

Product Description

SALSA® MLPA® Probemix P377-A3 Hematologic Malignancies

To be used with the MLPA General Protocol.

Version A3

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/>

PhenoGram Plot: <http://visualization.ritchielab.org/phenograms/plot>

Exon numbering

The LRG, RefSeq transcript NM, NG or NR sequences used for exon numbering in this P377-A3 Hematologic Malignancies product description are provided in Table 2. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content

The SALSA MLPA Probemix P377-A3 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 known to have a significant diagnostic or prognostic role 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-A3 probemix at MRC Holland and can be used as a positive control samples as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P377-A3	Expected CNA or mutation
NA01353	Coriell Institute	2p23.2-p24.3	<i>ALK, MYCN</i>	Heterozygous duplication
NA00945	Coriell Institute	2p24.3	<i>MYCN</i>	Heterozygous deletion
NA04409	Coriell Institute	2p24.3	<i>MYCN</i>	Heterozygous duplication
NA04371	Coriell Institute	5q33.3	<i>EBF1, MIR146A</i>	Heterozygous duplication
NA01221	Coriell Institute	6q21	<i>FYN</i>	Heterozygous duplication
NA09367	Coriell Institute	6q21-q23.3	<i>FYN, MYB</i>	Heterozygous duplication
NA07994	Coriell Institute	6q23.3-q27	<i>MYB, ESR1, SMOC2</i>	Heterozygous duplication
NA08386	Coriell Institute	6q27	<i>SMOC2</i>	Heterozygous deletion
NA07081	Coriell Institute	7p12.2	<i>IKZF1</i>	Heterozygous duplication
NA10925	Coriell Institute	7p12.2	<i>IKZF1</i>	Heterozygous deletion
NA10160	Coriell Institute	7q21.2	<i>CDK6</i>	Heterozygous deletion
NA12519	Coriell Institute	7q31.2	<i>MET</i>	Heterozygous triplication/homozygous duplication
NA10313	Coriell Institute	7q36.2	<i>DPP6</i>	Heterozygous deletion
NA02030	Coriell Institute	8q24.21	<i>MYC</i>	Heterozygous duplication
NA03999	Coriell Institute	8q24.21	<i>MYC</i>	Heterozygous deletion
NA03226	Coriell Institute	9p13.2-p21.3	<i>MTAP, CDKN2A, CDKN2B, PAX5</i>	Heterozygous duplication
NA05067	Coriell Institute	9p13.2-p21.3	<i>MTAP, CDKN2A, CDKN2B, PAX5</i>	Heterozygous duplication
NA01750	Coriell Institute	9p21.3	<i>MTAP, CDKN2A, CDKN2B</i>	Heterozygous duplication
NA02819	Coriell Institute	9p21.3	<i>MTAP, CDKN2A, CDKN2B</i>	Heterozygous duplication
NA20125	Coriell Institute	10q23.31	<i>PTEN</i>	Heterozygous duplication
NA08618	Coriell Institute	11q22.3	<i>ATM</i>	Heterozygous duplication
NA09596	Coriell Institute	11q22.3	<i>ATM</i>	Heterozygous deletion
NA07981	Coriell Institute	12p13.2-p13.32	<i>CCND2, ETV6</i>	Heterozygous triplication/homozygous duplication
NA08035	Coriell Institute	12p13.2-p13.32	<i>CCND2, ETV6</i>	Heterozygous duplication
NA05832	Coriell Institute	13q14.2-q14.3	<i>RB1, MIR15A, DLEU2, DLEU1</i>	Heterozygous duplication
NA12606	Coriell Institute	13q14.2-q14.3	<i>RB1, MIR15A, DLEU2, DLEU1</i>	Heterozygous duplication
NA14164	Coriell Institute	13q14.2-q14.3	<i>RB1, MIR15A, DLEU2, DLEU1</i>	Heterozygous deletion
NA06870	Coriell Institute	18p11.21	<i>RNMT</i>	Heterozygous triplication/homozygous duplication
NA50322	Coriell Institute	18p11.21	<i>RNMT</i>	Heterozygous deletion
NA01359	Coriell Institute	18p11.21-q21.2	<i>RNMT, DCC</i>	Heterozygous duplication
NA03623	Coriell Institute	18p11.21-q21.2	<i>RNMT, DCC</i>	Heterozygous duplication
NA07891	Coriell Institute	18q21.2	<i>DCC</i>	Heterozygous deletion

NA12722	Coriell Institute	9p21.3	<i>MTAP, CDKN2A, CDKN2B</i>	Heterozygous duplication
		18p11.21-q21.2	<i>RNMT, DCC</i>	Heterozygous duplication
NA23245	Coriell Institute	7p12.2-q36.2	<i>IKZF1, CDK6, RELN, MET, DPP6</i>	Heterozygous deletion
		8q24.21	<i>MYC</i>	Heterozygous duplication
		9p24.1	<i>JAK2</i>	p.V617F mutation
JAK2 p.V617F 50% reference standard [◊]	Horizon Discovery	7p12.2-q36.2	<i>IKZF1, CDK6, RELN, MET, DPP6</i>	Heterozygous duplication
		9p24.1	<i>JAK2</i>	p.V617F mutation
		18q21.2	<i>DCC</i>	Heterozygous duplication
JAK2 p.V617F 0% (wild-type) reference standard [§]	Horizon Discovery	7p12.2-q36.2	<i>IKZF1, CDK6, RELN, MET, DPP6</i>	Heterozygous duplication
		18q21.2	<i>DCC</i>	Heterozygous duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P377-A3 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 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 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	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication	$1.75 < FR < 2.15$

Ambiguous copy number	All other values
-----------------------	------------------

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

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 *MYB*, *MYCN* and *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

- 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-A3 Hematologic Malignancies

Length (nt)	SALSA MLPA probe	Chromosomal position	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information		
125	DCC probe 21566-L27817	18q21.2	18-048,959
132	IKZF1 probe 03340-L27816	7p12.2	07-050,338
137	ATM probe 02675-L01168	11q22.3	11-107,599
143	IKZF1 probe 13872-L27818	7p12.2	07-050,418
148 «	MYB probe 12500-L27820	6q23.3	06-135,549
154	MYC probe 20383-L27819	8q24.21	08-128,822
160	CDKN2A probe 01524-L27821	9p21.3	09-021,985
165	MIR15A probe 04019-L17530	13q14.3	13-049,521
169	RUNX1 probe 20384-L25345	21q22.12	21-035,343
173 «	MYCN probe 03028-L17950	2p24.3	02-016,000
178	MIR146A probe 15652-L17541	5q33.3	05-159,845
184	MYC probe 14869-L16611	8q24.21	08-128,822
190	DLEU2 probe 04020-L17532	13q14.3	13-049,554
196	ETV6 probe 14054-L15652	12p13.2	12-011,935
203	ATM probe 08426-L08309	11q22.3	11-107,659
208 §	JAK2 probe 05672-L17742	p.V617F=c.1849G>T	09-005,064
214	ESR1 probe 11996-L12824	6q25.1	06-152,424
220	MET probe 10329-L10843	7q31.2	07-116,211
226	EBF1 probe 12509-L13559	5q33.3	05-158,459
232 ¶	DPP6 probe 14027-L15625	7q36.2	07-154,227
239	ALK probe 08325-L28371	2p23.2	02-029,274
244	ETV6 probe 13874-L17160	12p13.2	12-011,883
252 ‡	TP53 probe 02376-L27832	17p13.1	17-007,519
256	SMOC2 probe 09380-L27831	6q27	06-168,809
262	FYN probe 12546-L27830	6q21	06-112,148
266	MIR146A probe 15653-L18125	5q33.3	05-159,845
274	UNC13D probe 11696-L17540	17q25.1	17-071,342
279	PAX5 probe 13870-L17534	9p13.2	09-036,993
285	MDM2 probe 07179-L17544	12q15	12-067,494
292	MIR145 probe 14248-L15086	5q33.1	05-148,789
297 «	RUNX1 probe 02840-L27829	21q22.12	21-035,094
303	IKZF3 probe 15461-L17667	17q12	17-035,202
313	CDK6 probe 03184-L28370	7q21.2	07-092,085
321	CACNA1A probe 09065-L28369	19p13.13	19-013,289
329	MTAP probe 01294-L28034	9p21.3	09-021,793
337	IKZF1 probe 13869-L15387	7p12.2	07-050,427
346 ±	TP53 probe 00345-L00171	17p13.1	17-007,514
355	CCND2 probe 00498-L00084	12p13.32	12-004,279
364	EBF1 probe 14059-L27828	5q33.3	05-158,137
375 +	PAX5 probe 12521-L27827	9p13.2	09-036,830
384	DLEU1 probe 01589-L27826	13q14.3	13-049,782
392	TP53 probe 01587-L17743	17p13.1	17-007,515
400	PRPF31 probe 06024-L05449	19q13.42	19-059,327
409	TP53 probe 02263-L01749	17p13.1	17-007,532
418	CDKN2B probe 20386-L28368	9p21.3	09-021,991
427	ATM probe 08443-L08330	11q22.3	11-107,722
436 «	MYCN probe 03327-L17744	2p24.3	02-016,003
445	PTEN probe 13684-L18623	10q23.31	10-089,614
453	ATM probe 20385-L27825	11q22.3	11-107,629
470	RB1 probe 01800-L28440	13q14.2	13-047,953
478	PTEN probe 13696-L28441	10q23.31	10-089,715
488	RB1 probe 12565-L28442	13q14.2	13-047,937

Length (nt)	SALSA MLPA probe	Chromosomal position	Location (hg18) in kb
499	RNMT probe 20552-L17745	18p11.21	18-013,724
507	RELN probe 20553-L18622	7q22.1	07-103,058

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

± SNPs rs80184930 and rs774269719 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.

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

+ More variable. This probe has a high standard deviation due to a strong quadruplex region on the probe target and surrounding sequence. Be cautious when interpreting the results and always confirm deviating *PAX5* exon 10 copy number results with a different MLPA probemix (e.g. P335 ALL-IKZF1) or with a different method.

‡ 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-A3 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon	Location/Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
2p gain (<i>MYCN</i> and <i>ALK</i> genes) Exon numbering for <i>MYCN</i> is according to NG_007457.1 RefSeq sequence. 2p gain, including <i>MYCN</i> and <i>ALK</i> , 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 <i>MYCN</i> and <i>ALK</i> probes are present in the P037 CLL-1 and P252 NB mix 2 probemixes.					
173 <	03028-L17950	MYCN , ex 2	NM_005378.6; 470-471	GGAAGAAGTTT-GAGCTGCTGCC	3.5 kb
436 <	03327-L17744	MYCN , ex 3	NM_005378.6; 1351-1352	TGCACCCCCACA-GAAGAAGATAAA	13 Mb
239	08325-L28371	ALK	2p23.2	TTTCTCTGGAT-ATATGCCATACC	-
Loss of 5q Exon numbering for <i>EBF1</i> is according to RefSeq transcript NM_001290360.3. Exon numbering for <i>MIR146A</i> is 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. <i>MIR145</i> and <i>MIR146A</i> were shown to be key mediators of 5q syndrome (Starczynowski et al. 2010). Deletions of <i>EBF1</i> 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 <i>EBF1</i> are present in the P335 ALL-IKZF1 probemix.					
292	14248-L15086	MIR145	5q33.1	CAGCCACTTGTG-ATGCTGGGAAG	9.4 Mb
364	14059-L27828	EBF1 , ex 10	NM_001290360.3; 1416-1417	GTTGTGGAAGTC-ACACTGCTCTAC	322.2 kb
226	12509-L13559	EBF1 , ex 1	NM_001290360.3; 278-279	ATTTGCTTTCCA-GCCCGCCTTGAT	1.4 Mb
178	15652-L17541	MIR146A , ex 1	NR_029701.1; 4 nt before exon 1	CACCATCTCTGA-AAAGCCGATGTG	0.1 kb
266	15653-L18125	MIR146A , ex 1	NR_029701.1; 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. <i>MYB</i> duplications have been described in leukemias (reviewed in Pattabiraman et al. 2012), e.g. <i>MYB</i> 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 and in the P462 Follicular Lymphoma probemix. More probes for <i>MYB</i> can be found in the P383 T-ALL Probemix.					
262	12546-L27830	FYN	6q21	GGTGTGAAGTCT-TCGTCTCATACG	23.4 Mb
148 <	12500-L27820	MYB	6q23.3	TGAGGACTTTGA-GATGTGTGACCA	16.9 Mb
214	11996-L12824	ESR1	6q25.1	TTCGACATGCTG-CTGGCTACATCA	16.4 Mb
256	09380-L27831	SMOC2	6q27	GTGCTTACAGCA-TTGTGGAATCAT	-

Length (nt)	SALSA MLPA probe	Gene/Exon	Location/Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
<p>IKZF1 gene, 7p12.2</p> <p>Exon numbering is according to LRG_1121 and is different from the exon numbering used by Mullighan et al. where exon 3-6 deletions correspond to exon 4-7 in the LRG exon numbering. Deletions of the <i>IKZF1</i> (<i>IKAROS</i>) gene are detected in ALL, especially in cases that also carry the <i>BCR-ABL1</i> gene fusion (Mullighan et al. 2008). <i>IKZF1</i> 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 <i>IKZF1</i> 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 <i>IKZF1</i> gene are present in P202 <i>IKZF1-ERG</i> and P335 <i>ALL-IKZF1</i> probemixes.</p>					
132	03340-L27816	IKZF1 , ex 3	NM_006060.6; 355-356	GGGAGGACAGCA-AAGCTCCAAGAG	79.9 kb
143	13872-L27818	IKZF1 , ex 5	NM_006060.6; 680-681	TGCGGGGCTCA-TTCACCCAGAAG	9.2 kb
337	13869-L15387	IKZF1 , ex 7	NM_006060.6; 994-995	CAAGATAGGATC-AGAGAGATCTCT	41.7 Mb
<p>Loss of 7q</p> <p>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 <i>MET</i> gene.</p>					
313	03184-L28370	CDK6	7q21.2	GAGAAGAAGACT-GCCTAGAGATG	11 Mb
507	20553-L18622	RELN	7q22.1	GGGCTATTGATG-AGATTATCATGA	13.2 Mb
220	10329-L10843	MET	7q31.2	AAGTGGATGGCT-TTGAAAGTCTG	38 Mb
232	14027-L15625	DPP6	7q36.2	ACCAAGATCCTA-GCCTACGATGAG	-
<p>MYC gene, 8q24.21</p> <p>Exon numbering for <i>MYC</i> is according to LRG_1397. Gains or amplifications of the <i>MYC</i> 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 <i>MYC</i> can be found in the P037 CLL-1 and P462 Follicular Lymphoma probemixes.</p>					
184	14869-L16611	MYC , ex 3	NM_002467.6; 1431-1432	AACAACCGAAAA-TGCCACGCCCC	0.1 kb
154	20383-L27819	MYC , ex 3	NM_002467.6; 1520-1521	GAACGAGCTAAA-ACGGAGCTTTTT	-
<p>JAK2 p.V617F mutation, 9p24.1</p> <p>Exon numbering for <i>JAK2</i> is according to LRG_612. <i>JAK2</i> p.V617F mutation, in exon 14, is a somatic mutation of G into T resulting in substitution of valine to phenylalanine and constitutive activation of cell proliferation. <i>JAK2</i> 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 <i>JAK2</i> mutations frequently found in MPN samples: <i>JAK2</i> p.V617F, <i>JAK2</i> p.E543-D544del and <i>JAK2</i> p.N542-E543del.</p>					
208	05672-L17742	JAK2 , ex 14; p.V617F (c.1849G>T)	NM_004972.4; 2316-2315 reverse	GTCTCCACAGAA-ACATACTCCATA	16.7 Mb
<p>9p21.3 deletions</p> <p>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 <i>CDKN2A/2B</i> 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 <i>MTAP</i> homozygous deletion could be treated with <i>MAT2A</i> inhibitors, which are currently tested in clinical trials (Konteaty et al. 2021). Deletions of 9p21.3 are detected also in other hematologic malignancies. More <i>CDKN2A/2B</i> probes are present in the P202 <i>IKZF1-ERG</i>, P335 <i>ALL-IKZF1</i> and P419 <i>CDKN2A/2B-CDK4</i> probemixes and in the ME024 9p21 <i>CDKN2A/2B</i> region probemix, which detects both copy number and methylation changes of these genes.</p>					
329	01294-L28034	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 Mb
<p>PAX5 gene, 9p13.2</p> <p>Exon numbering for <i>PAX5</i> is according to LRG_1384. <i>PAX5</i> deletions are frequent in B-ALL and in <i>BCR-ABL1</i> positive ALL cases (Coyaud et al. 2010). 9p deletions in ALL can be large and extend sometimes into the <i>CDKN2A/2B</i> genes. Note that amplifications of exon 2 & 5 have been suggested to be an alternative mechanism of <i>PAX5</i> inactivation and could define a novel subgroup in BCP-ALL (Schwab et al. 2017). More <i>PAX5</i> probes are present in the P335 <i>ALL-IKZF1</i> and ME024 9p21 <i>CDKN2A/2B</i> region probemixes.</p>					

Length (nt)	SALSA MLPA probe	Gene/Exon	Location/Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
375 +	12521-L27827	PAX5 , ex 10	NM_016734.3; 1461-1462	CCTATTGAGGGT-GACAGCCACCCA	162.2 kb
279	13870-L17534	PAX5 , ex 5	NM_016734.3; 756-757	GTGAGCACGGAT-TCGGCCGGCTCG	-
PTEN gene, 10q23.31 Exon numbering for <i>PTEN</i> is according to LRG_311. <i>PTEN</i> 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). <i>PTEN</i> deletions occur in 11% of diffuse large B-cell lymphoma (Wang et al. 2018). Note that <i>PTEN</i> point mutations are more frequent, but they cannot be detected with these MLPA probes. More probes for <i>PTEN</i> can be found in P294 Tumour Loss and P383 T-ALL probemixes. Probes to detect every exon of <i>PTEN</i> gene are present in the P225 <i>PTEN</i> probemix.					
445	13684-L18623	PTEN , ex 1	NM_000314.8; 5 nt after exon 1	TTGACCTGTATC-CATTTCTGCGGC	101.0 kb
478 #	13696-L28441	PTEN , ex 9	NM_000314.8; 2171-2170 reverse	AGAGAATTGTTC-CTATAACTGGTA	-
ATM gene, 11q22.3 Exon numbering for <i>ATM</i> is according to LRG_135. Deletion of 11q22-q23, including <i>ATM</i> , is associated with an aggressive course of B-cell chronic lymphocytic leukaemia (B-CLL) (Guarini et al. 2012). Deletions of <i>ATM</i> are also detected in ALL and is found in 6% of CLL cases (Guarini et al., 2012). More probes for <i>ATM</i> are found in P040 CLL Probemix, while probemixes P041 and P042 <i>ATM</i> contain probes to detect every exon of the <i>ATM</i> gene.					
137	02675-L01168	ATM , ex 1	NM_000051.4; 17nt before exon 1	CACGCAGGGTTT-GAACCGGAAGCG	29.8 kb
453	20385-L27825	ATM , ex 12	NM_000051.4; 1990-1991	AAATTCTTGTA-GTCTCACTATGA	29.9 kb
203	08426-L08309	ATM , ex 25	NM_000051.4; 3744-3745	GAGAAAGTTTCT-GAAACTTTTGGA	63.1 kb
427	08443-L08330	ATM , ex 58	NM_000051.4; 8671-8672	AAAAATTCTTGG-ATCCAGCTATTT	-
ETV6 gene, 12p13.2 & chromosome 12 gains Exon numbering for <i>ETV6</i> is according to LRG_609. <i>ETV6</i> deletions are frequent in childhood ALL and in AML/MDS with normal karyotype (Wall et al. 2012). <i>ETV6</i> deletions are also frequently associated with leukemic transformation of Philadelphia chromosome-negative myeloproliferative neoplasms. More <i>ETV6</i> 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 <i>MDM2</i> and <i>CCND2</i> probes are present in the P323 CDK4-HMGA2-MDM2 probemix. More <i>CCND2</i> probes are present in the P175 Tumour Gain Probemix.					
355	00498-L00084	CCND2	12p13.32	ATGCCAGTTGGG-CCGAAAGAGAGA	7.6 Mb
244	13874-L17160	ETV6 , ex 3	NM_001987.5; 641-642	TTTACTGGAGCA-GGGATGACGTAG	52.0 kb
196	14054-L15652	ETV6 , ex 8	NM_001987.5; 1959-1960	TCTTGCAGACCA-AGAGGGACCCTG	55.6 Mb
285	07179-L17544	MDM2	12q15	ACCAACAGACTT-TAATAACTTCAA	-
13q deletions Exon numbering for <i>RB1</i> is according to LRG_517. Deletions of 13q, especially on 13q14, occur in >50% in CLL and mantle cell lymphoma cases (Wolf et al. 2001). <i>RB1</i> 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 <i>RB1</i> 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 <i>RB1</i> are present in the P335 ALL-IKZF1 probemix, while P047 <i>RB1</i> covers the whole gene.					
488	12565-L28442	RB1 , ex 23	NM_000321.3; 2557-2558	CACCCTACGGA-TTCCTGGAGGGA	15.1 kb
470	01800-L28440	RB1 , ex 27	NM_000321.3; 3270-3271	GAGTCCTGATAA-CCCAGGCTGTC	1.6 Mb
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 & 17q deletions Exon numbering for <i>TP53</i> is according to LRG_321. Isochromosome of 17q, i(17)(q10), is common in AML and CML (20%), and results in loss of 17p and <i>TP53</i> (Kanagal-Chamanna et al. 2012). Moreover, deletion of 17p and <i>TP53</i> 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 to detect <i>TP53</i> copy number changes are present in the P038 CLL-2, P040 CLL and P056 <i>TP53</i> probemixes.					
346 ±	00345-L00171	TP53 , ex 11	NM_000546.6; 1269-1270	AAAGGGTCAGTC-TACCTCCGCCA	1.0 kb
392	01587-L17743	TP53 , ex 10	NM_000546.6; 1174-1175	TTCCGAGAGCTG-AATGAGGCCTTG	4.5 kb
252 ‡	02376-L27832	TP53 , ex 4b	NM_000546.6; 546-547	CAAGATGTTTTG-CCAACCTGGCCAA	12.4 kb

Length (nt)	SALSA MLPA probe	Gene/Exon	Location/Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
409	02263-L01749	TP53 , ex 1	NM_000546.6; 127 nt before exon 1	CTTCCTCCGGCA-GGCGGATTACTT	27.7 Mb
303	15461-L17667	IKZF3	17q12	AGCAGGCCAAC-AGTGGAAGATG	36.1 Mb
274	11696-L17540	UNC13D	17q25.1	GCACATCCAGAA-ACTGGTGGGCGT	-
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). More probes for 18q (MALT1 and BCL2 genes) can be found in the P462 Follicular lymphoma probemix.					
499	20552-L17745	RNMT	18p11.21	TACAATGAACTT-CAGGAAGTTGGT	35.2 Mb
125	21566-L27817	DCC	18q21.2	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 Mb
400	06024-L05449	PRPF31	19q13.42	GGATCGGGTCTT-GGCAGGGAGAAC	-
RUNX1 (AML1) , 21q22.1					
Exon numbering for <i>RUNX1</i> is according to LRG_482. High-level amplifications of <i>RUNX1</i> , 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 <i>RUNX1</i> and iAMP21 detection are present in the P327 iAMP21-ERG and for <i>RUNX1</i> in P437 Familial MDS-AML probemixes.					
297 ‹	02840-L27829	RUNX1 , ex 8	NM_001754.5; 1040-1041	TGGTCCTACGAT-CAGTCCTACCAA	249.5 kb
169	20384-L25345	RUNX1 , ex 2	NM_001754.5; 190-191	TTTTTCAGGAGGA-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.

± SNPs rs80184930 and rs774269719 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.

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

+ More variable. This probe has a high standard deviation due to a strong quadruplex region on the probe target and surrounding sequence. Be cautious when interpreting the results and always confirm deviating *PAX5* exon 10 copy number results with a different MLPA probemix (e.g. P335 ALL-*IKZF1*) or with a different method.

‡ 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.

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 and 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 (CE-marked):** 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 that have 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* V617F, *JAK2* E543-D544del, *JAK2* N542-E543del, *MPL* W515L, *MPL* W515K, *KIT* D816V, *CALR* L367fs*46, *CALR* 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.
- **P462 Follicular Lymphoma:** Contains probes for 14 regions frequently affected by copy number changes in follicular lymphoma, including 8q (*MYC*) and 18q (*MALT1*, *BCL2*).
- **P520 MPN mix 2:** Contains probes for high detection sensitivity (≥ 1 % allele burden) for the following mutations frequently found in MPN samples: *JAK2* V617F, *JAK2* E543-D544del, *JAK2* N542-E543del, *MPL* W515L, *MPL* W515K, *KIT* D816V, *CALR* L367fs*46, *CALR* K385fs*47.
- **ME024 9p21 CDKN2A/2B region:** Contains probes for the detection of copy number and methylation changes of genes located at 9p21.3.

References

- Bardelli V et al. (2021). MYB rearrangements and over-expression in T-cell acute lymphoblastic leukemia. *Genes Chromosom Cancer*. 60:482-8.
- Bertin R et al. (2003). CDKN2A, CDKN2B, and MTAP gene dosage permits precise characterization of mono- and bi-allelic 9p21 deletions in childhood acute lymphoblastic leukemia. *Genes Chromosom Cancer*. 37:44-57.
- Chen JA et al. (2021). Lymphoid blast transformation in an MPN with BCR-JAK2 treated with ruxolitinib: putative mechanisms of resistance. *Blood Adv*. 5:3492-6.
- Cordoba I et al. (2012). Better prognosis for patients with del(7q) than for patients with monosomy 7 in myelodysplastic syndrome. *Cancer*. 118:127-33.
- Coyaud E et al. (2010). Wide diversity of PAX5 alterations in B-ALL: a Groupe Francophone de Cytogenetique Hematologique study. *Blood*. 115:3089-97.
- Ferrero S et al. (2020). KMT2D mutations and TP53 disruptions are poor prognostic biomarkers in mantle cell lymphoma receiving high-dose therapy: a FIL study. *Haematologica*. 105:1604-12.
- Fizzotti M et al. (1995). Detection of homozygous deletions of the cyclin-dependent kinase 4 inhibitor (p16) gene in acute lymphoblastic leukemia and association with adverse prognostic features. *Blood*. 85:2685-90.
- Guarini A et al. (2012). ATM gene alterations in chronic lymphocytic leukemia patients induce a distinct gene expression profile and predict disease progression. *Haematologica*. 97:47-55.
- Gutierrez A et al. (2009). High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood*. 114:647-50.
- Harrison CJ et al. (2014). An international study of intrachromosomal amplification of chromosome 21 (iAMP21): cytogenetic characterization and outcome. *Leukemia*. 28:1015-21.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Iacobucci I et al. (2009). Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on

- behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). *Blood*. 114:2159-67.
- Johansson B et al. (2002). Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. *Acta Haematol*. 107:76-94.
 - Jones AV et al. (2009). JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet*. 41:446-9.
 - Jotta P et al. (2010). Negative prognostic impact of PTEN mutation in pediatric T-cell acute lymphoblastic leukemia. *Leukemia*. 24:239-42.
 - Kanagal-Shamanna R et al. (2012). Myeloid neoplasms with isolated isochromosome 17q represent a clinicopathologic entity associated with myelodysplastic/myeloproliferative features, a high risk of leukemic transformation, and wild-type TP53. *Cancer*. 118:2879-88.
 - Kim S et al. (2013). Clinical significance of cytogenetic aberrations in bone marrow of patients with diffuse large B-cell lymphoma: prognostic significance and relevance to histologic involvement. *J Hematol Oncol*. 6:76.
 - Konteatis Z et al. (2021). Discovery of AG-270, a First-in-Class Oral MAT2A Inhibitor for the Treatment of Tumors with Homozygous MTAP Deletion. *Journal of Medicinal Chemistry*. 64:4430-49.
 - Liu Y et al. (2017). The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. *Nat Genet*. 49:1211-18.
 - Malarikova D et al. (2020). Concurrent TP53 and CDKN2A Gene Aberrations in Newly Diagnosed Mantle Cell Lymphoma Correlate with Chemoresistance and Call for Innovative Upfront Therapy. *Cancers*. 12:2120.
 - Martinelli G et al. (2009). IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. *J Clin Oncol*. 27:5202-07.
 - Masir N et al. (2007). Follicular lymphoma with trisomy 18 exhibiting loss of BCL-2 expression on transformation to a large cell lymphoma. *J Clin Pathol*. 60:1061-64.
 - Merup M et al. (1998). 6q Deletions in Acute Lymphoblastic Leukemia and Non-Hodgkin's Lymphomas. *Blood*. 91:3397-400.
 - Mougalian SS and O'Brien S (2011) Adverse prognostic features in chronic lymphocytic leukemia. *Oncology (Williston Park)*. 25:692-6, 699.
 - Mullighan CG et al. (2008). BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature*. 453:110-4.
 - Mullighan CG et al. (2009). Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *New England Journal of Medicine*. 360:470-80.
 - Pattabiraman DR et al. (2013). Role and potential for therapeutic targeting of MYB in leukemia. *Leukemia*. 27:269-77.
 - Quesada AE et al. (2017). Increased MYC copy number is an independent prognostic factor in patients with diffuse large B-cell lymphoma. *Mod Pathol*. 30:1688-97.
 - Schieppati F et al. (2020). An increase in MYC copy number has a progressive negative prognostic impact in patients with diffuse large B-cell and high-grade lymphoma, who may benefit from intensified treatment regimens. *Haematologica*. 105:1369-78.
 - Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 30:e57.
 - Schwab C et al. (2017). Intragenic amplification of PAX5: a novel subgroup in B-cell precursor acute lymphoblastic leukemia? *Blood Adv*. 1:1473-77.
 - Schraders M et al. (2009). High-resolution genomic profiling of pediatric lymphoblastic lymphomas reveals subtle differences with pediatric acute lymphoblastic leukemias in the B-lineage. *Cancer Genet Cytogenet*. 191:27-33.
 - Schwab CJ et al. (2013). Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. *Haematologica*. 98:1081-8.
 - Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat*. 28:205.

- Sole F et al. (2005). Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. *Haematologica*. 90:1168-78.
- Starczynowski DT et al. (2010). Deregulation of innate immune signaling in myelodysplastic syndromes is associated with deletion of chromosome arm 5q. *Cell Cycle*. 9:855-6.
- Streich L et al. (2020). Aggressive morphologic variants of mantle cell lymphoma characterized with high genomic instability showing frequent chromothripsis, CDKN2A/B loss, and TP53 mutations: A multi-institutional study. *Genes Chromosom Cancer*. 59:484-94.
- Szarzyńska-Zawadzka B et al. (2019). PTEN abnormalities predict poor outcome in children with T-cell acute lymphoblastic leukemia treated according to ALL IC-BFM protocols. *Am J Hematol*. 94.
- 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.
- Vita M et al. (2006). The Myc oncoprotein as a therapeutic target for human cancer. *Semin Cancer Biol*. 16:318-30.
- Wall M et al. (2012). ETV6 deletion is a common additional abnormality in patients with myelodysplastic syndromes or acute myeloid leukemia and monosomy 7. *Haematologica*. 97:1933-36.
- Wang DM et al. (2011). Intermediate prognosis of 6q deletion in chronic lymphocytic leukemia. *Leuk Lymphoma*. 52:230-7.
- Wang X et al. (2018). Clinical Significance of PTEN Deletion, Mutation, and Loss of PTEN Expression in De Novo Diffuse Large B-Cell Lymphoma. *Neoplasia*. 20:574-93.
- Wolf S et al. (2001). B-cell neoplasia associated gene with multiple splicing (BCMS): the candidate B-CLL gene on 13q14 comprises more than 560 kb covering all critical regions. *Hum Mol Genet*. 10:1275-1285.
- Yamada Y et al. (1997). Deletions of p15 and/or p16 genes as a poor-prognosis factor in adult T-cell leukemia. *J Clin Oncol*. 15:1778-85.
- Zaliouva M et al. (2019). ERG deletions in childhood acute lymphoblastic leukemia with DUX4 rearrangements are mostly polyclonal, prognostically relevant and their detection rate strongly depends on screening method sensitivity. *Haematologica*. 104:1407-16.
- Zhang J et al. (2011). Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood*. 118:3080-87.

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

Selected publications using SALSA MLPA Probemix P377 Hematologic Malignancies

- Alhourani E et al. (2014). Comprehensive chronic lymphocytic leukemia diagnostics by combined multiplex ligation dependent probe amplification (MLPA) and interphase fluorescence in situ hybridization (iFISH). *Mol Cytogenet*. 7:79.
- Alhourani E et al. (2015). Isochromosome 17q in Chronic Lymphocytic Leukemia. *Leuk Res Treatment*. 2015:1-6.
- Alhourani E et al. (2016). BIRC3 alterations in chronic and B-cell acute lymphocytic leukemia patients. *Oncol Lett*. 11:3240-6.
- 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*.
- Konialis C et al. (2014) Routine application of a novel MLPA-based first-line screening test uncovers clinically relevant copy number aberrations in haematological malignancies undetectable by conventional cytogenetics. *Hematology*. 19:217-24.
- Othman MA et al (2015). High rates of submicroscopic aberrations in karyotypically normal acute lymphoblastic leukemia. *Mol Cytogenet*. 8(1).
- Othman MA et al. (2014) A Novel Cryptic Three-Way Translocation t(2;9;18)(p23.2;p21.3;q21.33) with Deletion of Tumor Suppressor Genes in 9p21.3 and 13q14 in a T-Cell Acute Lymphoblastic Leukemia. *Leuk Res Treatment*. 2014:357123.

- Othman MA et al. (2015). MLLT10 and IL3 rearrangement together with a complex four-way translocation and trisomy 4 in a patient with early T-cell precursor acute lymphoblastic leukemia: A case report. *Oncol Rep.* 33: 625-30.
- Othman MA et al. (2015). Novel Cryptic Rearrangements in Adult B-Cell Precursor Acute Lymphoblastic Leukemia Involving the MLL Gene. *J Histochem Cytochem.* 63:384-90.
- Othman MA et al. (2016). A novel IGH@ gene rearrangement associated with CDKN2A/B deletion in young adult B-cell acute lymphoblastic leukemia. *Oncol Lett.* 11:2117-22.
- Srinivasan VK et al (2020). Genomic alterations in chronic lymphocytic leukemia and their correlation with clinico-hematological parameters and disease progression. *Blood Research.* 55(3): 131-8.
- Tripon F et al. (2020). Co-occurrence of PML-RARA gene fusion, chromosome 8 trisomy, and FLT3 ITD mutation in a young female patient with de novo acute myeloid leukemia and early death. *Medicine.* 99:e19730.

P377 product history	
Version	Modification
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
<p>Version A3-01 – 26 October 2021 (04P)</p> <ul style="list-style-type: none"> - 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). <p>Version 12 – 30 June 2020 (T08)</p> <ul style="list-style-type: none"> - 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. <p>Version 11 – 06 February 2020 (T08)</p> <ul style="list-style-type: none"> - 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. <p>Version 10 – 27 March 2018 (T08)</p> <ul style="list-style-type: none"> - Warning added to Table 2 for probe relying on its specificity on a single nucleotide difference between target and related gene or pseudogene. <p>Version 09 – 01 February 2018 (T08)</p> <ul style="list-style-type: none"> - Exon numbering of IKZF1 probes adjusted according to NM_006060.5 <p>Version 08 – 22 December 2017 (T08)</p> <ul style="list-style-type: none"> - New references to articles citing use of this probemix were added. - New related probemixes were added.

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
 - Small changes in Table 1 and Table 2.
 - Mapview location has been removed from Table 2, as this information is already available in Table 1.
 - Various textual changes throughout the document.
- Version 07 – 10 May 2017 (T08)
- “SD046 Sample DNA” was replaced with “SALSA Binning DNA SD068”, and therefore throughout the text “SALSA Binning DNA SD068” is used.
 - Paragraph describing the SALSA Binning DNA SD068 has been modified on page 2.
 - Figure 2 replaced with a one of SD068 sample DNA.
- Version 06 – 13 January 2017 (T08)
- Warning added in Table 1 and Table 2, 297 nt probe 02840-L27829.
 - Several minor textual changes throughout.

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