Product Description SALSA® MLPA® Probemix P037-B1 CLL-1

To be used with the MLPA General Protocol.

Version B1. For complete product history see page 10.

Catalogue numbers:
- **P037-025R:** SALSA MLPA Probemix P037 CLL-1, 25 reactions.
- **P037-050R:** SALSA MLPA Probemix P037 CLL-1, 50 reactions.
- **P037-100R:** SALSA MLPA Probemix P037 CLL-1, 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 P037 CLL-1 is a **research use only (RUO)** assay for the detection of deletions or duplications in various genes and chromosomal regions implicated in B-cell chronic lymphocytic leukemia (B-CLL) such as: 2p (*MYCN*, *ALK*, *REL)*, 6q (*TNFAIP3*), 8p (*TNFRSF10A/B*), 8q (*EIF3H*, *MYC*), 9p21 (*CDKN2A/B*), 11q (*ATM*), chromosome 12, 13q14 (*MIR15A, DLEU2/7*) and 17p (*TP53*).

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 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 important prognostic markers of this disease.

The **P038 CLL-2** probemix contains more probes for the 11q region and different probes for chromosome 12, 13q14 and the *TP53* gene. Moreover, it contains probes targeting the *PTEN* gene, 14q, chromosome 19, and probes specific for *NOTCH1* p.P2514*fs, *SF3B1* p.K700E and *MYD88* p.L265P point mutations. The **P040 CLL** probemix contains a selection of target genes and regions from P037 and P038 for the detection of del13q14, trisomy 12, del11q22-q23, 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 P037-B1 CLL-1 contains 54 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 41 probes for 2p, 6q, 8p/q, 9p21, 11q, 12p/q, 13q and 17p chromosomal regions. 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).

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-fragment (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 NA09216, NA00945, NA04409, NA01353, NA10401, NA09367, NA07994, NA06802, NA14485, NA03255, NA02030, NA03999, NA02819, NA03226, NA08618, NA09596, NA07981, NA05832, NA12606, NA03330, NA14164, NA13721 from the Coriell Institute and ACC-203 (SK-N-MC) from the Leibniz Institute DSMZ have been tested with this P037-B1 probemix at MRC-Holland and can be used as a 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 P037-B1	Expected CNA
NA09216	Coriell Institute	2p24.3	*MYCN*	Heterozygous deletion
NA00945	Coriell Institute	2p24.3	*MYCN*	Heterozygous deletion
ACC203 SK-N-MC	Leibniz Institute DSMZ	2p24.3	*MYCN*	Heterozygous deletion
NA04409	Coriell Institute	2p24.3	*MYCN*	Heterozygous duplication
NA01353	Coriell Institute	2p24.3-p23.2	*MYCN, ALK*	Heterozygous duplication
NA10401	Coriell Institute	2p24.3-p16.1	*MYCN, ALK, REL*	Heterozygous duplication
NA09367	Coriell Institute	6q21-q23.3	*SEC63, TNFAIP3*	Heterozygous duplication
NA07994	Coriell Institute	6q23.3-q26	*TNFAIP3, LATS1, IGF2R, PARK2*	Heterozygous duplication
NA06802	Coriell Institute	6q25.3-q26	*IGF2R, PARK2*	Heterozygous deletion
NA14485	Coriell Institute	8p21.3	*TNFRSF10A, TNFRSF10B*	Heterozygous duplication
NA03255	Coriell Institute	8p21.3	*TNFRSF10A, TNFRSF10B*	Heterozygous duplication

Sample name	Source	Chromosomal position of CNA	Altered target genes in P037-B1	Expected CNA
NA02030	Coriell Institute	8p21.3-q24.21	*TNFRSF10A, TNFRSF10B, EIF3H, MYC*	Heterozygous duplication
ACC203 SK-N-MC	Leibniz Institute DSMZ	8p21.3-q24.21	*TNFRSF10A, TNFRSF10B, EIF3H, MYC*	Amplification
NA03999	Coriell Institute	8q24.21	*MYC*	Heterozygous deletion
NA02819	Coriell Institute	9p21.3	*CDKN2A, CDKN2B*	Heterozygous duplication
NA03226	Coriell Institute	9p21.3	*CDKN2A, CDKN2B*	Heterozygous duplication
NA08618	Coriell Institute	11q22.3	*ATM*	Heterozygous duplication
NA09596	Coriell Institute	11q22.3	*ATM*	Heterozygous deletion
NA07981	Coriell Institute	12p13.32-p12.1	*CCND2, LRMP*	Homozygous duplication
NA02819	Coriell Institute	12q24.33	*CHFR*	Heterozygous deletion
NA05832	Coriell Institute	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	Heterozygous duplication
NA12606	Coriell Institute	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	Heterozygous duplication
NA03330	Coriell Institute	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	Heterozygous duplication
NA14164	Coriell Institute	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	Heterozygous deletion
NA13721	Coriell Institute	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	Heterozygous deletion
ACC203 SK-N-MC	Leibniz Institute DSMZ	17p13.1	*TP53*	Heterozygous deletion

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 these criterion are 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 *MYCN* gene. 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 P037 CLL-1.
- 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 1 and 4b but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P037-B1 CLL-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)							
		reference	**2p**	**6q**	**8p/q**	**9p**	**11q**	**12p/q**	**13q**	**17p**
64-105	Control fragments – see table in probemix content section for more information									
130	Reference probe 00797-L19287	5q31								
136	Reference probe 13224-L14557	1p21								
142	**EIF3H probe** 13351-L22157				8q24.11					
148	**KCNRG probe** 04018-L04000								13q14.3	
154	Reference probe 11424-L22558	1q41								
160	**MYC probe** 00580-L22108				8q24.21					
167	**MIR15A probe** 04019-L22561								13q14.3	
173	**ATM probe** 02644-L02111						11q22.3			
178 «	**MYCN probe** 03028-L21406		2p24.3							
184 ∫	Reference probe 01217-L18058	4q35								
190 Δ ¬	**FNDC3A probe** 17896-L22175								13q14.2	
195	**DLEU2 probe** 04020-L21407								13q14.3	
200	Reference probe 04827-L22160	5p13								
205 «	**MYCN probe** 17473-L21265		2p24.3							
211	**TNFAIP3 probe** 17472-L22159			6q23.3						
217	**IFNG probe** 00472-L21249							12q15		
223	**REL probe** 17474-L21266		2p16.1							
229	**CDKN2B probe** 16059-L18233					9p21.3				
235	**IGF2R probe** 02798-L22562			6q25.3						
241 #	**TNFRSF10B probe** 17475-L21781				8**p**21.3					
247 «	**MYCN probe** 17476-L22557		2p24.3							
256	**CDKN2A probe** 15674-L17640					9p21.3				
263 ‡	**TP53 probe** 02376-L21409									17p13.1
267	Reference probe 12782-L15494	2q13								
274 «	**CDK4 probe** 17735-L22100							12q14.1		
281	**MYC probe** 17477-L22565				8q24.21					
285 ‡	**TP53 probe** 02384-L21411									17p13.1
292	**REL probe** 17478-L21270		2p16.1							
299	**TP53 probe** 17420-L21142									17p13.1
306 ¬	**ATP7B probe** 03242-L22875								13q14.3	
312	**TNFRSF10A probe** 17479-L22161				8**p**21.3					
321	**LRMP probe** 00495-L22559							12**p**12.1		
328	Reference probe 08115-L22104	11p15								
337	**ATM probe** 02663-L22102						11q22.3			
344	Reference probe 16871-L19664	9q34								
352	**PARK2 probe** 02182-L21780			6q26						
358	**CCND2 probe** 00498-L21253							12**p**13.32		
365 «	**MYCN probe** 02572-L21412		2p24.3							
373	**DLEU7 probe** 17480-L21272								13q14.3	
382	**RB1 probe** 01794-L01357								13q14.2	
391	Reference probe 07808-L22560	3p22								
400 «	**CHFR probe** 02684-L21413							12q24.33		
409	**TP53 probe** 02263-L01749									17p13.1
418	**ALK probe** 08323-L08192		2p23.2							
427	Reference probe 06435-L05961	6p22								
436	**AIM1 probe** 17481-L22106			6q21						
445	**SEC63 probe** 17736-L21863			6q21						
451	Reference probe 05026-L22184	2q32								
457	**LATS1 probe** 17483-L22569			6q25.1						
466	**DLEU7 probe** 03042-L21414								13q14.3	
472	Reference probe 11803-L12598	15q15								
481	**ALK probe** 15397-L08194		2p23.2							
495	**TNFAIP3 probe** 17484-L21276			6q23.3						
500	Reference probe 15203-L20113	3p12								

« 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.
¬ 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.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
∫ Frequent copy number alterations detected with this probe. Aberrant results should be treated with caution.
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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 2. P037-B1 probes arranged according to chromosomal location

Table 2a. 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
2p gain Gain of the short arm of chromosome 2 is a recurring chromosomal aberration in CLL. *MYCN*, *ALK* and *REL* oncogenes, located on 2p, are frequently gained in CLL (Jarosova et al. 2010). 2p gain is suggested to be a marker of disease progression and poor prognosis (Fabris et al. 2012, Chapiro et al., 2010). Ligation sites for *MYCN* (2p24.3), *ALK* (2p32.2) and *REL* (2p16.1) probes are indicated according to NM_005378.6, NM_004304.5 and NM_002908.4, and the exon numbering is according to NG_007457.1, LRG_488, NM_002908.4, respectively.						
247 «	17476-L22557	**MYCN**, exon 2	347-348	ATGCCGGGCATG-ATCTGCAAGAAC	02-016.000	0.1 kb
178 «	03028-L21406	**MYCN**, exon 2	470-471	TGGAAGAAGTTT-GAGCTGCTGCCC	02-016.000	3.4 kb
365 «	02572-L21412	**MYCN**, exon 3	1200-1201	CTGTCACCACAT-TCACCATCACTG	02-016.003	0.3 kb
205 «	17473-L21265	**MYCN**, exon 3	1452-1453	CGGAGGACAGTG-AGCGTCGCAGAA	02-016.003	13.3 **M**b
481	15397-L08194	**ALK**, exon 27	4906-4907	TTTCTCTTGGAT-ATATGCCATACC	02-029.274	334.5 kb
418	08323-L08192	**ALK**, exon 4	1909-1910	ACACCTCAGCTG-ACTCCAAGCACA	02-029.608	31.4 **M**b
223	17474-L21266	**REL**, exon 7	1034-1035	TATCACAGAACC-CGTAACAGTAAA	02-060.999	3.4 kb
292	17478-L21270	**REL**, exon 11	1506-1507	TCAAGCTGGTCA-TCAGTGGCCCAC	02-061.003	-
6q deletion 6q deletion is associated with intermediate prognosis in CLL patients (Wang et al. 2011). 6q deletion shows atypical morphology (Cuneo et al. 2004), higher white blood cell counts and more extensive lymphadenopathy (Stilgenbauer et al. 1999). Tumour suppressor genes such as *TNFAIP3*, *LATS1*, *AIM1* (Philipp et al. 2011, Lehmann et al. 2008) have been shown to be deleted in the del6q cases. Ligation sites for *TNFAIP3* (6q23.3) probes are indicated according to NM_006290.4 and the exon numbering is according to NG_032761.1.						
436	17481-L22106	**AIM1**	6q21	CTATGACCACGG-CTTTCAGTACTT	06-107.076	1.2 **M**b
445	17736-L21863	**SEC63**	6q21	CAGCAGGGTGAA-ACTAACAAGAAC	06-108.321	29.9 **M**b
495	17484-L21276	**TNFAIP3**, exon 2	412-413	GTTCAGAACTTG-CCAGTTTTGTCC	06-138.234	9.7 kb
211	17472-L22159	**TNFAIP3**, exon 9	2432-2433	ATCCTGGCCTGC-CGCAGCGAGGAG	06-138.244	11.8 **M**b
457	17483-L22569	**LATS1**	6q25.1	CAAAACCCATCT-GTTCCTCCATAC	06-150.046	10.3 **M**b
235	02798-L22562	**IGF2R**	6q25.3	TTCAACACAACA-GTGAGCTGTGAC	06-160.350	1.4 **M**b
352	02182-L21780	**PARK2**	6q26	TCTGCCGGGAAT-GTAAAGAAGCGT	06-161.728	-
8p loss and 8q amplification Loss of 8p (including *TNFRSF10A/B* genes) and amplification at 8q24 (including *MYC* oncogene) (Brown et al. 2012) are detected in CLL (Rinaldi et al. 2011, Ouillette et al. 2011) with higher frequency in a subset of CLL with 17p deletion. 8p loss was associated with shorter overall survival and time to treatment in 17p deletion subset (Forconi et al. 2008). Gains at 8q and deletions at 8p associate with resistance to alkylating agents and poor prognosis (Rinaldi et al. 2011). Ligation sites for *MYC* (8q24.21) probes are indicated according to NM_002467.5 and the exon numbering is according to NG_007161.2.						
241 #	17475-L21781	**TNFRSF10B**	8**p**21.3	GGTGATTGTACA-CCCTGGAGTGAC	08-022.942	196.4 kb
312	17479-L22161	**TNFRSF10A**	8**p**21.3	GAATCCCGGGAG-CGCAGCGAGTGG	08-023.138	94.7 **M**b
142	13351-L22157	**EIF3H**	8q24.11	TAGATGGCCTTG-TGAGTGCTGTTC	08-117.837	11.0 **M**b
160	00580-L22108	**MYC**, exon 3	2317-2318	GAACGAGCTAAA-ACGGAGCTTTTT	08-128.822	0.1 kb
281	17477-L22565	**MYC**, exon 3	2466-2467	AGGACTTGTTGC-GGAAACGACGAG	08-128.822	-

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
9p21 loss *CDKN2A* and *CDKN2B* located at 9p21.3 are cell cycle regulators involved in development of many tumour types. Loss of 9p21 (encompassing *CDKN2A/B*) is relatively infrequent in CLL patients (Ouillette et al. 2011; Fabris et al. 2011, Buijs et al. 2006). 30% of the cases of Richter's transformation can exhibit deletions at 9p21 (Fabbri et al. 2013, Chigrinova et al. 2013).						
256	15674-L17640	**CDKN2A**	9p21.3	TCCTTTCCGTCA-TGCCGGCCCCCA	09-021.961	34.6 kb
229	16059-L18233	**CDKN2B**	9p21.3	GCCTGTCTGAGA-CTCACAGGAAGG	09-021.996	-
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). Ligation sites for *ATM* probes are indicated according to NM_000051.3 and the exon numbering is according to LRG_135.						
173	02644-L02111	**ATM**, exon 14	2556-2557	TCTTTTGGTGGG-TGTCCTTGGCTG	11-107.632	16.5 kb
337	02663-L22102	**ATM**, exon 22	3642-3641 reverse	CAGCCAACATGC-GAACTTGGTGAT	11-107.649	-
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).						
358	00498-L21253	**CCND2**	12**p**13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	12-004.279	20.9 **M**b
321	00495-L22559	**LRMP**	12**p**12.1	GTCTCTAGAACA-TATCTTGTGGCC	12-025.152	31.3 **M**b
274 «	17735-L22100	**CDK4**	12q14.1	TCTCTGAGGCTA-TGGAGGGTCCTC	12-056.428	10.4 **M**b
217	00472-L21249	**IFNG**	12q15	GATGGCTGAACT-GTCGCCAGCAGC	12-066.835	65.1 **M**b
400 «	02684-L21413	**CHFR**	12q24.33	GACATGCCCTTT-ACAGACTGGGGA	12-131.959	-
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 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. The 13q14 deletion size is shown to indicate differential prognosis (Ouillette et al. 2011, Parker et al. 2011). Therefore, probes in two flanking regions (*FNDC3A* and *ATP7B*) are included to define the deletion size. The exon numbering of *DLEU7* is according to NM_198989.3.						
382	01794-L01357	**RB1**	13q14.2	TTTTGTTCTTTA-AACACACTTTGG	13-047.936	667.7 kb
190 Δ ¬	17896-L22175	**FNDC3A**	13q14.2	CGCCTCCACCAC-GTCATATGTACT	13-048.603	889.3 kb
148	04018-L04000	**KCNRG**	13q14.3	GCTTAAGCCATA-ATGCCTGCTGCT	13-049.493	28.5 kb
167	04019-L22561	**MIR15A**	13q14.3	TGGATTTTGAAA-AGGTGCAGGCCA	13-049.521	33.0 kb
195	04020-L21407	**DLEU2**	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	13-049.554	630.8 kb
466	03042-L21414	**DLEU7**, exon 1	13q14.3	AAGAAGATCGTG-ACAAATTCCCTA	13-050.185	130.3 kb
373	17480-L21272	**DLEU7**, exon 2	13q14.3	GACTTCGGAGCT-GGTCAGCGTGGA	13-050.315	1.1 **M**b
306 ¬	03242-L22875	**ATP7B**	13q14.3	TTCCCTGGCCCA-GAGAAACCCCAA	13-051.434	
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.						
285 ‡	02384-L21411	**TP53**, exon 7	1041-1042	CTGTCCTGGGAG-AGACCGGCGCAC	17-007.518	1.4 kb
263 ‡	02376-L21409	**TP53**, exon 4b	606-607	CAAGATGTTTTG-CCAACTGGCCAA	17-007.519	0.8 kb
299	17420-L21142	**TP53**, exon 3	511-510 reverse	TAGCTGCCCTGG-TAGGTTTTCTGG	17-007.520	11.6 kb
409	02263-L01749	**TP53**, exon 1	67 nt before exon 1	CTTCCTCCGGCA-GGCGGATTACTT	17-007.532	

a) The exon numbering and NG/NM_ sequence used have been retrieved on 10/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.
b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« 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.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
¬ 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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Location (hg18) in kb
136	13224-L14557	COL11A1	1p21	01-103.234
154	11424-L22558	USH2A	1q41	01-213.981
267	12782-L15494	EDAR	2q13	02-108.913
451	05026-L22184	COL3A1	2q32	02-189.573
500	15203-L20113	GBE1	3p12	03-081.775
391	07808-L22560	SCN5A	3p22	03-038.625
184 ∫	01217-L18058	KLKB1	4q35	04-187.390
200	04827-L22160	NIPBL	5p13	05-036.997
130	00797-L19287	IL4	5q31	05-132.038
427	06435-L05961	KIAA0319	6p22	06-024.653
344	16871-L19664	COL5A1	9q34	09-136.799
328	08115-L22104	ABCC8	11p15	11-017.406
472	11803-L12598	SPG11	15q15	15-042.647

∫ Frequent copy number alterations detected with this probe. Aberrant results should be treated with caution.
Probe sequences are available at www.mlpa.com.

Related SALSA MLPA probemixes

- **P038 CLL-2**: contains probes for 11q, 12p/q, 13q14, *TP53* & *PTEN* genes and probes specific for *NOTCH1* p.P2514*fs, *SF3B1* p.K700E and *MYD88* p.L265P point mutations.
- **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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P037 Product history	
Version	*Modification*
B1	13 target probes have been replaced and 12 new ones added. Moreover, 10 reference probes have been replaced and 2 new ones included. 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 — 29 November 2019 (02P)

- Product description rewritten and adapted to a new template.
- Product description separated from P038 probemix description.
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the *MYCN, ALK, REL, TNFAIP3 and MYC* genes updated according to new version of the NM_ reference sequence.
- Warning added to Table 1 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

Version 23 – 03 January 2018 (T08)

- Changed the name for NOTCH1 c.7544-7545delCT probe to c.7541-7542delCT in table 3b to more accurately reflect the location of the ligation site.
- Various minor textual changes.

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