Product Description
SALSA® MLPA® Probemix P496-A1 KMT2A

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 12.

Catalogue numbers:
- **P496-025R:** SALSA MLPA Probemix P496 KMT2A, 25 reactions.
- **P496-050R:** SALSA MLPA Probemix P496 KMT2A, 50 reactions.
- **P496-100R:** SALSA MLPA Probemix P496 KMT2A, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SD096 Binning DNA and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P496 KMT2A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *KMT2A* gene. This probemix can also be used to detect the presence of the *ASXL1* c.1934dupG mutation.

The histone methyltransferase KMT2A methylates lysine 3 on histone 4 (H3K4), thereby shaping the epigenetic landscape in cells, modulating chromatin accessibility and transcription. A subset of leukemias is characterised by *KMT2A* alterations, which typically include amplifications, partial tandem duplications (PTDs) and translocations (Rao et al. 2015). *KMT2A* alterations are observed in 5-20% of Acute Myeloid Leukemia (AML) cases, and are associated with poor outcome (Choi et al. 2018; Issa et al. 2021; Yuen et al. 2023). *KMT2A* PTDs (also known as *MLL* PTDs) are observed in 6-7% of Myelodysplastic Syndrome (MDS) as well, and alongside *TP53* and *FLT3* mutations are considered as top genetic predictors of adverse outcomes according to IPSS-M (Molecular International Prognostic Scoring System for Myelodysplastic Syndromes) (Bernard E et al. 2022; Choi et al. 2018). Accurate detection of *KMT2A* PTDs using next-generation sequencing (NGS) or fluorescence *in situ* hybridisation (FISH) approaches remains difficult (McKerrell et al. 2016; Afrin et al. 2018; Dai et al. 2021; Tsai et al. 2022), whereas MLPA technique was shown to yield robust and reliable results with a short turnaround time (Balgobind et al. 2010; Kentaro et al. 2013) and allows more accurate detection of *KMT2A* PTDs when combined with DNA structural analysis approaches (Capo-Chichi et al. 2022).

ASXL1 is a gene that is frequently mutated in various haematological malignancies, including AML and MDS. The most common genetic alteration found in the *ASXL1* gene is the c.1934dupG mutation, which leads to a premature stop codon in the *ASXL1* transcript, resulting in a truncated ASXL1 protein which is rapidly degraded. Importantly, loss-of-function *ASXL1* mutations were shown to promote myeloid transformation through loss of PRC2-mediated gene repression (Abdel-Wahab et al. 2012; Gelsi-Boyer et al. 2012). The *ASXL1* c.1934dupG mutation appears to be mostly restricted to the haematopoietic lineage, and is associated with poor prognosis in AML and MDS patients (Gelsi-Boyer et al. 2012).

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *KMT2A* exon numbering used in this P496-A1 KMT2A product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcript NM_001197104.2, which can be found in Tables 1 and 2. The *TP53* exon numbering is derived from the LRG_321 sequence; the exon numbering derived from MANE project for this gene can be found in between brackets in Table 2. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P496-A1 KMT2A contains 60 MLPA probes with amplification products between 64 and 505 nucleotides (nt). This includes 17 probes for the *KMT2A* gene and two probes upstream and downstream of *KMT2A* gene, namely for the *UBE4A* and *TMEM25* genes. This probemix also contains one probe specific for the *ASXL1* c.1934dupG mutation which will only generate a signal when the mutation is present. Furthermore, probes were added for copy number determination of other regions commonly affected by CNAs in AML and MDS. These include two probes targeting *CTNNA1* and *NPM1* (5q deletions), one flanking probe for 5p, two probes for *IKZF1*, *CUX1*, *KMT2E* and *EZH2* (chromosome 7 and 7q deletions); three probes for *ATM* (11q deletions), two flanking probes for the 11p arm, one flanking probe for 11q, three probes for *TP53* (17p deletions), two probes for *NF1* and one for *SUZ12* (17q deletions). In addition, 13 reference probes are included that detect autosomal chromosomal locations and target relatively copy number stable regions in various cancer types including hematological cancers. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3.

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals 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
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. Samples described in the table below from the Coriell Institute and Leibniz Institute DSMZ have been tested with this P496-A1 probemix at MRC Holland, and can be used as a positive control samples to detect copy number alterations or the *ASXL1* c.1934dupG mutation. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Chromosomal position of CNA or mutation (hg18)*	Altered target genes in P496-A1	Expected copy number alteration / mutation
Germline samples from Coriell Institute			
NA14523	5p13.2	*NIPBL*	Heterozygous duplication
NA04371	5q35.1	*NPM1*	Heterozygous duplication
NA07081	7p12.2	*IKZF1*	Heterozygous duplication
NA01059	7q22.1-q22.2	*CUX1, KMT2E*	Heterozygous deletion
NA12519	7q36.1	*EZH2*	Homozygous duplication
NA07412	7q36.1	*EZH2*	Heterozygous deletion
NA03435	11p15.4	*NUP98*	Heterozygous duplication
NA05518	11p15.1	*SLC6A5*	Heterozygous deletion
NA09596	11q23.3	*ATM*	Heterozygous deletion
Cancer cell line samples from Leibniz Institute DSMZ			
KASUMI-1◊ (ACC-220)	20q11.21	*ASXL1*	c.1934dupG mutation
	17p13.1	*TP53*	Heterozygous deletion
KASUMI-6◊ (ACC-686)	11q23.3	*KMT2A* (exon 2-7)+	PTD (gain)
	17p13.1	*TP53*	Heterozygous deletion
EOL-1◊ (ACC-386)	11q23.3	*KMT2A* (exon 2-7)+	PTD (gain)
UOC-M1◊ (ACC-775)	5q31.2	*CTNNA1*	Heterozygous deletion
	7p12.2-7q36.1	*IKZF1, CUX1, KMT2E, EZH2*	Heterozygous deletion
	11p15.1-p15.4	*NUP98, SLC6A5*	Heterozygous deletion
	11q22.3-q24.3	*ATM, UBE4A, KMT2A, TMEM25, ETS1*	gain

Sample name	Chromosomal position of CNA or mutation (hg18)*	Altered target genes in P496-A1	Expected copy number alteration / mutation
	17p13.1	*TP53*	Heterozygous deletion
ML-2◊ (ACC-15)	11p15.1	*SLC6A5*	Heterozygous deletion
	11q23.3	*KMT2A* (exon 9-36), *TMEM25*, *ETS1*	Heterozygous deletion
NOMO-1◊ (ACC-542)	5p13.2	*NIPBL*	gain
	7q22.1	*CUX1*	gain
	7q22.2-q36.1	*KMT2E, EZH2*	Heterozygous deletion
	11q23.3	*KMT2A* (exon 9)	Heterozygous deletion

* 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 this P496-A1 KMT2A probemix.

◊ Some of the reference probes are also affected by CNAs in this sample.

+ In the literature this cell line is described to have *KMT2A* exon 2 to 8 PTD. However, as probes for exon 8 are not included in P496-A1 a gain of *KMT2A* exon 8 is not noted here.

SALSA Binning DNA SD096

The SD096 Binning DNA provided with this probemix can be used for binning of all probes including the mutation-specific probe (ASXL1 probe 18261-SP0848-L2626 for the c.1934dupG mutation). SD096 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD096 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD096 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10. When 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 the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P496-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 AML and MDS, genetic defects affecting the *KMT2A* gene typically comprise CNAs and translocations. However, point mutations in *KMT2A* may also occur, most of which will not be detected by using SALSA MLPA Probemix P496-A1 KMT2A.
- 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 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 database

LOVD mutation database: https://databases.lovd.nl/shared/genes/KMT2A; COSMIC mutation database: https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KMT2A. We strongly encourage users to deposit positive results in the LOVD and COSMIC Databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *KMT2A* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P496-A1 KMT2A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			Location (hg18) in kb
		Reference	*KMT2A*, at 11q23.3	Target region / mutation	
64-105	Control fragments – see table in probemix content section for more information				
121	Reference probe 19616-L27455	4p13			04-042,278
126	Reference probe S0959-L26042	9q21			09-078,133
130 «	**KMT2A** probe 23318-L32964		Exon 1		11-117,812
136 ¬	**TMEM25** probe 23319-L32965			11q23.3	11-117,908
139	**TP53** probe 08304-L26800			17p13.1	17-007,518
145	**KMT2A** probe 23320-L32966		Exon 7		11-117,858
148	Reference probe 17858-L23867	2p21			02-044,401
154	**NF1** probe 12018-L32953			17q11.2	17-026,708
160 § ‡ Ж	**ASXL1** probe 18261-SP0848-L26260			c.1934dupG	20-030,486
166	**KMT2A** probe 23321-L32967		Exon 31		11-117,888
172	Reference probe 21100-L29489	1q32			01-199,330
177 ¬	**TMEM25** probe 23322-L32968			11q23.3	11-117,910
182 ¬	**ETS1** probe 09496-L25025			11q24.3	11-127,836
186	**KMT2A** probe 19885-L29240		Exon 36		11-117,898
193 ¬	**UBE4A** probe 23323-L32969			11q23.3	11-117,767
197	**KMT2A** probe 09499-L32954		Exon 3		11-117,848
203 ¬	**NIPBL** probe 04827-L32994			5p13.2	05-036,997
208	Reference probe 13384-L25019	6q12			06-064,999
215	**NUP98** probe 06675-L06253			11p15.4	11-003,669
228	**KMT2A** probe 23334-L33002		Exon 4		11-117,853
233	Reference probe 15154-L25342	18q21			18-056,191
240	**CUX1** probe 19886-L26753			7q22.1	07-101,527
246	**EZH2** probe 18267-L26041			7q36.1	07-148,161
251 ¬	**UBE4A** probe 23325-L32971			11q23.3	11-117,769
256	**TP53** probe 02376-L30912			17p13.1	17-007,519
264	**IKZF1** probe 13873-L15917			7p12.2	07-050,412
269 «	**CUX1** probe 09472-L23871			7q22.1	07-101,664
274	Reference probe 09792-L23872	15q15			15-042,654
280	**KMT2A** probe 19132-L25682		Exon 5		11-117,854
285	**NPM1** probe 18521-L25083			5q35.1	05-170,752
290	**ATM** probe 08445-L23873			11q22.3	11-107,741
296	**SLC6A5** probe 08936-L33053			11p15.1	11-020,586
303 Ø	**KMT2A** probe 23335-L32983		Intr. 1		11-117,824
308	**EZH2** probe 18272-L23874			7q36.1	07-148,143
315	Reference probe 14763-L24999	1q23			01-159,404
321	**KMT2A** probe 23326-L32972		Exon 3		11-117,850
328	**CTNNA1** probe 18523-L23814			5q31.2	05-138,188
337	**KMT2A** probe 23327-L32973		Exon 2		11-117,845
346	**ATM** probe 02663-L22101			11q22.3	11-107,649
353	**KMT2A** probe 23328-L32974		Exon 10		11-117,861
358 «	**KMT2A** probe 23329-L32998		Exon 1		11-117,813
364	**NPM1** probe 18527-L23818			5q35.1	05-170,750
371	**ATM** probe 08420-L22087			11q22.3	11-107,630
378	Reference probe 08141-L24356	14q11			14-020,863
384	**KMT2E** probe 18528-L24355			7q22.2	07-104,505
391	**KMT2A** probe 23330-L32996		Exon 6		11-117,856
401	**TP53** probe 17423-L32981			17p13.1	17-007,518
407	Reference probe 01237-L24913	10p14			10-012,019
412	**NF1** probe 18544-L24995			17q11.2	17-026,612
418	**KMT2E** probe 18796-L24994			7q22.2	07-104,490

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			Location (hg18) in kb
		Reference	*KMT2A*, at 11q23.3	Target region / mutation	
436	**KMT2A** probe 23331-L32977		Exon 13		11-117,866
443	**KMT2A** probe 19133-L32999		Exon 3		11-117,848
452	Reference probe 16286-L27983	13q14			13-050,429
459	**CTNNA1** probe 18533-L32980			5q31.2	05-138,117
466	**KMT2A** probe 23332-L32978		Exon 9		11-117,860
472	**IKZF1** probe 14061-L24990			7p12.2	07-050,423
484	**KMT2A** probe 23333-L32979		Exon 19		11-117,872
489	Reference probe 17939-L15290	3q25			03-157,716
498	**SUZ12** probe 18539-L23830			17q11.2	17-027,340
505	Reference probe 15203-L23403	3p12			03-081,775

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

§ Mutation-specific probe. This probe will only generate a signal when the *ASXL1* c.1934dupG mutation is present. It has been tested on artificial DNA **and on DNA from the KASUMI-1 cell line.**

‡ An unspecific peak might be detected at one nucleotide shorter length from the expected length of this *ASXL1* c.1934dupG mutation-specific probe at 160 nt. Please analyse the peak pattern carefully when making calls for this mutation-specific 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.

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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Table 2. *KMT2A* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location / ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 5q deletions; *NIPBL* (5p13.2), *CTNNA1* (5q31.2) and *NPM1* (5q35.1). Chromosome 5 CNAs are common in AML and MDS (Walter et al. 2009). Deletions affecting the 5q-arm are recurrent genetic aberrations observed in approximately 10-15% of AML and MDS cases. In MDS, isolated 5q deletions are associated with a good prognosis. In AML, 5q deletions often occur in conjunction with 7q deletions as part of a complex karyotype, associated with a poor outcome (Venugopal et al. 2021). Genes located on 5q with a described role in myeloid malignancies include *CTNNA1* (Mrozek et al. 2008; Huang et al. 2023) and *NPM1* (Mazzola et al. 2019; Garfinkle and Gruber 2019). *NPM1* is also affected by exon 12 mutations in AML patients (La Starza et al. 2010; Falini et al. 2005; Berger et al. 2006). This probemix contains two NPM1 probes for copy number detection, but not for point mutations.					
203 ¬	04827-L32994	***NIPBL***	5p13.2	CAACAAACCACT-ATCTCACATAGC	101,7 **M**b
459	18533-L32980	***CTNNA1***, ex 1	NM_001903.5; 85-86	CAGCTAGCCGCA-GGTAACTTCGTA	71,3 kb
328	18523-L23814	***CTNNA1***, ex 6	NM_001903.5; 938-939	ATGCACTCAATA-ACTTTGACGTAA	32,3 **M**b
364	18527-L23818	***NPM1***, ex 2	NM_002520.7; 7 nt before exon 2	CTTTTTTCTTCA-TTTACAGGTTGT	2,8 kb
285	18521-L25083	***NPM1***, ex 5	NM_002520.7; 551-552	GTGGTAGCAAGG-TTCCACAGGTAG	-
Chromosome 7 or 7q deletions; *IKZF1* (7p12.2), *CUX1* (7q22.1), *KMT2E* (7q22.2) and *EZH2* (7q36.1). Loss of chromosome 7 or deletion of 7q is one of the most common alterations found in myeloid malignancies, occurring in 10-20% of cases. Complete loss of chromosome 7 in AML and MDS is associated with a poor prognosis, whereas 7q deletions are associated with intermediate risk (El-Menoufy et al. 2018). Genes located on chr. 7 which have been postulated to be relevant in the context of AML include the tumour suppressors *IKZF1*, *CUX1, KMT2E* (also known as *MLL5*) and *EZH2* (Heuser et al. 2009; de Rooij et al. 2015; Skoda and Schwaller 2019).					
264	13873-L15917	***IKZF1***, ex 4	NM_006060.6; 477-478	TACGAATGCTTG-ATGCCTCGGGAG	10,7 kb
472	14061-L24990	***IKZF1***, ex 6	NM_006060.6; 812-813	TTTTCTGCAGTT-GGTAAACCTCAC	51,9 **M**b
240	19886-L26753	***CUX1***, ex 5	NM_181552.4; 367-368	GAACCAGAAACT-TAGGGAAACTCT	136,7 kb
269 «	09472-L23871	***CUX1***, ex 22	NM_181552.4; 3596-3595, reverse	AGCTTCTCCACA-TTGTTGGGGTCG	2,7 **M**b

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location / ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
418	18796-L24994	***KMT2E***, ex 4	NM_182931.3; 626-627	TGTGGTAGTTGA-GAAATCCAACAG	15,2 kb
384	18528-L24355	***KMT2E***, ex 11	NM_182931.3; 1612-1613	AGTTTATGCTGA-GAGAACAGTTTG	43,9 **M**b
308	18272-L23874	***EZH2***, ex 14	NM_004456.5; 1761-1762	TGCCCTTGTGTG-ATAGCACAAAAT	17,7 kb
246	18267-L26041	***EZH2***, ex 4	NM_004456.5; 483-484	TGGTCTCCCCTA-CAGCAGAATTTT	-
Chromosome 11q alterations; *ATM* and *KMT2A* at 11q22.3-q23.3 *KMT2A* (also known as *MLL*) alterations are observed in 5-20% of AML cases, typically comprising partial tandem duplications (PTDs), amplifications and translocations (Rao et al. 2015), and are associated with poor outcome (Choi et al. 2018; Issa et al. 2021; Yuen et al. 2023). *KMT2A* PTDs are most commonly duplications occurring within exon 2-10 of *KMT2A* gene and are associated with an unfavourable prognosis (Choi et al. 2018). Probes targeting PTDs are indicated by (+). *KMT2A* is flanked by *UBE4A* and *TMEM25* genes.					
215 ¬	06675-L06253	***NUP98***	11**p**15.4	CTTCCTTCGTAT-CTGGAGGGTTCT	16,5 **M**b
296 ¬	08936-L33053	***SLC6A5***	11**p**15.1	TGTTTGCCTCCT-TTGTGTCTGTAC	87,2 **M**b
371	08420-L22087	***ATM***, ex 13	NM_000051.4; 2174-2175	AGAAAAGCACCA-GTCCAGTATTGG	18,9 kb
346	02663-L22101	***ATM***, ex 22	NM_000051.4; 3407-3406, reverse	CAGCCAACATGC-GAACTTGGTGAT	92,6 kb
290	08445-L23873	***ATM***, ex 63	NM_000051.4; 9192-9193	ATGAGACTACAA-GAGAAACTGAAA	10,3 **M**b
193 ¬	23323-L32969	***UBE4A***, ex 18	NM_001204077.2; 2925-2926	CCAAGGATGGAC-GTTCCTATTCCC	2,1 kb
251 ¬	23325-L32971	***UBE4A***, ex 19	NM_001204077.2; 3064-3065	ACAGGAAGAGGA-AACCTATGCAGA	43,7 kb
KMT2A, at 11q23.3. Ligation site information for *KMT2A* is according to NM_001197104.2.					
130 «	23318-L32964	***KMT2A***, ex 1	4-3, reverse	CCCGTGAAGTGA-AGCAGCGAGAGG	0,5 kb
		Start codon	22-24		
358 «	23329-L32998	***KMT2A***, ex 1	92 nt after exon 1, reverse	GAGATGGTCCCA-GAATTGGGATGC	10,6 kb
303 Ø	23335-L32983	***KMT2A***, intr 1	10,7 kb after exon 1, reverse	TTCCAATCATAT-AATCATCTTTTG	21,1 kb
337 +	23327-L32973	***KMT2A***, ex 2	503-504	CAGAGTGCGAAG-TCCCACAAGGTC	3,5 kb
197 +	09499-L32954	***KMT2A***, ex 3	1220-1221	AAGAAAGGTGAA-GACACAGGTCAA	0,1 kb
443 +	19133-L32999	***KMT2A***, ex 3	1372-1373	CGAATAGTAGAT-TCAGTGCCCCGT	1,1 kb
321 +	23326-L32972	***KMT2A***, ex 3	2501-2502	GGCAGAGCCATT-TTCATCAAGTAG	3,2 kb
228 +	23334-L33002	***KMT2A***, ex 4	4 nt before exon 4, reverse	TCTTGACCCTGA-ATATGAGGCAAT	1,3 kb
280 +	19132-L25682	***KMT2A***, ex 5	3469-3468, reverse	CCTCGATCGACG-TCCTTTCTTTAC	2,1 kb
391 +	23330-L32996	***KMT2A***, ex 6	3623-3622, reverse	AGGCTTTGGAAG-GCATCCATTGTA	1,6 kb
145 +	23320-L32966	***KMT2A***, ex 7	3742-3743	TGGTGGACTCTA-GTCAGAAACCTA	2,4 kb
466 +	23332-L32978	***KMT2A***, ex 9	4150-4151	ATGCAGGCACTT-TGAACATCCTCA	0,7 kb
353 +	23328-L32974	***KMT2A***, ex 10	4336-4337	TTCTCTGTGCCA-GTAGTGGGCATG	5,2 kb
436	23331-L32977	***KMT2A***, ex 13	4636-4637	GCTGTGGATCCA-CAACTCCAGGCA	5,6 kb
484	23333-L32979	***KMT2A***, ex 19	5426-5427	ACCTTCACTTGA-CCATAATTATGC	16,2 kb
166	23321-L32967	***KMT2A***, ex 31	11114-11115	GTCATTGACAGA-TAAAGTCCAGGA	10,0 kb
186	19885-L29240	***KMT2A***, ex 36	11771-11772	GCCTAACTGCTA-TTCTCGGGTCAT	9,9 kb
		Stop codon	11938-11940		
136 ¬	23319-L32965	***TMEM25***, ex 2	NM_032780.4; 139-140	CTTCTGAGCTCA-GGTACACCCCTG	2,7 kb
177 ¬	23322-L32968	***TMEM25***, ex 9	NM_032780.4; 1160-1161	GCAGTGATGAGA-TCTGGCTCTGAG	9,5 **M**b
182 ¬	09496-L25025	***ETS1***	11q24.3	TGTGTATGCAAA-ATGAATGGCACA	-
Chromosome 17p and 17q deletions Deletion of 17p, harbouring *TP53*, is observed in around 5% of AML patients and can be present as a single aberration or in the context of a complex karyotype, the latter being associated with a poor outcome. Significant positive association between was identified between *TP53* deletion and loss of chromosome 5, 5q deletion and loss of chromosome 7, all high-risk aberrations (Seifert et al. 2009; Walter et al. 2009). Moreover, loss of 17p arm and gain of 17q can result from isochromosome 17q formation (i(17q)) - an intermediate prognostic marker according to IPSS-R and IPSS-M (Greenberg et al. 2012; Bernard et al. 2022). Deletion of the tumour suppressor *NF1* is observed in 4—11% of AML cases and may deregulate signalling pathways implicated in AML development and progression (Walter et al. 2009; Parkin et al. 2010; Boudry-Labis et al. 2013; Haferlach et al. 2009; Giraud et al. 2023). Another important target on 17q is *SUZ12* (Score et al. 2012).					

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location / ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
The *TP53 exon* numbering is derived from the LRG_321 sequence; the exon numbering derived from MANE project according to NM_000546.6 for this gene can be found in between brackets.					
401	17423-L32981	***TP53***, ex 8 [9]	1095-1096	CTCTCCCCAGCC-AAAGAAGAAACC	0,2 kb
139	08304-L26800	***TP53***, ex 7 [8]	981-982	CTGTCCTGGGAG-AGACCGGCGCAC	1,4 kb
256	02376-L30912	***TP53***, ex 4b [5]	546-547	CAAGATGTTTTG-CCAACTGGCCAA	19,3 **M**b
412	18544-L24995	***NF1***, ex 34	NM_001042492.3; 4829-4830	TCTTTCCTTCAT-AAGTGACGGCAA	96,6 kb
154	12018-L32953	***NF1***, ex 53	NM_001042492.3; 8120-8121	TTTACGTAAAGT-TTCAGTGTCTGA	631,4 kb
498	18539-L23830	***SUZ12***	17**q**11.2	CATCAGGAAAAC-AAGCCTGGTTCA	-
***ASXL1* c.1934dupG mutation on 20q11.21** The *ASXL1* c.1934dupG mutation is the most commonly-found genetic alteration in the *ASXL1* gene in AML. *ASXL1* loss-of-function mutations were shown to promote myeloid transformation, and are associated with a poor prognosis in AML and MDS (Abdel-Wahab et al. 2012; Gelsi-Boyer et al. 2012; Chou et al. 2010).					
160 § ‡ Ж	18261-SP0848-L26260	***ASXL1***, ex 13	NM_015338.6, 2376-2375 and 2352-2351, reverse	CGGGCCACCCC**C**- 24nt spanning oligo- CCTCTCTATGGC	-

[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.

§ Mutation-specific probe. This probe will only generate a signal when the *ASXL1* c.1934dupG mutation is present. It has been tested on artificial DNA **and on DNA from the KASUMI-1 cell line.**

‡ An unspecific peak might be detected at one nucleotide shorter length from the expected length of this *ASXL1* c.1934dupG mutation-specific probe at 160 nt. Please analyse the peak pattern carefully when making calls for this mutation-specific 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.

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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

+ This probe targets an exon in the *KMT2A* gene that is commonly affected by PTDs in AML.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
315	14763-L24999	*PPOX*	1q23	AGACTGTGCACA-GTTTTGCCCAGC	39,2 **M**b
172	21100-L29489	*CACNA1S*	1q32	AAGCCGCCATGA-AGATCATTGCCT	-
148	17858-L23867	*SLC3A1*	2p21	CAGCCCAGATCG-GCTTTGAAGTTA	-
505	15203-L23403	*GBE1*	3p12	GACCTAGAGGGA-CTCATGATCTTT	75,0 **M**b
489	17939-L15290	*KCNAB1*	3q25	CTTTTCCAGAGA-GAGAAAGTGGAG	-
121	19616-L27455	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	-
208	13384-L25019	*EYS*	6q12	ACAAGGGGTTGA-TACCATGTGGAC	-
126	S0959-L26042	*PCSK5*	9q21	AGAAAGGCCTGA-TCATGAACCCTC	-
407	01237-L24913	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	-
452	16286-L27983	*RNASEH2B*	13q14	TGACTGTTAATG-ACTACCTTTGGT	-
378	08141-L24356	*RPGRIP1*	14q11	TATTCCTTCTAT-GACTTTGAAACC	-
274	09792-L23872	*SPG11*	15q15	GCTGACAAGCAA-ATGCCTCCATGG	-
233	15154-L25342	*MC4R*	18q21	GACATTTACTCA-CAGCAGGCATGG	-

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

Related SALSA MLPA probemixes

- **P414 MDS**: This probemix contains two probes targeting *KMT2A,* other probes targeting chromosomal arms 5q and 7q, and also includes several target for revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes (chromosomes 3, 8q, 12p, 19 and Y).
- **P437 Familial MDS-AML**: This probemix contains probes targeting various genes, such as *GATA2*, *TERT* and *RUNX1*, that have been described to be relevant in the context of familial MDS and AML.
- **P056 TP53**: This probemix contains 15 probes for the detection of *TP53* CNAs and *TP53* flanking regions.
- **P335 ALL-IKZF1**: This probemix contains eight probes targeting *IKZF1*.
- **P202 IKZF1-ERG**: This probemix contains 21 probes for *IKZF1* copy number determination.

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P496 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 29 November 2023 (04P) - In the positive control sample DNA table on page 3 several corrections were made and for all the cell lines the ACC numbers were added in between brackets. - Minor typo corrections. Version A1-01 - 14 November 2023 (04P) - Not applicable, new document.

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