Product Description
SALSA® MLPA® Probemix P175-B1 Tumour Gain

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 12.

Catalogue numbers:
- **P175-025R:** SALSA MLPA Probemix P175 Tumour Gain, 25 reactions.
- **P175-050R:** SALSA MLPA Probemix P175 Tumour Gain, 50 reactions.
- **P175-100R:** SALSA MLPA Probemix P175 Tumour Gain, 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 P175 Tumour Gain is a **research use only (RUO)** assay for the detection of copy number aberrations in 24 genes, which are frequently gained or amplified in various tumour types. This probemix can also be used to detect the presence of the *BRAF* p.V600E (c.1799T>A) point mutation.

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 exon numbering used in this P175-B1 Tumour Gain product description is the exon numbering from the LRG, RefSeq transcript NM_ or NG_ sequence, as indicated in Table 2. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. 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 P175-B1 Tumour Gain contains 62 MLPA probes with amplification products between 115 and 504 nucleotides (nt). This includes two probes for each of the following genes: *ABL1*, *ALK*, *AR*, *AURKA/B*, *BRAF*, *CCND1/2*, *CDK4*, *DHFR*, *EGFR*, *ERBB2*, *FGFR1*, *KDR*, *KIT*, *MDM2/4*, *MET*, *MYC*, *MYCN*, *PDGFRA*, *RET*, *SMO* and *TOP2A*. Furthermore, this probemix also contains one probe specific for the *BRAF* p.V600E (c.1799T>A) point mutation which will only generate a signal when the mutation is present. In addition, 13 reference probes are included that detect autosomal chromosomal locations which are relatively stable in most tumour types. However, it should be noted that tumour karyotypes can harbour multiple numerical and structural aberrations, which can complicate interpretation of these reference probes. Complete

probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

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

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

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

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

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from healthy individuals without a history of cancer. It is recommended to use samples of the same sex to facilitate interpretation. 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 following samples from the Coriell Institute and Leibniz Institute DSMZ have been tested at MRC Holland with the P175-B1 probemix and can be used to detect copy number alterations (CNAs) in the genes mentioned in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P175-B1	Expected copy number alteration
NA05347	Coriell Institute	1q32.1	*MDM4*	Heterozygous duplication
NA10401 #	Coriell Institute	2p24.3	*MYCN*	Heterozygous duplication
		2p23.2	*ALK*	Heterozygous duplication
NA00945	Coriell Institute	2p24.3	*MYCN*	Heterozygous deletion
NA07081	Coriell Institute	7p11.2	*EGFR*	Heterozygous duplication
NA01059	Coriell Institute	7q31.2	*MET*	Heterozygous deletion
NA12519	Coriell Institute	7q31.2	*MET*	Homozygous duplication/ Heterozygous triplication
		7q32.1	*SMO*	
		7q34	*BRAF*	
NA07412	Coriell Institute	7q34	*BRAF*	Heterozygous deletion
NA02030	Coriell Institute	8p11.23	*FGFR1*	Heterozygous duplication
		8q24.21	*MYC*	Heterozygous duplication
NA03999	Coriell Institute	8q24.21	*MYC*	Heterozygous deletion
NA13685	Coriell Institute	9q34.12	*ABL1*	Heterozygous duplication
NA07981	Coriell Institute	12p13.32	*CCND2*	Homozygous duplication / Heterozygous triplication
NA08123	Coriell Institute	20q13.2	*AURKA*	Heterozygous duplication
NA03384	Coriell Institute	Xq12	*AR*	Homozygous duplication/ Heterozygous triplication
DU-4475 (= ACC-427) ±	DSMZ	1q32.1	*MDM4*	Homozygous duplication / Heterozygous triplication
		7q34	*BRAF* p.V600E (c.1799T>A)	Point mutation
SU-DHL-8 (= ACC-573) #	DSMZ	7p11.2	*EGFR*	Heterozygous duplication
		7q31.2	*MET*	Heterozygous duplication
		7q32.1	*SMO*	Heterozygous duplication
		7q34	*BRAF*	Heterozygous duplication
		12p13.32	*CCND2*	Heterozygous duplication
		12q14.1	*CDK4*	Heterozygous duplication
		20q13.2	*AURKA*	Homozygous duplication/ Heterozygous triplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by this P175-B1 Tumour Gain probemix.

In this sample CNAs are observed for one or more reference probes.

± In this sample ambiguous ratios are observed for a gain of 7q32.1-7q34 (including *MET*, *SMO* and *BRAF* genes).

SALSA Binning DNA SD029

The SD029 Binning DNA provided with this probemix can be used for binning of all probes including the one mutation-specific probe (*BRAF* probe 08780-SP0039-L08904 for the p.V600E (c.1799T>A) point mutation). SD029 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above-mentioned probe. Inclusion of one reaction with 5 µl SD029 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of the mutation signal. It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD029 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis

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

Interpretation of results

The standard deviation of each individual reference probe over all the reference samples should be ≤0.10. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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. 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.

P175 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 copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.
- Please note that due to high nucleotide sequence similarity of mutated V600E (GTG to GAG single nucleotide variation) and V600K (GTG to AAG double nucleotide variation) codons, the BRAF V600E probe included in this probemix might give a small signal on a sample with V600K mutation.

Limitations of the procedure

- In most populations, the major cause of genetic defects in cancer are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P175 Tumour Gain.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results

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

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

COSMIC mutation database

https://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC mutation 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 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P175-B1 Tumour Gain

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			
115 *	Reference probe S0864-L24551	21q22		21-037,920
121	**DHFR probe** S0428-L27347		5q14.1	05-079,986
124	**AURKA probe** S0429-L27348		20q13.2	20-054,379
131 *	**AR probe** 21771-L13680		Xq12	X-066,823
136 *	Reference probe 13867-L30857	16p13		16-008,765
143 ¥ «	**CDK4 probe** 03173-L30917		12q14.1	12-056,431
148 *	**ERBB2 probe** 21772-L30858		17q12	17-035,122
152 *	Reference probe 14199-L25033	2q13		02-108,894
157 ¥	**MYC probe** 20780-L30918		8q24.21	08-128,822
161 ¥	**MET probe** 20064-L27635		7q31.2	07-116,187
167 ¥	**ABL1 probe** 12502-L30479		9q34.12	09-132,579
172 ¥	**ALK probe** 08324-L30480		2p23.2	02-029,405
176 ¥	**CCND2 probe** 03177-L30859		12p13.32	12-004,253
182 *	**RET probe** 21776-L30860		10q11.21	10-042,942
187 ¥	**MDM4 probe** 03185-L30861		1q32.1	01-202,761
191 ¥	**AURKB probe** 12749-L30862		17p13.1	17-008,051
196 *	Reference probe 05703-L29853	3q21		03-123,456
202 ¥	**MET probe** 10314-L30481		7q31.2	07-116,167
208 ¥	**SMO probe** 12750-L30482		7q32.1	07-128,633
214	**BRAF probe** 04260-L14063		7q34	07-140,123
220 *	Reference probe 06714-L30959	15q24		15-070,433
226 § Ж	**BRAF probe** 08780-SP0039-L08904		p.V600E (c.1799T>A)	07-140,100
232 ¥	**EGFR probe** 06408-L31001		7p11.2	07-055,217
238 ¥	**MYC probe** 21646-L19746		8q24.21	08-128,822
244	**DHFR probe** 12753-L13869		5q14.1	05-079,986
251	**BRAF probe** 10507-L11060		7q34	07-140,099
257	**TOP2A probe** 01055-L00628		17q21.2	17-035,823
265 ¥ «	**CDK4 probe** 15904-L30865		12q14.1	12-056,429
273 ¥	**CCND1 probe** 05401-L30866		11q13.2	11-069,167
282 *	Reference probe 13392-L30484	6q12		06-065,358
292 ¥	**MDM2 probe** 07179-L30485		12q15	12-067,494
299 ¥	**CCND1 probe** 00583-L30869		11q13.2	11-069,175
305 ¥	**KDR probe** 12755-L30870		4q12	04-055,657
312 ¥	**ABL1 probe** 12516-L30871		9q34.12	09-132,749
319 *	Reference probe 06580-L30872	2q24		02-165,907
325 ¥	**AR probe** 12604-L30873		Xq12	X-066,860
330 ¥	**MDM4 probe** 03186-L30874		1q32.1	01-202,779
337 *	Reference probe 20864-L28882	14q24		14-072,684
344 ¥	**ERBB2 probe** 00717-L30875		17q12	17-035,137
351 ¥	**KIT probe** 21774-L30876		4q12	04-055,257
357 *	**FGFR1 probe** 04439-L30877		8p12	08-038,393
363 *	Reference probe 14835-L29122	1p34		01-045,252
370 ¥	**RET probe** 18546-L30919		10q11.21	10-042,928
376 ¥ «	**MYCN probe** 02572-L30879		2p24.3	02-016,003
385 ¥	**FGFR1 probe** 01046-L24278		8p12	08-038,434
391	**PDGFRA probe** 12762-L13878		4q12	04-054,851
399 *	**CCND2 probe** 03178-L30880		12p13.32	12-004,283
406 ¥	**SMO probe** 12757-L30881		7q32.1	07-128,640
412 ¥	**MDM2 probe** 07180-L30490		12q15	12-067,497
418 *	Reference probe 20960-L30882	6p12		06-052,049

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
426 ¥	**ALK probe** 08323-L30883		2p23.2	02-029,608
430 ¥	**EGFR probe** 02063-L30920		7p11.2	07-055,191
438 *	**PDGFRA probe** 18756-L24124		4q12	04-054,826
445 ¥ «	**MYCN probe** 03327-L20117		2p24.3	02-016,003
454 ¥	**KDR probe** 12758-L31062		4q12	04-055,663
462 ¥	**AURKB probe** 12759-L30885		17p13.1	17-008,052
469 *	Reference probe 19978-L30964	4p16		04-005,637
475 ¥	**KIT probe** 12761-L30887		4q12	04-055,298
481 ¥	**TOP2A probe** 01056-L30888		17q21.2	17-035,801
489	**AURKA probe** 10236-L14068		20q13.2	20-054,390
496 *	Reference probe 17940-L30958	19p13		19-013,255
504 *	Reference probe 21229-L30802	10p11		10-032,800

* New in version B1.

¥ Changed in version B1. Minor alteration, no change in sequence detected.

§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E (c.1799T>A) point mutation is present. It has been tested on artificial DNA and on cell line DU-4475 (ACC427), **but not on positive human samples!** Please note that this probe might give a small signal on a sample with the *BRAF* p.V600K mutation.

« 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.

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. P175 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Exon[a] / mutation	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
MDM4 gene at **1q32.1**; NM_002393.5; 11 exons. Frequently gained or amplified in for example glioblastoma. One more MDM4 probe is present in the P462 Follicular Lymphoma probemix.					
187	03185-L30861	*MDM4*	Exon 2	TTCACTACCAAA-ATGACATCATTT	17.3 kb
330	03186-L30874	*MDM4*	Exon 8	GGAGTGGGATGT-AGCTGGCCTGCC	-
MYCN gene at **2p24.3**; NG_007457; 3 exons. Frequently gained or amplified in for example neuroblastoma. More MYCN probes are present in the P037 CLL-1 and P377 Hematologic Malignancies probemixes.					
376 «	02572-L30879	*MYCN*	Exon 3	CTGTCACCACAT-TCACCATCACTG	0.2 kb
445 «	03327-L20117	*MYCN*	Exon 3	TGCACCCCCACA-GAAGAAGATAAA	13.4 **M**b to *ALK* gene
ALK gene at **2p23.2**; LRG_488; 29 exons. Gains and amplification detected in for example testicular tumours. More ALK probes are present in the P252 NB mix 2 probemix.					
172	08324-L30480	*ALK*	Exon 6	TCACTTGTTGGA-ATGGGACAGTCC	203.7 kb
426	08323-L30883	*ALK*	Exon 4	ACACCTCAGCTG-ACTCCAAGCACA	79.3 **M**b to *EDAR* gene (ref)
PDGFRA gene at **4q12**; LRG_309; 23 exons. Frequently gained or amplified in for example glial cancers. One more PDGFRA probe is present in the P105 Glioma-2 probemix.					
438	18756-L24124	*PDGFRA*	Exon 5	ACCTGTGCTGTT-TTTAACAATGAG	25.4 kb
391	12762-L13878	*PDGFRA*	Exon 22	ACAATGCATACA-TTGGTGTCACCT	405 kb to *KIT* gene
KIT gene at **4q12**; LRG_307; 21 exons. Frequently gained or amplified in for example epithelial cancers. More KIT probes are present in the P354 KIT SNAI2 probemix.					
351	21774-L30876	*KIT*	Exon 2	CGTGCACCAACA-AACACGGCTTAA	41.5 kb
475	12761-L30887	*KIT*	Exon 20	ACATAATGAAGA-CTTGCTGGGATG	359 kb to *KDR* gene
KDR gene at **4q12**; LRG_1198; 30 exons. Frequently gained or amplified in for example epithelial cancers. No other KDR probes are present in our collection at this moment.					
305	12755-L30870	*KDR*	Exon 19	TGGTGACCAATA-TGAATGAGGATC	6.2 kb
454	12758-L31062	*KDR*	Exon 14	GAAACCTGGAGA-ATCAGACGACAA	-
DHFR gene at **5q14.1**; NG_023304; 6 exons. Gains and amplifications detected in for example skin and kidney cancers. No other DHFR probes are present in our collection at this moment.					
121	S0428-L27347	*DHFR*	Exon 2	CGCTGTTTCTCT-AACTTGTAGGAA	0.8 kb
244	12753-L13869	*DHFR*	Exon 1	GGCTTCCCGTAG-ACTGGAAGAATC	-
EGFR gene at **7p11.2**; LRG_304; 28 exons. Frequently gained or amplified in various tumour types, for example in glial, breast and lung cancers. More EGFR probes are present in the P105 Glioma-2 and P315 EGFR probemixes.					
430	02063-L30920	*EGFR*	Exon 8	AGCTATGAGATG-GAGGAAGACGGC	25.5 kb
232	06408-L31001	*EGFR*	Exon 20	CCTCCTGGACTA-TGTCCGGGAACA	61.0 **M**b to *MET* gene
MET gene at **7q31.2**; LRG_662; 21 exons. Frequently gained or amplified in for example glial and kidney cancers. More MET probes are present in the P308 MET probemix.					
202	10314-L30481	*MET*	Exon 4	TATCACTGGGAA-GAAGGTAAGCTG	19.3 kb
161	20064-L27635	*MET*	Exon 10	AGCACAATAACA-GGTGTTGGGAAA	12.4 **M**b to *SMO* gene
SMO gene at **7q32.1**; LRG_1393 12 exons. Frequently gained or amplified in for example melanoma. No other SMO probes are present in our collection at this moment.					
208	12750-L30482	*SMO*	Exon 4	CCCTGCTGTTAT-TCTCTTCTACGT	6.8 kb
406	12757-L30881	*SMO*	Exon 12	TCGGTGAGGAAG-AAGAGCCTTGAA	11.5 **M**b to *BRAF* gene

Length (nt)	SALSA MLPA probe	Gene	Exon[a] / mutation	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
BRAF gene at **7q34**; LRG_299; 18 exons. Frequently gained, amplified or mutated in for example melanoma. More BRAF probes are present in the P298 BRAF-HRAS-KRAS-NRAS and P370 BRAF-IDH1-IDH2 probemixes.					
251	10507-L11060	*BRAF*	Exon 15	TATTTTTCCACT-GATTAAATTTTT	0.1 kb
226 § Ж	08780-SP0039-L08904	*BRAF*	p.V600E (c.1799T>A)	TTCTTCATGAAG-ACCTCACAG TAAAAATAGGTGATTTTGGTCT AGCTACAG**A**-GAAATCTCGATG	23.6 kb
214 #	04260-L14063	*BRAF*	Exon 13	CTTGTATCACCA-TCTCCATATCAT	-
FGFR1 gene at **8p12**; LRG_993; 18 exons. Frequently gained or amplified in various tumour types, for example in breast and lung cancers. More FGFR1 probes are present in the P370 BRAF-IDH1-IDH2 and P133 Kallmann-2 probemixes.					
357	04439-L30877	*FGFR1*	Exon 13	ACCCCAGCCACA-ACCCAGAGGAGC	41.5 kb
385	01046-L24278	*FGFR1*	Exon 2	CAACCTCTAACT-GCAGAACTGGGA	90.4 **M**b to *MYC* gene
MYC gene at **8q24.21**; LRG_1397; 3 exons. Frequently gained or amplified in various tumour types, for example in ovarian, breast and lung cancers. More MYC probes are present in the P458 Gastric cancer probemix.					
238	21646-L19746	*MYC*	Exon 3	AGGACTATCCTG-CTGCCAAGAGGG	0.2 kb
157	20780-L30918	*MYC*	Exon 3	GAACGAGCTAAA-ACGGAGCTTTTT	-
ABL1 gene at **9q34.12**; LRG_769; 12 exons. *ABL1* is frequently involved in translocations (e.g. *BCR/ABL1* fusion gene) in different hematologic malignancies, and sometimes in subsequent amplifications of these fusion genes. One more ABL1 probe is present in the P383 T-ALL probemix.					
167	12502-L30479	*ABL1*	Exon 1	CTTTATGTGTGA-GAATTGAAATGA	170.1 kb
312	12516-L30871	*ABL1*	Exon 12	TCGAAAAGAGCG-AGGTCCCCCGGA	-
RET gene at **10q11.21**; LRG_518; 20 exons. Gains and amplifications detected in for example lung cancer. More RET probes are present in the P169 Hirschsprung probemix.					
370	18546-L30919	*RET*	Exon 8	TGCAGTCAGCAA-GAGACGGCTGGA	14.5 kb
182	21776-L30860	*RET*	Exon 19	CCTCCCTTCCAC-ATGGATTGAAAA	-
CCND1 gene at **11q13.2**; LRG_990; 5 exons. Frequently gained or amplified in various tumour types, for example in breast cancer. In both P078 Breast tumour and P458 Gastric cancer probemixes one more CCND1 probe is present.					
273	05401-L30866	*CCND1*	Exon 2	TCGCTGGAGCCC-GTGAAAAAGAGC	8.1 kb
299	00583-L30869	*CCND1*	Exon 5	CCCTGCTGGAGT-CAAGCCTGCGCC	-
CCND2 gene at **12p13.32**; NG_034254; 5 exons. Frequently gained or amplified in for example testicular tumours. One more CCND2 probe is present in the P037 CLL-1, P040 CLL and P377 Hematologic Malignancies probemixes.					
176	03177-L30859	*CCND2*	Exon 1	AGACCAGTTTTA-AGGGGAGGACCG	29.9 kb
399	03178-L30880	*CCND2*	Exon 5	TAACAGCCAAGA-AGCCTGCAGGAG	52.1 **M**b to *CDK4* gene
CDK4 gene at **12q14.1**; LRG_490; 8 exons. Frequently gained or amplified in various tumours, for example in glial cancers, melanoma and soft tissue tumours. More CDK4 probes are present in the P419 CDKN2A/2B-CDK4 probemix.					
265 «	15904-L30865	*CDK4*	Exon 8	TGCTGACTTTTA-ACCCACACAAGC	2.7 kb
143 «	03173-L30917	*CDK4*	Exon 3	AACCCTGGTGTT-TGAGCATGTAGA	11.1 **M**b to *MDM2* gene
MDM2 gene at **12q15**; NG_016708; 11 exons. Frequently gained or amplified in various tumour types, for example in soft tissue tumours. More MDM2 probes are present in the P323 CDK4-HMGA2-MDM2 probemix.					
292	07179-L30485	*MDM2*	Exon 3	ACCAACAGACTT-TAATAACTTCAA	3.4 kb
412	07180-L30490	*MDM2*	Exon 4	TGGACTAAACTG-AAGAATTACCTG	-
AURKB gene at **17p13.1**; NM_004217; 9 exons. Gains and amplifications detected in for example soft tissue tumours. No other AURKB probes are present in our collection at this moment.					
191	12749-L30862	*AURKB*	Exon 5	CCTTCCTCCACT-TTCTAAGCAGGC	0.2 kb
462	12759-L30885	*AURKB*	Exon 4	GCACTTACGTTA-AGATGTCGGGTG	27.1 **M**b to *ERBB2* gene

Length (nt)	SALSA MLPA probe	Gene	Exon[a] / mutation	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
ERBB2 gene, also known as *HER-2/NEU*, at **17q12**; LRG_724; 32 exons. Frequently gained or amplified in various tumour types, for example in breast and gastric cancers. More ERBB2 probes are present in the P004 ERBB2 and P078 Breast tumour probemixes.					
148	21772-L30858	*ERBB2*	Exon 13	AGGTGACAGCAG-AGGATGGAACAC	14.9 kb
344	00717-L30875	*ERBB2*	Exon 30	TCACTGCTGGAG-GACGATGACATG	665 kb to *TOP2A* gene
TOP2A gene at **17q21.2**; NG_027678; 35 exons. Frequently gained or amplified in for example breast and stomach cancers. More TOP2A probes are present in the P004 ERBB2, P078 Breast tumour and P458 Gastric cancer probemixes.					
481	01056-L30888	*TOP2A*	Exon 33	TAAGGGCAGTGT-ACCACTGTCTTC	21.3 kb
257	01055-L00628	*TOP2A*	Exon 7	AAGCCCTTCAAT-GGAGAAGATTAT	-
AURKA gene at **20q13.2**; NG_012133; 11 exons. Frequently gained or amplified in for example gastrointestinal cancers. More AURKA probes are present in the P078 Breast tumour probemix.					
124	S0429-L27348	*AURKA*	Exon 10	TACAAAAGAATA-TCACGGGTAAGA	11.1 kb
489	10236-L14068	*AURKA*	Exon 8	AGGCATCCTAAT-ATTCTTAGACTG	-
AR gene at **Xq12**; LRG_1406; 8 exons. Gains and amplifications detected in for example prostate cancer. More AR probes are present in the P074 AR probemix.					
131	21771-L13680	*AR*	Exon 3	AGCAGGGATGAC-TCTGGGAGGTAA	37.6 kb
325	12604-L30873	*AR*	Exon 8	CATCAGTTCACT-TTTGACCTGCTA	-

[a] See section Exon numbering on page 1 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.

§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E (c.1799T>A) point mutation is present. It has been tested on artificial DNA and on cell line DU-4475 (ACC427), **but not on positive human samples!** Please note that this probe might give a small signal on a sample with the *BRAF* p.V600K mutation.

« 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.

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
363	14835-L29122	*UROD*	1p34	AAGCACCATGGC-TCAGGCCAAGCG	01-045,252
152	14199-L25033	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108,894
319	06580-L30872	*SCN2A*	2q24	AACTTGGTTTGG-CAAATGTGGAAG	02-165,907
196	05703-L29853	*CASR*	3q21	GTGGCTTCCAAA-GACTCAAGGACC	03-123,456
469	19978-L30964	*EVC2*	4p16	AGACTCTGTCGG-CCTACACCGCCC	04-005,637
418	20960-L30882	*PKHD1*	6p12	TTTATCCACCAA-GTGGTGTTCCAG	06-052,049
282	13392-L30484	*EYS*	6q12	AGCCAGCTGGTA-TGCACTAATGGG	06-065,358
504	21229-L30802	*CCDC7*	10p11	ATCGCCTTAAAC-AGAGGTCTAAAT	10-032,800
337	20864-L28882	*PSEN1*	14q24	TTTCTGTGAAAC-AGTATTTCTATA	14-072,684
220	06714-L30959	*HEXA*	15q24	TAGCCAGCTTGT-TTGGAAATCTGC	15-070,433
136	13867-L30857	*ABAT*	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008,765
496	17940-L30958	*CACNA1A*	19p13	GCCATTACATCC-TGAACCTGCGCT	19-013,255
115	S0864-L24551	*KCNJ6*	21q22	AGCTCCTACATC-ACCAGTGAGATC	21-037,920

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

Related SALSA MLPA probemixes

P294 Tumour Loss	▪ Contains probes for 1p36, 13q14 *(RB1)*, *AMER1*, *APC*, *BRCA1/2*, *CDKN2A/B*, *FHIT*, *FKBP8*, *NF1*, *PTCH1*, *PTEN*, *SMAD4*, *SMARCB1*, *STK11*, *TP53*, *TSC1/2*, *VHL* and *WT1*.
Selected genes	▪ See information in Table 2 for more related probemixes.

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P175 product history	
Version	*Modification*
B1	Six target probes have been replaced for the *AR*, *CCND2*, *ERBB2*, *FGFR1*, *PDGFRA* and *RET* genes. One target probe for the *CCND1* gene has been removed. Several probes have been changed in length. In addition, 13 reference probes have been added and the data analysis method has been modified.
A3	Several probes have been changed in length.
A2	One target probe for *CDK4* gene has been replaced and one probe for *RET* gene has been changed in length.
A1	First release.

Implemented changes in the product description
Version B1-04 – 10 January 2023 (04P) - Added information about possible small signal for BRAF V600E mutation probe on a sample with V600K mutation to P175 specific notes section and Tables 1 and 2. - Removed sample NA08035 from table of Positive control DNA samples. Version B1-03 – 29 March 2022 (04P) - Product description rewritten and adapted to a new template. - Several selected publications using probemix P175 Tumour Gain have been added. - Several minor textual changes throughout the document - Added information on additional positive samples on page 3. - Source of exon numbering updated to include LRG and/or NG information (when available). Version B1-02 – 25 September 2018 (01P) - P175 specific note added on page 3. - New reference added for P175 probemix on page 10. Version B1-01 – 06 June 2018 (01P) - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2a and 2b) and restructured to a new template. - New references added for the P175 probemix on pages 9-10. Version 10 – 13 January 2017 (T08) - Warning added in Table 1 and Table 2, 436 nt probe 03327-L02466.

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