Product Description SALSA® MLPA® Probemix P040-B2 CLL

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, several probes have a small change in length, but no change in the sequence detected. For complete product history see page 8.

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.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 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 are 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 highly 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.

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/

Exon numbering:
The *ATM, TP53* and *RB1* exon numbering used in this P040-B2 CLL product description is the exon numbering from the LRG_135, LRG_321 and LRG_517 sequences, respectively, and for *KCNRG* according to NM_173605.2 sequence. The exon numbering used has been retrieved on 06/2019. As changes to the LRG and NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

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 that target relatively copy number stable regions in CLL. Complete probe sequences are available online (www.mlpa.com) and the identity of the genes detected by the reference probes is available in the Table 2.

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, 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 healthy without a history of CLL. 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. Samples from the Coriell Institute have been tested with this P040-B2 probemix at MRC-Holland and can be used as a positive control samples as indicated 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 P040-B2	Expected CNA
NA00959	Coriell Institute	11q13.3-q25	*CTTN, PICALM, ATM, DDX10, PCSK7* and *NCAPD3*	Heterozygous duplication
NA08618	Coriell Institute	11q22.3	*ATM* and *DDX10*	Heterozygous duplication
NA09596	Coriell Institute	11q14.1-q23.1	*PICALM, ATM* and *DDX10*	Heterozygous deletion
NA07981	Coriell Institute	12p13.33-p11.1	*CCND2, CD27* and *LRMP*	Amplification
NA03330	Coriell Institute	13q11-q34	*RB1, KCNRG, MIR15A, DLEU2, DLEU1, DLEU7* and *ATP7B*	Heterozygous duplication
NA13721	Coriell Institute	13q14.11-q32.1	*RB1, KCNRG, MIR15A, DLEU2, DLEU1, DLEU7* and *ATP7B*	Heterozygous deletion
SK-N-MC	DSMZ	17p13.1	*TP53*	Homozygous deletion of exon 2 and heterozygous deletion of other exons

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

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

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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, many genetic alterations in chromosomal regions included in this probemix 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. 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 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: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC mutation 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 SNPs and unusual results (e.g. a deletion of TP53 exons 6 and 11 but not exon 10) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P040-B2 CLL

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				
		Reference	Chr. 11/ATM	Chr. 12	Chr. 13	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 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 4b**
259	**ATM probe** 00435-L22589		**Exon 63**			
266	Reference probe 10728-L22588	6p12				
274	**TP53 probe** 17419-L21141					**Exon 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			12**p**12.1		
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			12**p**13.32		
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			12**p**13.31		
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				

¥ Changed in version B2. Minor alteration, no change in sequence detected.
« 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.

Table 2. P040-B2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon	Chr. band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Reference probes on chromosomes 1, 3, 5, 6, 7 and 9.						
454	13254-L22584	COL11A1	1p21	CCAATGGGTCTA-ACTGGAAGACCA	96.4 **M**b	01-103.251
172	06556-L19388	TNNT2	1q32	AGCGGAAGAGGA-TGCTGAAGCAGA	-	01-199.604
497	15203-L22591	GBE1	3p12	GACCTAGAGGGA-CTCATGATCTTT	48.2 **M**b	03-081.775
131	16316-L22397	RAB7A	3q21	CACAATAGGAGC-TGACTTTCTGAC	-	03-130.000
154	05751-L05189	HCN1	5p12	GTCTTCAGTTCT-TAGTACCACTAC	80.7 **M**b	05-045.498
192	09224-L21967	LMNB1	5q23	AAATATACCTCA-AGATATGTGCTG	-	05-126.186
266	10728-L22588	PKHD1	6p12	ATTCTACCAAAT-GCAGAGAACAGT	-	06-051.720
208	04732-L22394	ABCB4	7q21	TTTCGATTTGGT-GCATATCTCATT	-	07-086.879
418	08665-L08675	ALDOB	9q31	GCTCTCAGAAAT-TGCCCAGAGCAT	-	09-103.233
ATM at **11q22.3**. Indicated exons for *ATM* are according to LRG_135. 11q deletion, which results in loss of *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.						
137	03896-L21555	CTTN	11q13.3	AGGCAGAGCTGA-GCTACAGAGGCC	15.4 **M**b	11-069.957
364	17617-L21621	PICALM	11q14.2	CCTGTAATGACG-CAACCAACCTTA	22.2 **M**b	11-085.363
238	02657-L21624	ATM, ex 4	11q22.3	AGCCTCAACACA-AGCCTCCAGGCA	24.7 kb	11-107.605
371	08420-L22087	ATM, ex 13	11q22.3	AGAAAAGCACCA-GTCCAGTATTGG	14.6 kb	11-107.630
292	08422-L08319	ATM, ex 18	11q22.3	AACTACTGCTCA-GACCAATACTGT	34.4 kb	11-107.644
328	08431-L08322	ATM, ex 36	11q22.3	AATCATGACATT-TGGATAAAGACA	18.4 kb	11-107.679
311	19808-L27211	ATM, ex 45	11q22.3	TGTATTCGCTCT-ATCCCACACTTA	24.5 kb	11-107.697
436	08443-L21628	ATM, ex 58	11q22.3	AAAAATTCTTGG-ATCCAGCTATTT	19.6 kb	11-107.722
259	00435-L22589	ATM, ex 63	11q22.3	CAGGCCATAGAC-CCCAAAAATCTC	575.3 kb	11-107.741
226	17614-L21618	DDX10	11q22.3	TCATTGGAAACA-CTGCCTTTGTCT	8.3 **M**b	11-108.317
283	17615-L21619	PCSK7	11q23.3	AGCCGGGCTCTT-CTTACTGGTTCC	17.0 **M**b	11-116.606
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	12**p**13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	2.2 **M**b	12-004.279
462	00678-L22089	CD27	12**p**13.31	GTGGAGCCTGCA-GAGCCTTGTCGT	1.9 **M**b	12-006.431
319	00495-L03128	LRMP	12**p**12.1	GTCTCTAGAACA-TATCTTGTGGCC	13.8 **M**b	12-025.152
427	04279-L16051	LRRK2	12q12	TCTTCTCATGTA-AACTGTTTTGGT	15.7 **M**b	12-038.932
409	14405-L21970	CDK2	12q13.2	CATTGTTTCAAG-TTGGCCAAATTG	1.8 **M**b	12-054.647
142 «	03173-L02512	CDK4	12q14.1	AACCCTGGTGTT-TGAGCATGTAGA	10.4 **M**b	12-056.431
232	00472-L22093	IFNG	12q15	GATGGCTGAACT-GTCGCCAGCAGC	35.0 **M**b	12-066.835
178	16488-L22395	PAH	12q23.2	AGTTAGATGCAA-TGAAAAGAACAC	19.0 **M**b	12-101.831
298	17616-L21620	PSMD9	12q24.31	GCCCACAAAGAG-GCCATGAGCCGC	445.2 kb	12-120.822
202	04752-L04100	DIABLO	12q24.31	TGAAGTGTGGCA-GGTGATCATAGG	10.7 **M**b	12-121.267
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). Indicated exons for *RB1* are according to LRG_517 and for *KCNRG* according to NM_173605.2 sequence.						
479	04502-L22091	RB1, ex 1	13q14.2	GAAGGCGCCTGG-ACCCACGCCAGG	77.7 kb	13-047.776
160	00845-L00378	RB1, ex 17	13q14.2	CTTGATTCTGGA-ACAGATTTGTCT	1.6 **M**b	13-047.853
221	04017-L03414	KCNRG, ex 1	13q14.3	CTCTAGTTTGAA-GTGAGGGAAGAA	5.1 kb	13-049.488
148	04018-L04000	KCNRG, ex 3	13q14.3	GCTTAAGCCATA-ATGCCTGCTGCT	28.5 kb	13-049.493
166	04019-L03416	MIR15A	13q14.3	TGGATTTTGAAA-AGGTGCAGGCCA	33.0 kb	13-049.521
196	04020-L22084	DLEU2	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	228.1 kb	13-049.554
378	01589-L12435	DLEU1	13q14.3	CCTTTTAATAGG-ATCTCTCCTGGA	91.0 kb	13-049.782
342	01590-L22586	DLEU1	13q14.3	ACTCTCCCTTGT-ACAGTTAGCTGT	311.6 kb	13-049.873
471	03042-L22590	DLEU7	13q14.3	AAGAAGATCGTG-ACAAATTCCCTA	1.2 **M**b	13-050.185
244	16307-L22396	ATP7B	13q14.3	GAACCTTCCTGA-GGGGCAGTGTGG	-	13-051.416
Reference probe on chromosome 15.						
394	09770-L12865	SPG11	15q21	GAGCTGATACCA-GCATTGGATTTA	-	15-042.709

Length (nt)	SALSA MLPA probe	Gene/Exon	Chr. band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
TP53 at **17p13.1**. Indicated exons for *TP53* are according to LRG_321. *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.						
445	17424-L21146	TP53, ex 11	17p13.1	CTCATGTTCAAG-ACAGAAGGGCCT	1.0 kb	17-007.514
348	17422-L22585	TP53, ex 10	17p13.1	TGAGGCCTTGGA-ACTCAAGGATGC	3.6 kb	17-007.515
274	17419-L21141	TP53, ex 6	17p13.1	CTCTGACTGTAC-CACCATCCACTA	0.9 kb	17-007.518
251 ‡	02376-L17746	TP53, ex 4b	17p13.1	CAAGATGTTTTG-CCAACTGGCCAA	1.2 kb	17-007.519
214	02375-L21623	TP53, ex 2d	17p13.1	TTCCTGAAAACA-ACGTTCTGGTAA	11.0 kb	17-007.520
186	01588-L21622	TP53, ex 1	17p13.1	TCCGGGGACACT-TTGCGTTCGGGC	-	17-007.531
Reference probes on chromosomes 18, 19 and 21.						
304	16436-L18889	MYO5B	18q21	AACTGCAGCTTA-GCGTGTTGCTTT	-	18-045.659
334	21112-L22587	CACNA1A	19p13	GTGCTGACTGTT-TTCCAGTGCATA	-	19-013.331
486	14884-L22092	KCNJ6	21q22	CTCGAAGCTCCT-ACATCACCAGTG	-	21-037.920

‡ 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.
« 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.

a) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P037/P038 CLL: contain additional probes targeting more genomic regions implicated in CLL.
- P041/P042 ATM: contain additional probes for *ATM*.
- P056 TP53: contains additional probes for *TP53*.

References

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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 B2-02 – 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).

Version 11 – 11 December 2015 (T08)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification products.
- Footnote added for Table 1 and 2 regarding the TP53 probe 02376-L17746.
- Various minor textual changes.
- Manufacturer's address adjusted.

Version 10 – 12 August 2015 (54)
- Various minor textual changes.
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.
- "Peak area" replaced with "peak height".

Version 09 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 08 (48)
- Sample picture 2 and 3 are modified (arrows added) and accordingly the captions are adapted.

Version 07 (48)
- Header row repeated in second page of Table 2.

Version 06 (48)
- Product description adapted to a new product version (version number changed, lot number added, Table 1 and Table 2 modified, new pictures included).
- Various minor textual changes on the whole document, various minor layout changes.
- References added on page 1 and 2.
- Data analysis method has been modified.

Version 05 (45)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1, various minor layout changes, tables have been numbered.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New reference added on page 1, data analysis section has been modified.

Version 04
- New picture (figure 2) has been included on p.6
- Data analysis – paragraph 'When only a small…' has been changed.
- "*Complete probe sequences are available on request"* added on page 4.

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