

Product Description SALSA® MLPA® Probemix P041-B1 ATM-1 & P042-B2 ATM-2

To be used with the MLPA General Protocol.

P041 Version B1. Released November 2014.

P042 Version B2. As compared to version B1, one probe has a small change in length. No change in sequence detected. For complete product history see page 10.

Catalogue numbers:

- **P041-025R:** SALSA MLPA probemix P041 ATM-1, 25 reactions.
- **P041-050R:** SALSA MLPA probemix P041 ATM-1, 50 reactions.
- **P041-100R:** SALSA MLPA probemix P041 ATM-1, 100 reactions.

- **P042-025R:** SALSA MLPA probemix P042 ATM-2, 25 reactions.
- **P042-050R:** SALSA MLPA probemix P042 ATM-2, 50 reactions.
- **P042-100R:** SALSA MLPA probemix P042 ATM-2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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.

Intended use: The SALSA MLPA probemixes P041 ATM-1 and P042 ATM-2 are in vitro diagnostic (IVD)¹ or research use only (RUO) assays for the detection of deletions or duplications in the human *ATM* gene in order to confirm a potential cause and clinical diagnosis for Ataxia-Telangiectasia or hereditary predisposition to develop cancer, including but not limited to breast cancer. This product can also be used for molecular genetic testing of at-risk family members.

This assay is for use with human DNA extracted from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P041/P042 probemixes must be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in *ATM* are point mutations, none of which will be detected by MLPA. It is therefore recommended to use these SALSA MLPA probemixes in combination with sequence analysis of the *ATM* gene.

This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must be interpreted by a molecular geneticist or equivalent.

¹Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Mutations in the *ATM* gene cause Ataxia-Telangiectasia (A-T, also known as Louis-Bar syndrome). A-T is an autosomal recessive disorder affecting the nervous system, immune system and several other organs. It is characterised by progressive cerebellar ataxia, telangiectases, and a predisposition to malignancy, particularly leukaemia and lymphoma. A-T patients often have a weakened immune system and develop chronic lung infections. It occurs in 1 in 40,000 to 100,000 people worldwide.

The ATM protein is a member of the phosphatidylinositol-3 kinase family of proteins that respond to DNA damage by phosphorylating key substrates involved in DNA repair and/or cell cycle control. This could

explain the increased risk in ATM-heterozygotes of developing malignancies, in particular breast cancer. Around 1% of breast cancer patients harbour mutations in *ATM* (Buys et al. 2017, Lerner-Ellis et al. 2015). The relative risk for developing breast cancer is estimated to be two to four fold compared to the general population (Tavtigian et al. 2009, Thompson et al. 2005). Germline heterozygous pathogenic *ATM* variants have also been reported in several types of leukaemia and lymphoma and hereditary pancreatic cancer (Bullrich et al. 1999, Oguchi et al. 2003, Roberts et al. 2012).

Gene structure: The *ATM* gene spans ~146 kilobases (kb) on chromosome 11q22.3. The *ATM* LRG_135 is available at www.lrg-sequence.org and is identical to Genbank NG_009830.1.

Transcript variants: For *ATM*, multiple transcript variants have been described. Transcript variant 2 (NM_000051.3; 13147 nt; coding sequence 386-9556; <https://www.ncbi.nlm.nih.gov/gene/472>) is a reference standard in the NCBI RefSeq project. The ATG start site is located in exon 2 (386-388) and the stop codon is located in exon 63 (9554-9556). Transcript variant 1 differs from transcript variant 2 in the 5' UTR. Both transcripts encode the same isoform (a).

Exon numbering: The exon numbering used in this P041-B1 P042-B2 product description is the exon numbering from RefSeq transcript NM_000051.3, which is identical to the LRG_135 sequence (exon 1-63). The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

Probemix content: The P041-B1 and the P042-B2 probemixes each contain 34 probes for the *ATM* gene. When used together, a probe for each *ATM* exon is present, including one probe for intron 1, two probes for exon 1 and 61 and two probes for intron 61.

The P041-B1 probemix contains 45 MLPA probes with amplification products between 130 and 485 nt in length including 11 reference probes. The P042-B2 probemix contains 45 probes with amplification products between 131 and 485 nt in length, 11 of which are reference probes. The identity of the genes detected by the reference probes is available online (www.mlpa.com).

These probemixes contain 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 or 96 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).

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 from peripheral blood, 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: 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 unrelated individuals who are from families without a history of A-T or *ATM*-related cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (<https://catalog.coriell.org>) and DSMZ (<https://www.dsmz.de/home.html>) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA08618 from the Coriell Institute has been tested at MRC-Holland and can be used as a positive control sample to detect a partial duplication of the 11q arm, which includes the whole *ATM* gene. The quality of cell lines can change, therefore samples should be validated before use.

Performance characteristics: The frequency of *ATM* deletions or duplications in A-T is around 2-5% (<https://www.ncbi.nlm.nih.gov/books/NBK26468/>, Cavalieri et al. 2008, Podralska et al. 2014), whereas in breast cancer this is less than 0.1% (Susswein et al. 2016, Tung et al. 2015). The analytical sensitivity and specificity for the detection of deletions or duplications in the *ATM* gene is very high and can be considered >99% (based on a 2008-2016 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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 expected results for *ATM* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or 4 (homozygous duplication).

The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- 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 (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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Notes ATM results:

- Especially deletions of the last exons (exon 62-63) are frequently encountered (own validation observations, Micol et al. 2011, Nakamura et al. 2012, Podralska et al. 2014, Susswein et al. 2016, Tung et al. 2015). *Duplication* of these exons is suggested not to be associated with an increased risk of hereditary breast cancer (LaBreche et al. 2017). Therefore, duplication of the last exons of *ATM* should be interpreted with caution.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *ATM* gene is small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P041/P042 ATM.
- 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.

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 one or 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.

ATM mutation database: <http://chromium.lovd.nl/LOVD2/home.php>. We strongly encourage users to deposit positive results in the ATM 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 copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *ATM* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P041-B1 ATM-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^(a)	
		Reference	ATM
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 16316-L18705	3q21	
136	ATM probe 19701-L26473		Exon 10
142	ATM probe 19703-L26475		Exon 17
148	ATM probe 03414-L03275		Exon 46
154	Reference probe 13816-L15310	2q13	
160	Reference probe 07992-L07773	7q21	
167	ATM probe 19708-L27071		Exon 26
173	ATM probe 19806-L27072		Intron 1
178	ATM probe 02643-L02110		Exon 31
184	ATM probe 02653-L02120		Exon 49
192	ATM probe 03416-L26741		Exon 33
196	ATM probe 02648-L04595		Exon 2
205	ATM probe 02654-L27331		Exon 16
209	Reference probe 16261-L27376	20q11	
217	ATM probe 02656-L02123		Exon 53
223	ATM probe 19712-L26484		Exon 27
229	Reference probe 15079-L26739	15q25	
234	ATM probe 02657-L02124		Exon 4
242	ATM probe 19714-L27329		Exon 14
249	ATM probe 19715-L26995		Exon 62
255	ATM probe 02658-L26745		Exon 34
261	ATM probe 02659-L26744		Exon 56
268	Reference probe 03075-L19995	5p15	
274	ATM probe 19719-L26491		Exon 40
280	ATM probe 02660-L26996		Exon 5
287	ATM probe 03418-L26743		Exon 57
292 +	ATM probe 02647-L02114		Exon 37
301	Reference probe 04570-L23473	16q13	
310	ATM probe 19720-L26492		Exon 29
319 ±	ATM probe 02662-L02129		Exon 6
328	ATM probe 02663-L02130		Exon 22
337	ATM probe 02664-L02131		Exon 38
345	ATM probe 19809-L02132		Exon 59
355	Reference probe 01045-L00615	8q22	
362	ATM probe 19721-L26493		Exon 1
373	ATM probe 02667-L04984		Exon 23
382	ATM probe 19723-L26495		Exon 8
391	ATM probe 02642-L02109		Exon 61
409	Reference probe 07455-L07103	17q21	
418	ATM probe 19725-L27158		Exon 1
427	ATM probe 02671-L27157		Exon 42
436	ATM probe 19726-L26498		Exon 19
445	Reference probe 16286-L18578	13q14	
463	ATM probe 02674-L02141		Exon 44
485	Reference probe 16456-L18909	18q21	

(a) The exon numbering used in this P041-B1 P042-B2 product description is the exon numbering from RefSeq transcript NM_000051.3, which is identical to the LRG_135 sequence (exon 1-63). The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

+ SNP rs587780628 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

* SNP rs201159454 could influence the probe signal. Frequencies of 4-16% have been reported, however, we have never encountered deviations for this probe due to this SNP. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 1b. SALSA MLPA Probemix P042-B2 ATM-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^(a)	
		Reference	ATM
64-105	Control fragments – see table in probemix content section for more information		
131	Reference probe 16316-L22397	3q21	
136	Reference probe 07905-L27802	7p14	
142	ATM probe 19704-L26476		Exon 30
150	ATM probe 08415-L26617		Exon 3
156	ATM probe 19705-L26878		Exon 21
161	ATM probe 19706-L26478		Exon 32
170 Ж	ATM probe S1099-SP0950-L27632		Exon 28
178	ATM probe 19709-L26481		Exon 43
184	Reference probe 06658-L06231	9p24	
191	ATM probe 19803-L26627		Exon 24
198	ATM probe 08437-L26618		Exon 48
203	ATM probe 19710-L26882		Exon 35
209	ATM probe 08426-L26619		Exon 25
215	ATM probe 19711-L27805		Exon 11
220	ATM probe 08436-L27803		Exon 47
232	ATM probe 08444-L27210		Exon 60
243 Я	ATM probe 08442B-L27951		Exon 55
250	ATM probe 19713-L27950		Exon 7
256	Reference probe 10808-L27953	4q25	
262	ATM probe 19717-L27949		Exon 15
273	ATM probe 19718-L27948		Exon 61
280	ATM probe 08439-L27947		Exon 51
286	ATM probe 08445-L26621		Exon 63
292	ATM probe 08422-L26622		Exon 18
301	Reference probe 14941-L16674	6q22	
311	ATM probe 19808-L27211		Exon 45
319	Reference probe 12552-L13602	3q13	
328	ATM probe 08431-L08322		Exon 36
341	ATM probe 08433-L26623		Exon 41
346	ATM probe 09367-L26624		Exon 52
358	ATM probe 19350-L08325		Exon 50
365	Reference probe 14059-L26885	5q33	
373	ATM probe 08420-L08326		Exon 13
382	ATM probe 19722-L26494		Exon 39
394	ATM probe 09667-L26625		Exon 54
401	Reference probe 10638-L12897	8q12	
412	ATM probe 19802-L26626		Exon 12
419	ATM probe 19724-L26496		Intron 61
427	ATM probe 08443-L08330		Exon 58
436	Reference probe 14775-L16472	1q23	
448	ATM probe 19727-L26499		Exon 20
454	Reference probe 02144-L01619	13q13	
463	ATM probe 19728-L26500		Exon 9
474	ATM probe 19729-L26501		Intron 61
485	Reference probe 16456-L18909	18q21	

(a) The exon numbering used in this P041-B1 P042-B2 product description is the exon numbering from RefSeq transcript NM_000051.3, which is identical to the LRG_135 sequence (exon 1-63). The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

‡ Changed in version B2 (from lot B2-0217 onwards). Small change in length, no change in sequence detected.

* This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

Table 2. ATM probes arranged according to chromosomal location

Length (nt)		SALSA MLPA probe	ATM Exon ^(a)	Ligation site ^(b) NM_000051.3	Partial sequence ^(c) (24 nt adjacent to ligation site)	Distance to next probe
P041	P042					
			<i>start codon</i>	<i>386-388 (exon 2)</i>		
418		19725-L27158	Exon 1	88-89	TCCGACGGGCCG-AATGTTTTGGGG	0.1 kb
362		19721-L26493	Exon 1	235-236	GGAAGCGGGAGT-AGGTAGTGTGCGT	1.8 kb
173		19806-L27072	Intron 1	1673 nt after exon 1 reverse	CCTCATGAAGTC-ATATCTGGGCAG	2.8 kb
196		02648-L04595	Exon 2	431-430 reverse	TCTATCATGTTT-TAGTTGACGGCA	0.1 kb
	150	08415-L26617	Exon 3	485-486	TTAAGCGCCTGA-TTCGAGATCCTG	1.5 kb
234		02657-L02124	Exon 4	645-646	AGCCTCAACACA-AGCCTCCAGGCA	6.5 kb
280		02660-L26996	Exon 5	834-835	CAAAGACATTCT-TTCTGTGAGAAA	8.2 kb
319 ±		02662-L02129	Exon 6	954-955	AGTGGCTAGAAT-AATTCATGCTGT	0.9 kb
	250	19713-L27950	Exon 7	1183-1184	CTTTATATTTGG-ACTCAACATAGG	2.1 kb
382		19723-L26495	Exon 8	1348-1349	CTGCTAGTGAAT-GAGATAAGTCAT	2.0 kb
	463	19728-L26500	Exon 9	1511-1512	CTACACAAAGAG-AATCTAGTGATT	1.9 kb
136		19701-L26473	Exon 10	1795-1796	GACAAGAGGTCA-AACCTAGAAAGC	1.2 kb
	215	19711-L27805	Exon 11	2187-2188	AATTCTTCACAG-GTAATTTAAGTT	0.8 kb
	412	19802-L26626	Exon 12	2225-2226	AAATCTTGTTGA-GTCTCACTATGA	1.1 kb
	373	08420-L08326	Exon 13	2409-2410	AGAAAAGCACCA-GTCCAGTATTGG	2.3 kb
242		19714-L27329	Exon 14	2593-2594	GGTGAATAGCT-GAAGAGGAAGCA	1.3 kb
	262	19717-L27949	Exon 15	2720-2719 reverse	TAGTCGCATCAT-ATTTCTCAAGGA	1.5 kb
205		02654-L27331	Exon 16	2789-2790	TTGCATCTGGCT-TTTTCTGCGAT	8.2 kb
142		19703-L26475	Exon 17	2925-2926	TGGAAATCTAAT-GGAGGTGGAGGA	1.3 kb
	292	08422-L26622	Exon 18	3120-3121	AACTACTGCTCA-GACCAATACTGT	2.6 kb
436		19726-L26498	Exon 19	3243-3244	GCTTTTAAAGGA-GCTTCTGGAGA	0.3 kb
	448	19727-L26499	Exon 20	3428-3429	CAAGGGATGCTC-AAGGACAGTTTC	1.2 kb
	156	19705-L26878	Exon 21	3462-3463	ACTTGATTTTCA-GCATCTAACAAA	0.3 kb
328		02663-L02130	Exon 22	3642-3641 reverse	CAGCCAACATGC-GAACTGGTGAT	6.7 kb
373		02667-L04984	Exon 23	3701-3702	GAGATTCTTCCA-GGTTACTGAAAG	1.6 kb
	191	19803-L26627	Exon 24	3889-3890	AGCCCTATCTGC-GAAAAACAGGCT	1.6 kb
	209	08426-L26619	Exon 25	3979-3980	GAGAAAGTTTCT-GAAACTTTTGGA	1.6 kb
167		19708-L27071	Exon 26	4224-4225	AGAGGACTGGAA-AAGTCTTCTAAC	3.4 kb
223		19712-L26484	Exon 27	1 nt after exon 27 reverse	AAAATGTACATA-CCCTGAAAAGTC	1.4 kb
	170 Ж #	S1099-SP0950-L27632	Exon 28	4611-4612 and 31 nt after exon 28	AGAAATCTTTC-41 nt spanning oligo-CATTCTCTTT	0.7 kb
310		19720-L26492	Exon 29	19 nt after exon 29 reverse	GCTTATATATTG-GTCTAAATATGT	2.9 kb
	142	19704-L26476	Exon 30	4899-4900	TTGCCAGACAGC-CGTGACTTACTG	0.7 kb
178		02643-L02110	Exon 31	5137-5136 reverse	AAGGGTCCTCTA-CTGTATTTGATT	1.6 kb
	161	19706-L26478	Exon 32	5292-5293	GAGAGCTTCTCA-GGGTGCTAATTT	2.3 kb
192		03416-L26741	Exon 33	5350-5349 reverse	ATTGCCATCTTG-GATAACTGCAAC	2.4 kb
255		02658-L26745	Exon 34	5409-5410	TGGAAGCTGCTT-GGGAGAAGTGGG	2.0 kb
	203	19710-L26882	Exon 35	5609-5610	AAAACATTTTAG-CCACAAGACTG	1.3 kb
	328	08431-L08322	Exon 36	5797-5798	AATCATGACATT-TGGATAAAGACA	1.9 kb
292 +		02647-L02114	Exon 37	6025-6024 reverse	GTGGATCGGCTC-GTTTGCAGAGAAG	3.1 kb
337		02664-L02131	Exon 38	6082-6083	TTTTCCGATGC-TGTTTGATAAAA	2.2 kb
	382	19722-L26494	Exon 39	8 nt before exon 39 reverse	AGGTCTAAAGAA-AAATGAGATATA	2.3 kb
274		19719-L26491	Exon 40	1 nt before exon 40 reverse	ATGCAAGACTTC-TGTTTGTCAAA	3.5 kb
	341	08433-L26623	Exon 41	6430-6431	ATAGGGGAGCCA-GATAGTTTGTAT	0.2 kb
427		02671-L27157	Exon 42	6532-6533	CTAGTAACATAT-GACCTCGAAACA	1.4 kb
	178	19709-L26481	Exon 43	6682-6683	GAAGAATTCAT-TACCAAGCAGCA	2.5 kb
463		02674-L02141	Exon 44	6755-6756	AAGGAACCAGTT-ACCATGAATCAT	1.4 kb
	311	19808-L27211	Exon 45	6893-6894	TGTATTGCTCT-ATCCCACTTA	4.0 kb

148		03414-L03275	Exon 46	7022-7023	CCCAGCTTCTCA-AGGACAGTGATT	0.8 kb
	220	08436-L27803	Exon 47	7323-7324	GAGTATTCTCAA-GCAAATGATCAA	1.5 kb
	198	08437-L26618	Exon 48	7421-7422	GCAACTGGTTAG-CAGAAACGTGCT	1.5 kb
184		02653-L02120	Exon 49	7648-7647 reverse	CCTACTTCCTCT-TTGGCTCTTTTC	1.1 kb
	358	19350-L08325	Exon 50	7740-7741	TGAATTAGCCCT-GCGTGCACTGAA	1.2 kb
	280	08439-L27947	Exon 51	7965-7966	GGCTGCTAGAAT-GGGGACCAAGAT	0.5 kb
	346	09367-L26624	Exon 52	8092-8093	GCAAACAGAGAT-GAATTTCGACT	0.8 kb
217		02656-L02123	Exon 53	8206-8207	AGAATAATATGT-ACTATCAGAAGT	1.1 kb
	394	09667-L26625	Exon 54	8355-8356	TACTAAACTTAA-GAATTTAGAAGA	1.1 kb
	243	08442B-L27951	Exon 55	8421-8422	AGAATATGGAAA-TCTGGTACTAT	0.9 kb
261		02659-L26744	Exon 56	8574-8575	TGTCATGCAACA-GGTCTCCAGAT	7.4 kb
287		03418-L26743	Exon 57	8682-8683	GCGAAGTGGTGT-TCTTGAATGGTG	2.6 kb
	427	08443-L08330	Exon 58	8906-8907	AAAAATTCTTGG-ATCCAGCTATTT	1.5 kb
345		19809-L02132	Exon 59	9027-9026 reverse	GTTCTGCTGACT-GCTCATTATCA	6.5 kb
	232	08444-L27210	Exon 60	9081-9082	ACAGGGCAAAT-CCTTCTACTCC	1.0 kb
391		02642-L02109	Exon 61	9194-9195	AAACCATGGAAG-TGATGAGAACT	0.6 kb
	273	19718-L27948	Exon 61	525 nt after exon 61 reverse	GTTTGCTCTGCA-GGCCCAAACCT	7.9 kb
	474	19729-L26501	Intron 61	1758 nt before exon 62	CAGAGCACTTTA-ACCTGGGTGTAT	0.7 kb
	419	19724-L26496	Intron 61	1053 nt before exon 62	ACTTAACGGACA-AGCTACATGTAA	1.1 kb
249		19715-L26995	Exon 62	9251-9250 reverse	GTCAAAGAGTGG-ATCATATAGAAG	0.3 kb
	286	08445-L26621	Exon 63	9427-9428	ATGAGACTACAA-GAGAACTGAAA	
			stop codon	9554-9556 (exon 63)		

(a) The exon numbering used in this P041-B1 P042-B2 product description is the exon numbering from RefSeq transcript NM_000051.3, which is identical to the LRG_135 sequence (exon 1-63). The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

(b) Ligation sites of the P041/P042 ATM MLPA probes are indicated according to RefSeq sequence NM_000051.3 containing 63 exons.

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

* This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

± SNP rs201159454 could influence the probe signal. Frequencies of 4-16% have been reported, however, we have never encountered deviations for this probe due to this SNP. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

+ SNP rs587780628 could influence the probe signal. 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.

Related SALSA MLPA probemixes

- P002/P087 BRCA1 Hereditary breast cancer, screening *BRCA1*.
- P037/P038/P040 CLL Tumour mixes that contain probes for genes involved in chronic lymphocyte leukaemia. Several ATM probes are included.
- P045/P090/P077 BRCA2 Hereditary breast cancer, screening *BRCA2*.
- P190 CHEK2 Contains probes for *CHEK2*, *ATM* and *TP53* involved in predisposition to cancer.
- P316 Recessive Ataxias Contains probes for *APTX*, *SETX*, *FXN*, involved in recessive ataxias.
- P377 Hematologic Malignancies Tumour mix that contains probes for genes involved in hematologic malignancies. Several ATM probes are included.

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P041 Product history	
<i>Version</i>	<i>Modification</i>
B1	12 ATM probes and most reference probes have been replaced. One target probe has been removed. Also, the lengths of some probes have been adjusted.
A3	The control fragments have been adjusted (QDX2).
A2	Control fragments at 88, 96, 100 and 105 nt have been added.
A1	First release

P042 Product history	
<i>Version</i>	<i>Modification</i>
B2	One target probe has a small change in length.
B1	3 ATM probes have been added and 12 ATM probes have been replaced. Also, most reference probes have been replaced.
A3	The control fragments have been adjusted (QDX2).
A2	Control fragments at 88, 96, 100 and 105 nt have been added.
A2b	One probe for exon 54 at 382 nt has been added.
A1	First release

Implemented changes in the product description	
<i>Version B1/B2-02 – 1 August 2018 (04)</i>	
<ul style="list-style-type: none"> - Product description restructured and adapted to a new template. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Positive Coriell sample was added. - Morocco and Israel were added as countries where product has IVD status. - Number of gene transcripts have been adjusted, NM sequence used in this product description was NOT changed. 	
<i>Version B1/B2-01 – 28 March 2017 (03)</i>	
<ul style="list-style-type: none"> - Product description restructured and adapted to a new template. - SNP remarks added to probes 02647-L02114 and 02662-L02129. - Product description adapted to a new version for P042 (version number changed, small changes in Table 1 and Table 2). 	
<i>Version 13 – 21 September 2016 (55)</i>	
<ul style="list-style-type: none"> - Warning added on probe 19705-L26878 and small changes in Table 1 and 2. - Minor textual changes. - References on page 1 adjusted. 	
<i>Version 12 (53)</i>	
<ul style="list-style-type: none"> - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). 	

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<p>IVD</p>	<p>EUROPE* CE MOROCCO ISRAEL</p>
<p>RUO</p>	<p>ALL OTHER COUNTRIES</p>

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA).
The product is for RUO in all other European countries.