# Product Description
# SALSA® MLPA® Probemix P202-C1 IKZF1-ERG

To be used with the MLPA General Protocol.

**Version C1**
For complete product history see page 11.

**Catalogue numbers:**
- **P202-025R:** SALSA MLPA Probemix P202 IKZF1-ERG, 25 reactions.
- **P202-050R:** SALSA MLPA Probemix P202 IKZF1-ERG, 50 reactions.
- **P202-100R:** SALSA MLPA Probemix P202 IKZF1-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 P202 IKZF1-ERG is a **research use only (RUO)** assay for the detection of deletions or duplications in the *IKZF1* (7p12.2) and *ERG* (21q22.2) genes, which are frequently altered in acute lymphoblastic leukemia (ALL). In addition, this probemix can be used to detect copy number aberrations of the *CDKN2A/2B* genes (9p21.3) and the 14q32.33 chromosomal regions, which are frequently altered in ALL.

Partial or complete deletions of the *IKZF1* (also known as *IKAROS*) gene are detected in ALL, especially in cases that also carry the *BCR-ABL1* gene fusion (Philadelphia chromosome). In ALL, *IKZF1* deletions have been associated with relapse and poor clinical outcome (Mullighan et al. 2009, Martinelli et al. 2009, and Iacobucci et al. 2009). Partial or complete gene deletions of *IKZF1* are detected in ~80% of paediatric and 60-90% of adult *BCR-ABL1* positive ALL cases. Partial gene deletions of *IKZF1* frequently affect exons 4-7, but smaller intragenic deletions, down to single exon deletions, have been reported and have been suggested to be associated with unfavourable prognosis in paediatric B-cell precursor (BCP) ALL (Boer et al. 2016).

Short intragenic deletions of *ERG* have been described in BCP-ALL patients and have been shown to be associated with good outcome and, moreover, *ERG* deletion is suggested to define a subgroup of superior outcome among patients with *IKZF1* deletions (Clappier et al. 2014 and Zaliova et al. 2014).

In chronic-phase chronic myeloid leukemia (CML), *IKZF1* copy number changes are rare. However, in CML-blast crisis, deletions of *IKZF1* are more frequent (25-66%) and might therefore have a role in CML transformation from chronic phase to blast crisis (Alpár et al. 2012).

In common variable immunodeficiency (CVID) disorder, characterized by late-onset hypogammaglobulinemia and a poor antibody response to infectious and vaccine antigens, families with germline heterozygous *IKZF1* deletions have been detected (Kuehn et al. 2016).

**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 *IKZF1*, *ERG*, *CDKN2A* and *CDKN2B* exon numbering used in this P202-C1 IKZF1-ERG product description is the exon numbering from the MANE sequence. The *ERG* and *CDKN2A* 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 release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

**Probemix content**
The SALSA MLPA Probemix P202-C1 IKZF1-ERG contains 59 MLPA probes with amplification products between 118 and 504 nucleotides (nt). This includes 21 probes for the *IKZF1* gene, 13 probes for the *ERG* gene, three probes for the *CDKN2A/2B* genes, four probes for the 14q32.33 chromosomal region, and for both *IKZF1* and *ERG* a telomeric and a centromeric flanking probe. In addition, 14 reference probes are included that detect relatively copy number stable regions in acute lymphoblastic leukemia. Complete probe sequences are available online (www.mrcholland.com) and the identity of the genes detected by the reference probes is available in Table 3.

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

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

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

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

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

**Reference samples**

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

**Positive control DNA samples**

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers from the Coriell Institute (described in the table below) have been tested with this P202-C1 probemix at MRC Holland and can be used as positive control sample. The quality of cell lines can change; therefore samples should be validated before use.

| Coriell sample | Chromosomal position (hg18) of copy number aberration* | Altered target genes in P202-C1 | Expected copy number alteration |
|---|---|---|---|
| NA10925 | 7p12.2 | *ZPBP*, *IKZF1* and *FIGNL1* | Heterozygous deletion |
| NA07081 | 7p12.2 | *ZPBP*, *IKZF1* and *FIGNL1* | Heterozygous duplication |
| NA01750 | 9p21.3 | *CDKN2A* and *CDKN2B* | Heterozygous duplication |
| NA08123 | 14q32.33 | *CEP170B*, *MTA1*, *CRIP2* and *IGHD* | Heterozygous deletion |
| NA09868 | 21q22.13-q22.2 | *KCNJ6*, *ERG* and *ETS2* | Heterozygous deletion |

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P202-C1 IKZF1-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 probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the final ratio (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 | 1.30 < FR < 1.65 |
| Heterozygous triplication/homozygous duplication | 1.75 < FR < 2.15 |
| Ambiguous copy number | All other values |

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

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *IKZF1* 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.

**P202 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 copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

**Limitations of the procedure**

- Most genetic alterations in cancer are small (point) mutations. If present, these type of mutations in *IKZF1*, *CDKN2A/2B* and *ERG* will not be detected by using SALSA MLPA Probemix P202 IKZF1-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 copy number alteration 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. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a deletion of *IKZF1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P202-C1 IKZF1-ERG

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *IKZF1* | *ERG* | Other regions |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | |
| 118 | Reference probe 20126-L20708 | 11p11 | | | |
| 124 ¬ | **KCNJ6 probe** 21195-L31204 | | | 21q22.13 | |
| 129 « | **IKZF1 probe** 21892-L30651 | | Exon 1 | | |
| 136 | Reference probe 13224-L31079 | 1p21 | | | |
| 142 | **IKZF1 probe** 21511-L30640 | | Exon 5 | | |
| 148 « | **IKZF1 probe** 17215-L31078 | | Exon 1 | | |
| 154 | **IKZF1 probe** 15407-L17580 | | Exon 8 | | |
| 160 ¬ | **ZPBP probe** 15408-L17236 | | 7p12.2 | | |
| 166 | Reference probe 07394-L20506 | 12q13 | | | |
| 172 | **IKZF1 probe** 15410-L31063 | | Exon 7 | | |
| 177 | **ERG probe** 18151-L22661 | | | upstream | |
| 184 | **ERG probe** 18152-L22662 | | | Exon 10 | |
| 190 | **IKZF1 probe** 15409-L30639 | | Exon 6 | | |
| 196 | Reference probe 07815-L30637 | 3p22 | | | |
| 202 | **IKZF1 probe** 15424-L17583 | | Exon 4 | | |
| 207 Δ | **IGHD probe** 16524-L28592 | | | | 14q32.33 |
| 213 « | **IKZF1 probe** 14056-L20508 | | Exon 2 | | |
| 220 | Reference probe 08940-L20509 | 11p15 | | | |
| 226 ¬ | **FIGNL1 probe** 20418-L28594 | | 7p12.2 | | |
| 231 | **IKZF1 probe** 15416-L17244 | | Exon 3 | | |
| 236 | **IKZF1 probe** 21893-L30652 | | Exon 3 | | |
| 244 | Reference probe 18056-L22446 | 16q23 | | | |
| 250 | **ERG probe** 18155-L22665 | | | Exon 7 | |
| 256 | **IKZF1 probe** 15426-L17587 | | Exon 6 | | |
| 262 | **CDKN2A probe** 15675-L18954 | | | | 9p21.3 |
| 269 « | **IKZF1 probe** 13877-L15918 | | Exon 1 | | |
| 275 | Reference probe 16270-L30644 | 20q11 | | | |
| 283 | **ERG probe** 21887-L31064 | | | upstream | |
| 288 | **IKZF1 probe** 17109-L20256 | | Exon 8 | | |
| 296 ¬ | **ETS2 probe** 09515-L31066 | | | 21q22.2 | |
| 301 | **ERG probe** 21894-L31065 | | | Exon 3 | |
| 308 « | **IKZF1 probe** 21895-L30654 | | Exon 2 | | |
| 315 | **ERG probe** 21885-L31210 | | | Exon 4 | |
| 322 | **CDKN2A probe** 21890-L22800 | | | | 9p21.3 |
| 329 | Reference probe 03918-L30417 | 15q21 | | | |
| 336 | **ERG probe** 02833-L30411 | | | Exon 2 | |
| 343 | **IKZF1 probe** 13869-L30647 | | Exon 7 | | |
| 352 | **ERG probe** 20881-L22666 | | | Exon 1 | |
| 358 | **IKZF1 probe** 21896-L30655 | | Exon 5 | | |
| 366 « | **CEP170B probe** 21897-L30656 | | | | 14q32.33 |
| 373 | **ERG probe** 18157-L31067 | | | Exon 5 | |
| 382 | **ERG probe** 18158-L22668 | | | Exon 8 | |
| 389 | Reference probe 08835-L30638 | 2p13 | | | |
| 395 « Ø | **IKZF1 probe** 21883-L17250 | | Intron 1 | | |
| 402 Ŭ | **IKZF1 probe** 21898-L31074 | | Intron 3 | | |
| 409 | **CDKN2B probe** 03814-L03851 | | | | 9p21.3 |
| 417 | Reference probe 03073-L15904 | 5p15 | | | |
| 423 | **IKZF1 probe** 21886-L31075 | | Exon 4 | | |
| 427 « | **MTA1 probe** 14071-L31076 | | | | 14q32.33 |
| 436 | Reference probe 13809-L31211 | 5q14 | | | |
| 443 | Reference probe 12790-L31073 | 2q13 | | | |

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *IKZF1* | *ERG* | Other regions |
| 449 « | **CRIP2 probe** 21901-L31116 | | | | 14q32.33 |
| 459 ð | **IKZF1 probe** 21903-L31254 | | Intron 3 | | |
| 468 | Reference probe 13538-L31070 | 19p13 | | | |
| 475 | **ERG probe** 18159-L22669 | | | Exon 9 | |
| 481 | **ERG probe** 18160-L22670 | | | upstream | |
| 490 | **IKZF1 probe** 21904-L31069 | | upstream | | |
| 497 | **ERG probe** 19022-L25058 | | | Exon 6 | |
| 504 | Reference probe 15203-L22928 | 3p12 | | | |

[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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø This probe is targeting an alternative exon of *IKZF1* present in NM_001291837.2 transcript variant 14.

ð This probe is targeting an alternative exon of *IKZF1* present in NM_001291845.2 transcript variant 15.

Ù This probe is targeting an alternative exon of *IKZF1* present in NM_001291846.2 transcript variant 16.

Δ More variable. This probe may be more variable, as a high number of variations is identified in healthy control samples, see further details in DGV database. Aberrant results should be treated with caution.

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 2a. *IKZF1* probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene, exon[a] | Chromosomal band (hg18) / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| ***IKZF1* gene, 7p12.2**<br>Indicated ligation sites are in NM_006060.6, unless otherwise noted. | | | | | |
| 160 ¬ | 15408-L17236 | *ZPBP* | 7p12.2 | CCACACGTGTTA-TGTGTAACGCAA | 193.0 kb |
| 490 | 21904-L31069 | ***IKZF1***, up | 30 kb upstream of ex 1 | TCATGTTCACAA-AATCTTGGGCAT | 29.3 kb |
| 129 « | 21892-L30651 | ***IKZF1***, ex 1 | 539 nt before ex 1 reverse; NM_001291839.2: 63 nt after exon 1 reverse | GAAAACTTTGCA-ATCGCGCCGGGC | 0.2 kb |
| 148 « | 17215-L31078 | ***IKZF1***, ex 1 | 316 nt before ex 1 | GCAGGTCGAGCA-GGGACCGCCAGC | 0.5 kb |
| 269 « | 13877-L15918 | ***IKZF1***, ex 1 | 187-188 | TCTTGGCCCCAA-AGCGCGACGCAC | 4.0 kb |
| 395 « Ø | 21883-L17250 | ***IKZF1***, intr 1 | 3.9 kb after ex 1; NM_001291837.2; 172-173 | TGGAAAAGGCAG-CTCTCACTTGGC | 10.1 kb |
| 308 « | 21895-L30654 | ***IKZF1***, ex 2 | 13 nt before ex 2 reverse | TGAGAAAGAGAG-GAAGGGATTTTA | 0.1 kb |
| 213 « | 14056-L20508 | ***IKZF1***, ex 2 | 253-254 | AGACATGTCCCA-AGTTTCAGGTGA | 8.6 kb |
| 231 | 15416-L17244 | ***IKZF1***, ex 3 | 288-289 | CTGTAAGCGATA-CTCCAGATGAGG | 0.1 kb |
| 236 | 21893-L30652 | ***IKZF1***, ex 3 | 362-361 reverse | CTGTCACTCTTG-GAGCTTTGCTGT | 65.4 kb |
| 459 ð | 21903-L31254 | ***IKZF1***, intr 3 | 8.4 kb before ex 4; NM_001291845.2: 498-499 | TCCTATCATGTA-AATATCGTACGT | 1.4 kb |
| 402 Ù | 21898-L31074 | ***IKZF1***, intr 3 | 7.0 kb before ex 4; NM_001291846.2; 469-470 | ATCTTCTCACAC-AAGCGGCTACTT | 7.0 kb |
| 202 | 15424-L17583 | ***IKZF1***, ex 4 | 0 nt before ex 4 | TGTTTCTTTCAG-CCAGTAATGTTA | 0.2 kb |
| 423 | 21886-L31075 | ***IKZF1***, ex 4 | 590-591 | GATATCTGTGGG-ATCATTTGCATC | 5.8 kb |
| 142 | 21511-L30640 | ***IKZF1***, ex 5 | 680-681 | TGCGGGGCCTCA-TTCACCCAGAAG | 0.1 kb |
| 358 | 21896-L30655 | ***IKZF1***, ex 5 | 797-796 reverse | GAGTGCGTCCTC-AGGTGGCCAGTG | 4.7 kb |
| 256 | 15426-L17587 | ***IKZF1***, ex 6 | 812-813 | TTTTCTGCAGTT-GGTAAACCTCAC | 0.1 kb |
| 190 | 15409-L30639 | ***IKZF1***, ex 6 | 897-898 | GCTGCCACAACT-ACTTGGAAAGCA | 4.3 kb |
| 172 | 15410-L31063 | ***IKZF1***, ex 7 | 26 nt before ex 7 | AAGCCTTTCTAA-ACTGGCCTCTCT | 0.1 kb |
| 343 | 13869-L30647 | ***IKZF1***, ex 7 | 994-995 | CAAGATAGGATC-AGAGAGATCTCT | 8.5 kb |
| 154 | 15407-L17580 | ***IKZF1***, ex 8 | 1441-1442 | CAACGAGGAGCA-GCGCAGCGGTCT | 0.8 kb |
| 288 | 17109-L20256 | ***IKZF1***, ex 8 | 2271-2272 | GGTGTGCCGCCA-CCCAAGTGCCAA | 44.9 kb |

| Length (nt) | SALSA MLPA probe | Gene, exon[a] | Chromosomal band (hg18) / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 226 ¬ | 20418-L28594 | *FIGNL1* | 7p12.2 | AAAGCCACCATA-AAGGAAATAGTT | - |

« 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø This probe is targeting an alternative exon of *IKZF1* present in NM_001291837.2 transcript variant 14.

ð This probe is targeting an alternative exon of *IKZF1* present in NM_001291845.2 transcript variant 15.

Ʊ This probe is targeting an alternative exon of *IKZF1* present in NM_001291846.2 transcript variant 16.

Clinical and/or diagnostic significance of copy number alterations in the above mentioned three alternative exons is not yet established.

## Table 2b. *CDKN2A/2B* probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene, exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| ***CDKN2A* and *CDKN2B* genes, 9p21.3**<br>Indicated ligation sites for *CDKN2A* are in NM_000077.5 and NM_058195.4. Indicated ligation site for *CDKN2B* is for NM_004936.4. | | | | | |
| 262 | 15675-L18954 | ***CDKN2A***, ex 3 (4) | NM_000077.5; 182 nt after ex 3; NM_058195.4; 182 nt after ex 3 | TGAAATGCGGTT-AAAATGATGAAT | 7.4 kb |
| 322 | 21890-L22800 | ***CDKN2A,*** ex 1 (2) | NM_000077.5; 71 nt before ex 1; NM_058195.4; 3.7 kb before ex 2 | GCACCGGAGGAA-GAAAGAGGAGGG | 33.9 kb |
| 409 | 03814-L03851 | ***CDKN2B***, ex 1 | NM_004936.4; 462-463 | CCTGGAAGCCGG-CGCGGATCCCAA | - |

## Table 2c. 14q32.33 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene | Chromosomal band (hg18) | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| **14q32.33 region**<br>Chromosome 14, which contains immunoglobulin heavy locus (IGH), is frequently trisomic in high hyperdiploid B-cell ALL. This can lead to a higher number of *IGH* rearrangements than in cases with disomy 14. Four probes are included in this probemix targeting the 14q32.33 chromosomal region. Information about 14q32.33 copy number is suggested to be used in combination with *IGH* rearrangements for minimal residual disease detection in BCP-ALL (Csinady et al. 2009). | | | | | |
| 366 « | 21897-L30656 | ***CEP170B*** | 14q32.33 | CCCCTGAACTCT-CCAGGGCATCTT | 579.0 kb |
| 427 « | 14071-L31076 | ***MTA1*** | 14q32.33 | CACACAGTCTTA-CCAGTGGTATTC | 9.0 kb |
| 449 « | 21901-L31116 | ***CRIP2*** | 14q32.33 | CACCCTGCAGCC-ACTGCCATTTCC | 372.2 kb |
| 207 Δ | 16524-L28592 | ***IGHD*** | 14q32.33 | TCCGTGACTGTC-ACCTGGTACATG | - |

« 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.

Δ More variable. This probe may be more variable, as high number of variation is identified in healthy control samples, see further details in DGV database. Aberrant results should be treated with caution.

## Table 2d. *ERG* probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene, exon[a] | Chromosomal band (hg18) / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| **ERG gene, 21q22.2**<br>Indicated ligation sites are in NM_182918.4, unless otherwise noted. | | | | | |
| 124 ¬ | 21195-L31204 | *KCNJ6* | 21q22.13 | AGCTCCTACATC-ACCAGTGAGATC | 757.6 kb |
| 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 |
| 382 | 18158-L22668 | ***ERG***, ex 8 (10) | 931-932 | CCATCTCCTTCC-ACAGTGCCCAAA | 0.7 kb |
| 250 | 18155-L22665 | ***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 |
| 373 | 18157-L31067 | ***ERG***, ex 5 (7) | 702-703 | TCCTCTTCCACA-TTTGACTTCAGA | 0.9 kb |
| 315 | 21885-L31210 | ***ERG***, ex 4 (6) | 632-633 | ACTTCCAGAGGC-TCACCCCCAGCT | 19.9 kb |

| Length (nt) | SALSA MLPA probe | Gene, exon[a] | Chromosomal band (hg18) / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 301 | 21894-L31065 | ***ERG***, ex 3 (5) | 408-407 reverse | TGTAGCTGCCGT-AGTTCATCCCAA | 22.1 kb |
| 336 | 02833-L30411 | ***ERG***, ex 2 (4) | 152-153 | ACCAGTCGTTGT-TTGAGTGTGCCT | 52.9 kb |
| 352 | 20881-L22666 | ***ERG***, ex 1 (intr 3) | 23 nt before exon 1 | TGGCTGACTTCA-TTTCCCAGACTT | 77.2 kb |
| 283 | 21887-L31064 | ***ERG***, up (3) | 77 kb before exon 1; NM_001136154.1; 270-271 | GCTTACTGAAGG-ACATGATTCAGA | 9.2 kb |
| 481 | 18160-L22670 | ***ERG***, up (2) | 86 kb before exon 1; NM_001136154.1; 169-170 | CGTGTTGACCAA-AAGCAAGACAAA | 76.8 kb |
| 177 | 18151-L22661 | ***ERG***, up (1) | 163 kb before exon 1; NM_001136154.1; 76-77 | CGCTCCGGGACG-GTCGTGACGGCC | 151.4 kb |
| 296 ¬ | 09515-L31066 | *ETS2* | 21q22.2 | AAATGAAGAGCA-AACACTGCAAGA | - |

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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 |
|---|---|---|---|---|---|
| 136 | 13224-L31079 | *COL11A1* | 1p21 | CAGATGGTGTCA-GAGGTCTCAAGG | - |
| 389 | 08835-L30638 | *DYSF* | 2p13 | GACTGAGAGCAA-AATCCCAGCACG | 37.1 **M**b |
| 443 | 12790-L31073 | *EDAR* | 2q13 | AGAATCAAGGCT-TTTGTGATATGT | - |
| 196 | 07815-L30637 | *SCN5A* | 3p22 | TGGTTCGAGACA-TTCATCATCTTC | 43.1 **M**b |
| 504 | 15203-L22928 | *GBE1* | 3p12 | GACCTAGAGGGA-CTCATGATCTTT | - |
| 417 | 03073-L15904 | *CTNND2* | 5p15 | CATCAGCCTCAG-AGAAGACGAGTT | 78.3 **M**b |
| 436 | 13809-L31211 | *ADGRV1* | 5q14 | ATGCGAGACGAA-CAGTCTGCAGTC | - |
| 220 | 08940-L20509 | *SLC6A5* | 11p15 | TTGCCTCTCAGG-TGTGGAAAGATG | 26.7 **M**b |
| 118 | 20126-L20708 | *MYBPC3* | 11p11 | ACGTCTCTGACA-CCACGGTCTCCC | - |
| 166 | 07394-L20506 | *COL2A1* | 12q13 | TCACTTCCTTCT-TGCTCACAGGGT | - |
| 329 | 03918-L30417 | *FBN1* | 15q21 | CCTACAGATGTG-AATGCTTCCCTG | - |
| 244 | 18056-L22446 | *PLCG2* | 16q23 | GATCCAGCAGTA-CTTCCCATCCAA | - |
| 468 | 13538-L31070 | *CACNA1A* | 19p13 | TGTGCAGTCCTT-CAAGGTGAGTCC | - |
| 275 | 16270-L30644 | *SAMHD1* | 20q11 | TGACGACATGGA-AGCCTATACTAA | - |

Complete probe sequences are available at www.mrcholland.com.

## Related SALSA MLPA probemixes

- **P335 ALL-IKZF1:** Contains probes for *IKZF1, CDKN2A/2B, EBF1*, PAR1-region, *PAX5, ETV6, BTG1* and *RB1*.
- **P327 iAMP21-ERG:** Contains probes for *RUNX1, ERG* and iAMP21 detection in ALL.
- **P383 T-ALL:** Contains probes for the *STIL-TAL1, LEF1, CASP8AP2, MYB, EZH2, CDKN2A/B, MTAP, MLLT3, NUP214-ABL1, PTEN, LMO1, LMO2, NF1, SUZ12, PTPN2* and *PHF6* genes involved in T-ALL.
- **P329 CRLF2-CSF2RA-IL3RA:** Contains probes for the *CLRF2, CSF2RA* and *IL3RA* genes involved in ALL.
- **P377 Hematologic Malignancies:** Contains probes for screening DNA samples on the most common copy number changes associated with ALL, AML, CLL, CML, MDS and lymphomas.
- **P419 CDKN2A/2B-CDK4:** Contains several probes for each exon of *CDKN2A/2B* and at least one probe for each exon of *CDK4*.
- **P037 CCL-1, P038 CLL-2 and P040 CLL:** Contain probes for various genes involved in CLL.
- **ME024 9p21 CDKN2A/2B region:** Contains probes for detection of methylation and/or copy number status of the chromosomal region 9p21.3 (*CDKN2A/2B, CDKN2B-AS1, MTAP, MIR31 and PAX5*).

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## Selected publications using SALSA MLPA Probemix P202 IKZF1-ERG


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| **P202 product history** | |
|---|---|
| *Version* | *Modification* |
| C1 | Probemix content completely revised. Probes have been added for the *ERG* gene and flanking regions, and removed for the *IKZF2* and *IKZF3* genes. In addition, several probes for *IKZF1* and several reference probes have been replaced. |
| B2 | A few probes have a change in length but no change in sequences detected. |
| B1 | Two new IKZF1 probes, three new IKZF2 probes, one new IKZF3 probe and four probes for 14q32.33 added. New QDX2 fragments added. |
| A1 | First release. |

**Implemented changes in the product description**

Version C1-04 – 18 October 2022 (04P)
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Exon numbering of the *ERG* and *CDKN2A* genes has been changed according to MANE.
- Ligation sites of the probes targeting the *CDKN2A/2B* and *ERG* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version C1-03 – 21 April 2021 (01P)
- Warning added to Tables 1 & 2c about possible higher variability of the IGHD probe (16524-L28592) at 207 nt.

Version C1-02 – 05 February 2020 (01P)
- Gene name GPR98 has been changed to ADGRV1 in Table 2e, following HUGO Gene Nomenclature.
- Various minor layout changes.
- New references added in Selected publications on page 9.

Version C1-01 – 16 January 2019 (01P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Tables 2) and to a new template.
- For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36).

Version 10 – 01 February 2018 (T08)
- Publication information of the references updated on page 2.
- Exon and ligation site information corrected for IKZF1 probes (17215-L20539, 15422-L17250 and 15424-L17583).
- Shortened version of the chromosomal bands introduced for reference probes in Table 1 and 2.
- Typos corrected.

Version 09 – 15 September 2017 (T08)
- Warning added in Table 1, 296 nt probe 04606-L17670, 373 nt probe 13657-L15111, 427 nt probe 14071-L18749, and 445 nt probe 16357-L15668.

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