Product Description
SALSA® MLPA® Probemix P335-C2 ALL-IKZF1

To be used with the MLPA General Protocol.

Version C2
As compared to version C1, lengths of several probes are adjusted but no changes in the sequences detected. For complete product history see page 15.

Catalogue numbers:
- **P335-025R:** SALSA MLPA Probemix P335 ALL-IKZF1, 25 reactions.
- **P335-050R:** SALSA MLPA Probemix P335 ALL-IKZF1, 50 reactions.
- **P335-100R:** SALSA MLPA Probemix P335 ALL-IKZF1, 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.

Intended purpose
The SALSA MLPA Probemix P335 ALL-IKZF1 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative assay[2] for the detection of deletions of the *IKZF1* gene for stratification of patients with acute lymphoblastic leukemia (ALL) into prognostic subgroups. The SALSA MLPA Probemix P335 ALL-IKZF1 is a RUO assay[2] for the detection of deletions or duplications in B-cell differentiation and cell cycle control genes (*EBF1*, *CDKN2A/B*, *PAX5*, *ETV6*, *BTG1* and *RB1*) and in the PAR1 region. This assay is for use on genomic DNA isolated from human peripheral whole blood and bone marrow specimens.

Copy number alterations (CNAs) detected with P335 ALL-IKZF1 should be confirmed with a different technique. In particular, CNAs detected by only a single probe always require confirmation by another method. In the majority of patients, defects in the *IKZF1* gene are deletions, but point mutations can occur which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis of the *IKZF1* gene.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes or population screening.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Note that the clinical relevance of some genes in the P335 ALL-IKZF1 probemix is not yet fully established. Therefore, the **CE mark for diagnostic use only applies to the *IKZF1* gene**, and all other genes in the probemix are meant to be used in a research setting only.

Clinical background
The overall incidence rate of ALL amounts to 1.6 in 100,000 per year (Malard and Mohty 2020). The peak incidence lies in childhood at an age of less than 5 years; thereafter, the incidence rate declines continually until the age of 50 years. After that, incidence slightly rises a second time. In patients over 50 years it rises a second time and reaches another peak at the age of over 80 years). There is a slight predominance of males (1.2:1). B-cell ALL accounts for 75% of all cases of ALL and T-cell ALL accounts for the remaining 25% of cases (https://www.lls.org/leukemia/acute-lymphoblastic-leukemia/diagnosis/all-subtypes).

Partial or complete deletions of the *IKZF1* (IKAROS family zinc finger 1) gene are frequently detected in ALL cases (Mullighan et al. 2008), especially in those patients who also carry the *BCR-ABL1* gene fusion (Philadelphia chromosome). *IKZF1* deletions can be identified in approximately 70% of the children with Philadelphia chromosome-positive (Ph+) ALL (2-4% of all paediatric ALL cases), in 10-15% of Philadelphia chromosome-negative (Ph−) paediatric ALL, and in 40% of adult B-cell precursor ALL cases (Bernt and Hunger 2014; Lejman et al. 2022; van der Sligte et al. 2015). Deletion of *IKZF1* is associated with a poor prognosis in B-ALL patients (Mullighan et al. 2009a) and a higher chance of relapse (Kuiper et al. 2010).

Several other (partial) gene deletions and duplications, such as in *PAX5*, *ETV6*, *RB1*, *BTG1*, *EBF1* and *CDKN2A/2B*, have also been described in ALL patients. Prognostic profiles combining these aberrations, such as the *IKZF1*^{plus} profile (*IKZF1* deletions that co-occur with deletions in *CDKN2A*, *CDKN2B*, *PAX* or PAR1 in the absence of *ERG* deletion; Stanulla et al. 2018), have been described in recent years. See Table 2 for more information on all genes and regions covered in this probemix.

Gene structure
The *IKZF1* gene, located on chromosome 7p12.2, spans ~86 kb of genomic DNA and contains 8 exons. The *IKZF1* MANE select transcript sequence is available at https://www.ncbi.nlm.nih.gov/refseq/MANE/ and is identical to NM_006060.6.

Transcript variants
For *IKZF1,* the longest isoform, also known as Ik-1 as described by Ezzat et al. (2003) is encoded by transcript variant NM_006060.6 (6255 nt; coding sequence 222-1781; http://www.ncbi.nlm.nih.gov/gene/10320). This is the MANE Select transcript sequence and also a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 8. In addition to this major transcript, multiple other minor transcript variants have been described.

Exon numbering
The *IKZF1*exon numbering used in this P335-C2 ALL-IKZF1 product description is the exon numbering from the MANE Select transcript sequence (https://www.ncbi.nlm.nih.gov/refseq/MANE/). From product description version C2-02 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. Exon numbering used here may differ from literature.

Probemix content
The SALSA MLPA Probemix P335-C2 ALL-IKZF1 contains 57 MLPA probes with amplification products between 120 and 504 nucleotides (nt). This includes one probe for each of the eight exons of the *IKZF1* gene (7p12.2). Furthermore, this probemix also contains seven probes for *PAX5* (9p13.2), six probes for *ETV6* (12p13.2), five probes for *RB1* (13q14.2), four probes for *BTG1* and the *BTG1* downstream region (12q21.33), four probes for *EBF1* (5q33.3), three probes for *CDKN2A*/*CDKN2B* (9p21.3) and five probes for the Xp22.33 region (PAR1 region; *SHOX* area, *CRLF2*, *CSF2RA*, *IL3RA* and *P2RY8* genes). In addition, one probe at Yp11.31 (*ZFY*) and one probe at 9p24.1 (*JAK2*) have been included to help determine the extent of a deletion/duplication detected in patient samples. See page 6 and 7 for more information about interpretation of results of the *ZFY* probe. Finally, 13 reference probes are included that target relatively copy number stable regions 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 male, 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 from peripheral whole blood or bone marrow, 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. The minimum percentage of tumour cells required for reliable analysis is 30% (Al Zaabi et al. 2010, Coll-Mulet et al. 2008). We advise using tumour samples with at least 50% tumour cell content. Therefore, tumour samples should be evaluated by a pathologist before the extraction of DNA.

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. For example, when testing DNA samples derived from bone marrow, use reference samples from bone marrow in the same experiment for optimal data normalisation. Reference samples should be derived from healthy **male** individuals who are from families without a history of ALL. For more information on the need of male reference samples refer to section P335 specific note below. 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. The quality of cell lines can change; therefore samples should be validated before use.

The following cell lines available from Coriell and DSMZ have been tested with P335-C2 probemix and can be used as positive DNA samples:

Sample name	Source	Chromosomal position of CNA*	Altered target (and flanking) genes in P335-C2	Expected copy number alteration
NA01353	Coriell Institute	Xp22.33-PAR1 region	*SHOX, CRLF2, CSF2RA* (part of PAR1 region)	Heterozygous deletion
NA01750	Coriell Institute	9p21.3-p24.1	*JAK2, CDKN2A, CDKN2B*	Heterozygous duplication
NA04371	Coriell Institute	5q33.3	*EBF1*	Heterozygous duplication
		Xp22.33-PAR1 region	*CSF2RA*	Heterozygous duplication
NA05067	Coriell Institute	9p13.2-p24.1	*JAK2, CDKN2A, CDKN2B, PAX5* (all exons)	Heterozygous duplication
NA07081	Coriell Institute	7p12.2	*IKZF1* (all exons)	Heterozygous duplication
NA07981	Coriell Institute	12p13.2	*ETV6* (all exons)	Heterozygous triplication/homozygous duplication
NA09403	Coriell Institute	Xp22.33-PAR1 region	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8* (PAR1 region)	Heterozygous deletion
NA10925	Coriell Institute	7p12.2	*IKZF1*	Heterozygous deletion
		Xp22.33-PAR1 region	*CRLF2, CSF2RA* (part of PAR1 region)	Heterozygous duplication
NA12606	Coriell Institute	13q14.2	*RB1*	Heterozygous duplication
NA12722 ◊	Coriell Institute	9p21.3-p24.1	*JAK2, CDKN2A, CDKN2B*	Heterozygous duplication
NA14164	Coriell Institute	13q14.2	*RB1*	Heterozygous deletion
BV-173 ◊	DSMZ	7p12.2	*IKZF1* (exons 1-7)	Heterozygous deletion
		9p21.3	*CDKN2A* (exon 4)	Heterozygous deletion
		9p21.3	*CDKN2A* (exon 2), *CDKN2B* (exon 2)	Homozygous deletion
		Xp22.33-PAR1 region	PAR1 region	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P335-C2 ALL-IKZF1 probemix.

◊ In this indicated cell line sample some of the reference probes are also affected by CNAs.

Performance characteristics

IKZF1 deletions can be identified in approximately 70% of children with *BCR-ABL1* positive (Philadelphia chromosome, Ph+) ALL (2% of all pediatric ALL cases), in 10-15% of Philadelphia chromosome negative (Ph−) pediatric ALL cases and in 40% of adult B-ALL cases.

Genomic deletions in *IKZF1* are either whole gene deletions (25-50% of all *IKZF1* deletions; Mullighan et al. 2009a; Palmi et al. 2013) or intragenic deletions, with the most frequent intragenic deletion (exons 4-7) comprising 30-55% of all deletions (Kastner et al. 2013, Iacobucci et al. 2009). The analytical sensitivity and specificity for the detection of deletions or duplications in the *IKZF1* gene is very high and can be considered >99%.

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for *IKZF1* gene specific MLPA probes are allele copy number of 2 (normal), 1 (heterozygous deletion) and 0 (homozygous deletion). Duplication of the *IKZF1* gene is not expected in ALL patient samples, except in the context of larger duplications within or of chromosome arm 7p (observed in less than 5% of ALL cases according to the Progenetix.org database). Duplication of *IKZF1* is not expected to impact patient stratification.

For all other (pseudo)autosomal genes in the P335 ALL-IKZF1 probemix, the expected results are allele copy number of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous gain) or ≥4 (heterozygous triplication/homozygous gain). More information on expected deletions or duplications per gene can be found in Table 2.

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

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous gain	1.75 < FR < 2.15
Ambiguous	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 affected both by percentage of tumour cells and by possible subclonality.

In case of a heterozygous deletion that is subclonal and/or the sample contains a lower percentage of tumour cells, the FR might not be in the expected range of 0.40-0.65 (as indicated in the table above). For example, in case of ~50% tumour cell content or a copy number alteration present in ~50% of the tumour cells, the FR will be ~0.75. However, the same FR (0.75) will also be found for a sample with a tumour cell percentage of 25% or a subclone comprising 25% of all tumour cells that harbours a homozygous deletion. The MLPA technique cannot discriminate between these two scenarios.

- 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 in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich 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: 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.

P335 specific note:

In this probemix, the 120 nt Y-specific target probe (*ZFY*) is included as a flanking probe to help determine the extent of a deletion in the PAR1 region and to detect loss of the whole Y chromosome in male samples. To ensure that the comparative analysis can be completed for all samples, **only male reference samples must be used**. The comparative analysis will fail if female reference samples are used.

- In male samples, the 120 nt Y-specific target probe will be counted as an "additional" probe when using Coffalyser.Net software (see Figure 1). In healthy male individuals a final ratio of ~1 will be obtained for the Y-specific target probe after the comparative analysis. If the Y chromosome is lost in male samples, rather than deletion of the PAR1 region only, this can also be confirmed by visual examination of the 105 nt Y-fragment peak in the electropherogram (fragment analysis).
- In female samples, the 120 nt Y-specific target probe will be reported as "absent", i.e. will have a final ratio of 0, after the comparative analysis.

sample name	sample type	bin smpl	FRSS	FMRS	probes	DNA	DD	X	Y
Reference sample 1	reference	☐	✓	✓	57/57	✓	✓	✓	✓
Reference sample 2	reference	☐	✓	✓	57/57	✓	✓	✓	✓
Reference sample 3	reference	☐	✓	✓	57/57	✓	✓	✓	✓
Sample	sample	☐	✓	✓	56/56	✓	✓	✓	●
noDNA	no DNA	☐	✓		0/0	●	●	●	●

Figure 1. Example fragment analysis overview in Coffalyser.Net with three male reference samples, a female test sample and a no DNA reaction. Note that the number of detected/expected probes differs between male (57/57) and female (56/56) samples.

Limitations of the procedure

- In the majority of patients, defects in the *IKZF1* gene are deletions, but point mutations can occur which will not be detected using SALSA MLPA Probemix P335 ALL-IKZF1.
- 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.

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.

***IKZF1* mutation databases**

https://databases.lovd.nl/shared/genes/IKZF1, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=IKZF1, and http://cancer.sanger.ac.uk/cosmic/.

We strongly encourage users to deposit positive results in one of these databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a deletion of *IKZF1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P335-C2 ALL-IKZF1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
120 ¬	**ZFY probe** S0135-L27684		Yp11.31	Y-002.889
124	Reference probe 15370-L13762	7q11		07-075.448
130	Reference probe 13867-L15385	16p13		16-008.765
136	**CRLF2 probe** 13889-L15427		Xp22.33-PAR1 region	X-001.281
142	**IKZF1 probe** 21511-L30001		7p12.2	07-050.418
149 ¥	**SHOX-area probe** 05648-L32510		Xp22.33-PAR1 region	X-000.771
157 ¥ ¬	**JAK2 probe** 07452-L32509		9p24.1	09-005.113
161 ¥	**PAX5 probe** 12501-L32513		9p13.2	09-037.024
166	**CSF2RA probe** 13892-L16221		Xp22.33-PAR1 region	X-001.374
172	**PAX5 probe** 14647-L15394		9p13.2	09-037.011
178 ¬	**BTG1-area probe** 18021-L22630		12q21.33	12-090.658
184	**P2RY8 probe** 17837-L15740		Xp22.33-PAR1 region	X-001.545
192 ¥	Reference probe 06941-L32511	11q12		11-061.484
196	**ETV6 probe** 17838-L22035		12p13.2	12-011.936
202	**PAX5 probe** 17839-L22036		9p13.2	09-036.957
208 «	**IKZF1 probe** 14056-L15654		7p12.2	07-050.329
214 ¥	Reference probe 13265-L32718	1p21		01-103.204
220	**RB1 probe** 01782-L01346		13q14.2	13-047.821
226	**EBF1 probe** 12509-L13559		5q33.3	05-158.459
232	**BTG1 probe** 21378-L30126		12q21.33	12-091.063
239	**CDKN2B probe** 16059-L30167		9p21.3	09-021.996
244	**ETV6 probe** 13874-L17160		12p13.2	12-011.883
252	**CDKN2A probe** 10333-L30127		9p21.3	09-021.965
258	Reference probe 04534-L22019	2q24		02-166.606
264	**IKZF1 probe** 13873-L15917		7p12.2	07-050.412
269 «	**IKZF1 probe** 13877-L15918		7p12.2	07-050.315
274	**PAX5 probe** 17840-L22037		9p13.2	09-036.872
282	**PAX5 probe** 13870-L15920		9p13.2	09-036.993
288	**IKZF1 probe** 17109-L20256		7p12.2	07-050.436
295 +	Reference probe 10435-L22110	9q34		09-136.850
301 «	**ETV6 probe** 14058-L15656		12p13.2	12-011.694
309	**CDKN2A probe** 17814-L22631		9p21.3	09-021.958
315	**RB1 probe** 01789-L22025		13q14.2	13-047.851
324	Reference probe 03918-L20270	15q21		15-046.585
330	**BTG1 probe** 12553-L22632		12q21.33	12-091.062
337	**PAX5 probe** 17841-L22038		9p13.2	09-036.913
344 ¥	**IKZF1 probe** 13869-L32515		7p12.2	07-050.427
351	**IL3RA probe** 13907-L22294		Xp22.33-PAR1 region	X-001.416
358	**RB1 probe** 01792-L22295		13q14.2	13-047.928
364	Reference probe 14675-L16327	3p25		03-010.142
372	**EBF1 probe** 14059-L30509		5q33.3	05-158.137
379	**IKZF1 probe** 15427-L22113		7p12.2	07-050.338
389 ¥ «	**ETV6 probe** 14060-L32514		12p13.2	12-011.694
394	**PAX5 probe** 17842-L22633		9p13.2	09-036.830
401	**ETV6 probe** 13875-L22014		12p13.2	12-011.797
409 ¬	**BTG1-area probe** 18022-L22363		12q21.33	12-091.006
418	Reference probe 10063-L30260	8q22		08-100.251
427	**RB1 probe** 01797-L16909		13q14.2	13-047.945
436	**EBF1 probe** 13868-L22053		5q33.3	05-158.072
445	**RB1 probe** 01799-L01362		13q14.2	13-047.949
454	Reference probe 18691-L02476	5p15		05-009.094

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
463 ¥	**EBF1 probe** 23047-L32673		5q33.3	05-158.058
470	**IKZF1 probe** 14061-L22112		7p12.2	07-050.422
478	Reference probe 14846-L22111	3q11		03-099.783
485	**ETV6 probe** 13871-L22009		12p13.2	12-011.914
494	Reference probe 15203-L16978	3p12		03-081.775
504	Reference probe 09870-L19465	2p15		02-061.126

¥ Changed in version C2. 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
± SNP rs556167410 could influence the 295 nt probe signal.

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. P335-C2 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
EBF1 gene, 5q33.3. Indicated locations are according to NM_001290360.3 and exon numbering according to MANE Select transcript. *EBF1* deletions are suggested to be an important factor in the relapse of ALL as 25% of relapsed ALL cases have deletions in the *EBF1* locus (Yang et al. 2008). Deletions of *EBF1* are suggested to be associated with a higher risk of relapse (Olsson et al. 2014).					
463	23047-L32673	***EBF1***, ex 16	3043-3044	ATGGCCTCTCGA-GAGCTTGGTGGC	14.0 kb
436	13868-L22053	***EBF1***, ex 14	1888-1889	CCAACTATAACT-CCGTCACCACGA	65.2 kb
372	14059- L30509	***EBF1***, ex 10	1416-1417	GTTGTGGAAGTC-ACACTGTCCTAC	322.2 kb
226	12509-L13559	***EBF1***, ex 1	278-279	ATTTGCTTTCCA-GCCCGCCTTGAT	–
IKZF1 gene, 7p12.2. Indicated locations are according to NM_006060.6 and exon numbering according to MANE Select transcript. Exon numbering is different from the exon numbering used by Mullighan et al. (2009a), where exon 3-6 deletions correspond to exon 4-7 in the MANE exon numbering. Deletions or mutations of *IKZF1* are significantly associated with an increased risk of relapse and adverse events in ALL patients (Mullighan et al. 2009a; Martinelli et al. 2009; Iacobucci et al. 2009; Stanulla et al. 2020). In the absence of *ERG* deletions and in combination with *CDKN2A, CDKN2B, PAX5 and* PAR1 deletions, deletions of *IKZF1* (IKZF1^{plus} profile) are associated with a very poor prognosis (Stanulla et al. 2018). Additional IKZF1 probes as well as probes for the *ERG* gene are present in the P202 IKZF1-ERG probemix.					
269 «	13877-L15918	***IKZF1***, ex 1	187-188	TCTTGGCCCCAA-AGCGCGACGCAC	14.2 kb
208 «	14056-L15654	***IKZF1***, ex 2	253-254	AGACATGTCCCA-AGTTTCAGGTGA	8.6 kb
379	15427-L22113	***IKZF1***, ex 3	355-356	GGGAGGACAGCA-AAGCTCCAAGAG	74.0 kb
264	13873-L15917	***IKZF1***, ex 4	477-478	TACGAATGCTTG-ATGCCTCGGGAG	5.9 kb
142	21511-L30001	***IKZF1***, ex 5	680-681	TGCGGGGCCTCA-TTCACCCAGAAG	4.8 kb
470	14061-L22112	***IKZF1***, ex 6	812-813	TTTTCTGCAGTT-GGTAAACCTCAC	4.4 kb
344	13869-L32515	***IKZF1***, ex 7	994-995	CAAGATAGGATC-AGAGAGATCTCT	9.3 kb
288	17109-L20256	***IKZF1***, ex 8	2271-2272	GGTGTGCCGCCA-CCCAAGTGCCAA	25 **Mb** to *POR* (reference probe)
CDKN2A/CDKN2B genes, 9p21.3. Exon numbering of *CDKN2A* is based on the MANE Select transcript (NM_000077.5, p16^{INK4a}) and the MANE Plus Clinical transcript (NM_058195.4, p14^{ARF}). The *CDKN2A* exon numbering has been changed; the exon numbering (LRG) used in previous versions of this product description can be found in between brackets. Deletions of *CDKN2A* are present in 50-81% of T-ALL, in 15-35% of childhood B-ALL and in 30-45% of adult B-ALL cases (Gonzáles-Gil et at. 2021). *CDKN2A/2B* deletions are more frequently found in high-risk patients of all ages, and the majority of studies on relapse ALL suggests that (homozygous) *CDKN2A/2B* deletions are more frequent at relapse than at diagnosis. In combination with *IKZF1* deletions and in the absence of *ERG* deletions (IKZF1^{plus} profile), deletions of the *CDKN2A* and/or *CDKN2B* genes are associated with a very poor prognosis (Stanulla et al. 2018). Additional CDKN2A/2B probes are present in the P419 CDKN2A/2B-CDK4 and ME024 9p21 probemixes. The latter probemix detects both copy number and methylation changes of the *CDKN2A/2B* genes.					

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
157 ¬	07452-L32509	***JAK2***	9p24.1	GAATCACTGACA-GAGAGCAAGTTT	17 **Mb**
309	17814-L22631	***CDKN2A***, ex 3 (4)	NM_000077.5 830-831; NM_058195.4; 904-905	TTGCGAGCCTCG-CAGCCTCCGGAA	7.1 kb
252	10333- L30127	***CDKN2A***, ex 1 (2)	NM_000077.5; 138 nt before exon 1; NM_058195.4; 3.8 kb before exon 2	GCCTGGAAAGAT-ACCGCGGTCCCT	30.9 kb
239	16059- L30167	***CDKN2B***, ex 2	9p21.3	GCCTGTCTGAGA-CTCACAGGAAGG	15 **Mb** to *PAX5*
PAX5 gene, 9p13.2. Indicated locations are according to NM_016734.3 and exon numbering according to MANE Select transcript. *PAX5* deletions in T-ALL are large, involving other genes and sometimes even extending into the *CDKN2A/2B* genes (Schwab and Harrison 2018). *PAX5* deletions are present in 30% of B-progenitor ALL cases and in 50% of Ph+ ALL (Lejman et al. 2022; Li et al. 2021). Moreover, intragenic *PAX5* amplifications were suggested to be present in a new subgroup of B-cell precursor ALL (~1%) and in 3% of B-other ALL cases, which is associated with poor outcome (Schwab et al. 2017). In combination with *IKZF1* deletions and in the absence of *ERG* deletions (IKZF1^{plus} profile), deletions of the *PAX5* gene are associated with a very poor prognosis (Stanulla et al. 2018).					
394	17842-L22633	***PAX5***, ex 10	2018-2019	CTCCTTCTTTAG-TATCTTTACGAG	42.0 kb
274	17840-L22037	***PAX5***, ex 8	2 nt after exon 8	ATGGTGCCTGGT-GAGTTTGCACTG	41.4 kb
337	17841-L22038	***PAX5***, ex 7	1108-1109	CTGACATCGGGA-GCAGTGTGCCAG	43.2 kb
202	17839-L22036	***PAX5***, ex 6	968-969	GTTTGAGAGGCA-GCACTACTCAGA	36.1 kb
282	13870-L15920	***PAX5***, ex 5	756-757	GTGAGCACGGAT-TCGGCCGGCTCG	18.0 kb
172	14647-L15394	***PAX5***, ex 2	378-379	CTTGCTCATCAA-GGTGTCAGGCCC	13.6 kb
161	12501-L32513	***PAX5***, ex 1	43 nt before exon 1	CATCTTGTGATG-TTGGCGAGAACA	100 **Mb** to *COL5A1 (reference probe)*
ETV6 gene, 12p13.2. Indicated locations are according to NM_001987.5 and exon numbering according to MANE Select transcript. *ETV6* deletions are more frequent in B-ALL (51%) as compared to T-ALL (4%), and *ETV6* is often involved in rearrangements and fusions detected in ALL patients (e.g. in *ETV6-RUNX1* fusions) (Schwab et al. 2013). It is suggested that native *ETV6* deletions in *ETV6-RUNX1+* childhood ALL is associated with better prognosis (Ko et al. 2011). Although microdeletions often occur at the translocation breakpoints, this MLPA probemix will not detect all microdeletions in which *ETV6* is involved.					
301 «	14058-L15656	***ETV6***, ex 1	366-367	AATGACCGCGTC-TGGCTGGCCGTG	0.1 kb
389 «	14060-L32514	***ETV6***, ex 1	480-481	TGCTCAGTGTAG-CATTAAGGTAAA	102.4 kb
401	13875-L22014	***ETV6***, ex 2	563-564	TTCATGTTCCAG-TGCCTCGAGCGC	86.6 kb
244	13874-L17160	**ETV6**, ex 3	641-642	TTTACTGGAGCA-GGGATGACGTAG	30.3 kb
485	13871-L22009	***ETV6***, ex 5	967-968	AATGTGCACCAT-AACCCTCCCACC	22.3 kb
196	17838-L22035	***ETV6***, ex 8	2530-2531	AGTCTTGGGGAT-TGTTGGCACCTA	79 **Mb** to *BTG1*
BTG1 gene, 12q21.33. Indicated locations are according to NM_001731.3 and exon numbering according to MANE Select transcript. *BTG1* deletions appeared to be more frequent in high risk ALL cases and are often combined with other deletions (Fang et al. 2018). *BTG1* deletions are detected with high frequency in ALL patients with Down syndrome (Lundin et al. 2012). As many deletions of *BTG1* extend into the centromeric area of *BTG1* into a gene poor region (Waanders et al. 2012), two downstream probes were included for the *BTG1*-area.					
178 ¬	18021-L22630	***BTG1*-area**	Downstream of *BTG1*	CACTAAAAATGT-GCATACTTCAAC	348.3 kb
409 ¬	18022-L22363	***BTG1*-area**	Downstream of *BTG1*	TGGAAAATGGGA-ATGTTCAGGGTG	55.7 kb
330 #	12553-L22632	***BTG1***, ex 2	1074-1075	TTGCTAGGGAGG-GAAGTCCTAGGG	1.6 kb
232	21378-L30126	***BTG1***, ex 1	394-395	GTTTCTCCGCAC-CAAGGGGCTCAC	–
RB1 gene, 13q14.2. Indicated locations are according to NM_000321.3 and exon numbering according to MANE Select transcript. *RB1* deletions have been reported to be more frequent in high risk ALL as compared to non-selected cases and often co-occur with other CNAs, in particular iAMP21 (Fang et al. 2018; Schwab et al. 2013; Steeghs et al. 2019). Deletions often involve one or more of the last 10 exons of this 27-exon gene (Schwab et al. 2013). Additional RB1 probes are available in the P047 RB1 probemix.					
220	01782-L01346	***RB1***, ex 6	83 nt after exon 6	ATTCCCCAATTT-TTATTGAGTAAT	30.2 kb

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
315	01789-L22025	***RB1***, ex 14	268 nt before exon 14	GCTTTTGTGTTG-TCTTGGCGGCCA	76.9 kb
358	01792-L22295	***RB1***, ex 19	2041-2042	ATTCTACTGCAA-ATGCAGAGACAC	16.8 kb
427	01797- L16909	***RB1***, ex 24	265 nt before exon 24	GAAACTTGCCTT-TGCCCTCCCTAA	3.8 kb
445	01799-L01362	***RB1***, ex 26	2852-2853	AGAGTCCAAATT-TCAGCAGAAACT	–
Xp22.33 / Yp11.32 (PAR1) region. With exception of the ZFY probe, all these probes target the PAR1 region, which is present in two copies per cell, irrespective of gender. This region shows recurrent aberrations in ALL (Harvey et al. 2010). *CSF2RA*/*IL3RA* deletions are frequent in ALL (7% of ALL cases; up to 55% in Down syndrome-associated ALL). Focal deletions within the *P2RY8* and *CRLF2* genes, resulting in a fusion gene, are associated with a poor prognosis (Mullighan et al. 2009b). In combination with *IKZF1* deletions and in the absence of *ERG* deletions (IKZF1^{plus} profile), deletions in the PAR1 region (deletion of *CSF2RA* and *IL3RA* and retention of the CRLF2 probe associated with *P2RY8-CRLF2* fusion) are associated with a very poor prognosis (Stanulla et al. 2018). Please note that loss in signal may also be due to the loss of the Y chromosome in male samples, which can be detected by comparing the PAR1 results to the ZFY probe at 120 nt and to the Y-specific control fragment at 105 nt. The SHOX-area probe is located in between the *SHOX and CRLF2* genes, to enable determination of the extent of a deletions within the PAR1 region. More probes for the PAR1 region are included in the P329 CRLF2-CSF2RA-IL3RA and P018 SHOX probemixes.					
149	05648-L32510	***SHOX*-area**	Xp22.33	TGGTGCTGAAAT-GAGGAAGCCCTG	510.7 kb
136	13889-L15427	***CRLF2***	Xp22.33	GGATCTCCTCTA-TGAGGTTCAGTA	93.0 kb
166	13892-L16221	***CSF2RA***	Xp22.33	TTTCACTTACCA-GTAGGTTTTCCG	41.4 kb
351	13907- L22294	***IL3RA***	Xp22.33	GGAAGATATCAG-AAACATCCTAGG	129.4 kb
184	17837-L15740	***P2RY8***	Xp22.33	TTTACGCAAACA-TGTATTCCAGCA	1.3 **Mb**
120 ¬	S0135- L27684	***ZFY***	Yp11.31	TCATAGAGGAGG-ATGTTCAGTGCT	–

[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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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
214	13265-L32718	*COL11A1*	1p21	CCTCCTTAGGGA-TTTCAAGGCAAG	01-103.204
504	09870-L19465	*PEX13*	2p15	TGAGGATGACCA-TGTAGTTGCCAG	02-061.126
258	04534-L22019	*SCN1A*	2q24	ACTGTTCTCCAT-ATTGGTTAAAAG	02-166.606
364	14675-L16327	*BRK1*	3p25	GCAACACTAAAC-GAGAAATTGACA	03-010.142
494	15203-L16978	*GBE1*	3p12	GACCTAGAGGGA-CTCATGATCTTT	03-081.775
478	14846-L22111	*CPOX*	3q11	CAGAATTGAAAG-TATCTTGATGTC	03-099.783
454	18691-L02476	*SEMA5A*	5p15	GTCCATCACTGT-GTAGCTACCGTT	05-009.094
124	15370-L13762	*POR*	7q11	GATGGGAAGTGA-GTGCCCACCCTG	07-075.448
418	10063-L30260	*VPS13B*	8q22	TCTTTATGGGAA-ACTTCTGAAACT	08-100.252
295 ^ +	10435-L22110	*COL5A1*	9q34	AGGGCCTTCCAA-GCCGGCTTCTCC	09-136.850
192	06941-L32511	*BEST1*	11q12	GCACCAGGACCT-GCCTCGGATGGA	11-061.484
324	03918-L20270	*FBN1*	15q21	CCTACAGATGTG-AATGCTTCCCTG	15-046.585
130	13867-L15385	ABAT	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008.765

^ In comparison to focal chromosome 9p deletions, including the *JAK2*, *CDKN2A/2B* and *PAX5* genes, the COL5A1 probe at 9q34 is also affected in case of complete chromosome 9 deletions.

± SNP rs556167410 could influence the 295 nt probe signal.

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 all exons 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.
P327 iAMP21-ERG	Contains probes for *RUNX1*, *ERG* genes and iAMP21 detection in ALL.
P329 CRLF2-CSF2RA-IL3RA	Contains probes for *CRLF2*, *CSF2RA*, *IL3RA* and *SHOX* genes, involved in B-ALL.
P377 Hematologic malignancies	Contains probes for the most common copy number alterations 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.
D007 Acute Lymphoblastic Leukemia ^	digitalMLPA probemix that contains probes for 52 target genes and three chromosomal regions associated with ALL, including 16 probes for *IKZF1*.

* Please note that probemix P202 can only be used to obtain additional information, but cannot be used to confirm results of single probe aberrations detected in the *IKZF1* gene.

^ For probes that have different ligation sites than the P335 probes this probemix can be used for confirmation. This concerns the majority but not all of the IKZF1 probes in D007. For more information, please contact info@mrcholland.com.

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P335 product history	
Version	*Modification*
C2	Lengths of several probes are adjusted, but no changes in the sequences detected.
C1	One target probe and two reference probes replaced, length of several probes changed.
B2	Length of one probe changed.
B1	Several target probes replaced, one new probe included for *PAX5* exon 7 and two new flanking probes included for 9p and Yp chromosome arms. All reference probes replaced.
A4	The 88 and 96 nt DNA denaturation control fragments replaced.
A3	Length of two probes changed.
A2	Two reference probes replaced.
A1	First release.

Implemented changes in the product description

Version C2-02 – 03 March 2023 (04P)
- Section Clinical background and background information in Table 2 updated for multiple genes.
- In sections Gene structure, Exon numbering and in Table 2, exon numbering is now according to MANE. Exon numbering for *CDKN2A* is now MANE transcripts.
- Reference to the P335 specific note added to the section References samples.
- In section Interpretation of results, the term “duplication” was replaced with “gain” as this is the term commonly used in ALL diagnostics.
- P335 specific note rephrased to better explain the need for male reference samples and result interpretation.
- In section *IKZF1* mutation databases, link to LOVD database updated.
- Warning about SNP added for the 295 nt reference probe in Table 1 and 3.
- digitalMLPA probemix D007 added to the list of related probemixes.
- List of references updated.
- Various minor textual and layout changes.

Version C2-01 – 27 October 2021 (04P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Intended purpose adapted to a new template
- First point in “limitations of the procedure” adjusted to match text in intended use: “in the majority of patients, defects in the *IKZF1* gene are deletions, but point mutations can occur which will not be detected by MLPA”.
- Term "amplification" changed to "heterozygous triplication/homozygous duplication" to be consistent with Copy number status table in section Interpretation of results.
- Term "dosage quotient" changed to "final ratio"
- Sentence added to section Reference samples: “ For example, when testing DNA samples derived from bone marrow, use reference samples from bone marrow in the same experiment for optimal data normalisation.”

- All probemix and positive sample data presented are from internal tests with the P335-C2 version.
- Explanation added to Table 2 for location of the *SHOX* area probe.
- Footnote added to table "Related SALSA MLPLA probemixes" regarding the use of the P202 probemix.
- (More recent) references added in clinical background, Table 2, and sections References and Selected Publications
- Costa Rica added to countries where P335 is registered as in-vitro diagnostic device.
- UK has been added to the list of countries in Europe that accept the CE mark.
- Various minor textual or layout changes.

Version C1-03 – 04 November 2020 (02P)
- Positive sample information added on page 3.
- Ligation sites of the probes targeting the *PAX5*, *BTG1*, *ETV6*, *CDKN2A*, *RB1* and *EBF1* genes updated according to new version of the NM_ reference sequences.
- Probemix P018 SHOX added to related probemixes on page 11.
- Selected publications using P335 list shortened to highlight the most informative publications.
- Additional publication added in Table 2 to clarify rearrangements in the PAR1 region.
- Product description adapted to a new template.
- Intended use was updated to a new template.
- Israel added as countries with IVD status.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual or layout changes.

Version C1-02 – 17 January 2019 (04)
- Regulatory status section updated.
- In Table 2 and throughout the document, the NM sequence and the ligation sites for the *IKZF1* gene were updated according to NM_006060.6.
- Information and the name of the related mix P202-IKZF1 (IKAROS) has been changed to P202-IKZF1-ERG as this probemix has been revised (from version C1 onwards).

Version C1-01 – 21 March 2018 (04)
- Product description completely rewritten and adapted to a new template
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Small changes of probe lengths in Tables 1 and 2 in order to better reflect the true lengths of the amplification products.
- Added information about the 120 nt target-specific Y chromosome probe to improve data interpretation.
- Added information concerning the minimum percentage of tumour cells needed for reliable data analysis.
- Warning added to Table 2 for probe relying on its specificity on a single nucleotide difference between target and related gene or pseudogene.

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