Product Description
SALSA® MLPA® Probemix P202-C2 IKZF1-ERG

To be used with the MLPA General Protocol.

Version C2
As compared to version C1, a few probes have a small change in length but no changes in the sequence detected. For complete product history see page 12.

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

SALSA® MLPA® Probemix P202 IKZF1-ERG (hereafter: P202 IKZF1-ERG) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P202-025R	**P202-050R**	**P202-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding quality tests 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
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 determine the copy number of the *CDKN2A/2B* genes (9p21.3) and the 14q32.33 chromosomal region, recurrently 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. 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 product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering
The *IKZF1, ERG, CDKN2A,* and *CDKN2B* exon numbering used in this P202-C2 IKZF1-ERG product description is the exon numbering derived from MANE project (release version 1.4) based on MANE Select transcript NM_006060.6, NM_182918.4, NM_000077.5 (and MANE Plus Clinical transcript NM_058195.4), and NM_004936.4, respectively. 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
P202-C2 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 target relatively copy number stable regions in ALL. Partial 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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes (except IGHD probe 16524-L28592 at 207 nt) over the experiment.

Required specimens

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

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

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families 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. Samples from the Coriell Institute (described in the table below) have been tested with P202-C2 IKZF1-ERG at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Sample name	Chromosomal position (hg18) of CNA*	Altered target genes in P202-C2 IKZF1-ERG	Expected CNA
Germline samples from Coriell Institute.			
NA07081	7p12.2	*ZPBP*, *IKZF1*, and *FIGNL1*	Heterozygous duplication
NA10925			Heterozygous deletion
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
Cancer cell line samples from Leibniz Institute DSMZ.			
SEM (ACC 546)	7p12.2	*ZPBP*, *IKZF1*, and *FIGNL1*	Heterozygous deletion
TOM-1 (ACC 578)	7p12.2	*IKZF1* (intron 3-exon 8)	Heterozygous deletion
	9p21.3	*CDKN2A*	Homozygous deletion
	14q32.33	*IGHD*	Heterozygous deletion
ALL-SIL (ACC 511)	9p21.3	*CDKN2A* and *CDKN2B*	Homozygous deletion
HSB-2 (ACC 435)	9p21.3	*CDKN2A*	Homozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by P202-C2 IKZF1-ERG.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 (except IGHD probe 16524-L28592 at 207 nt) 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	FR
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	1.75 < FR < 2.15
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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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 FRs 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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 and 14q32.33 region. 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: https://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 IKZF1-ERG specific notes:

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. 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

- 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 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 FR 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 in sequence data 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 https://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. P202-C2 IKZF1-ERG

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				Location (hg18) in kb
		Reference	*IKZF1*	*ERG*	Other	
64-105	Control fragments – see table in probemix content section for more information					
118	Reference probe 20126-L20708	11p11				11-047,314
124 ¬	**KCNJ6 probe** 21195-L31204			21q22.13		21-037,920
129 «	**IKZF1 probe** 21892-L30651		Upstream			07-050,314
136	Reference probe 13224-L31079	1p21				01-103,234
142	**IKZF1 probe** 21511-L30640		Exon 5			07-050,418
148 «	**IKZF1 probe** 17215-L31078		Upstream			07-050,315
154	**IKZF1 probe** 15407-L17580		Exon 8			07-050,435
160 ¬	**ZPBP probe** 15408-L17236		7p12.2			07-050,092
166	Reference probe 07394-L20506	12q13				12-046,670
172	**IKZF1 probe** 15410-L31063		Exon 7			07-050,427
177	**ERG probe** 18151-L22661			Upstream		21-038,955
184	**ERG probe** 18152-L22662			Exon 10		21-038,677
190	**IKZF1 probe** 15409-L30639		Exon 6			07-050,423
196	Reference probe 07815-L30637	3p22				03-038,592
202	**IKZF1 probe** 15424-L17583		Exon 4			07-050,412
207 Δ	**IGHD probe** 16524-L28592				14q32.33	14-105,383
213 «	**IKZF1 probe** 14056-L20508		Exon 2			07-050,329
220	Reference probe 08940-L20509	11p15				11-020,606
226 ¬	**FIGNL1 probe** 20418-L28594		7p12.2			07-050,481
231	**IKZF1 probe** 15416-L17244		Exon 3			07-050,338
236	**IKZF1 probe** 21893-L30652		Exon 3			07-050,338
244	Reference probe 18056-L22446	16q23				16-080,518
250	**ERG probe** 18155-L22665			Exon 7		21-038,686
256	**IKZF1 probe** 15426-L17587		Exon 6			07-050,423
262	**CDKN2A probe** 15675-L18954				9p21.3	09-021,958
269 «	**IKZF1 probe** 13877-L15918		Exon 1			07-050,315
275	Reference probe 16270-L30644	20q11				20-034,973
283	**ERG probe** 21887-L31064			Upstream		21-038,869
288	**IKZF1 probe** 17109-L20256		Exon 8			07-050,436
296 ¬	**ETS2 probe** 09515-L31066			21q22.2		21-039,107
301	**ERG probe** 21894-L31065			Exon 3		21-038,717
308 «	**IKZF1 probe** 21895-L30654		Exon 2			07-050,329
315	**ERG probe** 21885-L31210			Exon 4		21-038,697
322	**CDKN2A probe** 21890-L22800				9p21.3	09-021,965
329	Reference probe 03918-L30417	15q21				15-046,585
336	**ERG probe** 02833-L30411			Exon 2		21-038,739
343	**IKZF1 probe** 13869-L30647		Exon 7			07-050,427
352	**ERG probe** 20881-L22666			Exon 1		21-038,792
358	**IKZF1 probe** 21896-L30655		Exon 5			07-050,418
366 «	**CEP170B probe** 21897-L30656				14q32.33	14-104,423
373	**ERG probe** 18157-L31067			Exon 5		21-038,696
382	**ERG probe** 18158-L22668			Exon 8		21-038,685
389 ¥	Reference probe 08835-L33177	2p13				02-071,755
395 « Ø	**IKZF1 probe** 21883-L17250		Intron 1			07-050,319
402 Ŭ	**IKZF1 probe** 21898-L31074		Intron 3			07-050,405
409	**CDKN2B probe** 03814-L03851				9p21.3	09-021,999
417	Reference probe 03073-L15904	5p15				05-011,785
423	**IKZF1 probe** 21886-L31075		Exon 4			07-050,412
427 «	**MTA1 probe** 14071-L31076				14q32.33	14-105,002
436	Reference probe 13809-L31211	5q14				05-090,110
443	Reference probe 12790-L31073	2q13				02-108,880

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				Location (hg18) in kb
		Reference	*IKZF1*	*ERG*	Other	
450 ¥ «	**CRIP2 probe** 21901-L33192				14q32.33	14-105,011
459 ð	**IKZF1 probe** 21903-L31254		Intron 3			07-050,403
468	Reference probe 13538-L31070	19p13				19-013,207
475	**ERG probe** 18159-L22669			Exon 9		21-038,685
481	**ERG probe** 18160-L22670			Upstream		21-038,879
489 ¥	**IKZF1 probe** 21904-L33176		Upstream			07-050,285
497	**ERG probe** 19022-L25058			Exon 6		21-038,694
504	Reference probe 15203-L22928	3p12				03-081,775

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

¥ Changed in version C2. Minor alteration, no change in sequence detected.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. CNA of only the flanking or reference probes are unlikely to be related to the condition tested.
« 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.
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.
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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. ***IKZF1*** (7p12.2)

Length (nt)	MLPA probe	Gene/ exon[a]	Chromosomal band (hg18) / Ligation site[b] NM_006060.6	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
160 ¬	15408-L17236	*ZPBP*	7p12.2	CCACACGTGTTA-TGTGTAACGCAA	193.0 kb
489	21904-L33176	Upstream	30 kb upstream	TCATGTTCACAA-AATCTTGGGCAT	29.3 kb
129 «	21892-L30651	Upstream	539 nt before exon 1, reverse; NM_001291839.2: 63 nt after exon 1, reverse	GAAAACTTTGCA-ATCGCGCCGGGC	0.2 kb
148 «	17215-L31078	Upstream	316 nt before exon 1	GCAGGTCGAGCA-GGGACCGCCAGC	0.5 kb
269 «	13877-L15918	Exon 1	187-188	TCTTGGCCCCAA-AGCGCGACGCAC	4.0 kb
395 « Ø	21883-L17250	Intron 1	3.9 kb nt after exon 1; NM_001291837.2: 172-173	TGGAAAAGGCAG-CTCTCACTTGGC	10.1 kb
308 «	21895-L30654	Exon 2	13 nt before exon 2, reverse	TGAGAAAGAGAG-GAAGGGATTTTA	0.1 kb
213 «	14056-L20508	Exon 2	253-254	AGACATGTCCCA-AGTTTCAGGTGA	8.6 kb
231	15416-L17244	Exon 3	288-289	CTGTAAGCGATA-CTCCAGATGAGG	0.1 kb
236	21893-L30652	Exon 3	362-361, reverse	CTGTCACTCTTG-GAGCTTTGCTGT	65.4 kb
459 ð	21903-L31254	Intron 3	8.4 kb before exon 4; NM_001291845.2: 498-499	TCCTATCATGTA-AATATCGTACGT	1.4 kb
402 Ʊ	21898-L31074	Intron 3	7.0 kb before exon 4; NM_001291846.2: 469-470	ATCTTCTCACAC-AAGCGGCTACTT	7.0 kb
202	15424-L17583	Exon 4	Intron 3-382	TGTTTCTTTCAG-CCAGTAATGTTA	0.2 kb
423	21886-L31075	Exon 4	590-591	GATATCTGTGGG-ATCATTTGCATC	5.8 kb
142	21511-L30640	Exon 5	680-681	TGCGGGGCCTCA-TTCACCCAGAAG	0.1 kb
358	21896-L30655	Exon 5	797-796, reverse	GAGTGCGTCCTC-AGGTGGCCAGTG	4.7 kb

Length (nt)	MLPA probe	Gene/ exon[a]	Chromosomal band (hg18) / Ligation site[b] NM_006060.6	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
256	15426-L17587	Exon 6	812-813	TTTTCTGCAGTT-GGTAAACCTCAC	0.1 kb
190	15409-L30639	Exon 6	897-898	GCTGCCACAACT-ACTTGGAAAGCA	4.3 kb
172	15410-L31063	Exon 7	26 nt before exon 7	AAGCCTTTCTAA-ACTGGCCTCTCT	0.1 kb
343	13869-L30647	Exon 7	994-995	CAAGATAGGATC-AGAGAGATCTCT	8.5 kb
154	15407-L17580	Exon 8	1441-1442	CAACGAGGAGCA-GCGCAGCGGTCT	0.8 kb
288	17109-L20256	Exon 8	2271-2272	GGTGTGCCGCCA-CCCAAGTGCCAA	44.9 kb
226 ¬	20418-L28594	*FIGNL1*	7p12.2	AAAGCCACCATA-AAGGAAATAGTT	

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

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

Table 2b. ***CDKN2A/2B*** (9p21.3)

Length (nt)	MLPA probe	Gene/ exon[a]	Ligation site[b]	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
262	15675-L18954	***CDKN2A*** Downstream	NM_000077.5/NM_058195.4: 182 nt after exon 3	TGAAATGCGGTT-AAAATGATGAAT	7.4 kb
322	21890-L22800	***CDKN2A*** Exon 1	NM_000077.5: 71 nt before exon 1; NM_058195.4: 3.7 kb before exon 2	GCACCGGAGGAA-GAAAGAGGAGGG	33.9 kb
409	03814-L03851	***CDKN2B*** Exon 1	NM_004936.4; 462-463	CCTGGAAGCCGG-CGCGGATCCCAA	

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

Table 2c. **14q32.33**

Length (nt)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
14q32.33 region 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
450 «	21901-L33192	***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 a high number of variations is identified in healthy control samples, see further details in DGV database. Aberrant results should be treated with caution.

Table 2d. ***ERG*** (21q22.2)

Length (nt)	MLPA probe	Gene/ exon[a]	Chromosomal band (hg18) / Ligation site[b] NM_182918.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
124 ¬	21195-L31204	*KCNJ6*	21q22.13	AGCTCCTACATC-ACCAGTGAGATC	757.6 kb
184	18152-L22662	Exon 10	1462-1463	TACTGGAATTCA-CCAACTGGGGGT	7.5 kb
475	18159-L22669	Exon 9	991-992	ATTCTTGGACCA-ACAAGTAGCCGC	0.7 kb
382	18158-L22668	Exon 8	931-932	CCATCTCCTTCC-ACAGTGCCCAAA	0.7 kb
250	18155-L22665	Exon 7	905-906	CCACGCCCCAGT-CGAAAGGTACAG	8.2 kb
497	19022-L25058	Exon 6	791-792	CTTTTATTTTCC-CAAATACTTCAG	2.0 kb
373	18157-L31067	Exon 5	702-703	TCCTCTTCCACA-TTTGACTTCAGA	0.9 kb
315	21885-L31210	Exon 4	632-633	ACTTCCAGAGGC-TCACCCCCAGCT	19.9 kb
301	21894-L31065	Exon 3	408-407 reverse	TGTAGCTGCCGT-AGTTCATCCCAA	22.1 kb
336	02833-L30411	Exon 2	152-153	ACCAGTCGTTGT-TTGAGTGTGCCT	52.9 kb
352	20881-L22666	Exon 1	23 nt before exon 1	TGGCTGACTTCA-TTTCCCAGACTT	77.2 kb
283	21887-L31064	Upstream	77 kb before exon 1; NM_001136154.1; 270-271	GCTTACTGAAGG-ACATGATTCAGA	9.2 kb
481	18160-L22670	Upstream	86 kb before exon 1; NM_001136154.1; 169-170	CGTGTTGACCAA-AAGCAAGACAAA	76.8 kb
177	18151-L22661	Upstream	163 kb before exon 1; NM_001136154.1; 76-77	CGCTCCGGGACG-GTCGTGACGGCC	151.4 kb
296 ¬	09515-L31066	*ETS2*	21q22.2	AAATGAAGAGCA-AACACTGCAAGA	

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

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

SNVs located in the target sequence of a probe can influence probe hybridisation 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)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)
136	13224-L31079	*COL11A1*	1p21	CAGATGGTGTCA-GAGGTCTCAAGG
389	08835-L33177	*DYSF*	2p13	GACTGAGAGCAA-AATCCCAGCACG
443	12790-L31073	*EDAR*	2q13	AGAATCAAGGCT-TTTGTGATATGT
196	07815-L30637	*SCN5A*	3p22	TGGTTCGAGACA-TTCATCATCTTC
504	15203-L22928	*GBE1*	3p12	GACCTAGAGGGA-CTCATGATCTTT
417	03073-L15904	*CTNND2*	5p15	CATCAGCCTCAG-AGAAGACGAGTT
436	13809-L31211	*ADGRV1*	5q14	ATGCGAGACGAA-CAGTCTGCAGTC
220	08940-L20509	*SLC6A5*	11p15	TTGCCTCTCAGG-TGTGGAAAGATG
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

Related products
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P202 product history	
Version	*Modification*
C2	Small changes in length but no changes in sequence detected.
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 C2-01 – 3 October 2025 (05P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1, Table 2 and Table 3). - List of positive samples is supplemented with DSMZ cancer cell lines. - New references added to 'Selected publications using P202 IKZF1-ERG' section. 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.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200