Product Description
SALSA® MLPA® Probemix P377-B1 Hematologic Malignancies

To be used with the MLPA General Protocol.

Version B1
As compared to A3, one *PAX5* specific probe is replaced. Changes in the probe length of several probes but no change in the sequences detected. For complete product history see page 15.

Catalogue numbers:
- **P377-025R:** SALSA MLPA Probemix P377 Hematologic Malignancies, 25 reactions.
- **P377-050R:** SALSA MLPA Probemix P377 Hematologic Malignancies, 50 reactions.
- **P377-100R:** SALSA MLPA Probemix P377 Hematologic Malignancies, 100 reactions.

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

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

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

General information
The SALSA MLPA Probemix P377 Hematologic Malignancies is a **research use only (RUO)** assay, and is intended for screening DNA samples derived from blood or bone marrow for the most common and diagnostically significant copy number changes associated with hematologic malignancies, including acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphoid leukaemia (CLL), chronic myeloid leukaemia (CML), myelodysplastic syndrome (MDS) and various lymphomas. The probemix can also be used to detect the *JAK2* p.V617F point mutation which is commonly detected in myeloproliferative neoplasm (MPN). This probemix is intended to be used in combination with karyotype analysis. Suggestions on MLPA probemixes that can be used to confirm results or to get a better resolution on genes or chromosomal areas of interest can be found in Table 2.

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/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

Exon numbering
The exon numbering used in this P377-B1 product description is the exon numbering derived from MANE project (release version 1.0), unless otherwise specified, see 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 in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P377-B1 Hematologic Malignancies contains 54 MLPA probes with amplification products between 125 and 507 nucleotides (nt). This includes 53 probes for the detection of deletions or duplications in the chromosomal regions 2p (*MYCN*, *ALK*), 5q (*MIR145*, *EBF1* and *MIR146A*), 6q, 7p12 (*IKZF1*), 7q, 8q24 (*MYC*), 9p (*MTAP*, *CDKN2A*, *CDKN2B*, *PAX5*), 10q23 (*PTEN*), 11q22 (*ATM*), 12p13 (*ETV6*, *CCND2*, *MDM2*), 12q, 13q14 (*RB1*, *MIR15A*, *DLEU2*, *DLEU1*), 17p13 (*TP53*), 17q, Chr 18, Chr 19 and 21q22 (*RUNX1*) which are suggested to have a diagnostic or prognostic role in the analysis of samples in hematologic malignancies.

Furthermore, this probemix also contains one probe specific for the *JAK2* p.V617F (c. 1849G>T) point mutation, which will only generate a signal when the mutation is present. In this probemix, 53 out of 54 MLPA probes are used as reference probes, as they are spread over a number of different chromosomal regions and it is expected that the majority of these probes will have a normal copy number in most samples. Complete probe sequences are available in Table 2 and online (www.mrcholland.com).

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 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. 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 from Coriell Institute and Horizon Discovery have been tested with this P377-B1 probemix at MRC Holland and can be used as a positive control samples for the copy number alterations (CNAs) and mutations as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Chromosomal position of CNA*	Altered target genes in P377-B1	Expected CNA or mutation
Coriell Institute samples			
NA01353	2p23.2-p24.3	*ALK, MYCN*	Heterozygous duplication
NA00945	2p24.3	*MYCN*	Heterozygous deletion
NA04409	2p24.3	*MYCN*	Heterozygous duplication
NA04371	5q33.3	*EBF1, MIR146A*	Heterozygous duplication
NA01221	6q21	*FYN*	Heterozygous duplication
NA09367	6q21-q23.3	*FYN, MYB*	Heterozygous duplication
NA07994	6q23.3-q27	*MYB, ESR1, SMOC2*	Heterozygous duplication
NA08386	6q27	*SMOC2*	Heterozygous deletion
NA07081	7p12.2	*IKZF1*	Heterozygous duplication
NA10925	7p12.2	*IKZF1*	Heterozygous deletion
NA10160	7q21.2	*CDK6*	Heterozygous deletion
NA12519	7q31.2	*MET*	Heterozygous triplication /homozygous duplication
NA10313	7q36.2	*DPP6*	Heterozygous deletion
NA02030	8q24.21	*MYC*	Heterozygous duplication
NA03999	8q24.21	*MYC*	Heterozygous deletion
NA03226	9p13.2-p21.3	*MTAP, CDKN2A, CDKN2B, PAX5*	Heterozygous duplication
NA05067	9p13.2-p21.3	*MTAP, CDKN2A, CDKN2B, PAX5*	Heterozygous duplication
NA01750	9p21.3	*MTAP, CDKN2A, CDKN2B*	Heterozygous duplication
NA02819	9p21.3	*MTAP, CDKN2A, CDKN2B*	Heterozygous duplication
NA20125	10q23.31	*PTEN*	Heterozygous duplication
NA08618	11q22.3	*ATM*	Heterozygous duplication
NA09596	11q22.3	*ATM*	Heterozygous deletion
NA07981	12p13.2-p13.32	*CCND2, ETV6*	Heterozygous triplication /homozygous duplication
NA08035	12p13.2-p13.32	*CCND2, ETV6*	Heterozygous duplication
NA05832	13q14.2-q14.3	*RB1, MIR15A, DLEU2, DLEU1*	Heterozygous duplication
NA14164	13q14.2-q14.3	*RB1, MIR15A, DLEU2, DLEU1*	Heterozygous deletion
NA06870	18p11.21	*RNMT*	Heterozygous triplication /homozygous duplication
NA50322	18p11.21	*RNMT*	Heterozygous deletion
NA01359	18p11.21-q21.2	*RNMT, DCC*	Heterozygous duplication
NA03623	18p11.21-q21.2	*RNMT, DCC*	Heterozygous duplication
NA07891	18q21.2	*DCC*	Heterozygous deletion
NA12722	9p21.3	*MTAP, CDKN2A, CDKN2B*	Heterozygous duplication
	18p11.21-q21.2	*RNMT, DCC*	Heterozygous duplication
NA23245	7p12.2-q36.2	*IKZF1, CDK6, RELN, MET, DPP6*	Heterozygous deletion
	8q24.21	*MYC*	Heterozygous duplication
	9p24.1	*JAK2*	p.V617F mutation

Sample name	Chromosomal position of CNA*	Altered target genes in P377-B1	Expected CNA or mutation
Horizon Diagnostics reference standard samples			
JAK2 p.V617F 50% reference standard◊	7p12.2-q36.2	*IKZF1, CDK6, RELN, MET, DPP6*	Heterozygous duplication
	9p24.1	*JAK2*	p.V617F mutation
	18q21.2	*DCC*	Heterozygous duplication
JAK2 p.V617F 0% reference standard§	7p12.2-q36.2	*IKZF1, CDK6, RELN, MET, DPP6*	Heterozygous duplication
	18q21.2	*DCC*	Heterozygous duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by P377-B1 Hematologic Malignancies probemix.

◊ DNA from SW28 cell line with SW48 background (Catalogue no.: HD649)

§ DNA from SW48 cell line (Catalogue no.: HD652)

SALSA Binning DNA SD068

The SD068 Binning DNA provided with this probemix can be used for binning of all probes including the *JAK2* p.V617F mutation-specific probe (208 nt probe 05672-L17742). SD068 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 SD068 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 signals. 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 SD068 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 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 or in or near the *RUNX1* genes. 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.
- 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.

P377 specific notes

- In case data analysis is performed with a different method than Coffalyser.Net, we recommend treating each probe as a reference probe with the exception of the *JAK2* mutation-specific probe.
- 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 most populations, the majority of genetic alterations in the genes included in this probemix are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P377 Hematologic Malignancies.
- 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.

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 deletion of *IKZF1* exons 3 and 7 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P377-B1 Hematologic Malignancies

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Used as a reference probe	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information			
125	**DCC** probe 21566-L27817	18q21.2	Yes	18-048,959
132	**IKZF1** probe 03340-L27816	7p12.2	Yes	07-050,338
137	**ATM** probe 02675-L01168	11q22.3	Yes	11-107,599
143	**IKZF1** probe 13872-L27818	7p12.2	Yes	07-050,418
148	**MYB** probe 12500-L27820	6q23.3	Yes	06-135,549
154	**MYC** probe 20383-L27819	8q24.21	Yes	08-128,822
160	**CDKN2A** probe 01524-L27821	9p21.3	Yes	09-021,985
165	**MIR15A** probe 04019-L17530	13q14.3	Yes	13-049,521
169	**RUNX1** probe 20384-L25345	21q22.12	Yes	21-035,343
173	**MYCN** probe 03028-L17950	2p24.3	Yes	02-016,000
178	**MIR146A** probe 15652-L17541	5q33.3	Yes	05-159,845
184	**MYC** probe 14869-L16611	8q24.21	Yes	08-128,822
190	**DLEU2** probe 04020-L17532	13q14.3	Yes	13-049,554
196	**ETV6** probe 14054-L15652	12p13.2	Yes	12-011,935
203	**ATM** probe 08426-L08309	11q22.3	Yes	11-107,659
208 §	**JAK2** probe 05672-L17742	p.V617F=c.1849G>T	**No**	09-005,064
214	**ESR1** probe 11996-L12824	6q25.1	Yes	06-152,424
220	**MET** probe 10329-L10843	7q31.2	Yes	07-116,211
226	**EBF1** probe 12509-L13559	5q33.3	Yes	05-158,459
232 ¶	**DPP6** probe 14027-L15625	7q36.2	Yes	07-154,227
239	**ALK** probe 08325-L28371	2p23.2	Yes	02-029,274
244	**ETV6** probe 13874-L17160	12p13.2	Yes	12-011,883
252 ‡	**TP53** probe 02376-L27832	17p13.1	Yes	17-007,519
256	**SMOC2** probe 09380-L27831	6q27	Yes	06-168,809
262	**FYN** probe 12546-L27830	6q21	Yes	06-112,148
266	**MIR146A** probe 15653-L18125	5q33.3	Yes	05-159,845
274	**UNC13D** probe 11696-L17540	17q25.1	Yes	17-071,342
279	**PAX5** probe 13870-L17534	9p13.2	Yes	09-036,993
285	**MDM2** probe 07179-L17544	12q15	Yes	12-067,494
292	**MIR145** probe 14248-L15086	5q33.1	Yes	05-148,789
297 «	**RUNX1** probe 02840-L27829	21q22.12	Yes	21-035,094
303	**IKZF3** probe 15461-L17667	17q12	Yes	17-035,202
313	**CDK6** probe 03184-L28370	7q21.2	Yes	07-092,085
321	**CACNA1A** probe 09065-L28369	19p13.13	Yes	19-013,289
328 ¥	**MTAP** probe 01294-L13278	9p21.3	Yes	09-021,793
337	**IKZF1** probe 13869-L15387	7p12.2	Yes	07-050,427
346 ±	**TP53** probe 00345-L00171	17p13.1	Yes	17-007,514
355	**CCND2** probe 00498-L00084	12p13.32	Yes	12-004,279
364	**EBF1** probe 14059-L27828	5q33.3	Yes	05-158,137
373 *	**PAX5** probe 23224-L22633	9p13.2	Yes	09-036,830
384	**DLEU1** probe 01589-L27826	13q14.3	Yes	13-049,782
392	**TP53** probe 01587-L17743	17p13.1	Yes	17-007,515
400	**PRPF31** probe 06024-L05449	19q13.42	Yes	19-059,327
409	**TP53** probe 02263-L01749	17p13.1	Yes	17-007,532
418	**CDKN2B** probe 20386-L28368	9p21.3	Yes	09-021,991
427	**ATM** probe 08443-L08330	11q22.3	Yes	11-107,722
436	**MYCN** probe 03327-L17744	2p24.3	Yes	02-016,003
445	**PTEN** probe 13684-L18623	10q23.31	Yes	10-089,614
453	**ATM** probe 20385-L27825	11q22.3	Yes	11-107,629
470	**RB1** probe 01800-L28440	13q14.2	Yes	13-047,953

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Used as a reference probe	Location (hg18) in kb
478	**PTEN** probe 13696-L28441	10q23.31	Yes	10-089,715
488	**RB1** probe 12565-L28442	13q14.2	Yes	13-047,937
499	**RNMT** probe 20552-L17745	18p11.21	Yes	18-013,724
507	**RELN** probe 20553-L18622	7q22.1	Yes	07-103,058

* New in version B1.

¥ Changed in version B1. Minor alteration, no change in sequence detected.

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

± SNVs rs80184930 and rs774269719 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¶ SNV rs367797577 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this 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.

‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the table above. Single probe aberration(s) must be confirmed by another method.

Table 2. P377-B1 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
2p gain (*MYCN* and *ALK* genes), indicated ligation sites and exon numbering for *MYCN* are according to the MANE Select transcript NM_005378.6. 2p gain, including *MYCN* and *ALK*, is detected in CLL specimens and is suggested to be a marker of disease progression and poor prognosis (Fabris et al. 2012; Chapiro et al. 2010). 2p gains and amplifications are detected in lymphomas as well. More *MYCN* and *ALK* probes are present in the P037 CLL-1 and P252 NB mix 2 probemixes.					
173	03028-L17950	***MYCN***, ex 2	470-471	GGAAGAAGTTT-GAGCTGCTGCCC	3.5 kb
436	03327-L17744	***MYCN***, ex 3	1351-1352	TGCACCCCCACA-GAAGAAGATAAA	13 **M**b
239	08325-L28371	***ALK***	2p23.2	TTTCTCTTGGAT-ATATGCCATACC	-
Loss of 5q (*MIR145, EBF1* and *MIR146A* genes), indicated ligation sites and exon numbering for *EBF1* are according to the MANE Select transcript NM_024007.5 and for *MIR146A* according to RefSeq transcript NR_029701.1. Loss of 5q, especially 5q33.1-q33.3, is a recurrent cytogenetic change in MDS and in AML. *MIR145* and *MIR146A* were shown to be key mediators of 5q syndrome (Starczynowski et al. 2010). Deletions of *EBF1* have been reported in up to 9% of ALL cases in specific subtypes (Schwab et al. 2013). More probes targeting the 5q arm can be found in the P414 MDS probemix and more probes for *EBF1* are present in the P335 ALL-IKZF1 probemix.					
292	14248-L15086	***MIR145***	5q33.1	CAGCCACTTGTG-ATGCTGGGGAAG	9.4 **M**b
364	14059-L27828	***EBF1***, ex 10	1413-1414	GTTGTGGAAGTC-ACACTGTCCTAC	322.2 kb
226	12509-L13559	***EBF1***, ex 1	278-279	ATTTGCTTTCCA-GCCCGCCTTGAT	1.4 **M**b
178	15652-L17541	***MIR146A***, ex 1	4 nt before exon 1	CACCATCTCTGA-AAAGCCGATGTG	0.1 kb
266	15653-L18125	***MIR146A***, ex 1	8 nt after exon 1	TCGTGGGCTTGA-GGACCTGGAGAG	-
Copy number alterations at 6q Chromosome 6q deletions are commonly found in various lymphoid malignancies such as CLL (Wang et al. 2011), ALL, non-Hodgkins lymphoma (NHL) (Merup et al. 1998), T-cell lymphoblastic lymphoma (T-LBL), multiple myeloma (MM), and mantle zone lymphoma (MZL). Note that deletion of 6q has prognostic implications in some of these entities. *MYB* duplications have been described in leukemias (reviewed in Pattabiraman et al. 2012), e.g. *MYB* is amplified in 5-12% of pediatric ALL cases (Liu et al. 2017, Bardelli et al. 2020). More probes for detection of deletion on chromosome 6q are present in the P037 CLL-1 probemix. More probes for *MYB* can be found in the P383 T-ALL probemix.					
262	12546-L27830	***FYN***	6q21	GGTGTGAACTCT-TCGTCTCATACG	23.4 **M**b
148	12500-L27820	***MYB***	6q23.3	TGAGGACTTTGA-GATGTGTGACCA	16.9 **M**b
214	11996-L12824	***ESR1***	6q25.1	TTCGACATGCTG-CTGGCTACATCA	16.4 **M**b
256	09380-L27831	***SMOC2***	6q27	GTGCTTACAGCA-TTGTGGAATCAT	-

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
IKZF1 gene, 7p12.2. indicated ligation sites and exon numbering for *IKZF1* are according to the MANE Select transcript NM_006060.6. Deletions of the *IKZF1* (*IKAROS*) gene are detected in ALL, especially in cases that also carry the *BCR-ABL1* gene fusion (Mullighan et al. 2008). *IKZF1* deletions in ALL have been associated with relapse and very poor clinical outcome (Mullighan et al. 2009; Martinelli et al. 2009; Iacobucci et al. 2009). Deletions of *IKZF1* might be involved also in other hematologic malignancies as Ikaros proteins are active throughout human B-cell differentiation, e.g. in MPN (Chen et al. 2021). Probes targeting all exons of the *IKZF1* gene are present in P202 *IKZF1-ERG* and P335 *ALL-IKZF1* probemixes.					
132	03340-L27816	***IKZF1***, ex 3	355-356	GGGAGGACAGCA-AAGCTCCAAGAG	79.9 kb
143	13872-L27818	***IKZF1***, ex 5	680-681	TGCGGGGCCTCA-TTCACCCAGAAG	9.2 kb
337	13869-L15387	***IKZF1***, ex 7	994-995	CAAGATAGGATC-AGAGAGATCTCT	41.7 **M**b
Loss of 7q Monosomy 7 or interstitial deletions in 7q are frequent in myeloid disorders (MDS and AML). In MDS, monosomy 7 or del(7q) associates with progression to acute leukaemia and poor prognosis (Sole et al. 2005). Recent findings suggest a better prognosis for del(7q) as compared to monosomy 7 in MDS (Cordoba et al. 2012). More probes targeting chromosome 7 can be found in the P414 MDS. Probemix P308 MET contains probes covering all exons of the *MET* gene.					
313	03184-L28370	***CDK6***	7q21.2	GAGAAGAAGACT-GGCCTAGAGATG	11 **M**b
507	20553-L18622	***RELN***	7q22.1	GGGCTATTGATG-AGATTATCATGA	13.2 **M**b
220	10329-L10843	***MET***	7q31.2	AAGTGGATGGCT-TTGGAAAGTCTG	38 **M**b
232 ¶	14027-L15625	***DPP6***	7q36.2	ACCAAGATCCTA-GCCTACGATGAG	-
MYC gene, 8q24.21, indicated ligation sites and exon numbering for *MYC* are according to the MANE Select transcript NM_002467.6. Gains or amplifications of the *MYC* gene are detected in various hematologic malignancies, including ALL, AML, CLL, CML, MPNs and various lymphomas (reviewed in Vita et al. 2006) and could predict overall survival in diffuse large B-cell lymphoma (Quesada et al. 2017; Schieppati et al. 2020). More probes for *MYC* can be found in the P037 CLL-1.					
184	14869-L16611	***MYC***, ex 3	1431-1432	AACAACCGAAAA-TGCACCAGCCCC	0.1 kb
154	20383-L27819	***MYC***, ex 3	1520-1521	GAACGAGCTAAA-ACGGAGCTTTTT	-
***JAK2* p.V617F mutation**, 9p24.1, indicated ligation site and exon numbering for *JAK2* are according to the MANE Select transcript NM_004972.4. *JAK2* p.V617F mutation, in exon 14, is a somatic mutation of G into T resulting in substitution of valine to phenylalnine and constitutive activation of cell proliferation. *JAK2* p.V617F mutation is detected throughout myeloid malignancies – in AML, MDS and myeloproliferative neoplasms (MPNs) – and the highest frequencies (~95%) are found in polycythemia vera (PV) (Jones et al. 2009). P520 MPN mix 2 contains probes for high detection sensitivity (≥1 % allele burden) for the following *JAK2* mutations frequently found in MPN samples: *JAK2* p.V617F, *JAK2* p.E543-D544del and *JAK2* p.N542-E543del.					
208 §	05672-L17742	***JAK2***, ex 14; **p.V617F** (c.1849G>T)	2316-2315, reverse	GTCTCCACAGAA-ACATACTCCATA	16.7 **M**b
9p21.3 deletions 9p21.3 deletions are especially frequent in ALL, in 20% of B-cell precursor ALL and in 50% of T-ALL patients (Bertin et al. 2003). Deletions of *CDKN2A/2B* have been shown to associate with unfavourable outcome in both pediatric and adult ALL (Fizzotti et al. 1995; Yamada et al. 1997) and are also frequent in mantle cell lymphoma (Streich et al. 2020; Malarikova et al. 2020). Additionally, preliminary studies suggest that patients with *MTAP* homozygous deletion could be treated with MAT2A inhibitors, which are currently tested in clinical trials (Konteatis et al. 2021). Deletions of 9p21.3 are detected also in other hematologic malignancies. More *CDKN2A/2B* probes are present in the P202 IKZF1-ERG, P335 ALL-IKZF1 and P419 CDKN2A/2B-CDK4 probemixes and in the ME024 9p21 CDKN2A/2B region probemix, which detects both copy number and methylation changes of these genes.					
328	01294-L13278	***MTAP***	9p21.3	GGTGGTGGTGCC-AGAGGCCATGTC	192.6 kb
160	01524-L27821	***CDKN2A***	9p21.3	AAGCGCTCAGAT-GCTCCGCGGCTG	5.3 kb
418	20386-L28368	***CDKN2B***	9p21.3	CCTAGGAAAGGT-GATAGAGCTTAG	14.8 **M**b
PAX5 gene, 9p13.2, indicated ligation sites and exon numbering for *PAX5* are according to the MANE Select transcript NM_016734.3. *PAX5* deletions are frequent in B-ALL and in *BCR-ABL1* positive ALL cases (Coyaud et al. 2010). 9p deletions in ALL can be large and extend sometimes into the *CDKN2A/2B* genes. Note that amplifications of exon 2 & 5 have been suggested to be an alternative mechanism of *PAX5* inactivation and could define a novel subgroup in BCP-ALL (Schwab et al. 2017). More *PAX5* probes are present in the P335 ALL-IKZF1 and ME024 9p21 CDKN2A/2B region probemixes.					
373	23224-L22633	***PAX5***, ex 10	2019-2020	CCTATTGAGGGT-GACAGCCACCCA	162.8 kb
279	13870-L17534	***PAX5***, ex 5	756-757	GTGAGCACGGAT-TCGGCCGGCTCG	-

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
PTEN gene, 10q23.31, indicated ligation sites and exon numbering for *PTEN* are according to the MANE Select transcript NM_000314.8. *PTEN* deletions occurring in 9% of T-ALL patients are associated with early treatment failure and may contribute to increased resistance to chemotherapy and increased relapse (Gutierrez et al. 2009; Jotta et al. 2010; Szarzyńska-Zawadzka et al. 2018). *PTEN* deletions occur in 11% of diffuse large B-cell lymphoma (Wang et al. 2018). Note that *PTEN* point mutations are more frequent, but they cannot be detected with these MLPA probes. More probes for *PTEN* can be found in P294 Tumour Loss and P383 T-ALL probemixes. Probes to detect every exon of *PTEN* gene are present in the P225 PTEN probemix.					
445	13684-L18623	***PTEN***, ex 1	5 nt after exon 1	TTGACCTGTATC-CATTTCTGCGGC	101.0 kb
478 #	13696-L28441	***PTEN***, ex 9	2171-2170, reverse	AGAGAATTGTTC-CTATAACTGGTA	-
ATM gene, 11q22.3, indicated ligation sites and exon numbering for *PTEN* are according to the MANE Select transcript NM_000051.4. Deletion of 11q22-q23, including *ATM*, is associated with an aggressive course of B-cell chronic lymphocytic leukaemia (B-CLL) (Guarini et al. 2012). Deletions of *ATM* are also detected in ALL and is found in 6% of CLL cases (Guarini et al., 2012). More probes for *ATM* are found in P040 CLL Probemix, while probemixes P041 and P042 ATM contain probes to detect every exon of the *ATM* gene.					
137	02675-L01168	***ATM***, ex 1	17 nt before exon 1	CACGCAGGGTTT-GAACCGGAAGCG	29.8 kb
453	20385-L27825	***ATM***, ex 12	1990-1991	AAATTCTTGTGA-GTCTCACTATGA	29.9 kb
203	08426-L08309	***ATM***, ex 25	3744-3745	GAGAAAGTTTCT-GAAACTTTTGGA	63.1 kb
427	08443-L08330	***ATM***, ex 58	8671-8672	AAAAATTCTTGG-ATCCAGCTATTT	-
ETV6 gene, 12p13.2 & **chromosome 12 gains,** indicated ligation sites and exon numbering for *ETV6* are according to the MANE Select transcript NM_001987.5. *ETV6* deletions are frequent in childhood ALL and in AML/MDS with normal karyotype (Wall et al. 2012). *ETV6* deletions are also frequently associated with leukemic transformation of Philadelphia chromosome-negative myeloproliferative neoplasms. More *ETV6* probes are present in the P335 ALL-IKZF1 and P414 MDS probemixes. Trisomy 12 is the most common chromosomal abnormality in CLL and gains of chr 12 are detected in Non-Hodgkin and Hodgkin lymphoma, follicular lymphoma and diffuse large B-cell lymphoma. More *MDM2* and *CCND2* probes are present in the P323 CDK4-HMGA2-MDM2 probemix. More *CCND2* probes are present in the P175 Tumour Gain probemix.					
355	00498-L00084	***CCND2***	12p13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	7.6 **M**b
244	13874-L17160	***ETV6***, ex 3	641-642	TTTACTGGAGCA-GGGATGACGTAG	52.0 kb
196	14054-L15652	***ETV6***, ex 8	1959-1960	TCTTGCAGACCA-AGAGGGACCCTG	55.6 **M**b
285	07179-L17544	***MDM2***	12**q**15	ACCAACAGACTT-TAATAACTTCAA	-
13q deletions *(RB1 gene),* indicated ligation sites and exon numbering for *RB1* are according to the MANE Select transcript NM_000321.3. Deletions of 13q, especially on 13q14, occur in >50% in CLL and mantle cell lymphoma cases (Wolf et al. 2001). *RB1* deletions have been reported to be more frequent in high-risk ALL as compared to non-selected cases (Zhang et al. 2011). In T-cell lymphoblastic lymphoma deletions of *RB1* often involve one or more of the last 10 exons of this 27-exon gene (Schraders et al. 2009). More probes to detect 13q copy number changes are present in the P037, P038 and P040 CLL probemixes. More probes for *RB1* are present in P335 ALL-IKZF1 probemix, while P047 RB1 covers the whole gene.					
488	12565-L28442	***RB1***, ex 23	2557-2558	CACCCTTACGGA-TTCCTGGAGGGA	15.1 kb
470	01800-L28440	***RB1***, ex 27	3270-3271	GAGTCCTGATAA-CCCAGGCCTGTC	1.6 **M**b
165	04019-L17530	***MIR15A***	13q14.3	TGGATTTTGAAA-AGGTGCAGGCCA	33.0 kb
190	04020-L17532	***DLEU2***	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	228.1 kb
384	01589-L27826	***DLEU1***	13q14.3	CCTTTTAATAGG-ATCTCTCCTGGA	-
17p *(TP53 gene)* & 17q deletions, indicated ligation sites for *TP53* are according to the MANE Select transcript NM_000546.6. Exon numbering is according to LRG_321; the MANE exon numbering can be found in between brackets (if different than LRG). Isochromosome of 17q, i(17)(q10), is common in AML and CML (20%), and results in loss of 17p and *TP53* (Kanagal-Chamanna et al. 2012). Moreover, deletion of 17p and *TP53* is characteristic for a rare, aggressive, subset of CLL (5-10%) with a poor clinical outcome (Mougalian and O'Brien 2011) and is also found in patients with MCL (Ferrero et al. 2020). More probes for *TP53* are present in the P038 CLL-2, P040 CLL and P056 TP53 probemixes.					
346 ±	00345-L00171	***TP53***, ex 11	1269-1270	AAAGGGTCAGTC-TACCTCCCGCCA	1.0 kb
392	01587-L17743	***TP53***, ex 10	1174-1175	TTCCGAGAGCTG-AATGAGGCCTTG	4.5 kb
252 ‡	02376-L27832	***TP53***, ex 4b [5]	546-547	CAAGATGTTTTG-CCAACTGGCCAA	12.4 kb
409	02263-L01749	***TP53***, ex 1	127 nt before exon 1	CTTCCTCCGGCA-GGCGGATTACTT	27.7 **M**b
303	15461-L17667	***IKZF3***	17**q**12	AGCAGGCCAACC-AGTGGAAAGATG	36.1 **M**b
274	11696-L17540	***UNC13D***	17**q**25.1	GCACATCCAGAA-ACTGGTGGGCGT	-

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 18 Gains of chromosome 18 are common in ALL and lymphomas (especially in follicular lymphoma, diffuse large B-cell lymphoma and extranodal marginal zone B-cell lymphoma of MALT type) (Kim et al. 2013 and Masir et al. 2007).					
499	20552-L17745	***RNMT***	18p11.21	TACAATGAACTT-CAGGAAGTTGGT	35.2 **M**b
125	21566-L27817	***DCC***	18**q**21.2	GAGTTGTGGCTT-ACAATGAATGGG	-
Chromosome 19 Gains of chromosome 19 are common in CML (~13%), however occurring as a late event in the molecular genetic evolution of CML (Johansson et al. 2002).					
321	09065-L28369	***CACNA1A***	19p13.13	CTCAGGCCTTCT-ACTGGACTGTAC	46 **M**b
400	06024-L05449	***PRPF31***	19**q**13.42	GGATCGGGTTCT-GGCAGGGAGAAC	-
RUNX1 (*AML1*), 21q22.1, indicated ligation sites and exon numbering for *RUNX1* are according to the MANE Select transcript NM_001754.5. High-level amplifications of *RUNX1*, associated with intrachromosomal amplification of chromosome 21 (iAMP21), have been reported in childhood ALL and are associated with high risk of relapse and poor clinical outcome (Harrison et al. 2014). More probes for *RUNX1* and iAMP21 detection are present in the P327 iAMP21-ERG and for *RUNX1* in P437 Familial MDS-AML probemixes.					
297 «	02840-L27829	***RUNX1***, ex 8	1040-1041	TGGTCCTACGAT-CAGTCCTACCAA	249.5 kb
169	20384-L25345	***RUNX1***, ex 2	190-191	TTTTCAGGAGGA-AGCGATGGCTTC	-

[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 mutation is present.

± SNVs rs80184930 and rs774269719 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¶ SNV rs367797577 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this 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.

‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence theregion targeted by this probe.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the table above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- **P037/P038/P040 CLL**: Contain probes for detecting characteristic copy number changes in CLL.
- **P041/P042 ATM:** Together these probemixes contain probes for every exon of the *ATM* gene.
- **P056 TP53:** Contains probes for every exon of *TP53*, and probes for *CHEK2*.
- **P202 IKZF1-ERG:** Contains probes for all *IKZF1* and *ERG* exons, 9p21.3 (*CDKN2A/2B*) and 14q32 regions.
- **P225 PTEN:** Contains at least two probes for every exon of *PTEN*.
- **P252 NB mix 2:** Contains probes for chromosomes 2 (*MYCN* and *ALK*, among others) and 17.
- **P323 CDK4-HMGA2-MDM2:** Contains probes for *CDK4*, *HMGA2* and *MDM2* genes.
- **P327 iAMP21-ERG:** Contains probes for *RUNX1* and *ERG* and probes for iAMP21 detection involved in ALL.
- **P329 CLRF2-CSF2RA-IL3RA:** Contains probes for *CLRF2*, *CSF2RA*, *IL3RA* region, involved in ALL.
- **P335 ALL-IKZF1:** Contains probes for all *IKZF1* exons, *CDKN2A*/*CDKN2B*, *EBF1*, Chr. X PAR-region, *PAX5*, *ETV6*, *BTG1* and *RB1*.
- **P383 T-ALL:** Contains probes for genomic regions with diagnostic and/or prognostic importance in T-ALL.
- **P414 MDS:** Contains probes for detecting characteristic copy number changes in MDS.
- **P419 CDKN2A/2B-CDK4:** Contains probes for *CDKN2A*/*CDKN2B* and *CDK4*.

- **P420 MPN mix1:** Contains probes for the following mutations frequently found in MPN samples: *JAK2* p.V617F, *JAK2* p.E543-D544del, *JAK2* p.N542-E543del, *MPL* p.W515L, *MPL* p.W515K, *KIT* p.D816V, *CALR* p.L367fs*46, *CALR* p.K385fs*47.
- **P425 Multiple Myeloma:** Contains probes targeting genes/regions that are suggested to be of prognostic relevance in multiple myeloma.
- **P437 Familial MDS-AML:** Contains probes for *RUNX1*, *CEBPA*, *GATA2*, *TERT* and *TERC*, including point mutations of *GATA2* p.R398W, p.T354M and *TERT* p.A10662T.
- **P520 MPN mix 2:** Contains probes for high detection sensitivity (≥1 % allele burden) for the following mutations frequently found in MPN samples: *JAK2* p.V617F, *JAK2* p.E543-D544del, *JAK2* p.N542-E543del, *MPL* p.W515L, *MPL* p.W515K, *KIT* p.D816V, *CALR* p.L367fs*46, *CALR* p.K385fs*47.
- **ME024 9p21 CDKN2A/2B region:** Contains probes for the detection of copy number and methylation changes of genes located at 9p21.3.

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See detailed information and references on included chromosomal areas and genes in Table 2.

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P377 product history	
Version	*Modification*
B1	One *PAX5* specific probe is replaced. Changes in the probe length of several probes but no change in the sequences detected.
A3	The length of several probes has been changed, but no change in the sequence was detected.
A2	One flanking probe is replaced and the lengths of several probes are adjusted without changing the sequence detected.
A1	First release.

Implemented changes in the product description
Version B1-01 – 07 March 2024 (04P) - Product description adapted to a new product version (version number changed, changes in Tables 1 and 2). - Ligation sites of the probes targeting EBF1 are updated according to the MANE Select transcript NM_024007.5. - Various minor textual or layout changes. - Positive sample table adjusted for layout and to the latest results. Version A3-01 – 26 October 2021 (04P) - Product description rewritten and adapted to a new template (version number changed, changes in Table 1 and Table 2). - Added results of tests on positive samples and reference standards. - Various minor textual or layout changes. - Transcript numbers of the probes targeting genes *MYC, ATM, RB1* and *TP53* updated according to the newest version. - Ligation sites of the probes targeting genes *ATM* and *TP53* updated according to new version of the NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). Version 12 – 30 June 2020 (T08) - Warning below Table 1 and 2 modified for the PAX5 exon 10 probe (12521-L27827) at 374 nt – results should be interpreted cautiously and always confirmed with a different MLPA probemix (e.g. P335 ALL-IKZF1) or with a different method. Version 11 – 06 February 2020 (T08) - Related probemix information updated on page 1. - Ligation sites of the probes targeting the *MYCN, EBF1, IKZF1, MYC, JAK2, PAX5* and *ETV6* genes updated according to newest versions of the NM_ reference sequences in Table 2. - Information about P520 MPN mix 2 for high detection sensitivity (≥1 % allele burden) for the following mutations *JAK2* V617F, *JAK2* E543-D544del and *JAK2* N542-E543del added to Table 2.

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