Product Description
SALSA® MLPA® Probemix P114-C1 Long-QT

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 11.

Catalogue numbers:
- **P114-025R:** SALSA MLPA Probemix P114 Long-QT, 25 reactions.
- **P114-050R:** SALSA MLPA Probemix P114 Long-QT, 50 reactions.
- **P114-100R:** SALSA MLPA Probemix P114 Long-QT, 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 P114 Long-QT is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and *KCNJ2* genes in order to confirm a potential cause for and clinical diagnosis of congenital Long-QT syndrome (LQTS) types, 1, 2, 5, 6 and 7, respectively. In addition, this probemix can be used to confirm a potential cause for and clinical diagnosis of Jervell Lange-Nielsen syndrome (JLNS); a recessive form of LQTS associated with mutations in *KCNQ1* or *KCNE1*. P114 Long-QT can also be used for molecular genetic testing of at-risk family members. This assay is for use with human DNA extracted from peripheral whole blood specimens.

Copy number variations (CNVs) detected with P114 Long-QT 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 in *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and *KCNJ2* genes 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, newborn or population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations. This assay is not for use with DNA extracted from dried blood spots.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description.
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background

Congenital Long-QT Syndrome (LQTS) is a hereditary disease that predisposes patients to cardiac arrhythmias, which can result in recurrent syncopes, seizure and sudden death. LQTS patients are electrocardiographically characterized by a prolonged QT interval resulting in a predisposition to develop the ventricular tachycardia *torsade de pointes*. The cumulative mortality is 6-8% before the age of 40, and therefore it is a leading cause of sudden death in young people. LQTS occurs in an estimated 1:2500 live births and is generally caused by mutations in cardiac sodium or potassium channel genes which result in the prolongation of the ventricular action potential. LQTS can be diagnosed based on prolonged QT intervals and/or abnormal T-waves in an ECG. There are presently 15 types of LQTS known, each linked to a distinct gene. The most common causes of LQTS are mutations in the genes *KCNQ1*, *KCNH2*, and *SCN5A*. Generally, LQTS is inherited in an autosomal dominant fashion but some exceptions are discussed below. LQTS is also referred to as Romano-Ward syndrome (RWS).

40-55% of LQTS patients have type 1; an autosomal dominant disease caused by defects in the *KCNQ1* gene. Mutations in *KCNQ1* are not only associated with long QT syndrome: homozygous or compound heterozygous mutations in *KCNQ1* are associated with the recessive disorder Jervell and Lange-Nielsen syndrome (JLNS). Patients with JLNS present a much more severe phenotype. They have more extended long-QT intervals and suffer from sensorineural hearing loss. 50% of patients with this syndrome have cardiac events before the age of three and more than half of untreated children die before the age of 15.

30-45% of LQTS patients have LQTS type 2, which is also autosomal dominant and caused by defects in the *KCNH2* gene. <1% of LQTS patients have type 5 which is associated with *KCNE1*; and ~1% have type 6, which is associated with *KCNE2*. Both types are autosomal dominant traits. *KCNE1* and *KCNE2* are located closely together on chromosome 21q22. As of yet, no exon CNVs or whole gene deletions/duplications of *KCNE1* and *KCNE2* have been found in LQTS patients (Williams et al. 2015). Like *KCNQ1*, homozygous or compound heterozygous mutations in *KCNE1* are associated with JLNS. LQTS type 7, also autosomal dominant, is associated with mutations in *KCNJ2*. Less than 1% of LQTS patients have this type, but there is evidence that CNVs of *KCNJ2* occur in LQTS patients (Marquis-Nicholson et al. 2014). LQTS type 7 is also known as Andersen-Tawil syndrome. Besides a long-QT interval and ventricular arrhythmias, these patients experience periodic paralysis due to flaccid muscle weakness and can have a variety of congenital or developmental abnormalities including low-set ears, widely spaced eyes, small mandible, fifth-digit clinodactyly, syndactyly, short stature, scoliosis and in some cases mental retardation.

Notably, 5-10% of LQTS patients have type 3, which is associated with gain-of-function variants of the *SCN5A* gene. All known gain-of-function mutations are point mutations, which cannot be detected with MLPA. Because CNVs in *SCN5A* are not expected to cause LQTS, no probes for this gene are included in this probemix. Loss-of-function variants in *SCN5A* result in a different disease: Brugada syndrome, for which the SALSA MLPA Probemix P108 is available.

More information on LQTS can be found here: https://www.ncbi.nlm.nih.gov/books/NBK1129/.

Gene structure

The *KCNQ1* gene spans 404 kilobases (kb) on chromosome 11p15 and contains 17 exons. The *KCNQ1* LRG_287 is available at www.lrg-sequence.org and is identical to GenBank NG_008935.1.

The *KCNH2* gene spans 33 kb on chromosome 7q36 and contains 16 exons. The *KCNH2* LRG_288 is available at www.lrg-sequence.org and is identical to GenBank NG_008916.1.

The *KCNJ2* gene spans 11 kb on chromosome 17q24 and contains 2 exons. The *KCNJ2* LRG_328 is available at www.lrg-sequence.org and is identical to GenBank NG_008798.1.

The *KCNE1* gene spans 65 kb and contains 4 exons, while *KCNE2* spans 7 kb and contains 2 exons. *KCNE1* and *KCNE2* are positioned tail-to-tail close together (76 kb apart) on chromosome 21q22. The *KCNE1* LRG_290 and the *KCNE2* LRG_291 are available at www.lrg-sequence.org and are identical to GenBank NG_009091.1 and NG_008804.1, respectively.

Transcript variants

For *KCNQ1,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes the longest isoform (1) (NM_000218.3; 3224 nucleotides (nt); coding sequence (CDS): 92-2122). *KCNQ1* transcript variant 2 (NM_181798.2; 3021 nt, CDS: 270-1919) starts with exon 2, while transcript variant 1 starts with exon 1 and skips exon 2. More information can be found on the NCBI *KCNQ1* gene page: https://www.ncbi.nlm.nih.gov/gene/3784.

For *KCNH2,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes the longest isoform (a) (NM_000238.4; 4292 nt; CDS: 409-3888). Transcript variant 1 skips exon 6, while this exon is included in other variants. More information can be found on the NCBI *KCNH2* gene page: https://www.ncbi.nlm.nih.gov/gene/3757.

For *KCNJ2,* one transcript variant has been described encoding the full length protein (NM_000891.3; 5391 nt; CDS: 387-1670). More information can be found on the NCBI KCNJ2 gene page: https://www.ncbi.nlm.nih.gov/gene/3759.

For *KCNE1,* multiple variants have been described. All variants encode the same protein but transcript variant 2 is the longest transcript (NM_000219.6; 3505 nt; CDS: 561-950). More information can be found on the NCBI *KCNE1* gene page: https://www.ncbi.nlm.nih.gov/gene/3753.

For *KCNE2,* one transcript variant has been described encoding the full length protein (NM_172201.2; 1061 nt; CDS: 159-530). More information can be found on the NCBI *KCNE2* gene page: https://www.ncbi.nlm.nih.gov/gene/9992.

Exon numbering

In this P114-C1 product description the *KCNQ1* exon numbering is from the LRG_287 sequence; the *KCNH2* exon numbering is from LRG_288; the *KCNJ2* exon numbering is from LRG_328; the *KCNE1* exon numbering is from LRG_290; and the *KCNE2* exon numbering is from LRG_291.

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 P114-C1 Long-QT contains 52 MLPA probes with amplification products between 124 and 500 nt. This includes 18 probes for *KCNQ1*, 16 probes for *KCNH2*, three probes for *KCNJ2*, four probes for *KCNE1*, and two probes for *KCNE2*. 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 from 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 LQTS. 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. Listed in the table below are the samples from the Coriell Institute that have been tested with this P114-C1 probemix at MRC Holland and can be used as positive control samples to detect the indicated copy number alterations. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P114-C1	Expected copy number alteration
NA03435	Coriell Institute	11p15.5-p15.4	*KCNQ1*	Heterozygous whole gene duplication
NA12519	Coriell Institute	7q36-q31.1	*KCNH2*	Homozygous whole gene duplication
NA01220	Coriell Institute	7q34-q36.3	*KCNH2*	Heterozygous whole gene duplication
NA07412	Coriell Institute	7q32.3-q36.3	*KCNH2*	Heterozygous whole gene deletion
NA08808	Coriell Institute	7q33-q36.2	*KCNH2*	Heterozygous whole gene deletion
NA13031	Coriell Institute	21q22.11-q22.12	*KCNE1-KCNE2*	Heterozygous whole gene duplication

* Indicated chromosomal bands accommodate the gene(s) targeted by MLPA probes, however, the whole extent of the copy number alteration (CNA) present in this cell line cannot be determined by this P114-C1 Long-QT probemix.

Performance characteristics

Approximately 1% of all LQTS patients have deletions or duplications in *KCNQ1*, *KCNH2*, or *KCNJ2* (Barc et al. 2011; Marquis-Nicholson et al. 2014). Mutations in *KCNE1*, *KCNE2* and *KCNJ2* only cause a few percent of LQTS cases, and CNVs are rare among these mutations: to our knowledge, no LQTS cases have been reported for *KCNE1* and *KCNE2*, and for *KCNJ2* only a few instances have been reported. Analytical performance for the detection of deletions/duplications in *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2,* and *KCNJ2* is very high and can be considered >99% (based on a 2006-2023 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 all target probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (heterozygous triplication or homozygous duplication). 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.
- 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.

P114 specific notes

- Deletions/duplications spanning both whole *KCNE1* and *KCNE2* genes have been found in individuals not affected with LQTS.
- In the GRCh38/(hg38) genome build the putative pseudogene *KCNE1b* has been added to the short arm of chromosome 21 as part of contig CU633980.13. This putative pseudogene has 100% homology with *KCNE1* exons 2-4. The pseudogene was not annotated in previous genome builds and currently the true presence of *KCNE1b* in the human genome is not confirmed. Experiments performed at MRC Holland on a positive sample carrying a *KCNE1-KCNE2* duplication suggest that *KCNE1b* is not present in the human genome.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *KCNQ1*, *KCNH2*, *KCNJ2*, *KCNE1* and *KCNE2* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P114 Long-QT.
- 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.

LQTS mutation databases

Databases for the *KCNQ1*, *KCNH2*, *KCNJ2*, *KCNE1*, *KCNE2* genes (and other LQTS associated genes) can be found at https://www.lovd.nl/. We strongly encourage users to deposit positive results in these databases. 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 *KCNQ1* exons 9 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P114-C1 Long-QT

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*KCNQ1*	*KCNH2*	*KCNJ2*	*KCNE1*	*KCNE2*
64-105	Control fragments – see table in probemix content section for more information						
124	Reference probe 18709-L21056	5q					
131	**KCNJ2** probe 22089-L15549				**Exon 2**		
136 +	**KCNE1** probe 05070-L04470					**Exon 4**	
142	**KCNQ1** probe 03535-L02901		**Exon 1**				
148	**KCNQ1** probe 03545-L04801		**Exon 10**				
154	**KCNH2** probe 03526-L02892			**Exon 1**			
160	Reference probe 15974-L18507	8q					
165	**KCNH2** probe 15850-L17933			**Exon 16**			
171	**KCNQ1** probe 16243-L18501		**Exon 11**				
178	**KCNH2** probe 03531-L02897			**Exon 7**			
184	**KCNE2** probe 05072-L04472						**Exon 1**
190	**KCNJ2** probe 13979-L31068				**Exon 1**		
199 Δ	**KCNQ1** probe 22117-L31489		**Exon 6**				
202	Reference probe 10697-L31832	6p					
209	**KCNH2** probe 22361-L31523			**Exon 2**			
215	**KCNH2** probe 15847-L31833			**Exon 9**			
220	**KCNQ1** probe 03539-L02905		**Exon 3**				
226	**KCNH2** probe 15846-L29772			**Exon 8**			
232	Reference probe 16428-L25931	18q					
238	**KCNH2** probe 03532-L02898			**Exon 10a**			
244	**KCNH2** probe 15849-L17932			**Exon 13**			
250	**KCNE1** probe 16247-L18505					**Exon 1**	
256	**KCNQ1** probe 03540-L18356		**Exon 4**				
263	**KCNH2** probe 15845-L29770			**Exon 5**			
268	**KCNQ1** probe 03550-L18357		**Exon 13**				
274	**KCNH2** probe 03528-L02894			**Exon 3**			
282	**KCNE2** probe 05073-L04473						**Exon 2**
288	Reference probe 15880-L30312	2p					
294	**KCNQ1** probe 22163-L31834		**Exon 5**				
301	**KCNQ1** probe 03551-L02917		**Exon 14**				
310	**KCNQ1** probe 22164-L31192		**Exon 12**				
319 +	**KCNE1** probe 15854-L17947					**Exon 2**	
328	**KCNH2** probe 15851-L18358			**Exon 16**			
337	**KCNQ1** probe 03542-L20369		**Exon 7**				
346	**KCNH2** probe 04097-L20370			**Exon 11**			
355	Reference probe 11614-L12374	12p					
364	**KCNQ1** probe 16248-L19751		**Exon 8**				
373	**KCNQ1** probe 14793-L16504		**Exon 15**				
382	**KCNQ1** probe 14064-L18359		**Exon 16**				
391	**KCNH2** probe 04099-L25306			**Exon 4**			
400	Reference probe 01237-L00568	10p					

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*KCNQ1*	*KCNH2*	*KCNJ2*	*KCNE1*	*KCNE2*
409	**KCNQ1** probe 03544-L19823		**Exon 9**				
418	**KCNQ1** probe 03555-L29768		**Exon 17**				
427	**KCNH2** probe 04403-L19825			**Exon 15**			
436	**KCNQ1** probe 03537-L02903		**Exon 2**				
445	Reference probe 16286-L31835	13q					
454	**KCNQ1** probe 03554-L02920		**Exon 17**				
463 +	**KCNE1** probe 05071-L04471					**Exon 4**	
472	**KCNH2** probe 15848-L17931			**Exon 12**			
481	**KCNJ2** probe 22090-L31320				**Exon 2**		
490	**KCNH2** probe 22119-L18503			**Exon 6**			
500	Reference probe 19675-L27812	4p					

[a] See section Exon numbering on page 3 for more information.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
+ In the GRCh38/(hg38) genome build a new pseudogene, *KCNE1b*, has been added to the short arm of Chr21 as part of contig CU633980.13. The putative *KCNE1b* pseudogene has 100% homology with *KCNE1* exon 2-4. This probe also potentially targets *KCNE1b*. The pseudogene was not annotated in previous genome builds and currently the presence of *KCNE1b* in the human genome is not yet confirmed. Experiments performed at MRC Holland on a duplication positive sample suggest that *KCNE1b* is in fact not present in the human genome.

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. P114-C1 probes arranged according to chromosomal location

Table 2a. *KCNQ1*

Length (nt)	SALSA MLPA probe	*KCNQ1* exon[a]	Ligation site NM_000218.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*92-94 (exon 1)*		
142	03535-L02901	Exon 1	428-429	GCGTCTACAACT-TCCTCGAGCGTC	16.2 kb
436	03537-L02903	Exon 2	NM_181798.2; 212-213	TTTGAGGCCTGT-GGCTGCTGTGGA	66.3 kb
220	03539-L02905	Exon 3	506-507	TCTGCCTCATCT-TCAGCGTGCTGT	42.7 kb
256	03540-L18356	Exon 4	639-640	CTGCCGCAGCAA-GTACGTGGGCCT	0.6 kb
294	22163-L31834	Exon 5	720-721	CGTGGCCTCCAT-GGTGGTCCTCTG	0.7 kb
199 Δ	22117-L31489	Exon 6	869-868 reverse	GCCACCCACCTG-GCGGTGGATGAA	0.8 kb
337	03542-L20369	Exon 7	914-915	TGGGCCTCATCT-TCTCCTCGTACT	10.6 kb
364	16248-L19751	Exon 8	1082-1081 reverse	GACAGAGAAGCA-GGAGGCGATGGT	1.7 kb
409	03544-L19823	Exon 9	1155-1156	TGCCCTGAAGGT-GCAGCAGAAGCA	2.4 kb
148	03545-L04801	Exon 10	1246-1247	TATGCTGCCGAG-AACCCCGACTCC	1.2 kb
171	16243-L18501	Exon 11	1393-1394	GTGACTCCTGGA-GAGAAGATGCTC	73.2 kb
310	22164-L31192	Exon 12	1514-1513 reverse	ATGGGGCATGCT-CACTTCCAGCAG	106.9 kb
268	03550-L18357	Exon 13	1616-1617	GGCTGCGGGAAC-ACCATCGGGCCA	7.2 kb
301	03551-L02917	Exon 14	1749-1750	CCTCAACCTCAT-GGTGCGCATCAA	1.0 kb
373	14793-L16504	Exon 15	1791-1792	GGACCAGTCCAT-TGGGAAGCCCTC	1.0 kb
382	14064-L18359	Exon 16	1851-1852	CGGCAGCAACAC-GATCGGCGCCCG	69.9 kb
454	03554-L02920	Exon 17	2041-2042	TCCGTCGACCCT-GAGCTCTTCCTG	0.9 kb
		Stop codon	*2120-2122 (exon 17)*		
418	03555-L29768	Exon 17	2908-2909	CCAAACACACAG-AAGGGGACTGCC	

Table 2b. *KCNH2*

Length (nt)	SALSA MLPA probe	*KCNH2* exon[a]	Ligation site NM_000238.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*409-411 (exon 1)*		
154	03526-L02892	Exon 1	448-449	CGCAGAACACCT-TCCTGGACACCA	3.1 kb
209	22361-L31523	Exon 2	695-696	CAAAGTGGAAAT-CGCCTTCTACCG	15.0 kb
274	03528-L02894	Exon 3	741-742	TGTCTGGTGGAT-GTGGTGCCCGTG	1.4 kb
391	04099-L25306	Exon 4	1052-1053	CCTGGACGAAGT-GACAGCCATGGA	0.9 kb
263	15845-L29770	Exon 5	1357-1356 reverse	CGAGGTGGAGTT-GAGCAAGCCGCT	2.2 kb
490	22119-L18503	Exon 6	NM_172057.3; 95 nt after exon 6, reverse	TACTTCCCAGCA-GCCCTCTCCCCA	2.7 kb
178 #	03531-L02897	Exon 7	1819-1820	TCCTCATCAACT-TCCGCACCACCT	0.9 kb
226	15846-L29772	Exon 8	2151-2152	CACATGGACTCA-CGCATCGGCTGG	0.7 kb
215 #	15847-L31833	Exon 9	2535-2536	TCCTACACCAAC-GGCATCGACATG	0.7 kb
238	03532-L02898	Exon 10a	2749-2750	CCGCCCTGTACT-TCATCTCCCGGG	1.2 kb
346	04097-L20370	Exon 11	2840-2841	GCCTCTGAACCT-GTATGCAAGGCC	0.5 kb
472	15848-L17931	Exon 12	3054-3055	GGTGGCTTCAGT-CGGCAACGCAAG	0.9 kb
244	15849-L17932	Exon 13	3358-3359	GCAGCGACACTT-GCAACCCCCTGT	0.6 kb
427	04403-L19825	Exon 15	3602-3603	CACTGTCCTGCA-GCTGCTACAGAG	1.5 kb
165	15850-L17933	Exon 16	3753-3754	TCCCAGTTCATG-GCGTGTGAGGAG	0.1 kb
328	15851-L18358	Exon 16	3866-3867	CCTGCACAGACA-CGGCTCGGACCC	
		Stop codon	*3886-3888 (exon 16)*		

Table 2c. *KCNJ2*

Length (nt)	SALSA MLPA probe	*KCNJ2* exon[a]	Ligation site NM_000891.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
190	13979-L31068	Exon 1	93-92 reverse	AGTGGTTTGTAA-AAAGCGAGTGAG	5.6 kb
		Start codon	*387-389 (exon 2)*		
131	22089-L15549	Exon 2	527-526 reverse	TCTTTCTTCACA-AAGCGGCTCCTG	0.8 kb
481 #	22090-L31320	Exon 2	1363-1364	GGGCCACCGCTA-TGAGCCTGTGCT	
		Stop codon	*1668-1670 (exon 2)*		

Table 2d. *KCNE1 – KCNE2*

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		KCNE2	**NM_172201.2**		
184	05072-L04472	Exon 1	94-95	AGCAAGAAGGTT-CAGAACAGCCTG	6.5 kb
		Start codon	*159-161 (exon 2)*		
282	05073-L04473	Exon 2	249-250	GCCAGAACACAA-CAGCTGAGCAAG	78.8 kb
		Stop codon	*528-530 (exon 2)*		
		KCNE1	**NM_000219.6**		
		Stop codon	*948-950 (exon 4)*		
463 +	05071-L04471	Exon 4	799-800	CCCATTCAACGT-CTACATCGAGTC	0.2 kb
136 +	05070-L04470	Exon 4	561-562	TAATGCCCAGGA-TGATCCTGTCTA	9.0 kb
		Start codon	*561-563 (exon 4)*		
319 +	15854-L17947	Exon 2	492-493	TATCCAGAGGAA-ATAGCCAAGGAT	52.5 kb
250	16247-L18505	Exon 1	374-375	CGCAGTGTGCTT-GAGGAGACTTCA	

[a] See section Exon numbering on page 3 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.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
+ In the GRCh38/(hg38) genome build a new pseudogene, *KCNE1b*, has been added to the short arm of Chr21 as part of contig CU633980.13. The putative *KCNE1b* pseudogene has 100% homology with *KCNE1* exon 2-4. This probe also potentially targets *KCNE1b*. The pseudogene was not annotated in previous genome builds and currently the presence of *KCNE1b* in the human genome is not yet confirmed. Experiments performed at MRC Holland on a duplication positive sample suggest that *KCNE1b* is in fact not present in the human genome.
# 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.

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

Related SALSA MLPA probemixes

- P108 SCN5A contains probes for the *SCN5A* gene. Gain-of-function point mutations in *SCN5A* are associated with LQTS, but loss-of-function mutations – including CNVs – are associated with Brugada Syndrome.
- P350 CLCN1-KCNJ2 contains five probes for *KCNJ2*, three of which target the same sequences as the three *KCNJ2* probes present in this P114-C1 probemix.
- ME030 BWS/RSS contains probes for the *KCNQ1* gene located in the 11p15 BWS/RSS locus. Aberrant methylation and copy number aberrations of this locus are associated with Beckwith-Wiedemann syndrome.

References

- Barc J et al. (2011). Screening for copy number variation in genes associated with the long QT syndrome: clinical relevance. *J Am Coll Cardiol*. 57:40-47.
- Marquis-Nicholson R et al. (2014). Array comparative genomic hybridization identifies a heterozygous deletion of the entire KCNJ2 gene as a cause of sudden cardiac death. *Circ Cardiovasc Genet*. 7:17-22.
- 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.
- Williams VS et al. (2015). Multiplex ligation-dependent probe amplification copy number variant analysis in patients with acquired long QT syndrome. *Europace*. 17:635-641.

Selected publications using SALSA MLPA Probemix P114 Long-QT

- Barc J et al. (2011). Screening for copy number variation in genes associated with the long QT syndrome: clinical relevance. *J Am Coll Cardiol*. 57:40-47.
- Campuzano O et al. (2014). Identification of Genetic Alterations, as Causative Genetic Defects in Long QT Syndrome, Using Next Generation Sequencing Technology. *PLoS One*. 9:e114894.
- Christiansen M et al. (2014). Mutations in Danish patients with long QT syndrome and the identification of a large founder family with p.F29L in KCNH2. *BMC Med Genet.* 15:31.
- Coll M et al. (2017). Targeted next-generation sequencing provides novel clues for associated epilepsy and cardiac conduction disorder/SUDEP. *PLoS One*. 12:e0189618.
- Eddy CA et al. (2008). Identification of large gene deletions and duplications in KCNQ1 and KCNH2 in patients with long QT syndrome. *Heart Rhythm*. 5:1275-1281.
- Gladding PA et al. (2010). Posthumous diagnosis of long QT syndrome from neonatal screening cards. *Heart Rhythm*. 7:481-486.

- Hertz CL et al. (2015). Next-generation sequencing of 34 genes in sudden unexplained death victims in forensics and in patients with channelopathic cardiac diseases. *Int J Legal Med*. 129:793-800.
- Koopmann TT et al. (2006). Long QT syndrome caused by a large duplication in the KCNH2 (HERG) gene undetectable by current polymerase chain reaction-based exon-scanning methodologies. *Heart Rhythm.* 3:52-55.
- Marquis-Nicholson R et al. (2013). Array-based Identification of Copy Number Changes in a Diagnostic Setting: Simultaneous gene-focused and low resolution whole human genome analysis. *Sultan Qaboos Univ Med J*. 13:69-79.
- Marschall C et al. (2019). Variant panorama in 1,385 index patients and sensitivity of expanded next-generation sequencing panels in arrhythmogenic disorders. *Cardiovasc Diagn Ther*. 9:S292-S298.
- Stattin EL et al. (2012). Founder mutations characterise the mutation panorama in 200 Swedish index cases referred for Long QT syndrome genetic testing. *BMC Cardiovasc Disord*. 12:95.
- Sung JY et al. (2014). Large deletion in KCNQ1 identified in a family with Jervell and Lange-Nielsen syndrome. *Ann Lab Med*. 34:395-398.
- Tester DJ et al. (2010). Prevalence and spectrum of large deletions or duplications in the major long QT syndrome-susceptibility genes and implications for long QT syndrome genetic testing. *Am J Cardiol*. 106:1124-1128.
- Valente FM et al. (2019). Transcription alterations of KCNQ1 associated with imprinted methylation defects in the Beckwith-Wiedemann locus. *Genet Med*. 21:1808–1820.
- Williams VS et al. (2015). Multiplex ligation-dependent probe amplification copy number variant analysis in patients with acquired long QT syndrome. *Europace*. 17:635-641.

P114 product history	
Version	*Modification*
C1	Three new target probes for *KCNJ2* and one new target probe for *KCNQ1* exon 6 were included, one target probe was replaced, and four target probes were adjusted. Six reference probes were replaced and one reference probe length was changed.
B3	One reference probe was replaced and several probe lengths were adjusted.
B2	Three reference probes were replaced.
B1	Six *KCNQ1* and two *KCNE1* probes were replaced. The number of *KCNH2* probes was increased from 9 to 16. Probes for *SCN5A* and the ICR region in intron 12 of *KCNQ1* were removed. The number of reference probes was increased.
A2	DNA denaturation control fragments (D-fragments) at 88, 96, 100 and 105 nt were added.
A1	First release.

Implemented changes in the product description

Version C1-04 – 09 February 2024 (04P)
- Salt warnings removed from Table 1 and 2 as the probes are no longer considered salt sensitive.
- Removed Warning "*New in version C1" and "¥ Changed in version C1. Minor alteration, no change in sequence detected" in Table 1 and 2.
- Clinical Background information updated.
- Minor textual changes.

Version C1-03 – 22 August 2022 (04P)
- Correction in Table 1; symbol for variability is moved from the 190 nt probe to the 199 nt probe.
- Ligation site of the probe targeting *KCNQ1* transcript variant 2 updated according to new version of the NM_ reference sequence.
- Minor textual changes were made throughout the document.

Version C1-02 – 03 March 2021 (04P)
- Product description rewritten and adapted to a new template.

- Minor textual changes were made to the *clinical background* and *performance characteristics* sections.
- Ligation sites of the probes targeting the *KCNQ1, KCNH2, KCNE1, KCNE2,* and *KCNJ2* genes updated according to new version of the NM_ reference sequences.
- Warning added to Table 1 and 2 for variability of probe 22117-L31489 (199nt).
- One new article is included in *Selected publications*.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version C1-01 — 26 September 2019 (02P)
- Product description rewritten and adapted to a new template.
- P114-C1 is now CE marked.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Warning added to Table 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

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.