Product Description
SALSA® MLPA® Probemix P410-A2 GRIN2A-GRIN2B

To be used with the MLPA General Protocol.

Version A2
Compared to version A1, four reference probes have been replaced and one reference probe has been added. For complete product history see page 7.

Catalogue numbers:
- **P410-025R:** SALSA MLPA Probemix P410 GRIN2A-GRIN2B, 25 reactions.
- **P410-050R:** SALSA MLPA Probemix P410 GRIN2A-GRIN2B, 50 reactions.
- **P410-100R:** SALSA MLPA Probemix P410 GRIN2A-GRIN2B, 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 P410 GRIN2A-GRIN2B is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GRIN2A* and *GRIN2B* genes, which are associated with neurodevelopmental disorders.

N-methyl-D-aspartate (NMDA) receptors are glutamate-activated ion channels found at excitatory synapses throughout the brain. They play an important role in various processes in the brain such as excitatory neurotransmission and synaptic plasticity. NMDA receptors are composed of multiple subunits including at least one NR1 subunit, one or more NR2 subunits and occasionally an NR3 subunit. The NR2 subunits are encoded by the *GRIN2A-D* genes. NR2 subunits are expressed differentially across various cell types and are important for the control of electrophysiological properties of the NMDA receptor. Genetic variation in either the *GRIN2A* or *GRIN2B* genes has been implicated in various neurodevelopmental disorders, such as epilepsy, intellectual disability and encephalopathy.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK385627 and https://www.ncbi.nlm.nih.gov/books/NBK501979.

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 *GRIN2A* and *GRIN2B* exon numbering used in this P410-A2 GRIN2A-GRIN2B product description is the exon numbering from the NG_011812.2 and NG_031854.2 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 P410-A2 GRIN2A-GRIN2B contains 43 MLPA probes with amplification products between 130 and 463 nucleotides (nt). This includes 17 probes for the *GRIN2A* gene and 16 probes for the *GRIN2B* gene, ten 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 neurodevelopmental disorders. 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 *GRIN2A* and *GRIN2B* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P410 GRIN2A-GRIN2B.
- 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.

***GRIN2A* and *GRIN2B* mutation database**

https://databases.lovd.nl/shared/genes/GRIN2A and https://databases.lovd.nl/shared/genes/GRIN2B. 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 *GRIN2A* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P410-A2 GRIN2A-GRIN2B

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*GRIN2A*	*GRIN2B*
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 19616-L26704	4p		
136	**GRIN2B probe** 17433-L21189			**Exon 10**
142	**GRIN2A probe** 17434-L21499		**Exon 5**	
148	**GRIN2B probe** 17435-L21191			**Exon 11**
154	Reference probe 09745-L10153	2q		
160	**GRIN2A probe** 17436-L21192		**Exon 11**	
166 Ж	**GRIN2B probe** 17437-SP0503-L21193			**Exon 9**
173 Ж	**GRIN2A probe** 17438-SP0504-L21194		**Exon 13**	
178 Ж	**GRIN2B probe** 17439-SP0505-L21195			**Exon 4**
190 *	Reference probe 10710-L31035	6p		
197 Ж	**GRIN2B probe** 17441-SP0506-L21197			**Exon 5**
203	**GRIN2A probe** 17442-L21500		**Exon 10**	
208	**GRIN2A probe** 17443-L21501		**Exon 6**	
214	Reference probe 10338-L22006	11q		
220	**GRIN2A probe** 17444-L21200		**Exon 14**	
226	**GRIN2B probe** 17445-L21201			**Exon 3**
234 Ж	**GRIN2A probe** 17446-SP0507-L21502		**Exon 5**	
241 Ж	**GRIN2B probe** 17447-SP0508-L22007			**Exon 4**
247	Reference probe 05988-L05413	20p		
256	**GRIN2A probe** 17448-L21204		**Exon 12**	
265 Ж	**GRIN2B probe** 17449-SP0509-L21205			**Exon 7**
276	**GRIN2A probe** 17450-L21504		**Exon 14**	
283	**GRIN2A probe** 17451-L21505		**Exon 3**	
292	Reference probe 13325-L14751	18q		
301	**GRIN2B probe** 17452-L21208			**Exon 1**
310	**GRIN2A probe** 17453-L21209		**Exon 7**	
319	**GRIN2B probe** 17454-L21210			**Exon 3**
328	**GRIN2A probe** 17455-L21211		**Exon 8**	
337	Reference probe 03264-L02701	3q		
346	**GRIN2B probe** 17456-L21506			**Exon 8**
355	**GRIN2A probe** 17457-L21213		**Exon 4**	
364	**GRIN2B probe** 17458-L21508			**Exon 2**
373	**GRIN2A probe** 17459-L21509		**Exon 1**	
385 *	Reference probe 12451-L32242	22q		
390 Ж	**GRIN2B probe** 17460-SP0510-L21216			**Exon 1**
400	**GRIN2A probe** 17461-L21217		**Exon 4**	
409	**GRIN2B probe** 17462-L21218			**Exon 12**
419	**GRIN2A probe** 17463-L21219		**Exon 9**	
427 *	Reference probe 17156-L20348	1p		
436	**GRIN2B probe** 17464-L21220			**Exon 2**
444 Ж	**GRIN2A probe** 17465-SP0511-L21221		**Exon 3**	
454	**GRIN2B probe** 17466-L21222			**Exon 13**
463 *	Reference probe 13155-L14444	7q		

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

* New in version A2.

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

Table 2. P410-A2 probes arranged according to chromosomal location

Table 2a. *GRIN2A*

Length (nt)	SALSA MLPA probe	*GRIN2A* exon[a]	Ligation site NM_000833.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*465-467 (Exon 3)*		
373	17459-L21509	Exon 1	178-179	GGTGAGCGCTGA-GAATCGCGGCCG	2.3 kb
	No Probe	Exon 2			
283	17451-L21505	Exon 3	481-482	CAGAGTGGGCTA-TTGGACCCTGCT	0.2 kb
444 Ж	17465-SP0511-L21221	Exon 3	685-686 & 712-713	AGCTCTGCTGAT-27 nt spanning oligo-CACGCACGTGTG	241.9 kb
400	17461-L21217	Exon 4	1164-1165	ACTGTTCCAAAG-ACGAGGCTGTTC	0.2 kb
355	17457-L21213	Exon 4	1396-1397	GGAGAAGTTCTC-CTACATCCCCGA	47.0 kb
142	17434-L21499	Exon 5	1501-1502	ATGGGATGGCAA-AGACTTATCCTT	0.1 kb
234 Ж	17446-SP0507-L21502	Exon 5	28 nt & 55 nt after exon 5	GTGCTGAATGCA-27 nt spanning oligo-AGGGGCTCACAG	41.0 kb
208	17443-L21501	Exon 6	1624-1625	GAGCCTGAGGCA-CGCCGTGTGGCC	8.9 kb
310	17453-L21209	Exon 7	1819-1820	GGGGATGAATGT-GAAGAAATGCTG	0.3 kb
328	17455-L21211	Exon 8	1981-1982	TCAACGGGCAGT-CATGGCAGTTGG	6.5 kb
419	17463-L21219	Exon 9	2117-2118	TTATTTGCAGAA-CCATTCAGCGCC	4.6 kb
203	17442-L21500	Exon 10	3 nt before exon 10	TTTCATCTTCTG-CAGCACCCCATG	7.4 kb
160	17436-L21192	Exon 11	2596-2597	ATTTAATCAGAA-AGGAGTAGAGGA	23.9 kb
256	17448-L21204	Exon 12	2727-2728	GGAGTGGGTACA-TCTTTGCCACCA	29.3 kb
173 Ж	17438-SP0504-L21194	Exon 13	24 nt before exon 13 & 2827-2828	GGTCCTCACTGC-31 nt spanning oligo-GGAGGAGCTGGA	4.6 kb
220	17444-L21200	Exon 14	3526-3527	CGTGGATTCCAT-ACGCCAGGATTC	4.7 kb
276	17450-L21504	Exon 14	8256-8257	TGCAGGATTATA-ATCTCACAATCT	
		stop codon	*4857-4859 (Exon 14)*		

Table 2b. *GRIN2B*

Length (nt)	SALSA MLPA probe	*GRIN2B* exon[a]	Ligation site NM_000834.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*448-450 (Exon 2)*		
301	17452-L21208	Exon 1	164-165	CATGCACACGGA-ATAATTACTCTG	0.2 kb
390 Ж	17460-SP0510-L21216	Exon 1	343-344 & 370-371	CTTATGTGGATA-27 nt spanning oligo-TCCCAACATGCT	113.9 kb
364	17458-L21508	Exon 2	608-609	CAAGGATGCCCA-CGAGAAAGATGA	0.1 kb
436	17464-L21220	Exon 2	708-709	CGCATCTGTGAT-CTCATGTCTGAC	112.2 kb
226	17445-L21201	Exon 3	1029-1030	GAGAATAGCTTT-GTGGGCTGGGAG	0.4 kb
319	17454-L21210	Exon 3	1401-1402	CCTGAGCCCAAA-AGCAGTTGTTAC	77.6 kb
178 Ж	17439-SP0505-L21195	Exon 4	1489-1490 & 1522-1523	TTGAGGGGAGGA-33 nt spanning oligo-ACCCGAAACTGG	0.2 kb
241 Ж	17447-SP0508-L22007	Exon 4	88 nt & 118 nt after exon 4	GTCCTTTGGCTT-30 nt spanning oligo-ATACCTTGATAC	59.1 kb
197 Ж	17441-SP0506-L21197	Exon 5	1674-1675 & 1701-1702	CTGAGCATTGTG-27 nt spanning oligo-GTGGAAAGTGTG	1.4 kb
	No Probe	Exon 6			
265 Ж	17449-SP0509-L21205	Exon 7	2043-2044 & 2070-2071	TTCATAGAGACA-27 nt spanning oligo-AATGGGACTGTC	3.4 kb
346	17456-L21506	Exon 8	2209-2210	GTTATAACAGGT-GCCTCGCTGATG	3.1 kb
166 Ж	17437-SP0503-L21193	Exon 9	2356-2357 & 2388-2389	CAGTGTGGGCCT-32 nt spanning oligo-GCCAACTTAGCT	36.7 kb
136	17433-L21189	Exon 10	2481-2482	AATGACTTCTCA-CCCCCTTTCCGC	1.9 kb

Length (nt)	SALSA MLPA probe	*GRIN2B* exon[a]	Ligation site NM_000834.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
148	17435-L21191	Exon 11	2621-2622	CCTGTACAGGAA-ACTGGATGCCTT	3.0 kb
409	17462-L21218	Exon 12	3017-3018	CTGTTCTGGCAA-GCCTGGCATGGT	2.4 kb
454	17466-L21222	Exon 13	3063-3064	TACAGCTGCATC-CATGGGGTGGCG	
		stop codon	*4900-4902 (Exon 13)*		

[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.

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. 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.

P410 product history	
Version	*Modification*
A2	Four reference probes have been replaced and one reference probe has been added.
A1	First release.

Implemented changes in the product description
Version A2-02 –20 November 2024 (04P) - The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate. Version A2-01 – 10 August 2022 (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 *GRIN2A* gene updated according to new version of the NM_ reference sequence.

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