polyposis Syndrome	MAP	MUTYH	P378, P043, P072
		AFAP	APC	P043
		FAP	APC	P043

References

- Jaeger E et al. (2012). Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. *Nat Genet*. 44:699-703.
- Rohlin A et al. (2016). GREM1 and POLE variants in hereditary colorectal cancer syndromes. *Genes Chromosomes Cancer.* 55:95-106.
- 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 P378 MUTYH

- Aimé A et al. (2015). Somatic c. 34G>T KRAS mutation: a new prescreening test for MUTYH-associated polyposis? *Canc Genet.* 7:390-395.
- Castillejo A et al. (2014). Prevalence of germline MUTYH mutations among Lynch-like syndrome patients. *Eur J Cancer.* 50:2241-2250.
- Guarinos C et al. (2014). Prevalence and characteristics of MUTYH-associated polyposis in patients with multiple adenomatous and serrated polyps. *Clin Cancer Res.* 20:1158-1168.
- Morak M et al. (2014). Biallelic MUTYH mutations can mimic Lynch syndrome. *Eur J Hum Genet.* 22:1334-1337.
- Rohlin A et al. (2016). GREM1 and POLE variants in hereditary colorectal cancer syndromes. *Genes Chromosomes Cancer.* 55:95-106.
- Taki K et al. (2016). Mutation analysis of MUTYH in Japanese colorectal adenomatous polyposis patients. *Fam Cancer.* 15:261-265.

P378 Product history	
Version	*Modification*
D1	One new reference probe has been added and 11 have been replaced; *MUTYH* exon 8, 11, 12, and exon 16 probes have been replaced, *MUTYH* exon 8 probe has been added, and *MUTYH* exon 7 probe has been removed; *SCG5* exon 5 probe has been replaced and *SCG5* downstream probe (enhancer probe upstream *GREM1*) has been added; and several probes have a small change in length.
C1	A target probe for *MUTYH* exon 10 has been included.
B1	Seven target probes have been replaced and 12 new target probes have been added (*MUTYH*, *GREM1* and *SCG5*), including mutation specific probes for *MUTYH* Y179C and G396D; all reference probes have been replaced.
A2	The 88 and 96 nt control fragments have been replaced (QDX2); the 258 nt probe has a small change in length.
A1	First release.

Implemented changes in the product description
Version D1-01 – 23 February 2018 (04) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Various minor textual changes. *Version 14 – 30 October 2017 (T08)* - Warning added in Table 1 and 2, 135 nt probe 18483-L23305, 160 nt probe 18350-L23692, and 310 nt probe 18354-L23308. *Version 13 – 23 November 2016 (T08)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

- Two new references added on page 2.
- Ligation sites of probes for SCG5 gene updated due to changing to another transcript as a reference sequence (from NM_001144757.1 to NM_001144757.2).
- Various minor textual changes.

Version 12 – 15 January 2015 (T07)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Various minor textual changes and corrections throughout the document.
- Updated link for "Database of Genomic Variants".

Version 11 – 16 September 2014 (T06)
- Various minor textual changes on page 1.
- New related probemix ME043 added on page 2.
- New reference added on page 2.
- Table 2b adjusted to contain information on which probes are expected to be duplicated in case of 40kb upstream duplication of GREM1.

Version 10 – 13 June 2014 (T06)
- Exon numbering of the MUTYH gene has been updated in Table 1 and 2a due to changing to the new NG exon numbering (NG_008189.1).
- New references added on page 2.

Version 09 – 02 October 2013 (T03)
- Adjusted SD text on page 1 & 2.

Version 08 – 16 August 2013 (T03)
- Adjusted SD text on page 2.

Version 07 – 02 July 2013 (T03)
- Product description is adapted to a new product version (version number changed, lot number added, tables 1 and 2 adapted and new pictures included).
- Various textual changes and information on the GREM1 and SGC5 gene added on page 1.
- Information about Sample DNA is added on page 2.
- Analysis method has been adapted for use on tumour DNA on page 2.
- Warning added on page 2 about consequences of low tumour cell percentage for MLPA analysis.

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