Product Description
SALSA® MLPA® Probemix P339-B2 SHANK3

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 9.

Catalogue numbers:
- **P339-025R:** SALSA MLPA Probemix P339 SHANK3, 25 reactions.
- **P339-050R:** SALSA MLPA Probemix P339 SHANK3, 50 reactions.
- **P339-100R:** SALSA MLPA Probemix P339 SHANK3, 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 P339 SHANK3 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SHANK3* gene, which is associated with Phelan–McDermid syndrome and autism spectrum disorder (ASD).

SHANK3 is a synaptic scaffolding protein enriched in the postsynaptic density of excitatory synapses, and plays important roles in the formation, maturation, and maintenance of synapses. Haploinsufficiency of the *SHANK3* gene causes a developmental disorder, 22q13.3 deletion syndrome (known as Phelan–McDermid syndrome), which is characterized by severe expressive language and speech delay, hypotonia, global developmental delay, and autistic behaviour. Since several *SHANK3* mutations have been identified in a particular phenotypic group in patients with ASD, SHANK3 is strongly suspected of being involved in the pathogenesis and neuropathology of ASD.

Warning: All **SHANK3 *probes*** are located in an extremely GC-rich chromosomal area. Such CpG islands are more difficult to denature than the rest of the human genome. The presence of salt in DNA samples can result in incomplete denaturation of these CpG islands, 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 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration).

Most severely affected will be probes for exons 11-17; see figure 1 on page 4. In particular, any deletions that include exons 11-13 should be treated with caution (be aware that the exon numbering has been adjusted in this Product description version B2-02).

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/

Exon numbering
The *SHANK3* exon numbering used in this P339-B2 SHANK3 product description is the exon numbering from the NG_070230.1 sequence. The *SHANK3* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version B2-02 onwards, we have adopted the NG sequence exon numbering. 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 P339-B2 SHANK3 contains 44 MLPA probes with amplification products between 133 and 481 nucleotides (nt). This includes 30 probes for the *SHANK3* gene. Furthermore, four probes are added as flanking probes for the 22q13.33 region. 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 Phelan–McDermid syndrome and autism spectrum disorder. 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

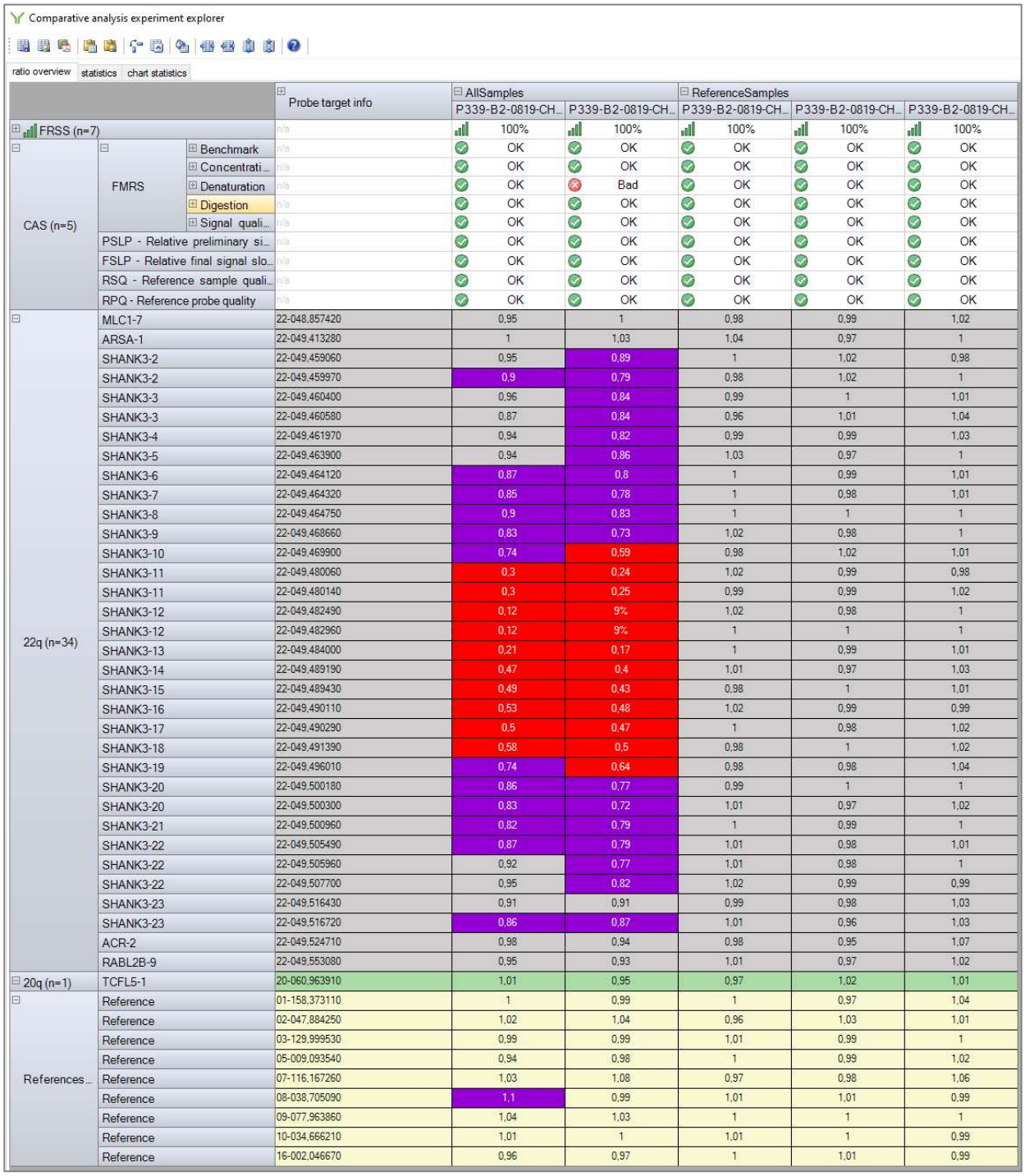
The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can 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 or in or near the *SHANK3* gene (figure 1). In case of apparent (partial) deletions please check if the 238 nt probe, located on chromosome 20q13, also gives a lower ratio, and if the control D-fragments (the 88 and 96) are going down. If this is the case the sample probably contains a high salt concentration. 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.



Comparative analysis experiment explorer

ratio overview | statistics | chart statistics

		Probe target info	AllSamples		ReferenceSamples		
			P339-B2-0819-CH...	P339-B2-0819-CH...	P339-B2-0819-CH...	P339-B2-0819-CH...	P339-B2-0819-CH...
FRSS (n=7)		n/a	100%	100%	100%	100%	100%
CAS (n=5)	FMRS - Benchmark	n/a	OK	OK	OK	OK	OK
	FMRS - Concentrati...	n/a	OK	OK	OK	OK	OK
	FMRS - Denaturation	n/a	OK	Bad	OK	OK	OK
	FMRS - Digestion	n/a	OK	OK	OK	OK	OK
	FMRS - Signal quali...	n/a	OK	OK	OK	OK	OK
	PSLP - Relative preliminary si...	n/a	OK	OK	OK	OK	OK
	FSLP - Relative final signal slo...	n/a	OK	OK	OK	OK	OK
	RSQ - Reference sample quali...	n/a	OK	OK	OK	OK	OK
	RPQ - Reference probe quality	n/a	OK	OK	OK	OK	OK
22q (n=34)	MLC1-7	22-048,857420	0,95	1	0,98	0,99	1,02
	ARSA-1	22-049,413280	1	1,03	1,04	0,97	1
	SHANK3-2	22-049,459060	0,95	0,89	1	1,02	0,98
	SHANK3-2	22-049,459970	0,9	0,79	0,98	1,02	1
	SHANK3-3	22-049,460400	0,96	0,84	0,99	1	1,01
	SHANK3-3	22-049,460580	0,87	0,84	0,96	1,01	1,04
	SHANK3-4	22-049,461970	0,94	0,82	0,99	0,99	1,03
	SHANK3-5	22-049,463900	0,94	0,86	1,03	0,97	1
	SHANK3-6	22-049,464120	0,87	0,8	1	0,99	1,01
	SHANK3-7	22-049,464320	0,85	0,78	1	0,98	1,01
	SHANK3-8	22-049,464750	0,9	0,83	1	1	1
	SHANK3-9	22-049,468660	0,83	0,73	1,02	0,98	1
	SHANK3-10	22-049,469900	0,74	0,59	0,98	1,02	1,01
	SHANK3-11	22-049,480060	0,3	0,24	1,02	0,99	0,98
	SHANK3-11	22-049,480140	0,3	0,25	0,99	0,99	1,02
	SHANK3-12	22-049,482490	0,12	9%	1,02	0,98	1
	SHANK3-12	22-049,482960	0,12	9%	1	1	1
	SHANK3-13	22-049,484000	0,21	0,17	1	0,99	1,01
	SHANK3-14	22-049,489190	0,47	0,4	1,01	0,97	1,03
	SHANK3-15	22-049,489430	0,49	0,43	0,98	1	1,01
	SHANK3-16	22-049,490110	0,53	0,48	1,02	0,99	0,99
	SHANK3-17	22-049,490290	0,5	0,47	1	0,98	1,02
	SHANK3-18	22-049,491390	0,58	0,5	0,98	1	1,02
	SHANK3-19	22-049,496010	0,74	0,64	0,98	0,98	1,04
	SHANK3-20	22-049,500180	0,86	0,77	0,99	1	1
	SHANK3-20	22-049,500300	0,83	0,72	1,01	0,97	1,02
	SHANK3-21	22-049,500960	0,82	0,79	1	0,99	1
	SHANK3-22	22-049,505490	0,87	0,79	1,01	0,98	1,01
	SHANK3-22	22-049,505960	0,92	0,77	1,01	0,98	1
	SHANK3-22	22-049,507700	0,95	0,82	1,02	0,99	0,99
	SHANK3-23	22-049,516430	0,91	0,91	0,99	0,98	1,03
	SHANK3-23	22-049,516720	0,86	0,87	1,01	0,96	1,03
	ACR-2	22-049,524710	0,98	0,94	0,98	0,95	1,07
	RABL2B-9	22-049,553080	0,95	0,93	1,01	0,97	1,02
20q (n=1)	TCFL5-1	20-060,963910	1,01	0,95	0,97	1,02	1,01
References...	Reference	01-158,373110	1	0,99	1	0,97	1,04
	Reference	02-047,884250	1,02	1,04	0,96	1,03	1,01
	Reference	03-129,999530	0,99	0,99	1,01	0,99	1
	Reference	05-009,093540	0,94	0,98	1	0,99	1,02
	Reference	07-116,167260	1,03	1,08	0,97	0,98	1,06
	Reference	08-038,705090	1,1	0,99	1,01	1,01	0,99
	Reference	09-077,963860	1,04	1,03	1	1	1
	Reference	10-034,666210	1,01	1	1,01	1	0,99
	Reference	16-002,046670	0,96	0,97	1	1,01	0,99

Figure 1. Coffalyser.Net analysis of samples contaminated with a) 30mM of salt (first column) and b) 40mM of salt (second column) compared with three reference samples. Please note that Coffalyser.Net already gives a warning of denaturation problems (low 88 and 96 nt D-fragments) at 40mM salt.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *SHANK3* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P339 SHANK3.
- 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.

***SHANK3* mutation database**

https://databases.lovd.nl/shared/genes/SHANK3. 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 *SHANK3* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P339-B2 SHANK3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*SHANK3*	Other
64-105	Control fragments – see table in probemix content section for more information			
133	Reference probe 16316-L28699	3q		
139 «	**SHANK3 probe** 14165-L15775		**Exon 11**	
144 «	**SHANK3 probe** 20465-L28701		**Exon 3**	
148 «	**SHANK3 probe** 20480-L28702		**Exon 22**	
154 «	**SHANK3 probe** 20467-L28045		**Exon 7**	
160 « Ж Δ	**SHANK3 probe** 14168-SP0145-L15778		**Exon 12**	
166 « Δ	**SHANK3 probe** 14719-L15794		**Exon 13**	
173 «	**SHANK3 probe** 14169-L17279		**Exon 2**	
178 «	**SHANK3 probe** 14170-L17280		**Exon 5**	
184 «	**SHANK3 probe** 19609-L28703		**Exon 12**	
190	Reference probe 19368-L25761	8p		
202 «	**SHANK3 probe** 20464-L28042		**Exon 2**	
208 «	**SHANK3 probe** 14721-L15799		**Exon 22**	
214 «	**SHANK3 probe** 20469-L28047		**Exon 9**	
220 « Ж	**SHANK3 probe** 14176-SP0146-L28986		**Exon 20**	
226 «	**SHANK3 probe** 20470-L28048		**Exon 11**	
233	Reference probe 19624-L26861	10p		
238 «	TCFL5 probe 10533-L28704			20q salt sensitive probe
246	Reference probe 08715-L30393	9q		
254 « Ж	**SHANK3 probe** 20468-SP0959-L28046		**Exon 8**	
260 «	**SHANK3 probe** 14181-L28987		**Exon 4**	
266 «	**SHANK3 probe** 14182-L15792		**Exon 10**	
273 «	**SHANK3 probe** 20477-L28706		**Exon 20**	
283	Reference probe 12005-L29785	2p		
298 ¬	**ARSA probe** 02707-L28707			**Upstream**
304 «	**SHANK3 probe** 14185-L15795		**Exon 23**	
310 «	**SHANK3 probe** 20479-L28988		**Exon 22**	
325	Reference probe 18454-L23630	7q		
336 « Ж	**SHANK3 probe** 20466-SP0958-L28044		**Exon 6**	
346 « Δ	**SHANK3 probe** 20475-L28053		**Exon 18**	
355 «	**SHANK3 probe** 20478-L28056		**Exon 21**	
360 «	**SHANK3 probe** 14187-L28942		**Exon 14**	
367 « Ж	**SHANK3 probe** 20473-SP0960-L28943		**Exon 16**	
375 «	**SHANK3 probe** 14718-L28944		**Exon 3**	
382 ¬	**RABL2B probe** 06734-L05558			**Downstream**
402	Reference probe 16738-L20619	16p		
409 « ¬	**MLC1 probe** 06336-L14622			**Upstream**
418 «	**SHANK3 probe** 14192-L15802		**Exon 23**	
433 «	**SHANK3 probe** 20476-L28054		**Exon 19**	
445 «	**SHANK3 probe** 20472-L28050		**Exon 15**	
454	Reference probe 18691-L02476	5p		
463 « Ж	**SHANK3 probe** 20474-SP0961-L28052		**Exon 17**	
472 ¬	**ACR probe** 05112-L04496			**Downstream**
481	Reference probe 13595-L15052	1q		

[a] See section Exon numbering on page 2 for more information.

« 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. All *SHANK3* probes are very sensitive to higher salt concentrations. In case of apparent (partial) deletions please check if the 238 nt probe, located on chromosome 20q13, also gives a lower ratio, and if the control D-fragments (the 88 and 96) are going down. If this is the case the sample probably contains a high salt concentration. Exons 11-19 are even more sensitive than the other probes for high salt concentrations. Apparent deletions of these exons should be handled with care!

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

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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. *SHANK3* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SHANK3* exon[a]	Ligation site NM_001372044.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
409 « ¬	06336-L14622	*MLC1*		CAGCGCCAACAT-TCTGGACGAAGT	555.9 kb
298 ¬	02707-L28707	*ARSA*		GGAGGATCAGAT-CTCCGCTCGAGA	45.8 kb
		start codon	*371-373 (Exon 2)*		
173 «	14169-L17279	Exon 2 (Upstream)	296 nt before exon 2	CAAACTCCCCAA-ACTAGGCTGGCG	0.9 kb
202 «	20464-L28042	Exon 2 (1)	636-637	CGTGCGCGTCGG-CATCCCGGACCT	0.4 kb
144 «	20465-L28701	Exon 3 (2)	736-735, reverse	AGCGCGTCCTGG-AGGCTGTGGTTG	0.2 kb
375 «	14718-L28944	Exon 3 (2)	51 nt after exon 3	GGTGGATCACCA-AGCCCCGTGGCG	1.4 kb
260 «	14181-L28987	Exon 4 (3)	931-932	AAGCTTCACACA-AAGGTAAAGGAT	1.9 kb
178 «	14170-L17280	Exon 5 (4)	971-972	ACGTCCAGCTGC-ATAGCACGGACA	0.2 kb
336 « Ж	20466-SP0958-L28044	Exon 6 (5)	1114-1115 and 1138-1139	AAGAATGGTGGT-24 nt spanning oligo-GATGGGCTCACT	0.2 kb
154 «	20467-L28045	Exon 7 (6)	1222-1221, reverse	TCCTTGTAGTCA-GGTGAAGCCCCC	0.4 kb
254 « Ж #	20468-SP0959-L28046	Exon 8 (7)	56 nt and 80 nt after exon 8	TATACTTGCCTC-24 nt spanning oligo-GCACACAGGTGA	3.9 kb
214 «	20469-L28047	Exon 9 (8)	1520-1521	GTGGAGCTAACA-GGGATGTCCGCA	1.2 kb
266 «	14182-L15792	Exon 10 (9)	1588-1589	AACTTTGAGCTT-GCAGAGGTTATC	10.2 kb
139 «	14165-L15775	Exon 11 (10)	1630-1631	ACCCCAGTACCA-TTCAGGGAAACC	0.1 kb
226 «	20470-L28048	Exon 11 (10)	1711-1712	CCTCTGCAGCGC-TCAGCCAGCGAT	2.4 kb
160 « Ж Δ	14168-SP0145-L15778	Exon 12 (11)	601 nt and 571 nt before exon 12	TGTCCAGGACGA-30 nt spanning oligo-CCCAGCGATCCG	0.5 kb
184 «	19609-L28703	Exon 12 (11)	2056-2057	CCGCAGGGTGAA-GGCGAGATCCCG	1.0 kb
166 « Δ	14719-L15794	Exon 13 (12)	2128-2129	TTCTGGGAGGGA-ACCGTGAAAGGC	5.2 kb
360 «	14187-L28942	Exon 14 (13)	2251-2252	CGGCACTACACA-GTGGGCTCCTAC	0.2 kb
445 «	20472-L28050	Exon 15 (14)	9 nt before exon 15	CTCCCTGCTTTC-CTTCATCAGCGA	0.7 kb
367 « Ж	20473-SP0960-L28943	Exon 16 (15)	2456-2457 and 2478-2479	GTGTGGCCTGGA-22 nt spanning oligo-CTTCCTCATCGA	0.2 kb
463 « Ж	20474-SP0961-L28052	Exon 17 (16)	2578-2577 and 2537-2536, reverse	GTCACAGACACA-41 nt spanning oligo-CACCACCTGCTT	1.1 kb
346 « Δ	20475-L28053	Exon 18 (17)	2686-2687	AAGTCCATGACA-GCTGAGCTCGAG	4.6 kb
433 «	20476-L28054	Exon 19 (18)	876 nt before exon 19	CAGAACACAGAG-GTGACCACCTTA	4.2 kb
220 « Ж	14176-SP0146-L28986	Exon 20 (19)	21 nt before exon 20 and 2732-2733	AGGCAGCTGAGA-24 nt spanning oligo-AGCTGGACGAGA	0.1 kb
273 «	20477-L28706	Exon 20 (19)	2834-2835	GGCCCACCAGTC-GGAGGATCACAC	0.7 kb
355 «	20478-L28056	Exon 21 (20)	2880-2879, reverse	GGCCTGGGAGGC-CCTGGCGTTCAA	4.5 kb
310 «	20479-L28988	Exon 22 (21)	2965-2966	GAGAAGCTGGCG-TCCCTGCTGGAA	0.5 kb
148 «	20480-L28702	Exon 22 (21)	3441-3442	CAAGCCGCAGCG-CCGCAAGAGCCC	1.7 kb
208 «	14721-L15799	Exon 22 (21)	5178-5179	CCCTGCCAAGAA-GTCGCCCATCGC	8.7 kb
304 «	14185-L15795	Exon 23 (22)	5631-5632	CTGGCTGGAGAG-CATCCACCTAGG	0.3 kb
418 «	14192-L15802	Exon 23 (22)	5920-5921	AATCGTCCTGTT-TGCTGTTGCTCG	8.0 kb

Length (nt)	SALSA MLPA probe	*SHANK3* exon[a]	Ligation site NM_001372044.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*5789-5791 (Exon 23)*		
472 ¬	05112-L04496	*ACR*		AGCTTGCTGAAT-TCACGATGGGTG	28.4 kb
382 ¬ #	06734-L05558	*RABL2B*		AATACACAAGCC-GTAAAATCGAGT	

[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.

« 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. All *SHANK3* probes are very sensitive to higher salt concentrations. In case of apparent (partial) deletions please check if the 238 nt probe, located on chromosome 20q13, also gives a lower ratio, and if the control D-fragments (the 88 and 96) are going down. If this is the case the sample probably contains a high salt concentration. Exons 11-19 are even more sensitive than the other probes for high salt concentrations. Apparent deletions of these exons should be handled with care!

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

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P188 22q13	Contains numerous probes in the 22q13 region.
P250 DiGeorge	Contains numerous probes in the 22q11 region.

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.
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Selected publications using SALSA MLPA Probemix P339 SHANK3

- Leblond CS et al (2014). Meta-analysis of SHANK Mutations in Autism Spectrum Disorders: A Gradient of Severity in Cognitive Impairments. *PLoS Genet*. 4;10(9).
- Liu C et al. (2021). Altered striatum centered brain structures in SHANK3 deficient Chinese children with genotype and phenotype profiling. *Prog Neurobiol*, 200, 101985.
- Macedoni-Lukšič M et al (2013). Deletion of the last exon of SHANK3 gene produces the full Phelan-McDermid phenotype: a case report. *Gene*. 524:386-9.
- Meguid NA et al. (2020). Copy number variations of SHANK3 and related sensory profiles in Egyptian children with autism spectrum disorder. *Res Autism Spectr Disord*, 75, 101558.
- Nevado J et al. (2022). Variability in phelan-McDermid syndrome in a cohort of 210 individuals. *Front Genet*, 13.
- Shin S et al (2015). Routine chromosomal microarray analysis is necessary in Korean patients with unexplained developmental delay/mental retardation/autism spectrum disorder. *Ann Lab Med*. 35:510-8.

P339 product history	
Version	*Modification*
B2	Five reference probes have been replaced and two reference probes have been removed.
B1	A major part of the content of the probemix has been redesigned.
A1	First release.

Implemented changes in the product description
Version B2-02 – 24 January 2023 (04P) - Product description rewritten and adapted to a new template. - Exon numbering of the SHANK3 gene has been changed. - Figure 1 has been updated. - Data analysis method has been changed. - Ligation sites of the probes targeting the SHANK3 gene updated according to new version of the NM_ reference sequence. Version B2-01 — 7 October 2019 (02P) - 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). - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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