# Product Description
## SALSA® MLPA® Probemix P437-B1 Familial MDS-AML

To be used with the MLPA General Protocol.

**Version B1**
For complete product history see page 10.

**Catalogue numbers:**
- **P437-025R:** SALSA MLPA Probemix P437 Familial MDS-AML, 25 reactions.
- **P437-050R:** SALSA MLPA Probemix P437 Familial MDS-AML, 50 reactions.
- **P437-100R:** SALSA MLPA Probemix P437 Familial MDS-AML, 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 P437 Familial MDS-AML is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GATA2* (3q21.3), *TERC* (3q26.2), *TERT* (5p15.33), *CEBPA* (19q13.11) and *RUNX1* (21q22.12) genes, which are suggested to be of diagnostic relevance in familial MDS/AML. This probemix can also be used to detect the presence of three mutations, namely, the *GATA2* p.R398W (c.1192C>T), *GATA2* p.T354M (c.1061C>T) and *TERT* p.A1062T (c.3184G>A).

While the majority of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) cases are sporadic, familial MDS/AML cases have also been identified and reported in literature. Inherited mutations in the *GATA2*, *TERC*, *TERT*, *CEBPA* and *RUNX1* genes have been shown to associate with familial MDS/AML (for review see Holme et al. 2012). Although most of the germline aberrations in these genes are point mutations, deletions have been described as well, e.g. in the *GATA2* and *RUNX1* genes (Hsu et al. 2011, Kazenwadel et al. 2012, and Liew & Owen 2011). The most recurrent *GATA2* mutations identified in MonoMAC patients are p.R398W (c.1192C>T) and p.T354M (c.1061C>T) (Hsu et al. 2011). Furthermore, in the *TERT* gene the p.A1062T (c.3184G>A) mutation is shown to be a negative prognostic factor in younger AML patients (Both et al. 2017).

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

**Gene structure and transcript variants:**
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

**Exon numbering**
The *GATA2* exon numbering used in this P473-B1 Familial MDS-AML product description is from the LRG_295 sequence, the *TERC* exon numbering is from the LRG_347 sequence, the *TERT* exon numbering is from the LRG_343 sequence, the *CEBPA* exon numbering is from the NM_004364.4 and the *RUNX1* exon numbering is from the LRG_482.

The *GATA2, CEPBA* and *RUNX1* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2.

The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. From description version 1 onwards, we have adopted the exon numbering. 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 P478-B1 Familial MDS-AML contains 59 MLPA probes with amplification products between 124 and 505 nucleotides (nt). This includes 42 probes for the genes *GATA2*, *TECR*, *TERT*, *CEPBA* and *RUNX1*. Furthermore, this probemix also contains 3 probes specific for the *GATA2* p.R398W, *GATA2* p.T354M and *TERT* p.A1062T point mutations which will only generate a signal when the mutation is present. In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types including familial MDS-AML. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 2b 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, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

**Reference samples**
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

**Positive control DNA samples**

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Samples from the Coriell Institute have been tested with this P437-B1 probemix at MRC Holland and can be used as a positive control samples as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

| Sample name | Source | Chromosomal position (hg18) of copy number alteration* | Altered target genes in P437-B1 | Expected copy number alteration |
|---|---|---|---|---|
| NA10175 | Coriell Institute | 3q26.2 | *TERC* | Heterozygous duplication |
| NA11428 | Coriell Institute | 3q26.2 | *TERC* | Heterozygous duplication |
| NA20022 | Coriell Institute | 3q26.2 | *TERC* | Heterozygous duplication |
| NA14523 | Coriell Institute | 5p15.33 | *TERT* | Heterozygous duplication |
| NA14131 | Coriell Institute | 5p15.33 | *TERT* | Heterozygous deletion |
| NA01201 | Coriell Institute | 21q22.12 | *RUNX1* | Heterozygous deletion |
| MFE-280◊ | Leibniz Institute DSMZ | 3q21.3 | *GATA2* | Homozygous duplication |
| | | 3q26.2 | *TERC* | Amplification |
| | | 5p15.33 | *TERT* | Heterozygous duplication |
| | | 19q13.1 | *CEBPA* | Amplification |
| SK-N-MC◊ | Leibniz Institute DSMZ | 3q21.3-3q26.2 | *GATA2 & TERC* | Heterozygous duplication |

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P437-B1 Familial MDS-AML probemix.

◊ In the indicated cell line samples some of the reference probes are affected by CNAs.

**SALSA Binning DNA SD070**

The SD070 Binning DNA provided with this probemix can be used for binning of all probes including the three mutation-specific probes: *GATA2* probe 19052-SP0847-L24939 (p.R398W = c.1192C>T) at 168 nt, *GATA2* probe 19053-SP0738-L25512 (p.T354M = c.1061C>T) at 190 nt and *TERT* probe 19697-SP0859-L30075 (p.A1062T = c.3184G>A) at 200 nt. SD070 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD070 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD070 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

**Data analysis**

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

**Interpretation of results**

The standard deviation of each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the finale 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 *CEBPA* 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.

**P437 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**

- In most populations, the major cause of genetic defects in the *GATA2, TERC, TERT, CEBPA or RUNX1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P437 Familial MDS-AML.
- 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 solid tumours 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.

**LOVD mutation databases**

https://databases.lovd.nl/shared/genes/GATA2
https://databases.lovd.nl/shared/genes/TERC
https://databases.lovd.nl/shared/genes/TERT
https://databases.lovd.nl/shared/genes/CEBPA
https://databases.lovd.nl/shared/genes/RUNX1
We strongly encourage users to deposit positive results in the databases mentioned above. 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 duplication of *GATA2* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P437-B1 Familial AML-MDS

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Reference | *GATA2* | *TERC* | *TERT* | *CEBPA* | *RUNX1* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | | | |
| 124 | Reference probe 19616-L26241 | 4p13 | | | | | |
| 130 | **TERC probe** 08640-L08656 | | | **Exon 1** | | | |
| 136 « | **CEBPA probe** 19049-L24936 | | | | | **Exon 1** | |
| 142 | **GATA2 probe** 19050-L24937 | | **Exon 6** | | | | |
| 148 | **TERT probe** 19696-L29831 | | | | **Exon 15** | | |
| 157 Ø | **RUNX1 probe** 19051-L25507 | | | | | | **Intron 7** |
| 161 | **TERT probe** 10346-L29829 | | | | **Exon 2** | | |
| 168 § Ж | **GATA2 probe** 19052-SP0847-L24939 | | **p.R398W= c.1192C>T** | | | | |
| 173 | Reference probe 21100-L29832 | 1q3 | | | | | |
| 178 | **TERT probe** 15272-L26713 | | | | **Exon 1** | | |
| 184 | Reference probe 08731-L29833 | 9q21 | | | | | |
| 190 § Ж | **GATA2 probe** 19053-SP0738-L25512 | | **p.T354M = c.1061C>T** | | | | |
| 195 | **TERT probe** 08647-L29834 | | | | **Exon 7** | | |
| 200 * § Ж | **TERT probe** 19697-SP0859-L30075 | | | | **p.A1062T= c.3184G>A** | | |
| 207 | **TERT probe** 19055-L30074 | | | | **Exon 6** | | |
| 212 | **RUNX1 probe** 19057-L29836 | | | | | | **Exon 1** |
| 219 « | **GATA2 probe** 19058-L25516 | | **Exon 3** | | | | |
| 224 | **TERT probe** 19059-L25517 | | | | **Exon 9** | | |
| 229 | **TERT probe** 08652-L25343 | | | | **Exon 12** | | |
| 233 | Reference probe 15154-L25342 | 18q21 | | | | | |
| 241 | **RUNX1 probe** 19014-L30135 | | | | | | **Exon 4** |
| 246 | **GATA2 probe** 21373-L30136 | | **Exon 8** | | | | |
| 253 | Reference probe 10716-L30137 | 6p12 | | | | | |
| 258 | **TERT probe** 19061-L30138 | | | | **Exon 4** | | |
| 265 « | **CEBPA probe** 19062-L24949 | | | | | **Exon 1** | |
| 270 | **TERC probe** 19299-L24943 | | | **Exon 1** | | | |
| 276 Δ | **GATA2 probe** 19063-L30052 | | **Exon 7** | | | | |
| 283 | **RUNX1 probe** 21375-L29826 | | | | | | **Exon 2** |
| 288 | Reference probe 02877-L24219 | 1p33 | | | | | |
| 296 | **TERT probe** 19065-L26112 | | | | **Exon 3** | | |
| 301 « | **RUNX1 probe** 02840-L25520 | | | | | | **Exon 8** |
| 309 « | **CEBPA probe** 19066-L26430 | | | | | **Exon 1** | |
| 316 « | **GATA2 probe** 21376-L29827 | | **Exon 1** | | | | |
| 324 « | **RUNX1 probe** 19017-L25523 | | | | | | **Exon 9** |
| 331 | **TERT probe** 08651-L26433 | | | | **Exon 11** | | |
| 337 | Reference probe 20864-L28882 | 14q24 | | | | | |
| 346 « | **GATA2 probe** 19588-L26183 | | **Exon 2** | | | | |
| 355 | **GATA2 probe** 21377-L29828 | | **Exon 4** | | | | |
| 360 | **RUNX1 probe** 19069-L30005 | | | | | | **Exon 3** |
| 366 | **TERT probe** 08653-L30006 | | | | **Exon 13** | | |
| 373 | **TERC probe** 19070-L30007 | | | **Exon 1** | | | |
| 381 | Reference probe 19749-L26532 | 9q34 | | | | | |
| 388 | **RUNX1 probe** 19019-L25441 | | | | | | **Exon 7** |
| 395 | **TERT probe** 08656-L25442 | | | | **Exon 16** | | |
| 402 | Reference probe 01237-L25675 | 10p14 | | | | | |
| 409 | **GATA2 probe** 19071-L24958 | | **Exon 5** | | | | |
| 418 | **TERT probe** 19072-L24959 | | | | **Exon 10** | | |
| 425 | **RUNX1 probe** 19237-L25317 | | | | | | **Exon 5** |
| 431 Δ | Reference probe 15541-L25346 | 2q23 | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 439 | **RUNX1 probe** 02838-L25676 | | | | | | **Exon 6** |
| 445 Ø ʃ | **GATA2 probe** 19298-L25506 | | **Intron 6** | | | | |
| 454 | **TERT probe** 08645-L25504 | | | | **Exon 5** | | |
| 460 | Reference probe 16287-L29839 | 20q11 | | | | | |
| 469 | **TERT probe** 19073-L29840 | | | | **Exon 14** | | |
| 475 | **TERT probe** 19074-L29841 | | | | **Exon 8** | | |
| 481 | Reference probe 09772-L10187 | 15q21 | | | | | |
| 489 Ø | **TERC probe** 19295-L25478 | | | **Upstream** | | | |
| 497 | Reference probe 19555-L30008 | 2p13 | | | | | |
| 505 | Reference probe 06676-L29849 | 11p15 | | | | | |

[a] See section Exon numbering on page 1 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.

§ Mutation-specific probe. This probe will only generate a signal when; in *GATA2* gene the p.R398W= c.1192C>T or p.T354M = c.1061C>T and in *TERT* gene the p.A1062T = c.3184G>A mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

ʃ Important information on this probe can be found in and below 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. P437-B1 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene/Exon | Location / Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| ***GATA2* gene, at 3q21.3**. Ligation sites are indicated, unless noted otherwise, according to NM_001145661.2 (CDS: 436-1878), which is a reference standard in the NCBI RefSeqGene project. | | | | | |
| 246 | 21373-L30136 | *GATA2*, ex 8 | 1700-1701 | AAAGTGCATGCA-GGAGAAGTCATC | 0,1 kb |
| 168 § Ж | 19052-SP0847-L24939 | *GATA2*, ex 8, **c.1192C>T= p.R398W** | 1627-1626 and 1591-1590 rev. | GGACATCTTCC**A**-36nt spanning oligonucleotide-TGGCCTGTTAAC | 0,6 kb |
| 276 Δ | 19063-L30052 | *GATA2*, ex 7 | 1578-1579 | AAGCTGCACAAT-GTGAGTGCGCCC | 0,1 kb |
| 190 § Ж | 19053-SP0738-L25512 | *GATA2*, ex 7 **c.1061C>T= p.T354M** | 1496-1495 and 1470-1469 rev. | TGGTGGTTGTC**A**-26nt spanning oligonucleotide-GCTCTTCTGGCG | 1,4 kb |
| 445 Ø ʃ | 19298-L25506 | *GATA2*, intr 6 | 540 nt after ex 6 | ACATCTGCAGCC-TGAAGATAAGGA | 0,6 kb |
| 142 | 19050-L24937 | *GATA2*, ex 6 | 1428-1429 | CAGAACCGACCA-CTCATCAAGCCC | 2,5 kb |
| 409 | 19071-L24958 | *GATA2*, ex 5 | 684-685 | ACCGGAGGCCAG-ATGTGCCGCCCA | 0,5 kb |
| 355 | 21377-L29828 | *GATA2*, ex 4 | 600-601 | CTCGACTCGCAG-GGCAACCCCTAC | 0,9 kb |
| 219 « | 19058-L25516 | *GATA2*, ex 3 | 355-356 | CCTTCACTCTCA-GAGGCCGAGTCC | 0,6 kb |
| 346 « | 19588-L26183 | *GATA2*, ex 2 | 166-167 | CGAGCTAGGGGA-GGGAACGGTCTG | 4,8 kb |
| 316 « | 21376-L29827 | *GATA2*, ex 1 | 2,6 kb before ex 2; NM_032638.5: 28-29 | GAGCGCCAGGAA-GGTAGCGAGGCC | 41.3 **Mb** to *TERC* |
| ***TERC* gene, at 3q26.2.** Ligation sites are indicated according to NR_001566.1, which is a reference standard in the NCBI RefSeqGene project. | | | | | |
| 270 | 19299-L24943 | *TERC*, ex 1 | 100 nt after ex 1 rev. | TGCAGCACACTG-GCCCAGTCAGTC | 0,2 kb |
| 130 | 08640-L08656 | *TERC*, ex 1 | 314-315 | GAGTTGGGCTCT-GTCAGCCGCGGG | 0,4 kb |
| 373 | 19070-L30007 | *TERC*, ex 1 | 68 nt before ex 1 rev. | GGCCAAGCTGAC-TCTCGCGGCTCT | 0,3 kb |
| 489 | 19295-L25478 | *TERC*, upstream | 421 nt before ex 1 | GAGCCGAGACAA-GATTCTGCTGTA | - |

| | | | | | |
|---|---|---|---|---|---|
| ***TERT* gene, at 5p15.33**. Ligation sites are indicated according to NM_198253.3 (CDS: 80-3478), which is a reference standard in the NCBI RefSeqGene project. | | | | | |
| 395 | 08656-L25442 | *TERT*, ex 16 | 13 nt before ex 16 | ACGGAGTCTGAT-TTTGGCCCCGCA | 0,5 kb |
| 148 | 19696-L29831 | *TERT*, ex 15 | 3364-3365 | CTCCTGGGGTCA-CTCAGGACAGGC | 0,1 kb |
| 200 § Ж | 19697-SP0859-L30075 | *TERT*, ex 15, **c.3184G>A = p.A1062T** | 3263-3264 and 3291-3292 | CCAAGGGCGCC**A**-28nt spanning oligonucleotide-GTGGCTGTGCCA | 0,8 kb |
| 469 | 19073-L29840 | *TERT*, ex 14 | 3228-3229 | CCTGAAAGCCAA-GAACGCAGGTAT | 3,3 kb |
| 366 | 08653-L30006 | *TERT*, ex 13 | 3083-3084 | GCACCAACATCT-ACAAGATCCTCC | 1,9 kb |
| 229 | 08652-L25343 | *TERT*, ex 12 | 3000-3001 | GCGTCGCAAACT-CTTTGGGGTCTT | 4,0 kb |
| 331 | 08651-L26433 | *TERT*, ex 11 | 2809-2810 | TTCCCTGTAGAA-GACGAGGCCCTG | 1,9 kb |
| 418 | 19072-L24959 | *TERT*, ex 10 | 1 nt after ex 10 | ACCTTCCTCAGG-TGAGGCCCGTGC | 2,0 kb |
| 224 | 19059-L25517 | *TERT*, ex 9 | 99 nt after ex 9 | ACCATGACTGCT-CTGTCTTGAGGA | 2,8 kb |
| 475 | 19074-L29841 | *TERT*, ex 8 | 3 nt before ex 8 | CGTCTGCTTTCG-CAGAGCTCCTCC | 1,0 kb |
| 195 | 08647-L29834 | *TERT*, ex 7 | 2405-2406 | ACATGCGACAGT-TCGTGGCTCACC | 6,4 kb |
| 207 | 19055-L30074 | *TERT*, ex 6 | 2345-2344 rev. | GAAGGCCTTGCG-GACGTGCCCATG | 0,7 kb |
| 454 | 08645-L25504 | *TERT*, ex 5 | 2134-2135 | GGCCTGGACGAT-ATCCACAGGGCC | 1,0 kb |
| 258 | 19061-L30138 | *TERT*, ex 4 | 70 nt before ex 4 | GTGGGGGCTTGT-GGCTTCCCGTGA | 2,2 kb |
| 296 | 19065-L26112 | *TERT*, ex 3 | 1700-1701 | GTGAGGAGATCC-TGGCCAAGTTCC | 12,1 kb |
| 161 | 10346-L29829 | *TERT*, ex 2 | 321-322 | GAAGGAGCTGGT-GGCCCGAGTGCT | 0,3 kb |
| 178 | 15272-L26713 | *TERT*, ex 1 | 152-153 | TGCTGCCGCTGG-CCACGTTCGTGC | - |
| ***CEBPA* gene, at 19q13.11**. Ligation sites are indicated according to NM_004364.5 (CDS: 121-1197), which is a reference standard in the NCBI RefSeqGene project. | | | | | |
| 265 « | 19062-L24949 | *CEBPA*, ex 1 | 743-744 | GCACCTGCAGTT-CCAGATCGCGCA | 0,4 kb |
| 136 « | 19049-L24936 | *CEBPA*, ex 1 | 359-360 | GTTCCTGGCCGA-CCTGTTCCAGCA | 0,2 kb |
| 309 « | 19066-L26430 | *CEBPA*, ex1 | 114-115 | GAGAACTCTAAC-TCCCCCATGGAG | - |
| ***RUNX1* gene, at 21q22.12**. Ligation sites are indicated, unless noted otherwise, according to NM_001754.5 (CDS: 195-1637), which is a reference standard in the NCBI RefSeqGene project. | | | | | |
| 324 « | 19017-L25523 | *RUNX1*, ex 9 (10) | 1409-1410 | TCCTACCACCTG-TACTACGGCGCC | 7,1 kb |
| 301 « | 02840-L25520 | *RUNX1*, ex 8 (9) | 1040-1041 | TGGTCCTACGAT-CAGTCCTACCAA | 22,3 kb |
| 157 | 19051-L25507 | *RUNX1*, int 7 (ex 7) | 12,7 kb after ex 7; NM_001122607.2: 2321-2322 | AGAGGAAGACAC-AGCACCCTGGAG | 12,8 kb |
| 388 | 19019-L25441 | *RUNX1*, ex 7 | 982-981 reverse | TCTGACTCTGAG-GCTGAGGGTTAA | 25,1 kb |
| 439 | 02838-L25676 | *RUNX1*, ex 6 | 743-744 | TTCACAAACCCA-CCGCAAGTCGCC | 21,1 kb |
| 425 | 19237-L25317 | *RUNX1*, ex 5 | 627-628 | CGGCTGAGCTGA-GAAATGCTACCG | 6,5 kb |
| 241 | 19014-L30135 | *RUNX1*, ex 4 | 294-295 | TCCCCGTAGATG-CCAGCACGAGCC | 5,8 kb |
| 360 | 19069-L30005 | *RUNX1*, ex 3 | 284-285 | CCTTCTAGAGAC-GTCCACGGTATG | 155,9 kb |
| 283 | 21375-L29826 | *RUNX1*, ex 2 | 239-240 | TCGTACCCACAG-TGCTTCATGAGA | 0,4 kb |
| 212 | 19057-L29836 | *RUNX1*, ex 1 | 75-74 rev. | CTGACCACTATG-CTGGGTTCAGAC | - |

[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.

« 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.

§ Mutation-specific probe. This probe will only generate a signal when the described mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

∫ This probe covers a conserved intronic element (consisting of E-box and GATA motifs) that is under haploinsufficiency suggested to lead to MonoMAC syndrome (see Hsu et al. 2013).

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) | Location (hg18) in kb |
|---|---|---|---|---|---|
| 288 | 02877-L24219 | *FAF1* | 1p33 | GGACCTGCATTT-AATCCAGCAAGT | 01-051,026 |
| 173 | 21100-L29832 | *CACNA1S* | 1q32 | AAGCCGCCATGA-AGATCATTGCCT | 01-199,330 |
| 497 | 19555-L30008 | *DYSF* | 2p13 | CCATTGCCAAGA-AGGTCAGTGTCC | 02-071,750 |
| 431 Δ | 15541-L25346 | *ACVR2A* | 2q23 | TGTGTAGGTGAA-AGAATTACCCAG | 02-148,401 |
| 124 | 19616-L26241 | *ATP8A1* | 4p13 | CAGATTCTTCTT-CGAGGAGCTCAG | 04-042,278 |
| 253 | 10716-L30137 | *PKHD1* | 6p12 | GCCATCCTTGTT-TCTGATGGTGGA | 06-051,907 |
| 184 | 08731-L29833 | *PCSK5* | 9q21 | GTCCTGGATTCT-ATGGTGACCAAG | 09-078,101 |
| 381 | 19749-L26532 | *STXBP1* | 9q34 | CAAAGCTTCTAC-AGTCCCCACAAG | 09-129,465 |
| 402 | 01237-L25675 | *UPF2* | 10p14 | TGCCATTCCTTT-GCATCTCAAAAG | 10-012,019 |
| 505 | 06676-L29849 | *SMPD1* | 11p15 | CTGCTGAAGATA-GCACCACCTGCC | 11-006,369 |
| 337 | 20864-L28882 | *PSEN1* | 14q24 | TTTCTGTGAAAC-AGTATTTCTATA | 14-072,684 |
| 481 | 09772-L10187 | *SPG11* | 15q21 | TTTCTTCAGGAT-TGATAGTCATTC | 15-042,706 |
| 233 | 15154-L25342 | *MC4R* | 18q21 | GACATTTACTCA-CAGCAGGCATGG | 18-056,191 |
| 460 | 16287-L29839 | *SAMHD1* | 20q11 | CCGACTACAAGA-CATGGGGTCCGG | 20-035,013 |

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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

## Related SALSA MLPA probemixes

- **P257 TERT-DKC1**: Contains other probes for the *TERT* and *TERC* genes.
- **P327 iAMP21-ERG**: Contains probes for the *RUNX1* gene and its flanking regions.
- **P373 Microdeletion-7**: Contains six probes for the *TERT* gene.

## References


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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

## Selected publications using SALSA MLPA Probemix P437 Familial MDS-AML


- Almazni I et al. (2021). A novel RUNX1 exon 3-7 deletion causing a familial platelet disorder. *Platelets*. 22:1-4.
- Banescu C et al. (2019). Presence of copy number aberration and clinical prognostic factors in patients with acute myeloid leukemia: an analysis of effect modification. *Pol Arch Intern Med*. 23: 898-906.
- Duployez N et al. (2019). Germline RUNX1 Intragenic Deletion: Implications for Accurate Diagnosis of FPD/AML. *Hemasphere*. 3: e203.
- de Andrade Silva MC et al. (2018). Deletion of RUNX1 exons 1 and 2 associated with familial platelet disorder with propensity to acute myeloid leukemia. *Cancer Genetics*. 222–223:32-7.
- Rio-Machin A et al. (2020). The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. *Nat Commun*. 11:1044.


| **P437 product history** | |
|---|---|
| *Version* | *Modification* |
| B1 | A new mutation specific probe for *TERT* has been included, five target probes have been replaced, one new reference probe has been included, six reference probes have been replaced and several probes have been adjusted in length. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version B1-01 – 18 May 2021 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Warning added under Table 1 and 2b for 15541-L25346, 431 nt probe and 19063-L30052, 276 nt probe.<br>- Positive samples added on page 3.<br>- Ligation sites of the probes targeting the GATA2, TERT, CEBPA and RUNX1 genes updated according to newest versions of the NM_ reference sequences.<br>- New references added in section 'selected publications' on page 10. |
| Version 07 – 07 December 2018 (T08)<br>- For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36).<br>- Several minor textual changes.<br>Version 06 -22 March 2018 (T08)<br>- One new reference added on page 2.<br>- Warnings about the salt sensitivity removed from Table 1 and 2 according to the newest information from quality tests. |
| Version 05 – 31 August 2017 (T08)<br>- Warning added under Table 1 and Table 2, 161 nt probe 10346-L29829, 178 nt probe 15272-L26713, and 454 nt probe 08645-L25504.<br>Version 04 – 18 May 2017 (T08)<br>- Product description adapted to a new product version (version number changed, lot number added, changes in Tables 1, 2a and 2b, new pictures included).<br>- Small changes of probe lengths in Tables 1, 2a and 2b in order to better reflect the true lengths of the amplification products.<br>- Various minor textual and layout changes throughout the document. |
| Version 03 – 08 March 2017 (T08)<br>- Exon numbering has been changed for GATA2 and RUNX1 in Table 1 and 2a according to LRG_295 (NM_001145661.1 and NM_032638.4) and NM_001754.4, respectively.<br>- Probe number of the 179 nt probe corrected in Table 1 and Table 2a.<br>- Correction of chromosomal band location of RUNX1 in Table 2a.<br>- Two probes at 179 nt and 502 nt have a small adjustment in length to better reflect the true length of the amplification products (Table 1, 2a and 2b).<br>- MV locations in Tables 2a and 2b updated.<br>- Various minor textual and layout changes throughout the document. |

| **More information: www.mrcholland.com; www.mrcholland.eu** | |
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