# Product Description
# SALSA® MLPA® Probemix P327-B2 iAMP21-ERG

To be used with the MLPA General Protocol.

**Version B2**
For complete product history see page 10.

**Catalogue numbers:**
- **P327-025R:** SALSA MLPA Probemix P327 iAMP21-ERG, 25 reactions.
- **P327-050R:** SALSA MLPA Probemix P327 iAMP21-ERG, 50 reactions.
- **P327-100R:** SALSA MLPA Probemix P327 iAMP21-ERG, 100 reactions.

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

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

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

**General information**
The SALSA MLPA Probemix P327 iAMP21-ERG is a **research use only (RUO)** assay for the detection of deletions, duplications or amplifications of specific sequences on chromosome 21, which are associated with acute lymphoblastic leukemia (ALL), myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML).

ALL is a heterogeneous disease at the cytogenetic level. This can be utilized in defining clinically significant genetic subgroups to aid in risk stratification and in the treatment selection of ALL. Intrachromosomal amplification of chromosome 21 (iAMP21) is a recurrent chromosomal abnormality detected in ~2% of childhood B-lineage ALL patients (Harrison 2015). iAMP21 is more common in older children and adolescents with ALL (Soulier et al. 2003). Although the level and extent of the amplified region on chromosome 21 seems to vary between ALL patients, the common region of amplification contains the *RUNX1 (AML1)* gene, at 21q22.12. Clinical studies have shown that ALL patients with iAMP21 have an increased risk of relapse and significantly inferior survival compared to patients without this genetic change (Robinson et al. 2003; Moorman et al. 2010; Harrison 2015). Thereby ALL patients with iAMP21 are suggested to be treated with a more intense regimen (Moorman et al. 2013).

Similar chromosome 21 amplifications have also been reported in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), and are suggested to associate with poor survival (Marcucci et al. 2005; Thol et al. 2012; Weber et al. 2016).

Short intragenic deletions of *ERG*, at 21q22.12, are also frequently described in B-cell precursor (BCP) ALL patients. BCP-ALL patients with intragenic *ERG* deletion are suggested to have a favourable clinical outcome and, moreover, *ERG* deletion is suggested to define a subgroup of superior outcomes among patients with *IKZF1* Δ4-7 deletions (Clappier et al. 2014; Zaliova et al. 2014).

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

**Gene structure and transcript variants:**
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/ and http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

**Exon numbering**
The *RUNX1* and *EGR* exon numbering used in this P327-B2 iAMP21-ERG product description is the exon numbering from the MANE sequence. The *ERG* exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets in Table 2. **From description version 04 onwards, we have adopted the MANE exon numbering. Please be aware that the MANE and LRG exon numbering do not always correspond, and MANE exon numbering used here may differ from literature.** As changes to the databases can occur after the release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

**Probemix content**
The SALSA MLPA Probemix P327-B2 iAMP21-ERG contains 59 MLPA probes with amplification products between 126 and 504 nucleotides (nt). This includes 13 probes for the *ERG* gene, covering each exon of transcript variants 1-6 and 8, and six probes specific for the *RUNX1* gene. Several other probes are located in the regions 21q11.2-q22.3 that are also frequently duplicated, amplified or deleted in iAMP21 cases. In addition, 13 reference probes are included, detecting chromosomal regions that are relatively copy number stable in ALL. Partial probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

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

**MLPA technique**
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

**MLPA technique validation**
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

**Required specimens**
Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

**Reference samples**

A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated healthy individuals without a history of ALL. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

**Positive control DNA samples**

MRC Holland cannot provide positive DNA samples. The inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Samples with ID numbers described in the table below from the Coriell Institute have been tested with this P327-B2 probemix at MRC Holland and can be used as positive control samples to detect the below-mentioned copy number alterations (CNA). The quality of cell lines can change; therefore samples should be validated before use.

| Sample name | Source | Chromosomal position of CNA* | Altered target genes in P327-B2 | Expected CNA |
|---|---|---|---|---|
| NA00692 | Coriell Institute | 21q11.2-q21.3 | *HSPA13, SAMSN1, MIR99A, BTG3, TMPRSS15, NCAM2, MIR155* and *APP* | Heterozygous deletion |
| NA02571 | Coriell Institute | 21q11.2-q22.3 | *All genes on chromosome 21* | Trisomy 21 |
| NA03503 | Coriell Institute | 21q11.2-q21.1 | *HSPA13, SAMSN1, MIR99A, BTG3, TMPRSS15,* and *NCAM2* | Heterozygous duplication |
| NA05881 | Coriell Institute | 21q11.2-q22.11 | *HSPA13, SAMSN1, MIR99A, BTG3, TMPRSS15, NCAM2, MIR155, APP, CYYR1, ADAMTS5, BACH1* and *TIAM1* | Heterozygous duplication |
| | | 21q22.3 | *PRMT2* | Heterozygous duplication |
| NA08331 | Coriell Institute | 21q21.3 | *APP, CYRR1* and *ADAMTS5* | Heterozygous deletion |
| NA09868 | Coriell Institute | 21q22.13-q22.3 | *SIM2, HLCS, DYRK1A, KCNJ6, ERG, ETS2, PSMG1, TMPRSS2, RIPK4, TFF1, ITGB2, SLC19A1, COL6A2* and *PRMT2* | Heterozygous deletion |
| NA13031 | Coriell Institute | 21q22.11 | *KCNE2* | Heterozygous duplication |

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by this P327-B2 iAMP21-ERG probemix.

**Data analysis**

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

**Interpretation of results**

The standard deviation of each individual reference probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

| Copy number status | Final ratio (FR) |
|---|---|
| Normal | 0.80 < FR < 1.20 |
| Homozygous deletion | FR = 0 |
| Heterozygous deletion | 0.40 < FR < 0.65 |
| Heterozygous duplication/gain | 1.30 < FR < 1.65 |
| Heterozygous triplication/homozygous duplication/gain | 1.75 < FR < 2.15 |

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

**Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.**

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *RUNX1* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

**P327 specific note**

In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples. When regions targeted by reference probes are affected by CNAs, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

**Limitations of the procedure**

- In most populations, the majority of genetic alterations in chromosome 21 are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P327 iAMP21-ERG.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the

probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a CNA in a patient sample, especially in samples with more chaotic karyotypes.

**Confirmation of results**
Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long-range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

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

Please report false positive results due to SNVs and unusual results (e.g., a deletion of *ERG* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P327-B2 iAMP21-ERG

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18) | | Location (hg18) in kb |
|---|---|---|---|---|
| | | Reference | Target region | |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 126 | Reference probe 18709-L21698 | 5q31 | | 05-132,038 |
| 130 ¥ | Reference probe 20879-L29296 | 12q24 | | 12-116,137 |
| 136 ◊ | **TMPRSS15** probe 19012-L25527 | | 21q21.1 | 21-018,647 |
| 142 | Reference probe 16543-L25049 | 11q13 | | 11-070,536 |
| 147 « | **SIM2** probe 00815-L24793 | | 21q22.13 | 21-037,044 |
| 152 | **OLIG2** probe 12827-L25094 | | 21q22.11 | 21-033,321 |
| 157 | Reference probe 12416-L24822 | 22q12 | | 22-032,042 |
| 161 | **RIPK4** probe 12741-L25051 | | 21q22.3 | 21-042,050 |
| 167 ¥ | **RUNX1** probe 20384-L17531 | | 21q22.12 | 21-035,343 |
| 171 | **SAMSN1** probe 18163-L22868 | | 21q11.2 | 21-014,811 |
| 177 | **ERG** probe 18151-L22661 | | 21q22.2 | 21-038,955 |
| 184 | **ERG** probe 18152-L22662 | | 21q22.2 | 21-038,677 |
| 190 | **CYYR1** probe 19013-L25052 | | 21q21.3 | 21-026,775 |
| 195 ¥ | **ITGB2** probe 20880-L14405 | | 21q22.3 | 21-045,146 |
| 202 | **APP** probe 19010-L25053 | | 21q21.3 | 21-026,434 |
| 207 | Reference probe 09882-L10295 | 16p13 | | 16-003,801 |
| 214 | **ERG** probe 18153-L22663 | | 21q22.2 | 21-038,869 |
| 220 | **DYRK1A** probe 03791-L22920 | | 21q22.13 | 21-037,714 |
| 226 | **ERG** probe 18154-L22664 | | 21q22.2 | 21-038,697 |
| 232 | **HLCS** probe 03794-L14698 | | 21q22.13 | 21-037,191 |
| 238 | **RUNX1** probe 19014-L24827 | | 21q22.12 | 21-035,181 |
| 243 | **KCNJ6** probe 19134-L25333 | | 21q22.13 | 21-037,920 |
| 249 | Reference probe 06712-L25773 | 15q24 | | 15-070,436 |
| 254 | **ERG** probe 18155-L22921 | | 21q22.2 | 21-038,686 |
| 260 | **ADAMTS5** probe 07592-L14699 | | 21q21.3 | 21-027,229 |
| 265 | **BACH1** probe 19015-L25096 | | 21q21.3 | 21-029,624 |
| 272 | **ETS2** probe 09516-L25095 | | 21q22.2 | 21-039,115 |
| 277 | Reference probe 13796-L24815 | 3q25 | | 03-157,716 |
| 284 | **KCNE2** probe 18162-L22923 | | 21q22.11 | 21-034,665 |
| 292 « | **RUNX1** probe 02840-L02271 | | 21q22.12 | 21-035,094 |
| 299 ¥ | **ERG** probe 20883-L22635 | | 21q22.2 | 21-038,717 |
| 307 | **TFF1** probe 19016-L24829 | | 21q22.3 | 21-042,656 |
| 314 | Reference probe 12925-L25054 | 2p23 | | 02-031,604 |
| 320 « | **RUNX1** probe 19017-L25055 | | 21q22.12 | 21-035,086 |
| 328 | **ERG** probe 02833-L02262 | | 21q22.2 | 21-038,739 |
| 335 | **TMPRSS2** probe 19011-L22003 | | 21q22.3 | 21-041,782 |
| 342 | Reference probe 09073-L24818 | 19p13 | | 19-013,235 |
| 348 | **MIR155** probe 19018-L25059 | | 21q21.3 | 21-025,868 |
| 355 | **ERG** probe 18156-L22666 | | 21q22.2 | 21-038,792 |
| 364 ¥ | **ERG** probe 18157-L25525 | | 21q22.2 | 21-038,696 |
| 369 | Reference probe 16279-L25100 | 20q11 | | 20-034,993 |
| 378 | **MIR99A** probe 19135-L25379 | | 21q21.1 | 21-016,833 |
| 386 | **ERG** probe 18158-L25097 | | 21q22.2 | 21-038,685 |
| 393 | **RUNX1** probe 19019-L24832 | | 21q22.12 | 21-035,129 |
| 400 ¥ | **SLC19A1** probe 12745-L29350 | | 21q22.3 | 21-045,760 |
| 407 | **NCAM2** probe 19020-L24833 | | 21q21.1 | 21-021,580 |
| 415 | Reference probe 00963-L22682 | 2p16 | | 02-055,068 |
| 422 | **BTG3** probe 18164-L23401 | | 21q21.1 | 21-017,899 |
| 429 * | **TIAM1** probe 21297-L29691 | | 21q22.11 | 21-031,435 |
| 437 | **RUNX1** probe 02838-L24817 | | 21q22.12 | 21-035,154 |
| 445 ¥ ◊ | **HSPA13** probe 05916-L29504 | | 21q11.2 | 21-014,668 |

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18) | | Location (hg18) in kb |
|---|---|---|---|---|
| | | Reference | Target region | |
| 454 | Reference probe 17129-L25056 | 11p11 | | 11-047,311 |
| 461 | **COL6A2** probe 12727-L24814 | | 21q22.3 | 21-046,356 |
| 468 | **PRMT2** probe 02586-L25057 | | 21q22.3 | 21-046,888 |
| 475 | **ERG** probe 18159-L22669 | | 21q22.2 | 21-038,685 |
| 481 | **ERG** probe 18160-L22670 | | 21q22.2 | 21-038,879 |
| 490 | **PSMG1** probe 19137-L25693 | | 21q22.2 | 21-039,471 |
| 497 | **ERG** probe 19022-L25058 | | 21q22.2 | 21-038,694 |
| 504 | Reference probe 15203-L22928 | 3p12 | | 03-081,775 |

* New in version B2.

¥ Changed in version B2. Minor alteration, no change in sequence detected.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

◊ The name of the gene targeted by this probe was changed. The previous name can be found between parentheses in Table 2.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

## Table 2. P327 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene (exon)[a] | Location (hg18) / Ligation site | Partial sequence (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 445 | 05916-L29504 | *HSPA13 (=STCH)* | 21q11.2 | ATTCAGCAAGTA-TTGAAAGAAGGC | 143.1 kb |
| 171 | 18163-L22868 | *SAMSN1* | 21q11.2 | CCCACAAATGGA-AGTGGAGAACAA | 2.0 **M**b |
| 378 | 19135-L25379 | *MIR99A* | 21q21.1 | AGCTTGTGCGGT-CCACTTCACCAC | 1.1 **M**b |
| 422 | 18164-L23401 | *BTG3* | 21q21.1 | TTGTAGATGTAT-TCGTGTCAATAA | 748.0 kb |
| 136 | 19012-L25527 | *TMPRSS15 (=PRSS7)* | 21q21.1 | ATTTAGATCCTG-GACCCAGAAACA | 2.9 **M**b |
| 407 | 19020-L24833 | *NCAM2* | 21q21.1 | CCACAAGAATTC-AAACAAGGAGAA | 4.3 **M**b |
| 348 | 19018-L25059 | *MIR155* | 21q21.3 | GTGCTGAAGGCT-TGCTGTAGGCTG | 566.4 kb |
| 202 | 19010-L25053 | *APP* | 21q21.3 | GAAGTTGGGTTA-GTGTTGACATGC | 340.0 kb |
| 190 | 19013-L25052 | *CYYR1* | 21q21.3 | CAGATGCATATG-GCAATCCCAGCA | 454.4 kb |
| 260 | 07592-L14699 | *ADAMTS5* | 21q21.3 | GACCTACCACGA-AAGCAGATCCTG | 2.4 **M**b |
| 265 | 19015-L25096 | *BACH1* | 21q21.3 | ATGCACAAGCTT-ACTCCAGAACAG | 1.8 **M**b |
| 429 | 21297-L29691 | *TIAM1* | 21q22.11 | ATCCATGAAGAG-TTTGGGGCTGTG | 1.9 **M**b |
| 152 | 12827-L25094 | *OLIG2* | 21q22.11 | GGCCCGGAGTAA-GGGCAGCAGCGG | 1.3 **M**b |
| 284 | 18162-L22923 | *KCNE2* | 21q22.11 | AACACTCCAATG-ACCCCTACCACC | 421.6 kb |
| ***RUNX1* gene** at 21q22.12<br>Ligation sites are according to NM_001754.5 and exon numbering according to MANE. The *RUNX1* gene is located in a common region of highest-level amplification among iAMP21 patients (Rand et al. 2011). | | | | | |
| 320 « | 19017-L25055 | *RUNX1*, ex 9 | 1409-1410 | TCCTACCACCTG-TACTACGGCGCC | 7.1 kb |
| 292 « | 02840-L02271 | *RUNX1*, ex 8 | 1040-1041 | TGGTCCTACGAT-CAGTCCTACCAA | 35.0 kb |
| 393 | 19019-L24832 | *RUNX1*, ex 7 | 982-981 reverse | TCTGACTCTGAG-GCTGAGGGTTAA | 25.1 kb |
| 437 | 02838-L24817 | *RUNX1*, ex 6 | 743-744 | TTCACAAACCCA-CCGCAAGTCGCC | 27.6 kb |
| 238 | 19014-L24827 | *RUNX1*, ex 4 | 294-295 | TCCCCGTAGATG-CCAGCACGAGCC | 161.8 kb |
| 167 | 20384-L17531 | *RUNX1*, ex 2 | 190-191 | TTTTCAGGAGGA-AGCGATGGCTTC | 1.7 **M**b |
| | | | | | |
| 147 « | 00815-L24793 | *SIM2* | 21q22.13 | GATTCTTGAAAG-GTGTAGGTTTGA | 147.2 kb |
| 232 | 03794-L14698 | *HLCS* | 21q22.13 | AACATTTCAACT-TAGAGATCTATC | 523.4 kb |
| 220 | 03791-L22920 | *DYRK1A* | 21q22.13 | TCTTCCCTCCCT-TCCCCCACCCCA | 205.1 kb |
| 243 | 19134-L25333 | *KCNJ6* | 21q22.13 | CTCGAAGCTCCT-ACATCACCAGTG | 757.6 kb |
| ***ERG* gene** at 21q22.2<br>Ligation sites are according to NM_182918.4, unless otherwise specified and exon numbering is according to MANE. Intragenic deletions of *ERG* characterize a distinct oncogenic subtype of BCP-ALL with a good prognosis, especially among patients with an intragenic *IKZF1* deletion (Clappier et al. 2014, and Zaliova et al. 2014). In AML and MDS, increased expression of *ERG* has been associated with an adverse prognosis (Marcucci et al. 2005; Thol et al. 2012) and, in a subset of these cases genomic amplification of *ERG* has been shown as a mechanism driving ERG overexpression (Weber et al. 2016). | | | | | |

| Length (nt) | SALSA MLPA probe | Gene (exon)[a] | Location (hg18) / Ligation site | Partial sequence (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 184 | 18152-L22662 | *ERG*, ex 10 (12) | 1462-1463 | TACTGGAATTCA-CCAACTGGGGGT | 7.5 kb |
| 475 | 18159-L22669 | *ERG*, ex 9 (11) | 991-992 | ATTCTTGGACCA-ACAAGTAGCCGC | 0.7 kb |
| 386 | 18158-L25097 | *ERG*, ex 8 (10) | 931-932 | CCATCTCCTTCC-ACAGTGCCCAAA | 0.7 kb |
| 254 | 18155-L22921 | *ERG*, ex 7 (9) | 905-906 | CCACGCCCCAGT-CGAAAGGTACAG | 8.2 kb |
| 497 | 19022-L25058 | *ERG*, ex 6 (8) | 791-792 | CTTTTATTTTCC-CAAATACTTCAG | 2.0 kb |
| 364 | 18157-L25525 | *ERG*, ex 5 (7) | 702-703 | TCCTCTTCCACA-TTTGACTTCAGA | 0.9 kb |
| 226 | 18154-L22664 | *ERG*, ex 4 (6) | 632-633 | ACTTCCAGAGGC-TCACCCCCAGCT | 19.9 kb |
| 299 | 20883-L22635 | *ERG*, ex 3 (5) | 459-458 reverse | CTCTGCGCTCGT-TCGTGGTCATGT | 22.1 kb |
| 328 | 02833-L02262 | *ERG*, ex 2 (4) | 152-153 | ACCAGTCGTTGT-TTGAGTGTGCCT | 52.9 kb |
| 355 | 18156-L22666 | *ERG*, ex 1 (int 3) | 23 nt before ex 1 | TGGCTGACTTCA-TTTCCCAGACTT | 77.2 kb |
| 214 | 18153-L22663 | *ERG*, up (3) | 77 kb before ex 1; NM_001136154.1; 270-271 | GCTTACTGAAGG-ACATGATTCAGA | 9.2 kb |
| 481 | 18160-L22670 | *ERG*, up (2) | 86 kb before ex 1; NM_001136154.1; 169-170 | CGTGTTGACCAA-AAGCAAGACAAA | 76.8 kb |
| 177 | 18151-L22661 | *ERG*, up (1) | 163 kb before ex 1; NM_001136154.1; 76-77 | CGCTCCGGGACG-GTCGTGACGGCC | 159.9 kb |
| | | | | | |
| 272 | 09516-L25095 | *ETS2* | 21q22.2 | GAGCTGCTATCA-GACAAATCCTGC | 355.8 kb |
| 490 | 19137-L25693 | *PSMG1* | 21q22.2 | TGGAAGCTTTTA-AGCCTATACTTT | 2.3 **M**b |
| 335 | 19011-L22003 | *TMPRSS2* | 21q22.3 | TCCTCAGGTACC-TGCATCAACCCC | 267.7 kb |
| 161 | 12741-L25051 | *RIPK4* | 21q22.3 | AAGCCAAGAAGA-TGGAGATGGCCA | 605.7 kb |
| 307 | 19016-L24829 | *TFF1* | 21q22.3 | AGGCAGATCCCT-GCAGAAGTGTCT | 2.5 **M**b |
| 195 | 20880-L14405 | *ITGB2* | 21q22.3 | CAACAACTCCAA-CCAGTTTCAGAC | 614.1 kb |
| 400 | 12745-L29350 | *SLC19A1* | 21q22.3 | CTGGTGTCAGCA-AGCTGGGTTTGC | 595.8 kb |
| 461 | 12727-L24814 | *COL6A2* | 21q22.3 | GCAGGACTTCAG-GGCCACAGGTGC | 532.1 kb |
| 468 | 02586-L25057 | *PRMT2* | 21q22.3 | ACAGCCAGAGGA-GTTTGTGGCCAT | - |

[a] See section Exon numbering on page 2 for more information.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

◊ The name of the gene targeted by this probe was changed. The previous name can be found between parentheses in this Table 2.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

## Table 3. Reference probes arranged according to chromosomal location.

| Length (nt) | SALSA MLPA probe | Gene | Chromosomal band (hg18) | Partial sequence (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 314 | 12925-L25054 | *SRD5A2* | 2p23 | AGACCCAGAGCA-AACCCACTCCCA | 23.5 **M**b |
| 415 | 00963-L22682 | *RTN4* | 2p16 | CTGGAGAGACAT-TAAGAAGACTGG | - |
| 504 | 15203-L22928 | *GBE1* | 3p12 | GACCTAGAGGGA-CTCATGATCTTT | 7.6 **M**b |
| 277 | 13796-L24815 | *KCNAB1* | 3q25 | CTTTTCCAGAGA-GAGAAAGTGGAG | - |
| 126 | 18709-L21698 | *IL4* | 5q31 | ATCGACACCTAT-TAATGGGTCTCA | - |
| 454 | 17129-L25056 | *MYBPC3* | 11p11 | CACCCAACTATA-AGGCCCTGGACT | 23.2 **M**b |
| 142 | 16543-L25049 | *SHANK2* | 11q13 | GACAGCTCCAAA-GAAGAGACCATC | - |
| 130 | 20879-L29296 | *NOS1* | 12q24 | ACTGCTGAACCT-TTCCTCTGGGAC | - |
| 249 | 06712-L25773 | *HEXA* | 15q24 | GAATGTGTTGGT-TGTCTCTGTAGT | - |
| 207 | 09882-L10295 | *CREBBP* | 16p13 | CACAAGTCCATT-TGGACAGCCCTT | - |
| 342 | 09073-L24818 | *CACNA1A* | 19p13 | GAAAAGACATCA-ACACGATTAAAT | - |
| 369 | 16279-L25100 | *SAMHD1* | 20q11 | AGTGAACGAGAT-GTTCTCTGTGTT | - |
| 157 | 12416-L24822 | *LARGE1* | 22q12.3 | AGCAGCTGTCTG-AGCTGGACGAGG | - |

## Related SALSA MLPA probemixes

| | |
|---|---|
| **P018 SHOX** | Contains probes for the *SHOX* gene and Xp22 regions. |
| **P047 RB1** | Contains probes for 26 out of 27 exons of the *RB1* gene. |
| **P202 IKZF1-ERG** | Contains two probes for each exon and the regulatory regions of *IKZF1* transcript variant 1 (NM_006060.6), one probe for each exon of the *ERG* gene, and probes for *CDKN2A/2B* genes and the 14q32.33 region. |
| **P329 CRLF2-CSF2RA-IL3RA** | Contains probes for *CRLF2*, *CSF2RA*, *IL3RA* and *SHOX* genes, involved in B-ALL. |
| **P335 ALL-IKZF1** | Contains probes for *IKZF1, EBF1, CDKN2A/B, PAX5, ETV6, BTG1* and *RB1* genes and in the PAR1 region involved in ALL. |
| **P377 Hematologic malignancies** | Contains probes for the most common CNAs in ALL, AML, CLL, CML, MDS and various lymphomas. |
| **P383 T-ALL** | Contains probes for *STIL-TAL1*, *LEF1*, *CASP8AP2*, *MYB*, *EZH2*, *MLLT3*, *MTAP*, *CDKN2A/2B*, *NUP214-ABL1*, *PTEN*, *LMO1/2*, *NF1*, *SUZ12*, *PTPN2* and*PHF6* genes, involved in T-ALL. |
| **P419 CDKN2A/2B-CDK4** | Contains probes for *CDKN2A*, *CDKN2B* and *CDK4* genes. |
| **ME024 9p21 CDKN2A/2B region** | Contains probes for the 9p21 region, including *CDKN2A* and *CDKN2B* genes for detection of both copy number and methylation status. |

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| **P327 product history** | |
|---|---|
| *Version* | *Modification* |
| B2 | One 21q22 probe has been replaced and several probe lengths have been adjusted. |
| B1 | Twelve new probes for the *ERG* gene and three additional probes for chromosome 21 have been included. Fifteen chromosome 21-specific probes and most of the reference probes have been replaced. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version B2-05 – 17 October 2025 (04P)<br>- Adjusted remarks regarding availability of complete probe sequences.<br>- Added "gain" next to the heterozygous/triplication/homozygous duplication in 'Interpretation of results section' table.<br>- Various minor textual changes.<br><br>Version B2-04 – 07 December 2022 (04P)<br>- Exon numbering of the *ERG* gene has been changed according to MANE in Table 2.<br>- Ligation sites of the probes targeting the *ERG* gene updated according to new version of the NM_ reference sequence (MANE Select transcript) in Table 2.<br><br>Version B2-03 – 05 April 2022 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Various minor textual or layout changes.<br>- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.<br>- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).<br>- List of related probemixes updated.<br>- New references added using P327 iAMP21-ERG to page 10. |

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