Product Description
SALSA® MLPA® Probemix P040-B2 CLL

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 9.

Catalogue numbers:
- **P040-025R:** SALSA MLPA Probemix P040 CLL, 25 reactions.
- **P040-050R:** SALSA MLPA Probemix P040 CLL, 50 reactions.
- **P040-100R:** SALSA MLPA Probemix P040 CLL, 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 P040 CLL is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TP53* gene on 17p13, the *RB1/DLEU/MIR15A-16*-region on 13q14, the *ATM* gene on 11q22 as well the presence of trisomy 12 in DNA samples obtained from chronic lymphocytic leukemia patients.

B cell chronic lymphocytic leukemia (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 prognostic markers of this disease.

SALSA MLPA probemixes P037 and P038 contain probes for several genomic regions and genes that are recurrently imbalanced in B-CLL. This P040 probemix contains a selection of targeted genes and regions from P037 and P038.

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 *ATM*, *RB1*, *TP53* and *KCNRG* exon numbering in this P040-B2 product description is the exon numbering derived from MANE project based on the MANE Select transcripts, as indicated in Table 2. DLEU1 exon

numbering is based on NR_109973.1. The exon numbering used in previous versions of this product description can be found in between brackets in Table 1 and 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 exon numbering for the same gene might be different in other MRC Holland product descriptions, where other resources for exon numbering was used.

Probemix content

The SALSA MLPA Probemix P040-B2 CLL contains 52 MLPA probes with amplification products between 131 and 497 nucleotides (nt). This includes six probes for the *TP53* gene on 17p13, 10 probes for the *RB1/DLEU/MIR15A-16*-region on 13q14, seven probes for the *ATM* gene on 11q22 as well 11 probes on chromosome 12. In addition, 13 reference probes are included targeting relatively copy number stable regions in various cancer types including CLL. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and 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 who are from families 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

See the section Positive samples on the P040 CLL product page on our website.

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 these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/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 *CDK4* and *CHFR* 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.

P040 specific note:

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

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 P040 CLL.
- 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 patient samples 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.

Mutation databases

We strongly encourage users to deposit positive results in the COSMIC (http://cancer.sanger.ac.uk/cosmic), LOVD (https://databases.lovd.nl) and the TP53 (https://tp53.isb-cgc.org/) 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 duplication of *TP53* exons 2d and 6 but not exon 4b) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P040-B2 CLL

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				
		Reference	**11q/*ATM***	**12p/q**	**13q14**	***TP53***
64-105	Control fragments – see table in probemix content section for more information					
131	Reference probe 16316-L22397	3q21				
137	**CTTN probe** 03896-L21555		11q13.3			
142 «	**CDK4 probe** 03173-L02512			12q14.1		
148	**KCNRG probe** 04018-L04000				13q14.3	
154	Reference probe 05751-L05189	5p12				
160	**RB1 probe** 00845-L00378				13q14.2	
166	**MIR15A probe** 04019-L03416				13q14.3	
172	Reference probe 06556-L19388	1q32				
178	**PAH probe** 16488-L22395			12q23.2		
186	**TP53 probe** 01588-L21622					**Exon 1**
192 ∫	Reference probe 09224-L21967	5q23				
196	**DLEU2 probe** 04020-L22084				13q14.3	
202	**DIABLO probe** 04752-L04100			12q24.31		
208	Reference probe 04732-L22394	7q21				
214	**TP53 probe** 02375-L21623					**Exon 3 (2d)**
221	**KCNRG probe** 04017-L03414				13q14.3	
226	**DDX10 probe** 17614-L21618		11q22.3			
232	**IFNG probe** 00472-L22093			12q15		
238	**ATM probe** 02657-L21624		**Exon 4**			
244	**ATP7B probe** 16307-L22396				13q14.3	
251 +	**TP53 probe** 02376-L17746					**Exon 5 (4b)**
259	**ATM probe** 00435-L22589		**Exon 63**			
266	Reference probe 10728-L22588	6p12				
274	**TP53 probe** 17419-L21141					**Exon 7 (6)**
283	**PCSK7 probe** 17615-L21619		11q23.3			
292	**ATM probe** 08422-L08319		**Exon 18**			
298	**PSMD9 probe** 17616-L21620			12q24.31		
304	Reference probe 16436-L18889	18q21				
311	**ATM probe** 19808-L27211		**Exon 45**			
319	**LRMP probe** 00495-L03128			12p12.1		
328	**ATM probe** 08431-L08322		**Exon 36**			
334 ∫	Reference probe 21112-L22587	19p13				
342 ±	**DLEU1 probe** 01590-L22586				13q14.3	
348	**TP53 probe** 17422-L22585					**Exon 10**
355	**CCND2 probe** 00498-L00084			12p13.32		
364	**PICALM probe** 17617-L21621		11q14.2			
371	**ATM probe** 08420-L22087		**Exon 13**			
378	**DLEU1 probe** 01589-L12435				13q14.3	
386	**NCAPD3 probe** 13859-L15378		11q25			
394	Reference probe 09770-L12865	15q21				
400 «	**CHFR probe** 02684-L03126			12q24.33		
409	**CDK2 probe** 14405-L21970			12q13.2		
418	Reference probe 08665-L08675	9q31				
427	**LRRK2 probe** 04279-L16051			12q12		
436	**ATM probe** 08443-L21628		**Exon 58**			
445	**TP53 probe** 17424-L21146					**Exon 11**
454	Reference probe 13254-L22584	1p21				
462	**CD27 probe** 00678-L22089			12p13.31		
471	**DLEU7 probe** 03042-L22590				13q14.3	
479	**RB1 probe** 04502-L22091				13q14.2	
486	Reference probe 14884-L22092	21q22				
497	Reference probe 15203-L22591	3p12				

± SNP rs537991557 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ 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.
« 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.
∫ Important information on this probe can be found in and below Table 3.

Table 2. P040-B2 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
ATM at **11q22.3**. 11q deletion, which results in loss of the *ATM* gene, is found in 15-20% of CLL cases. Deletion of 11q22-q23 as well as *ATM* point mutations are associated with aggressive disease and short median survival (Döhner et al. 1997; Neilson et al. 1997; Guarini et al. 2012). All exons of the *ATM* gene are covered by P041 and P042 SALSA MLPA probemixes. The exon numbering and ligation sites of the ATM probes are indicated according to MANE Select transcript NM_000051.4.						
137	03896-L21555	***CTTN***	11q13.3	AGGCAGAGCTGA-GCTACAGAGGCC	11-069,957	15.4 **M**b
364	17617-L21621	***PICALM***	11q14.2	CCTGTAATGACG-CAACCAACCTTA	11-085,363	22.2 **M**b
238	02657-L21624	***ATM***, exon 4	410-411	AGCCTCAACACA-AGCCTCCAGGCA	11-107,605	24.7 kb
371	08420-L22087	***ATM***, exon 13	2174-2175	AGAAAAGCACCA-GTCCAGTATTGG	11-107,630	14.6 kb
292	08422-L08319	***ATM***, exon 18	2885-2886	AACTACTGCTCA-GACCAATACTGT	11-107,644	34.4 kb
328	08431-L08322	***ATM***, exon 36	5562-5563	AATCATGACATT-TGGATAAAGACA	11-107,679	18.4 kb
311	19808-L27211	***ATM***, exon 45	6658-6659	TGTATTCGCTCT-ATCCCACACTTA	11-107,697	24.5 kb
436	08443-L21628	***ATM***, exon 58	8671-8672	AAAAATTCTTGG-ATCCAGCTATTT	11-107,722	19.6 kb
259	00435-L22589	***ATM***, exon 63	9273-9274	CAGGCCATAGAC-CCCAAAAATCTC	11-107,741	575.3 kb
226 #	17614-L21618	***DDX10***	11q22.3	TCATTGGAAACA-CTGCCTTTGTCT	11-108,317	8.3 **M**b
283	17615-L21619	***PCSK7***	11q23.3	AGCCGGGCTCTT-CTTACTGGTTCC	11-116,606	17 **M**b
386	13859-L15378	***NCAPD3***	11q25	TGGGCAATCTGA-TTAACCTCTGTT	11-133,596	-
Trisomy 12 is a frequent aberration in CLL (10-20% of patients). Treatment response and overall survival is favourable (Hallek et al. 2010) or intermediate (Gunnarsson et al. 2011) in the cases with trisomy 12. Atypical lymphocyte morphology is observed in some cases of CLL with trisomy 12 (Matutes et al. 1996).						
355	00498-L00084	***CCND2***	12p13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	12-004,279	2.2 **M**b
462	00678-L22089	***CD27***	12p13.31	GTGGAGCCTGCA-GAGCCTTGTCGT	12-006,431	18.7 **M**b
319	00495-L03128	***LRMP***	12p12.1	GTCTCTAGAACA-TATCTTGTGGCC	12-025,152	13.8 **M**b
427	04279-L16051	***LRRK2***	12q12	TCTTCTCATGTA-AACTGTTTTGGT	12-038,932	15.7 **M**b
409	14405-L21970	***CDK2***	12q13.2	CATTGTTTCAAG-TTGGCCAAATTG	12-054,647	1.8 **M**b
142 «	03173-L02512	***CDK4***	12q14.1	AACCCTGGTGTT-TGAGCATGTAGA	12-056,431	10.4 **M**b
232	00472-L22093	***IFNG***	12q15	GATGGCTGAACT-GTCGCCAGCAGC	12-066,835	35 **M**b
178	16488-L22395	***PAH***	12q23.2	AGTTAGATGCAA-TGAAAAGAACAC	12-101,831	19 **M**b
298	17616-L21620	***PSMD9***	12q24.31	GCCCACAAAGAG-GCCATGAGCCGC	12-120,822	445.2 kb
202	04752-L04100	***DIABLO***	12q24.31	TGAAGTGTGGCA-GGTGATCATAGG	12-121,267	10.7 **M**b
400 «	02684-L03126	***CHFR***	12q24.33	GACATGCCCTTT-ACAGACTGGGGA	12-131,959	-
13q14 deletion is the most common (~50%) chromosomal aberration in CLL and is characterized by favourable outcome when present as sole abnormality. Larger 13q deletion size predicts poorer outcome (Gunnarsson et al. 2011). *DLEU2/MIR15A/16-1* gene cluster, as well as *RB1* gene are important tumour suppressor candidates within 13q14 deletion region (Klein et al. 2010). The exon numbering and ligation sites of the RB1 and KCNRG probes are according to MANE Select Transcripts NM_000321.3 and NM_173605.2, respectively. The exon numbering of DLEU1 probes is based on NR_109973.1.						
479	04502-L22091	***RB1***, upstream	161 nt before exon 1	GAAGGCGCCTGG-ACCCACGCCAGG	13-047,776	77.7 kb
160	00845-L00378	***RB1***, exon 17	1689-1690	CTTGATTCTGGA-ACAGATTTGTCT	13-047,853	1.6 **M**b
221	04017-L03414	***KCNRG***, exon 1	39-40	CTCTAGTTTGAA-GTGAGGGAAGAA	13-049,488	5.1 kb
148	04018-L04000	***KCNRG***, exon 3	1028-1029	GCTTAAGCCATA-ATGCCTGCTGCT	13-049,493	28.5 kb
166	04019-L03416	***MIR15A***	13q14.3	TGGATTTTGAAA-AGGTGCAGGCCA	13-049,521	33.0 kb
196	04020-L22084	***DLEU2***	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	13-049,554	228.1 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
378	01589-L12435	***DLEU1***, downstream	203 kb after exon 3	CCTTTTAATAGG-ATCTCTCCTGGA	13-049,782	91.0 kb
342 ±	01590-L22586	***DLEU1***, downstream	294 kb after exon 3	ACTCTCCCTTGT-ACAGTTAGCTGT	13-049,873	311.6 kb
471	03042-L22590	***DLEU7***	13q14.3	AAGAAGATCGTG-ACAAATTCCCTA	13-050,185	1.2 **M**b
244	16307-L22396	***ATP7B***	13q14.3	GAACCTTCCTGA-GGGGCAGTGTGG	13-051,416	-
TP53 at **17p13.1**. *TP53* is the most frequently mutated/deleted gene in CLL cases with 17p deletion. Del(17p), and also *TP53* mutations are associated with more aggressive clinical course, worse prognosis, short overall survival, thus belong to ultra-high risk CLL (Mougalian and O'Brien, 2011). Detection of TP53 locus deletion/mutation is important for therapy strategy (Stilgenbauer and Zenz, 2010; Schetelig et al. 2008; Dreger et al. 2010). All exons of *TP53* gene are covered by the P056 SALSA MLPA probemix. Ligation sites and exon numbering for TP53 (17p13.1) probes are indicated according to MANE Select transcript NM_000546.6. The exon numbering used in previous versions of this product description can be found in between brackets.						
445	17424-L21146	***TP53***, exon 11	1300-1301	CTCATGTTCAAG-ACAGAAGGGCCT	17-007,514	1.0 kb
348	17422-L22585	***TP53***, exon 10	1188-1189	TGAGGCCTTGGA-ACTCAAGGATGC	17-007,515	3.6 kb
274	17419-L21141	***TP53***, exon 7 (6)	831-832	CTCTGACTGTAC-CACCATCCACTA	17-007,518	0.9 kb
251 +	02376-L17746	***TP53***, exon 5 (4b)	546-547	CAAGATGTTTTG-CCAACTGGCCAA	17-007,519	1.2 kb
214	02375-L21623	***TP53***, exon 3 (2d)	230-231	TTCCTGAAAACA-ACGTTCTGGTAA	17-007,520	11.0 kb
186	01588-L21622	***TP53***, exon 1	58-59	TCCGGGGACACT-TTGCGTTCGGGC	17-007,531	-

[a] See section Exon numbering on page 1 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.

± SNP rs537991557 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
+ 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.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Location (hg18) in kb
454	13254-L22584	*COL11A1*	1p21	01-103,251
172	06556-L19388	*TNNT2*	1q32	01-199,604
497	15203-L22591	*GBE1*	3p12	03-081,775
131	16316-L22397	*RAB7A*	3q21	03-130,000
154	05751-L05189	*HCN1*	5p12	05-045,498
192 ∫	09224-L21967	*LMNB1*	5q23	05-126,186
266	10728-L22588	*PKHD1*	6p12	06-051,720
208	04732-L22394	*ABCB4*	7q21	07-086,879
418	08665-L08675	*ALDOB*	9q31	09-103,233
394	09770-L12865	*SPG11*	15q21	15-042,709
304	16436-L18889	*MYO5B*	18q21	18-045,659
334 #	21112-L22587	*CACNA1A*	19p13	19-013,331
486	14884-L22092	*KCNJ6*	21q22	21-037,920

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

∫ 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.

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 aberration(s) must be confirmed by another method.

Related products

For related products, see the product page on our website.

References

- Döhner H et al. (1997) 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood.* 89:2516-22.
- Dreger P et al. (2016) Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood.* 116:2438-47.
- Guarini A et al. (2012) ATM gene alterations in chronic lymphocytic leukemia patients induce a distinct gene expression profile and predict disease progression. *Haematologica.* 97:47-55.
- Gunnarsson R et al. (2011) Array-based genomic screening at diagnosis and during follow-up in chronic lymphocytic leukemia. *Haematologica.* 96:1161-9.
- Hallek M et al. (2010) Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet.* 376:1164-74.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Klein U et al. (2010) The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell.* 17:28-40.
- Neilson JR et al. (1997) Deletions at 11q identify a subset of patients with typical CLL who show consistent disease progression and reduced survival. *Leukemia.* 11:1929-32.
- Matutes E et al. (1996) Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. *Br J Haematol*. 92:382-8.
- Mougalian SS and O'Brien S (2011) Adverse prognostic features in chronic lymphocytic leukemia. *Oncology (Williston Park).* 25:692-6, 699.
- Schetelig J et al. (2008) Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European Group for Blood and Marrow Transplantation analysis. *J Clin Oncol*. 26:5094-100.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Stilgenbauer S and Zenz T (2010) Understanding and managing ultra high-risk chronic lymphocytic leukemia. *Hematology. Am Soc Hematol Educ Program*. 2010:481-8.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P040 CLL

- Abdool A et al. (2010) Detection, analysis and clinical validation of chromosomal aberrations by multiplex ligation-dependent probe amplification in chronic leukemia. *PLoS One.* 25;5:e15407.
- Al Zaabi E et al. (2010) Multiplex Ligation-Dependent Probe Amplification Versus Multiprobe Fluorescence in Situ Hybridization To Detect Genomic Aberrations in Chronic Lymphocytic Leukemia. A Tertiary Center Experience. *J Mol Diagn*. 12:197-203.

- Bănescu C et al. (2019) Presence of copy number aberrations and clinical prognostic factors in patients with acute myeloid leukemia: an analysis of effect modification. *Pol Arch Intern Med*. 129:898-906.
- Buijs A et al. (2006) Detection of risk-identifying chromosomal abnormalities and genomic profiling by multiplex ligation-dependent probe amplification in chronic lymphocytic leukemia. *Haematologica.* 91:1434-5.
- Coll-Mulet L et al. (2008) Multiplex ligation-dependent probe amplification for detection of genomic alterations in chronic lymphocytic leukaemia. *Br J Haematol*. 142:793-801.
- Fabris S et al. (2011) Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization to detect chromosomal abnormalities in chronic lymphocytic leukemia: a comparative study. *Genes Chromosomes Cancer*. 50:726-34.
- Groenen P et al. (2011) High prevalence of adverse prognostic genetic aberrations and unmutated IGHV genes in small lymphocytic lymphoma as compared to chronic lymphocytic leukemia. *J Hematopathol*. 4:189-97.
- Hanlon K et al. (2009) Evaluation of 13q14 status in patients with chronic lymphocytic leukemia using single nucleotide polymorphism-based techniques. *J Mol Diagn*. 11:298-305.
- Mareckova A et al. (2019) ATM and TP53 mutations show mutual exclusivity but distinct clinical impact in mantle cell lymphoma patients. *Leuk Lymphoma.* 60:1420-8.
- Rani L et al. (2019) Comparative assessment of prognostic models in chronic lymphocytic leukemia: evaluation in Indian cohort. *Ann Hematol*. 98:437-43.
- Santidrián AF et al. (2007) The potential anticancer agent PK11195 induces apoptosis irrespective of p53 and ATM status in chronic lymphocytic leukemia cells. *Haematologica*. 92:1631-8.
- Stevens-Kroef M et al. (2009) Identification of chromosomal abnormalities relevant to prognosis in chronic lymphocytic leukemia using multiplex ligation-dependent probe amplification. *Cancer Genet Cytogenet*. 195:97-104.

P040 product history	
Version	*Modification*
B2	Several probes have a small change in length, but no change in the sequence detected.
B1	Majority target probes and all reference probes are replaced. All probes on 11p are removed and the 88 and 96 nt control fragments have been replaced.
A3	Two extra control fragments have been added.
A2	First unrestricted release.

Implemented changes in the product description

Version B2-04 – 28 July 2025 (04P)
- Positive control DNA samples section: information moved to product page on website.
- Removed Related SALSA MLPA products section.
- Exon numbering of the *TP53* gene is now reported based on MANE Select Transcript NM_000546.6 in Table 1 and 2 (removed LRG_321).

Version B2-03 – 06 June 2023 (04P)
- Product description rewritten and adapted to a new template.
- Added new samples in the ‘ Positive control DNA samples’ section on page 3.
- Exon numbering of the *TP53* gene is now also reported based on MANE Select Transcript NM_000546.6 in addition to LRG_321.
- Ligation sites of probes targeting *ATM*, *RB1*, *KCNRG* and *TP53* genes have been added based on the MANE Select NM_ sequence.
- 04502-L22091 RB1 probe exon information has changed from ‘exon 1’ to ‘upstream’.
- Ligation site information added for RB1 and DLEU1 probes in Table 2.
- Warning added to Tables 2 and 3 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- A new reference added on page 10.

Version B2-02 – 12 November 2019 (02P)
- Minor layout changes.

- One new reference added to the selected publications using P040 on page 8.

Version B2-01 - 26 June 2019 (02P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2) and adapted to a new template.
- New positive control DNA samples added on page 2.
- Various minor textual or layout changes.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

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