Product Description
SALSA® MLPA® Probemix P137-C1 SCN1A

To be used with the MLPA General Protocol.

Version C1
As compared to version B3, probes for *SCN1A* exon 3 and 15 and two reference probes have been replaced. For complete product history see page 10.

Catalogue numbers:
- **P137-025R:** SALSA MLPA Probemix P137 SCN1A, 25 reactions.
- **P137-050R:** SALSA MLPA Probemix P137 SCN1A, 50 reactions.
- **P137-100R:** SALSA MLPA Probemix P137 SCN1A, 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.

Intended purpose
The SALSA MLPA Probemix P137 SCN1A is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in *SCN1A* gene in genomic DNA isolated from human peripheral whole blood specimens. P137 SCN1A is intended to confirm a potential cause for and clinical diagnosis of Dravet syndrome (DS) and other *SCN1A*-related seizure disorders, and for Familial hemiplegic migraine 3 (FHM3).

Copy number variations (CNVs) detected with P137 SCN1A should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *SCN1A* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for In Vitro Diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
SCN1A-related seizure disorders include at the severe end of the spectrum DS and intractable childhood epilepsy with generalized tonic-clonic seizures (ICE-GTC), and at the mild end simple febrile seizures (FS) and generalized epilepsy with febrile seizures plus (GEFS+). Myoclonic-astatic epilepsy (MAE or Doose syndrome),

Lennox-Gastaut syndrome (LGS), infantile spasms, and vaccine-related encephalopathy and seizures are the less commonly observed phenotypes.

SCN1A-related seizure disorders are inherited in an autosomal dominant manner. Pathogenic variants may be inherited or *de novo*. The percentage of cases with *de novo* mutations increases as the severity of the phenotype increases. The *SCN1A*-related seizures phenotype varies even among family members with the same pathogenic variant.

DS (OMIM # 607208, also known as severe myoclonic epilepsy in infancy (SMEI), early infantile epileptic encephalopathy 6 (EIEE6), and polymorphic myoclonic epilepsy in infancy (PMEI)) is a rare lifelong form of epilepsy that begins in the first year of life. DS is associated with heterozygous mutations in the *SCN1A* gene. The frequency of *SCN1A* mutations in DS patients is approximately 70-80%, most of the mutations being *de novo* (Marini et al. 2009). Apparent *de novo* sporadic mutations may also be the result of germline mosaicism in apparently unaffected parents (de Lange et al. 2018). Somatic mosaic deletions of the *SCN1A* gene have also been identified in individuals with DS (Nakayama et al. 2018). DS is defined by prolonged and frequent seizures that do not remit, and normally evolve to include myoclonic seizures. Other symptoms of DS are behavioural and developmental delay, movement and balance problems, hyperactivity and sleep difficulties.

ICE-GTC is characterized by generalized seizures including absence seizures and generalized tonic-clonic seizures with onset in infancy or childhood. Children with frequent generalized tonic-clonic seizures often develop cognitive impairment. FS are characterized by childhood seizures that occur only in association with fever (higher than 38°C). The age of onset is 6 months, and the seizures resolve by the age of 5 years old. Generalized epilepsy with febrile seizures plus (GEFS+) refers to findings in a family instead of an individual. The seizure phenotypes tend toward the mild end of the spectrum. Affected individuals within a family with GEFS+ often have FS in early childhood, followed by occasional tonic, clonic, myoclonic, or absence seizures which respond to medication and remit by late childhood or early adolescence.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1318/.

Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura. FHM3 is caused by mutations in the *SCN1A* gene. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1388/.

Gene structure
The *SCN1A* gene spans ~143 kb on chromosome 2q24.3 and contains 26 exons. The *SCN1A* LRG_8 is available at http://www.lrg-sequence.org/ and is identical to GenBank NG_011906.1.

Transcript variants
For *SCN1A*, multiple transcript variants have been described. Transcript variant 2 is the most predominant and encodes isoform 2 (NM_006920.6; 13079 nt; coding sequence 479-6475; https://www.ncbi.nlm.nih.gov/gene/6323). *SCN1A* transcript variant 4 (NM_001202435.3) contains an alternate 5' untranslated exon 1 (exon hA) compared to variant 2.

Exon numbering
The *SCN1A* exon numbering used in this P137-C1 SCN1A product description is the exon numbering from the LRG_8 sequence, which uses the RefSeq transcript NM_006920.4, and has 26 exons. NM_006920.6 is available at NCBI, and has the same 26 coding exons and three additional non-coding exons at the beginning of the *SCN1A* gene. 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 LRG 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 P137-C1 SCN1A contains 40 MLPA probes with amplification products between 142 and 454 nucleotides (nt). This includes 30 probes for the *SCN1A* gene, covering all 26 coding exons of

SCN1A, the first non-coding exon of NM_006920.6 (exon hB), and the upstream region of *SCN1A* (exon hA; exon 1 of the transcript variant 4 (NM_001202435.3)). In addition, 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 from human peripheral blood, 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 *SCN1A*-Related Seizure Disorders or FHM3. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA10401 and NA10607 from the Coriell Institute have been tested with this P137-C1 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID number	Source	Altered target genes in P137-C1	Expected copy number alteration
NA10401	Coriell Institute	*SCN1A*	Heterozygous duplication of *SCN1A*, including the upstream exons
NA10607	Coriell Institute	*SCN1A*	Heterozygous deletion of *SCN1A*, including the upstream exons

Performance characteristics

Approximately 2-3% of all mutations in DS are expected to be deletions/duplications (Marini et al. 2009), which can be detected with the P137-C1 probemix. In *SCN1A*-point mutation negative patients this percentage is approximately 16% (Madia et al. 2006, Wang et al. 2008, https://www.ncbi.nlm.nih.gov/books/NBK1318/). The percentage of deletions/duplications for other *SCN1A*-related seizures disorders and FHM3 is unknown. However, the association between these diseases and the *SCN1A* gene is well established. Analytical performance for the detection of deletions and duplications in the *SCN1A* gene is very high and can be considered >99% (based on a 2006-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the *SCN1A* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). Copy numbers of 4 (heterozygous triplication/homozygous duplication) or 0 (homozygous deletion) may occur, but are extremely rare.

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 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.

P137 specific notes:
- According to the updated NM_006920.6 sequence, three non-coding exons are described in the upstream region of the *SCN1A* gene. The P137-C1 probemix has two probes termed as exon hB, which correspond to exon 1 of NM_006920.6, and two probes termed as exon hA targeting the upstream region (exon 1 of the transcript variant 4 (NM_001202435.3)). The clinical significance of mutations/copy number changes in these non-coding exons is not fully known. However, microdeletions in exons hA and hB have been found in two DS patients, and a point mutation in exon hA has been described in a patient with partial epilepsy with antecedent febrile seizures (Gao et al. 2017, Nakayama et al. 2010).
- The *SCN1A* gene is located in a complicated 2q24 region, since several highly homologous genes are present in this region (*SCN2A*, *SCN3A*, *SCN7A* and *SCN9A*). In rare cases, apparent duplications might therefore be due to sequence changes in the other similar genes.
- Mosaicism has been reported in individuals with DS (de Lange et al. 2018, Nakayama et al. 2018). Mosaic *SCN1A* deletions obtained with the P137 SCN1A probemix must be confirmed by analysis of a second, independently collected DNA sample or a different technique, in order to exclude a false positive mosaic result.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *SCN1A* are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P137 SCN1A.
- 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.

***SCN1A* mutation database**

https://databases.lovd.nl/shared/genes/SCN1A. We strongly encourage users to deposit positive results in the LOVD 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 *SCN1A* exons 7 and 9 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P137-C1 SCN1A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SCN1A*
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 09258-L11422	7q	
149	**SCN1A probe** 04540-L03929		**Exon 19**
154	**SCN1A probe** 04531-L05030		**Exon 10**
160	**SCN1A probe** 04523-L18517		**Exon 1**
166	**SCN1A probe** 04524-L03913		**Exon 2**
172 ±	**SCN1A probe** 04541-L03930		**Exon 20**
178	**SCN1A probe** 04532-L03921		**Exon 11**
183	**SCN1A probe** 15940-L18066		**Exon hA**
190	Reference probe 06743-L06347	8q	
196 *	**SCN1A probe** 22541-L31721		**Exon 3**
202	**SCN1A probe** 04542-L03931		**Exon 21**
211	**SCN1A probe** 04533-L03922		**Exon 12**
221	Reference probe 07223-L21127	3p	
229	**SCN1A probe** 04526-L03915		**Exon 4**
236	**SCN1A probe** 15941-L18067		**Exon hA**
243	**SCN1A probe** 04543-L18518		**Exon 22**
250	**SCN1A probe** 04534-L30856		**Exon 13**
256 *	Reference probe 10808-L27953	4q	
265	**SCN1A probe** 15942-L18068		**Exon hB**
274	**SCN1A probe** 04527-L04899		**Exon 5**
280	**SCN1A probe** 04544-L04900		**Exon 23**
292	**SCN1A probe** 04535-L03924		**Exon 14**
301	Reference probe 08313-L08182	11q	
310	**SCN1A probe** 04528-L03917		**Exon 6**
319	**SCN1A probe** 04545-L18535		**Exon 24**
328 *	**SCN1A probe** 22542-L31769		**Exon 15**
337	**SCN1A probe** 07367-L07014		**Exon 9**
346 *	Reference probe 05982-L05407	20p	
355	**SCN1A probe** 04529-L03918		**Exon 7**
364	**SCN1A probe** 17380-L04745		**Exon 25**
373	**SCN1A probe** 04537-L03926		**Exon 16**
382	Reference probe 04278-L23577	12q	
391	**SCN1A probe** 04530-L03919		**Exon 8**
400	**SCN1A probe** 04538-L03927		**Exon 17**
409	**SCN1A probe** 15943-L18069		**Exon hB**
418	Reference probe 11057-L11726	14q	
427	**SCN1A probe** 04539-L03928		**Exon 18**
436	Reference probe 14775-L16472	1q	
445	**SCN1A probe** 04547-L18519		**Exon 26**
454	Reference probe 14954-L16687	6q	

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

* New in version C1.

± SNP rs149579028 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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. *SCN1A* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SCN1A* exon[a]	Ligation site NM_006920.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*479-481*		
236 ◑	15941-L18067	Exon hA	14-15 in NM_001202435.3	GTGTAGGAGACA-CACTGCTGGCCT	0.1 kb
183 ◑	15940-L18066	Exon hA	108-107 reverse in NM_001202435.3	TTACCCCCAAAA-GATGTAGTAAAA	21.1 kb
409 ◑	15943-L18069	Exon hB	37-38	CCGAGAGGATAC-TGCAGAGGTCTC	0.2 kb
265 ◑	15942-L18068	Exon hB	218-219	GATGCTGTTCCT-CACTGCAGATGG	54.3 kb
160	04523-L18517	Exon 1	565-566	GAAAGACGCATT-GCAGAAGAAAAG	14.9 kb
166	04524-L03913	Exon 2	779-780	AGGCCATCTTCC-GGTTCAGTGCCA	2.2 kb
196 #	22541-L31721	Exon 3	916-915 reverse	GGGTTACTCATT-GTCATAAACACA	1.8 kb
229	04526-L03915	Exon 4	1037-1038	TTACTTTCCTTC-GGGATCCATGGA	1.7 kb
274	04527-L04899	Exon 5	1090-1091	AGGTACGTCACA-GAGTTTGTGGAC	1.0 kb
310 #	04528-L03917	Exon 6	1266-1267	CGTATTTGCTCT-AATTGGGCTGCA	3.0 kb
355	04529-L03918	Exon 7	1470-1471	GGAGGGTTTTTT-AGATGCACTACT	1.2 kb
391	04530-L03919	Exon 8	1546-1547	AAAGCTGGTAGA-AATCCCAATTAT	1.0 kb
337	07367-L07014	Exon 9	1848-1849	GCAACAGGAGGC-AGCTCAGGTAAA	1.5 kb
154	04531-L05030	Exon 10	1903-1904	GAGCCCAGTGCA-GCAGGCAGGCTC	1.4 kb
178	04532-L03921	Exon 11	2293-2294	GAGAGCCGTAGA-GATTCCTTGTTT	1.5 kb
211	04533-L03922	Exon 12	2517-2518	TGAAATGAGAAA-GAGAAGGTCAAG	1.0 kb
250	04534-L30856	Exon 13	2702-2703	ACTGTTCTCCAT-ATTGGTTAAAAG	1.9 kb
292	04535-L03924	Exon 14	2988-2989	GGTAGAACTTGG-ACTCGCCAATGT	1.4 kb
328	22542-L31769	Exon 15	3121-3120 reverse	ACGAGGGTTAAA-TTTCCCAGAGCC	1.9 kb
373	04537-L03926	Exon 16	3795-3796	GACTGTACCAAT-TGCTGTAGGAGA	20.5 kb
400	04538-L03927	Exon 17	3948-3949	AGAACAGCCCGT-AGTGGAACCTGA	1.9 kb
427	04539-L03928	Exon 18	4079-4080	GAAGGACGTGTT-TCCGAATAGTTG	1.7 kb
149 #	04540-L03929	Exon 19	4274-4275	CATATGGCTATC-AAACATATTTCA	2.4 kb
172 ±	04541-L03930	Exon 20	4393-4394	AAATCTCTCAGG-ACACTAAGAGCT	7.2 kb
202	04542-L03931	Exon 21	4588-4589	AACACCACAACT-GGTGACAGGTTT	2.9 kb
243	04543-L18518	Exon 22	4737-4738	ATAGGCCACATT-CAAAGGATGGAT	1.6 kb
280	04544-L04900	Exon 23	4825-4826	CTGTACATGTAT-CTTTACTTTGTT	2.1 kb
319	04545-L18535	Exon 24	5005-5006	AAAAAACCGCAA-AAGCCTATACCT	1.8 kb
364	17380-L04745	Exon 25	5178-5179	ACGCATCAATCT-GGTGTTCATTGT	2.5 kb
445	04547-L18519	Exon 26	5970-5971	TCTCAATCTGCC-ACAACCAAACAA	
		stop codon	*6473-6475*		

[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.

◑ The significance of deletions/duplications of exons hA and hB is not clear as these exons are non-coding. However, microdeletions in exons hA and hB have been found in two DS patients, and a point mutation in exon hA has been described in a patient with partial epilepsy with antecedent febrile seizures (Gao et al. 2017, Nakayama et al. 2010).

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.

± SNP rs149579028 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

Related SALSA MLPA probemixes

P138 SLC2A1-STXBP1	GLUT1 deficiency syndrome, *STXBP1*-Encephalopathy with epilepsy, Ohtahara syndrome, contains probes for the *SLC2A1* and *STXBP1* genes.
P166 KCNQ2	Benign familial neonatal convulsions, contains probes for the *KCNQ2* gene.
P197 KCNQ3	Familial neonatal convulsions type 2, Epilepsy benign neonatal type 2, contains probes for the *KCNQ3* gene.

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Selected publications using SALSA MLPA Probemix P137 SCN1A

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P137 product history	
Version	*Modification*
C1	Probes for SCN1A exon 3 and 15 and two reference probes have been replaced.
B3	Two reference probes have been replaced and two reference probes have been removed. In addition, several probe lengths have been adjusted.
B2	The 88 and 96 control fragments have been replaced (QDX2).
B1	Four probes for two new upstream exons have been included. In addition, several reference probes have been replaced.
A2	DNA denaturation control fragments (D-fragments) and X-, and Y- specific control fragments have been included.
A1	A probe for *SCN1A* exon 9 has been added. One control probe has been replaced.
A	First release.

Implemented changes in the product description
Version C1-02 – 03 November 2021 (04P) - Product description rewritten and adapted to a new template. - Sections Intended purpose, Gene structure, *SCN1A* Mutation database, Related SALSA MLPA probemixes and Selected publications using SALSA MLPA Probemix P137 SCN1A have been updated. - UK has been added to the list of countries in Europe that accept the CE mark. Version C1-01 – 19 November 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).

- Ligation sites of the probes targeting the *SCN1A* gene updated according to new versions of the NM reference sequences.
- P137-C1 is now CE marked.

Version B3-03 – 09 July 2019 (01P)
- Warning in Table to and 2 for More variable probe was updated. Aberrant results should be treated with caution was replaced by Results should be treated with caution.

Version B3-02 – 18 January 2019 (01P)
- The sections general information and positive control DNA samples were updated.
- Ligation sites in Table 2 adjusted according to the updated NM sequence.
- Warning added to Table 1 and 2 for SNP rs149579028 that influences the probe signal: 172 nt probe 04541-L03930.
- Warnings added for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene to interpretation of results section and Table 2.
- Warning added to Table 1 and Table 2 for more variable probe, 328 nt probe 04536-L03925.
- Several references using the P137 probemix have been removed and added.
- Various minor textual changes throughout the document.

Version B3-01 – 08 March 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual or layout changes.

Version 15 – 11 January 2017 (55)
- Warning added in Table 1, 383 nt probe 03091-L07348.

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.