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

To be used with the MLPA General Protocol.

P041 Version B1
P042 Version B2
For complete product history see page 11.

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 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.

Intended purpose
The SALSA MLPA Probemixes P041 ATM-1 and P042 ATM-2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assays[2] for the detection of deletions or duplications in the *ATM* gene in genomic DNA isolated from human peripheral whole blood specimens. P041 ATM-1 and P042 ATM-2 are intended to confirm a potential cause for and clinical diagnosis of Ataxia-Telangiectasia or hereditary predisposition to develop cancer, including but not limited to breast cancer, and for molecular genetic testing of at-risk family members.

In order to cover all *ATM* exons, both P041 ATM-1 and P042 ATM-2 should be used. Copy number variations (CNVs) detected with P041 ATM-1/P042 ATM-2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *ATM* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

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 and contains 63 exons. 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 is a reference standard in the NCBI RefSeq project (NM_000051.4; 12915 nt; coding sequence 151-9321; https://www.ncbi.nlm.nih.gov/gene/472). The ATG translation start site is located in exon 2 and the stop codon is located in exon 63. *ATM* transcript variant 1 differs from transcript variant 2 in the 5' UTR. Both transcripts encode the same isoform (a).

Exon numbering

The *ATM* exon numbering used in this P041-B1 P042-B2 ATM product description is the exon numbering from the LRG_135 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. 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.

Probemix content

The SALSA MLPA Probemixes P041-B1 ATM-1 and P042-B2 ATM-2 each contain 34 probes for the *ATM* gene. When used together, a probe for each *ATM* exon is present. Probemix P041-B1 ATM-1 contains 45 MLPA probes with amplification products between 130 and 485 nucleotides (nt). Probemix P042-B2 ATM-2 contains 45 MLPA probes with amplification products between 131 and 485 nucleotides (nt). In each probemix, 11 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

Both 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.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).

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 human peripheral whole blood specimens, 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 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 (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA00959, NA08618, NA09596, NA15099 and HG03694 from the Coriell Institute have been tested with the P041-B1 and P042-B2 probemixes at MRC Holland and can be used as positive control samples to detect the copy number alterations described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P041-B1/P042-B2	Expected copy number alteration
NA00959	Coriell Institute	11q22.3	*ATM*	Heterozygous *ATM* duplication
NA08618	Coriell Institute	11q22.3	*ATM*	Heterozygous *ATM* duplication
NA09596	Coriell Institute	11q22.3	*ATM*	Heterozygous *ATM* deletion
NA15099	Coriell Institute	11q22.3	*ATM*	Heterozygous *ATM* duplication
HG03694	Coriell Institute	11q22.3	*ATM*	Heterozygous exon 62+63 duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by these P041-B1 ATM-1 or P041-B2 ATM-2 probemixes.

Performance characteristics
The frequency of *ATM* deletions or duplications in A-T is around 1-5% (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 2008-2020 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms 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 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 expected results for the *ATM* 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 each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. 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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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.

- 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. 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. 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.

P041/P042 specific note:

- Deletions of the last *ATM* exons (exon 62-63) are encountered frequently (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 might not result in inactivation of that gene copy and should therefore be interpreted with caution.
- For the P041 probemix, we observed a prominent peak with a length of approximately 480 nt in a No-DNA control. This peak has a height <25% of the median of the four Q-fragments and is therefore not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *ATM* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemixes P041 ATM-1/P042 ATM-2.
- 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.

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.

***ATM* mutation database**
We strongly encourage users to deposit positive results in the ATM mutation database (https://www.LOVD.nl/ATM). 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 SNVs and unusual results (e.g., a duplication of *ATM* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.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	3q	
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	2q	
160	Reference probe 07992-L07773	7q	
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	20q	
217	**ATM probe** 02656-L02123		**Exon 53**
223	**ATM probe** 19712-L26484		**Exon 27**
229	Reference probe 15079-L26739	15q	
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	5p	
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	16q	
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	8q	
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	17q	
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	13q	
463	**ATM probe** 02674-L02141		**Exon 44**
485	Reference probe 16456-L18909	18q	

+ 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. Unusually large differences in frequency (0.001%-16%) have been reported for this SNP and the nearby rs79075295 SNP, which might indicate that sequence analysis of this region is prone to mistakes. 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	3q	
136	Reference probe 07905-L27802	7p	
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	9p	
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 ¥	**ATM probe** 08442-L27951		**Exon 55**
250	**ATM probe** 19713-L27950		**Exon 7**
256	Reference probe 10808-L27953	4q	
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	6q	
311	**ATM probe** 19808-L27211		**Exon 45**
319	Reference probe 12552-L13602	3q	
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	5q	
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	8q	
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	1q	
448	**ATM probe** 19727-L26499		**Exon 20**
454	Reference probe 02144-L01619	13q	
463	**ATM probe** 19728-L26500		**Exon 9**
474	**ATM probe** 19729-L26501		**Intron 61**
485	Reference probe 16456-L18909	18q	

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

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

Ж 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. When this occurs in reference samples, it can look like an increased signal in the test samples.

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.

Table 2. ATM probes arranged according to chromosomal location

Length (nt)		SALSA MLPA probe	*ATM* exon[a]	Ligation site NM_000051.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
P041	P042					
			start codon 151-153 (Exon 2)			
418		19725-L27158	Exon 1	147 nt before exon 1	TCCGACGGGCCG-AATGTTTTGGGG	0.1 kb
362		19721-L26493	Exon 1	1 nt before exon 1-1	GGAAGCGGGAGT-AGGTAGCTGCGT	1.8 kb
173		19806-L27072	Intron 1	1673 nt after exon 1 reverse	CCTCATGAAGTC-ATATCTGGGCAG	2.8 kb
196		02648-L04595	Exon 2	196-195 reverse	TCTATCATGTTC-TAGTTGACGGCA	0.1 kb
	150	08415-L26617	Exon 3	250-251	TTAAGCGCCTGA-TTCGAGATCCTG	1.5 kb
234		02657-L02124	Exon 4	410-411	AGCCTCAACACA-AGCCTCCAGGCA	6.5 kb
280		02660-L26996	Exon 5	599-600	CAAAGACATTCT-TTCTGTGAGAAA	8.2 kb
319 ±		02662-L02129	Exon 6	719-720	AGTGGCTAGAAT-AATTCATGCTGT	0.9 kb
	250	19713-L27950	Exon 7	948-949	CTTTATATTTGG-ACTCAACATAGG	2.1 kb
382		19723-L26495	Exon 8	1113-1114	CTGCTAGTGAAT-GAGATAAGTCAT	2.0 kb
	463	19728-L26500	Exon 9	1276-1277	CTACACAAAGAG-AATCTAGTGATT	1.9 kb
136		19701-L26473	Exon 10	1560-1561	GACAAGAGGTCA-AACCTAGAAAGC	1.2 kb
	215	19711-L27805	Exon 11	1952-intron 11	AATTCTTCACAG-GTAATTTAAGTT	0.8 kb
	412	19802-L26626	Exon 12	1990-1991	AAATTCTTGTGA-GTCTCACTATGA	1.1 kb
	373	08420-L08326	Exon 13	2174-2175	AGAAAAGCACCA-GTCCAGTATTGG	2.3 kb
242		19714-L27329	Exon 14	2358-2359	GGTGTAATAGCT-GAAGAGGAAGCA	1.3 kb
	262	19717-L27949	Exon 15	2485-2484 reverse	TAGCTGCATCAT-ATTTCTCAAGGA	1.5 kb
205		02654-L27331	Exon 16	2554-2555	TTGCATCTGGCT-TTTTCCTGCGAT	8.2 kb
142		19703-L26475	Exon 17	2690-2691	TGGAAATCTAAT-GGAGGTGGAGGA	1.3 kb
	292	08422-L26622	Exon 18	2885-2886	AACTACTGCTCA-GACCAATACTGT	2.6 kb
436		19726-L26498	Exon 19	3008-3009	GCTTTTAAAGGA-GCTTCCTGGAGA	0.3 kb
	448	19727-L26499	Exon 20	3193-3194	CAAGGGATGCTC-AAGGACAGTTTC	1.2 kb
	156	19705-L26878	Exon 21	intron 20-3228	ACTTGATTTCAG-GCATCTAACAAA	0.3 kb
328		02663-L02130	Exon 22	3407-3406 reverse	CAGCCAACATGC-GAACTTGGTGAT	6.7 kb
373		02667-L04984	Exon 23	3466-3467	GAGATTCTTCCA-GGTTACTGAAAG	1.6 kb
	191	19803-L26627	Exon 24	3654-3655	AGCCCTATCTGC-GAAAAACAGGCT	1.6 kb
	209	08426-L26619	Exon 25	3744-3745	GAGAAAGTTTCT-GAAACTTTTGGA	1.6 kb
167		19708-L27071	Exon 26	3989-3990	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	4376-4377 and 31 nt after exon 28	AGAAATTCTTTC-41 nt spanning oligo-CATTCCTTCTTT	0.7 kb
310		19720-L26492	Exon 29	19 nt after exon 29 reverse	GCTTATATATTG-GTCTAAATATGT	2.9 kb
	142	19704-L26476	Exon 30	4664-4665	TTGCCAGACAGC-CGTGACTTACTG	0.7 kb
178		02643-L02110	Exon 31	4902-4901 reverse	AAGGGTCCTCTA-CTGTATTTGATT	1.6 kb
	161	19706-L26478	Exon 32	5057-5058	GAGAGCTTCTCA-GGGTGCTAATTT	2.3 kb
192		03416-L26741	Exon 33	5115-5114 reverse	ATTGCCATCTTG-GATAACTGCAAC	2.4 kb
255		02658-L26745	Exon 34	5174-5175	TGGAAGCTGCTT-GGGAGAAGTGGG	2.0 kb
	203	19710-L26882	Exon 35	5374-5375	AAAACATTTTAG-CCACAAAGACTG	1.3 kb
	328	08431-L08322	Exon 36	5562-5563	AATCATGACATT-TGGATAAAGACA	1.9 kb

Length (nt)		SALSA MLPA probe	*ATM* exon[a]	Ligation site NM_000051.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
P041	P042					
292 +		02647-L02114	Exon 37	5790-5789 reverse	GTGGATCGGCTC-GTTTGCGAGAAG	3.1 kb
337		02664-L02131	Exon 38	5847-5848	TTTTTCCGATGC-TGTTTGGATAAA	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-TGTTTGTCACAA	3.5 kb
	341	08433-L26623	Exon 41	6195-6196	ATAGGGGAGCCA-GATAGTTTGTAT	0.2 kb
427		02671-L27157	Exon 42	6297-6298	CTAGTAACATAT-GACCTCGAAACA	1.4 kb
	178	19709-L26481	Exon 43	6447-6448	GAAGAACTTCAT-TACCAAGCAGCA	2.5 kb
463		02674-L02141	Exon 44	6520-6521	AAGGAACCAGTT-ACCATGAATCAT	1.4 kb
	311	19808-L27211	Exon 45	6658-6659	TGTATTCGCTCT-ATCCCACACTTA	4.0 kb
148		03414-L03275	Exon 46	6787-6788	CCCAGCTTCTCA-AGGACAGTGATT	0.8 kb
	220	08436-L27803	Exon 47	7088-7089	GAGTATTCTCAA-GCAAATGATCAA	1.5 kb
	198	08437-L26618	Exon 48	7186-7187	GCAACTGGTTAG-CAGAAACGTGCT	1.5 kb
184		02653-L02120	Exon 49	7413-7412 reverse	CCTACTTCCTCT-TTGGCTCTTTTC	1.1 kb
	358	19350-L08325	Exon 50	7505-7506	TGAATTAGCCCT-GCGTGCACTGAA	1.2 kb
	280	08439-L27947	Exon 51	7730-7731	GGCTGCTAGAAT-GGGGACCAAGAT	0.5 kb
	346	09367-L26624	Exon 52	7857-7858	GCAAACAGAGAT-GAATTTCTGACT	0.8 kb
217		02656-L02123	Exon 53	7971-7972	AGAATAATATGT-ACTATCAGAAGT	1.1 kb
	394	09667-L26625	Exon 54	8120-8121	TACTAAACTTAA-GAATTTAGAAGA	1.1 kb
	243	08442-L27951	Exon 55	8186-8187	AGAATATGGAAA-TCTGGTGACTAT	0.9 kb
261		02659-L26744	Exon 56	8339-8340	TGTCATGCAACA-GGTCTTCCAGAT	7.4 kb
287		03418-L26743	Exon 57	8447-8448	GCGAAGTGGTGT-TCTTGAATGGTG	2.6 kb
	427	08443-L08330	Exon 58	8671-8672	AAAAATTCTTGG-ATCCAGCTATTT	1.5 kb
345		19809-L02132	Exon 59	8792-8791 reverse	GTTCTGCTGACT-GCTCATTTATCA	6.5 kb
	232	08444-L27210	Exon 60	8846-8847	ACAGGGCAAAAT-CCTTCCTACTCC	1.0 kb
391		02642-L02109	Exon 61	8959-8960	AAACCATGGAAG-TGATGAGAAACT	0.6 kb
	273	19718-L27948	Exon 61	525 nt after exon 61 reverse	GTTTGCTCTGCA-GGCCCAAACCCT	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	9016-9015 reverse	GTCAAAGAGTGG-ATCATATAGAAG	0.3 kb
	286	08445-L26621	Exon 63	9192-9193	ATGAGACTACAA-GAGAAACTGAAA	
			stop codon 9319-9321 (exon 63)			

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

+ 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. Unusually large differences in frequency (0.001%-16%) have been reported for this SNP and the nearby rs79075295 SNP, which might indicate that sequence analysis of this region is prone to mistakes. 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.
Ж 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
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.

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

Related SALSA MLPA probemixes

- P002/P087 BRCA1 — Hereditary breast cancer, screening *BRCA1*.
- P045/P090/P077 BRCA2 — Hereditary breast cancer, screening *BRCA2*.
- P037/P038/P040 CLL — Tumour mixes that contain probes for genes involved in chronic lymphocyte leukaemia. Several ATM probes are included.
- 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.
- P376 BRCA1ness — Contains probes for *BRCA1*, *BRCA2* and several chromosomal regions associated with BRCA1ness
- P489 BARD1 — Contains probes for *BARD1*, involved in hereditary breast cancer

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P041 product history	
Version	*Modification*
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	
Version	*Modification*
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.
A1b	One probe for exon 54 at 382 nt has been added.
A1	First release.

Implemented changes in the product description
Version B1/B2-03 – 25 March 2021 (04P) - Product description rewritten and adapted to a new template. - UK has been added to the list of countries in Europe that accept the CE mark - Ligation sites of the probes targeting the *ATM* gene updated according to new version of the NM_ reference sequence. - Added 4 Coriell samples to the list of positive DNA samples. - Added note about non-specific peak appearing in No-DNA control reactions of P041. - SNP remark for probe 02662-L02129 was updated. - Probemixes P376 and P489 have been added as related products. Version B1/B2-02 – 1 August 2018 (04) - 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. Version B1/B2-01 – 28 March 2017 (03) - 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). Version 13 – 21 September 2016 (55) - Warning added on probe 19705-L26878 and small changes in Table 1 and 2. - Minor textual changes. - References on page 1 adjusted.

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

IVD	EUROPE* CE MOROCCO ISRAEL
RUO	ALL OTHER COUNTRIES

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