Product Description
SALSA® digitalMLPA™ Probemix D007-B1 Acute Lymphoblastic Leukemia

To be used with the digitalMLPA General Protocol.

Version B1

Check the version of your product on the probemix label to ensure you are reading the appropriate product description. As compared to version A1, one probe has been removed, 16 probes have been replaced, and 194 new probes targeting additional regions and genes of (emerging) interest have been added. Additions include new probes for copy number detection of *DNMT3A*, *MEF2C*, *CDKN1B*, *KRAS*, *EPOR*, 6q15-q16.1 deletion region (incl *CASP8AP2*, *BACH2* and other genes), 13q12 microdeletion region (*CDX2, FLT3, PAN3, FLT1*), 17q21 deletion region (*UBTF*, *ATXN7L3*), 20q11.21 (dic(9;20) breakpoint, incl *DNMT3B*) and 22q11.22 microdeletion region (up and downstream of *VPREB1*). New probes are also included for *MEF2D* fusion indication including partners *BCL9*, *HNRNPH1*, *HNRNPUL1*, and *DAZAP1*and for *TCF3* fusion indication including partners *PBX1* and *HLF*. Details about the added and changed probes can be found in the probemix specific Probe Information File (PIF). For complete product history see page 23.

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-B1 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. 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 quality tests 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.

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 or gains in 73 genes and eight chromosomal regions, as specified in Table 2, which are associated with acute lymphoblastic leukemia (ALL).

ALL is the most common childhood cancer comprising 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). The genetic alterations in 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. The digitalMLPA technique is covered by US patent 6,955,901 and corresponding patents outside the US and digitalMLPA products are sold under a license of InVitae corporation on patent US 9,624,533. The purchase of this product includes a license on these patents to use only this amount of product solely for the purchaser's own use.

Probemix content
A total number of 951 probes is included in D007-B1 Acute Lymphoblastic Leukemia, this consists of:
- 524 target probes detecting copy number alterations involved in ALL. See the Probe Information File and Table 2 for more details.
- 250 karyotyping probes, covering all existing chromosome arms (at the middle, near the centromeres and near the telomeres). See Table 2 for all chromosomal regions and genes included. A set of these karyotyping probes are used as reference probes, as indicated in Table 3 and 4.
- More than 160 control probes and fragments: these include 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.

The total number of probes can be used to calculate the number of reactions that can be combined into one sequencer run. See chapter "Amplicon Quantification by Illumina Sequencers" in the digitalMLPA General Protocol or the calculator tool available at support.mrcholland.com.

Reference probes
The 95 selected reference probes as defined in the *D007-B1 default product sheet* are a subset of karyotyping probes 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), 84 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-B1 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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 reference probes.

Required specimens
Extracted DNA peripheral blood and/or bone marrow, free from impurities known to affect digitalMLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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, Benard-Slagter et al. 2017). We would advise to use tumour samples with at least 50% tumour cell content. Therefore, tumour samples should be evaluated by a pathologist before extraction of DNA.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each digitalMLPA experiment for data normalisation. 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 deviations to the indicated copy number alteration (CNA) findings might occur. Table 1 contains a list of positive control samples that have been tested with D007-B1 Acute Lymphoblastic Leukemia at MRC Holland.

Table 1. Positive samples from biobanks tested by MRC Holland

Coriell sample ID	Genomic aberration
NA00501	Heterozygous duplication of 4q25-q35.2, including *LEF1* and *NR3C2*
NA00526	Trisomy 13, including *RB1*
NA00782	Heterozygous duplication of 4q13.1-q31.21, including *LEF1* Heterozygous duplication of *CRLF2* on Xp22.33
NA00803	Heterozygous deletion of 1q22-q23.3, including *MEF2D* and *PBX1*
NA00981	Heterozygous duplication of 20p chromosomal arm
NA01221	Heterozygous duplication 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 duplication 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 duplication of 9p21.3-p24.3, including *JAK2*, *MLLT3*, *MTAP*, *CDKN2A* and *CDKN2B*
NA03184	Trisomy 15, including *SPRED1*
NA03226	Heterozygous duplication of 9p, including *JAK2*, *MLLT3*, *MTAP*, *CDKN2A*, *CDKN2B* and *PAX5 and ZCCHC7*
NA03330	Trisomy 13, including *CDX2*, *FLT3*, *PAN3*, *FLT1* and *RB1*
NA03384	Heterozygous duplication of 11p15.4-q25, including *LMO1*, *LMO2*, *CD44*, *SLC1A2* and *RAG2* Four copies of chromosome X, and mosaic loss of chromosome Y
NA03493	Heterozygous deletion of 5q35.3, including *HNRNHPH1* Heterozygous duplication of 6p25.3
NA03563	Heterozygous duplication of 3q13.2-q29, including *CD200*, *BTLA*, *TBL1XR1* and *LINC00501* Heterozygous deletion of 9p24.3
NA04371	Heterozygous duplication of 5q33.3-q35.3, including *EBF1* Heterozygous duplication of *CSF2RA* on Xp22.33
NA04626	Trisomy X, including the PAR1 region, *DMD* and *PHF6*
NG04823	Trisomy 21, including the iAMP21 region and *RUNX1* and *ERG*
NA06226	Heterozygous duplication of 16p13.3-p13.12, including *CREBBP*
NA06803	Heterozygous deletion of 11p12-p13, including *LMO2*, *CD44*, *SLC1A2* and *RAG2*
NA07081	Heterozygous duplication of 7p, including *IKZF1*
NA07106	Trisomy 22, including *VPREB1*
NA07412	Heterozygous deletion of 7q34-q36.3, including *EPHA1* and *EZH2*
NA07994	Heterozygous duplication of 6q23.3-q27, including *MYB* Heterozygous deletion of 10q26.3
NA08146	Heterozygous deletion of *VPREB1* on 22q11.22 Heterozygous duplication of *CRLF2* and *CSF2RA* on Xp22.33 Heterozygous deletion of 17p11.2

Coriell sample ID	Genomic aberration
NA08778	Heterozygous deletion of 3q11.2-q13.2, including *CD200* and *BTLA*
NA09216	Heterozygous deletion of 2p23.3, including *DNMT3A* Gain for *ASMT* on Xp22.33
NA10401	Trisomy 2, including *IKZF2*
NA10925	Heterozygous deletion of 7p12.2-p12.3, including *IKZF1*
NA10946	Heterozygous deletion of 6q14.3-q16.1, including *CASP8AP2*, *BACH2*, *MAP3K7*, *EPHA7*. Heterozygous duplication of *SWSAP1*, *EPOR*, *RGL3*, *GCDH* on 19p13.2 Ambiguous ratios (duplication) for *ATXN7L3* and *UBTF* on 17q21.31.
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*
NA13031	Heterozygous deletion of 17q22, including *HLF* Heterozygous duplication of *KCNE2* on 21q22.11 Ambiguous ratios (deletion) for *IGHM* on 14q32.33
NA13284	Heterozygous deletion of 22q13.31-q13.33
NA13410	Heterozygous duplication of 14q32.31-q32.33, including *IGHM* Homozygous duplication of *ASMT* on Xp22.33
NA13685	Heterozygous duplication of 9q34.12-q34.3, including *ABL1*, *NUP214* and *NOTCH1* Heterozygous deletion of *IGHM* on 14q32.33 Heterozygous deletion of *DYNC2I1* on 7q36.3
NA14523	Heterozygous deletion of Xp11.22-p22.33, including the PAR1 region and *DMD*
NA16362	Heterozygous duplication of 22q11.1-q11.21 Heterozygous deletion of 22q11.22, including *TOP3B*, *PRAMENP*, *IGLV8-61* and *IGLV4-60*) Heterozygous duplication of 22q13.31-q13.33
NA17941	Heterozygous duplication of 1q21.2-q44, including *BCL9*, *MEF2D* and *PBX1* Heterozygous deletion of 13q32.33, including *IGHM* Heterozygous deletion of Xq22.1-Xq28, including *PHF6*
NA20027	Heterozygous deletion of chromosome X Heterozygous deletion of *IGHM* on 14q32.33
NA20125	Heterozygous duplication 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

Performance characteristics

The expected number of chromosomal rearrangements that can be detected with this probemix strongly depends on the ALL patient (sub)group tested and on the population. The analytical sensitivity and specificity of digitalMLPA for the detection of deletions/gains in the target sequences covered is very high and can be considered >99%. Performance can be compromised by: impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples or a poor data normalisation procedure and other technical errors.

Data analysis

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

<u>Separate product sheet for near haploid or low hypodiploid samples</u>

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-B1 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 in high hyperdiploid samples. However, for ALL samples that would be classified as near haploid or low hypodiploid samples by conventional karyotyping or DNA indexing, loss of the majority of chromosomes would result in wrong baseline detection using the *default* 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-B1 *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-B1 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 or 0 (deletion), ≥ 3 (gain). The same results can be expected for the X-chromosome-specific probes in female samples. For the 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	Inter ratio	
	(pseudo)autosomal probes / X-chromosome-specific probes in female samples	X- and Y-chromosome-specific probes in male samples
Normal	0.85 < ratio < 1.15	0.60 < ratio < 1.40
Deletion *	ratio < 0.75	ratio < 0.40
Gain ±	ratio > 1.25	ratio > 1.60
Ambiguous copy number	All other values	All other values

* Ratios might indicate a (subclonal) biallelic deletion when autosomal inter ratios are ≤0.30.
± Ratios might indicate an amplification when inter ratios are ≥2.15.

Please note that these above mentioned inter ratios are affected both by percentage of tumour cells and by possible subclonality. In case of a deletion that is subclonal and/or a lower percentage of tumour cells, the inter ratio may be higher than expected. For example, a monoallelic deletion in a sample with 50% tumour cell content or a monoallelic deletion present in 50% of the tumour cells, will result in an inter ratio around 0.75. However, the same (ambiguous) inter ratio of 0.75 will also be found in a sample with a biallelic deletion and a tumour cell percentage of 25%, or a subclone harbouring a biallelic deletion comprising 25% of all tumour cells. The digitalMLPA technique cannot discriminate between these two scenarios.

More information on this can be found on our website.

General notes on digitalMLPA interpretation:

- Arranging probes according to chromosomal location facilitates interpretation of the results. Analysis of parental samples may be necessary for correct interpretation of complex results.

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe read count of several consecutive probes, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or an additional purification step (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. Sequence changes (e.g. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can also lead to false-positive results.
- 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 inactivation of that gene copy.
- Copy number changes detected by reference or flanking probes are unlikely to have any relation to the condition tested for.

D007-B1 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.
- The IGHM and IGLV locus undergo recombination during B cell maturation. Hence, final ratios of probes targeting these two loci may be affected by the population of B cell clones present in a sample, which can change with age, disease, or clonal expansion during infection.

Limitations of the procedure

- DNA indexing or karyotyping should be done to determine the ploidy of the sample. digitalMLPA cannot distinguish haploid or complete triploid or tetraploid samples (with a loss of gain of all chromosomes) as compared to diploid samples, as the Coffalyser software determines a baseline based on the reference probes in each individual sample. In addition, to select the correct Coffalyser product sheet to properly analyse hyperdiploid (*default* product sheet) and near-haploid or low-hypodiploid samples (*near haploid_low hypodiploid* product sheet), pre-existing knowledge is needed regarding the ploidy status.
- Other techniques (e.g. karyotyping and FISH) are more suitable to detect cases with masked hypodiploidy.
- 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.
- **Warning:** Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.

- 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, 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 of multiple consecutive probes detected with D007-B1 Acute Lymphoblastic Leukemia should be verified by another method when possible. MLPA probemixes are available for many genes and chromosomal regions in D007-B1 Acute Lymphoblastic Leukemia. The MLPA probemixes mentioned in section 'Related SALSA MLPA probemixes' in this product description contain probes with a different ligation site that can be used for initial confirmation of results. Alternatively, copy number changes can be confirmed by another independent technique such as long range PCR, qPCR, array CGH, FISH 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/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 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-B1 Acute Lymphoblastic Leukemia probe targets

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
1p33	*TAL1*	NM_001290403.2	4/5	***STIL-TAL1* fusion ¥ in 10-30% of T-ALL** Cavé et al. 2004, Patra et al. 2021
1p33	*STIL*	NM_001048166.1	4/17	
1q21.2	*BCL9** ¥	NM_004326.4	6/10	***MEF2D-BCL9* fusion¥ in 7% in B-ALL** Gu et al. 2016, Ohki et al. 2019, Suzuki et al. 2016
	*GJA5**	-	1/2	*Included as flanking probe for BCL9*
1q22	*MEF2D** ¥	NM_005920.4	9/12	***MEF2D* rearrangements ¥in up to 7% of B-ALL** Gu et al. 2016, Yuki et al. 2004
1q23.3	*PBX1** ¥	NM_002585.4	9/9	***TCF3-PBX1* fusion ¥ in 6% of B-ALL** Barbosa et al. 2018, Paulsson et al. 2007 Rowsey et al. 2019
2p23.3	*DNMT3A**	NM_022552.5	5/23	***DNMT3A* deletions in 1% of T-ALL** Bond et al. 2019
2q34	*IKZF2*	NM_001387220.1	3/9	**Deletions of *IKZF2* in 50% of low-diploid ALL** Holmfeldt et al. 2013
3p14.2	*FHIT* «	NM_002012.4	3/10	**Deletions of *FHIT* in 4% childhood and 7% adult ALL** Okamoto et al. 2010
3q13.2	*CD200* «	NM_005944.7	3/6	***CD200*/*BTLA* deletions in 5% B-ALL** Ghazavi et al. 2015
3q13.2	*BTLA*	NM_181780.4	3/5	
3q26.32	*TBL1XR1*	NM_024665.7	4/16	**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 probes for TBL1XR1*
4q25	*LEF1*	NM_016269.5	5/12	***LEF1* deletions in 11% of T-ALL** Gutierrez et al. 2010

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
4q31.23	*NR3C2*	NM_000901.5	3/9	**Deletions of *NR3C1* and *NR3C2* are associated with relapsed ALL** Safavi et al. 2014, Kuster et al. 2011
5q14.3	*RASA1**	-	1/25	*Included as flanking probe for MEF2C*
	*MEF2C**	NM_002397.5	5/11	***MEF2C* deletions in 10% of T-ALL** Homminga et al. 2011
5q extent of deletion - 5q31.2	*EGR1, CTNNA1*	-	1 probe for each gene	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
5q31.3	*NR3C1* «	NM_000176.3	5/9	**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	***EBF1-PDGFRB* fusion ¥ in B-cell precursor ALL, sensitive to tyrosine kinase inhibitors** Lengline et al. 2013, Weston et al. 2013
5q33.1	*RPS14, SPARC*	-	1 probe for each gene	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
5q33.3	*EBF1*¥	NM_024007.5	5/16	***EBF1* deletions in 25% of relapsed ALL** Yang et al. 2008
5q35.3	*HNRNPH1** ¥	NM_001257293.2	4/14	***MEF2D* fusion ¥ partner in B-ALL** Ohki et al. 2019
5q extent of deletion – 5q35.3	*SQSTM1, FLT4*	-	1 probe for each gene	**Recurrent 5q (terminal) deletions T-ALL (5q31.2-q35.3)** La Starza et al. 2016
6q extent of deletion - 6q14.3	*HTR1E**	-	1 probe for each gene	**6q15-q16.1 deletions in 10% of T-ALL** Cordas et al. 2018
6q15	*SLC35A1*, ORC3**			
6q15	*CASP8AP2*«	NM_001137667.2	5/11	***CASP8AP2* deletions in 12% of T-ALL** Remke et al. 2009
6q15	*BACH2**	NM_021813.4	3/9	**6q15-q16.1 deletions in 10% of T-ALL** Cordas et al. 2018
6q15	*MAP3K7**	NM_145331.3	5/17	
6q15	*CASC6**		1/4	
6q16.1	*EPHA7**	NM_004440.4	5/17	
6q extent of deletion - 6q16.1	*MANEA*, KLHL32*, MMS22L*, POU3F2**	-	1 probe for each gene	
6q23.3	*MYB*	NM_001130173.2	3/16	**Gain in 8-15% of T-ALL** Clappier et al. 2007, O'Neil et al. 2007
7p12.2	*IKZF1*	NM_006060.6	15/8	**Poor prognostic factor, alone or in combination with other CNAs** Boer et al. 2016, Mullighan et al. 2008a (note: different exon numbering), Stanulla et al. 2018, Lopes et al. 2023 Specific **intragenic deletions might lead to different clinical outcomes.** Chang et al. 2024
		NM_001291837.2	1 probe in alternative exon 1	
		NM_001291845.2 / NM_001291846.2	2 probes in alternative exon 4	
	IKZF1 regulatory region	NM_006060.6 / NM_001410879.1	7 probes upstream of exon 1	**Deletions of *IKZF1 exon* 1 and 5' untranscribed regulatory regions result in haploinsufficiency** Stanulla et al. 2020
7q34-q35	*EPHA1*	NM_005232.5	3/18	***EPHA1* is thought to be an important factor in T-lymphocyte development** Charmsaz et al. 2013
7q36.1	*EZH2*	NM_004456.5	3/20	**Deletions of *EZH2* in T-ALL** Ntziachristos et al. 2012, Zhang et al. 2012
8q12.1	*TOX*	NM_014729.3	3/9	**Recurrent deletions of *TOX* in T-ALL** Mullighan et al. 2009a, Yu et al. 2015

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
9p24.1	*JAK2*	NM_004972.4	8/25	**Deletions of *JAK2* reported in up to 7% of (high-risk) B-ALL cases** Bhandari et al. 2017, Salmoiraghi et al. 2013, Roberts et al. 2012
9p21.3	*MLLT3*	NM_004529.4	4/11	**Deletions of *MLLT3* in 26% of T-ALL** Mullighan et al. 2007
9p21.3	*MTAP*	NM_002451.4	3/8	**Deletion of *MTAP* in 11% of B-ALL** Mirebeau et al. 2006, Bertin et al. 2003
9p21.3	*CDKN2A*	NM_000077.5 (p16INK4a) NM_058195.4 (P14ARF)	10/3	***CDKN2A* and *CDKN2B* deletions in 35-50% of ALL** Sulong et al. 2009 **Included in *IKZF1*[plus] profile – frequently co-deleted in ALL, unfavorable prognosis** Stanulla et al. 2018
		NM_001195132.2 (p16gamma)	2 probes in exon 3 of alternative transcript	
		NM_001363763.2 (isoform 6)	1 probe in alternative exon 1	
9p21.3	*CDKN2B*	NM_004936.4	3/2	
9p13.2	*PAX5* «	NM_016734.3	11/10	**Large deletions of *PAX5*, may include *CDKN2A/2B*. PAX5alt, including Intragenic *PAX5* amplifications is associated with a high incidence of relapse .** Schwab et al. 2013, Schwab et al. 2017, Chang et al. 2024 **Included in *IKZF1*[plus] profile – frequently co-deleted in ALL, associated with poor prognosis** Stanulla et al. 2018
	PAX5 flanking	NM_016734.3	1 probe upstream of *PAX5*	**To determine the extent of the *PAX5* deletion.** Mullighan et al. 2007
		NM_032226.3	2 probes in *ZCCHC7* «	
9q34.12	*ABL1* ¥	NM_005157.6	5/11	***NUP214-ABL1* fusion gene ¥ in 6-8% ALL** Craux et al. 2009, Roberts et al. 2012, Patra et al. 2021
9q34.13	*NUP214* ¥	NM_005085.4	5/36	
9q34.3	*NOTCH1*	NM_017617.5	7/34	**Activating intragenic deletions in *NOTCH1*** Haydu et al. 2012
10q23.31	*PTEN*	NM_000314.8	20/9	**Deletions of *PTEN* in 9% of ALL cases; resistance to chemotherapy and early treatment failure** Zuurbier et al. 2012, Mendes et al. 2014
10q25.1-q25.2	*ADD3*	NM_016824.5	3/15	**Recurrent deletion of *ADD3* in B-ALL** Mullighan et al. 2009a, Olsson et al. 2014
11p15.4	*LMO1*	NM_002315.3	3/4	**Oncogenic transcription factor, *LMO1* overexpressed in T-ALL** Oram et al. 2013
11p13	*LMO2* ¥	NM_005574.4	4/6	***RAG2-LMO2* fusion ¥ in 4% of pediatric T-ALL** Van Vlierberghe et al. 2006
11p13	*CD44*	NM_000610.4	1/18	
11p13	*SLC1A2*	NM_004171.4	1/11	
11p12	*RAG2* « ¥	NM_000536.4	2/2	
12p13.2	*ETV6*	NM_001987.5	8/8	**Rearrangements and fusions (e.g. in *ETV6-RUNX1* fusion); microdeletions at breakpoint** Ko et al. 2011
12p13.1	*CDKN1B**	NM_004064.5	3/3	**Deletions of *CDKN1B* in 12% of T-all** Colomer-Lahiguera et al. 2017
12p12.1	*KRAS**	NM_004985.5	5/5	**Focal deletions of *KRAS* in 6% of adult Philadelphia chromosome-positive ALL** Fedullo et al. 2019
12q21.33	*BTG1*	NM_001731.3	2/2	

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
		-	3 probes downstream of BTG1	***BTG1* deletions extend to downstream area; associated with Down syndrome (DS-)ALL** Waanders et al. 2012
13q12.2	*CDX2**	NM_001265.6	2/3	**13q12.2-q12.3 deletions in up to 2% of B-ALL** Yang et al. 2020
	*URAD**	-	1/2	
	*FLT3**	NM_004119.3	8/24	
	*PAN3**	NM_175854.8	16/19	
	*PAN3-AS1**	NR_029383.1	1/2	
13q12.3	*FLT1**	NM_002019.4	4/30	
13q14.2	*RB1*	NM_000321.3	9/27	**Deletions of *RB1* in ALL, especially associated with iAMP21** Schwab et al. 2013
	RB1 flanking	-	5' region: 1 probe, *ITM2B* 3' region: 4 probes, *RCBTB2*, *CDADC1*, *DLEU2*, *DLEU1*	
14q32.33	*IGHM*	NG_001019.6	2/6	**Non-functional rearrangements/deletions in *BCR-ABL*-positive B-ALL** Trageser et al. 2009, Chen et al. 2016
15q14	*SPRED1*	NM_152594.3	3/7	**Recurrent (focal) deletions of *SPRED1* in relapse cases** Olsson et al. 2014, Mullighan et al. 2008b
16p13.3	*CREBBP*	NM_004380.3	3/31	**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	**Deletions of *CTCF* in 2-3% of T-ALL** Vicente et al. 2015
17p13.1	*TP53*	NM_000546.6	13/11 1 probe downstream of TP53	**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	**Deletions of *NF1* in 11% of T-ALL; poor response to induction therapy** Balgobind et al. 2008
17q11.2	*SUZ12*	NM_015355.4	3/16	**Deletions of *SUZ12* in T-ALL** Zhang et al. 2012, Ntziachristos et al. 2012
17q12	*IKZF3*	NM_012481.5	3/8	**Deletions of *IKZF3* especially in hypodiploid ALL** Mullighan et al. 2007, Holmfeldt et al. 2013
17q21.31	*ATXN7L3** ¥	NM_001382309.1	4/13	**17q21 deletions in up to 9% of B-ALL cases, involving UBTF-ATXN7L3 fusions ¥** Kimura et al. 2022, Fioretos et al. 2022, Passet et al. 2022
	*UBTF** ¥	NM_0142233.4	11/21	
17q22	*HLF** ¥	NM_002126.5	4/4	**(Rare) *TCF3-HLF* fusion ¥ described in B-ALL** Panagopoulos et al. 2012, Lejman et al. 2020
18p11.21	*PTPN2*	NM_002828.4	4/9	**Deletions of *PTPN2* in 6% of T-ALL** Kleppe et al. 2010
19p13.3	*DAZAP1** ¥	NM_018959.4	6/12	***MEF2D-DAZAP1* fusion ¥ in B-ALL** Gu et al. 2016, Yuki et al. 2004
19p13.3	*TCF3** ¥	NM_003200.5	7/19	***(Rare) TCF3-HLF* fusion ¥ in B-ALL** Panagopoulos et al. 2012, Lejman et al. 2020
19p13.2	*SWSAP1**		1/2	*Included as flanking probe for EPOR*

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
	*EPOR**	NM_000121.4	8/8	**Rearrangements involving *EPOR* and *IGH* in B-ALL** Iacobucci et al. 2016, Roberts et al. 2012, Zur Stadt et al. 2019
	*RGL3**		1/19	*Included as flanking probe for EPOR*
19q13.2	*HNRNPUL1** ¥	NM_007040.6	6/15	**Fusion partner of *MEF2D* ¥ in B-ALL** Gu et al. 2016, Ohki et al. 2019
20q11.21	*BCL2L1**	NM_138578.3	2/3	**20q11.21 (dic(9;20) breakpoint)** An et al. 2008, An et al. 2009, Antic et al. 2023
	*ASXL1**		1/13	
	*DNMT3B**	NM_006892.4	2/23	
	*SUN5**		1/13	
21q11.2	*HSPA13*	-	1 probe for each gene	**iAMP21, a region of amplification on chromosome 21, which contains at least *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*			
21q21.3	*MIR155*			
21q21.3	*APP*			
21q21.3	*CYYR1*			
21q21.3	*ADAMTS5*			
21q21.3	*BACH1*			
21q22.11	*TIAM1*			
21q22.11	*OLIG2*			
21q22.11	*KCNE2*			
21q22.12	***RUNX1*** ±	NM_001754.5	6/9	
21q22.13	*SIM2*	-	1 probe for each gene	
21q22.13	*HLCS*			
21q22.13	*DYRK1A*			
21q22.13	*KCNJ6*			
21q22.2	***ERG*** ≠	NM_182918.4	12/10	**Short intragenic deletions of *ERG* in BCP-ALL** Clappier et al. 2014 ***IKZF1*** **plus profile (unfavorable prognosis): absence of *ERG* deletions** Stanulla et al. 2018
21q22.2	*ETS2*	-	1 probe for each gene	**iAMP21, a region of amplification on chromosome 21, which contains at least *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* «			
22q11.22	*TOP3B**	-	1/18	**22q11.22 microdeletion region in 40% of B-ALL** Mangum et al. 2021
	*PRAMENP**	NR_135291.1	2/7	

Chromosomal position (hg38)	Gene	NM sequence [a]	# probes / # exons in gene	Remarks and literature
	IGLV8-61, IGLV4-60**	-	4 probes in or next to these genes	
	VPREB1	NM_007128.4	2/2	**Focal deletions of *VPREB1* in ~30% of B-ALL cases** Mangum et al. 2014, Chen et al. 2016
	BMS1P20, IGLV7-43**	-	2 probes in or around these genes	
22q11.23	*IGLL1*	-	1/3	*Included as flanking probe for 22q11.22 microdeletion region*
Xp22.33	*SHOX*	NM_000451.4	5/5	**Frequent rearrangements in PAR1 region** Mullighan et al. 2009b, Russell et al. 2009 **Included in *IKZF1*^{plus} profile – frequently co-deleted in ALL, unfavorable prognosis** Stanulla et al. 2018
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-p21.2	*DMD*	NM_004006.3	7/79	**Recurrent deletion of *DMD*, present in relapse cases** Kawamata et al. 2008, Mullighan et al. 2008b
Xq26.2	*PHF6* «	NM_001015877.2	4/11	**Deletions of *PHF6* in 3% of T-ALL** Van Vlierberghe et al. 2010

(a) NM sequence and MANE: **We have adopted the MANE exon numbering. Please be aware that the MANE and LRG exon numbering does not always correspond.** When MANE is not available, then the exon numbering is based on the NM or NG sequence. The exon numbering and NM_ sequence used have been retrieved on 09/2024. 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.

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

* New in version B1.
« Changed in version B1.
¥ 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.

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	*ADSS2* §
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* §
3p12.3	*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	*MARCHF8*
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* §
11q21	*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* *
15q26.1	*BLM* *
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-B1 default* product sheet. A different selection of reference probes is present in the *D007-B1 near haploid_low hypodiploid* product sheet (see Table 4).
* New in version B1.
Note: No karyotyping probes are present in the acrocentric chromosome p arms.

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 near haploidy and low hypodiploidy

Chromosome	Number of reference probes	Gene names
Retained in near haploidy and low hypodiploidy		
Chromosome 8	6	*CLN8, RBPMS, GSR, SNTG1, 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*
Chromosome X and Y	30	*PAR1* region including *SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, AKAP17A, ZBED1, CD99*
Additionally retained in 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	3	*GAB4, ZMAT5, SFI1*

Table 5. Related SALSA® MLPA® probemixes

Related probemix	Gene	Coverage ±	Can be used for confirmation ^
P047 RB1	*RB1*	Contains probes for each exon of *RB1*, except for exon 15.	Yes
P056 TP53	*TP53*	Contains probes for each exon of *TP53*.	No
P081/P082 NF1	*NF1*	Contain probes for each exon of *NF1*.	Yes
P105 Glioma	*CDKN2A, PTEN, TP53*	Contains one probe for each exon of *CDKN2A* and *PTEN*. Also contains 9 probes for *TP53*.	No
P175 Tumour Gain	*ABL1*	Contains probes for exons 1 and 12 of *ABL1*.	Yes
P202 IKZF1-ERG	*IKZF, ERG*	Contains probes for each exon of *IKZF1* and *ERG*.	Yes
P225 PTEN	*PTEN*	Contains at least two probes for each exon of *PTEN*.	No
P298 BRAF-HRAS-KRAS-NRAS	*BRAF, HRAS, KRAS, NRAS*	Contains 9 probes targeting *KRAS*.	Yes
P323 CDK4-HMGA-MDM2	*CDK4, HMGA, MDM2*	Contains probes on chromosomal arms 12p and 12q.	Yes
P327 iAMP21-ERG	*iAMP21, ERG*	Contains probes for the iAMP21 region, including probes for *RUNX1* and for each exon of *ERG*.	Yes
P329 CRLF2-CSF2RA-IL3RA	*CRLF2, CSF2RA, IL3RA*	Contains probes for *SHOX*, *CRLF2*, *CSF2RA*, *IL3RA* and *P2RY8*.	Yes
P335 ALL-IKZF1	*IKZF1*	Contains probes for *EBF1*, *IKZF1*, *PAX5*, *ETV6*, *BTG1*, *RB1* and the PAR1 region.	Yes
P370 BRAF-IDH1-IDH2	*MYB*	Contains several probes for *MYB*.	Yes
P377 Hematologic Malignancies	*IKZF1, RUNX1, RB1*	Contains several probes for *IKZF1*, *RUNX1*, *RB1* and flanking probes *DLEU1* and *DLEU2*.	Yes
P383 T-ALL	Multiple	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	*EGR1, SPARC*	Contains seven probes for 5q31.2-q33.3 (including additional probes for *EGR1* and *SPARC*).	Yes
P419 CDKN2A/2B-CDK4	*CDKN2A, CDKN2B*	Contains multiple probes for each exon of *CDKN2A* and *CDKN2B*.	Yes
P437 Familial MDS-AML	*RUNX1*	Contains one probe for each exon of *RUNX1*.	Yes
P474 CD274-PDCD1LG2-JAK2	*JAK2*	Contains several probes for *JAK2*.	Yes
ME024 9p21 CDKN2A/2B region	*CDKN2A, CDKN2B, MTAP*	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 most ligation sites are different between D007-B1 Acute Lymphoblastic Leukemia probes and the probes in the corresponding probemixes. Of note, this statement concerns the majority of the probes in

a probemix and does not mean that all probes always have a different ligation site. For more information, please contact info@mrcholland.com.

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D007 Acute Lymphoblastic Leukemia product history	
Version	*Modification*
A1	First release.
B1	One target probe removed, 16 probes replaced, and 194 new probes have been added targeting regions and genes of (emerging) interest.

Implemented changes in the product description
Version B1-02 – 24 Januari 2025 (04) - Added inter ratio values for interpretation of X- and Y-chromosome-specific probe results in 'Interpretation of results' section on page 6. Version B1-01 – 08 November 2024 (04) - Product description adapted to a new product version. - Tables in this product description updated with newest information. - New cut off values for deletions and gains introduced on page 6. Please note that these borders are different than those in the D007-A1 product description. - New positive samples added to Table 1.

- New references added on pages 18-23.
- Various minor textual changes.

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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