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

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 11.

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.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 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 copy number determination of 11q22-q23, chromosome 12, 13q14 and 17p13. Other related probemixes can be found on page 9.

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
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

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

Exon numbering
The *MYCN*, *ALK*, *REL*, *TNFAIP3*, *MYC*, *ATM*, *DLEU7* exon numbering in this P037-B1 product description is the exon numbering derived 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 project for this gene 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 have been used for exon numbering.

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. 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 heathy 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 NA00945, NA09216, NA04409, NA01353, NA01221, NA06802, NA09367, NA07994, NA03255, NA14485, NA02030, NA09888, NA03999, NA02819, NA03226, NA08618, NA09596, NA07891, NA02718, NA13721, NA14164, NA03330 and NA05832 from the Coriell Institute and SK-N-MC (ACC 203) from Leibniz Institute DSMZ have been tested with this P037-B1 probemix at MRC Holland and can be used as a positive control sample to detect copy number alterations (CNAs) of various target regions/genes. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Chromosomal position of CNA (hg18)*	Altered target genes in P037-B1	Expected copy number alteration
Germline samples from Coriell Institute			
NA00945	2p24.3	*MYCN*	heterozygous deletion
NA09216			
NA04409			heterozygous duplication
NA01353	2p24.3-p23.2	*MYCN, ALK*	heterozygous duplication
NA01221	6q21	*AIM1, SEC63*	heterozygous duplication
NA06802	6q25.3-q26	*IGF2R, PARK2*	heterozygous deletion
NA09367	6q21-q23.3	*SEC63, TNFAIP3*	heterozygous duplication
NA07994	6q23.3-q26	*TNFAIP3, LATS1, IGF2R, PARK2*	heterozygous duplication
NA03255	8p21.3	*TNFRSF10B, TNFRSF10A*	heterozygous duplication
NA14485			
NA02030	8p21.3-q24.21	*TNFRSF10B, TNFRSF10A, EIF3H, MYC*	heterozygous duplication
NA09888	8q24.11	*EIF3H*	heterozygous deletion
NA03999	8q24.21	*MYC*	heterozygous deletion
NA02819 +	9p21.3	*CDKN2A, CDKN2B*	heterozygous duplication
NA03226			
NA08618	11q22.3	*ATM*	heterozygous duplication
NA09596			heterozygous deletion
NA02819 +	12q24.33	*CHFR*	heterozygous deletion
NA07891			heterozygous duplication
NA02718	13q14.2-q14.3	*RB1, FNDC3A, KCNRG, MIR15A, DLEU2, DLEU7, ATP7B*	heterozygous deletion
NA13721			
NA14164			
NA03330			heterozygous duplication
NA05832			
Cancer cell line sample from Leibniz Institute DSMZ			
SK-N-MC◊	2p24.3	*MYCN*	ambigous #
	8p21.3-q24.21	*TNFRSF10B, TNFRSF10A, EIF3H, MYC*	gain
	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 P037-B1 CLL-1 probemix.
+ CNAs are present in two target regions in this sample: 9p21.3 (*CDKN2A/B*) and 12q24.33 (*CHFR*).
◊ CNAs detected by reference probes are not reported for this sample.
Ratios between 0.65 and 0.80 found for all probes targeting *MYCN* thus indicating a potential subclonal loss.

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	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 *MYCN, CHFR and CDK4* 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.

P037 specific note:

- Chr6p KIAA0319 reference probe (06435-L05961) and chr6q target probes AIM1 (17481-L22106) and SEC63 (17736-L21863) are consecutive in probe length (at 427, 436 and 445 nt, respectively) and in genomic location on chromosome 6. Aberrant results detected by these probes should be treated with caution and fragment separation should be carefully examined.

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 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. 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 (http://cancer.sanger.ac.uk/cosmic) and LOVD (https://databases.lovd.nl) mutation databases. Database. 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., deletion/duplication of only one probe for *MYCN* exon 2) to MRC Holland: info@mrcholland.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 « Δ	**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 the NCI TP53 Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
± SNP rs41303285 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberrations must be confirmed by another method.

Table 2. P037-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
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. 2013, Chapiro et al. 2010). Ligation sites for MYCN (2p24.3), ALK (2p32.2) and REL (2p16.1) probes are indicated according to MANE Select transcripts NM_005378.6, NM_004304.5 and NM_001291746.2, respectively.						
247 « Δ	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 10	1410-1411	TCAAGCTGGTCA-TCAGTGGCCCAC	02-061,003	47.9 **M**b to ref probe-
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* and *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 MANE Select transcript NM_001270508.2**.** **Note**: Chr6p KIAA0319 reference probe and chr6q target probes AIM1 and SEC63 (at 427, 436 and 445 nt, respectively) are consecutive in probe length and in genomic location on chromosome 6. Aberrant results detected by these probes should be treated with caution and fragment separation should be carefully examined.						
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	460-461	GTTCAGAACTTG-CCAGTTTTGTCC	06-138,234	9.7 kb
211	17472-L22159	***TNFAIP3***, exon 9	2480-2481	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) are detected in CLL (Brown et al. 2012; Rinaldi et al. 2011, Ouillette et al. 2011) with higher frequency in a subset of CLL with 17p deletion. 8p loss was						

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
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 MANE Select transcript NM_002467.6.						
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	1520-1521	GAACGAGCTAAA-ACGGAGCTTTTT	08-128,822	0.1 kb
281	17477-L22565	***MYC***, exon 3	1669-1670	AGGACTTGTTGC-GGAAACGACGAG	08-128,822	-
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 MANE Select transcript NM_000051.4.						
173	02644-L02111	***ATM***, exon 14	2321-2322	TCTTTTGGTGGG-TGTCCTTGGCTG	11-107,632	16.5 kb
337	02663-L22102	***ATM***, exon 22	3407-3406 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 MANE Select transcript NM_001306135.2.						
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***, downstream	110 Mb after exon 2	AAGAAGATCGTG-ACAAATTCCCTA	13-050,185	130.3 kb
373	17480-L21272	***DLEU7***, exon 1	438-439	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, Schetelig et al. 2008, Dreger et al. 2010).						

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
Ligation sites for TP53 (17p13.1) probes are indicated according to MANE Select transcript NM_000546.6. The *TP53* exon numbering is derived from the LRG_321 sequence; the exon numbering derived from MANE project according to NM_000546.6 for this gene can be found in between brackets.						
285 ‡	02384-L21411	***TP53***, exon 7 (8)	981-982	CTGTCCTGGGAG-AGACCGGCGCAC	17-007,518	1.4 kb
263 ‡	02376-L21409	***TP53***, exon 4b (5)	546-547	CAAGATGTTTTG-CCAACTGGCCAA	17-007,519	0.8 kb
299	17420-L21142	***TP53***, exon 3 (4)	451-450 reverse	TAGCTGCCCTGG-TAGGTTTTCTGG	17-007,520	11.6 kb
409	02263-L01749	***TP53***, exon 1 (upstream)	127 nt before exon 1	CTTCCTCCGGCA-GGCGGATTACTT	17-007,532	-

[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.

« 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.
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 the NCI TP53 Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 3. P037-B1 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
391	07808-L22560	*SCN5A*	3p22	03-038,625
500	15203-L20113	*GBE1*	3p12	03-081,775
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.
± SNP rs41303285 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberrations must be confirmed by another method.

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 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 *(RB1*).

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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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 B1-02 – 06 April 2023 (04P) - Product description rewritten and adapted to a new template. - *TP53* database name and hyperlink change in the footnotes of Table 1 and 2. - Ligation sites of the probes targeting the *REL, TNFAIP3*, *MYC*, *ATM* and *TP53* genes are updated according to recent MANE select NM_ transcripts. - Exon numbering updated and ligation site information added for DLEU7 probes in Table 2. - New references added in 'Selected publications using SALSA MLPA Probemix P037 CLL-1' section on page 11. - Added footnote about variable results for MYCN probe 17476-L22557 in Table 1 and 2. - Added footnote about SNP on DNA target for Reference probe 11424-L22558 in Table 1 and 3. - Removed P098 from the list of related probemixes, and added P377 probemix on page 10. *Version B1-01 — 23 January 2020 (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. - Notification added about data interpretation of KIAA0319 reference probe (06435-L05961) and target probes AIM1 (17481-L22106) and SEC63 (17736-L21863) on page 4 and in Table 2a. - 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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