

Product Description

SALSA® MLPA® Probemix P040-B3 CLL

To be used with the MLPA General Protocol.

Version B3

As compared to version B2, two reference probes have been replaced. For complete product history see page 10.

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

SALSA® MLPA® Probemix P040 CLL (hereafter: P040 CLL) is to be used in combination with:

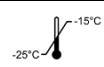

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P040-025R	P040-050R	P040-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		
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A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis

Information regarding 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

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 (CLL) 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 product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <https://www.ncbi.nlm.nih.gov/gene>

For NM_ mRNA reference sequences: <https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide>

Matched Annotation from NCBI and EMBL-EBI (MANE): <https://www.ncbi.nlm.nih.gov/refseq/MANE>

Tark – Transcript Archive: <https://tark.ensembl.org>

Exon numbering

The *ATM*, *RB1*, *TP53*, and *KCNRG* exon numbering used in this P040-B3 CLL product description is the exon numbering derived from MANE project (release version 1.4) based on MANE Select transcripts as indicated in Table 2. *DLEU1* exon numbering is based on NR_109973.1. 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 used for exon numbering are indicated.

Probemix content

P040-B3 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 as five probes upstream and downstream of the *ATM* gene, and 11 probes on chromosome 12. In addition, 13 reference probes are included that target relatively copy number stable regions in various cancer types including CLL. Partial probe sequences and the identity of the genes detected by the reference probes 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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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 formalin-fixed, paraffin-embedded (FFPE) tissues, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples

A sufficient number (≥ 3) of different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from healthy individuals without a history of cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (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 should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region *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: <https://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, none of which will be detected by using 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 in 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 in sequence data 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 <https://varnomen.hgvs.org>.

Please report false positive results due to SNVs and unusual results (e.g., a deletion of *TP53* exons 7 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

Table 1. P040-B3 CLL

Length (nt)	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	
193 *	Reference probe 17863-L31585	19q13				
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	
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	
259	ATM probe 00435-L22589		Exon 63			
266	Reference probe 10728-L22588	6p12				
274	TP53 probe 17419-L21141				Exon 7	
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 21423-L33230	5p13				
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				

^a See section Exon numbering on page 2 for more information.

* New in version B3.

+ 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P040-B3 probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene/Exon ^a	Location / Ligation site ^b	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
<p>ATM at 11q22.3. 11q deletion, which results in loss of the <i>ATM</i> gene, is found in 15-20% of CLL cases. Deletion of 11q22-q23 as well as <i>ATM</i> 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 <i>ATM</i> gene are covered by P041 and P042 SALSA MLPA probemixes. The exon numbering and ligation sites of the <i>ATM</i> probes are indicated according to MANE Select transcript NM_000051.4.</p>						
137	03896-L21555	CTTN	11q13.3	AGGCAGAGCTGA-GCTACAGAGGCC	11-069,957	15.4 Mb
364	17617-L21621	PICALM	11q14.2	CCTGTAATGACG-CAACCAACCTTA	11-085,363	22.2 Mb
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-GACCAACTACTGT	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	TCATTGGAACA-CTGCCTTGTCT	11-108,317	8.3 Mb
283	17615-L21619	PCSK7	11q23.3	AGCCGGGCTCTT-CTTACTGGTTCC	11-116,606	17 Mb
386	13859-L15378	NCAPD3	11q25	TGGCAATCTGA-TTAACCTCTGTT	11-133,596	-
<p>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).</p>						
355	00498-L00084	CCND2	12p13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	12-004,279	2.2 Mb
462	00678-L22089	CD27	12p13.31	GTGGAGCCTGCA-GAGCCTTGTCGT	12-006,431	18.7 Mb
319	00495-L03128	LRMP	12p12.1	GTCTCTAGAACA-TATCTTGTTGCC	12-025,152	13.8 Mb
427	04279-L16051	LRRK2	12q12	TCTTCTCATGTA-AACTGTTTTGGT	12-038,932	15.7 Mb
409	14405-L21970	CDK2	12q13.2	CATTGTTTCAAG-TTGGCCAAATTG	12-054,647	1.8 Mb
142 «	03173-L02512	CDK4	12q14.1	AACCCTGGTGT-TGAGCATGTAGA	12-056,431	10.4 Mb
232	00472-L22093	IFNG	12q15	GATGGCTGAACT-GTCGCCAGCAGC	12-066,835	35 Mb
178	16488-L22395	PAH	12q23.2	AGTTAGATGCAA-TGAAAAGAACAC	12-101,831	19 Mb
298	17616-L21620	PSMD9	12q24.31	GCCCACAAGAG-GCCATGAGCCGC	12-120,822	445.2 kb
202	04752-L04100	DIABLO	12q24.31	TGAAGTGTGGCA-GGTGATCATAGG	12-121,267	10.7 Mb
400	02684-L03126	CHFR	12q24.33	GACATGCCCTTT-ACAGACTGGGGA	12-131,959	-
<p>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). <i>DLEU2/MIR15A/16-1</i> gene cluster, as well as <i>RB1</i> gene are important tumour suppressor candidates within 13q14 deletion region (Klein et al. 2010). The exon numbering and ligation sites of the <i>RB1</i> and <i>KCNRG</i> probes are according to MANE Select Transcripts NM_000321.3 and NM_173605.2, respectively. The exon numbering of <i>DLEU1</i> probes is based on NR_109973.1.</p>						
479	04502-L22091	RB1 , upstream	161 nt before exon 1	GAAGGCGCTGG-ACCCACGCCAGG	13-047,776	77.7 kb
160	00845-L00378	RB1 , exon 17	1689-1690	CTTGATTCTGGA-ACAGATTGTCT	13-047,853	1.6 Mb
221	04017-L03414	KCNRG , exon 1	39-40	CTCTAGTTTGAA-GTGAGGGAAGAA	13-049,488	5.1 kb
148	04018-L04000	KCNRG , exon 2	1028-1029	GCTTAAGCCATA-ATGCCTGCTGCT	13-049,493	28.5 kb
166	04019-L03416	MIR15A	13q14.3	TGGATTTGAAA-AGGTGCAGGCCA	13-049,521	33.0 kb

Length (nt)	MLPA probe	Gene/Exon ^a	Location / Ligation site ^b	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
196	04020-L22084	DLEU2	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	13-049,554	228.1 kb
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-ACAAATTCCTA	13-050,185	1.2 Mb
244	16307-L22396	ATP7B	13q14.3	GAACCTCCTGA-GGGGCAGTGTGG	13-051,416	-
<p>TP53 at 17p13.1. <i>TP53</i> is the most frequently mutated/deleted gene in CLL cases with 17p deletion. Del(17p), and also <i>TP53</i> 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 <i>TP53</i> locus deletion/mutation is important for therapy strategy (Stilgenbauer and Zenz, 2010; Schetelig et al. 2008; Dreger et al. 2010). All exons of <i>TP53</i> gene are covered by the P056 SALSA MLPA probemix.</p> <p>Ligation sites and exon numbering for <i>TP53</i> probes are indicated according to MANE Select transcript NM_000546.6.</p>						
445	17424-L21146	TP53 , exon 11	1300-1301	CTCATGTTCAAG-ACAGAAGGGCCT	17-007,514	1.0 kb
348	17422-L22585	TP53 , exon 10	1188-1189	TGAGGCCTTGA-ACTCAAGGATGC	17-007,515	3.6 kb
274	17419-L21141	TP53 , exon 7	831-832	CTCTGACTGTAC-CACCATCCACTA	17-007,518	0.9 kb
251 +	02376-L17746	TP53 , exon 5	546-547	CAAGATGTTTTG-CCAACTGGCCAA	17-007,519	1.2 kb
214	02375-L21623	TP53 , exon 3	230-231	TTCCTGAAAACA-ACGTTCTGGTAA	17-007,520	11.0 kb
186	01588-L21622	TP53 , exon 1	58-59	TCCGGGGACACT-TTGCCTTCGGGC	17-007,531	-

^a See section Exon numbering on page 2 for more information.

^b Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

+ 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.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene	Chromosomal band (hg18)	Location (hg18) in kb
454	13254-L22584	<i>COL11A1</i>	1p21	01-103,251
172	06556-L19388	<i>TNNT2</i>	1q32	01-199,604
497	15203-L22591	<i>GBE1</i>	3p12	03-081,775
131	16316-L22397	<i>RAB7A</i>	3q21	03-130,000
334	21423-L33230	<i>NIPBL</i>	5p13	05-037,093
154	05751-L05189	<i>HCN1</i>	5p12	05-045,498
266	10728-L22588	<i>PKHD1</i>	6p12	06-051,720
208	04732-L22394	<i>ABCB4</i>	7q21	07-086,879
418	08665-L08675	<i>ALDOB</i>	9q31	09-103,233
394	09770-L12865	<i>SPG11</i>	15q21	15-042,709
304	16436-L18889	<i>MYO5B</i>	18q21	18-045,659
193	17863-L31585	<i>SLC7A9</i>	19q13	19-038,043
486	14884-L22092	<i>KCNJ6</i>	21q22	21-037,920

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

Related products

For related products, see the [product page](#) on our website.

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P040 product history	
Version	Modification
B3	Two reference probes have been replaced.
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 B3-01 – 18 May 2026 (05P)

- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Remark on SNP rs537991557 for DLEU1 probe 01590-L22586 in Table 1 and Table 2 removed.
- Remark regarding denaturation issues for CHFR probe 02684-L03126 in Table 1 and Table 2 removed.


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.

More information: www.mrcholland.com; www.mrcholland.eu

	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

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