Product Description
SALSA® MLPA® Probemix P093-C2 HHT/HPAH

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 10.

Catalogue numbers:
- **P093-025R:** SALSA MLPA Probemix P093 HHT/HPAH, 25 reactions.
- **P093-050R:** SALSA MLPA Probemix P093 HHT/HPAH, 50 reactions.
- **P093-100R:** SALSA MLPA Probemix P093 HHT/HPAH, 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 P093 HHT/HPAH 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 *ENG*, *ACVRL1* and *BMPR2* genes in genomic DNA isolated from human peripheral whole blood specimens. P093 HHT/HPAH is intended to confirm a potential cause for and clinical diagnosis of Hereditary Hemorrhagic Telangiectasia (HHT) or Heritable Pulmonary Arterial Hypertension (HPAH) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P093 HHT/HPAH should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *ENG*, *ACVRL1* and *BMPR2* genes are point mutations, none of which will be detected by MLPA. 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, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[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 and Coffalyser.Net analysis software.

Clinical background
Hereditary Hemorrhagic Telangiectasia (HHT) is a disease with an autosomal dominant inheritance pattern and is characterized by the presence of multiple arteriovenous malformations (AVMs). In AVMs, arteries connect directly to veins instead of through intervening capillaries, resulting in high blood pressure. AVMs occur on the skin, but also in the brain, lungs, liver and intestines. Depending on the location, rupture of these malformations can have catastrophic consequences for the patient. Diagnosis is based on the presence of

multiple AVMs in the skin, mucus membranes, or visceral organs. Recurrent nosebleeds are also a common finding in HHT patients. Molecular genetic testing is performed to confirm or establish a diagnosis in a proband. HHT is primarily caused by pathogenic variations in the genes endoglin (*ENG/HHT1*) and activin A receptor like type 1 (*ACVRL1/HHT2*). Both genes encode endothelial cell surface receptors that are part of a TGF-β/BMP signalling cascade, a pathway involved in angiogenesis, among multiple other developmental processes. Approximately 10% of pathogenic variation consists of large deletions/duplications. More information is available at: https://www.ncbi.nlm.nih.gov/books/NBK1351/.

Heritable Pulmonary Arterial Hypertension (HPAH) is inherited in an autosomal dominant manner. This disease is caused by loss or obstruction of the smallest pulmonary arteries, resulting in high blood pressure in the arteries of the lung. Diagnosis is based on the presence of pulmonary hypertension as confirmed through right heart catheterization, and subsequently by identification of a heterozygous pathogenic variant in a known associated gene (simplex cases) and/or confirmation of Pulmonary Arterial Hypertension (PAH) in one or more of the proband's family members. Approximately 75% of HPAH is caused by variation in the bone morphogenetic protein receptor type 2 (*BMPR2*) gene. Based on a review of four published studies (Aldred et al. 2006, Cogan et al. 2006, Liu et al. 2012, Sztrymf et al. 2008), 7-43% of identified *BMPR2* mutations in individuals with HPAH are large duplications/deletions. It is important to note that this range of percentages is derived from a limited number of studies, and one study has a particularly small cohort size (Cogan et al. 2006). Therefore, this range may not represent the true population-based prevalence of exonic deletions and duplications within *BMPR2* rearrangements associated with HPAH. Similar to *ENG* and *ACVRL1*, the *BMPR2* gene also encodes a cell surface receptor that is part of the TGF-β/BMP signalling pathway. Sporadically, PAH is observed as a symptom of HHT. The biological similarities between the causative genes suggests a similar aetiology between HPAH and HHT. This is supported by rare observations of mutations in *ACVRL1*, and even more infrequent in *ENG*, causing HPAH. In the literature, a patient has been described with a combined PAH and HHT phenotype carrying a deletion of exons 6 and 7 in *BMPR2* (Handa et al. 2014). In very rare cases, HPAH can be caused by mutations in the *KCNK3*, *SMAD9* or *CAV1* gene. To the best of our knowledge, no HPAH causing deletions or duplications have been reported in these genes. More information is available at: https://www.ncbi.nlm.nih.gov/books/NBK1485/.

Gene structure

The *BMPR2* gene (13 exons) spans ~191 kb of genomic DNA and is located on chromosome 2q33.1-2, about 203 Mb from the p-telomere. The *BMPR2* LRG_712 is identical to GenBank NG_009363.1 and is available at www.lrg-sequence.org. The *ACVRL1* (*ALK1*) gene (10 exons) spans ~16 kb of genomic DNA and is located on chromosome 12q13.3, about 51 Mb from the p-telomere. The *ACVRL1* LRG_543 is identical to GenBank NG_009549.1 and is available at www.lrg-sequence.org. The *ENG* gene (14 exons) spans ~40 kb of genomic DNA and is located on chromosome 9q34.11, about 130 Mb from the p-telomere. The *ENG* LRG_589 is identical to GenBank NG_009551.1 and is available at www.lrg-sequence.org.

Transcript variants

One *BMPR2* transcript variant has been described: NM_001204.7; 12068 nt; coding sequence 1149-4265; http://www.ncbi.nlm.nih.gov/gene/659. In Table 2a, the ligation sites of the BMPR2 MLPA probes are indicated according to this sequence which contains 13 exons. The ATG translation start site is located in exon 1 (1149-1151) and the stop codon is located in exon 13 (4263-4265).

Two *ACVRL1* transcript variants have been described of which NM_000020.3 (4263 nt; coding sequence 284-1795; http://www.ncbi.nlm.nih.gov/gene/94) is better conserved. In Table 2b, the ligation sites of the ACVRL1 MLPA probes are indicated according to this sequence which contains 10 exons. The ATG translation start site is located in exon 2 (198-200) and the stop codon is located in exon 10 (1707-1709).

Three *ENG* transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/2022. The sequence of *ENG* transcript variant 2 (NM_000118.4; 3201 nt; coding sequence 419-2296) is a reference standard in the NCBI RefSeqGene project. In Table 2c, the ligation sites of the ENG MLPA probes are indicated according to this sequence which contains 14 exons. The ATG translation start site is located in exon 1 (419-421) and the stop codon is located in exon 14(b) (2294-2296).

Exon numbering
The *BMPR2, ACVRL1* and *ENG* exon numbering used in this P093-C2 HHT/HPAH product description is the exon numbering from the RefSeq transcripts NM_001204.7, NM_000020.3 and NM_000118.4, respectively, which is identical to the exon numbering of the LRG_712, LRG_543 and LRG_589 sequences, respectively. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P093-C2 HHT/HPAH contains 51 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 14 probes for the *BMPR2* gene (one probe for each exon and one additional probe for exon 1), 11 probes for the *ACVRL1* gene (one probe for each exon and one additional probe for exon 1) and 18 probes for the *ENG* gene (one probe for each exon, and one additional probe for exons 1 and 2, and two additional probes for exon 14(b)) In addition, 8 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

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

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

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 human peripheral whole blood specimens, 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
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 unrelated individuals who are from families without a history of Hereditary Hemorrhagic Telangiectasia or Heritable Pulmonary Arterial Hypertension. 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/home.html) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA11213 (deletion *BMPR2*), NA01229 (duplication *BMPR2*) and NA10186 (duplication *ENG*) from the Coriell Institute have been tested with this P093-C2 probemix at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P093-C2	Expected copy number alteration
NA10186	Coriell Institute	Trisomy 9	*ENG*	Heterozygous duplication
NA01229	Coriell Institute	2q31-qter	*BMPR2*	Heterozygous duplication
NA11213	Coriell Institute	2q32.1-q33.2	*BMPR2*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P093-C2 HHT/HPAH probemix.

Performance characteristics

The majority of HHT patients have pathogenic variants that are easily detected by sequence analysis. It is estimated that ~5% of all HHT patients have large rearrangements in the *ACVRL1* or *ENG* gene, either involving part of the gene or the whole gene. When MLPA is used in addition to sequence analysis of the *ACVRL1* and *ENG* genes, the detection rate generally increases by ~10%.

The majority of HPAH patients have pathogenic variants that are easily detected by sequence analysis. It is estimated that approximately ~5-32% of all HPAH patients have large rearrangements in the *BMPR2* gene, either involving part of the gene or the whole gene. When MLPA is used in addition to sequence analysis of the *BMPR2* gene, the detection rate generally increases by ~20%.

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 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 the BMPR2, ACVRL1 and ENG probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). The standard deviation of each individual probe 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 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.

- 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. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BMPR2*, *ACVRL1* and *ENG* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P093 HHT/HPAH.
- 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.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

Mutation databases

BMPR2 mutation database https://databases.lovd.nl/shared/genes/BMPR2, *ACVRL1* mutation database https://databases.lovd.nl/shared/genes/ACVRL1 and *ENG* mutation database https://databases.lovd.nl/shared/genes/ENG. We strongly encourage users to deposit positive results in these respective databases. 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 *BMPR2* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P093-C2 HHT/HPAH

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*BMPR2*	*ENG*	*ACVRL1*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 00797-L00463	5q			
137	**BMPR2 probe** 04004-L03427		**Exon 1**		
142	**ENG probe** 05235-L04615			**Exon 5**	
148	**BMPR2 probe** 04010-L03433		**Exon 7**		
154	**BMPR2 probe** 08198-L11404		**Exon 1**		
160	**ACVRL1 probe** 03790-L03965				**Exon 10**
166	**ENG probe** 03005-L02445			**Exon 1**	
172	**BMPR2 probe** 04011-L03434		**Exon 8**		
178	**ACVRL1 probe** 05229-L04609				**Exon 1**
184	**ENG probe** 03006-L02446			**Exon 2**	
190	**BMPR2 probe** 04005-L03428		**Exon 2**		
197	Reference probe 10688-L11270	6p			
203	**ENG probe** 03007-L03614			**Exon 3**	
209	**ACVRL1 probe** 05230-L05738				**Exon 3**
214	**ACVRL1 probe** 03789-L05739				**Exon 1**
220	**BMPR2 probe** 04012-L03435		**Exon 9**		
226	**ACVRL1 probe** 05231-L04611				**Exon 5**
232	**ENG probe** 03008-L05740			**Exon 4**	
238	Reference probe 08858-L08980	3q			
244	**ACVRL1 probe** 03016-L20477				**Exon 2**
250	Reference probe 02869-L20478	1p			
256	**ENG probe** 03009-L02449			**Exon 6**	
265	**ACVRL1 probe** 03017-L02457				**Exon 4**
270	**BMPR2 probe** 04338-L03966		**Exon 3**		
276	**ACVRL1 probe** 08196-L11406				**Exon 7**
283	**ENG probe** 07680-L07402			**Exon 8**	
290	Reference probe 15677-L17643	9p			
297	**ACVRL1 probe** 03018-L20479				**Exon 6**
304	**BMPR2 probe** 04013-L20480		**Exon 10**		
312	**ENG probe** 03011-L20481			**Exon 9**	
319	**ACVRL1 probe** 03019-L02459				**Exon 8**
328	**BMPR2 probe** 04007-L03430		**Exon 4**		
337	**ENG probe** 03583-L03013			**Exon 11**	
346	**ACVRL1 probe** 03020-L28916				**Exon 9**
355	**BMPR2 probe** 04014-L03437		**Exon 11**		
364	**ENG probe** 03013-L02453			**Exon 13**	
376	**ENG probe** 05237-L28917			**Exon 10**	
385	Reference probe 16932-L28918	4q			
393	**ENG probe** 05236-L28919			**Exon 7**	
400	**ENG probe** 03788-L28920			**Exon 14b**	
409	**BMPR2 probe** 04008-L28921		**Exon 5**		
417	**BMPR2 probe** 04015-L28922		**Exon 12**		
426	Reference probe 16449-L28923	18q			
433	**BMPR2 probe** 04009-L28924		**Exon 6**		
441	**BMPR2 probe** 04016-L28925		**Exon 13**		
449	**ENG probe** 08501-L28926			**Exon 14b**	
457	**ENG probe** 05234-L28927			**Exon 2**	
465	**ENG probe** 05233-L28928			**Exon 1**	
472	**ENG probe** 08502-L28929			**Exon 14b**	
481	**ENG probe** 08500-L28930			**Exon 12**	
490	Reference probe 09966-L28931	17q			

[a] See section Exon numbering on page 3 for more information.

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

Table 2. P093-C2 probes arranged according to chromosomal location

Table 2a. *BMPR2*

Length (nt)	SALSA MLPA probe	BMPR2 exon[a]	Ligation site NM_001204.7	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1149-1151 (ex 1)*		
154	08198-L11404	Exon 1	573-574	TTGATCCAGTCA-AGGAAGAGGATT	0.5 kb
137	04004-L03427	Exon 1	1119-1120	ATTTCTTTTCTT-TGCCCTCCTGAT	87.4 kb
190	04005-L03428	Exon 2	1256-1257	CTATGTGCGTTT-AAAGATCCGTAT	2.7 kb
270	04338-L03966	Exon 3	1443-1444	ACTATGAAGAAT-GTGTAGTAACTA	46.2 kb
328	04007-L03430	Exon 4	1600-1601	AGATGAGACAAT-AATCATTGCTTT	1.2 kb
409	04008-L28921	Exon 5	1706-1707	CTTCACAGTATG-AACATGATGGAG	4.1 kb
433	04009-L28924	Exon 6	1930-1931	TGCCCGCTTTAT-AGTTGGAGATGA	1.2 kb
148	04010-L03433	Exon 7	2069-2070	CTTGCTCATTCT-GTTACTAGAGGA	10.7 kb
172	04011-L03434	Exon 8	2225-2226	ATGAGGCTGACT-GGAAATAGACTG	1.7 kb
220	04012-L03435	Exon 9	2308-2309	GGCACCAGAAGT-GCTAGAAGGAGC	9.8 kb
304	04013-L20480	Exon 10	2496-2497	TTGAGGATATGC-AGGTTCTCGTGT	10.4 kb
355	04014-L03437	Exon 11	2646-2647	GTGCTGAGGAAA-GGATGGCTGAAC	2.7 kb
417	04015-L28922	Exon 12	2938-2939	AGCACAAGCTCG-AATCCCCAGCCC	4.4 kb
441	04016-L28925	Exon 13	4154-4155	GAAGTCAACAAT-AATGGCAGTAAC	
		stop codon	*4263-4265 (ex 13)*		

[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.

Table 2b. *ACVRL1*

Length (nt)	SALSA MLPA probe	ACVRL1 exon[a]	Ligation site NM_000020.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*198-200 (ex 2)*		
214	03789-L05739	Exon 1	72 nt before ex 1	GAAACGGTTTAT-TAGGAGGGAGTG	>0.1 kb
178	05229-L04609	Exon 1	23 nt before ex 1 reverse	AGCAAAAATGTT-TCTTATTCCAGC	5.0 kb
244	03016-L20477	Exon 2	229-230	GAAAGGCCTTCT-GATGCTGCTGAT	0.8 kb
209	05230-L05738	Exon 3	471-472	ACTGCTGCGACA-GCCACCTCTGCA	0.4 kb
265	03017-L02457	Exon 4	673-674	CGAGCTGGGAGA-GTCCAGTCTCAT	0.3 kb
226	05231-L04611	Exon 5	811-812	GGTTGCCTTGGT-GGAGTGTGTGGG	0.4 kb
297	03018-L20479	Exon 6	874-875	CGGTGAGAGTGT-GGCCGTCAAGAT	0.7 kb
276	08196-L11406	Exon 7	975-976	ACACAGGCTTCA-TCGCCTCAGACA	0.8 kb
319	03019-L02459	Exon 8	1284-1285	GCGATTACCTGG-ACATCGGCAACA	2.9 kb
346	03020-L28916	Exon 9	1470-1471	ATAGACCACCCT-TCTATGATGTGG	1.9 kb
160	03790-L03965	Exon 10	1706-1707	AAAGTGATTCAA-TAGCCCAGGAGC	
		stop codon	*1707-1709 (ex 10)*		

[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.

Table 2c. *ENG*

Length (nt)	SALSA MLPA probe	ENG exon[a]	Ligation site NM_000118.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*304-306 (ex 1)*		
465	05233-L28928	Exon 1	426 nt before ex 1	GAGATCACTGGT-TCAAGAAGATGG	0.8 kb
166	03005-L02445	Exon 1	344-345	TGCCCTGCTGCT-GGCCAGCTGCAG	11.1 kb
457	05234-L28927	Exon 2	451-450 reverse	GCAGCCCTTCGA-GACCTGGCTAGT	0.0 kb
184	03006-L02446	Exon 2	480-481	CAGGCCCCCAAT-GCCATCCTTGAA	13.4 kb
203	03007-L03614	Exon 3	593-594	AGAGGTGCTTCT-GGTCCTCAGTGT	3.2 kb
232	03008-L05740	Exon 4	788-789	TGCTGCTGAGCT-GAATGACCCCCA	0.7 kb
142	05235-L04615	Exon 5	850-851	TGTCCTTCTGCA-TGCTGGAAGCCA	0.6 kb
256	03009-L02449	Exon 6	1060-1061	CGTCCTCATCCT-GCAGGGTCCCCC	0.4 kb
393	05236-L28919	Exon 7	1240-1241	GCATTGTGGCAT-CCTTCGTGGAGC	0.4 kb
283	07680-L07402	Exon 8	1300-1301	CCCACAGGTGGT-AGGCTGCAGACC	4.5 kb
312	03011-L20481	Exon 9 (9a)	1547-1548	CTTACTCCAGCT-GTGGCATGCAGG	0.3 kb
376	05237-L28917	Exon 10 (9b)	1602-1603	CTGTCGAGCTCA-TCACCACAGCGG	0.8 kb
337	03583-L03013	Exon 11 (10)	1655-1656	CCTCTCTTTCCA-GCTGGGCCTCTA	0.6 kb
481	08500-L28930	Exon 12 (11)	1913-1914	CCTCCACTTCTA-CACAGTACCCAT	1.0 kb
364	03013-L02453	Exon 13 (12)	2004-2005	GTCCATAGGACT-GTCTTCATGCGC	1.2 kb
449	08501-L28926	Exon 14b (13)	2143-2144	TCTGGTACATCT-ACTCGCACACGC	0.2 kb
472	08502-L28929	Exon 14b (13)	2361-2362	CAGCAGCACCAA-CCACAGCATCGG	0.4 kb
400	03788-L28920	Exon 14b (13)	2762-2763	GGAGAACTTGAA-ACAGATTCAGGC	
		stop codon	*2179-2181 (ex 14b)*		

[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.

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

Related SALSA MLPA probemixes

P158 Juvenile Polyposis Syndrome (JPS): Contains probes for the *SMAD4*, *BMPR1A* and *PTEN* genes.

References

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Selected publications using SALSA MLPA Probemix P093 HHT/HPAH

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- Kitayama K et al. (2021). Mutational and clinical spectrum of Japanese patients with hereditary hemorrhagic telangiectasia. *BMC Medical Genomics.* 14:1-9.
- Liang K-W et al. (2022). Whole Exome Sequencing of Patients With Heritable and Idiopathic Pulmonary Arterial Hypertension in Central Taiwan. *Frontiers in Cardiovascular Medicine.* 9.
- Momose Y et al. (2015). De novo mutations in the BMPR2 gene in patients with heritable pulmonary arterial hypertension. Ann Hum Genet. 79:85-91.
- Mutize TT et al. (2020). The clinical and genetic features of hereditary haemorrhagic telangiectasia (HHT) in central South Africa—three novel pathogenic variants. *Molecular Biology Reports.* 47:9967-9972.
- Van Der Bruggen CE et al. (2016). Bone Morphogenetic Protein Receptor Type 2 Mutation in Pulmonary Arterial Hypertension: A View on the Right Ventricle. Circulation. 115.020696.
- Xi Q, Liu Z, Zhao Z, Luo Q & Huang Z (2016). High Frequency of Pulmonary Hypertension-Causing Gene Mutation in Chinese Patients with Chronic Thromboembolic Pulmonary Hypertension. PLoS One. 11:e0147396.

P093 product history	
Version	*Modification*
C2	Length of several probes has been adjusted.
C1	Five reference probes have been replaced and two new reference probes have been added. The length of 9 probes have been changed without change in the sequence. Finally, new control fragments at 88, 96, 100 and 105 nt have been added.
B2	Two extra control fragments at 100 and 105 nt have been added. One probe has a small change in length (277 nt).
B1	Four target probes have been replaced (BMPR2: 1, ACVRL1: 1 and ENG: 2), 1 reference probe has been replaced, 1 reference probe has been added, 3 ENG target probes have been added and 1 ACVRL1 exon 10 probe has been removed.
(A)	First release

Implemented changes in the product description

Version C2-06 – 25 September 2025 (04P)
- Percentage of *BMPR2* rearrangements associated with HPAH that are CNVs updated in Clinical background section based on a recent literature review.
- Percentage of all HPAH patients found to have CNVs in *BMPR2* updated in section Performance Characteristics based on a recent literature review.
- Minor textual corrections were performed in section Clinical background.
- SNP warnings for BMPR2 probe at 270nt and ENG probes at 184nt, 203nt and 312nt were removed from Table 1, Table 2a and Table 2c.
- List of References updated with four publications.
- Selected publications list adjusted to include most relevant articles.

Version C2-05 – 23 April 2024 (04P)
- Minor textual changes/corrections in sections Clinical background and Transcript variants.
- NM_ sequence and ligation sites have been updated for *ENG* in Table 2c.

Version C2-04 – 23 December 2021 (04P)
- Warning added for SNP rs376641299 to Table 1 and 2c.
- Salt sensitivity warning for BMPR2 probes at 172 and 220 nt removed from Table 1 and 2a.
- Ligation sites of the probes targeting the *BMPR2* and *ACVRL1* genes updated according to new version of the NM_ reference sequence.
- NM_ sequence for *BMPR2* has been updated.
- UK has been added to the list of countries in Europe that accept the CE mark.

- All selected publications before 2011 have been removed.
- Remark on updated probes in Probemix version C2 removed from Table 1.

Version C2-03 – 11 March 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended Purpose adapted to a new template.
- Warning for additional SNPs added under Table 1 and Table 2c.
- Sample ID of positive sample NA10186 corrected.

Version C2-02 - 25 June 2020 (03)
- Israel was added to the list of countries where this product is registered as IVD.

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IVD	EUROPE* CE 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.