Product Description
SALSA® MLPA® Probemix P278-D1 PCCA-PCCB

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 7.

Catalogue numbers:
- **P278-025R:** SALSA MLPA Probemix P278 PCCA-PCCB, 25 reactions.
- **P278-050R:** SALSA MLPA Probemix P278 PCCA-PCCB, 50 reactions.
- **P278-100R:** SALSA MLPA Probemix P278 PCCA-PCCB, 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.

General information
The SALSA MLPA Probemix P278 PCCA-PCCB is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PCCA* and *PCCB* genes, which are associated with propionic acidemia.

Propionic acidemia (PA) is an autosomal recessive disorder. PA is a disease with a wide clinical spectrum, and can include growth impairment, intellectual disability, seizures, basal ganglia lesions, pancreatitis, and cardiomyopathy. Other rarely reported complications include optic atrophy, hearing loss, premature ovarian insufficiency, and chronic renal failure. The worldwide incidence of PA is estimated to be 1 in 50.000 to 100.000 live births and higher in some specific populations.

PA is caused by deficiency of the mitochondrial enzyme propionyl-CoA carboxylase (PCC) that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA. The enzyme PCC is composed of α- and β-subunits which are encoded by the *PCCA* and *PCCB* genes localized on chromosomes 13q32.3 and 3q22.3, respectively. Missense mutations are predominant in *PCC* genes. Exon deletions account for ~20% of *PCCA* disease-causing alleles, while only three large deletions in *PCCB* have been described causing PA (Chiu et al. 2014, Desviat et al. 2006, Kraus et al. 2012).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK92946.

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:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *PCCA* and *PCCB* exon numbering used in this P278-D1 PCCA-PCCB product description is the exon numbering from the NG_008768.1 and NG_008939.1 sequences, respectively. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P278-D1 PCCA-PCCB contains 51 MLPA probes with amplification products between 124 and 504 nucleotides (nt). This includes 27 probes for the *PCCA* gene, one probe for each exon and two probes for exons 1, 4, and 7, and 15 probes for the *PCCB* gene, one probe for each exon. In addition, nine 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 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 different unrelated individuals who are from families without a history of propionic acidemia. 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 NA06312 and NA22208 from the Coriell

Institute have been tested with the P278-D1 probemix at MRC Holland and can be used as positive control samples to detect heterozygous deletion of complete *PCCA* gene and heterozygous deletion of exons 13-20 of *PCCA* gene, respectively. 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 P278-D1	Expected copy number alteration
NA06312	Coriell Institute	13q32.3	*PCCA*	Heterozygous deletion of complete gene.
NA22208	Coriell Institute	13q32.3	*PCCA*	Heterozygous deletion of exons 13-20.

* 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 P278-D1 PCCA-PCCB probemix.

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 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 *PCCA* and *PCCB* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P278 PCCA-PCCB.
- 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.

PCCA and PCCB mutation database
https://databases.lovd.nl/shared/genes. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 SNVs and unusual results (e.g., a duplication of *PCCA* exons 11 and 13 but not exon 12) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P278-D1 PCCA-PCCB

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*PCCA*	*PCCB*
64-105	Control fragments – see table in probemix content section for more information			
124	Reference probe 19616-L26275	4p		
130	**PCCB probe** 21397-L29874			**Exon 10**
136	**PCCB probe** 21398-L31193			**Exon 2**
142	**PCCB probe** 21399-L29876			**Exon 12**
148	Reference probe 21413-L06969	1q		
154	**PCCA probe** 08686-L08698		**Exon 9**	
162	**PCCA probe** 08691-L29565		**Exon 14**	
166	**PCCB probe** 21400-L29877			**Exon 1**
172	**PCCA probe** 09864-L29566		**Exon 1**	
178	Reference probe 21417-L11672	4q		
185	**PCCA probe** 08692-L29568		**Exon 15**	
190	**PCCB probe** 21401-L31194			**Exon 3**
197	**PCCB probe** 21402-L29879			**Exon 7**
202	**PCCA probe** 08690-L29569		**Exon 13**	
208	**PCCA probe** 09865-L08705		**Exon 16**	
214	Reference probe 19623-L26860	10p		
220	**PCCA probe** 09868-L10283		**Exon 24**	
229	**PCCA probe** 08679-L09778		**Exon 4**	
233	**PCCA probe** 08696-L23671		**Exon 19**	
240	**PCCA probe** 21540-L29572		**Exon 23**	
248	**PCCA probe** 08677-L29570		**Exon 2**	
255	**PCCA probe** 08695-L29571		**Exon 18**	
263	**PCCB probe** 21403-L29880			**Exon 6**
267	**PCCB probe** 21404-L29881			**Exon 15**
274	**PCCA probe** 08697-L08709		**Exon 20**	
283	**PCCA probe** 08680-L08692		**Exon 4**	
290	**PCCA probe** 21105-L08700		**Exon 11**	
303	Reference probe 05697-L05139	12q		
310	**PCCA probe** 08676-L08688		**Exon 1**	
319	**PCCA probe** 21414-L12786		**Exon 10**	
329	**PCCA probe** 08694-L08706		**Exon 17**	
337	Reference probe 01659-L01241	17p		
346	**PCCA probe** 08684-L08696		**Exon 7**	
355	**PCCA probe** 08681-L08693		**Exon 5**	
364	**PCCA probe** 08685-L08697		**Exon 8**	
373	**PCCA probe** 21416-L29567		**Exon 21**	
383	**PCCA probe** 08678-L09777		**Exon 3**	
391	**PCCA probe** 21106-L29573		**Exon 12**	
400	Reference probe 13405-L14862	6q		
409	**PCCA probe** 08699-L08711		**Exon 22**	
416	**PCCB probe** 21405-L29882			**Exon 5**
424	**PCCA probe** 08682-L31196		**Exon 6**	
429	**PCCB probe** 21406-L31195			**Exon 9**
436	Reference probe 09614-L31197	20p		
445	**PCCA probe** 21415-L08695		**Exon 7**	
454	**PCCB probe** 21407-L29884			**Exon 8**
465	**PCCB probe** 21408-L29885			**Exon 11**
472	**PCCB probe** 21409-L29886			**Exon 14**
481	**PCCB probe** 21410-L29887			**Exon 4**
492	**PCCB probe** 21411-L29888			**Exon 13**
504	Reference probe 09870-L19465	2p		

[a] See section Exon numbering on page 2 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. P278-D1 probes arranged according to chromosomal location

Table 2a. *PCCA*

Length (nt)	SALSA MLPA probe	*PCCA* exon[a]	Ligation site NM_000282.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*29-31 (Exon 1)*		
172	09864-L29566	Exon 1	28-29	GCGGGGACAACA-ATGGCGGGGTTC	0.1 kb
310	08676-L08688	Exon 1	132-133	GCGGACCCTGAA-GGTGAGGAGCAA	13.7 kb
248	08677-L29570	Exon 2	162-163	AAGACAGTGCTT-AATGGTGTCCCG	8.9 kb
383	08678-L09777	Exon 3	237-238	TCTTGTTGCTAA-TAGAGGAGAAAT	0.2 kb
229	08679-L09778	Exon 4	288-289	GAAGATGGGCAT-TAAGACAGTTGC	0.1 kb
283	08680-L08692	Exon 4	53 nt after exon 4	GGTCAAGCAGAA-AAAGTGAGACAT	42.9 kb
355	08681-L08693	Exon 5	384-385	TCCCACCAGTAA-AAGCTACCTCAA	2.3 kb
424	08682-L31196	Exon 6	477-478	TTCAGAAAACAA-AGAATTTGCCAG	52.0 kb
445	21415-L08695	Exon 7	21 nt before exon 7	TTGTTATAAATT-TTGACTTGTTTT	0.1 kb
346	08684-L08696	Exon 7	561-562	GGGCGACAAGAT-TGAAAGCAAATT	26.4 kb
364	08685-L08697	Exon 8	6 nt before exon 8	CATTTCCTGCTT-TTACAGGATGCA	21.8 kb
154	08686-L08698	Exon 9	720-721	AGGCATGCGCAT-TGCTTGGGATGA	5.3 kb
319	21414-L12786	Exon 10	104 nt after exon 10	GAGTTGTGCCTT-TAGAATCTGTTG	5.8 kb
290	21105-L08700	Exon 11	892-893	CTTAATGAAAGA-GAGTGCTCAATT	4.5 kb
391	21106-L29573	Exon 12	990-991	AGAACAAGCTGT-AGCTCTTGCCAG	28.2 kb
202	08690-L29569	Exon 13	1120-1121	ACAGAATGCATT-ACTGGCCTGGAC	1.5 kb
162	08691-L29565	Exon 14	1299-1300	CCAAGAACCGTT-ACATCTACCTGG	4.2 kb
185	08692-L29568	Exon 15	1332-1333	GGACAGTGGCAT-CCAACCAGGAAG	2.7 kb
208	09865-L08705	Exon 16	1423-1424	GCACTGAAGAGA-ATGGCAGATGCA	20.8 kb
329	08694-L08706	Exon 17	1526-1527	AAGGAGACATCA-GCACTAAATTTC	9.6 kb
255	08695-L29571	Exon 18	1634-1635	TGTTTGTGGCAT-TCCAGTTAAGAG	28.3 kb
233	08696-L23671	Exon 19	1719-1720	GCTCTCAGTAAA-ATTGCATGATAA	57.1 kb
274	08697-L08709	Exon 20	1799-1800	GGTCGAAACTAA-ATGTGACCAGCA	23.6 kb
373	21416-L29567	Exon 21	1908-1909	AAACATGAGCAT-TCAGTTTCTTGG	66.2 kb
409	08699-L08711	Exon 22	1974-1975	ATTGAACAAATT-TATGCTGGAAAA	12.2 kb
240	21540-L29572	Exon 23	2098-2099	ATTTGTGTGATT-GAAGCCATGAAA	2.4 kb
220	09868-L10283	Exon 24	39 nt before exon 24	CTGGTTACTAAT-TCTTACTCTCCC	
		stop codon	*2213-2215 (Exon 24)*		

Table 2b. *PCCB*

Length (nt)	SALSA MLPA probe	*PCCB* exon[a]	Ligation site NM_000532.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*37-39 (Exon 1)*		
166	21400-L29877	Exon 1	138-139	CAGGCCACCTCT-GTTAACGAACGC	5.4 kb
136	21398-L31193	Exon 2	255-254, reverse	CCAGGGTCCAGC-AAGAGACTGATC	0.7 kb
190	21401-L31194	Exon 3	383-384	CCGAATCAATGG-AAGATTGGTTTA	3.9 kb
481	21410-L29887	Exon 4	430-429, reverse	TCCTGACAGACT-GCCTCCAAAAAC	1.5 kb
416	21405-L29882	Exon 5	539-540	ACGGATCCAAGA-AGGAGTGGAGTC	21.8 kb
263	21403-L29880	Exon 6	612-613	GTCATCCCTCAG-ATTTCTCTGATC	9.9 kb
197	21402-L29879	Exon 7	736-737	TTGTGAAGTCTG-TCACCAATGAGG	4.2 kb
454	21407-L29884	Exon 8	878-879	CCTGCCCCTGAG-CAGTCAGGACCC	3.1 kb
429	21406-L31195	Exon 9	989-988, reverse	AGTGTATGATGT-CCACCATGTTGT	15.9 kb
130	21397-L29874	Exon 10	1104-1103 , reverse	TTAGGTTGGTTG-CCAACAATTCCA	9.8 kb
465	21408-L29885	Exon 11	1213-1214	TCACTTTTGTTG-ATGTCCCTGGCT	0.3 kb

Length (nt)	SALSA MLPA probe	*PCCB* exon[a]	Ligation site NM_000532.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
142	21399-L29876	Exon 12	1306-1307	CTGAGGCAACTG-TACCCAAAGTCA	0.4 kb
492	21411-L29888	Exon 13	1376-1375, reverse	TATCACCACAAA-GGTGCTTAGAGC	1.1 kb
472	21409-L29886	Exon 14	1468-1469	AAGGGCATGAGA-ATGTGGAAGCTG	1.2 kb
267	21404-L29881	Exon 15	1613-1612, reverse	GACGTTGTACCT-TCTTGCTGGCCA	
		stop codon	*1654-1656 (Exon 15)*		

[a] See section Exon numbering on page 2 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.

References

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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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- Rivera-Barahona A, et al. (2018) Identification of 34 novel mutations in propionic acidemia: Functional characterization of missense variants and phenotype associations. *Mol Genet Metab,* 125(3), 266-275.
- Wang Y et al. (2018). A novel PCCA mutation in a patient with late-onset propionic acidemia identified by genetic diagnosis panel. *Front Pediatr*, 233.

P278 product history	
Version	*Modification*
D1	Probes for every exon of the *PCCB* gene have been added, three reference probes have been replaced and several probe lengths have been adjusted.
C2	Two reference probes have been replaced and one removed, in addition several lengths of probes have been adjusted.
C1	Two reference probes were removed and five replaced, control fragments have been adjusted (QDX2).
B2	Probe for *PCCA* exon 10 has been replaced and one reference probe has been removed.
B1	New *PCCA* exon 10 probe.
A1	First release.

Implemented changes in the product description
Version D1-02 – 25May 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting PCCA and PCCB genes 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. - New publications added using SALSA MLPA Probemix P278 PCCA-PCCB. Version D1-01 – 18 October 2018 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (product name changed, version number changed, changes in Table 1 and Table 2). - Various textual changes on page 1. Version 11 – 06 January 2017 (55) - Product description adapted to a new version (version number added, small changes in Table 1 and Table 2, new picture included). - Various minor textual changes on page 1. - Information about SNP rs76498271 removed. - Data analysis method has been modified.

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