Product Description
SALSA® MLPA® Probemix P329-B1 CRLF2-CSF2RA-IL3RA

To be used with the MLPA General Protocol.

Version B1
As compared to the previous A2 lot, one IL3RA probe and one flanking probe are removed, one flanking probe is replaced, the majority of the reference probes are revised, and several probes have a change in length but not in the sequence detected. For complete product history see page 9.

Catalogue numbers:
- **P329-025R:** SALSA MLPA Probemix P329 CRLF2-CSF2RA-IL3RA, 25 reactions.
- **P329-050R:** SALSA MLPA Probemix P329 CRLF2-CSF2RA-IL3RA, 50 reactions.
- **P329-100R:** SALSA MLPA Probemix P329 CRLF2-CSF2RA-IL3RA, 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 P329 CRLF2-CSF2RA-IL3RA is a **research use only (RUO)** assay for the detection of deletions or duplications in the *CRLF2, CSF2RA* and *IL3RA* genes, which are associated with B-cell acute lymphoblastic leukaemia (ALL).

The first 3 Mb of the X and Y chromosomes are homologous and are called the pseudoautosomal region 1 (PAR1) and among the genes in this region are *CRLF2, CSF2RA* and *IL3RA*. The chromosomal region containing these genes has been linked to lymphoid cell transformation in B-cell ALL. Frequent rearrangements in the PAR1 region are reported in ALL (Russell et al. 2009 and Mullighan et al. 2009), involving a deletion of the *IL3RA* and *CSF2RA* genes resulting in the overexpression of the *CRLF2* gene in 7% of individuals with B-progenitor ALL and 53% of individuals with ALL associated with Down syndrome. Deletions in the PAR1 region in ALL are an indication for poor prognosis (Mullighan et al. 2009), especially when combined with other gene deletions such as *IKZF1, CDKN2A, CDKN2B* and *PAX5* (Stanulla et al. 2018, Kiss et al. 2020).

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

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

Exon numbering
The *CSF2RA* exon numbering used in this P329-B1 CRLF2-CSF2RA-IL3RA product description is the exon numbering from the LRG_186 sequence. The *CRLF2* exon numbering is the exon numbering from the NG_034237.1, and *IL3RA* gene exon numbering is the exon numbering from the NM_002183.4 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always

correspond to the exon numbering obtained from the LRG or NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content

The SALSA MLPA Probemix P329-B1 CRLF2-CSF2RA-IL3RA contains 47 MLPA probes with amplification products between 124 and 494 nucleotides (nt). This includes five probes for *CRLF2*, 13 probes for *CSF2RA* and seven probes for *IL3RA* gene. Furthermore, this probemix also contains the following flanking probes: four probes telomeric from the *CRLF2* gene (for the *SHOX* gene and SHOX area) and four probes centromeric from the *IL3RA* gene (for *P2RY8, ZBED1* and *CD99* genes). In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types including acute lymphoblastic leukemia. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3.

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

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

MLPA technique

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

MLPA technique validation

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

Required specimens

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

Reference samples

A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals without a history of 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. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers indicated in the table below from the

Coriell Institute have been tested with this P329-B1 probemix at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position (hg18) of copy number alteration*	Altered target genes in P329-B1	Expected copy number alteration
NA03623	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	Heterozygous duplication
NA04626	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	Heterozygous duplication
NA09403	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	Heterozygous deletion
NA13019	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	Heterozygous deletion
NA14523	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	Heterozygous deletion
NA20027	Coriell Institute	Xp22.33/ Yp11.32	*SHOX, CRLF2, CSF2RA, IL3RA, P2RY8, ZBED1* and *CD99*	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 P329-B1 CRLF2-CSF2RA-IL3RA probemix.

Data analysis

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

Interpretation of results

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

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

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

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *IL3RA* 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.

P329 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, most genetic alterations in *CRLF2, CSF2RA* and *IL3RA* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P329 CRLF2-CSF2RA-IL3RA.
- 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 tumours with more chaotic karyotypes.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

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 *CSF2RA* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P329-B1 CRLF2-CSF2RA-IL3RA

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] [b]				
		Reference	*CRLF2*	*CSF2RA*	*IL3RA*	Other
64-105	Control fragments – see table in probemix content section for more information					
124 ¥	Reference probe 18709-L25925	5q31				
130 *	Reference probe 13867-L15385	16p13				
136	**CRLF2 probe** 13889-L15427		**Exon 4**			
142	**IL3RA probe** 13597-L15055				**Exon 6**	
149	**CSF2RA probe** 13890-L15428			**Exon 7**		
156 «	**IL3RA probe** 13891-L16341				**Exon 9**	
160 *	Reference probe 07394-L07041	12q13				
166	**CSF2RA probe** 13892-L16221			**Exon 10**		
179 *	Reference probe 13562-L15019	19p13				
184 ¬ ±	**SHOX-AREA probe** 06293-L06219					Xp22.33
191	**CSF2RA probe** 13894-L15432			**Exon 1**		
197	**CSF2RA probe** 13895-L16222			**Exon 13**		
202	**CRLF2 probe** 13896-L15434		**Exon 5**			
208 *	Reference probe 16261-L18553	20q11				
214 *	Reference probe 08940-L09035	11p15				
220	**CSF2RA probe** 13897-L15435			**Exon 9**		
232 «	**IL3RA probe** 13898-L16224				**Exon 7**	
238 ¬	**SHOX-AREA probe** 05650-L16223					Xp22.33
244 ¬	**P2RY8 probe** 14140-L15740					Xp22.33
250	**CRLF2 probe** 13899-L16225		**Exon 6**			
256 Ж	**CSF2RA probe** 13900-SP0138-L15438			**Exon 2a**		
265 ¥ ¬	**P2RY8 probe** 22820-L32443					Xp22.33
275	Reference probe 04489-L03878	1p34				
283	**CRLF2 probe** 13902-L15440		**Exon 3**			
292	**CSF2RA probe** 13903-L15441			**Exon 11**		
302	**IL3RA probe** 13904-L15442				**Exon 3**	
310 *	Reference probe 12783-L32182	2q13				
318	**CSF2RA probe** 13905-L15443			**Exon 4**		
328 «	**IL3RA probe** 13906-L15444				**Exon 8**	
337 ¥ ¬	**SHOX probe** 21538-L30066					Xp22.33
346	**IL3RA probe** 13907-L15445				**Exon 1**	
355	**CSF2RA probe** 13908-L15446			**Exon 8**		
364 ¬	**ZBED1 probe** 14142-L15742					Xp22.33
373 *	Reference probe 08831-L32190	2p13				
382	**CSF2RA probe** 13910-L15448			**Exon 14**		
392	**CRLF2 probe** 13911-L15449		**Exon 2**			
401 *	Reference probe 08544-L32189	3q24				
409 «	**IL3RA probe** 13912-L16228				**Exon 12**	
419	**CSF2RA probe** 13913-L15451			**Exon 5**		
427 *	Reference probe 06435-L27142	6p22				
436	**CSF2RA probe** 13915-L15453			**Exon 16**		
444 *	Reference probe 17129-L20312	11p11				
453 * ¬	**CD99 probe** 16859-L16226					Xp22.33
463	Reference probe 05950-L05394	2p22				
472 ¬	**SHOX-AREA probe** 14700-L16348					Xp22.33
481 ¥	**CSF2RA probe** 22821-L16342			**Exon 6**		
494 *	Reference probe 15203-L16978	3p12				

[a] **See section** Exon numbering on page 1 for more information.

[b] The homologous sequence of Xp22.33 band on X-chromosome corresponds to Yp11.32 band on Y-chromosome.

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.
± SNP rs143255564 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. CNAs 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 hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P329-B1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site / Chromosomal band[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
337 ¬	21538-L30066	*SHOX*	Xp22.33	ACAGCTAACCAC-CTAGACGCTTGC	233,5 kb
184 ¬ ±	06293-L06219	SHOX-AREA	Xp22.33	TAATTGATGAGA-TGCAGAAGCCAG	128,5 kb
238 ¬	05650-L16223	SHOX-AREA	Xp22.33	GAAATTCAGTTT-TAATAACACAGA	66,0 kb
472 ¬	14700-L16348	SHOX-AREA	Xp22.33	CTCTGGTGAGAT-GCCATCTAGAGA	325,2 kb
Telomeric from CRLF2 gene					
CRLF2 gene, Xp22.33. Indicated ligation sites are according to NM_022148.4.					
		end codon	*1129-1131 (Exon 8)*		
250	13899-L16225	Exon 6	696-697	CCTCCCAAACCA-AAGCTGTCCAAA	2,5 kb
202	13896-L15434	Exon 5	630-631	GACTGGTCAGAG-GTGACATGCTGG	3,9 kb
136	13889-L15427	Exon 4	458-459	GGATCTCCTCTA-TGAGGTTCAGTA	4,1 kb
283	13902-L15440	Exon 3	235-236	AGTGCACCAACT-ACCTTCTCCAGG	2,3 kb
392	13911-L15449	Exon 2	164-165	GAATGCCAGCAA-ATACTCCAGGAC	60,1 kb
		start codon	*16-18 (Exon 1)*		
CSF2RA gene, Xp22.33. Indicated ligation sites are according to NM_001161529.2.					
		start codon	*309-311 (Exon 4)*		
191	13894-L15432	Exon 1	24 nt after exon 1	CTTTCCTTCTGT-GGTCTTTGAGCA	5,8 kb
256 Ж	13900-SP0138-L15438	Exon 2a	45 nt and 10 nt before exon 2a	GTTTCCACTATA-35 nt spanning oligo-CCTTTCACAGTT	8,0 kb
318	13905-L15443	Exon 4	350-349 reverse	AATGCTGGGTGT-GGTAACTCACAG	3,1 kb
419	13913-L15451	Exon 5	430-431	TGTGAGGTTTGA-CTCCAGGACGAT	2,8 kb
481	22821-L16342	Exon 6	594-595	TCACATTTGAGG-TTCACGTGAATA	0,2 kb
149	13890-L15428	Exon 7	690-691	TCTCCTGTTTCA-TCTACAATGCGG	1,7 kb
355	13908-L15446	Exon 8	909-908 reverse	TTGGATGCCAAT-TTCTCGGCTGGT	4,0 kb
220	13897-L15435	Exon 9	1037-1038	CCCAGGACCTAT-CAGAAGCTGTCG	1,0 kb
166	13892-L16221	Exon 10	1117-1116 reverse	TTTCACTTACCA-GTAGGTTTTCCG	5,2 kb
292	13903-L15441	Exon 11	1238-1239	AGCTCCTGGAGT-GAAGCCATTGAA	3,3 kb
197	13895-L16222	Exon 13	1270-1269 reverse	CAGAGCCGAGGT-TCCCGTCGTCAG	1,6 kb
382	13910-L15448	Exon 14	1410-1411	ACAAACTGAATG-ATAACCATGAGG	3,9 kb
436	13915-L15453	Exon 16	24 nt before exon 16	TGAAGATCTGAC-AGCCTGAACCCT	27,5 kb
		end codon	*1509-1511 (Exon 16)*		
IL3RA gene, Xp22.33. Indicated ligation sites are according to NM_002183.4.					
		start codon	*181-183 (Exon 2)*		
346	13907-L15445	Exon 1	53-54	GGAAGATATCAG-AAACATCCTAGG	8,5 kb
302	13904-L15442	Exon 3	311-310 reverse	TCACATTTCTGT-TAAGGTCCCAGG	7,1 kb
142	13597-L15055	Exon 6	774-775	TGCACAGATAAG-TTTGTCGTCTTT	3,8 kb
232 «	13898-L16224	Exon 7	826-827	ACATGACTGCAA-AGTGTAATAAGA	2,6 kb
328 «	13906-L15444	Exon 8	3 nt after exon 8	ACAGAACAGGTG-AGTGTTCCCTAC	6,3 kb
156 «	13891-L16341	Exon 9	979-980	TCAATCCTGGAA-CGTACACAGTAC	17,3 kb

409 «	13912-L16228	Exon 12	1300-1301	CTGAAGTACAGG-TCGTGCAGAAAA	83,8 kb
		end codon	*1315-1317 (Exon 12)*		
Centromeric from IL3RA					
244 ¬	14140-L15740	*P2RY8*	Xp22.33	TTTACGCAAACA-TGTATTCCAGCA	83,0 kb
265 ¬	22820-L32443	*P2RY8*	Xp22.33	GAGAAGCCGAGT-GTATTTTGGGGG	790,3 kb
364 ¬	14142-L15742	*ZBED1*	Xp22.33	TCGTCAAGAGCA-ACACGGAGCAGA	250,5 kb
453 ¬	16859-L16226	*CD99*	Xp22.33	GGCGGATGATGT-TTACTAACGATG	-

[a] See section Exon numbering on page 1 for more information.
[b] The homologous sequence of Xp22.33 band on X-chromosome corresponds to Yp11.32 band on Y-chromosome.
[c] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs143255564 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. CNAs 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 hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. 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
275	04489-L03878	*SLC2A1*	1p34	CTATCTGAGCAT-CGTGGCCATCTT	01-043,166
463	05950-L05394	*SPAST*	2p22	ACTCGTAAGAAA-AAAGACTTGAAG	02-032,194
373	08831-L32190	*DYSF*	2p13	GAAGAAATCAGT-GAGTGACCAGGA	02-071,741
310	12783-L32182	*EDAR*	2q13	CTCCACACACGT-TGGCATACACAT	02-108,889
494	15203-L16978	*GBE1*	3p12	GACCTAGAGGGA-CTCATGATCTTT	03-081,775
401	08544-L32189	*ZIC1*	3q24	ATGCACTCTATG-TGTTCAGGAAGC	03-148,616
124	18709-L25925	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
427	06435-L27142	*KIAA0319*	6p22	AAAGCACGAGAT-GGAATGACCAAC	06-024,653
214	08940-L09035	*SLC6A5*	11p15	TTGCCTCTCAGG-TGTGGAAAGATG	11-020,606
444	17129-L20312	*MYBPC3*	11p11	CACCCAACTATA-AGGCCCTGGACT	11-047,311
160	07394-L07041	*COL2A1*	12q13	TCACTTCCTTCT-TGCTCACAGGGT	12-046,670
130	13867-L15385	*ABAT*	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008,765
179	13562-L15019	*CACNA1A*	19p13	TTTGGGATTCTG-GTAAGTACCACC	19-013,225
208	16261-L18553	*SAMHD1*	20q11	AGTAGACAATGA-GTTGCGTATTTG	20-034,979

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

Related SALSA MLPA probemixes

- **P018 SHOX (CE)**: Contains probes for *SHOX* gene and several other genes in the PAR1 region.
- **P202 IKZF1-ERG**: Contains probes for *IKZF1*, *ERG* and *CDKN2A/B* and for the 14q32.33 region.
- **P327 iAMP21-ERG**: Contains probes for *RUNX1*, *ERG* and iAMP21 detection in ALL.
- **P335 ALL-IKZF1 (CE)**: Contains probes for *IKZF1* and various other genes / regions that are often deleted in ALL.
- **P383 T-ALL**: Contains probes for *STIL-TAL1*, *LEF1*, *CASP8AP2*, *MYB*, *EZH2*, *MLLT3*, *MTAP*, *CDKN2A/B*, *NUP214-ABL1*, *PTEN*, *LMO1/2*, *NF1*, *SUZ12*, *PTPN2* and *PHF6*, which are involved in T-ALL.
- **P419 CDKN2A/2B-CDK4**: Contains probes for *CDKN2A*, *CDKN2B* and *CDK4*.

- **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.

References

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- Mullighan CG et al. (2009). Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet.* 41:1243-6.
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- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P329 CRLF2-CSF2RA-IL3RA

- Wrona E et al. (2019). Gene expression of ASNS, LGMN and CTSB is elevated in a subgroup of childhood BCP-ALL with PAX5 deletion. *Oncol Lett*. 18:6926-32.
- Yu CH et al. (2020). MLPA and DNA index improve the molecular diagnosis of childhood B-cell acute lymphoblastic leukemia. *Sci Rep.* 101:11501.

P329 product history	
Version	*Modification*
B1	One IL3RA probe and one flanking probe are removed, one flanking probe is replaced, the majority for the reference probes are revised, and several probes have a change in length but not in the sequence detected.
A1	First release.

Implemented changes in the product description
Version B1-01 – 14 January 2021 (04P) - Product description adapted to a new product version and to a new template (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *CRLF2, CSF2RA* and *IL3RA* genes are updated according to new versions of the NM_ reference sequence in Table 2. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). -Two new references for P329 probemix are added to page 9. - Warning for SNP rs143255564 added to the SHOX area probe at 184 nt in Tables 1 and 2. *Version 08 – 18 April 2017 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - NM_sequence information for CRLF2 in introduction and Table 2 is updated. - Replaced references into text. - Removed text about necessity of same sexes data analysis. - Added remark about salt sensitivity for 13906-L15444 probe. *Version 07 – 7 October 2015 (55)*

- Exon number and information for CSF2RA in introduction are updated according to NM_reference sequence. - Manufacturers address changed.

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