Product Description
SALSA® MLPA® Probemix P310-B4 TCOF1

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, four reference probes have been replaced, and one target probe has been adjusted in length, not in the sequence detected. For complete product history see page 7.

Catalogue numbers:
- **P310-025R:** SALSA MLPA Probemix P310 TCOF1, 25 reactions.
- **P310-050R:** SALSA MLPA Probemix P310 TCOF1, 50 reactions.
- **P310-100R:** SALSA MLPA Probemix P310 TCOF1, 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 P310 TCOF1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TCOF1* gene, which is associated with Treacher Collins-Franceschetti 1 syndrome.

Treacher Collins-Franceschetti 1 syndrome is characterized by abnormal craniofacial development. Defects in the *TCOF1* gene on chromosome 5 are the main cause of this disease. This gene encodes a nuclear protein with a LIS1 homology domain. The protein is involved in ribosomal DNA gene transcription through its interaction with upstream binding factor (UBF). The protein encoded by *TCOF1* is treacle. This protein is active during early embryonic development in structures that become bones and other tissues in the face.

The *TCOF1* gene (26 exons) spans ~43 kb of genomic DNA and is located on 5q33.1, about 150 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1532/.

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 *TCOF1* exon numbering used in this P310-B4 TCOF1 product description is the exon numbering from the NG_011341.1 sequence. 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 P310-B4 TCOF1 contains 35 MLPA probes with amplification products between 136 and 436 nucleotides (nt). This includes 25 probes for the *TCOF1* gene, one probe for each exon of the gene with the exception of exons 8, 19 and 20 and a probe each for intron 6 and 16. In addition, 10 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 Treacher Collins-Franceschetti syndrome. 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

- In most populations, the major cause of genetic defects in the *TCOF1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P310 TCOF1.

- 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.

***TCOF1* mutation database**

https://databases.lovd.nl/shared/genes/TCOF1. 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 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 *TCOF1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P310-B4 TCOF1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*TCOF1*
64-105	Control fragments – see table in probemix content section for more information		
136 *	Reference probe 21700-L30358	15q	
141	**TCOF1** probe 11635-L12399		**Exon 14**
146	**TCOF1** probe 15302-L17553		**Exon 2**
153 ±	**TCOF1** probe 11636-L17554		**Exon 22**
157	**TCOF1** probe 13673-L17057		**Exon 17**
164 ±	**TCOF1** probe 11637-L17058		**Exon 26**
169	**TCOF1** probe 11638-L17060		**Exon 4**
178	**TCOF1** probe 11639-L12403		**Exon 7**
183 *	Reference probe 06432-L26377	6p	
193	**TCOF1** probe 11641-L12405		**Exon 12**
202 Ж	**TCOF1** probe 13675-SP0128-L17502		**Exon 9**
208 Ж	**TCOF1** probe 13676-SP0129-L17501		**Exon 25**
214	**TCOF1** probe 12807-L12410		**Exon 23**
221	Reference probe 13173-L14918	3q	
229	**TCOF1** probe 13677-L15142		**Intron 16**
238	Reference probe 08791-L08815	10q	
247	**TCOF1** probe 13678-L15143		**Exon 24**
256	Reference probe 08812-L08872	2p	
265 Ж	**TCOF1** probe 13679-SP0130-L15144		**Exon 10**
273	**TCOF1** probe 11648-L12412		**Intron 6**
283	**TCOF1** probe 15306-L17079		**Exon 5**
292 Ж	**TCOF1** probe 13680-SP0131-L17556		**Exon 16**
301	**TCOF1** probe 11649-L17555		**Exon 1**
310	Reference probe 09065-L15938	19p	
317	**TCOF1** probe 11651-L12415		**Exon 11**
328	**TCOF1** probe 11652-L15939		**Exon 18**
337	Reference probe 14039-L15637	7q	
346	**TCOF1** probe 11653-L15940		**Exon 3**
364 ¥	**TCOF1** probe 23050-L15146		**Exon 13**
373 *	Reference probe 21994-L30832	4p	
391 *	Reference probe 12657-L14803	16q	
409	**TCOF1** probe 11658-L12422		**Exon 15**
418 ±	**TCOF1** probe 13683-L15148		**Exon 6**
427	**TCOF1** probe 11659-L16062		**Exon 21**
436	Reference probe 18191-L22778	1q	

[a] See section Exon numbering on page 1 for more information.

* New in version B4.

¥ Changed in version B4. Minor alteration, no change in sequence detected.

± SNP rs373075669 could influence the probe signal (153 nt - 11636-L17554). SNP rs183916761 could influence the probe signal (164 nt - 11637-L17058). SNP rs77741284 could influence the probe signal (148 nt - 13683-L15148). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.

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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. *TCOF1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*TCOF1* exon[a]	Ligation site NM_000356.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*51-53 (Exon 1)*		
301	11649-L17555	Exon 1	90-91	TACTTCCCCTGA-TCTACCACCATC	3.3 kb
146	15302-L17553	Exon 2	11 nt before exon 2	GCTTTCTCTTTA-CCTCTCTGCAGA	3.0 kb
346	11653-L15940	Exon 3	270-271	CACTGCAAGCTA-AGAAAACCCGTG	3.7 kb
169	11638-L17060	Exon 4	378-379	CATCTACCAACT-CCTCAGTCCTGG	0.9 kb
283	15306-L17079	Exon 5	467-468	GGGAATTCCATG-CCACACCCTGCC	0.9 kb
418 ±	13683-L15148	Exon 6	4 nt after exon 6	ACGTGGAGGTAA-TTGCCACCCATC	2.6 kb
273	11648-L12412	Intron 6	2.0 kb before exon 7 (NM_001135243.2: 884-885)	GAGGGATCTGAA-AGTGAGGAGGAG	1.9 kb
178	11639-L12403	Exon 7	44 nt before exon 7	CATCACCAGAGA-GTTTTCACAAGC	1.0 kb
	No probe	Exon 8			
202 Ж	13675-SP0128-L17502	Exon 9	1289-1290; 8 nt after exon 9	GAGGCACTGGCA-26 nt spanning oligo-GGAAGCCGCCCT	0.1 kb
265 Ж	13679-SP0130-L15144	Exon 10	46 nt before exon 10; 10 nt before exon 10	CCTCACTCACAT-36 nt spanning oligo-TGTCTCCCAGGT	0.4 kb
317	11651-L12415	Exon 11	1525-1526	CTCACTCCAGGA-AAAGTCCTTGGG	0.6 kb
193	11641-L12405	Exon 12	1960-1961	AACCGTGGGACA-GGTGAGGCCTGT	0.3 kb
364	23050-L15146	Exon 13	12 nt after exon 13	GTGAGGCCTAGA-AGGAGCAGGCCC	2.4 kb
141	11635-L12399	Exon 14	2277-2278	CTGCTCAAGCCA-AGCAGAGGTCTC	0.2 kb
409	11658-L12422	Exon 15	19 nt before exon 15	CCTCCAATACTA-TTATCCCCCTGC	0.5 kb
292 Ж	13680-SP0131-L17556	Exon 16	4 nt after exon 16; 29 nt after exon 16	CTGCCCAGGTAA-25 nt spanning oligo-ACACTCACTCCT	4.2 kb
229	13677-L15142	Intron 16	4.0 kb before exon 17 (NM_001008657.3: 3105-3106)	TGGTGTGGTCCA-AGCTTCTGTGTG	4.1 kb
157	13673-L17057	Exon 17	2763-2764	CCCAGGCTGCAA-GCACCCCGAGGA	2.0 kb
328	11652-L15939	Exon 18	2965-2966	GTCCAGTCGGAT-ATCAGATGGCAA	2.8 kb
	No probe	Exon 19			
	No probe	Exon 20			
427	11659-L16062	Exon 21	3385-3386	GACCGCAGCAGA-GTCCAGCGAGGA	0.6 kb
153 ±	11636-L17554	Exon 22	13 nt before exon 22	CCTCTTTCACAA-TGGGCTTCTTCA	3.1 kb
214	12807-L12410	Exon 23	3781-3782	GGTCCTGACTGA-GCTGCTGGAACA	2.0 kb
247	13678-L15143	Exon 24	43 nt after exon 24	AAACAGACCCAA-ACCCAAGCCCCC	0.6 kb
208 Ж	13676-SP0129-L17501	Exon 25	16 nt after exon 25; 40 nt after exon 25	GCTTCCCAATCA-24 nt spanning oligo-ACAGCTCTGGTG	0.8 kb
164 ± #	11637-L17058	Exon 26	4427-4428	GACAGCCAGCTT-CAGGGGTCCCTG	
		stop codon	*4284-4286 (Exon 25)*		

[a] See section Exon numbering on page 1 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.

± SNP rs373075669 could influence the probe signal (153 nt - 11636-L17554). SNP rs183916761 could influence the probe signal (164 nt - 11637-L17058). SNP rs77741284 could influence the probe signal (148 nt - 13683-L15148). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.

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.

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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

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 P310 TCOF1

- Beygo J et al. (2011). First report of single exon deletion in TCOF1 causing Treacher Collins syndrome. *Mol Syndromol.* 2:53-59.
- Bowman M et al. (2012). Gross deletions in TCOF1 are a cause of Treacher-Collins-Franceschetti syndrome. *Eur J Hum Genet.* 20:769-777.
- Li X et al. (2019). Genotype-phenotype variability in Chinese cases of Treacher Collins syndrome. *Acta Otolaryngol*. 139:567-575.
- Liu J et al. (2020). Identification of a novel gross deletion of TCOF1 in a Chinese prenatal case with Treacher Collins syndrome. *Mol Genet Genomic Med*. 8:e1313.
- Schaefer E et al. (2014). Autosomal recessive POLR1D mutations with decrease of TCOF1 mRNA is responsible for Treacher Collins syndrome. *Genet Med.* 16:720-724.
- Schlump J-U et al. (2012). Treacher Collins syndrome: clinical implications for the paediatrician—a new mutation in a severely affected newborn and comparison with three further patients with the same mutation, and review of the literature. *Eur J Pediatr*. 171:1611-1618.
- Vincent M et al. (2014). Large deletions encompassing the TCOF1 and CAMK2A genes are responsible for Treacher Collins syndrome with intellectual disability. *Eur J Hum Genet.* 22:52-56.

P310 product history	
Version	*Modification*
B4	Four reference probes have been replaced. One target probe has been adjusted in length, not in the sequence detected.
B3	Two reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Four *TCOF1* probes have been removed, three new *TCOF1* probes have been included and six reference probes have been replaced.
A1	First release.

Implemented changes in the product description

Version B4-02 – 10 August 2021 (04P)
- Warning added to Table 1 and 2 for the TCOF1 probe at 164 nt (11637-L17058) on the effect of SNP rs183916761.

Version B4-01 – 23 June 2021 (04P)
- 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).
- Ligation sites of the probes targeting the *TCOF1* gene updated according to new version of the 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.
- Warnings for the effect of SNPs adjusted; rs-numbers have now been included.

Version 13 –10 November 2020 (55)
- Various minor textual or layout changes.

- Warning added to Tables 1 and 2 for *TCOF1* exon 13 probe (M13681-L15146). This probe is sensitive to certain experimental variations. Aberrant results should be treated with caution.

Version 12 – 25 October 2017 (55)

- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual and layout changes throughout the document.

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