Product Description SALSA® MLPA® Probemix P038-B1 CLL-2

To be used with the MLPA General Protocol.

Version B1. For complete product history see page 10.

Catalogue numbers:
- **P038-025R:** SALSA MLPA Probemix P038 CLL-2, 25 reactions.
- **P038-050R:** SALSA MLPA Probemix P038 CLL-2, 50 reactions.
- **P038-100R:** SALSA MLPA Probemix P038 CLL-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.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P038 CLL-2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the genes/chromosomal regions implicated in B-cell chronic lymphocytic leukemia (B-CLL) such as: 10q23 (*PTEN*), 11q (*ATM*), chromosome 12, 13q14 (*RB1, DLEU1/2*), 14q, 17p13 (*TP53*) and chromosome 19. This probemix can also be used to detect the presence of the *NOTCH1* p.P2514*fs, *SF3B1* p.K700E and *MYD88* p.L265P point mutations.

B-CLL is the most common hematologic neoplasm in Western countries and results in the progressive accumulation of morphologically mature but functionally incompetent CD5(+) CD23(+) B lymphocytes in the bone marrow, blood, spleen and lymph nodes of the affected person. Chromosomal translocations are rare events in B-CLL. Copy number changes of certain chromosomal regions are, however, frequent. Some of these have been found to be highly prognostic markers of this disease.

More genomic regions affected by copy number alterations in CLL are targeted by the P037 CLL-1 probemix containing probes for 2p (*MYCN, ALK, REL*), 6q (*TNFAIP3*), 8p (*TNFRSF10A/B*), 8q (*EIF3H, MYC*), 9p21 (*CDKN2A/B),* 11q *(ATM)*, chromosome 12, 13q14 *(RB1, MIR15A, DLEU2/7)* and *TP53*. Moreover, the P040 CLL probemix contains a selection of targeted genes and regions from P037 and P038 allowing detection of del13q14, trisomy 12, del11q and del17p13. Other related probemixes can be found on page 8.

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/

Probemix content: The SALSA MLPA Probemix P038-B1 CLL-2 contains 51 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 35 probes for 10q23 (*PTEN*), 11q (*ATM*), chromosome 12, 13q14 (*RB1, DLEU1/2*), 14q, 17p13 (*TP53*) and chromosome 19. Furthermore, this probemix also contains three probes specific for the *NOTCH1* p.P2514*fs, *SF3B1* p.K700E and *MYD88* p.L265P mutations which will only generate a signal when the mutation is present. In addition, 13 reference probes are included that target relatively copy number stable regions in CLL. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com) and in Table 2b.

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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragments (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 healthy individuals without a history of cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA20125, NA09596, NA15099, NA00959, NA07981, NA05832, NA03330, NA13721, NA14164, NA08123 from the Coriell Institute have been tested with this P038-B1 probemix at MRC-Holland and can be used as positive control samples to detect various CNAs as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA	Altered target genes in P038-B1	Expected CNA
NA20125	Coriell Institute	10q23.31	*PTEN*	Heterozygous duplication
		14q32.33	*KIAA0125*	Heterozygous deletion
NA09596	Coriell Institute	11q22.3	*ATM, RDX*	Heterozygous deletion
		14q32.33	*KIAA0125*	Heterozygous deletion
NA15099	Coriell Institute	11q22.3-q23.2	*ATM, RDX, PPP2R1B, CADM1*	Heterozygous duplication
NA00959	Coriell Institute	11q22.3-q23.2	*ATM, RDX, PPP2R1B, CADM1*	Heterozygous duplication
NA07981	Coriell Institute	12p13.31	*CD27*	Homozygous duplication
NA05832	Coriell Institute	13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous duplication
NA03330	Coriell Institute	13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous duplication
NA13721	Coriell Institute	13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous deletion
NA14164	Coriell Institute	13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous deletion
NA08123	Coriell Institute	14q32.33	*AKT1, MTA1, KIAA0125*	Heterozygous deletion

SALSA Binning DNA SD009: The SD009 Binning DNA provided with this probemix can be used for binning of three mutation-specific probes (*SF3B1* probe 17802-SP0549-L21900 c.2098A>G=p.K700E, *MYD88* probe 17803-L22642 c.794T>C=p.L265P and *NOTCH1* probe 17801-SP0548-L22640 c.7544-7545delCT =p.P2514*fs). SD009 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD009 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 signals, as for this purpose true mutation positive patient samples or cell lines should be used. 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 SD009 Binning DNA product description, available online: www.mlpa.com.

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.mlpa.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. When this criterion is fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *CCNE1, HMGA2, MTA1* genes. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *ATM* and *TP53* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P038 CLL-2.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

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.

COSMIC mutation database: https://cancer.sanger.ac.uk/cosmic; and **LOVD mutation database**: https://databases.lovd.nl/. We strongly encourage users to deposit positive results in the above mentioned databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNPs and unusual results (e.g., a duplication of *TP53* exons 5 and 10 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P038-B1 CLL-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)								
		reference	mutation-specific	10q	11q	12p/q	13q	14q	17p	19p/q
64-105	Control fragments – see table in probemix content section for more information									
130	Reference probe 00797-L00463	5q31								
136	**STAT6 probe** 17492-L21300					12q13.3				
142	**CD27 probe** 16210-L18463					12p13.31				
148	**AKT1 probe** 17493-L21301							14q32.33		
154	Reference probe 06070-L06371	22q12								
160	**RB1 probe** 00845-L00378						13q14.2			
167	**TP53 probe** 01588-L06028								17p13.1	
172	Reference probe 07331-L06968	1q25								
178	**DLEU2 probe** 17494-L21302						13q14.3			
184	**ATM probe** 04044-L03849				11q22.3					
192	Reference probe 08181-L22377	10p13								
198	**TP53 probe** 01996-L22378								17p13.1	
203	**KIAA0125 probe** 16524-L21379							14q32.33		
211	Reference probe 04533-L03922	2q24								
216 § Ж	**NOTCH1 probe** 17801-SP0548-L22640		c.7541-7542delCT (p.P2514*fs)							
221	**KCNRG probe** 04017-L03414						13q14.3			
226	**ATM probe** 08442-L08312				11q22.3					
234 § Ж	**SF3B1 probe** 17802-SP0549-L21900		c.2098A>G (p.K700E)							
240	**PAH probe** 02334-L23006					12q23.2				
245 ¬	**ATP7B probe** 03240-L23007						13q14.3			
252 §	**MYD88 probe** 17803-L22642		c.794T>C (p.L265P)							
258	Reference probe 11232-L21790	7p15								
265	**CDKN2D probe** 17495-L21303									19p13.2
274	**TP53 probe** 17419-L21141								17p13.1	
281 #	**RDX probe** 01633-L01171				11q22.3					
288	**ATM probe** 08445-L21782				11q22.3					
294 «	Reference probe 01332-L16059	7q11								
301	**LDLR probe** 02320-L21325									19p13.2
310	**IGF1 probe** 09545-L22156					12q23.2				
319	**TP53 probe** 17421-L23008								17p13.1	
328	**AKT2 probe** 08238-L08116									19q13.2
338	**PPP2R1B probe** 17496-L21657				11q23.1					
346	**TP53 probe** 17422-L21144								17p13.1	
355	Reference probe 00965-L00552	2p13								
361	**CADM1 probe** 03816-L15763				11q23.2					
368 #	**PTEN probe** 03638-L08334			10q23.31						
373	**ATM probe** 08420-L08326				11q22.3					
381	**DLEU1 probe** 01589-L17521						13q14.3			
391 «	**CCNE1 probe** 15145-L22643									19q12
402	**DLEU1 probe** 00801-L21380						13q14.3			
409	Reference probe 13863-L15381	16p13								
418 «	**HMGA2 probe** 15074-L16832					12q14.3				
427	**MIR498 probe** 14249-L15099									19q13.41
436	Reference probe 13363-L14793	15q24								
445 «	**MTA1 probe** 16357-L15668							14q32.33		
454	Reference probe 13254-L21630	1p21								
463	**ATM probe** 02674-L02141				11q22.3					
472 #	**PTEN probe** 13696-L22095			10q23.31						
480	**RB1 probe** 01800-L22096						13q14.2			
490	Reference probe 14883-L22098	14q11								
500	Reference probe 15203-L20113	3p12								

§ Mutation-specific probe. This probe will only generate a signal when the corresponding mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of 13q14 deletion. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Table 2. P038-B1 probes arranged according to chromosomal location

Table 2a. Target probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene, exon	Location/ Ligation site	Partial sequence[a] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
***SF3B1* p.K700E mutation** Recurrent mutations of the splicing factor gene *SF3B1* occur mainly together with 11q deletions (Wang et al. 2011) and are associated with rapid disease progression and poor overall survival (Quesada et al. 2011, Oscier et al. 2013). *SF3B1* mutations are recurrent in fludarabine-refractory cases (Rossi et al. 2011). Recurrent somatic mutations of *SF3B1* have been found in other haematological malignancies including MDS (~28%) and AML (~5%) (Malcovati et al. 2011, Je et al. 2013, Damm et al. 2012). *SF3B1* K700E amino acid substitution is the most frequent mutation found in CLL. The ligation site for the SF3B1 (at 2q33.1) probe is indicated according to NM_012433.3, and the exon numbering is according to LRG_624.						
234 § Ж	17802-SP0549-L21900	SF3B1, exon 15 c.2098A>G (p.K700E)	2192-2191 and 22 nt before exon 15 reverse	GGTCCGAACTT**C**- 42nt spanning oligo-TAACTATGCCCC	02-197.975	-
***MYD88* p.L265P mutation** *MYD88* mutations (mainly p.L265P substitution) are found in 2-4% Caucasian and 8% Asian CLL patients predominantly in cases with a mutated immunoglobulin gene. Patients with the *MYD88* mutation are diagnosed at a younger age and there are controversial reports about the prognostic parameters for *MYD88*-mutated cases (Martínez-Trillos A et al. 2014, Qin SC et al. 2017, Improgo MR et al. 2019). Moreover, the *MYD88* p.L265P mutation has also been found in 29% of ABC subtype of DLBCL (not in other subtypes) (Ngo et al. 2011) and it is also a commonly recurring mutation in patients with Waldenström's macroglobulinemia and can be used in the differential diagnosis of this disease (Treon et al. 2012, Varettoni et al. 2013). The ligation site for the *MYD88* (at 3p22.2) probe is indicated according to NM_001172567.2, and the exon numbering is according to LRG_157.						
252 §	17803-L22642	MYD88, exon 5 c.794T>C (p.L265P)	819-820	TCAGAAGCGAC**C**-GATCCCCATCAA	03-038.158	43.6 **M**b to ref
***NOTCH1* p.2514*fs mutation** A 2 bp frameshift deletion of *NOTCH1* (c.7541-7542delCT, also known as c.7544-7545delCT, p.2514*fs) is found in ~5% of CLL patients and is correlated with unfavourable prognosis (Villamor et al. 2013, Del Giudice et al. 2012, Sportoletti et al. 2010, Rossi et al. 2012[1]). *NOTCH1* mutations are more prevalent in progressive and/or chemorefractory CLL (Fabbri et al. 2011) and might predict transformation of CLL into a clonally related Richter syndrome (Rossi et al. 2012[2]). *NOTCH1* mutations are associated with poor overall survival in mantle cell lymphoma as well (Kridel et al. 2012). The ligation site for the NOTCH1 probe is indicated according to NM_017617.5, and the exon numbering is according to LRG_1122.						
216 § Ж	17801-SP0548-L22640	NOTCH1, exon 34 c.7541-7542delCT (p. P2514*fs)	7804-7805 and 7849-7850	TCACCCCGTCCC-45 nt spanning oligo-TCCGACTGGTCC	09-138.510	-
***PTEN* loss at 10q23.31** The loss of the tumour suppressor *PTEN* is common in various kinds of tumours including leukemia. Low expression level of *PTEN* predicts poor prognosis in chronic lymphocytic leukemia (Zou et al. 2012). Ligation sites for *PTEN* probes are indicated according to NM_000314.8 and the exon numbering is according to LRG_311.						
368 #	03638-L08334	PTEN, exon 5	1250-1251	GGTGTAATGATA-TGTGCATATTTA	10-089.683	32.4 kb
472 #	13696-L22095	PTEN, exon 9	2171-2170 reverse	AGAGAATTGTTC-CTATAACTGGTA	10-089.715	-
11q deletion 11q deletion, which results in loss of the *ATM* gene at 11q22.3, is found in 15-20% of CLL cases. Deletion of 11q22-q23 as well as *ATM* mutations are associated with aggressive disease and short median survival (Döhner et al. 1997, Neilson et al. 1997, Guarini et al. 2012). Larger 11q deletions also occur, including tumour suppressor genes *PPP2R1B*, *CADM1* and *RDX* (Gunn et al. 2009, Döhner et al. 1997). Ligation sites for *ATM* probes are indicated according to NM_000051.3, and the exon numbering is according to LRG_135.						
184	04044-L03849	ATM, exon 1	300-301	GAGGAGTCGGGA-TCTGCGCTGCAG	11-107.599	30.8 kb
373	08420-L08326	ATM, exon 13	2409-2410	AGAAAAGCACCA-GTCCAGTATTGG	11-107.630	66.0 kb

Length (nt)	SALSA MLPA probe	Gene, exon	Location/ Ligation site	Partial sequence[a] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
463	02674-L02141	ATM, exon 44	6755-6756	AAGGAACCAGTT-ACCATGAATCAT	11-107.696	15.0 kb
226	08442-L08312	ATM, exon 55	8421-8422	AGAATATGGAAA-TCTGGTGACTAT	11-107.711	30.4 kb
288	08445-L21782	ATM, exon 63	9427-9428	ATGAGACTACAA-GAGAAACTGAAA	11-107.741	1.9 **M**b
281 ¬ #	01633-L01171	RDX	11q22.3	GAAGCAGAACGA-CTTGAAAAGGAG	11-109.613	1.5 **M**b
338 ¬	17496-L21657	PPP2R1B	11q23.1	TGAGACAGATCT-CCCAGGAGCATA	11-111.137	3.7 **M**b
361 ¬	03816-L15763	CADM1	11q23.2	CAGCGCATGTCA-TTAGCATCTCAT	11-114.880	-
Trisomy 12 Trisomy 12 is the third most common cytogenetic abnormality in CLL detected in 10-20% of patients; it confers intermediate or favourable treatment response and overall survival (Hallek et al. 2010). Atypical lymphocyte morphology is observed in some trisomy 12 cases (Matutes et al. 1996). A large portion of trisomy 12 cases harbouring *NOTCH1* mutations exhibit aggressive CLL (Balatti et al. 2012).						
142	16210-L18463	CD27	12p13.31	CCATCACTGCCA-ATGCTGAGTGTG	13-047.853	4.9 **M**b
136	17492-L21300	STAT6	12q13.3	CCGACGCCTTCT-GCTGCAACTTGG	13-047.953	8.7 **M**b
418 «	15074-L16832	HMGA2	12q14.3	GACCCAGGGGAA-GACCCAAAGGCA	13-049.455	3.7 **M**b
310	09545-L22156	IGF1	12q23.2	GAGGCTGGAGAT-GTATTGCGCACC	13-049.488	457.9 kb
240	02334-L23006	PAH	12q23.2	GGTTCCCAAGAA-CCATTCAAGAGC	13-049.577	-
13q14 deletion Interstitial deletion at 13q14 is the most common (~50%) chromosomal aberration in CLL. The *DLEU/miR15A/16-1* cluster, as well as the *RB1* gene, are important tumour suppressor candidates within the 13q14 deletion region (Klein et al. 2010, Palamarchuk et al. 2010). Deletion of 13q14 represents a CLL group with the best prognosis and, when it is the sole abnormality, also with the highest overall survival. In P037 CLL-1 probemix more probes on 13q14 are included to define the deletion size. Ligation sites for *RB1* (13q14.2) and *DLEU1* (13q14.3) probes are indicated according to NM_000321.2 and NR_109973.1, and the exon numbering is according to LRG_517 and NR_109973.1, respectively.						
160	00845-L00378	RB1, exon 17	1693-1694	CTTGATTCTGGA-ACAGATTTGTCT	13-047.853	99.1 kb
480	01800-L22096	RB1, exon 27	3274-3275	GAGTCCTGATAA-CCCAGGCCTGTC	13-047.953	1.5 **M**b
178	17494-L21302	DLEU2	13q14.3	CCAATCTCAAGC-CTGTACATTGTT	13-049.455	32.8 kb
221	04017-L03414	KCNRG	13q14.3	CTCTAGTTTGAA-GTGAGGGAAGAA	13-049.488	89.2 kb
402	00801-L21380	DLEU1, exon 3	474-475	GAAGAACAGAAC-CTTCAGGAATTG	13-049.577	205.5 kb
381	01589-L17521	DLEU1, downstream	203 kb after exon 3	CCTTTTAATAGG-ATCTCTCCTGGA	13-049.782	1.7 **M**b
245 ¬	03240-L23007	ATP7B	13q14.3	GGTTGCCTTGAT-GGCAGGAAAGGC	13-051.441	-
14q deletion 14q24.1-q32.33 loss is detected in ~8% of CLL patients (Kay et al. 2010, Pospisilova et al. 2007, Wlodarska et al. 2007). 14q deletions are frequently associated with trisomy 12, *NOTCH1* mutations and unmutated *IGHV* genes (Cosson et al. 2014). The co-occurrence of 14q deletion and trisomy 12 is significantly associated with shorter overall survival and time to treatment in comparison to non 14p deletion cases (Reindl et al. 2010).						
148	17493-L21301	AKT1	14q32.33	AGAATGATGGCA-CCTTCATTGGCT	14-104.318	665.3 kb
445 «	16357-L15668	MTA1	14q32.33	ACAGACGGCCAA-TGGGAACGTGGA	14-104.983	400.1 kb
203	16524-L21379	KIAA0125	14q32.33	TCCGTGACTGTC-ACCTGGTACATG	14-105.383	-
17p deletion 17p deletions are detected in 5-10% of newly diagnosed CLL resulting in a loss of *TP53* tumour suppressor gene at 17p13.1. Del(17p) and also *TP53* mutations are associated with a more aggressive clinical course, worse prognosis and short overall survival, and belong to ultra-high risk CLL (Mougalian and O'Brien 2011). *TP53* deletion/mutations predict no response to treatment with purine analogues and are thus important for therapy selection (Stilgenbauer and Zenz 2010, Scheteling et al. 2008, Dreger et al. 2010). Ligation sites for *TP53* (17p13.1) probes are indicated according to NM_000546.5, and the exon numbering is according to LRG_321.						
346	17422-L21144	TP53, exon 10	1248-1249	TGAGGCCTTGGA-ACTCAAGGATGC	17-007.515	3.6 kb
274	17419-L21141	TP53, exon 6	891-892	CTCTGACTGTAC-CACCATCCACTA	17-007.518	0.7 kb
319	17421-L23008	TP53, exon 5	795-796	TATCCGAGTGGA-AGGAAATTTGCG	17-007.519	1.7 kb
198	01996-L22378	TP53, exon 2a	177-178	CTCTTGCAGCAG-CCAGACTGCCTT	17-007.521	10.8 kb
168	01588-L06028	TP53, exon 1	118-119	TCCGGGGACACT-TTGCGTTCGGGC	17-007.531	-
Trisomy 19 Trisomy 19 has been detected in 1.6-5% of CLL cases and is concurrent with trisomy 12 (Gunnarsson et al. 2011, Groenen et al. 2011). In addition to trisomy 12, trisomy 19 has been associated with mutated *IGHV* genes (Sellmann et al. 2007).						
265	17495-L21303	CDKN2D	19p13.2	AGCACGGGGCTG-ATGTCAACGTGC	19-010.539	543.5 kb
301	02320-L21325	LDLR	19p13.2	AATGACCTTAAG-ATCGGCTACGAG	19-011.082	23.9 **M**b
391 «	15145-L22643	CCNE1	19q12	TTGTCTGAACAA-AATAGGGCTTCT	19-035.006	10.4 **M**b
328	08238-L08116	AKT2	19q13.2	CGACTATCTCAA-ACTCCTTGGCAA	19-045.440	13.4 **M**b
427	14249-L15099	MIR498	19q13.41	GCTGTGATTTCA-AGCCAGGGGGCG	19-058.869	

a) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.
§ Mutation-specific probe. This probe will only generate a signal when the corresponding mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of 13q14 deletion. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested. Included to facilitate the determination of the extent
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.

Table 2b. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Location (hg18) in kb
454	13254-L21630	COL11A1	1p21	01-103.251
172	07331-L06968	SERPINC1	1q25	01-172.145
355	00965-L00552	ZNF638	2p13	02-071.431
211	04533-L03922	SCN1A	2q24	02-166.607
500	15203-L20113	GBE1	3p12	03-081.775
130	00797-L00463	IL4	5q31	05-132.038
258	11232-L21790	GARS	7p15	07-030.609
294 «	01332-L16059	STX1A	7q11	07-072.755
192	08181-L22377	OPTN	10p13	10-013.208
490	14883-L22098	RPGRIP1	14q11	14-020.873
436	13363-L14793	SEMA7A	15q24	15-072.490
409	13863-L15381	ABAT	16p13	16-008.715
154	06070-L06371	CACNG2	22q12	22-035.290

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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

Related SALSA MLPA probemixes

P037 CLL-1: contains probes for 2p, 6q, 8p/q, 9p21, *ATM*, chromosome 12, 13q14 and *TP53*.
P040 CLL: contains probes for selected target genes and regions from P037 and P038.
P041/P042 ATM: contain probes for all *ATM* exons on 11q.
P056 TP53: contains probes for all *TP53* exons.
P047 RB1 & **P098 Wilson disease**: contain more probes for 13q14.3.
P252 Neuroblastoma: contains more probes for the 2p region.
P323 CDK4-HMGA2-MDM2: contains more probes for chromosome 12.

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P038 Product history	
Version	*Modification*
B1	19 target probes have been replaced and 11 new probes added. Moreover, 12 reference probes have been replaced. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).

A2	Extra control fragments at 88-96-100-105 nt have been added.
A1	First release.

Implemented changes in the product description
Version B1-01 — 06 February 2020 (02P) - Product description rewritten and adapted to a new template. - Product description separated from P037 probemix description. - Various minor textual or layout changes. - Ligation sites of the probes targeting the *SF3B1, MYD88* genes updated according to new version of the NM_ reference sequence in Table 2a. - Ligation sites and exon numbering added for *DLEU1* probes in Table 2a. - NM_ reference sequence version updated for *NOTCH1* and *PTEN* probes in Table 2a. - Warning added to Table 1 and Table 2a for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - New references added in Table 2a and on pages 8-10. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

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