Product Description
SALSA® MLPA® Probemix P038 CLL-2

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 13.

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.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 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.P2514Rfs*4 , *SF3B1* p.K700E and *MYD88* p.L265P (also known as p.L252P) 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 target genes and regions from P037 and P038 allowing copy number determination of 11q, chromosome 12, 13q14 and 17p13. Other related probemixes can be found on page 11.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK470433/

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
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering

The *SF3B1*, *MYD88*, *NOTCH1*, *PTEN*, *ATM*, *RB* and *DLEU1* exon numbering used in this P038-B1 CLL-2 product description is the exon numbering from MANE project (release version 1.0) based on MANE Select transcripts, as indicated in Table 2. The *TP53* exon numbering is derived from the LRG_321 sequence; the exon numbering derived from MANE Select for *TP53* can be found in between brackets in Table 2. 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. Please note, that in other MRC Holland product descriptions exon numbering for the same gene might differ in case other resources are indicated to be used for exon numbering.

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.P2514Rfs*4, *SF3B1* p.K700E and *MYD88* p.L265P (also known as p.L252P) 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. The identity of the genes detected by the reference probes is available in Table 3. Complete probe sequences 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). 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.

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 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 (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA20125, NA09596, NA15099, NA00959, NA07981, NA05832, NA03330, NA13721, NA14164 and NA08123 from the Coriell Institute, and HNT-34 (ACC-600), L-363 (ACC-049) and U-266 (ACC-009) from Leibniz Institute DSMZ 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 (hg18)*	Altered target genes in P038-B1	Expected copy number alteration / mutation
NA20125	Coriell Institute	10q23.31	*PTEN*	Heterozygous duplication
		14q.32.33	*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 duplication
NA14164	Coriell Institute	13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous deletion
NA08123	Coriell Institute	14q32.33	*AKT1, MTA1, KIAA0125*	Heterozygous deletion
HNT-34	DSMZ	2q33.1	*SF3B1* c.2098A>G (p.K700E)	Point mutation
L-363◊	DSMZ	14q32.33	*AKT1, MTA1*	Heterozygous deletion
		14q32.33	*KIAA0125*	Homozygous deletion
		17p13.1	*TP53*	Heterozygous deletion
U-266◊	DSMZ	11q22.3-q23.2	*ATM1, RDX, PPP2R1B, CADM1*	Heterozygous deletion
		12p13.31	*CD27*	Heterozygous deletion
		13q14.2-q14.3	*RB1, DLEU2, KCNRG, DLEU1, ATP7B*	Heterozygous deletion
		14q32.33	*KIAA0125*	Homozygous deletion
		17p13.1	*TP53*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P038-B1-CLL-2 probemix.
◊ CNAs detected by reference probes are not reported for this sample.

SALSA Binning DNA SD009

The SD009 Binning DNA provided with this probemix can be used for binning of all probes including the three mutation-specific probes (SF3B1 probe 17802-SP0549-L21900 c.2098A>G=p.K700E, MYD88 probe 17803-L22642 c.794T>C=p.L265P (also known as c.755T>C=p.L252P) and NOTCH1 probe 17801-SP0548-L22640 c.7541-7542delCT=p.P2514Rfs*4). SD009 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 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 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 SD009 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. When this criterion is fulfilled, the following cut-off values for the final ratio (FR) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status indication	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios 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 subclonal 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 or in or near the *CCNE1, HMGA2* and *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.
- 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 cancer 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. 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.
- 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 and LOVD mutation databases
We strongly encourage users to deposit positive results in the COSMIC (https://cancer.sanger.ac.uk/cosmic) and LOVD (https://databases.lovd.nl) mutation 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 SNVs and unusual results (e.g., a deletion of *ATM* exons 46 and 63, but not exon 55) to MRC Holland: info@mrcholland.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	17q	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					12**p**13.31				
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 § Δ Ж	**NOTCH1 probe** 17801-SP0548-L22640		c.7541-7542delCT (p.P2514Rfs*4)							
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									19**p**13.2
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									19**p**13.2
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			

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)								
		reference	**Mutation-specific**	**10q**	**11q**	**12p/q**	**13q**	**14q**	**17q**	**19p/q**
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.
± SNP rs566016487 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
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 a deletion/duplication. 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.

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. P038-B1 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
***SF3B1* c.2098A>G = 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 MANE Select transcript NM_012433.4.						
234 § Ж	17802-SP0549-L21900	***SF3B1***, exon 15 c.2098A>G (p.K700E)	2127-2126 and 22 nt before exon 15 reverse	GGTCCGAACTT**C**-42 nt spanning oligo-TAACTATGCCCC	02-197,975	-
***MYD88* c.794T>C = p.L265P (also known as c.755T>C = p.L252P) mutation** *MYD88* mutations (mainly p.L265P substitution, also known as p.L252P) 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 et al. 2014, Qin et al. 2017, Improgo 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 MANE Select transcript NM_002468.5.						
252 §	17803-L22642	***MYD88***, exon 5 c.794T>C (p.L265P)	795-796	TCAGAAGCGAC**C**-GATCCCCATCAA	03-038,158	43,6 **M**b to ref
***NOTCH1* c.7541-7542delCT = p.P2514Rfs*4 (also known as 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 MANE Select transcript NM_017617.5.						
216 § Δ Ж	17801-SP0548-L22640	***NOTCH1***, exon 34 c.7541-7542delCT (p.P2514Rfs*4)	7804-7805 and 7849-7850	TCACCCCGTCC**C**-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 MANE Select transcript NM_000314.8.						
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 MANE Select transcript NM_000051.4.						
184	04044-L03849	***ATM***, exon 1	65-66	GAGGAGTCGGGA-TCTGCGCTGCAG	11-107,599	30,8 kb
373	08420-L08326	***ATM***, exon 13	2174-2175	AGAAAAGCACCA-GTCCAGTATTGG	11-107,630	66,0 kb
463	02674-L02141	***ATM***, exon 44	6520-6521	AAGGAACCAGTT-ACCATGAATCAT	11-107,696	15,0 kb

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
226	08442-L08312	***ATM***, exon 55	8186-8187	AGAATATGGAAA-TCTGGTGACTAT	11-107,711	30,4 kb
288	08445-L21782	***ATM***, exon 63	9192-9193	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	12-006,430	4,9 **M**b
136	17492-L21300	***STAT6***	12q13.3	CCGACGCCTTCT-GCTGCAACTTGG	12-055,788	8,7 **M**b
418 «	15074-L16832	***HMGA2***	12q14.3	GACCCAGGGGAA-GACCCAAAGGCA	12-064,508	36,8 **M**b
310	09545-L22156	***IGF1***	12q23.2	GAGGCTGGAGAT-GTATTGCGCACC	12-101,337	457,9 kb
240	02334-L23006	***PAH***	12q23.2	GGTTCCCAAGAA-CCATTCAAGAGC	12-101,795	-
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) probes are indicated according to MANE Select transcript NM_000321.3. Ligation sites and exon numbering for DLEU1 (13q14.3) probes are indicated according to NR_109973.1.						
160	00845-L00378	***RB1***, exon 17	1689-1690	CTTGATTCTGGA-ACAGATTTGTCT	13-047,853	99,1 kb
480 ±	01800-L22096	***RB1***, exon 27	3270-3271	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 et al. 2010, Schetelig et al. 2008, Dreger et al. 2010). Ligation sites for TP53 (17p13.1) probes are indicated according to MANE Select transcript NM_000546.6, and the exon numbering is according to LRG_321. The MANE Select transcript NM_000546.6 exon numbering is indicated between brackets.						

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
346	17422-L21144	***TP53***, exon 10	1188-1189	TGAGGCCTTGGA-ACTCAAGGATGC	17-007,515	3,6 kb
274	17419-L21141	***TP53***, exon 6 (7)	831-832	CTCTGACTGTAC-CACCATCCACTA	17-007,518	0,7 kb
319	17421-L23008	***TP53***, exon 5 (6)	735-736	TATCCGAGTGGA-AGGAAATTTGCG	17-007,519	1,7 kb
198	01996-L22378	***TP53***, exon 2a (2)	117-118	CTCTTGCAGCAG-CCAGACTGCCTT	17-007,521	10,8 kb
168	01588-L06028	***TP53***, exon 1	58-59	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] 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 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.

± SNP rs566016487 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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 a deletion/duplication. 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.

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. P038-B1 reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Location (hg18) in kb	Distance to next probe
454	13254-L21630	*COL11A1*	1p21	01-103,251	68,9 **M**b
172	07331-L06968	*SERPINC1*	1q25	01-172,145	-
355	00965-L00552	*ZNF638*	2p13	02-071,431	95,2 **M**b
211	04533-L03922	*SCN1A*	2q24	02-166,607	31,4 **M**b
500	15203-L20113	*GBE1*	3p12	03-081,775	-
130	00797-L00463	*IL4*	5q31	05-132,038	-
258	11232-L21790	*GARS*	7p15	07-030,609	42,1 **M**b
294 «	01332-L16059	*STX1A*	7q11	07-072,755	-

192	08181-L22377	*OPTN*	10p13	10-013,208	76,5 **M**b
490	14883-L22098	*RPGRIP1*	14q11	14-020,873	83,4 **M**b
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 11q, 12p/q, 13q14 and *TP53*
P041 and P042 ATM	contain probes for all *ATM* exons on 11q
P056 TP53	contains probes for all *TP53* exons
P047 RB1	contain more probes for 13q14.2
P252 NB mix 2	contains more probes for the 2p region
P323 CDK4-HMGA2-MDM2	contains more probes for chromosome 12
P377 Hematologic Malignancies	contains more probes for 6q, 8q, 9p, 11q, 12p/q, 13q14 and *TP53*.

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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 B1-02 (04P) – 12 April 2023 (04P) - Product description rewritten and adapted to a new template. - Transcript numbers of the *SF3B1*, *MYD88*, *ATM*, *RB1* and *TP53* genes have been updated according to the new version of MANE Select. - Ligation sites of the probes targeting *SF3B1*, *MYD88*, *ATM*, *RB1* and *TP53* have been updated according to their respective NM_ sequence according to the new version of the MANE Select. - The annotation for the NOTCH1 mutation-specific probe was changed from *NOTCH1* p.P2514*fs to *NOTCH1* p.P251Rfs*4 and for the MYD88 mutation-specific probe alternative names c.755T>C and p.L252P were added next to existing annotations c.794T>C and p.L265P. - Footnotes were added to Tables 1 and 2 to indicate flanking probes targeting 11q. - New references added in the Selected publications section. - The related SALSA Probemixes section was updated (now includes P377 Hematologic malignancies). - Various minor textual or layout changes. *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).

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