Product Description
SALSA® MLPA® Probemix P188-C1 22q13

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 7.

Catalogue numbers:
- **P188-025R:** SALSA MLPA Probemix P188 22q13, 25 reactions.
- **P188-050R:** SALSA MLPA Probemix P188 22q13, 50 reactions.
- **P188-100R:** SALSA MLPA Probemix P188 22q13, 100 reactions.

Warning: All *SHANK3* probes are located in an extremely GC-rich region, which is more difficult to denature than the rest of the human genome. The presence of salt in DNA samples can result in incomplete denaturation of GC-rich regions, which may result in false positive results: apparent deletions of several consecutive *SHANK3* probes, while reference probes are normal. Such false positive results are even more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 system, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration). Please check the DNA denaturation control fragments (D-fragments) carefully.

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 P188 22q13 is a **research use only (RUO)** assay for the detection of deletions or duplications in the 22q12 and 22q13 chromosomal regions. A partial 22q13 deletion is associated with the Phelan-McDermid syndrome (22q13.3 deletion syndrome).

The Phelan-McDermid syndrome (PHMDS; OMIM 606232) is characterised by severe expressive language delay and mild intellectual disability. Most patients display hypotonia and normal to accelerated growth. PHMDS, caused by a deletion of 22q13.3 that includes at least part of *SHANK3* or a pathogenic variant in *SHANK3*, is inherited in an autosomal dominant manner. Most cases are however not inherited, but occur *de novo*. Haploinsufficiency of the *SHANK3* gene is likely the cause of the major neurological features associated with PHMDS. Deletion of additional genes probably causes more complex phenotypes in individuals with larger deletions. The most common cause of PHMDS are terminal chromosomal deletions (including *SHANK3*), which are variable in size: ranging from <50 kb to >9 Mb. Interstitial deletions, intragenic deletions/pathogenic variants in *SHANK3*, ring chromosomes, and unbalanced translocations have also been described as causes of PHMDS (Phelan et al. 2005)

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1198/.

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/

Probemix content
The SALSA MLPA Probemix P188-C1 22q13 contains 46 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 30 probes for the 22q13 chromosomal region and four probes for the 22q12 chromosomal region. Four of the 22q13 probes detect sequences in the *SHANK3* gene. In addition, 12 reference probes are included which detect 12 different 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 reference 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 PHMDS. 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. The quality of cell lines can change; therefore samples should be validated before use.

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

- According to Gene Reviews (https://www.ncbi.nlm.nih.gov/books/NBK1198/) <5% of PHMDS cases are the result of small (point) mutations in the *SHANK3* gene, most of which will not be detected by using SALSA MLPA Probemix P188 22q13.
- 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.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *ACR* exons 1 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P188-C1 22q13

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 19616-L26704	4p		04-042.278
136 « ¥	**EIF4ENIF1 probe** 22243-L31358		**22q12.2**	22-030.215
142 «	**SHANK3 probe** 06786-L06378		**22q13.33**	22-049.490
148	**LARGE1 probe** 06069-L06372		**22q12.3**	22-031.999
154 *	Reference probe 17907-L22212	8p		08-019.746
163 « ¥	**CDC42EP1 probe** 06072-L31359		**22q13.1**	22-036.292
170 ¥	**ARSA probe** 22244-L28185		**22q13.33**	22-049.413
175 ¥	**TAB1 probe** 06073-L31360		**22q13.1**	22-038.163
182 ¥	**GGA1 probe** 06074-L31362		**22q13.1**	22-036.335
189 ¥	**RANGAP1 probe** 22245-L31363		**22q13.2**	22-040.012
193 ¥	**ALG12 probe** 22246-L16423		**22q13.33**	22-048.683
202 *	Reference probe 03709-L03163	9q		09-097.258
208 « ¥	**CYB5R3 probe** 22247-L31364		**22q13.2**	22-041.373
216 « ¥	**SHANK3 probe** 22248-L31365		**22q13.33**	22-049.508
225 ¥	**GTSE1 probe** 06080-L31366		**22q13.31**	22-045.072
232 *	Reference probe 16429-L18882	18q		18-045.687
240 « ¥	**MAPK8IP2 probe** 22249-L28184		**22q13.33**	22-049.396
247 *	**CACNG2 probe** 22234-L31349		**22q12.3**	22-035.313
257 ¥	**RABL2B probe** 22250-L31367		**22q13.33**	22-049.553
265 *	Reference probe 14759-L16456	11q		11-118.467
274 *	**CERK probe** 22235-L31350		**22q13.31**	22-045.474
283 « *	**ACR probe** 22257-L31374		**22q13.33**	22-049.524
289 Δ *	**FOXRED2 probe** 22236-L31351		**22q12.3**	22-035.222
297 *	Reference probe 04570-L20036	16q		16-055.491
307 *	**ACR probe** 22237-L31352		**22q13.33**	22-049.525
314 « ¥	**SHANK3 probe** 06784-L31368		**22q13.33**	22-049.462
324 ¥	**RANGAP1 probe** 22252-L31369		**22q13.2**	22-039.972
330 ¥	**RABL2B probe** 06088-L31578		**22q13.33**	22-049.554
337 *	Reference probe 03264-L02701	3q		03-194.848
346 *	**TYMP probe** 22238-L31353		**22q13.33**	22-049.314
355 « *	**MLC1 probe** 06339-L14621		**22q13.33**	22-048.849
364 « *	**CARD10 probe** 22239-L31354		**22q13.1**	22-036.244
373 *	Reference probe 04278-L03682	12q		12-038.905
385 « ¥	**MLC1 probe** 22253-L31370		**22q13.33**	22-048.858
393 « *	**ACR probe** 22258-L31375		**22q13.33**	22-049.529
400 «	**SHANK3 probe** 06785-L06377		**22q13.33**	22-049.480
409 *	Reference probe 09999-L21378	20q		20-042.477
427	**EP300 probe** 12281-L14003		**22q13.2**	22-039.900
436 *	**ATXN10 probe** 22240-L31355		**22q13.31**	22-044.581
445 *	Reference probe 16286-L18578	13q		13-050.429
454 ¥	**SBF1 probe** 06099-L31372		**22q13.33**	22-049.249
463 *	**PLXNB2 probe** 22241-L31356		**22q13.33**	22-049.069
472 « *	Reference probe 09686-L10096	7q		07-150.325
481 ¥	**ARHGAP8 probe** 22255-L31373		**22q13.31**	22-043.561
494 *	**CPT1B probe** 22242-L31357		**22q13.33**	22-049.357
500 *	Reference probe 21229-L29604	10p		10-032.800

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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. 22q12 and 22q13 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
136 «	22243-L31358	*EIF4ENIF1*	CTTGACCTGAAG-AAGCCTCCTGCC	1.8 Mb
148	06069-L06372	*LARGE1*	TCCTCTGGTTCT-TGCCTGCTTCAC	3.2 Mb
289 Δ	22236-L31351	*FOXRED2*	GATACCTCCCCA-CCGGTGAGCAGG	91.5 kb
247	22234-L31349	*CACNG2*	CCAGAGGATGCA-GATTACGAAGCT	930.4 kb
364 «	22239-L31354	*CARD10*	CCACTCACCGAG-GATCATGGAGCA	48.3 kb
163 «	06072-L31359	*CDC42EP1*	GGCAAAGGAGCT-GAGCAGCCATCC	42.3 kb
182	06074-L31362	*GGA1*	TCCCTTGGGGTT-TCCATGGTGCTG	1.8 Mb
175	06073-L31360	*TAB1*	TGACTCTGGGGT-TCCTGGTTAGGA	1.7 Mb
427	12281-L14003	*EP300*	ATGCCCAATGTA-TCTAACGACCTC	71.9 kb
324	22252-L31369	*RANGAP1*	TGACTGCCCCAT-GCTTTCCCCTTT	40.5 kb
189	22245-L31363	*RANGAP1*	CCGCCATCATCC-GCCGCGGTGCGG	1.4 Mb
208 «	22247-L31364	*CYB5R3*	TGGAGTCTGCCA-GCCACACTGGGA	2.2 Mb
481	22255-L31373	*ARHGAP8*	TGCAGTGAGGAA-GAGGCCCTCGGT	1.0 Mb
436	22240-L31355	*ATXN10*	GTATCCCGTTGA-TCCTGGACAACT	490.4 kb
225	06080-L31366	*GTSE1*	CTCTCCATGGAA-GGAGGCGGCGGC	401.9 kb
274	22235-L31350	*CERK*	AAGGACAGTGAG-AAGAAACGGTGG	3.2 Mb
193	22246-L16423	*ALG12*	TCCAGCACAATT-ATGACAATTCAG	165.6 kb
355 «	06339-L14621	*MLC1*	ATGAGAATCGTG-GAGATGTTTAAG	9.0 kb
385 «	22253-L31370	*MLC1*	GCTCCTGCTGGA-GCTGCTCATGGC	211.3 kb
463	22241-L31356	*PLXNB2*	CTTGTGGAGATA-AACAAGAGAGTC	179.2 kb
454 #	06099-L31372	*SBF1*	ACTTTGTCGTCC-GTATGATGAACT	65.2 kb
346	22238-L31353	*TYMP*	GTCATCCAGAGC-CCAGAGCAGGTA	43.5 kb
494	22242-L31357	*CPT1B*	CCGGGCACTGCC-TGGGCAAACCGA	39.0 kb
240 «	22249-L28184	*MAPK8IP2*	CTGCAAACCTTA-TCCTCTATTCTT	16.9 kb
170	22244-L28185	*ARSA*	GGAGGATCAGAT-CTCCGCTCGAGA	48.6 kb
314 «	06784-L31368	*SHANK3*	AAGCGGCGAGTT-TATGCCCAGAAC	18.3 kb
400 «	06785-L06377	*SHANK3*	GAGGAGAAAGAT-CGTGACCGGGAT	10.0 kb
142 «	06786-L06378	*SHANK3*	GGTCGGACACAA-GCAGGTGGTGGC	17.3 kb
216 «	22248-L31365	*SHANK3*	ACCAACTGTGAT-CAGTGAGCTCAG	15.9 kb
283 «	22257-L31374	*ACR*	GCTACCAACTGC-CATTCTGCTGGT	1.5 kb
307	22237-L31352	*ACR*	CTCTGCAAGAGA-GATATGTGGAGA	4.3 kb
393 « #	22258-L31375	*ACR*	CCATCATCTATA-CTGATGGAGGCA	23.7 kb
257 #	22250-L31367	*RABL2B*	AATACACAAGCC-GTAAAATCGAGT	0.8 kb
330 #	06088-L31578	*RABL2B*	CTCCCACATTCT-AGGCCCGTGATA	

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. 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 or pseudogene.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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.

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

Related SALSA MLPA probemixes

P245 Microdeletion Syndromes-1A	Contains a probe for the *SHANK3* and *RABL2B* gene (22q13.33).
P250 DiGeorge	Contains a probe for the *ARSA* and *SHANK3* gene (22q13.33).
P339 SHANK3	Contains probes for the *SHANK3* gene (22q13.33) and some flanking probes.
P343 Autism-1	Contains a few probes for the *SHANK3* gene (22q13.33).

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.
- Phelan et al. (2005) [Updated 2018 Jun 7]. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1198/

Selected publications using SALSA MLPA Probemix P188 22q13

- Boccuto L et al. (2013). Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders. *Eur J of Hum Genet* 21(3):310-316.
- Bonaglia M et al. (2011). Molecular mechanisms generating and stabilizing terminal 22q13 deletions in 44 subjects with Phelan/McDermid syndrome. *PLOS Genet* 7(7):e1002173.
- Pohovski L et al. (2013). Multiplex ligation-dependent probe amplification workflow for the detection of submicroscopic chromosomal abnormalities in patients with developmental delay/intellectual disability. *Mol Cytogenetics* 6(1):7.
- Soorya L et al. (2013). Prospective investigation of autism and genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency. *Mol Autism* 4(1):18.
- Sykes N et al. (2009). Copy number variation and association analysis of SHANK3 as a candidate gene for autism in the IMGSAC collection. *Eur J Hum Genet* 17(10):1347-1353.

P188 product history	
Version	*Modification*
C1	Nine reference probes have been replaced by 12 new reference probes and 13 target probes have been replaced by 12 new target probes. 18 target probes have been changed in length, not in sequence detected.
B2	Two reference probes and the 88 and 96 nt control fragments have been replaced.
B1	Three variable probes have been replaced by one new 22q13 probe and two new reference probes. In addition, four extra control fragments at 88-96-100-105 nt have been added.
A1	First release.

Implemented changes in the product description
Version C1-02 – 15 March 2023 (04P) - Product description rewritten and adapted to a new template. - Warnings on salt-sensitivity added in Table 1 and 2. - Related SALSA MLPA probemixes section was updated. *Version C1-01 - 25 April 2019 (02P)* - Product description restructured, 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). - Updated gene name *LARGE* to *LARGE1* in Table 1 and 2. - For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36). - Warnings added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Related SALSA MLPA probemixes section was updated.

More information: www.mrcholland.com; www.mrcholland.eu	
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