Product Description
SALSA® MLPA® Probemix P378-D1 MUTYH

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 12.

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 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.

Intended purpose
The SALSA MLPA Probemix P378 MUTYH is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *MUTYH* gene, as well as the presence of the two most common point mutations among people of European descent, c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp), in order to confirm a potential cause and clinical diagnosis of *MUTYH*-Associated Polyposis (MAP). In addition, P378 MUTYH can be used to detect duplications in the *SCG5/GREM1* region in order to confirm a potential cause and clinical diagnosis of Hereditary Mixed Polyposis Syndrome type 1 (HMPS1). This assay is also intended for molecular genetic testing of at-risk family members. P378 MUTYH is for use with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P378 MUTYH should be confirmed with a different technique. In particular, CNVs detected by only a single probe as well as the two *MUTYH* point mutations always require confirmation by another method. Most defects in the *MUTYH* gene are point mutations, which will not be detected by MLPA, with the exception of the two aforementioned *MUTYH* point mutations. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, population screening, pre-implantation or prenatal testing. Only in a research setting can this device be used for the detection of, or screening for, acquired or somatic genetic aberrations, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit, Coffalyser.Net analysis software, and SALSA Binning DNA SD022.

Clinical background

Mutations in the *MUTYH* gene result in a hereditary predisposition to colon and gastric cancer, which is referred to as MAP. MAP is an autosomal recessive disorder. In MAP patients, ten to several hundred colonic adenomatous polyps develop, and these become evident at a mean age of 50 years. However, colon cancer can also develop in the absence of polyposis. A single defective copy of the *MUTYH* gene may result in no, or only a small increase in risk for colorectal cancer (CRC). There are two common *MUTYH* mutations, c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp) that are carried by ~1%-2% of the general population and account for ≥90% of all *MUTYH* pathogenic variants in northern European populations. Up to 70% of MAP patients harbor at least one of these variants (Aretz *et al.* 2013). Copy number variations of *MUTYH* are very rare; they account for <1% of pathogenic alleles. The most frequent CNV in *MUTYH* - a deletion of exon 4-16 - is reported in multiple patients (Castillejo *et al.* 2014). More information on MAP is available at http://www.ncbi.nlm.nih.gov/books/NBK107219/.

A recurrent duplication of ~40 kb directly upstream of the *GREM1* gene is known to lead to HMPS1. Patients with HMPS1 have a predisposition for developing CRC (Jaeger *et al.* 2012). Presence of this duplication is predicted to cause reduced bone morphogenetic protein (BMP) pathway activity, a mechanism that underlies tumorigenesis in juvenile polyposis of the large bowel. Several additional duplications in the *GREM1* upstream region have been found: *e.g.* a duplication of the upstream region and the whole *GREM1* gene of ~57 kb has been described in one patient with sigmoid colon carcinoma (Venkatachalam *et al.* 2011); a duplication of ~16 kb has been described in members of a family presenting with atypical FAP (Rohlin *et al.* 2016); and a duplication of ~24 kb in a patient with multiple colon polyps has been reported (McKenna *et al.* 2019). These different duplications can be detected by multiple probes in this P378-D1 probemix as is indicated in the table below. More information on HMPS1 is available at http://omim.org/entry/601228.

SALSA MLPA probe			Probes expected to be affected in P378-D1 probemix (+), for each published duplication			
length (nt)	**Probe number**	**Gene & exon**	**40 kb (Jaeger *et al*. 2012)**	**57 kb (Venkatachalam *et al*. 2011)**	**16 kb (Rohlin *et al*. 2016)**	**24 kb (McKenna *et al*. 2019)**
250	18353-L23307	*SCG5* exon 2				
202	18310-L14109	*SCG5* exon 3	+			
220	18352-L23306	*SCG5* exon 4	+	+		
391	21357-L29761	*SCG5* exon 5	+	+		
157	18309-L30392	*SCG5* exon 6	+	+	+	+
226	21353-L29757	*SCG5* downstream	+	+	+	+
345	18356-L23310	*GREM1* upstream	+	+	+	+
310	18354-L23308	*GREM1* upstream	+	+		+
136	18483-L23305	*GREM1* exon 1		+		+
161	18350-L23692	*GREM1* exon 1		+		+
363	18358-L23312	*GREM1* exon 2		+		
382	18360-L23314	*GREM1* exon 2		+		

Gene structure

The *MUTYH* gene spans ~11 kb on chromosome 1p34.1. *MUTYH* contains 16 exons. The *MUTYH* LRG_220 is available at www.lrg-sequence.org and is identical to GenBank NG_008189.1.

The *SCG5* gene spans ~55 kb and has six exons, and the *GREM1* gene spans ~27 kb and contains two exons. Both genes are located on chromosome 15q13.3; *SCG5* is located ~21 kb upstream of *GREM1*. The *GREM1* LRG_1365 is available at www.lrg-sequence.org and is identical to GenBank NG_033791.2 For *SCG5* the NG_051230.1 sequence is available, but no LRG is available.

Transcript variants
For *MUTYH,* multiple variants have been described. Transcript variant alpha5 (NM_001128425.2; 1900 nt; coding sequence (CDS) 187-1836) represents the longest transcript and encodes the long isoform 5. In Table 2a, the ligation sites of the *MUTYH* MLPA probes are indicated according to this sequence. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/4595.

For *SCG5,* multiple variants have been described. Transcript variant 1 is the longest transcript and encodes the longer isoform 1 (NM_001144757.3; 1198 nt; CDS 77-715). In Table 2b, the ligation sites of the *SCG5* MLPA probes are indicated according to this sequence. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/6447.

For *GREM1,* multiple variants have been described. Transcript variant 1 (NM_013372.7; 14575 nt; CDS 160-714) represents the longest transcript and encodes isoform 1. In Table 2b, the ligation sites of the *GREM1* MLPA probes are indicated in relation to this sequence. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/26585.

Exon numbering
The *MUTYH* exon numbering used in this P378-D1 MUTYH product description is the exon numbering from the LRG_220 sequence. For *GREM1* the exon numbering from the LRG_1365 sequence was used and for *SCG5* no LRG is available and therefore the exon numbering from the NG_051230.1 sequence was 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 NG sequences. 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 P378-D1 MUTYH contains 47 MLPA probes with amplification products between 116 and 471 nucleotides (nt). This includes 18 copy number probes for the *MUTYH* gene and twelve for the *SCG5-GREM1* region. Furthermore, two probes are included for the common c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp) *MUTYH* mutations, which will only generate a signal when the mutation is present. In addition, 15 reference probes are included that detect relatively copy number stable regions in various cancer types associated with MAP and HMPS1. 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 (www.mrcholland.com).

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten *et al.* 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment. Note that the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.

Required specimens
Extracted DNA from human peripheral blood or, in a research setting, DNA derived from fresh or FFPE tumour tissue, 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 MAP or HMPS1. 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 from the Coriell Institute described in the table below have been tested with this P378-D1 Probemix at MRC Holland and can be used as positive control samples. Note that the two *MUTYH* mutation-specific probes are only intended to determine the presence of the mutation and should not be used to determine zygosity. The quality of cell lines can change; therefore samples should be validated before use.

Coriell sample ID	Affected target genes	Expected result
HG01918	*MUTYH*	Positive for the *MUTYH* c.536A>G (p.Tyr179Cys) mutation
HG00097; HG01095; HG01500; HG01685; NA19789; NA20522	*MUTYH*	Positive for the *MUTYH* c.1187G>A (p.Gly396Asp) mutation
NA03184	*SCG5-GREM1* region	Heterozygous duplication of probes present in Table 2b

SALSA Binning DNA SD022
The SALSA Binning DNA SD022 provided with this probemix can be used for binning of all probes, including the two *MUTYH* mutation-specific probes: the 184 nt probe 18416-SP0654-L23441, detecting the c.536A>G (p.Tyr179Cys) mutation, and the 258 nt probe 18417-SP0655-L23442, detecting the c.1187G>A (p.Gly396Asp) mutation. This 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 Binning DNA SD022 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). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SALSA Binning DNA SD022 product description, available online: www.mrcholland.com.

Performance characteristics

Almost all MAP patients have biallelic point mutations in the *MUTYH* gene. Deletions in this gene have rarely been described: the percentage of cases explained by large deletions/duplications is estimated to be <1%. The two common *MUTYH* point mutations c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp) account for ≥90% of all *MUTYH* pathogenic variants in northern European populations. Up to 70% of MAP patients harbor at least one of these variants. The presence of these mutations, c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp), can be detected by this probemix. Based on the populations tested in scientific literature the diagnostic sensitivity of the P378-D1 MUTYH Probemix for MAP is estimated at ~70%.

The only known cause of HMPS1 are large duplications in the upstream region of *GREM1* that may, or may not, include the *GREM1* gene. The published duplications are readily detected by multiple probes in this Probemix. The diagnostic sensitivity for HMPS1 is therefore 100%.

Analytical performance can be compromised by: SNVs or other polymorphisms 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 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 expected results for all copy number probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) can be obtained. The standard deviation of each individual probe (with exception of the mutation-specific probes) over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the copy number probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

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

The above mentioned FR values do not apply to the mutations-specific probes. The peaks of the mutation-specific probes are expected to be absent in the majority of samples tested and therefore their standard deviation cannot be determined. Clear signal (at least 10% of the median peak height of all reference probes in that sample) for one of these probes indicates that the mutation is present.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic or subclonal cases. Analysis of parental samples may be necessary for correct interpretation of complex results for germline analysis.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga *et al*. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz *et al*. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P378 specific notes

- 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.
- The two *MUTYH* mutation-specific probes are only intended to determine the presence (or absence) of the mutation.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *MUTYH* are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P378 MUTYH with the exception of the presence of the two most common point mutations in the *MUTYH* gene among Europeans (c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp)).
- 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 as well as the two common *MUTYH* point mutations always require confirmation by another method. Because the two *MUTYH* mutation-specific probes are only intended to determine the presence of the mutation, positive results obtained for either of these probes need to be confirmed by sequence analysis to determine the zygosity of the mutation.

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.

***MUTYH* mutation database**

We strongly encourage users to deposit positive results in the *MUTYH* LOVD mutation database: https://databases.lovd.nl/shared/genes/MUTYH. 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 (e.g., a duplication of *MUTYH* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.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 ɷ	**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 ɷ	**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		**c.536A>G mutation-specific**		
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**		
244	**MUTYH probe** 15787-L18348		**Exon 15**		
250	**SCG5 probe** 18353-L23307				**Exon 2**
258 §	**MUTYH probe** 18417-SP0655-L23442		**c.1187G>A mutation-specific**		
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**		
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] See section Exon numbering on page 3 for more information.

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. Both mutation-specific probes consist of three parts and have 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. Masking of the mutation-specific signal due to another mutation or SNP in the probe target can only occur when both are present on the same allele.

± SNP rs529425621 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Ø Intron probe. Copy number alterations of only this probe are of unknown clinical significance. This probe targets an alternative transcript; the 238 nt probe has a ligation site in exon 1 of NM_001048172.2 and the 427 nt probe has a ligation site in exon 1 of NM_001048174.2.

ⓞ The significance of exon 1 deletions is not clear as this exon is non-coding.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P378-D1 probes arranged according to chromosomal location

Table 2a. *MUTYH*

Length (nt)	SALSA MLPA probe	*MUTYH* exon[a]	Ligation site NM_001128425.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
274	15791-L30765	Exon 1	20-21	CTCGTGGCTAGT-TCAGGCGGAAGG	0.4 kb
		start codon	*187-189 (exon 1)*		
238 Ø	15789-L17846	Intron 1	NM_001048172.2; 53-54	GCTAATTGCCTA-TTGGCCTGTGCT	0.1 kb
427 ± Ø	18422-L23447	Intron 1	NM_001048174.2; 43-44	GGGCCTCCGTGT-TCTGCTGTCTTC	5.5 kb
283	15792-L17849	Exon 2	322-323	ACAACAGTCAGG-CCAAGCCTTCTG	0.9 kb
148	15777-L29704	Exon 3	457-458	TCAGAGACGTAG-CTGAAGTCACAG	0.2 kb
172	15781-L17838	Exon 4	9 nt after exon 4	CTGGTCAGTACA-TCTCCTGAGAGC	0.1 kb
232	15788-L17845	Exon 5	605-606	GCTGCAGCAGAC-CCAGGTTGCCAC	0.2 kb
166	15780-L17837	Exon 6	3 nt before exon 6	TGCCTGTGGCTA-TAGAAGTGGCCT	0.2 kb
184 §	18416-SP0654-L23441	c.536A>G (p.Tyr179Cys) in exon 7	722-721 and 694-693 reverse	CACGAGAATAG**C**-28 nt spanning oligo-CTCCTGTGGGTA	0.1 kb
318	21356-L29760	Exon 8	37 nt before exon 8, reverse	TATAAGACACCC-AAGACTCCTGGG	0.1 kb
432	21359-L29763	Exon 8	806-807	TACAGCAGAGAC-CCTGCAGCAGCT	0.2 kb
400	18420-L23445	Exon 9	941-942	CATTGGTGCTGA-TCCCAGCAGCAC	0.2 kb
214	20514-L28229	Exon 10	1039-1040	CAGCCATGGAGC-TAGGGGCCACAG	0.2 kb
196	21351-L29755	Exon 11	1128-1127 reverse	AAGAGCTGTTCC-TGCTCCACCTGA	0.3 kb
292	21355-L29759	Exon 12	1253-1252 reverse	TGGGGAAGTTGA-CCACTCCCAGGG	0.3 kb
258 §	18417-SP0655-L23442	c.1187G>A (p.Gly396Asp) in exon 13	1373-1374 and 1408-1409	CTCCCTCTCAG**A**-35 nt spanning oligo-CCTGGGAGCCCT	0.1 kb
328	18355-L23309	Exon 13	1505-1504 reverse	CACTTACCTCCC-CAAGGTGCCGGA	0.1 kb
190	15783-L18347	Exon 14	1539-1540	CACATCAAGCTG-ACATATCAAGTA	0.7 kb
244	15787-L18348	Exon 15	intron 14-1663	CTTCTTGTCTAG-GTTTTCCGTGTG	1.3 kb
		stop codon	*1834-1836 (exon 16)*		
418	21358-L30391	Exon 16	1844-1843 reverse	ATGGGGGCTTTC-AGAGGTGTCACT	-

Table 2b. *SCG5-GREM1* region

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		SCG5	**NM_001144757.3**		
	No probe	Exon 1			
		start codon	*77-79 (exon 2)*		
250	18353-L23307	Exon 2	97-98	AGGATGGTCTCT-ACCATGCTATCT	36.2 kb
202	18310-L14109	Exon 3	380-381	TGACTGGAGACA-ACATTCCTAAGG	4.7 kb
220	18352-L23306	Exon 4	484-485	AACACCCCTGAC-ACTGCAGAGTTC	7.2 kb
391	21357-L29761	Exon 5	618-619	ACGAAAGCGGAG-GGTAACACGTGC	4.9 kb
		stop codon	*713-715 (exon 6)*		
157	18309-L30392	Exon 6	768-769	TCAGCATGGCTT-ATGTGCACGTGT	4.3 kb
226	21353-L29757	Downstream	3.9 kb after exon 6, reverse	AGGTAATTCCAC-CTTTCCCTCTGT	8.6 kb
		GREM1	**NM_013372.7**		
345	18356-L23310	Upstream	8.4 kb before exon 1	AGAAACAAACAC-TGCAGGCAAGGT	2.9 kb
310	18354-L23308	Upstream	5.6 kb before exon 1	ACAGGTTACCCT-GTCTGCAGACAA	5.6 kb
136 ◎	18483-L23305	Exon 1	4-5	TGCCTGGCACTC-GGTGCGCCTTCC	0.1 kb
161 ◎	18350-L23692	Exon 1	153-154	ACCCGCCGCACT-GACAGGTGAGCG	12.7 kb
		start codon	*160-162 (exon 2)*		
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 (exon 2)*		-

[a] See section Exon numbering on page 3 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.

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. Both mutation-specific probes consist of three parts and have 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. Masking of the mutation-specific signal due to another mutation or SNP in the probe target can only occur when both are present on the same allele.

± SNP rs529425621 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Ø Intron probe. Copy number alterations of only this probe are of unknown clinical significance. This probe targets an alternative transcript; the 238 nt probe has a ligation site in exon 1 of NM_001048172.2 and the 427 nt probe has a ligation site in exon 1 of NM_001048174.2.

◎ The significance of exon 1 deletions is not clear as this exon is non-coding.

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
372	05953-L28763	*SPAST*	2p22	GCAAGTTGTGCT-AGTTCTTTTTGG	02-032.222
267	21354-L29758	*DYSF*	2p13	GAACCAAAGTCA-TCAAGAACAGCG	02-071.562
152	14199-L25033	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108.894
337	07367-L07014	*SCN1A*	2q24	GCAACAGGAGGC-AGCTCAGGTAAA	02-166.611
301	02266-L01752	*GHRL*	3p25	GGCTTTTCGCTT-GCTTCTGCAGCA	03-010.302
130	16316-L18705	*RAB7A*	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130.000
123	21531-L30487	*KIAA0319*	6p22	GAGGAGGAACAA-GTGGGACGGCGA	06-024.754
116	S0472-L30486	*PKHD1*	6p12	GTAACCATCTCA-GGTCTCTGATGA	06-052.018

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
463	14955-L16688	*LAMA2*	6q22	CATGTCAATGTA-ATGGACACAGCA	06-129.691
452	19636-L26295	*PARD3*	10p11	CCTGCAGCAAAT-AAAGAGCAGTAT	10-034.712
208	21495-L16542	*UROS*	10q26	AGTGTATGTGGT-TGGAAATGCTAC	10-127.491
471	21532-L27372	*SMPD1*	11p15	CTGCTGAAGATA-GCACCACCTGCC	11-006.369
351	16520-L23853	*RAG2*	11p12	GTTTAGCGGCAA-AGATTCAGAGAG	11-036.576
445	16571-L19062	*SHANK2*	11q13	TCGAGGTACGAT-GCGAAGGCAGAA	11-070.014
409	17462-L21218	*GRIN2B*	12p13	CTGTTCTGGCAA-GCCTGGCATGGT	12-013.611

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

Related SALSA MLPA probemixes

Condition		Gene	SALSA MLPA Probemix
Lynch syndrome (HNPCC)		*MLH1*	P003 MLH1/MSH2 P248 MLH1-MSH2 Confirmation ME011 Mismatch Repair Genes ME042 CIMP
		MSH2	P003 MLH1/MSH2 P248 MLH1-MSH2 Confirmation ME011 Mismatch Repair Genes
		MSH6	P072 MSH6-MUTYH ME011 Mismatch Repair Genes
		PMS2	P008 PMS2 ME011 Mismatch Repair Genes
		EPCAM	P003 MLH1/MSH2 P072 MSH6-MUTYH ME011 Mismatch Repair Genes
Polyposis syndrome	MAP	*MUTYH*	P378 MUTYH P043 APC P072 MSH6-MUTYH
	FAP	*APC*	P043 APC
	AFAP	*APC*	P043 APC

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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 D1-07 – 24 July 2023 (04P) - Product is no longer registered as IVD in Morocco. *Version D1-06 – 06 October 2022 (04P)* - Sections *Clinical background* and *Performance characteristics* were updated according to recent literature. - New positive sample for the *GREM1* region included and table with positive samples was adjusted. - *References* and *Selected Publications* were curated and new literature was included. - Minor textual and lay-out changes throughout document. *Version D1-05 – 09 November 2021 (04P)* - Information in Table 2a has been updated (column *MUTYH* exon) for the two *MUTYH* mutation specific probes and the mutations detected, to align with related products. *Version D1-04 – 22 July 2021 (04P)* - Product description rewritten and adapted to a new template. - Intended use has become Intended purpose and was rewritten using new template. - Sections *Clinical background* and *Transcript variants* are rewritten for clarification and to include recent knowledge, including a new table indicating which probes detect which published duplication in the *SCG5-GREM1* region. - Performance characteristics updated. - Throughout document several statement are included/rephrased emphasizing that only the presence of the common *MUYTH* mutations can be detected and not the zygosity. - Ligation sites of the probes targeting *MUTYH*, *GREM1* and *SCG5* updated according to new version of the NM_ reference sequence. - Remarks in Table 1 and 2 for salt sensitivity and for increased variability were removed because no probes deviate from current criteria according to recent quality testing. - Table containing the related probemixes has been updated. - *References* and *Selected publications* were curated and new literature was included. - UK has been added to the list of countries in Europe that accept the CE mark. *Version D1-03 – 29 April 2020 (04)* - Product is now registered for IVD use in Colombia and Israel. *Version D1-02 – 29 January 2019 (04)* - Product is now registered for IVD use in Morocco. - Sentence on RUO/IVD status of SD in SALSA Binning DNA SD022 section was removed.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.