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. Complete 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 criteriom 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	1.30 < FR < 1.65

Copy number status	Final ratio (FR)
Heterozygous triplication/homozygous duplication	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)[a]		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)[a]		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[b] (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 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 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)					

Length (nt)	SALSA MLPA probe	Gene (exon)[a]	Location (hg18) / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
and, in a subset of these cases genomic amplification of *ERG* has been shown as a mechanism driving ERG overexpression (Weber et al. 2016).					
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.

[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.

◊ 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	-

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
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	-

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

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 B2-04 – 07 December 2022 (04P) - Exon numbering of the *ERG* gene has been changed according to MANE in Table 2. - 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. Version B2-03 – 05 April 2022 (04P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). - List of related probemixes updated. - New references added using P327 iAMP21-ERG to page 10. Version B2-02 — 20 July 2020 (02P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Ligation sites of the probes targeting the RUNX1 gene updated according to new version of the NM_ reference sequence. - Positive control DNA samples information added to the product description Version B2-01 – 26 July 2018 (01P) - Product description restructured and adapted to a new template. - Information on SD028 has been removed. Version 11 – 18 January 2017 (T08) - Product description adapted to a new product version (lot & version number changed, changes in Table 1, 2a and 2b, new picture included). - Exon numbering of the RUNX1 gene has been changed in Table 2a according to NM_001754.4 (LRG_482). - Ligation sites of the RUNX1 and ERG probes have been added to Table 2a. - New related products (P377, P383, P437 and SD028) added on page 2. P037, P038 and P040 removed from related products on page 1. - Two new references for probemix P327 added on page 2. - Various minor textual changes throughout the document.

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