Product Description
SALSA® MLPA® Probemix P242-D1 Pancreatitis

To be used with the MLPA General Protocol.

Version D1
As compared to version C1, nine target probes have been added (targeting/flanking *PRSS1*, *PRSS2* and *TRD-AS1*), three mutation-specific probes have been added (*PRSS1* and *SPINK1*), three probes have been replaced (*PRSS1* and *SPINK1*), the *CASP2* and *BRAF* flanking probes have been removed, eight reference probes have been replaced and six target probes have been changed in length and not in sequence detected. For complete product history see page 12.

SALSA MLPA Probemix P242 Pancreatitis contains probes for the *CTRC, SPINK1, PRSS1*, *PRSS2, and TRD-AS1* genes. *PRSS1* and *PRSS2* are located in a genomic region (the T cell receptor beta region) known to be excised during T cell maturation. The apparent copy number for *PRSS1* and *PRSS2* after normalisation in Coffalyser.net will therefore be dependent on the percentage of DNA derived from mature T cells. To correct for this bias and obtain accurate copy numbers for *PRSS1* and *PRSS2*, it is necessary to perform additional intra-normalisation steps in Excel, as described in Appendix 1. Signals from MLPA probes detecting the TRD-AS1 gene show a strong correlation to probes in the T cell receptor beta region in normal blood samples and are therefore included as an additional aid to detect copy numbers of *PRSS1* and *PRSS2* (see Appendix 1).

Catalogue numbers
- **P242-025R:** SALSA® MLPA® Probemix P242 Pancreatitis, 25 reactions
- **P242-050R:** SALSA® MLPA® Probemix P242 Pancreatitis, 50 reactions
- **P242-100R:** SALSA® MLPA® Probemix P242 Pancreatitis, 100 reactions

SALSA® MLPA® Probemix P242 Pancreatitis (hereafter: P242 Pancreatitis) is to be used in combination with:

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

P242 Pancreatitis can be used in combination with:

- SALSA® Binning DNA SD099 (Cat. No: SD099)

Volumes and ingredients

Volumes			Ingredients
P242-025R	**P242-050R**	**P242-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

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

Storage and handling

Recommended storage conditions		

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

in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding quality tests and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
SALSA® MLPA® Probemix P242 Pancreatitis is a **research use only (RUO)** assay for the detection of deletions, duplications or genomic rearrangements in the *CTRC*, *SPINK1*, *PRSS1* and *PRSS2* genes, which are associated with hereditary pancreatitis. This probemix includes probes for the *TRD-AS1* gene as an aid to interpret *PRSS1* and *PRSS2* copy numbers when larger regions are affected (see Appendix 1). This probemix also detects the presence of the *SPINK1* p.N34S (c.101A>G), *PRSS1* p.N29I (c.86A>T) and *PRSS1* p.R122H (c.365G>A) point mutations.

Hereditary Pancreatitis (HP; OMIM # 167800) is characterised by recurrent episodes of inflammation of the pancreas that progress to chronic pancreatitis. HP has an autosomal dominant pattern of inheritance and is defined by two or more individuals with pancreatitis in two or more generations of a family, or pancreatitis associated with a known germline pathogenic variant.

PRSS1 encodes trypsin-1 (cationic trypsinogen), a major pancreatic digestive serine peptidase enzyme, which is produced and secreted by the pancreas. *PRSS1* pathogenic variants typically result in a trypsin protein that is either prematurely activated, while it is still in the pancreas, or resistant to degradation. Duplication of *PRSS1*, leading to an increase of the protein, also causes HP. Duplication and triplication of a ~605-kb segment containing *PRSS1* and *PRSS2* have been described (Chauvin et al. 2009). Furthermore, gene conversion between *PRSS1* and *PRSS2* has been reported (Nemeth and Sahin-Tóth 2014, Rygiel et al. 2015). In 90% of the cases, hereditary pancreatitis is caused by two point mutations in the *PRSS1* gene, R122H (~65%) and N29I (~25%) (Nemeth and Sahin-Tóth 2014). Gene conversion between *PRSS1* and *PRSS2* exon 2 and intron 2 can occur, resulting in the N29I mutation together with N54S, a non-pathogenic mutation (Teich et al. 2005). Masson et al. (2008a) identified a hybrid *PRSS1*/*PRSS2* duplication in which exons 1 and 2 were derived from *PRSS2* and exons 3 to 5 from *PRSS1*, which also contained the *PRSS1* N29I mutation. In less than 6% of the cases, HP is caused by *PRSS1* (large) deletions/duplications.

SPINK1 encodes the serine protease inhibitor Kazal-type 1. Pathogenic variants in *SPINK1* are associated with increased risk of pancreatitis and have been identified in approximately 20% of families with hereditary pancreatitis, without a *PRSS1* germline pathogenic variant. The risk for developing acute pancreatitis is highly increased when the N34S mutation in *SPINK1* is present (Koziel et al. 2015).

CTRC encodes chymotrypsin C (CTRC), a low-abundance pancreatic digestive enzyme that is synthetized with PRSS1. CTRC is important for the degradation of the prematurely activated trypsin within the pancreas. Pathogenic variants of *CTRC* have been associated with chronic pancreatitis (Masson et al. 2008b).

While identification of a heterozygous *PRSS1* pathogenic variant confirms a diagnosis of hereditary pancreatitis, the presence of isolated pathogenic variants in the *SPINK1* or *CTRC* genes is insufficient to cause pancreatitis (Masson et al. 2013).

The *CTRC* gene (8 exons) spans ~11 kb of genomic DNA and is located on 1p36.21, about 15 Mb from the p-telomere. The *SPINK1* gene (4 exons) spans ~7.1 kb of genomic DNA and is located on 5q32, about 148 Mb from the p-telomere. The *PRSS1* gene (5 exons) spans ~3.6 kb of genomic DNA and is located on 7q34, about 143 Mb from the p-telomere. The *PRSS2* gene (5 exons) spans ~3.6 kb of genomic DNA and is located on

7q34, about 143 Mb from the p-telomere. The *TRD-AS1* gene (5 exons) spans ~104 kb of genomic DNA and is located on 14q11.2, about 22 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK190101/ and https://www.ncbi.nlm.nih.gov/books/NBK84399/.

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

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

Exon numbering
The exon numbering used in this P242-D1 Pancreatitis product description is the exon numbering derived from MANE project (release version 1.0) based on the following MANE Select transcripts (from description version D1-01 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for this gene): *CTRC* transcript NM_007272.3, *SPINK1* transcript NM_001379610.1, *PRSS1* transcript NM_002769.5 and *PRSS2* transcript NM_002770.4. The *TRD-AS1* exon numbering used in this P242-D1 Pancreatitis product description is the exon numbering from the RefSeq transcript NR_148361.1. The *SPINK1* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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
P242-D1 Pancreatitis contains 41 MLPA probes with amplification products between 124 and 409 nucleotides (nt). This includes eight probes for the *CTRC* gene, six probes for the *SPINK1* gene (one of which is a flanking probe), nine probes for the *PRSS1* gene (one of which is a flanking probe), five probes for the *PRSS2* gene (three of which are flanking probes) and three probes for the *TRD-AS1* gene. These include three probes specific for the *SPINK1* p.N34S (c.101A>G), *PRSS1* p.N29I (c.86A>T) and *PRSS1* p.R122H (c.365G>A) mutations which will only generate a signal when the mutation is present. In addition, ten 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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all reference 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, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

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

All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of pancreatitis. 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 NA11949, NA07412 and NA12519 from the Coriell Institute have been tested with P242-D1 Pancreatitis at MRC Holland and can be used as positive control samples (see table below; the samples are negative for the mutations targeted by the mutation-specific probes in this probemix). The quality of cell lines can change; therefore deviations to the indicated copy number variation (CNV) findings might occur.

Sample name	Source	Expected copy number alteration
NA11949; NA07412	Coriell Institute	Heterozygous deletion affecting all *PRSS1* and *PRSS2* probes.
NA12519	Coriell Institute	Heterozygous duplication affecting all *PRSS1* and *PRSS2* probes.

SALSA® Binning DNA SD099
The SALSA® Binning DNA SD099 provided with this probemix can be used for binning of all probes including the three mutation-specific probes for the *SPINK1* p.N34S (c.101A>G) mutation (156 nt), the *PRSS1* p.N29I (c.86A>T) mutation (178 nt) and the *PRSS1* p.R122H (c.365G>A) mutation (130 nt). SD099 is a mixture of human female genomic DNA from healthy individuals and a titrated amount of plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD099 in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net. 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 signals. For further details, please consult the SD099 product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For

more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual. For accurate detection of copy numbers affecting *PRSS1* and *PRSS2* additional normalization steps in Excel may be necessary (see Appendix 1).

Interpretation of results

The standard deviation of each individual reference 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	1.75 < FR < 2.15
Ambiguous copy number	All other values

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

The above mentioned FR values do not apply to the mutation-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.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases (or subtle changes due to T cell maturation; see Appendix 1). 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: https://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.

P242 Pancreatitis specific notes:

- The *PRSS1* and *PRSS2* genes are located within the VD region of the T cell receptor beta locus on chromosome 7 and get excised during VDJ rearrangements of this locus during T cell maturation. To obtain correct copy numbers of *PRSS1* and *PRSS2* it is therefore necessary to perform the extra intra - normalization steps using flanking probes, outlined in Appendix 1. For detection of larger CNVs affecting *PRSS1* and *PRSS2*, the extra intra-normalisation using the local flanking probes cannot be used. Therefore, from version D1 onwards this probemix contains three TRD-AS1 probes targeting the non-coding gene *TRD-AS1*.
  From our testing of blood samples from different donors against reference samples without T cell maturation, we found the decrease in ratios of the PRSS1/PRSS2 probes vs. the TRD-AS1 probes to be correlated. The TRD-AS1 probes can thus be used as an aid to interpret *PRSS1* and *PRSS2* copy numbers when larger regions are affected. For detailed information, see Appendix 1.
- The *SPINK1* p.N34S (c.101A>G), *PRSS1* p.N29I (c.86A>T) and *PRSS1* p.R122H (c.365G>A) mutation-specific probes are only intended to determine the presence (or absence) of the respective mutations and should not be used to determine zygosity.
- The PRSS1 N29I probe is designed to detect the N29I mutation if present in *PRSS1* but not the identical wildtype sequence in the homologous gene *PRSS2*. As a consequence it will generate a signal for the *PRSS2*-to-*PRSS1* N29I conversion mutation reported by Teich et al. (2005), but not for the N29I in the *PRSS1-PRSS2* gene hybrid identified by Masson et al. (2008). This has not been tested on positive human samples.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *CTRC*, *SPINK1*, *PRSS1* and *PRSS2* genes are small (point) mutations, most of which will not be detected by using P242 Pancreatitis, except for the here mentioned point mutations.
- 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 as well as point mutations always require confirmation by another method. Because the 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 in sequence data indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

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

CTRC, _SPINK1_ and _PRSS1_ mutation databases

https://databases.lovd.nl/shared/genes/CTRC
https://databases.lovd.nl/shared/genes/SPINK1
https://databases.lovd.nl/shared/genes/PRSS1

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

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *PRSS1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. P242-D1 Pancreatitis

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*CTRC*	*SPINK1*	*PRSS1*	*PRSS2*	*TRD-AS1*
64-105	Control fragments – see table in probemix content section for more information						
124 *	Reference probe 19616-L26275	4p					
130 * §	**PRSS1 probe** 23353-L33011				**p.R122H**		
136 * ¬	**PRSS2 probe** 23354-L33082					downstream	
142	**PRSS1 probe** 09213-L09483				**Exon 4**		
148	**CTRC probe** 22402-L32009		**Exon 1**				
156 * §	**SPINK1 probe** 23355-L33094			**p.N34S**			
162 * ≈	**TRD-AS1 probe** 23356-L33085						Intron 2
166 *	Reference probe 22982-L32421	2q					
172 ¥	**CTRC probe** 22403-L33165		**Exon 8**				
178 * § Ж	**PRSS1 probe** S1314-SP1016-L33015				**p.N29I**		
185 *	**SPINK1 probe** 23357-L33086			**Exon 4**			
191 * ≈	**TRD-AS1 probe** 23358-L33087						Intron 2
196	**CTRC probe** 22404-L31746		**Exon 6**				
202 *	Reference probe 19414-L25829	3p					
208 * ¬	**PRSS1 probe** 23359-L33018				upstream		
218	**CTRC probe** 22405-L31594		**Exon 4**				
224 ¥	**SPINK1 probe** 08412-L33088			**Exon 2**			
230 *	Reference probe 10721-L29208	6p					
235	**CTRC probe** 22406-L31595		**Exon 2**				
242 *	**PRSS1 probe** 23360-L33019				**Exon 3**		
247 *	Reference probe 15628-L17492	16q					
254	**CTRC probe** 22408-L31597		**Exon 3**				
260 ¥	**CTRC probe** 22407-L33166		**Exon 7**				
265 ¥	**PRSS1 probe** 09210-L33090				**Exon 1**		
273 ¥	**PRSS1 probe** 23361-L33091				**Exon 5**		
280	Reference probe 13350-L26120	9q					
287 * ¬	**PRSS2 probe** 23362-L33092					downstream	
295	**SPINK1 probe** 09215-L28282			**Exon 3**			
304	**PRSS1 probe** 09211-L28284				**Exon 1**		
310	Reference probe 15855-L17948	11p					
319 * ≈	**TRD-AS1 probe** 23363-L33111						Intron 2
328 *	**PRSS2 probe** 23397-L33080					Intron 3	
336 ¥	**PRSS1 probe** 23364-L33167				**Exon 2**		
346 *	Reference probe 21721-L30379	15q					
355 * ¬	**PRSS2 probe** 23365-L33023					downstream	
363 *	**PRSS2 probe** 23366-L33168					**Exon 1**	
374	**CTRC probe** 22409-L31598		**Exon 5**				
383 *	Reference probe 21008-L29226	8q					
391	**SPINK1 probe** 22581-L31780			**Exon 5**			
400 * ¬	**SPINK1 probe** 23371-L33027			upstream			
409 *	Reference probe 20245-L27576	12q					

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

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
§ Mutation-specific probe. These probes will only generate a signal when the *SPINK1* p.N34S (c.101A>G) mutation (156 nt), *PRSS1* p.N29I (c.86A>T) mutation (178 nt) or *PRSS1* p.R122H (c.365G>A) mutation (130 nt) is present. The *PRSS1* p.N29I (c.86A>T) and *PRSS1* p.R122H (c.365G>A) mutation-specific probes have been tested on artificial DNA **but not on positive human samples!**

Ж 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested. The flanking probes at 287 nt and 355 nt are flanking the 605-kb duplication/triplication of the trypsinogen locus containing *PRSS1* and *PRSS2* as reported by Chauvin et al. (2009).
≈ The TRD-AS1 probe facilitates the detection of large-scale duplications or deletions encompassing the entire *PRSS1*/*PRSS2* region and its flanking probes (see Appendix 1).

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

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

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. *CTRC*

Length (nt)	MLPA probe	*CTRC* exon[a]	Ligation site[b] NM_007272.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*23-25 (Exon 1)*		
148	22402-L32009	Exon 1	39-40	GGGCATCACTGT-CCTCGCTGCGCT	1.9 kb
235	22406-L31595	Exon 2	4 nt after exon 2	CCTGGCAGGTAA-GCCTGTGTAGGG	0.1 kb
254	22408-L31597	Exon 3	215-216	GGACTTTGATTG-CTAGCAACTTCG	1.9 kb
218	22405-L31594	Exon 4	292-293	GGAAAGAACAAC-CTGGAGGTGGAA	1.1 kb
374	22409-L31598	Exon 5	9 nt after exon 5	GGAGTGAGTATC-GTCCCTGGCAAA	1.2 kb
196	22404-L31746	Exon 6	661-intron 6	TCAGCCTGCAAT-GTGAGTGGCTAG	0.9 kb
260	22407-L33166	Exon 7	749-750	CCCGGCGGGGCT-GCAACACCCGCA	0.9 kb
		stop codon	*827-829 (Exon 8)*		
172	22403-L33165	Exon 8	830-831	TGCAGCTGTGAT-TTGTTGCTGGGA	

Table 2b. *SPINK1*

Length (nt)	MLPA probe	*SPINK1* exon[a]	Ligation site[b] NM_001379610.1	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
400 ¬	23371-L33027	upstream	10 kb before exon 1	GTATCCACTCCT-GCATGCATTGAA	10.3 kb
		start codon	*95-97 (Exon 1)*		
224	08412-L33088	Exon 1 (2)	63-64	GCGGTGCAGTTT-TCAACTGACCTC	2.0 kb
295	09215-L28282	Exon 2 (3)	152-153	TATTTCTAGGTA-ACACTGGAGCTG	1.5 kb
156 §	23355-L33094	Exon 3	195-194 *reverse*	CATTAAGTACAC-TGTAACATTTGG	0.1 kb
185	23357-L33086	Exon 3	248-249	GGACTGATGGAA-ATACTTATCCCA	3.4 kb
391	22581-L31780	Exon 4 (5)	301-302	AAACGCCAGACT-TCTATCCTCATT	
		stop codon	*332-334 (Exon 4)*		

Table 2c. *PRSS1* and *PRSS2* (T cell receptor beta region)

Length (nt)	MLPA probe	Exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		PRSS1 gene	**NM_002769.5**		
208 ¬ #	23359-L33018	upstream	3.4 kb before exon 1	AAGATGGAGTCC-CAGAGCATGAAC	3.4 kb
265	09210-L33090	Exon 1	36 nt before exon 1	CTGGATCCTCGT-GAGGTATAAAGA	0.1 kb
		start codon	*14-16 (Exon 1)*		
304 #	09211-L28284	Exon 1	53-54	TGGCAGCTGCTC-GTGAGTATCATG	0.9 kb
178 § Ж	S1314-SP1016-L33015	Exon 2	99-98; 69 nt before exon 2	TCTCCTCACAGA-59 nt spanning oligo-TGCTTTCTGCTA	0.2 kb
336 # +	23364-L33167	Exon 2	174-175	CTCCCTCATCAA-CGAACAGTGGGT	1.2 kb
242	23360-L33019	Exon 3	311-310 reverse	AGTCTTCCTGTC-GTATTGGGAGTG	0.1 kb
130 §	23353-L33011	Exon 3	378-379	AATCAACGTCCA-CGTGTCCACCAT	0.6 kb
142 #	09213-L09483	Exon 4	598-599	GGCAAGGATTCA-TGTCAGGTGATT	0.4 kb
273 #	23361-L33091	Exon 5	722-723	ACAACTATGTGA-AATGGATTAAGA	38.3 kb
		stop codon	*755-757 (Exon 5)*		
		PRSS2 gene	**NM_002770.4**		
363 #	23366-L33168	Exon 1	45 nt before exon 1 *reverse*	CTCCCGAGGATG-GGGAGAGGAGAT	2.8 kb
		start codon	*14-16 (Exon 1)*		
328 #	23397-L33080	Intron 3	178 nt before exon 4	ATGATCACTTCG-TGGGAGAGGTTC	5.8 kb
		stop codon	*755-757 (Exon 5)*		
136 ¬	23354-L33082	downstream	4.9 kb after exon 5 *reverse*	CCTGTGACAAAA-AACACTGCTGTG	4.8 kb
355 ¬	23365-L33023	downstream	9.8 kb after exon 5 *reverse*	AAGCCATGGTGA-ACATCAGAGGGT	0.9 kb
287 ¬	23362-L33092	downstream	11 kb after exon 5 *reverse*	ACAAGAGTGATG-ACCTATTAAGAA	

Table 2d. *TRD-AS1* (T cell receptor delta region)

Length (nt)	MLPA probe	*TRD-AS1* exon[a]	Ligation site[b] NR_148361.1	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
319 ≈	23363-L33111	Intron 2	47 kb after exon 2	TTGTTATTGGAA-AAGGTTGAGCTA	5.3 kb
191 ≈	23358-L33087	Intron 2	47 kb before exon 3	ACAGGAAGATGA-CAGCACTGATTA	1.5 kb
162 ≈	23356-L33085	Intron 2	45 kb before exon 3	CTGGAACACCAA-ATAACTTACTCT	

[a] See section Exon numbering on page 3 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. These probes will only generate a signal when the *SPINK1* p.N34S (c.101A>G) mutation (156 nt), *PRSS1* p.N29I (c.86A>T) mutation (178 nt) or *PRSS1* p.R122H (c.365G>A) mutation (130 nt) is present. The *PRSS1* p.N29I (c.86A>T) and *PRSS1* p.R122H (c.365G>A) mutation-specific probes have been tested on artificial DNA **but not on positive human samples!**
Ж 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested. The flanking probes at 287 nt and 355 nt are flanking the 605-kb duplication/triplication of the trypsinogen locus containing *PRSS1* and *PRSS2* as reported by Chauvin et al. (2009).
≈ The TRD-AS1 probe facilitates the detection of large-scale duplications or deletions encompassing the entire *PRSS1*/*PRSS2* region and its flanking probes (see Appendix 1).
This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene, pseudogene or highly similar region.
+ This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result: 1) an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene; 2) an apparent deletion can be the result of gene conversion between *PRSS1* and *PRSS2* in exon 2 (Teich et al. 2005).

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

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

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P242 product history	
Version	*Modification*
D1	Nine target probes have been added (targeting/flanking *PRSS1*, *PRSS2* and targeting *TRD-AS1*), three mutation-specific probes have been added (targeting *PRSS1* and *SPINK1*), three probes have been replaced (targeting *PRSS1* and *SPINK1*), the CASP2 and BRAF flanking probes have been removed, eight reference probes have been replaced and six target probes have been changed in length and not in sequence detected.
C1	Eight CTRC probes and two SPINK1 probes have been added. One SPINK1 probe has been removed. One probe has been adjusted in length. Seven reference probes have been replaced.
B3	One flanking probe has been removed and four reference probes have been replaced.
B2	Three reference probes have been replaced and the control fragments have been adjusted (QDX2).
B1	The PRSS2 probe at 165 nt has been removed because of its high standard deviation.
A1	First release.

Implemented changes in the product description
Version D1-01 16 July 2025 (05P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Exon numbering of the *SPINK1* gene has been changed. - Ligation sites of the probes targeting the *SPINK1* gene updated according to new NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. Version C1-03 – 03 August 2023 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *SPINK1* gene updated according to new version of the NM_ reference sequence. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version C1-02 — 23 February 2021 (02P) - Warning adjusted on page 1, interpretation of results section and below Table 2a regarding PRSS1 probe ratios of ~0.8. - Positive samples NA07412 and NA12519 added to the section Positive control DNA samples.

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Appendix 1. Additional analysis steps for PRSS1 and PRSS2 probes

After normalisation, probes located in the *PRSS1* and *PRSS2* genes even with normal copy number may show values that deviate from the baseline ratio of 1.0. This deviation is expected, as both genes are located in the T cell receptor beta VD region, which is subject to removal during T cell maturation. Therefore, observed differences in normalised signal between patient and reference samples may not be due to copy number alterations, but rather reflect differences in the amount of T cells present in the samples.

To adjust for this bias and facilitate the detection of both focal copy number changes and large deletions or amplifications affecting the *PRSS1* and *PRSS2* genes, we suggest performing three additional, separate analyses as outlined below. These calculations are not included in Coffalyser.net, but can be performed in Excel using exported normalised results from Coffalyser.net as starting values.

1. Normalise all 14 7q34 copy number and mutation-specific probes (Table 2c) to the median value of the flanking probes at 208, 136, 355, and 287 nt in each sample separately. This will shift the baseline to 1.0 for these probes and reveal focal aberrations involving *PRSS1* and *PRSS2*.
2. Similarly, normalise the 12 7q34 copy number probes to the flanking probes at 355 and 287 nt. These two probes are located telomeric to the 605 kb region reported by Chauvin (2009). Uniform amplification of the first 12 centromeric copy number probes may imply that the entire 605 kb region is affected; however, this would need to be confirmed by additional validation.
3. Calculate the median value from the *TRD-AS1* probes at 162, 191, and 319 nt (Table 2d), all targeting the T cell receptor delta VD region at 14q11, for each sample separately. Plot these values against the median values of the four flanking probes located in the T cell receptor beta VD region, as calculated in point 1 above. As seen in Figure 1, the values obtained from the T cell receptor delta and T cell receptor beta VD regions strongly correlate in peripheral blood-derived samples, and any deviation from this relationship would indicate a copy number change affecting large parts of either of the two loci. Determining which of the two loci is affected would, however, require additional investigations.

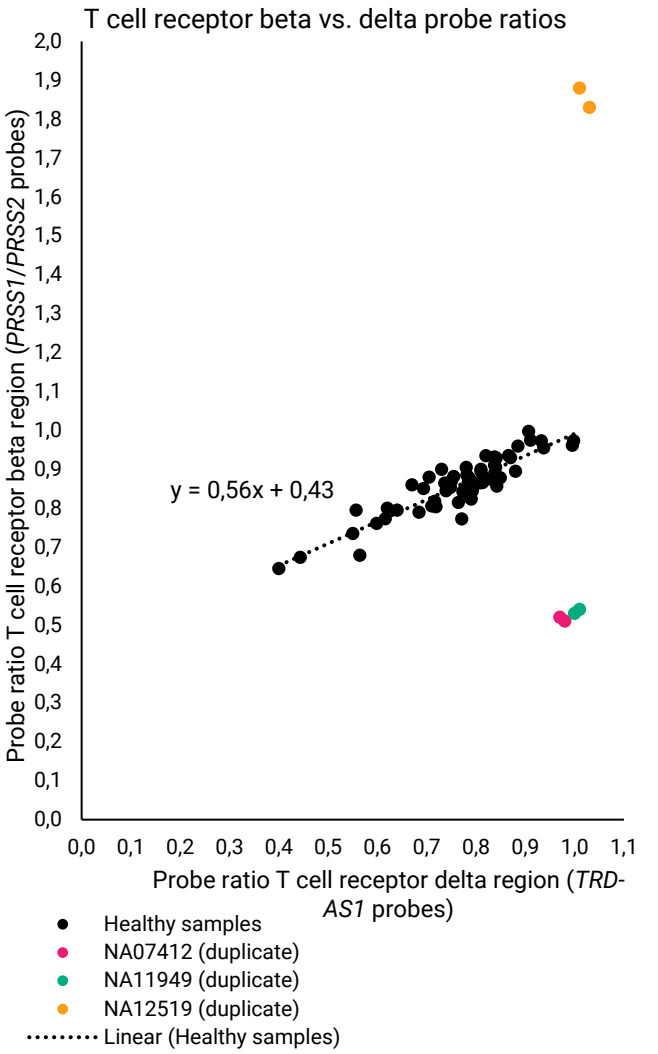

Figure 1. T cell receptor delta vs. T cell receptor beta probe ratios were analysed from 58 peripheral blood samples from healthy donors and three Coriell samples, as calculated in point 3. As shown in the graph, the deviating TRB copy number in the three Coriell samples (see the table of positive samples for details) clearly stands out from the pattern observed in the healthy donor samples.