Product Description
SALSA® MLPA® Probemix P125-C1 Mitochondrial DNA

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 9.

Catalogue numbers:

- **P125-025R:** SALSA MLPA Probemix P125 Mitochondrial DNA, 25 reactions.
- **P125-050R:** SALSA MLPA Probemix P125 Mitochondrial DNA, 50 reactions.
- **P125-100R:** SALSA MLPA Probemix P125 Mitochondrial DNA, 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.

RNAse treatment of the sample DNA is strongly recommended. See page 3.

The recommended amount of sample DNA to be used is lower as compared to other MLPA applications. See page 3.

General information
The SALSA MLPA Probemix P125 Mitochondrial DNA is a **research use only (RUO)** assay for the detection of deletions or duplications in the human mitochondrial DNA and the presence and allele fraction of six different point mutations as described in Tables 1 and 2.

Mitochondrial DNA (mtDNA) differs from nuclear DNA. The complete mitochondrial genome is circular and only 16,569 bp long. Only a small number of genes needed for mitochondrial functions are encoded by the mtDNA. The copy number of mitochondrial DNA is more than 100 fold higher as compared to genomic DNA. Most human cells contain over 200 copies of the mitochondrial genome.

The mutation rate in mtDNA is about 10 times higher than in nuclear DNA, probably due to an inferior repair system, the exposure to oxygen-free radicals generated by oxidative phosphorylation, and the lack of protective histones. The regions between nt 45-287 and between nt 16105-16348 are regarded as hypervariable. Mitochondrial DNA has no introns and hardly any intergenic regions. Most sequence changes will therefore affect a coding sequence. Transcription of mtDNA is polycistronic, which means that all genes encoded on the two (“heavy” and “light”) DNA strands are transcribed as two large precursor RNA strands. A deletion anywhere in the mitochondrial genome may affect the transcription or translation of other genes, even when their sequence is intact. As a result, deletions of various sizes can cause similar phenotypes. Inherited anomalies of mtDNA are always maternal as all mitochondria come from the ovum.

Deletions in the mitochondrial DNA have been identified in various diseases including Pearson Syndrome, Kearns-Sayre Syndrome and Progressive external ophthalmoplegia. These deletions vary in size (1.3-8 kb) and location. The most common deletion region is between positions 8469 and 13147. Diseases caused by

deletions and point mutations in mtDNA are characterised by heteroplasmy: a mixture of wild-type and mutant mtDNA is present in one cell. A deletion present in 35% of the mitochondria results in a 35% signal reduction of the affected MLPA probes. Please note that identical deletions can cause different diseases depending on the tissue in which they occur.

Some tandem duplications of parts of the mitochondrial genome have also been described, as well as a number of frequent mitochondrial point mutations resulting in a myopathy, such as MERRF and MELAS.

More information is available at:
https://www.ncbi.nlm.nih.gov/books/NBK1224/ (Mitochondrial Disorders Overview).
https://www.ncbi.nlm.nih.gov/books/NBK1203/ (Mitochondrial DNA deletion syndromes).
https://www.ncbi.nlm.nih.gov/books/NBK1233/ (MELAS).
https://www.ncbi.nlm.nih.gov/books/NBK1520/ (MERRF).
https://www.ncbi.nlm.nih.gov/books/NBK1173/ (Mitochondrial DNA-Associated Leigh Syndrome and NARP)
https://www.ncbi.nlm.nih.gov/books/NBK1174/ (Leber Hereditary Optic Neuropathy)

Mitochondrial DNA mutation database: https://www.mitomap.org/MITOMAP.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
The reference sequence used to locate the ligation sites of the probes contained within the Probemix P125-C1 is NC_012920.1, which is available at :
https://www.mitomap.org/foswiki/bin/view/MITOMAP/HumanMitoSeq.

Probemix content
The SALSA MLPA Probemix P125-C1 Mitochondrial DNA contains 46 MLPA probes with amplification products between 124 and 463 nt. 34 probes are copy number probes.

Six probes are specific for six different frequent mutations which will only generate a signal when the mutation is present. For each of these six point mutations, a second probe is present that generates a signal only on the wildtype sequence of the mutation. Analysis of both the mutation specific probe and the associated wildtype probe provides an estimate of the allelic fraction of the point mutation. The six point mutations that are targeted are:

- **m.3243A>G**; *TL1* gene (Most frequent cause of MELAS)
- **m.3460G>A**; *ND1* gene (p.A52T; associated with Leber Hereditary Optic Neuropathy / LHON)
- **m.8344A>G**; *TK* gene (Most frequent cause of MERRF)
- **m.8993T>G**; *ATP6* gene (associated with leigh syndrome and NARP)
- **m.11778G>A**; *ND4* gene (p.R340H; associated with Leber Hereditary Optic Neuropathy / LHON)
- **m.14484T>C**; *ND6* gene (p.M64V; associated with Leber Hereditary Optic Neuropathy / LHON)

This probemix contains four quality control fragments generating amplification products between 64 and 82 nt: the four DNA Quantity Fragments (Q-fragments, see table below). These will only be visible in reactions with extremely low amounts of sample DNA (< 1 ng). 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 quality control fragments
64-70-76-82	Q-fragments (only visible with <1 ng sample DNA)

N.B. In contrast to genomic DNA MLPA probemixes, this MLPA Probemix P125 Mitochondrial DNA does not contain the 92 nt benchmark fragment, nor the D-fragments, to check for denaturation, or X and Y fragments, to check for the gender of the sample. Therefore, Coffalyser.Net will not give a warning for problems with denaturation, nor will the software indicate the gender of the samples.

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

Modifications of the MLPA General protocol for P125 Mitochondrial DNA

1. RNAse sample treatment

Mitochondrial ribosomal RNA is encoded by the RNR1 and RNR2 genes on the mitochondrial genome. These genes are heavily expressed in every tissue. When DNA samples are not RNAse treated, the mitochondrial ribosomal RNA can affect the probe signal of the mtDNA probes that detect, partially overlap with, or are close to the RNR1 and RNR2 genes. The effect of RNA contamination of DNA samples will be small when less than 20 ng sample DNA is used. In general, a higher signal is obtained for these probes when large amounts of RNA and DNA are present as the mitochondrial ribosomal RNA binds to one mt DNA strand and thereby reduces the level of sample DNA reannealing for that region.

Many DNA purification methods include an RNAse treatment. Some however do not, including certain automatic DNA purification methods (e.g. Roche Magnapure, Chemagen). For samples that have not been RNAse treated during purification, we recommend the following procedure:

- Mix 4 µl sample DNA and 1 µl 0.5 mg/ml RNAse A. Incubate 30 minutes at 37 °C in a thermocycler with heated lid. Continue with the 5 minutes 98 C DNA denaturation step of the MLPA protocol.
- RNAse A is extremely stable; it can be diluted in TE and stored at -20 °C. We recommend RNAse A from Promega (A7973; 4 mg/ml solution), diluted 8 fold in TE (1 ml of 4 mg/ml RNAse A is sufficient for ~8000 samples). Do not use more than the recommended amount.

2. Amount of sample DNA used

We strongly recommend using only 5-50 ng sample DNA. Although samples containing less than 5 ng sample DNA may still contain sufficient copies of mitochondrial DNA to yield good MLPA results, very low sample DNA amounts can be lost by adherence to tube walls. DNA samples containing 100 ng or more of human DNA on the other hand, contain millions of copies of mitochondrial DNA. When this much sample DNA is used, reannealing of sample DNA results in more variable results, in particular when some RNA is still present

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, 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.

MRC-Holland has tested this product on DNA samples from healthy individuals, cell lines with four known point mutations and on artificial mutation positive samples; not on patient samples. Based on literature, a minimum percentage of 23-40% mutant mtDNA copies is needed to detect a deletion with this probemix (Mayorga et al. 2016, Tonska et al. 2012). DNA extracted from all tissues can be used, however, the percentage heteroplasmy found will vary between tissues. The muscle biopsy is considered as the optimal material (Mayorga et al. 2016, Tonska et al. 2012, Kwon et al. 2011). The liver biopsy may be quite useful, while blood can be less informative in some cases (Tonska et al. 2012). Please note that the sensitivity of gap-PCR assays for the detection of mitochondrial deletions will be higher. However, the use of this P125 MLPA assay will have advantages for the detection of unusual deletion sizes and will be more quantitative. When the size and location of a mitochondrial DNA deletion is known, suitable primers for a gap PCR assay can be more easily selected.

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 unrelated individuals who are from families without a history of disorders related to mitochondrial DNA. 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 NA11605, NA11906, NA11907, NA13411 and NA10744 from the Coriell Institute have been tested with this P125-C1 probemix at MRC Holland and can be used as a positive control samples to detect four of the six specific point mutations. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Mutation	WT probe	MUT probe	Comments
NA11605	Coriell Institute	m.3460G>A positive	267 nt	263 nt	95% mutation; 5% WT
NA11906	Coriell Institute	m.8344A>G positive	250 nt	242 nt	35% mutation; 65% WT
NA11907	Coriell Institute	m.8344A>G positive	250 nt	242 nt	98% mutation; 2% WT
NA13411	Coriell Institute	m.8993T>G positive	203 nt	197 nt	100% mutation
NA10744	Coriell Institute	m.11778G>A positive	223 nt	216 nt	100% mutation

MRC Holland is not aware of any cell line containing the m.3243A>G or the m.14484T>C mutations. The probes detecting these mutations have only been tested on the artificial positive SD085 binning DNA.

SALSA Binning DNA SD085

The SD085 Binning DNA provided with this probemix can be used for binning of the six mutation/SNP-specific probes (21433-L30223; 21436-L29939; 21440-L30473; 21443-L29946; 21446-L29949; 21452-L30472). SD085 Binning DNA is a mixture of genomic DNA from healthy individuals and a carefully titrated amount of plasmid DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD085 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s), as for this purpose true mutation/SNP positive patient samples or cell lines should be used. 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 SD085 Binning DNA product description, available online: www.mrcholland.com.
This product is for research use only (RUO).

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.

Due to the large difference in copy number between genomic and mitochondrial sequences, it is not possible to use reference probes targeting genomic sequences in this probemix. As a result, the ratio between genomic DNA and mitochondrial DNA (mitochondrial copy number) cannot be determined.

For data analysis, 14 probes are used as reference probes. These are probes that detect a sequence in mitochondrial DNA regions that are less frequently deleted. It is possible that in certain cases also one or more reference probes are involved in a deletion or duplication. In such a case, it may be necessary to reanalyse samples using a different selection of the P125 probes as reference probes.

Interpretation of results

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 of the P125 copy number probes:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Heteroplasmy sample with a deletion in 20% of all MT copies*	~ 0.80
Heteroplasmy sample with a deletion in 40% of all MT copies*	~ 0.60
Heteroplasmy sample with a deletion in 60% of all MT copies*	~ 0.40
Heteroplasmy sample with a deletion in 80% of all MT copies*	~ 0.20
Heteroplasmy sample with a duplication in 50% of all MT copies	~ 1.50
Homozygous deletion (unlikely) or polymorphism at, or very near, the probe ligation site in all mtDNA copies	FR= 0

* OR, in case of a single probe, a polymorphism or mutation very near the probe ligation site.

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.

For the six probe pairs that detect a specific point mutation, the presence of a probe signal for one of the mutation specific probes is proof that the mutation is present but cannot be used for quantification. In that case however, the decrease in probe signal for the corresponding wildtype specific probe indicates the percentage of mitochondrial genomes in which the mutation is present (% heteroplasmy). A decrease in signal for the wildtype specific probe, without a signal for the mutation specific probe, can be due to a small deletion, or to a different sequence change very near the probe ligation site.

- Arranging probes according to mitochondrial location facilitates interpretation of the results and may reveal more subtle changes such as those observed in heteroplasmy cases. DNA sequencing, or analysis of DNA samples extracted from other tissues, or analysis of maternal DNA samples, may in some cases be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe have a considerable chance of being a false positive result due to a polymorphism at, or very near the probe ligation site. 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 more GC-rich area. For this product, inclusion of denaturation control probes is not possible. The use of an additional purification step or an alternative DNA extraction method, or analysis of a diluted DNA sample may resolve such cases. Additionally, contamination of DNA samples with PCR amplicons, or cDNA, of mitochondrial DNA sequences, 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.
- Users should always consult the scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more genes 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.

P125 specific note(s):

- When the size and location of a deletion is known, gap-PCR will be a more sensitive technique to detect a low (<20%) heteroplasmy level in a sample.
- This product cannot be used to determine the number of mitochondrial DNA copies / cell. No probes are present for autosomal DNA sequences.

Limitations of the procedure

- 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 mitochondrial region *do* exist but remain undetected.
- This product can be used to detect 6 frequent mitochondrial point mutations but will not provide information on any other mitochondrial DNA sequence changes.
- 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 provides information on the *average* situation in the cells from which the DNA samples was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of heteroplasmy is low.

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 gap-PCR, whenever possible.

Mitochondrial DNA mutation database

We strongly encourage users to deposit positive results in the https://www.mitomap.org/MITOMAP. 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 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P125-C1 Mitochondrial DNA

Length (nt)	SALSA MLPA probe	Mitochondrial DNA position		
		Gene	MUT	WT
64-70-76-82 *	Control fragments; only visible when less than 1 ng sample DNA is used			
124	S1188-L31203	REF - *ND6*		
132	S1187-L31202	*ND5*		
139	S1177-L31201	REF - *ND1*		
142	21427-L29930	REF - *RNR1*		
149	21428-L30232	*CO1*		
155	04312-L30233	*ND4*		
160	21429-L29932	REF - *RNR1*		
166	21430-L30219	*CO2*		
172	04313-L30220	*ND4*		
178	21431-L29934	REF - *RNR1*		
184	21432-L30221	*CO2*		
190	04314-L30222	*ND5*		
197 § *	21433-L30223	*ATP6*	m.8993G	
203 ∞ +	21434-L30224	*ATP6*		m.8993T
210	21435-L29938	*CO2*		
216 §	21436-L29939	*ND4*	m.11778A	
223 ∞	21437-L29940	*ND4*		m.11778G
229	21438-L29941	REF - *RNR2*		
237	21439-L30225	*ATP6*		
242 §	21440-L30473	*TK*	m.8344G	
250 ∞	21441-L30226	*TK*		m.8344A
256	21442-L30227	*ND5*		
263 §	21443-L29946	*ND1*	m.3460A	
267 ∞	21444-L29947	*ND1*		m.3460G
274	21445-L30150	*ND5*		
281 §	21446-L29949	*TL1*	m.3243G	
289 ∞	21447-L30228			m.3243A
295	21448-L29951	REF - *ND1*		
302	21449-L29952	*ATP6*		
310	21450-L29953	REF - *ND2*		
319	21451-L30234	*CO3*		
326 §	21452-L30472	*ND6*	m.14484C	
337 ∞	21453-L30151	*ND6*		m.14484T
343	21454-L30302	REF - *RNR2*		
352	04306-L30301	*CO3*		
360	04308-L15254	REF - *CYB*		
372	04301-L30235	*TC*		
382	21456-L29959	*ND3*		
391	21457-L29960	REF - *CYB*		
402	21458-L29961	*CO1*		
411	21459-L30231	*ND4*		
420	21460-L29963	REF - *RNR2*		
436	21462-L29965	CO1		
442	21463-L29966	REF - *TP*		
454	21464-L29967	ND2		
463	21465-L29968	REF - *CYB*		

REF: Probes in regions that are less frequently part of a deletion or duplication are used as reference probes in data analysis by Coffalyser software. For certain samples, it may be necessary to use a different choice of reference probes.

***** Due to the high copy number of mitochondrial DNA, the four Q fragment peaks at 64-70-76-82 nt will usually not be visible.

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. For more details on the samples used by MRC for testing, please refer to the section *Positive control samples*.

∞ Wild type sequence detected. The presence of the indicated mutation will result in a decreased probe signal.

+ A decrease in signal for the 8993T wildtype probe (203 nt), without a signal for the 8993G probe (197 nt), can be due to the presence of a T8993C mutation which has also been reported as cause of Leight syndrome.

Table 2. Mitochondrial DNA probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Mitochondrial DNA position			Ligation site NC_012920.1	Distance to next probe
		Gene	MUT	WT		
142	21427-L29930	REF - RNR1			691-690, reverse	384 nt
160	21429-L29932	REF - RNR1			1076-1075, reverse	486 nt
178	21431-L29934	REF - RNR1			1563-1562, reverse	473 nt
420	21460-L29963	REF - RNR2			2037-2036, reverse	516 nt
343	21454-L30302	REF - RNR2			2552-2551, reverse	492 nt
229	21438-L29941	REF - RNR2			3041-3040, reverse	201 nt
281 §	21446-L29949	**TL1**	3243G		3243-3242, reverse	N.A.
289 ∞	21447-L30228	**TL1**		3243A	3243-3244	252 nt
263 §	21443-L29946	**ND1**	3460A		3460-3461	N.A.
267 ∞	21444-L29947	**ND1**		3460G	3460-3459, reverse	178 nt
139	S1177-L31201	REF - ND1			3651-3652	507 nt
295	21448-L29951	REF - ND1			4151-4152	720 nt
310	21450-L29953	REF - ND2			4865-4866	529 nt
454	21464-L29967	**ND2**			5406-5407	422 nt
372	04301-L30235	**TC**			5821-5822	281 nt
402	21458-L29961	**CO1**			6108-6109	508 nt
436	21462-L29965	**CO1**			6611-6612	526 nt
149	21428-L30232	**CO1**			7136-7137	479 nt
166	21430-L30219	**CO2**			7614-7615	325 nt
184	21432-L30221	**CO2**			7940-7941	297 nt
210	21435-L29938	**CO2**			8235-8236	95 nt
242 §	21440-L30473	**TK**	8344G		8344-8343, reverse	N.A.
250 ∞	21441-L30226	**TK**		8344A	8344-8345	336 nt
237	21439-L30225	**ATP6**			8641-8642	318 nt
197 §+	21433-L30223	**ATP6**	8993G		8993-8994	N.A.
203 ∞+	21434-L30224	**ATP6**		8993T	8993-8992, reverse	93 nt
302	21449-L29952	**ATP6**			9085-9086	322 nt
319	21451-L30234	**CO3**			9404-9405	517 nt
352	04306-L30301	**CO3**			9914-9915	423 nt
382	21456-L29959	**ND3**			10353-10354	509 nt
411	21459-L30231	**ND4**			10856-10857	681 nt
155	04312-L30233	**ND4**			11527-11528	212 nt
216 §	21436-L29939	**ND4**	11778A		11778-11779	N.A.
223 ∞	21437-L29940	**ND4**		11778G	11778-11777, reverse	301 nt
172	04313-L30220	**ND4**			12067-12068	399 nt
190	04314-L30222	**ND5**			12474-12475	440 nt
132	S1187-L31202	**ND5**			12919-12920	605 nt
274	21445-L30150	**ND5**			13522-13523	455 nt
256	21442-L30227	**ND5**			13976-13977	402 nt
124	S1188-L31203	REF - ND6			14379-14380	78 nt
326 §	21452-L30472	**ND6**	14484C		14484-14485	N.A.
337 ∞	21453-L30151	**ND6**		14484T	14484-14483, reverse	460 nt
463	21465-L29968	REF - CYB			14938-14939	412 nt
360	04308-L15254	REF - CYB			15341-15342	443 nt
391	21457-L29960	REF - CYB			15794-15795	164 nt

Length (nt)	SALSA MLPA probe	Mitochondrial DNA position			Ligation site NC_012920.1	Distance to next probe
		Gene	MUT	WT		
442	21463-L29966	REF - TP			15963-15964	1296 nt
142	*21427-L29930*	**The mitochondrial DNA is circular and only 16.569 bp long**				

REF: Probes in regions that are less frequently part of a deletion or duplication are used as reference probes in data analysis by Coffalyser software. For certain samples, it may be necessary to use a different choice of reference probes.

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. For more details on the samples used by MRC for testing, please refer to the section *Positive control samples*.

∞ Wild type sequence detected. The presence of the indicated mutation will result in a decreased probe signal.

+ A decrease in signal for the 8993T wildtype probe (203 nt), without a signal for the 8993G probe (197 nt), can be due to the presence of a T8993C mutation which has also been reported as cause of Leight syndrome.

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

Related SALSA MLPA probemixes

- P-010-POLG: Contains probes for *POLG*, *POLG2*, *TWNK* and *SLC24A5*
- P-089-TK2: Contains probes for *TK2*, *DGUOK*, *MPV17*, *RRM2B*, *SUCLA2* and *SUCLG*

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P125 Mitochondrial DNA

- Loos et al. (2021). Clinical and molecular characterization of mitochondrial DNA disorder in a group of Argentinian pediatric patients. *Mol Genet Metab Rep.*27: 100733.
- Mayorga L et al. (2016). Mitochondrial DNA deletions detected by Multiplex Ligation-dependent Probe Amplification. Mitochondrial DNA A DNA MappSeq Anal. 27:2864-7.
- Tonska K et al. (2012). Molecular investigations of mitochondrial deletions: Evaluating the usefulness of different genetic tests. Gene. 506:161-5.
- Kwon et al. (2011). Multiplex ligation-dependent probe amplification (MLPA) assay for the detection of mitochondrial DNA deletion in chronic progressive external ophthalmoplegia (CPEO). Ann Clin Lab Sci. 41:385-9.

P125 product history	
Version	*Modification*
C1	Almost all probes have been changed or replaced
B1	Four probes were replaced and six extra probes were added, including four mutation-specific probes.
A1	First release.

Implemented changes in the product description
Version C1-03 – 07 November2023 (04P) - Product description rewritten and adapted to a new template. Version C1-02 – 13 January 2020 (02P) - Information added about denaturation control and 100 nt X chromosome and 105 nt Y chromosome control fragments not being present in this probemix. Version C1-01 – 16 October 2019 (02P) - Product description completely rewritten.

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