Product Description

SALSA® MLPA® Probemixes P321-B3/P322-C3 VPS13B

To be used with the MLPA General Protocol.

P321 version B3
For complete product history see page 9.

P322 version C3
As compared to version C2, one reference probe has been replaced. For complete product history see page 9.

Catalogue numbers:

- **P321-025R:** SALSA MLPA Probemix P321 VPS13B mix 1, 25 reactions.
- **P321-050R:** SALSA MLPA Probemix P321 VPS13B mix 1, 50 reactions.
- **P321-100R:** SALSA MLPA Probemix P321 VPS13B mix 1, 100 reactions.

- **P322-025R:** SALSA MLPA Probemix P322 VPS13B mix 2, 25 reactions.
- **P322-050R:** SALSA MLPA Probemix P322 VPS13B mix 2, 50 reactions.
- **P322-100R:** SALSA MLPA Probemix P322 VPS13B mix 2, 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 P321/P322 VPS13B is a **research use only (RUO)** assay for the detection of deletions or duplications in the *VPS13B* gene, which is associated with Cohen syndrome. The P321 probemix can also be used to detect the presence of 2-bp (CT) deletion (c.3348_3349delCT/C1117fs).

Cohen syndrome is a rare autosomal recessive disorder that is overrepresented in the Finnish and Amish population. The phenotype consists of nonprogressive mild to severe psychomotor retardation, motor clumsiness, microcephaly, characteristic facial features, childhood hypotonia and joint laxity, progressive retinochoroidal dystrophy, myopia, intermittent isolated neutropenia and a cheerful disposition. Characteristic facial features include high-arched or wave-shaped eyelids, a short philtrum, thick hair, and low hairline. Defects in the vacuolar protein sorting 13 homolog B (*VPS13B*) gene are the main cause of Cohen syndrome.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1482/.

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 *VPS13B* exon numbering used in this P321-B3/P322-C3 VPS13B product description is the exon numbering from the LRG_351 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the 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 P321-B3 VPS13B mix 1 contains 45 MLPA probes with amplification products between 130 and 472 nucleotides (nt). The SALSA MLPA Probemix P322-C3 VPS13B mix 2 contains 43 MLPA probes with amplification products between 130 and 465 nucleotides (nt). The P321 and P322 probemixes include probes for 60 of the 64 exons of the *VPS13B* gene. Furthermore, the P321 probemix contains one probe specific for the c.3348_3349delCT mutation which will only generate a signal when the mutation is present. In addition, the P321 probemix contains nine reference probes and the P322 probemix contains ten reference probes, detecting different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

These probemixes each contain 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 Cohen syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

SALSA Binning DNA SD081

The SD081 Binning DNA provided with the P321 probemix can be used for binning of all probes including the mutation-specific probe (355 nt probe 10072-L10541 c.3348_3349delCT/C1117fs mutation). SD081 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD081 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD081 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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 *VPS13B* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P321/P322 VPS13B.
- 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.

***VPS13B* mutation database**

https://databases.lovd.nl/shared/genes/VPS13B. 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 *VPS13B* exons 9 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P321-B3 VPS13B mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*VPS13B*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 21303-L29709	3p	
136	**VPS13B probe** 10073-L10497		**Exon 25**
142	**VPS13B probe** 10113-L10537		**Exon 61**
149	**VPS13B probe** 10056-L10480		**Exon 10**
154	**VPS13B probe** 10096-L10520		**Exon 44**
160	**VPS13B probe** 12675-L13750		**Exon 63**
166	Reference probe 04825-L04209	5p	
172	**VPS13B probe** 10077-L10501		**Exon 28**
178	**VPS13B probe** 10107-L10531		**Exon 55**
184	**VPS13B probe** 10067-L10491		**Exon 20**
190	Reference probe 09780-L10195	15q	
196	**VPS13B probe** 10076-L10500		**Exon 27**
203	**VPS13B probe** 10046-L10470		**Exon 1**
209	**VPS13B probe** 10065-L15992		**Exon 17**
216	**VPS13B probe** 10109-L10533		**Exon 57**
226	**VPS13B probe** 11889-L12689		**Exon 12**
232	**VPS13B probe** 10069-L10493		**Exon 22**
238	**VPS13B probe** 10085-L10509		**Exon 36**
244	**VPS13B probe** 11890-L12690		**Exon 59**
250	Reference probe 10716-L30003	6p	
256	**VPS13B probe** 10098-L10522		**Exon 46**
265	**VPS13B probe** 10062-L10486		**Exon 16**
274	**VPS13B probe** 10079-L10503		**Exon 31**
283	**VPS13B probe** 10105-L10529		**Exon 53**
292	Reference probe 09883-L10296	16p	
300	**VPS13B probe** 10095-L10519		**Exon 43**
310	**VPS13B probe** 10049-L10473		**Exon 3**
319	**VPS13B probe** 10084-L10508		**Exon 35**
326	**VPS13B probe** 22115-L10481		**Exon 11**
336	Reference probe 09027-L09281	1q	
346	**VPS13B probe** 11892-L12692		**Exon 4**
355 §	**VPS13B probe** 10072-L10541		**C1117fs mutation**
364	**VPS13B probe** 10088-L10512		**Exon 37**
371	**VPS13B probe** 22114-L13111		**Exon 9**
382	Reference probe 21221-L29596	9p	
391	**VPS13B probe** 10083-L10507		**Exon 34**
400	**VPS13B probe** 10100-L10524		**Exon 48**
409	**VPS13B probe** 10063-L10487		**Exon 16**
418	**VPS13B probe** 10112-L10536		**Exon 60**
427	Reference probe 10781-L11396	19p	
436	**VPS13B probe** 10093-L10517		**Exon 41**
445	**VPS13B probe** 10102-L10526		**Exon 50**
454	**VPS13B probe** 10061-L10485		**Exon 15**
463	**VPS13B probe** 10108-L10532		**Exon 56**
472	Reference probe 13474-L11731	14q	

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

§ Mutation-specific probe. This probe will only generate a signal when the c.3348_3349delCT/C1117fs mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

Table 2. SALSA MLPA Probemix P322-C3 VPS13B mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*VPS13B*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 16316-L18705	3q	
136	**VPS13B probe** 10106-L10530		**Exon 54**
142	**VPS13B probe** 10078-L10502		**Exon 30**
154	**VPS13B probe** 10051-L10475		**Exon 5**
160	Reference probe 11004-L11675	4q	
166	**VPS13B probe** 10110-L10534		**Exon 58**
172	**VPS13B probe** 10071-L10495		**Exon 24**
178	**VPS13B probe** 10081-L10505		**Exon 33**
184	**VPS13B probe** 10104-L10528		**Exon 52**
190	Reference probe 16293-L18585	7q	
196	**VPS13B probe** 10048-L10472		**Exon 3**
202	**VPS13B probe** 10097-L10521		**Exon 45**
208	**VPS13B probe** 10080-L10504		**Exon 32**
220	**VPS13B probe** 10059-L10483		**Exon 13**
226	Reference probe 18543-L24863	2p	
232	**VPS13B probe** 10075-L10499		**Exon 26**
238	**VPS13B probe** 10089-L10513		**Exon 38**
244	**VPS13B probe** 10099-L10523		**Exon 47**
250	Reference probe 16267-L18559	20q	
256	**VPS13B probe** 10092-L10516		**Exon 40**
265	**VPS13B probe** 11891-L12691		**Exon 21**
274	**VPS13B probe** 10087-L10511		**Exon 37**
283	**VPS13B probe** 10114-L10538		**Exon 62**
292	Reference probe 11900-L12706	6p	
301	**VPS13B probe** 10074-L10498		**Exon 25**
310	**VPS13B probe** 10066-L10490		**Exon 19**
319	**VPS13B probe** 10082-L10506		**Exon 33**
328	**VPS13B probe** 10064-L10488		**Exon 17**
336	Reference probe 09027-L09281	1q	
346	**VPS13B probe** 10101-L10525		**Exon 49**
355	**VPS13B probe** 10086-L10510		**Exon 36**
363	**VPS13B probe** 10070-L10494		**Exon 23**
373	**VPS13B probe** 10047-L10471		**Exon 2**
382	Reference probe 09798-L10213	15q	
392	**VPS13B probe** 10116-L13096		**Exon 64**
400	**VPS13B probe** 10091-L10515		**Exon 39**
409	**VPS13B probe** 10053-L10477		**Exon 7**
418	**VPS13B probe** 10094-L10518		**Exon 42**
427 *	Reference probe 10781-L11396	19p	
436	**VPS13B probe** 10103-L10527		**Exon 51**
445	**VPS13B probe** 10054-L10478		**Exon 8**
454	**VPS13B probe** 10090-L10514		**Exon 38**
465	Reference probe 19747-L26530	9q	

* New in version C3.

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 3. *VPS13B* probes arranged according to chromosomal location

Length (nt) P321/P322	SALSA MLPA probe	*VPS13B* exon[a]	Ligation site NM_017890.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*104-106 (exon 2)*		
203	10046-L10470	Exon 1	47-48	GGACTTGGAGGT-GGAGGGGACGCG	0.5 kb
373	10047-L10471	Exon 2	104-105	TACCTTAAAAGA-TGCTGGAGTCAT	24.7 kb
196	10048-L10472	Exon 3	322-323	CCATGGACAAAA-CTGGGTTCAGAA	0.1 kb
310	10049-L10473	Exon 3	14 nt after exon 3	AAGAAATTATTG-AACCAAGTTGTT	57.8 kb
346	11892-L12692	Exon 4	487-488	ATGCAGCAGGCT-GCTCCTACAGAT	6.7 kb
154	10051-L10475	Exon 5	7 nt after exon 5	TTCTGGTGAGTA-AATATGGAGAAT	12.7 kb
	no probe	Exon 6			
409	10053-L10477	Exon 7	975-976	AGAAATAGGCAA-TTTTAAAGAAGG	5.5 kb
445	10054-L10478	Exon 8	1171-1172	GTGCCTGCAATT-GTGAGTTATGAC	13.4 kb
371	22114-L13111	Exon 9	1376-1375, reverse	TTGTTCCCAACA-CAATACTTCTTT	0.4 kb
149	10056-L10480	Exon 10	1442-1443	TTGATTGCCAGA-TTGGGTTTGTTG	0.6 kb
326	22115-L10481	Exon 11	1560-1561	TGGTGACAATTT-GAGTACGAAAGG	1.1 kb
226	11889-L12689	Exon 12	7 nt after exon 12	CAAAGGTATTGG-CTTCTTTCCTTT	6.3 kb
220	10059-L10483	Exon 13	1842-1843	CCTTGTTATAGG-TCCTCTTGATTT	13.6 kb
	no probe	Exon 14			
454	10061-L10485	Exon 15	2242-2243	CAGTTGGTGCAT-GTGGTCAGCAGC	13.3 kb
265	10062-L10486	Exon 16	61 nt before exon 16	ACTGACGTATTT-CATCCTGTGCTT	0.2 kb
409	10063-L10487	Exon 16	2412-2413	TCTTTATGGGAA-ACTTCTGAAACT	22.7 kb
328	10064-L10488	Exon 17	2441-2442	TGTGCAGGACCA-AAAGATCTCAGA	0.1 kb
209	10065-L15992	Exon 17	2538-2539	GCAAGCAATATA-TCAAAGTTGGTC	81.3 kb
	no probe	Exon 18			
310	10066-L10490	Exon 19	2693-2694	ACTGCAGCACAT-CATTGGTCAAAT	0.9 kb
184	10067-L10491	Exon 20	2835-2836	CAAGTGGCTCAA-TGAGAGTAGAAA	109.1 kb
265	11891-L12691	Exon 21	2961-2962	ACAAGGACTAGC-AGTTAATATTGA	7.4 kb
232	10069-L10493	Exon 22	3107-3108	TGTCTATTGGAA-GTGCCCCCTTGG	39.9 kb
363	10070-L10494	Exon 23	3224-3225	AAGCCATGTTGA-ATATATCTGAAA	10.9 kb
172	10071-L10495	Exon 24	3375-3376	CCCAAGTACAAT-TGTATCTGGTGA	0.1 kb
355 §	10072-L10541	Exon 24	3450-3453	AACACTTGTCCT-GTTTGCCTCAAA	25.0 kb
136	10073-L10497	Exon 25	3633-3634	TTGCCTAGTGGA-ACCTATGGGTTG	0.1 kb
301	10074-L10498	Exon 25	3712-3713	CGACATTCATTT-GTTGTCTGTCTC	14.1 kb
232	10075-L10499	Exon 26	3886-3887	GGGCCTGTTCCT-ACTTCTCCAGTT	20.0 kb
196	10076-L10500	Exon 27	4039-4040	GAACAAACTACA-AGTAATATTGGA	1.1 kb
172	10077-L10501	Exon 28	4183-4184	GATCTCAGTGCT-TCCATAGATGTC	5.0 kb
	no probe	Exon 29			
142	10078-L10502	Exon 30	4320-4321	TTCCTGTACTGA-CAAGCTGAACAG	3.6 kb
274	10079-L10503	Exon 31	4704-4705	TGGTCAGCCCAT-GAGGACCCATAC	9.5 kb
208	10080-L10504	Exon 32	4845-4846	CACTGTGGTTTT-GAAGATTGGCTC	35.5 kb
178	10081-L10505	Exon 33	4937-4938	GAGCCTTGAACT-TAGGAATTCTTC	0.1 kb
319	10082-L10506	Exon 33	5046-5047	AAAACCAGAGAA-GGAAAGTGTCTC	19.1 kb
391	10083-L10507	Exon 34	5158-5159	AGGAGAGCAATT-TTGACCCCCGTT	1.8 kb
319	10084-L10508	Exon 35	5284-5285	CATTCCTTAGAA-GTGAATATAACC	64.3 kb
238	10085-L10509	Exon 36	5451-5452	AGGCATGGCTGA-AACCTCATCTCG	0.1 kb
355	10086-L10510	Exon 36	5554-5555	GCCAGTCAGCAT-CGCATTGCCCGT	19.4 kb
274	10087-L10511	Exon 37	6122-6123	CCCTTGATTATT-GCACTGTTTGGC	0.1 kb
364	10088-L10512	Exon 37	6224-6225	TGAATGGACCAG-GTAAGAAAGTCA	38.2 kb
238	10089-L10513	Exon 38	6346-6347	CATAGTGAAGAG-ACTTCAGCCATG	0.2 kb
454	10090-L10514	Exon 38	6573-6574	ACCATGCCTGTT-AGCATCTCTCTC	17.4 kb
400	10091-L10515	Exon 39	6721-6722	ATTCTGATAGGA-CCATGTTGTGCT	3.2 kb
256	10092-L10516	Exon 40	6936-6937	ACAAGTTTCTGA-ACCAGTGCCTCA	0.6 kb
436	10093-L10517	Exon 41	7189-7190	ACCACAGAGGAT-CCAGATATTAGC	45.9 kb
418	10094-L10518	Exon 42	7348-7349	AATCTCGTGAAT-GACCAGAAGAAA	9.9 kb

Length (nt) P321/P322	SALSA MLPA probe	*VPS13B* exon[a]	Ligation site NM_017890.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
300	10095-L10519	Exon 43	7462-7463	AGCACTTGTGAT-CCACTTGTGACT	2.0 kb
154	10096-L10520	Exon 44	7712-7713	ATCTTCGACAGT-GGAATAATGGTT	5.6 kb
202	10097-L10521	Exon 45	8065-8066	GAAAATATTCTG-CTGGCGAGTCTC	25.0 kb
256	10098-L10522	Exon 46	8204-8205	CTTTTATTAGAA-CAATTCAGTACA	8.2 kb
244	10099-L10523	Exon 47	8365-8366	GGTCAAGATGGA-CAAGCTGTAGTT	0.8 kb
400	10100-L10524	Exon 48	8566-8567	AGTTCCATTATT-TATGTCTGGTGC	0.4 kb
346	10101-L10525	Exon 49	8767-8768	AACATAGAACCT-GACCTTGTACAT	0.7 kb
445	10102-L10526	Exon 50	8902-8903	AATCAGATCCTT-GACGAATTCTAT	0.5 kb
436	10103-L10527	Exon 51	9112-9113	TGGCTATTTGAA-GGAGAGAAAATT	1.3 kb
184	10104-L10528	Exon 52	9269-9270	CTCCAAAGTGGA-AAGATGGAGGTA	2.5 kb
283	10105-L10529	Exon 53	9391-9392	TCCTCCATGGTA-CAGCAAGGTATA	8.5 kb
136	10106-L10530	Exon 54	9543-9544	TACTTTTAGCAT-TTGCCCAGGTGG	2.8 kb
178	10107-L10531	Exon 55	9802-9803	AGGGCTATAGTG-CTGACATATCAA	0.4 kb
463	10108-L10532	Exon 56	10032-10033	CAAAGACTTACT-TCCAAGCCTACT	13.2 kb
216	10109-L10533	Exon 57	10147-10148	GGCTTTGGCTAT-GTGTATGTGGAT	5.1 kb
166	10110-L10534	Exon 58	10716-10717	AGACACATTTGT-ATACTACATCAA	5.5 kb
244	11890-L12690	Exon 59	11182-11183	GTGAGTGGCGTC-TCCAGAGGGACC	2.3 kb
418	10112-L10536	Exon 60	10 nt before exon 60	CCCCATGCTCTT-GTTCCCTCAGGT	6.5 kb
142	10113-L10537	Exon 61	11423-11424	TTGATCAGCCGA-TGCAGAACTTCC	2.5 kb
283	10114-L10538	Exon 62	11598-11599	TGGACTTTCTCA-GCTTCCCAAACA	0.8 kb
160	12675-L13750	Exon 63	11863-11864	GCACAGGACAGC-AAGCAGAACAAC	3.8 kb
392	10116-L13096	Exon 64	11959-11960	TCAGAGCAACAG-TACAACAGACTG	
		stop codon	*12170-12172 (exon 64)*		

[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.

§ Mutation-specific probe. This probe will only generate a signal when the c.3348_3349delCT/C1117fs mutation is present. It has been tested on artificial DNA **but not on positive human 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.

Selected publications using SALSA MLPA Probemix P321/P322 VPS13B

- Parri V et al. (2010) High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. Eur J Hum Genet. 18:1133-1140
- Jezela-Stanek A et al. (2016). Malan syndrome (Sotos syndrome 2) in two patients with 19p13. 2 deletion encompassing NFIX gene and novel NFIX sequence variant. *Biomed Pap*, 160(1), 161-167.

P321 product history	
Version	*Modification*
B3	Four reference probes have been replaced and one has been added. In addition several probe lengths have been adjusted.
B2	One reference probe has been replaced. In addition, the control fragments have been adjusted (QDX2).
B1	Several reference probes have been replaced and one target probe has been added.
A1	First release.

P322 product history	
Version	*Modification*
C3	One reference probe has been replaced.
C2	Four reference probes have been replaced and two have been added.
C1	Four reference probes have been replaced and the control fragments have been adjusted (QDX2).
B1	Several reference probes have been replaced.
A1	First release.

Implemented changes in the product description

Version B3/C3-01 – 29 July 2022 (04P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version for P322 VPS13B mix 2 (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *VPS13B* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version B3/C2-01 – 5 September 2018 (01P)
- Product description adapted to new product versions for P321 and P322 (version number changed, changes in Table 1 and Table 2).
- Product description restructured and adapted to a new template.

Version 09 – 6 October 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and 2, new pictures included).
- Manufacturer's address adjusted.
- Various minor textual changes.

Version 08 (53)
- Product description adapted to new product versions (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).

Version 07 (48)
- Warning added in Table 1, 427 nt probe 09880-L10293.

Version 06 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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