Product Description SALSA® MLPA® Probemix P414-C1 MDS

To be used with the MLPA General Protocol.

Version C1. As compared to version B1, five reference probes are replaced and several probes have a change in length but no change in the sequence targeted. 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 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.mlpa.com).

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

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

General information: The SALSA MLPA Probemix P414 MDS is a **research use only (RUO)** assay for the detection of deletions or duplications in the different chromosomal regions that are suggested to be of diagnostic or prognostic relevance in myelodysplastic syndromes (MDS) and are used in the revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes: 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 *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, which are either altered in their copy number or mutated in MDS. The purpose of this probemix is to detect the majority of these clinically and prognostically important changes.

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
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The exon numbering used in this P414-C1 MDS product description is the exon numbering from the RefSeq transcript NM_001964.3 for *EGR1,* LRG_531 for *EZH2*, LRG_613 for *KMT2A*, LRG_609 for *ETV6*, LRG_321 for *TP53*, and the RefSeq transcript NM_003118.4 for *SPARC*. The exon numbering used has been retrieved on 03/2020. As changes to the NCBI database can occur after the release of this product description, 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, 5q *(EGR1, MIR145, SPARC, MIR146A)* (+one flanking probe at 5p), 7q *(EZH2)* (+one flanking probe at 7p), 8q *(MYC)* (+one flanking probe at 8p), 11q *(KMT2A),* 12p *(ETV6),* chromosome

17 *(TP53, NF1, SUZ12),* chromosome 19, 20q *(ASXL1)* and Y-chromosome. Furthermore, this probemix also contains one probe specific for the *JAK2* p.V617F 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.mlpa.com) and the identity of the genes detected by the reference probes is available in Table 2b.

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.mlpa.com.

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

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com). 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 healthy individuals who are from families without a history of MDS. It is strongly recommended to use male samples to facilitate interpretation, see more information on page 4 in *P414 MDS specific notes* section. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Samples from the Coriell Institute have been tested with this P414-C1 probemix at MRC-Holland and can be used as a positive control samples, see table below for details. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P414-C1	Expected CNA
NA03563	Coriell Institute	3q21.3-q26.2	*GATA2, MECOM*	Heterozygous duplication
NA14234	Coriell Institute	5q22.2	*APC*	Heterozygous deletion
NA04371	Coriell Institute	5q33.3	*MIR146A*	Heterozygous duplication
NA10160	Coriell Institute	7q21.2-q21.2	*CDK6, SAMD9L*	Heterozygous deletion

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P414-C1	Expected CNA
NA01059	Coriell Institute	7q22.2-q31.2	*KMT2E, MET*	Heterozygous deletion
NA07412	Coriell Institute	7q36.1	*EZH2*	Heterozygous deletion
NA01220	Coriell Institute	7q36.1	*EZH2*	Heterozygous duplication
NA02030	Coriell Institute	8p12-q24.3	*FGFR1, NCOA2, RUNX1T1, MYC, PTK2*	Heterozygous duplication
NA14485	Coriell Institute	8p12	*FGFR1*	Heterozygous duplication
NA03999	Coriell Institute	8q24.21	*MYC*	Heterozygous deletion
NA20263	Coriell Institute	8q24.3	*PTK2*	Heterozygous duplication
NA00959	Coriell Institute	11q23.3-q24.3	*KMT2A, TIRAP, ETS1*	Heterozygous duplication
NA15099	Coriell Institute	11q23.3-q24.3	*KMT2A, TIRAP, ETS1*	Heterozygous duplication
NA09102	Coriell Institute	11q24.2-q24.3	*TIRAP, ETS1*	Heterozygous deletion
NA07981	Coriell Institute	12p13.1-p13.2	*ETV6, CDKN1B*	Triplication
NA02587	Coriell Institute	17q11.2-q12	*NF1, SUZ12, AATF*	Heterozygous deletion (mosaic)
NA07945	Coriell Institute	20q11.21-q13.12	*SRC, HNF4A*	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 P414-C1 MDS probemix.

SALSA Binning DNA SD029: The SD029 Binning DNA provided with this probemix can be used for binning of *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, as for this purpose true mutation/SNP positive patient samples or cell lines should be used. 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.mlpa.com.

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.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients are affected both by the 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

P414 MDS specific notes:

- 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 mutation.
- In this probemix, the 118 nt Y-specific target probe is included as a target probe to help detection of the loss of the whole Y-chromosome in male samples. **To ensure that the comparative analysis can be completed for all samples, only male reference samples should be used.** In male samples, the 118 nt Y-specific target probe will be counted as an "additional" probe when using Coffalyser.Net software. In female samples, the 118 nt Y-specific target probe will be reported as "absent" after the comparative analysis. Loss of the Y-chromosome in male samples can also be confirmed by visual examination of the 105 nt Y-fragment peak in the electropherogram (fragment analysis).

Limitations of the procedure:

- In most populations, the majority 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- 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 those 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 SNPs and unusual results (e.g., a duplication of *TP53* exons 1 and 10 but not exon 4b) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P414-C1 MDS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference Target region Mutation-specific	Location (hg18) in kb
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	20q11.23	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	20q11.21	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
348	**RUNX1T1 probe** 09487-L24873	8q21.3	08-093.099
355	**ZMYND8 probe** 14661-L16313	20q13.12	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	20q13.12	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

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference Target region Mutation-specific	Location (hg18) in kb
490	**ETV6 probe** 13871-L24874	12p13.2	12-011.914
496	Reference probe 09772-L25949	15q21	15-042.706

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in the sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *JAK2* p.V617F mutation is present.
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.

Table 2a. 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. Del Y is associated with a 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 probe. When using Coffalyser.Net software for data analysis, the presence of both Y-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	Yp11.31	TCATAGAGGAGG-ATGTTCAGTGCT	-
Loss of 3q Loss of 3q is associated with a decreased overall survival of MDS patients and it 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-39nt spanning oligonucleotide-TTGCACAGGTAG	40.6 **M**b
445	18573-L24179	MECOM	3q26.2	AGAGCGAAGACT-ATCCCCATGAAA	-
Chromosome 5q deletions 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).					
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	1.0 **M**b
155	16292-L18584	RPS14	5q33.1	TGCGACTCGTAC-CTATTTCCTCCT	1.2 **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 pinpointed including e.g. *SAMDL9, CDK6* (Asou et al. 2009), *EZH2* (Ernst et al. 2010; Nikoloski et al. 2010).					
133 ¬	03340-L20689	IKZF1	7**p**12.2	GGGAGGACAGCA-AAGCTCCAAGAG	41.7 **M**b
361	18558-L02523	CDK6	7q21.2	GAGAAGAAGACT-GGCCTAGAGATG	514.2 kb
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-44nt spanning oligonucleotide-TTGTCCTTGTTG	9.4 kb
308	22673-L23019	EZH2, ex 12	7q36.1	TGCCTCTTGTCA-GGTGTATGAGTT	-

Length (nt)	SALSA MLPA probe	Gene (exon[a])	Location	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
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	21.8 **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** *JAK2* p.V617F (c.1849G>T) mutation is detected in 2-5% of MDS and the V617F status is suggested to identify RARS-T subtype of MDS (Zipperrer et al. 2008; Schmitt-Graeff et al. 2008) which is 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 suggested to associate with poor response to therapy and a poor prognosis (Streubel et al. 2000). Other target genes in 11q amplification have also been suggested like the TIRAP (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 suggested to carry 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* gene can be found in P335 ALL-IKZF1 probemix and more probes for chromosome 12 can be found in P323 CDK4-HMGA2-MDM2 probemix.					
392	13875-L19638	ETV6, ex 2	12**p**13.2	TTCATGTTCCAG-TGCCTCGAGCGC	117.0 kb
490	13871-L24874	ETV6, ex 5	12**p**13.2	AATGTGCACCAT-AACCCTCCCACC	848.2 kb
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). Loss of 17p arm and gain of 17q can result from isochromosome 17q formation and i(17q) is an intermediate prognostic marker in IPSS-R (Greenberg et al. 2012). More probes for *TP53* gene can be found in P056 TP53 probemix.					
454	08785-L19640	TP53, ex 10	17**p**13.1	TTCCGAGAGCTG-AATGAGGCCTTG	4.5 kb
256	02376-L24176	TP53, ex 4b	17**p**13.1	CAAGATGTTTTG-CCAACTGGCCAA	12.2 kb
167	01588-L06028	TP53, ex 1	17**p**13.1	TCCGGGGACACT-TTGCGTTCGGGC	19.0 **M**b
341	02507-L25021	NF1	17q11.2	GGATCATGAAGA-ATTACTACGTAC	768.7 kb
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, associated with an intermediate prognostic risk (Greenberg et al. 2012; Haase et al. 2007).					
463	09980-L10439	SMARCA4	19**p**13.2	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 above section on exon numbering for more information.
b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

§ Mutation-specific probe. This probe will only generate a signal when the *JAK2* p.V617F mutation is present.
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.

Notes:

- Exon numbering might be different as compared to literature! Please notify us of any mistakes: info@mlpa.com.
- Chromosomal p-arms are indicated in bold for easier visualisation of target probes.

Table 2b. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Location (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
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.mlpa.com.

Related SALSA MLPA probemixes

- **P056 TP53**: This probemix contains probes for each exon of *TP53* gene.
- **P323 CDK4-HMGA2-MDM2**: This probemix contains more probes for chromosome 12.
- **P335 ALL-IKZF1**: This probemix contains more probes for *ETV6* gene.
- **P377 Hematologic malignancies**: This probemix contains more probes for 5q, chr. 7, 8q *(MYC),* 11q *(ATM)* and chr.17.
- **P520 MPN mix 2**: Contains probes for e.g. *JAK2* p.V617F mutation detection with only 1% allele burden.

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.
- 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.
- 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). JAK2V617F 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

- 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.
- 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 C1-02 – 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. *Version 06 – 05 August 2016 (T08)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). - Related SALSA® MLPA® probemixes updated on page 1. - Warning on SNP (rs143651717) for 355 nt probe removed as the frequency of this SNP is low (2/4550) according to the dbSNP build 146 database. - Information on the evaluation of the Y-chromosome-specific probes added to Table 2. - Ligation site location information removed from Table 2, as the 24 nt sequence adjacent to the ligation site is sufficient to locate the probe on corresponding NM_sequences. - Footnote added to Table 1 and 2 about the MLL5 gene being renamed to KMT2E. - Footnote added to Table 2 about frequent mutation in the TP53 gene for the 256 nt probe. *Version 05 – 23 February 2015 (T07)* - New sample picture included in product description.

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