Product Description
SALSA® digitalMLPA™ Probemix D007-A1 Acute Lymphoblastic Leukemia

To be used with the digitalMLPA General Protocol.

Version A1
First release.

Catalogue numbers

- **D007-025R:** SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia, 25 reactions
- **D007-050R:** SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia, 50 reactions
- **D007-100R:** SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia, 100 reactions

SALSA® digitalMLPA™ Probemix D007-A1 Acute Lymphoblastic Leukemia (hereafter: D007 Acute Lymphoblastic Leukemia) is to be used in combination with:

1. SALSA® digitalMLPA™ Reagent Kit (Cat No: DRK01-IL, DRK05-IL, DRK20-IL)

2. One or multiple barcode plates:
   SALSA® digitalMLPA™ Barcode Plate 1 (Cat No: BP01-IL (from lot 03-009-xxxxxx and higher))
   SALSA® digitalMLPA™ Barcode Plate 2 (Cat No: BP02-IL (from lot 03-008-xxxxxx and higher))

   N.B. The three-digit number between dashes (e.g. -008-) will increase with every new barcode plate lot.

3. Data analysis software Coffalyser digitalMLPA™ (Cat No: n.a.)

Volumes and ingredients

Volumes			Ingredients
D007-025R	**D007-050R**	**D007-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The digitalMLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding storage conditions and quality tests 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 digitalMLPA 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.

Due to high numbers of copy number alterations (CNAs) in acute lymphoblastic leukemia samples, the control probes included for sample identification will not be able to indicate sample DNA contamination, but can still be used for reliable sample identification (SNP code in Coffalyser digitalMLPA reports).

Two Coffalyser digitalMLPA product sheets are available for data analysis, a default product sheet and a near haploid_low hypodiploid product sheet. Different reference probe selections ensure correct data normalisation in samples with gross chromosomal aberrations. Prior knowledge of the ploidy status based on DNA indexing and/or karyotyping is necessary for proper analysis of (low) hypodiploid, near haploid and hyperdiploid samples using digitalMLPA data obtained with D007 Acute Lymphoblastic Leukemia.

General information

SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia is a **research use only (RUO)** assay for the detection of deletions, gains or amplifications in 52 specific target genes and three chromosomal regions (5q, iAMP21 and PAR1), which are associated with acute lymphoblastic leukemia (ALL) as mentioned in Table 2.

This assay is for use with genomic DNA isolated from peripheral whole blood or bone marrow specimens and is not intended to be used with genomic DNA extracted from formalin-fixed paraffin-embedded or fresh-frozen tumour materials. CNAs detected with this probemix should be confirmed with a different technique or with SALSA® MLPA® probemixes when possible. In particular, CNAs detected by only a single probe always require confirmation by another method. The majority of defects in the genes included in this probemix are deletions, gains or amplifications, but point mutations can occur which will not be detected by digitalMLPA. It is therefore recommended to use this assay in combination with sequence analysis for the gene(s) of interest.

ALL is the most common childhood cancer and includes multiple genetically heterogeneous subtypes of malignant clonal expansions of immature T- or B-cells. Although current treatment regimens have resulted in 5-year event-free survival rates of >90% in children, disease relapse is associated with a poor outcome (Roberts and Mullighan 2015). Genetic alterations in the different subtypes of ALL are well characterised and include gross chromosomal aberrations such as hyper-/hypodiploidy, but also fusion genes, gene deletions, gains and amplifications. SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia is designed to detect these key genetic copy number alterations in childhood ALL.

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

Probemix content

A total number of ~760 probes is included in D007 Acute Lymphoblastic Leukemia, consisting of:
- 347 target probes detecting copy number alterations involved in ALL (Table 2).
- 249 karyotyping probes, covering most chromosome arms at the centromeres, middle of the chromosome arms and telomere regions. No probes are present for the short p-arm of the five acrocentric chromosomes (13, 14, 15, 21 and 22). See Table 3 for all chromosomal regions and genes included.
- More than 160 control probes and fragments: these include probes to aid in normalisation in case of copy number changes in tumour samples, probes for sample identification and probes for detection of errors or deviations when performing digitalMLPA assays, impurities in and fragmentation of the DNA samples, ligase and polymerase activity and extent of hybridisation.

More information on the location and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available at www.mrcholland.com.

Reference probes

The selected reference probes as defined in the *D007-A1 default product sheet* are a subset of karyotyping probes (n=96) in regions that show minimal copy number changes in ALL, as indicated in Table 3. This was determined using information from the Progenetix oncogenomic online resource, Database of Genomic Variants (DGV), Broad Institute TCGA Copy Number Portal, and Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census. This selection is applicable for most ALL subtypes with (small) CNAs, chromosomal (arm) gains and losses, and for samples with (high) hyperdiploidy.

As the number of chromosomes with a normal diploid copy number retained in samples with low hypodiploidy and near haploidy is limited, a different selection of reference probes is required for optimal data normalisation of these sample types. Based on several publications (Harrison et al. 2004, Nachman et al. 2007, Holmfeldt et al. 2013, Safavi et al. 2017, Caroll et al. 2019), 86 suitable reference probes were selected on the chromosomes retained in both low hypodiploidy and near haploidy (chromosomes 8, 10, 14, 18, 21 and X / Y). In addition, a small number of reference probes was added for additional chromosomes reported to be retained in low hypodiploidy (chromosomes 1, 5, 6, 11 and 22). The number of selected reference probes per chromosome in this product sheet is indicated in Table 4. More information on the use of the *D007-A1 near haploid_low hypodiploid product sheet* can be found in section "Data analysis" below.

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

digitalMLPA technique
SALSA® digitalMLPA™ (Benard-Slagter et al. 2017) combines the robustness and simplicity of the trusted SALSA® MLPA® technology (Schouten et al. 2002) with next-generation sequencing. The principles of digitalMLPA are described in the digitalMLPA General Protocol (www.mrcholland.com).

digitalMLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using digitalMLPA for the first time, or when pre-analytical steps, DNA extraction method or the instruments used are changed. This validation experiment should result in a standard deviation ≤0.10 for all probes with the exception of SNP- and mutation-specific probes.

Required specimens
Extracted DNA from human peripheral blood and/or bone marrow, free from impurities known to affect digitalMLPA reactions. For more information see the digitalMLPA General Protocol, section DNA sample treatment. The minimum percentage of tumour cells required for reliable analysis is 30% (Al Zaabi et al. 2010, Coll-Mulet et al. 2008). We advise using tumour samples with at least 50% tumour cell content. Therefore, tumour samples should be evaluated by a pathologist before the extraction of DNA.

Reference samples
As X- and Y-chromosome specific probes are included in this probemix, at least three male reference samples AND three female reference samples need to be used per experiment. In instances where an experiment only contains patient samples of one gender, at least three reference samples of the same gender should be used. Pooled DNA from different genders can never be used as reference samples for D007 Acute Lymphoblastic Leukemia analysis.

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. More information regarding the selection and use of reference samples can be found in the digitalMLPA General Protocol.

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. The quality of cell lines can change, therefore positive samples should be either acquired from quality assessed biological sample repositories or validated before use. Table 1 contains a list of positive control samples that have been tested with D007 Acute Lymphoblastic Leukemia at MRC Holland.

Table 1. Positive samples from biobanks tested by MRC Holland

Coriell sample ID	Genomic aberration
NA00501	Heterozygous gain of 4q25-q35.2, including *LEF1* and *NR3C2*
NA00526	Trisomy 13, including *RB1*
NA00782	Heterozygous gain of 4q13.1-q31.21, including *LEF1* Heterozygous gain of *CRLF2* on Xp22.33
NA00981	Heterozygous gain of 20p chromosomal arm
NA01221	Heterozygous gain of 6q13-q22.33, including *CASP8AP2*
NA01359	Trisomy 18, including *PTPN2*
NA02030	Trisomy 8, including *TOX*
NA02587	Heterozygous deletion of 17q11.2, including *NF1* and *SUZ12* Heterozygous gain of the PAR1 region
NA02718	Heterozygous deletion of *RB1* and RB1 flanking probes on 13q14.2 Heterozygous deletion of Xp21.1-p22.11, including *DMD*
NA02819	Heterozygous gain of 9p21.3-p24.3, including *JAK2*, *MLLT3*, *MTAP*, *CDKN2A* and *CDKN2B*
NA03184	Trisomy 15, including *SPRED1*
NA03226	Heterozygous gain of 9p, including *JAK2*, *MLLT3*, *MTAP*, *CDKN2A*, *CDKN2B* and *PAX5*
NA03384	Heterozygous gain of 11p15.4-q25, including *LMO1*, *LMO2*, *CD44*, *SLC1A2* and *RAG2* Four copies of chromosome X, and mosaic loss of chromosome Y
NA03563	Heterozygous gain of 3q13.2-q29, including *CD200*, *BTLA*, *TBL1XR1* and *LINC00501* Heterozygous deletion of 9p24.3
NA04371	Heterozygous gain of 5q33.3-q35.3, including *EBF1* Heterozygous gain of *CSF2RA* on Xp22.33
NG04823	Trisomy 21, including the iAMP21 region and *RUNX1* and *ERG*
NA06226	Heterozygous gain of 16p13.3-p13.12, including *CREBBP*
NA06803	Heterozygous deletion of *LMO2*, *CD44*, *SLC1A2* and *RAG2* on 11p12-p13
NA07081	Heterozygous gain of 7p, including *IKZF1*
NA07106	Trisomy 22, including *VPREB1*
NA07412	Heterozygous deletion of 7q34-q36.3, including *EPHA1* and *EZH2*
NA07994	Heterozygous gain of 6q23.3-q27, including *MYB* Heterozygous deletion of 10q26.3
NA08035	Heterozygous gain of 12p arm, including *ETV6*
NA08146	Heterozygous deletion of *VPREB1* on 22q11.22 Heterozygous gain of *CRLF2* and *CSF2RA* on Xp22.33 Heterozygous deletion of 17p11.2
NA08778	Heterozygous deletion of 3q11.2-q13.2, including *CD200* and *BTLA*
NA10401	Trisomy 2, including *IKZF2*
NA10925	Heterozygous deletion of 7p12.2-p12.3, including *IKZF1*
NA10946	Heterozygous deletion of *CASP8AP2* on 6q15
NA10989	Heterozygous deletion of 9p24.1-p24.3, including *JAK2* Heterozygous deletion of *IGHM* on 14q32.33
NA12722	Trisomy 9, with a heterozygous deletion of *PAX5* and flanking probe *ZCCHC7* on one of the chromosomes Trisomy 18, including *PTPN2*
NA13685	Heterozygous gain of 9q34.12-q34.3, including *ABL1*, *NUP214* and *NOTCH1* Heterozygous deletion of *IGHM* on 14q32.33
NA14523	Heterozygous deletion of Xp11.22-p22.33, including the PAR1 region and *DMD*
NA20027	Heterozygous deletion of chromosome X Heterozygous deletion of *IGHM* on 14q32.33
NA20125	Heterozygous gain of 10q23.31-q26.1, including *PTEN* and *ADD3* Heterozygous deletion of *IGHM* on 14q32.33
NA50322	Heterozygous deletion of *PTPN2* on 18p11.21 Heterozygous deletion of *IGHM* on 14q32.33

Data analysis

Coffalyser digitalMLPA must be used for data analysis in combination with the appropriate lot-specific product sheet. Coffalyser digitalMLPA software is freely downloadable at www.mrcholland.com[1]. Use of other non-proprietary software may lead to inconclusive or false results. Normalisation of results should be performed within one experiment. The digitalMLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Separate product sheet for near haploid or low hypodiploid samples

As long as more than 50% of the sequences targeted by the reference probes have a normal copy number (CN=2) in the test sample, the correct baseline will be detected and data will be correctly normalised using the Coffalyser digitalMLPA *default product sheet*. The reference probe selection in the *D007-A1 default product sheet* is also suitable for use on high hyperdiploid samples, as >50% of the reference probes are expected to have a normal diploid copy number. However, for ALL samples that would be classified as near haploid or low hypodiploid samples by conventional karyotyping or DNA indexing, loss of too many chromosomes would result in wrong baseline detection using this reference probe selection. A separate product sheet is available that should be used for correct baseline detection and ratio determination in these low hypodiploid or near haploid ALL samples. The reference probe selection in this *D007-A1 near haploid_low hypodiploid product sheet* is based on chromosomes frequently retained in these near haploid or low hypodiploid sample types (see Table 4).

Please note that prior knowledge of the ploidy status based on DNA indexing and/or karyotyping is necessary for proper analysis of (low) hypodiploid, near haploid and hyperdiploid samples using digitalMLPA data obtained with D007 Acute Lymphoblastic Leukemia.

Use of the wrong product sheet for analysis with Coffalyser digitalMLPA will result in incorrect baseline detection and therefore incorrect normalization for all probes in the probemix. For example, a near haploid sample wrongfully analysed with the *D007-A1 default product sheet* could be interpreted as a sample with gains of a few chromosomes and or genes/regions.

Interpretation of results

The expected results for (pseudo)autosomal probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous gain), 4 (heterozygous triplication / homozygous gain) or ≥5 (amplification). The same results can be expected for the X-chromosome-specific probes in female samples. For the (non-PAR) X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or ≥2 (gain).

The standard deviation of all probes in the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the inter ratio of the probes can be used to interpret digitalMLPA results when **reference samples of the same sex** have been used:

Copy number status indication		Inter ratio
Autosomal sequences and X-chromosome sequences in females	**X-chromosome sequences in males**	
Normal	Normal	0.80 < ratio < 1.20
Homozygous deletion	Deletion	ratio = 0
Heterozygous deletion		0.40 < ratio < 0.65
Heterozygous gain		1.30 < ratio < 1.65
Heterozygous triplication/ Homozygous gain	Gain	1.75 < ratio < 2.15
Ambiguous*		All other values

Please note that these above mentioned inter ratios are affected both by the percentage of tumour cells and by possible subclonality. In case of a heterozygous deletion that is subclonal and/or the sample contains a lower percentage of tumour cells, the inter ratio might not be in the expected range of 0.40-0.65. For example, in case of

[1] In case a reference sample quality warning or error is reported due to high standard deviation for X and Y probes, more information can be found on our website.

50% tumour cell content or a copy number alteration present in 50% of the tumour cells, the inter ratio of a gene or region with a heterozygous deletion will be around 0.75. However, the same (ambiguous) inter ratio (0.75) will also be found for a sample harbouring a homozygous deletion with a tumour cell percentage of 25% or a subclone comprising 25% of all tumour cells. The digitalMLPA technique cannot discriminate between these two scenarios.

* Ambiguous ratios might indicate a (subclonal) homozygous deletion when inter ratios are < 0.30 or an amplification when inter ratios are > 2.15.

General notes on digitalMLPA interpretation:

- 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 result in false-positive results. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe read count, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or sample clean up (e.g. with ethanol precipitation or silica column based-kits) may resolve such cases. Control probes are present in all digitalMLPA probemixes that provide a warning for incomplete DNA denaturation.
- False-positive results (gains): Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe read count (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 digitalMLPA are pathogenic. 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. In some genes, intragenic deletions are known that result in very mild, or no disease (Schwartz et al. 2007). Gains that include the first or last exon of a gene (e.g. exons 1-3) might in some cases not result in the inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

D007 Acute Lymphoblastic Leukemia specific notes

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples, which could result in warnings in Coffalyser digitalMLPA output about reference probe quality.
- The SNP-specific control probes included in D007 Acute Lymphoblastic Leukemia can be used for sample identification. However, they will not indicate contamination of sample DNA.

Limitations of the procedure

- digitalMLPA cannot distinguish between hyperdiploid and low hypodiploid or near haploid samples. DNA indexing or karyotyping should be done to determine the ploidy of the sample, upon which the correct Coffalyser product sheet can be selected for data analysis.
- The majority of the defects in the genes included in this D007 Acute Lymphoblastic Leukemia Probemix are deletions, gains or amplifications, but point mutations can occur which will not be detected by this probemix.
- digitalMLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most copy number neutral inversions or translocations. Even when digitalMLPA 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, small indels) 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 read count by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- digitalMLPA 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, the subclonality of the aberration affects the inter ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample especially in samples with more chaotic karyotypes.

Confirmation of results

Copy number changes detected with D007 Acute Lymphoblastic Leukemia must be verified by another method. SALSA® MLPA® probemixes are available for many genes / chromosomal regions present in D007 Acute Lymphoblastic Leukemia. Most of these SALSA® MLPA® probemixes contain probes with a different ligation site that can be used for confirmation of results (see section 'Related SALSA® MLPA® probemixes' in this product description). Alternatively, copy number changes can be confirmed by another independent technique such as long-range PCR, qPCR, array CGH, FISH, WES/WGS/NGS-based methods or Southern blotting.

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/SNV/other variation that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or SNVs are present in the probe-target sequence. The finding of a heterozygous mutation or SNVs indicates that two different alleles of the sequence are present in the sample DNA and that a false-positive MLPA result was obtained.

COSMIC mutation database

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

Please report false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 2. D007-A1 Acute Lymphoblastic Leukemia

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Gene or region length (kb)	Literature
1p33	*TAL1*	NM_003189.5	4/5	15 kb	***STIL-TAL1* fusion ¥ in 10-30% T-ALL** Cavé et al. 2004
1p33	*STIL*	NM_001048166.1	4/17	64 kb	
2q34	*IKZF2*	NM_001387220.1	3/9	151 kb	**Deletions of *IKZF2* in 50% of low-diploid ALL** Holmfeldt et al. 2013
3p14.2	*FHIT*	NM_002012.4	3/10	1.5 **M**b	**Deletions of *FHIT* in 4% childhood and 7% adult ALL** Okamoto et al. 2010
3q13.2	*CD200*	NM_005944.7	3/6	30 kb	***CD200/BTLA* deletions in 5% B-ALL** Ghazavi et al. 2015
3q13.2	*BTLA*	NM_181780.4	3/5	33 kb	
3q26.32	*TBL1XR1*	NM_024665.7	4/16	304 kb	**Deletions in 15% of *ETV6-RUNX1* positive ALL; associated with glucocorticoid therapy resistance** Parker et al. 2008, Jones et al. 2014
	LINC00501	NR_047465.1	2/2		*Included as flanking probe for TBL1XR1*
4q25	*LEF1*	NM_016269.5	5/12	119 kb	***LEF1* deletions in 11% of T-ALL** Gutierrez et al. 2010
4q31.23	*NR3C2*	NM_000901.5	3/9	364 kb	**Deletions of *NR3C1* and *NR3C2* are associated with relapsed ALL** Safavi et al. 2014, Kuster et al. 2011
5q extent of deletion - 5q31.2	*EGR1, CTNNA1*	-	1 probe for each gene	42 **M**b	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
5q31.3	*NR3C1*	NM_000176.3	5/9	169 kb	**Deletions of *NR3C1* and *NR3C2* are associated with relapsed ALL** Safavi et al. 2014, Kuster et al. 2011
5q32	*PDGFRB*	NM_002609.4	4/23	42 kb	***EBF1-PDGFRB* fusion ¥ in B-cell precursor ALL, sensitive to tyrosine kinase inhibitors** Lengline et al. 2013, Weston et al. 2013
5q extent of deletion – 5q33.1	*RPS14, SPARC*	-	1 probe for each gene	42 **M**b	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
5q33.3	*EBF1*	NM_024007.5	5/16	404 kb	***EBF1* deletions in 25% of relapsed ALL** Yang et al. 2008
5q extent of deletion – 5q35.3	*SQSTM1, FLT4*	-	1 probe for each gene	42 **M**b	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
6q15	*CASP8AP2*	NM_012115.4	4/10	42 kb	***CASP8AP2* deletions in 12% of T-ALL** Remke et al. 2009
6q23.3	*MYB*	NM_001130173.2	3/16	38 kb	**Gain in 8-15% of T-ALL** Clappier et al. 2007, O'Neil et al. 2007
7p12.2	*IKZF1*	NM_006060.6	16/8	100 kb	**Associated with *BCR-ABL1* fusion, poor prognostic factor** Boer et al. 2016, Mullighan et al. 2008a (note: different exon numbering)
	IKZF1 regulatory region	NM_006060.6	7 probes upstream of exon 1	30 kb	**Deletions of *IKZF1 exon* 1 and 5' untranscribed regulatory regions result in haploinsufficiency** Stanulla et al. 2018
		NM_001291845.2 / NM_001291846.2	2 probes in alternative exon 4	-	
7q34-q35	*EPHA1*	NM_005232.5	3/18	18 kb	***EPHA1* is thought to be an important factor in T-lymphocyte development** Charmsaz et al. 2013
7q36.1	*EZH2*	NM_004456.5	3/20	77 kb	**Deletions of *EZH2* in T-ALL** Ntziachristos et al. 2012, Zhang et al. 2012
8q12.1	*TOX*	NM_014729.3	3/9	314 kb	**Recurrent deletions of *TOX* in T-ALL** Mullighan et al. 2009a, Yu et al. 2015
9p24.1	*JAK2*	NM_004972.4	4/25	145 kb	**Deletions of *JAK2* reported in up to 7% of B-ALL cases** Bhandari et al. 2017, Salmoiraghi et al. 2013
9p21.3	*MLLT3*	NM_004529.4	4/11	281 kb	**Deletions of *MLLT3* in 26% of T-ALL** Mullighan et al. 2007
9p21.3	*MTAP*	NM_002451.4	3/8	64 kb	**Deletion of *MTAP* in 11% of B-ALL** Mirebeau et al. 2006, Bertin et al. 2003

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Gene or region length (kb)	Literature
9p21.3	*CDKN2A*	NM_000077.5 (p16INK4a) NM_058195.4 (P14ARF)	12/3	27 kb	***CDKN2A* and *CDKN2B* deletions in 35-50% of ALL** Sulong et al. 2009
		NM_001363763.2	1 probe in alternative exon 1	1 kb	
9p21.3	*CDKN2B*	NM_004936.4	3/2	6.4 kb	
9p13.2	*PAX5*	NM_016734.3	10/10	208 kb	**Large deletions of *PAX5*, may include *CDKN2A/2B*. Intragenic *PAX5* amplifications also reported.** Schwab et al. 2013, Schwab et al. 2017
	PAX5 flanking		1 probe upstream of *PAX5*	94 kb	**To determine the extent of the *PAX5* deletion.** Mullighan et al. 2007
			1 probe in *ZCCHC7*		
9q34.12	*ABL1*	NM_005157.6	3/11	52 kb	***NUP214-ABL1* fusion gene [¥] in 6% ALL** Craux et al. 2009
9q34.13	*NUP214*	NM_005085.4	3/36	109 kb	
9q34.3	*NOTCH1*	NM_017617.5	7/34	52 kb	**Activating intragenic deletions in *NOTCH1*** Haydu et al. 2012
10q23.31	*PTEN*	NM_000314.8	20/9	108 kb	**Deletions of *PTEN* in 9% of ALL cases; resistance to chemotherapy and early treatment failure** Zuurbier et al. 2012, Mendes et al. 2014
10q25.2	*ADD3*	NM_016824.5	3/15	128 kb	**Recurrent deletion of *ADD3* in B-ALL** Mullighan et al. 2009a, Olsson et al. 2014
11p15.4	*LMO1*	NM_002315.3	3/4	40 kb	**Oncogenic transcription factor, *LMO1* overexpressed in T-ALL** Oram et al. 2013
11p13	*LMO2*	NM_005574.4	4/6	11 kb	***RAG2-LMO2* fusion [¥] in 4% of pediatric T-ALL** Van Vlierberghe et al. 2006
11p13	*CD44*	NM_000610.4	1/18	93 kb	
11p13	*SLC1A2*	NM_004171.4	1/11	168 kb	
11p12	*RAG2*	NM_000536.4	2/2	6 kb	
12p13.2	*ETV6*	NM_001987.5	8/8	246 kb	**Rearrangements and fusions (e.g. in *ETV6-RUNX1* fusion); microdeletions at breakpoint** Ko et al. 2011
12q21.33	*BTG1*	NM_001731.3	5/2	6 kb	***BTG1* deletions extend to downstream area; associated with Down syndrome (DS-)ALL** Waanders et al. 2012
13q14.2	*RB1*	NM_000321.3	9/27	178 kb	**Deletions of *RB1* in ALL, especially associated with iAMP21** Schwab et al. 2013
	RB1 flanking		5' region: 1 probe, *ITM2B*	43 kb	
			3' region: 4 probes, *RCBTB2*, *CDADC1*, *DLEU2*, *DLEU1*	1.8 **M**b	
14q32.33	*IGHM*	NG_001019.6	2/6	4.5 kb	**Non-functional rearrangements/deletions in *BCR-ABL*-positive B-ALL** Trageser et al. 2009
15q14	*SPRED1*	NM_152594.3	3/7	104 kb	**Recurrent (focal) deletions of *SPRED1* in relapse cases** Olsson et al. 2014, Mullighan et al. 2008b
16p13.3	*CREBBP*	NM_004380.3	3/31	156 kb	**Deletions of *CREBBP* in 1-2% of relapsed ALL cases** Mullighan et al. 2011, Vicente et al. 2015
16q22.1	*CTCF*	NM_006565.4	3/12	77 kb	**Deletions of *CTCF* in 2-3% of T-ALL** Vicente et al. 2015
17p13.1	*TP53*	NM_000546.6	14/11	19 kb	**Single allele deletions of *TP53* or combined with mutated other allele in 2-14% of ALL cases** Agirre et al. 2003
17q11.2	*NF1*	NM_001042492.3	3/58	283 kb	**Deletions of *NF1* in 11% of T-ALL; poor response to induction therapy** Balgobind et al. 2008

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Gene or region length (kb)	Literature
17q11.2	*SUZ12*	NM_015355.4	3/16	64 kb	**Deletions of *SUZ12* in T-ALL** Zhang et al. 2012, Ntziachristos et al. 2012
17q12	*IKZF3*	NM_012481.5	3/8	107 kb	**Deletions of *IKZF3* especially in hypodiploid ALL** Mullighan et al. 2007, Holmfeldt et al. 2013
18p11.21	*PTPN2*	NM_002828.4	4/9	91 kb	**Deletions of *PTPN2* in 6% of T-ALL** Kleppe et al. 2010
21q11.2	*HSPA13*		1 probe for each gene	20 **M**b	**iAMP21, a region of amplification on chromosome 21, which at least contains *RUNX1*** Robinson et al. 2003, Moorman et al. 2007, Harrison et al. 2014, Koleilat et al. 2022
21q11.2	*SAMSN1*				
21q21.1	*MIR99A*				
21q21.1	*BTG3*				
21q21.1	*TMPRSS15*				
21q21.1	*NCAM2*				
21q21.2	*MIR155*				
21q21.3	*APP*				
21q21.3	*CYYR1*				
21q21.3	*ADAMTSS*				
21q21.3	*BACH1*				
21q22.11	*TIAM1*				
21q22.11	*OLIG2*				
21q22.11	*KCNE2*				
21q22.12	***RUNX1*** ±	NM_001754.5	6/9	1.1 **M**b	
21q22.13	*SIM2*		1 probe for each gene	1.2 **M**b	
21q22.13	*HLCS*				
21q22.13	*DYRK1A*				
21q22.13	*KCNJ6*				
21q22.2	***ERG*** ≠	NM_182918.4	12/10	118 kb	**Short intragenic deletions of *ERG* in BCP-ALL** Clappier et al. 2014
21q22.2	*ETS2*		1 probe for each gene	7.9 **M**b	**iAMP21, a region of amplification on chromosome 21, which at least contains RUNX1** Robinson et al. 2003, Moorman et al. 2007, Harrison et al. 2014, Koleilat et al. 2022
21q22.2	*PSMG1*				
21q22.3	*TMPRSS2*				
21q22.3	*RIPK4*				
21q22.3	*TFF1*				
21q22.3	*ITGB2*				
21q22.3	*SLC19A1*				
21q22.3	*COL6A2*				
21q22.3	*PRMT2*				
22q21.22	*VPREB1*	NM_007128.4	2/2	730 **b**p	**Focal deletions of VPREB1 in ~30% of B-ALL cases** Mangum et al. 2014
22q21.23	*IGLL1*		1/3	7.2 kb	
Xp22.33	*SHOX*	NM_000451.4	3/5	2.2 **M**b	**Frequent rearrangements in PAR1 region** Mullighan et al. 2009b, Russell et al. 2009
Xp22.33	*CRLF2*	NM_022148.4	6/8		
Xp22.33	*CSF2RA*	NM_172245.4	15/13		
Xp22.33	*IL3RA*	NM_002183.4	8/12		
Xp22.33	*P2RY8*	NM_178129.5	3/2		
Xp22.33	*AKAP17A*		1 probe for each gene		
Xp22.33	*ASMT*				
Xp22.33	*ZBED1*				
Xp22.33	*CD99*				
Xp22.33	Flanking probes for PAR1 region		1 probe for *GYG2*		
Yp11.2			1 probe for *SRY*		
Xp21.1	*DMD*	NM_004006.3	8/79	2.1 **M**b	**Recurrent deletion of DMD, present in relapse cases** Kawamata et al. 2008, Mullighan et al. 2008b
Xq26.2	*PHF6*	NM_001015877.2	4/11	55 kb	**Deletions of PHF6 in 3% of T-ALL** Van Vlierberghe et al. 2010

(a) NM sequence and MANE: The exon numbering and NM_ sequence used have been retrieved from the MANE project release version 1.0 on 10/2022. As changes to the MANE database can occur after release of this product description, exon numbering may not be up-to-date. Exon numbering used here may differ from literature. When a MANE transcript is not available, then the exon numbering is based on the NM or NG sequence.

Thicker lined boxes in the table indicate the three regions included in D007 Acute Lymphoblastic Leukemia: 5q region, iAMP21 region and PAR1 region.

¥ D007 Acute Lymphoblastic Leukemia can provide an *indication* for the presence of fusion genes. These should always be confirmed with other methods.
± This gene is highlighted within this region as historically three or more extra copies of RUNX1 on a single abnormal chromosome 21 (a total of five or more RUNX1 signals per cell) was used to define iAMP21 (Harrison et al. 2014). It is now known that changes in this region might be more complex (Koleilat et al. 2022).
≠ This gene is highlighted within this region as it is important within the iAMP21 region, but also has relevance in combination with other CNAs outside the context of the iAMP21 region.

More information on the location and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available at www.mrcholland.com.

Table 3. Karyotyping probes

Chromosomal position (hg38)	Gene
1p36.33	*TMEM240*
1p36.33	*CFAP74*
1p32.3	*ACOT11* §
1p32.2	*PLPP3* §
1p13.1	*SLC22A15* §
1p12	*SPAG17* §
1q21.3	*RPRD2* §
1q21.3	*NUP210L* §
1q31.3	*KCNT2* §
1q31.3	*DENND1B*
1q44	*ADSS* §
1q44	*DESI2*
2p25.3	*TMEM18*
2p25.3	*COLEC11* §
2p22.3	*SPAST* §
2p22.2	*VIT*
2q11.2	*FER1L5*
2q11.2	*CNNM3*
2q11.2	*NPAS2* §
2q24.3	*SCN1A*
2q31.1	*ABCB11* §
2q37.3	*CAPN10*
2q37.3	*KIF1A*
3p26.2	*TRNT1* §
3p26.1	*SUMF1* §
3p24.2	*NR1D2* §
3p24.1	*NEK10* §
3p21.3	*CNTN3* §
3p11.1	*HTR1F* §
3q11.2	*EPHA6* §
3q12.1	*CPOX* §
3q23	*XRN1* §
3q24	*SLC9A9* §
3q29	*OPA1* §
3q29	*ACAP2*
4p16.3	*LETM1*
4p16.3	*ADD1*
4p15.31	*KCNIP4*
4p15.2	*DHX15*
4p12	*COMMD8*
4p12	*NFXL1*
4q13.1	*TECRL*
4q13.2	*UGT2A1*
4q31.21	*INPP4B*
4q31.22	*ZNF827*
4q35.2	*CYP4V2*
4q35.2	*TRIML1*

Chromosomal position (hg38)	Gene
5p15.33	*IRX4*
5p15.31	*NSUN2* §
5p15.2	*DNAH5*
5p13.3	*DROSHA*
5p13.3	*NPR3* §
5q11.2	*ITGA2* §
5q11.2	*DHX29* §
5q11.2	*IL31RA* §
5q11.2	*MIER3*
5q23.2	*MEGF10* §
5q31.2	*MYOT* §
5q35.3	*COL23A1* §
5q35.3	*MAPK9* §
6p25.2	*SERPINB6*
6p25.2	*ECI2*
6p22.1	*ZFP57*
6p21.33	*ATAT1*
6p12.1	*RAB23*
6p11.2	*PRIM2*
6q13	*COL19A1*
6q13	*RIMS1*
6q22.31	*TBC1D32*
6q22.33	*LAMA2*
6q27	*SMOC2*
6q27	*ERMARD*
7p22.2	*SDK1* §
7p22.1	*RADIL*
7p15.3	*RAPGEF5* §
7p15.3	*STK31* §
7p12.3	*ADCY1* §
7p12.3	*ABCA13* §
7p11.2	*LANCL2*
7p11.2	*SUMF2*
7q11.21	*KCTD7* §
7q11.22	*GALNT17* §
7q31.1	*PNPLA8* §
7q31.1	*IFRD1* §
7q36.3	*RBM33* §
7q36.3	*DYNC2I1*
8p23.3	*FBXO25*
8p23.3	*CLN8*
8p21.3	*GFRA2*
8p21.2	*CDCA2*
8p12	*RBPMS*
8p12	*GSR*

Chromosomal position (hg38)	Gene
8q11.21	*SNTG1*
8q12.2	*CHD7*
8q21.3	*RMDN1*
8q21.3	*CPNE3*
8q24.3	*TSNARE1*
8q24.3	*SLC39A4*
9p24.3	*DOCK8*
9p24.3	*KANK1*
9p24.1	*GLDC*
9p22.3	*FREM1*
9p22.2	*BNC2* §
9p13.2	*FBXO10* §
9p13.2	*DCAF10* §
9q21.11	*PGM5*
9q21.12	*TRPM3* §
9q22.33	*ANKS6* §
9q31.1	*ALDOB* §
9q34.3	*GRIN1*
9q34.3	*EHMT1* §
10p15.3	*DIP2C*
10p15.2	*PFKP*
10p13	*NMT2*
10p13	*ITGA8*
10p11.21	*CUL2*
10p11.21	*ZNF25*
10q11.21	*MARCH8*
10q11.22	*ARHGAP22*
10q22.2	*KAT6B*
10q22.3	*LRMDA*
10q26.3	*INPP5A*
10q26.3	*KNDC1*
11p15.5	*RIC8A*
11p15.5	*DEAF1*
11p14.3	*ANO5* §
11p14.2	*SLC5A12* §
11p11.2	*LRP4* §
11p11.2	*NUP160* §
11q12.1	*SERPING1* §
11q12.3	*BEST1* §
11q14.3	*FAT3* §
11q22	*MTMR2* §
11q25	*NTM*
11q25	*NCAPD3* §
12p13.33	*WNK1* §
12p13.33	*CACNA2D4*
12p12.3	*PTPRO* §
12p12.3	*AEBP2* §
12p11.22	*FAR2* §
12p11.22	*TMTC1* §

Chromosomal position (hg38)	Gene
12q12	*KIF21A* §
12q12	*NELL2* §
12q23.1	*NEDD1* §
12q23.1	*SLC17A8* §
12q24.33	*GALNT9*
12q24.33	*PGAM5*
13q12.3	*KATNAL1* §
13q14.3	*VPS36* §
13q21.33	*KLHL1*
13q34	*ARHGEF7* §
13q34	*GRK1* §
14q11.2	*TEP1*
14q11.2	*CHD8*
14q22.1	*DDHD1*
14q22.2	*SAMD4A*
14q32.31	*DYNC1H1*
14q32.33	*COA8*
15q12	*ATP10A* §
15q13.1	*OCA2* §
15q22.2	*VPS13C* §
15q22.31	*USP3*
15q26.3	*CHSY1* §
15q26.3	*TM2D3*
16p13.3	*DECR2*
16p13.3	*IFT140* §
16p13.13	*TXNDC11* §
16p13.12	*CPPED1* §
16p11.2	*HIRIP3*
16p11.2	*ITGAL* §
16q11.2	*GPT2* §
16q12.1	*LONP2* §
16q22.1	*SLC12A4*
16q22.1	*DUS2* §
16q24.3	*ANKRD11* §
16q24.3	*GAS8*
17p13.3	*VPS53*
17p13.3	*NXN*
17p13.1	*PIK3R6*
17p13.1	*USP43*
17p11.2	*RAI1*
17p11.2	*ULK2*
17q11.2	*MYO18A*
17q11.2	*PSMD11*
17q22	*ANKFN1*
17q23.2	*MED13*
17q25.3	*CCDC57*
17q25.3	*CSNK1D*

Chromosomal position (hg38)	Gene
18p11.32	*CLUL1*
18p11.31	*LPIN2*
18p11.31	*MYOM1*
18p11.21	*GNAL*
18p11.21	*SPIRE1*
18p11.21	*LDLRAD4*
18p11.21	*FAM210A*
18q11.2	*RBBP8*
18q11.2	*NPC1*
18q21.1	*LOXHD1*
18q21.1	*LIPG*
18q23	*CTDP1*
18q23	*TXNL4A*
19p13.3	*PLPP2*
19p13.3	*CDC34*
19p13.2	*GCDH*
19p13.2	*STX10*
19p13.11	*JAK3*
19p13.11	*GMIP*
19q12	*POP4*
19q13.11	*SLC7A9*
19q13.31	*KCNN4*
19q13.32	*FKRP*
19q13.42	*DNAAF3* §
19q13.43	*SLC27A5*
20p13	*RSPO4*
20p13	*TGM6* §
20p12.3	*TRMT6* §
20p12.2	*PLCB4*
20p11.23	*RIN2*
20p11.21	*APMAP* §

Chromosomal position (hg38)	Gene
20q11.22	*ACSS2* §
20q11.22	*EDEM2* §
20q13.12	*SLC13A3* §
20q13.13	*STAU1*
20q13.33	*OSBPL2*
20q13.33	*UCKL1*
21q11.2	*RBM11*
21q22.11	*ITSN1*
21q22.3	*PDE9A*
21q22.3	*TRAPPC10*
21q22.3	*TSPEAR*
22q11.1	*GAB4* §
22q11.21	*CECR2* §
22q12.2	*ZMAT5* §
22q12.2	*SFI1* §
22q13.31	*TRMU* §
22q13.33	*BRD1* §
Xp22.31	*ANOS1*
Xp22.11	*ACOT9*
Xp11.22	*FGD1*
Xq11.1	*ARHGEF9*
Xq13.3	*ZDHHC15*
Xq22.1	*NXF3*
Xq28	*CLIC2*
Xq28	*TMLHE*
Yp11.2	*PCDH11Y*
Yp11.2	*TBL1Y*
Yq11.221	*USP9Y*
Yq11.223	*KDM5D*
Yq11.223	*RPS4Y2*

§ Used as reference probe for normalisation purposes in data analysis using the Coffalyser digitalMLPA *D007-A1 default product sheet*.

More information on the location and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available at www.mrcholland.com.

Table 4. Reference probe selection for low hypodiploidy and near haploidy

Reference probes for low hypodiploidy and near haploidy are selected from the total set of karyotyping probes (Table 3). Selection is based on chromosomes retained in these ALL subtypes (see section "Reference probes" on page 2). This information is also present in the Probe Information File (PIF).

Chromosome	Number of reference probes	Gene names
Retained in near haploidy and low hypodiploidy		
Chromosome 8	7	*CLN8, RBPMS, GSR, SNTG1, CHD7, RMDN1, CPNE3*
Chromosome 10	9	*DIP2C, NMT2, CUL2, ZNF25, MARCHF8, ARHGAP22, KAT6B, LRMDA, INPP5A*
Chromosome 14	6	*TEP1, CHD8, DDHD1, SAMD4A, DYNC1H1, COA8*
Chromosome 18	10	*LPIN2, GNAL, SPIRE1, LDLRAD4, FAM210A, RBBP8, NPC1, LOXHD1, LIPG, CTDP1*
Chromosome 21	4	*RBM11, ITSN1, PDE9A, TRAPPC10*
Chromosomes X and Y	30	*PAR1* region including *SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, AKAP17A, ZBED1, CD99*
Additionally retained in low hypodiploidy		
Chromosome 1	4	*PLPP3, SLC22A15, NUP210L, ADSS2*
Chromosome 5	4	*NSUN2, NPR3, IL31RA, COL23A1*
Chromosome 6	4	*ECI2, PRIM2, LAMA2, ERMARD*
Chromosome 11	4	*ANO5, BEST1, MTMR2, NCAPD3*
Chromosome 22	4	*GAB4, ZMAT5, SFI1, BRD1*

Table 5. Related SALSA® MLPA® probemixes

Related probemix	Coverage ±	Can be used for confirmation ^
P018 SHOX	Contains probes for PAR1 region genes *SHOX, CRLF2, CSF2RA, IL3RA, ASMT* and *ZBED1*.	Yes *
P034-P035 DMD	Contain probes for each exon of *DMD*.	Yes *
P047 RB1	Contains probes for each exon of *RB1*, except exon 15.	Yes
P056 TP53	Contains probes for each exon of *TP53*.	No
P081/P082 NF1	Contain probes for each exon of *NF1*.	Yes
P105 Glioma-2	Contains one probe for each exon of *CDKN2A* and *PTEN*. Also contains 9 probes for *TP53*.	No
P175 Tumour Gain	Contains probes for exons 1 and 12 of *ABL1*.	Yes
P202 IKZF1-ERG	Contains probes for each exon of *IKZF1* and *ERG*.	Yes
P225 PTEN	Contains at least two probes for each exon of *PTEN*.	No
P295 SPRED1	Contains at least two probes for each exon of *SPRED1*.	Yes *
P313 CREBBP	Contains probes for each exon of *CREBBP*.	Yes *
P323 CDK4-HMGA-MDM2	Contains probes on chromosomal arms 12p and 12q.	Yes
P327 iAMP21-ERG	Contains probes for the iAMP21 region, including probes for *RUNX1* and for each exon of *ERG*.	Yes
P329 CRLF2-CSF2RA-IL3RA	Contains probes for *SHOX*, *CRLF2*, *CSF2RA*, *IL3RA* and *P2RY8*.	Yes
P335 ALL-IKZF1	Contains probes for *EBF1*, *IKZF1*, *PAX5*, *ETV6*, *BTG1*, *RB1* and the PAR1 region.	Yes
P370 BRAF-IDH1-IDH2	Contains several probes for *MYB*.	Yes
P377 Hematologic Malignancies	Contains several probes for *IKZF1*, *RUNX1*, *RB1* and flanking probes *DLEU1* and *DLEU2*.	Yes
P383 T-ALL	Contains probes for *TAL1*, *STIL*, *LEF1*, *CASP8AP2*, *MYB*, *EZH2*, *MLLT3*, *ABL1*, *NUP214*, *LMO1/2*, *RAG2/CD44/SLC1A2*, *NF1*, *SUZ12*, *PTPN2* and *PHF6*.	Yes
P414 MDS	Contains seven probes for 5q31.2-q33.3 (including additional probes for *EGR1* and *SPARC*).	Yes
P419 CDKN2A/2B-CDK4	Contains multiple probes for each exon of *CDKN2A* and *CDKN2B*.	Yes
P437 Familial MDS-AML	Contains one probe for each exon of *RUNX1*.	Yes

Related probemix	Coverage ±	Can be used for confirmation ^
P474 CD274-PDCD1LG2-JAK2	Contains several probes for *JAK2*.	Yes
ME024 9p21 CDKN2A/2B region	Contains probes for each exon of *CDKN2A*, *CDKN2B* and *MTAP*.	Yes

± Only genes or chromosomal regions included in D007 Acute Lymphoblastic Leukemia are mentioned in this table. SALSA® MLPA® probemixes additionally contain probes for genes not mentioned in this table.
^ Probemixes can be used for confirmation when ligation sites are different between D007 Acute Lymphoblastic Leukemia probes and the probes in the corresponding SALSA® MLPA® probemixes. Of note, this statement concerns the majority of the probes in a SALSA® MLPA® probemix and does not mean that all probes have a different ligation site. For more information, please contact info@mrcholland.com.
* The reference probes included in this SALSA® MLPA® probemix have not been optimised for tumour samples. If the tumour samples of interest harbour multiple copy number alterations on the genomic locations of the reference probes, the normalisation can be compromised and reliable result interpretation will not be possible with this probemix.

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D007 Acute Lymphoblastic Leukemia history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 08 January 2024 (03) - Replaced "SALSA digitalMLPA" with "SALSA® digitalMLPA™" where applicable. - To be used with: section restructured and reagent kits (Cat No: DRK05-IL, DRK20-IL) added. - Barcode plate names and lot numbers updated. - Added sections: Ingredients, SDS note, Storage and handling, information on shelf life and safe disposal. - Updated links to our website to https://www.mrcholland.com throughout the document. - Various minor textual changes. Version A1-01 – 3 November 2022 (03) - Not applicable, new document.

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