Product Description
SALSA® MLPA® Probemix P414-C1 MDS

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 10.

Catalogue numbers:
- **P414-025R:** SALSA MLPA Probemix P414 MDS, 25 reactions.
- **P414-050R:** SALSA MLPA Probemix P414 MDS, 50 reactions.
- **P414-100R:** SALSA MLPA Probemix P414 MDS, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SD029 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 P414 MDS is a **research use only (RUO)** assay for the detection of deletions or duplications in different chromosomal regions described to be of potential diagnostic or prognostic relevance in myelodysplastic syndromes (MDS), and are used in the International Prognostic Scoring System–Revised (IPSS-R) and Molecular International Prognostic Scoring System for Myelodysplastic Syndromes (IPSS-M) (Bernard E et al. 2022): chromosome 3, 5q (*EGR1*, *MIR145*, *SPARC*, *MIR146A*), 7q (*EZH2*), 8q (*MYC*), 11q (*KMT2A*), 12p (*ETV6*), chromosome 17 (*TP53*, *NF1*, *SUZ12*), chromosome 19, 20q (*ASXL1*) and Y-chromosome. This probemix can also be used to detect the presence of the *JAK2* p.V617F (c.1849G>T) point mutation.

Myelodysplastic syndromes are a heterogeneous collection of hematologic disorders, which are characterized by dysplastic hematopoietic differentiation. In 30% of all cases, MDS progresses to acute myeloid leukemia (AML). There are several oncogenes and tumour suppressor genes, that have mutations or copy number alterations (CNAs) in MDS (Ogawa S. 2019), many of which can be detected with this P414 probemix.

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 *EGR1*, *SPARC*, *EZH2*, *KMT2A*, *TP53* and *ETV6* exon numbering used in this P414-C1 MDS product description is the exon numbering from MANE project based on MANE Select transcripts as indicated 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.

Probemix content

The SALSA MLPA Probemix P414-C1 MDS contains 58 MLPA probes with amplification products between 118 and 496 nucleotides (nt). This includes 46 probes for the following chromosomal regions: chromosome 3 (*MLH1*, *GATA2*, *MECOM*), 5q *(APC, EGR1, MIR145, RPS14*, *SPARC, MIR146A)* & one flanking probe targeting *NIPBL* at 5p, 7q (*CDK6*, *SAMD9L*, *EPO*, *KMT2E*, *MET*, *EZH2*) & one flanking probe at 7p targeting *IKZF1*, 8q *(FGFR1, NCOA2, RUNX1T1, MYC, PTK2)* & one flanking probe targeting *FGFR1* at 8p, 11q *(KMT2A, TIRAP*, *ETS1),* 12p *(ETV6, CDKN1B),* chromosome 17 *(TP53, NF1, SUZ12, AATF),* chromosome 19 (*SMARCA4*, *PRPF31*), 20q *(ASXL1, SRC, HNF4A, ZMYND8)* and the Y-chromosome (*ZFY*). Furthermore, this probemix also contains one probe specific for the *JAK2* p.V617F (c.1849G>T) mutation which will only generate a signal when the mutation is present. In addition, 12 reference probes are included that target relatively copy number stable regions in various cancer types including MDS. Complete probe sequences are available online (www.mrcholland.com) and the identity of the genes detected by the reference probes is available 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 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples

A sufficient number (≥3) of 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 MDS. It is strongly recommended to use male samples to facilitate interpretation, see more information on page 5 in *P414 specific notes* section. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

See the section Positive samples on the P414 MDS product page on our website.

SALSA Binning DNA SD029

The SD029 Binning DNA provided with this probemix can be used for binning of all probes including the *JAK2* p.V617F (c.1849G>T) mutation-specific probe (JAK2 probe 05672-L17742). SD029 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 SD029 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 SD029 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 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.

P414 specific notes:

- 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.
- The presence of a clear signal for the 208 nt probe (at least 10% of the mean peak height of all reference probes in the sample), indicates the presence of the *JAK2* p.V617F (c.1849G>T) mutation.
- In this probemix, the 118 nt Y-specific probe is included as a target probe to help detection of the loss of the Y-chromosome in male samples. **To ensure that the comparative analysis can be completed for all samples, only male reference samples should be used**. When using Coffalyser.Net software for data analysis, the "#Y probes+fragments" section in 'Sample Results Explorer' or in 'Comparative analysis sample results' can be used for determining the presence of signal of the Y-chromosome targeting probes (at 105 and 118 nt), where '2/2' is indicated for male samples with intact Y-chromosome, and '0/2' is indicated for female or male samples with a Y-chromosome loss. SD029 will have the indication of '1/2'. Loss of the Y-chromosome in male samples can also be confirmed by visual examination of the 105 and 118 nt Y-fragment peaks in the electropherogram (fragment analysis).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the MDS samples are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P414 MDS.
- 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 solid 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.

COSMIC mutation database
http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *TP53* exons 1 and 10 but not exon 4b) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P414-C1 MDS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			Location (hg18) in kb
		Reference	Target	Mutation-specific	
64-105	Control fragments – see table in probemix content section for more information				
118	**ZFY probe** S0135-L13810		Yp11.31		Y-002,889
122	Reference probe 19616-L27299	4p13			04-042,278
127 +	**MIR145 probe** 19041-L29241		5q33.1		05-148,790
133 ¬	**IKZF1 probe** 03340-L20689		7p12.2		07-050,338
138	**NCOA2 probe** 09938-L20198		8q13.3		08-071,250
143	Reference probe 14199-L15813	2q13			02-108,894
151	**AATF probe** 21021-L29242		17q12		17-032,463
155	**RPS14 probe** 16292-L18584		5q33.1		05-149,807
160	**MYC probe** 20780-L29239		8q24.21		08-128,822
167	**TP53 probe** 01588-L06028		17p13.1		17-007,531
172	Reference probe 08726-L25038	9q21			09-078,038
176	**SRC probe** 22671-L19475		2q11.23		20-035,448
182	**ETS1 probe** 09496-L25025		11q24.3		11-127,836
186	**KMT2A probe** 19885-L29240		11q23.3		11-117,898
192	**SAMD9L probe** 16293-L24868		7q21.2		07-092,600
197	**KMT2A probe** 17085-L24869		11q23.3		11-117,853
202	**PTK2 probe** 18560-L24870		8q24.3		08-141,739
208 §	**JAK2 probe** 05672-L17742			**p.V617F (c.1849G>T)**	09-005,064
214	**ASXL1 probe** 18515-L25024		2q11.21		20-030,480
220	**SUZ12 probe** 18518-L23809		17q11.2		17-027,345
227	Reference probe 05282-L31924	14q22			14-050,130
232	**SPARC probe** 16294-L24866		5q33.1		05-151,047
238	**EPO probe** 17089-L20201		7q22.1		07-100,158
244	**MET probe** 10323-L10837		7q31.2		07-116,199
250	**MLH1 probe** 18556-L24871		3p22.2		03-037,031
256 ‡	**TP53 probe** 02376-L24176		17p13.1		17-007,519
263	Reference probe 14738-L20398	4q22			04-089,187
268	**MIR146A probe** 15653-L24279		5q33.3		05-159,845
274	**CDKN1B probe** 07949-L24280		12p13.1		12-012,762
282 Ж	**EZH2 probe** 18271-SP0635-L23385		7q36.1		07-148,135
286	**EGR1 probe** 22557-L32018		5q31.2		05-137,829
294	Reference probe 04570-L25187	16q13			16-055,491
301	**EGR1 probe** 17091-L20203		5q31.2		05-137,832
308	**EZH2 probe** 22673-L23019		7q36.1		07-148,145
315	**SPARC probe** 19042-L25092		5q33.1		05-151,027
324	Reference probe 22672-L26274	18q21			18-045,659
332	**TIRAP probe** 18557-L25022		11q24.2		11-125,666
341	**NF1 probe** 02507-L25021		17q11.2		17-026,576

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	Target	Mutation-specific	Location (hg18) in kb
348	**RUNX1T1 probe** 09487-L24873		8q21.3		08-093,099
355	**ZMYND8 probe** 14661-L16313		2q13.12		20-045,308
361	**CDK6 probe** 18558-L02523		7q21.2		07-092,085
368	**PRPF31 probe** 06016-L29420		19q13.42		19-059,319
377	Reference probe 10693-L19115	6p12			06-051,721
385 ¬	**FGFR1 probe** 01046-L24278		8p12		08-038,434
392	**ETV6 probe** 13875-L19638		12p13.2		12-011,797
400 ¬	**NIPBL probe** 04837-L24177		5p13.2		05-037,039
409	Reference probe 13405-L31765	6q12			06-065,393
415	**HNF4A probe** 09999-L29451		2q13.12		20-042,477
421	**APC probe** 01807-L29244		5q22.2		05-112,201
427	**KMT2E probe** 18796-L29272		7q22.2		07-104,490
436	Reference probe 08839-L32008	2p13			02-071,767
445	**MECOM probe** 18573-L24179		3q26.2		03-170,332
454	**TP53 probe** 08785-L19640		17p13.1		17-007,515
463	**SMARCA4 probe** 09980-L10439		19p13.2		19-011,000
472	Reference probe 00979-L31258	10p14			10-012,019
481 Ж	**GATA2 probe** 18576-SP0668-L23908		3q21.3		03-129,685
490	**ETV6 probe** 13871-L24874		12p13.2		12-011,914
496	Reference probe 09772-L25949	15q21			15-042,706

[a] See section Exon numbering on page 1 for more information.

§ Mutation-specific probe. This probe will only generate a signal when the *JAK2* p.V617F (c.1849G>T) mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the NCI TP53 Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ This probe showed reduced ratios (0.70-0.84) in multiple commercial germline DNA samples at MRC Holland. Aberrant results should be treated with caution.

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

Table 2. P414-C1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene, exon[a]	Location	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Loss of chromosome Y Loss of the Y-chromosome is detected in ~5% of MDS cases. Del Y is associated with significantly increased overall survival and it can be used as a marker for the low-risk prognostic subgroup (Greenberg et al. 2012). **Note** that the loss of the Y-chromosome can be confirmed in a patient sample by evaluating results obtained with the 105 nt probe. When using Coffalyser.Net software for data analysis, the presence of both Y-specific probes (105 and 118 nt) can be verified by visually inspecting the electropherograms. Please note that it can be difficult to distinguish female samples from male samples in which the Y chromosome was lost. When in doubt, additional gender confirmation is advised.					
118	S0135-L13810	***ZFY***	Y**p**11.31	TCATAGAGGAGG-ATGTTCAGTGCT	-
Loss of 3q Loss of 3q, as well as other chromosome 3 abnormalities, are associated with decreased overall survival of MDS patients and can be used as a marker for the poor prognostic subgroup (Greenberg et al. 2012).					
250	18556-L24871	***MLH1***	3**p**22.2	ATGGTCCCATAA-AATTCCCTGTGG	92.7 **M**b
481 Ж	18576-SP0668-L23908	***GATA2***	3q21.3	TTGATGAGTGGT-39 nt spanning oligo-TTGCACAGGTAG	40.7 **M**b
445	18573-L24179	***MECOM***	3q26.2	AGAGCGAAGACT-ATCCCCATGAAA	-
Chromosome 5q deletions					

Length (nt)	SALSA MLPA probe	Gene, exon[a]	Location	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Deletion of 5q is one of the most frequent alterations detected in MDS, occurring in 10-30% of MDS cases (Haase et al. 2007; Tothova et al. 2013), and it is a marker for increased survival in MDS (Greenberg et al. 2012). 5q minus syndrome, a subtype of MDS, is characterised by the deletion of the 5q arm. This chromosomal arm harbours several genes that are suggested to be the mediators of 5q phenotype including *MIR145, MIR146* (Starczynowski et al. 2010), *EGR1* (Joslin et al. 2007), RPS14 (Ebert et al. 2008), and *SPARC* (Jaju et al. 1998; Lehmann et al. 2007). *EGR1* and *SPARC* exon numbering is according to MANE Select transcript NM_001964.3 and NM_003118.4, respectively.					
400 ¬	04837-L24177	***NIPBL***	5**p**13.2	TCCTCAGGAACT-GCTCTTAGGAAA	75.2 **M**b
421	01807-L29244	***APC***	5q22.2	TGCTGCAGCTTT-AAGGAATCTCAT	25.6 **M**b
286	22557-L32018	***EGR1***, ex 1	5q31.2	GCTCGTCCAGGA-TGGCCGCGGCCA	2.4 kb
301	17091-L20203	***EGR1***, ex 2	5q31.2	TCTTAGGTCAGA-TGGAGGTTCTCA	11.0 **M**b
127 +	19041-L29241	***MIR145***	5q33.1	TTTCACAGCTGG-ATTTGCCTCCTT	10.2 **M**b
155	16292-L18584	***RPS14***	5q33.1	TGCGACTCGTAC-CTATTTCCTCCT	12.2 **M**b
315	19042-L25092	***SPARC***, ex 7	5q33.1	ACCCTGTATGAG-AGGGATGAGGAC	19.4 kb
232	16294-L24866	***SPARC***, ex 1	5q33.1	AACCCCTCCACA-TTCCCGCGGTCC	8.8 **M**b
268	15653-L24279	***MIR146A***	5q33.3	TCGTGGGCTTGA-GGACCTGGAGAG	-
Monosomy 7 and 7q deletions Monosomy of chromosome 7 or interstitial deletions of the 7q arm are commonly found in MDS and are associated with poor prognosis (Greenberg et al. 2012). Several candidate genes have been proposed as biomarkers in this context, including *SAMDL9, CDK6* (Asou et al. 2009) and *EZH2* (Ernst et al. 2010; Nikoloski et al. 2010). *EZH2* exon numbering is according to MANE Select transcript NM_004456.5.					
133 ¬	03340-L20689	***IKZF1***	7**p**12.2	GGGAGGACAGCA-AAGCTCCAAGAG	41.7 **M**b
361	18558-L02523	***CDK6***	7q21.2	GAGAAGAAGACT-GGCCTAGAGATG	0.5 **M**b
192	16293-L24868	***SAMD9L***	7q21.2	TCACCACAGAAG-TCCCAGAGACGA	7.6 **M**b
238	17089-L20201	***EPO***	7q22.1	TGGATAAAGCCG-TCAGTGGCCTTC	4.3 **M**b
427	18796-L29272	***KMT2E***	7q22.2	TGTGGTAGTTGA-GAAATCCAACAG	11.7 **M**b
244	10323-L10837	***MET***	7q31.2	AACAGCACTGTT-ATTACTACTTGG	31.9 **M**b
282 Ж	18271-SP0635-L23385	***EZH2***, ex 20	7q36.1	TTTTGCAATAAT-44 nt spanning oligonucleotide-TTGTCCTTGTTG	9.4 kb
308	22673-L23019	***EZH2***, ex 12	7q36.1	TGCCTCTTGTCA-GGTGTATGAGTT	-
Chromosome 8 gains A gain of chromosome 8 is found in 5-10% of MDS cases and is associated with an intermediate prognostic risk (Greenberg et al. 2012; Schanz et al. 2011; Haase et al. 2007).					
385 ¬	01046-L24278	***FGFR1***	8**p**12	CAACCTCTAACT-GCAGAACTGGGA	32.8 **M**b
138	09938-L20198	***NCOA2***	8q13.3	ACAGGGCAGGGT-GTCATCGACAAG	21.9 **M**b
348	09487-L24873	***RUNX1T1***	8q21.3	TCACCTGTGGAT-GTGAAGACGCAA	35.7 **M**b
160	20780-L29239	***MYC***	8q24.21	GAACGAGCTAAA-ACGGAGCTTTTT	12.9 **M**b
202	18560-L24870	***PTK2***	8q24.3	CTATTGAACTCT-GACCTGGGTGAG	-
***JAK2* p.V617F mutation, 9p24.1** *JAK2* p.V617F (c.1849G>T) mutation is detected in 2-5% of MDS cases and the V617F status is suggested to identify the RARS-T subtype of MDS (Zipperrer et al. 2008; Schmitt-Graeff et al. 2008), which was shown to correlate with better prognosis (Atallah et al. 2008). For detection of *JAK2* p.V617F down to 1% mutation burden P520 MPN mix 2 can be used.					
208 §	05672-L17742	***JAK2***	**c.1849G>T=p.V617F** NM_004972.4; 2315-2314 *reverse*	GTCTCCACAGA**A**-ACATACTCCATA	-
11q deletions and amplifications Deletion of the 11q arm is a marker of significantly better survival for MDS according to the IPSS-R (Greenberg et al. 2012). In contrast, amplification of the 11q arm, and especially of the *MLL (KMT2A)* gene, is associated with poor response to therapy and a poor prognosis (Streubel et al. 2000). Moreover, partial tandem duplications in KMT2A gene (KMT2A-PTD, also known as MLL-PTD) most commonly occurring within exons 2-11, are among the top predictors of adverse outcome according to IPSS-M (Bernard E et al. 2022). KMT2A exon 4 probe locates in the PTD region. P496 KMT2A probemix contains 17 probes for *KMT2A* and can be used for KMT2A-PTD detection. *TIRAP*, often overexpressed in MDS, is located on 11q, as well (Starczynowski et al. 2008; Starczynowski et al. 2010).					
197	17085-L24869	***KMT2A***, ex 4	11q23.3	GGACCCCGGATT-AAACATGTCTGC	45.1 kb
186	19885-L29240	***KMT2A***, ex 36	11q23.3	GCCTAACTGCTA-TTCTCGGGTCAT	7.8 **M**b
332	18557-L25022	***TIRAP***	11q24.2	CCAGATCCCGAA-TATCCTCCTGGC	2.2 **M**b
182	09496-L25025	***ETS1***	11q24.3	TGTGTATGCAAA-ATGAATGGCACA	-
Loss of 12p Loss of the 12p arm, which is a recurrent cytogenetic abnormality in MDS, is associated with a good prognosis (Greenberg et al. 2012). The minimal region of deletion in 12p includes the *ETV6* and *CDKN1B* genes (Sato et al. 1995). More probes for *ETV6*					

Length (nt)	SALSA MLPA probe	Gene, exon[a]	Location	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
gene can be found in P335 ALL-IKZF1 probemix and more probes for chromosome 12 can be found in P323 CDK4-HMGA2-MDM2 probemix. *ETV6* exon numbering is according to MANE Select transcript NM_001987.5.					
392	13875-L19638	***ETV6***, ex 2	12**p**13.2	TTCATGTTCCAG-TGCCTCGAGCGC	0,1 **M**b
490	13871-L24874	***ETV6***, ex 5	12**p**13.2	AATGTGCACCAT-AACCCTCCCACC	0,9 **M**b
274	07949-L24280	***CDKN1B***	12**p**13.1	CGCGCTCCTAGA-GCTCGGGCCGTG	
Loss of 17p and gain of 17q Well-known tumour suppressor gene *TP53* locates on 17p and is deleted in ~5% of MDS patients with a complex karyotype (Jasek et al. 2010). 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). More probes for *TP53* gene can be found in P056 TP53 probemix. *TP53* exon numbering is according to MANE Select transcript NM_000546.6. The exon numbering used in previous versions of this product description can be found in between brackets.					
454	08785-L19640	***TP53***, ex 10	17**p**13.1	TTCCGAGAGCTG-AATGAGGCCTTG	4.5 kb
256 ‡	02376-L24176	***TP53***, ex 5 (4b)	17**p**13.1	CAAGATGTTTTG-CCAACTGGCCAA	12.2 kb
167	01588-L06028	***TP53***, ex 1	17**p**13.1	TCCGGGGACACT-TTGCGTTCGGGC	19.1 **M**b
341	02507-L25021	***NF1***	17q11.2	GGATCATGAAGA-ATTACTACGTAC	0.8 **M**b
220	18518-L23809	***SUZ12***	17q11.2	TGCCCTTGGTGT-ACTCTGAACTGC	5.1 **M**b
151	21021-L29242	***AATF***	17q12	CAAGCTACTGAG-TTTCATGGCACC	-
Chromosome 19 gain A gain of chromosome 19 is found in ~15% of MDS patients and is associated with an intermediate prognostic risk (Greenberg et al. 2012; Haase et al. 2007).					
463	09980-L10439	***SMARCA4***	19p13.2	GTATGAGCCAGT-GAGGCGTTTCTT	48.3 **M**b
368	06016-L29420	***PRPF31***	19q13.42	ACAAGTGCAAGA-ACAATGAGAACC	
Loss of 20q Loss of the 20q arm is frequently found in MDS, and this loss alone is associated with a good prognosis (Greenberg et al. 2012; Martinez-Ramirez et al. 2005).					
214	18515-L25024	***ASXL1***	20q11.21	AGTGGTCTCGCC-ATCCAGCTACAG	5.0 **M**b
176	22671-L19475	***SRC***	20q11.23	CTATGACTATGA-GTCTAGGACGGA	7.0 **M**b
415	09999-L29451	***HNF4A***	20q13.12	CATGAAGGAGCA-GCTGCTGGTTCT	2.8 **M**b
355	14661-L16313	***ZMYND8***	20q13.12	CGAAAAGGCAAA-ACCTTCACCTCA	

[a] See section Exon numbering on page 1 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 *JAK2* p.V617F (c.1849G>T) mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the NCI TP53 Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ This probe showed reduced ratios (0.70-0.84) in multiple commercial germline DNA samples at MRC Holland. Aberrant results should be treated with caution.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.
Chromosomal p-arms are indicated in bold for easier visualisation of target probes

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
436	08839-L32008	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT	02-071,767
143	14199-L15813	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108,894
122	19616-L27299	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
263	14738-L20398	*PKD2*	4q22	TGTCACAACTTT-TGATTTCTTCCT	04-089,187

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
377	10693-L19115	*PKHD1*	6p12	TTGTACTCATCT-GTTGAATTCAGT	06-051,721
409	13405-L31765	*EYS*	6q12	GACTACTAATCA-AGTTTTAAGCAA	06-065,393
172	08726-L25038	*PCSK5*	9q21	ACACCTGCCAGA-GATGCCAAGGAA	09-078,038
472	00979-L31258	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
227	05282-L31924	*ATL1*	14q22	TTTATTTTCTTT-TTTGTGTATCTG	14-050,130
496	09772-L25949	*SPG11*	15q21	TTTCTTCAGGAT-TGATAGTCATTC	15-042,706
294	04570-L25187	*SLC12A3*	16q13	CAAGTTCCGACT-GGGATTCCATGA	16-055,491
324	22672-L26274	*MYO5B*	18q21	AACTGCAGCTTA-GCGTGTTGCTTT	18-045,659

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

Related products

For related products, see the product page on our website.

References

- Asou H et al. (2009). Identification of a common microdeletion cluster in 7q21.3 subband among patients with myeloid leukemia and myelodysplastic syndrome. *Biochem Biophys Res Commun*. 383:245-51.
- Atallah E et al. (2008). Prognostic interaction between thrombocytosis and JAK2 V617F mutation in the WHO subcategories of myelodysplastic/myeloproliferative disease-unclassifiable and refractory anemia with ringed sideroblasts and marked thrombocytosis. *Leukemia*. 22:1295-8.
- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Bernard E et al. (2022). Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. *N Engl J Med*. 1:1-14
- Ebert BL et al. (2008). Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. *Nature.* 451:335-9.
- Ernst T et al. (2010). Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 42:722-6.
- Greenberg PL et al. (2012). Revised international prognostic scoring system for myelodysplastic syndromes. *Blood.* 120:2454-65.
- Haase D et al. (2007). New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood.* 110:4385-95.
- Hömig-Hölzel C and Savola S (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol.* 21:189-206.
- Jaju RJ et al. (1998). Molecular cytogenetic delineation of the critical deleted region in the 5q- syndrome. *Genes Chromosomes Cancer.* 22:251-6.
- Jasek M et al. (2010). TP53 mutations in myeloid malignancies are either homozygous or hemizygous due to copy number-neutral loss of heterozygosity or deletion of 17p. *Leukemia.* 24:216-9.
- Joslin JM et al. (2007). Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. *Blood.* 110:719-26.
- Lehmann S et al. (2007). Common deleted genes in the 5q- syndrome: thrombocytopenia and reduced erythroid colony formation in SPARC null mice. *Leukemia*. 21:1931-6.
- Martinez-Ramirez A et al. (2005). Analysis of myelodysplastic syndromes with complex karyotypes by high-resolution comparative genomic hybridization and subtelomeric CGH array. *Genes Chromosomes Cancer*. 42:287-98.
- Nikoloski G et al. (2010). Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet.* 42:665-7.
- Ogawa S. (2019). Genetics of MDS. *Blood*. 133:1049-59.
- Sato Y et al. (1995). TEL and KIP1 define the smallest region of deletions on 12p13 in hematopoietic malignancies. *Blood.* 86:1525-33.
- Schanz J et al. (2011). Coalesced multicentric analysis of 2,351 patients with myelodysplastic syndromes indicates an underestimation of poor-risk cytogenetics of myelodysplastic syndromes in the international prognostic scoring system. *J Clin Oncol*. 29:1963-70.
- Schmitt-Graeff AH et al. (2008). JAK2 V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Haematologica*. 93:34-40.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.

- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Starczynowski DT et al. (2008). High-resolution whole genome tiling path array CGH analysis of CD34+ cells from patients with low-risk myelodysplastic syndromes reveals cryptic copy number alterations and predicts overall and leukemia-free survival. *Blood.* 112:3412-24.
- Starczynowski DT et al. (2010). Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat Med*. 16:49-58.
- Streubel B et al. (2000). Amplification of the MLL gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. *Genes Chromosomes Cancer.* 27:380-6.
- Tothova Z et al. (2013). New strategies in myelodysplastic syndromes: application of molecular diagnostics to clinical practice. *Clin Cancer Res.* 19:1637-43.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.
- Zipperrer E et al. (2008). MPL 515 and JAK2 mutation analysis in MDS presenting with a platelet count of more than 500 x 10(9)/l. *Ann Hematol*. 87:413-5.

Selected publications using SALSA MLPA Probemix P414 MDS

- Ai X et al. (2021). Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization for detecting chromosome abnormalities in myelodysplastic syndromes: A retrospective study. *Medicine (Baltimore)*. 100:e25768
- Chicano M et al. (2021) Next Generation Cytogenetics in Myeloid Hematological Neoplasms: Detection of CNVs and Translocations. *Cancers (Basel)*. 13:3001.
- Bănescu C et al. (2019). Presence of copy number aberration and clinical prognostic factors in patients with acute myeloid leukemia: an analysis of effect modification. *Pol Arch Intern Med.* 129:898-906.
- Kampa-Schittenhelm KM et al. (2020). Epigenetic activation of O-linked β-N-acetylglucosamine transferase overrides the differentiation blockage in acute leukemia. *EBioMedicine.* 54: 102678.
- Ma J et al. (2021). Multiplex ligation-dependent probe amplification identifies copy number changes in normal and undetectable karyotype MDS patients. *Ann Hematol*. 100:2207-14.
- Wang J et al. (2017). Multiplex ligation-dependent probe amplification assay identifies additional copy number changes compared with R-band karyotype and provide more accuracy prognostic information in myelodysplastic syndromes. *Oncotarget.* 8:1603-12.
- Yip BH et al. (2017). Amplification of mixed lineage leukemia gene perturbs hematopoiesis and cooperates with partial tandem duplication to induce acute myeloid leukemia. *Haematologica.* 102:e300-e304.

P414 product history	
Version	*Modification*
C1	Five reference probes are replaced and several probes have a change in length but no change in the sequence targeted.
B1	One reference and two target probes are replaced, and several probes have a small change in length but not in the sequence detected.
A1	First release.

Implemented changes in the product description
Version C1-04 – 29 July 2025 (04P) - Positive control DNA samples section: information moved to product page on website. - Removed Related SALSA MLPA products section. - Exon numbering of the *TP53* gene is now reported based on MANE Select Transcript NM_000546.6 in Table 2. *Version C1-03 – 15 January 2024 (04P)* - Product description rewritten and adapted to a new template. - Cancer cell line sample data added to Positive samples section and removed NA00959 sample data. - Indicated NM-sequences for MANE select transcripts used for exon numbering in Table 2. - Exon numbering of the TP53 according to MANE database added in brackets in Table 2.

- Footnote added to Table 1 and 2 for two probes: MIR145 probe 19041-L29241 and TP53 probe 02376-L24176.
- Added information about P496 KMT2A probemix in Table 2.
- Two articles added to References section.
- New references added in the Selected publications section.
- The related SALSA Probemixes section was updated (now includes P437 and P496 probemixes).

Version C1-02 – 03 June 2020 (02P)

- Table on Positive samples (page 2-3) has been adjusted to correct a typo for chromosomal location of CNA for NA09102.
- New reference is added to selected publications using SALSA MLPA Probemix P414 MDS on page 10.

Version C1-01 — 14 April 2020 (02P)

- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2a/b).
- Various minor textual or layout changes.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Related SALSA MLPA probemixes section revised on page 9.

Version 07 – 19 March 2019 (T08)

- Information about related MLPA probemixes updated on page 1.
- New reference for probemix P414 added on page 1.

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