Product Description
SALSA® MLPA® Probemix P315-C2 EGFR

To be used with the MLPA General Protocol.

Version C2
As compared to version C1, two probes have a small change in length, but no change in the sequence detected. For complete product history see page 9.

Catalogue numbers:
- **P315-025R:** SALSA MLPA Probemix P315-C2-EGFR, 25 reactions.
- **P315-050R:** SALSA MLPA Probemix P315-C2-EGFR, 50 reactions.
- **P315-100R:** SALSA MLPA Probemix P315-C2-EGFR, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SD006 Binning DNA 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 P315 EGFR is a **research use only (RUO)** assay for the detection of deletions or duplications in the human *EGFR* gene. This probemix can also be used to detect the presence of *EGFR* c.2573T>G=p.L858R and c.2369C>T=p.T790M point mutations.

The epidermal growth factor receptor (EGFR) is a cell surface tyrosine kinase (TK) enzyme involved in controlling cell growth. Ligand-induced stimulation of EGFR followed by subsequent formation of homodimers or heterodimers with other receptors, results in the activation of downstream signalling cascades that regulate cell proliferation, differentiation, survival and DNA synthesis (Kovacs et al., 2015; Wee et al., 2017). Various genetic alterations resulting in oncogenic activity of *EGFR* have been described to be pivotal in tumour development and progression in multiple cancer types (Lynch et al., 2004; Bhargava et al., 2005; Brennan et al., 2013). Moreover, patients with tumours harbouring *EGFR* alterations tend to have a more aggressive form of the disease, and expression levels of EGFR are highly predictive of clinical outcome for cancer patients (Uribe et al., 2021).

Genetic alterations that result in oncogenic activity of *EGFR* in cancer include copy number amplifications and deletions, structural rearrangements of the gene, and activating mutations (Lynch et al., 2004; Bhargava et al., 2005; Brennan et al., 2013). One of the most common *EGFR* deletions detected in various tumour types is the *EGFR* deletion variant III (EGFRvIII), resulting from an in-frame deletion of exons 2-7 leading to a ligand-independent receptor (Gan et al., 2013). In addition, numerous other *EGFR* (exon) deletions and duplications are found in tumour biopsies. *EGFR* mutations cluster in the kinase domain of *EGFR* (exons 18-21), and cause ligand-independent activation of the receptor. Therefore, these activating mutations represent possible targets for therapeutic intervention. In this regard, certain somatic *EGFR* mutations as well as gene amplification in patients with non-small cell lung cancer (NSCLC) highly correlate with the clinical response to TK inhibitors. Two frequent *EGFR* mutations, c.2573T>G=p.L858R and c.2369C>T=p.T790M, are shown to be an important mechanism of resistance to drugs acting on the TK domain of EGFR (Uribe et al. 2021).

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
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/ and http://tark.ensembl.org/
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 *EGFR* exon numbering used in this P315-C2 EGFR product description is the exon numbering from the MANE Select transcript (NM_005228.5), as indicated in Table 2. **From product description version C2-01 onwards, we have adopted the MANE exon numbering for the *EGFR* gene. Please be aware that the MANE and previous exon numbering do not always correspond.** 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 P315-C2 EGFR contains 47 MLPA probes with amplification products between 124 and 472 nucleotides (nt). This includes 30 probes for the *EGFR* gene on 7p11.2, covering all *EGFR* exons except for exon 11. Furthermore, this probemix also contains 2 probes specific for the c.2369C>T= p.T790M and c.2573T>G= p.L858R *EGFR* mutations which will only generate a signal when the respective mutation is present. In addition, 14 reference probes are included that detect autosomal chromosomal locations, targeting relatively copy number stable regions in various cancer types. 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, 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 different healthy individuals without a history of cancer. 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 NA07081 (*EGFR* duplication) from the Coriell Institute and ACC-444 (FU-OV-1, *EGFR* loss) from the Leibniz Institute DSMZ have been tested with this P315 probemix at MRC Holland and can be used as a positive control samples to detect *EGFR* copy number alterations. The quality of cell lines can change; therefore samples should be validated before use.

SALSA Binning DNA SD006

The SD006 Binning DNA provided with this probemix can be used for binning of all probes including the *EGFR* mutation-specific probes 22215-SP0449-L21566 for the c.2573T>G=p.L858R mutation and 17162-SP0448-L21565 for the c.2369C>T=p.T790M mutation. SD006 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD006 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 mutation signal(s). 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 SD006 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 probe over all the reference samples should be ≤10. When this criterion is fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
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 mosaic or 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.

P315-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 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.

Limitations of the procedure

- In most populations, most genetic alterations in the *EGFR* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P315 EGFR.
- 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.

Mutation databases

LOVD mutation database: https://databases.lovd.nl/shared/genes/EGFR; COSMIC mutation database**:** https://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 copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *EGFR* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P315-C2-EGFR

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*EGFR*	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information			
124	Reference probe 19616-L26275	4p13		04-042,278
130	Reference probe 20879-L29296	12q24		12-116,137
137	**EGFR** probe 06121-L20393		Exon 6	07-055,188
143	**EGFR** probe 05955-L20394		Exon 9	07-055,192
149	**EGFR** probe 12961-L20662		Exon 1	07-055,055
157	**EGFR** probe 05438-L20424		Exon 5	07-055,186
162	**EGFR** probe 06405-L31525		Exon 1	07-055,054
169	Reference probe 09267-L31348	10q21		10-053,746
173	**EGFR** probe 05956-L20559		Exon 10	07-055,192
179	**EGFR** probe 05965-L20425		Exon 19	07-055,210
184 ¥	**EGFR** probe 23288-L32941		Exon 7	07-055,189
192	Reference probe 14964-L16700	6q22		06-129,413
197 § Ж	**EGFR** probe 22215-SP0449-L21566		**c.2573T>G=p.L858R**	07-055,227
205	**EGFR** probe 17511-L21356		Exon 21	07-055,227
211	Reference probe 07019-L20663	14q11		14-020,839
218	**EGFR** probe 17208-L21000		Exon 4	07-055,182
226	**EGFR** probe 06408-L31296		Exon 20	07-055,217
231	**EGFR** probe 06407-L20428		Exon 18	07-055,209
239	Reference probe 08193-L31307	11q12		11-057,136
246 § Ж	**EGFR** probe 17162-SP0448-L21565		**c.2369C>T=p.T790M**	07-055,216
254	**EGFR** probe 05959-L31294		Exon 13	07-055,197
260	Reference probe 07592-L14699	21q21		21-027,229
268	**EGFR** probe 05969-L21001		Exon 23	07-055,234
274	**EGFR** probe 05960-L05377		Exon 14	07-055,199
281	Reference probe 18668-L24022	11p14		11-022,238
291 ¬	**POR** probe 12687-L31300		7q11.23	07-075,448
295	**EGFR** probe 05970-L20431		Exon 24	07-055,236
301 ¥	**EGFR** probe 23296-L32950		Exon 15	07-055,201
310	**EGFR** probe 17209-L20443		Exon 12	07-055,195
317 ¬	**DDC** probe 15419-L31298		7p12.2	07-050,579
323	Reference probe 05272-L31851	2p22		02-032,207
331	**EGFR** probe 05971-L31581		Exon 25	07-055,236
337	**EGFR** probe 17210-L20444		Exon 16	07-055,206
348	**EGFR** probe 05972-L20437		Exon 26	07-055,237
357	**EGFR** probe 05963-L20438		Exon 17	07-055,208
367	Reference probe 15524-L25197	16q13		16-055,478
380	**EGFR** probe 12901-L20439		Exon 28	07-055,241
391	**EGFR** probe 05436-L04852		Exon 3	07-055,178
404	**EGFR** probe 12900-L20440		Exon 27	07-055,238
414	Reference probe 12787-L20671	2q13		02-108,914
421	**EGFR** probe 21280-L27637		Exon 2	07-055,178
429	**EGFR** probe 02063-L21569		Exon 8	07-055,191
439	Reference probe 16538-L21570	15q15		15-042,672
447	**EGFR** probe 22216-L31527		Exon 22	07-055,228
454 ¬	**GBAS** probe 22218-L31297		7p11.2	07-056,013
462	Reference probe 10685-L31301	6p12		06-051,876
472	Reference probe 16938-L19881	4q12		04-054-663

¥ Changed in version C2. Minor alteration, no change in sequence detected.

§ Mutation-specific probe. This probe will only generate a signal when the *EGFR* p.L858R or p.T790M mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

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. Copy number alterations 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. Single probe aberration(s) must be confirmed by another method.

Table 2. EGFR probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*Gene* / exon [a]	Location / Ligation site	Partial sequence (24 nt adjacent to ligation site) [b]	Distance to next probe
317 ¬	15419-L31298	*DDC*	7p12.2	ACACCATGAACG-CAAGTGAATTCC	4.5 **M**b
		Telomeric to *EGFR*			
EGFR, at 7p11.2. Indicated ligation sites are in NM_005228.5.					
162	06405-L31525	Exon 1	223-224	CCGTCCAGTATT-GATCGGGAGAGC	0.1 kb
		start codon	*262-264 (Exon 1)*		
149	12961-L20662	Exon 1	325-326	TCTGCCCGGCGA-GTCGGGCTCTGG	123.0 kb
421	21280-L27637	Exon 2	440-441	TAACTGTGAGGT-GGTCCTTGGGAA	1.0 kb
391	05436-L04852	Exon 3	533-534	TTATGTCCTCAT-TGCCCTCAACAC	3.4 kb
218	17208-L21000	Exon 4	817-818	ACCACCTGGGCA-GCTGTAAGTGTC	4.6 kb
157	05438-L20424	Exon 5	852-853	TGTCCCAATGGG-AGCTGCTGGGGT	1.2 kb
137	06121-L20393	Exon 6	914-915	CTGTGCCCAGCA-GTGCTCCGGGCG	1.5 kb
184	23288-L32941	Exon 7	1117-1118	AGGGCAAATACA-GCTTTGGTGCCA	1.8 kb
429	02063-L21569	Exon 8	1215-1216	AGCTATGAGATG-GAGGAAGACGGC	0.7 kb
143	05955-L20394	Exon 9	1360-1361	CCTCCATCAGTG-GCGATCTCCACA	0.2 kb
173	05956-L20559	Exon 10	1434-1435	GATCCACAGGAA-CTGGATATTCTG	3.4 kb
310	17209-L20443	Exon 12	1609-1610	CATCCTTGGGAT-TACGCTCCCTCA	1.4 kb
254	05959-L31294	Exon 13	1862-1863	CCGAGGCAGGGA-ATGCGTGGACAA	2.2 kb
274	05960-L05377	Exon 14	1928-1929	CTCTGAGTGCAT-ACAGTGCCACCC	1.6 kb
301	23296-L32950	Exon 15	2068-2069	TCATGGGAGAAA-ACAACACCCTGG	5.8 kb
337	17210-L20444	Exon 16	2169-2170	CTTGAAGGCTGT-CCAACGAATGGG	1.9 kb
357	05963-L20438	Exon 17	2268-2269	CTCTTCATGCGA-AGGCGCCACATC	0.9 kb
231	06407-L20428	Exon 18	2346-2347	CTTACACCCAGT-GGAGAAGCTCCC	0.8 kb
179	05965-L20425	Exon 19	2474-2475	AGGTGAGAAAGT-TAAAATTCCCGT	6.6 kb
246 § Ж	17162-SP0448-L21565	**c.2369C>T= p.T790M**	2630-2629, 2585-2584 reverse	GCATGAGCTGC**A**-45 nt spanning oligo-ACACGTGGGGGT	0.1 kb
226	06408-L31296	Exon 20	2663-2664	CCTCCTGGACTA-TGTCCGGGAACA	10.3 kb
205	17511-L21356	Exon 21	2743-2742 reverse	ACGGTCCTCCAA-GTAGTTCATGCC	0.1 kb
197 § Ж	22215-SP0449-L21566	**c.2573T>G= p.L858R**	2834-2835, 2865-2866	AGATTTTGGGC**G**-31 nt spanning oligo-TACCATGCAGAA	1.0 kb
447	22216-L31527	Exon 22	10 nt after exon 22	CGGTGAGTCATA-ATCCTGATGCTA	5.9 kb
268	05969-L21001	Exon 23	3037-3038	AGATCTCCTCCA-TCCTGGAGAAAG	1.6 kb
295	05970-L20431	Exon 24	3139-3140	ATAGTCGCCCAA-AGTTCCGTGAGT	0.9 kb
331	05971-L31581	Exon 25	3265-3266	ACCGTGCCCTGA-TGGATGAAGAAG	0.5 kb
348	05972-L20437	Exon 26	3397-3398	GCAACAATTCCA-CCGTGGCTTGCA	0.8 kb
404	12900-L20440	Exon 27	3466-3467	TCTTGCAGCGAT-ACAGCTCAGACC	2.8 kb
380	12901-L20439	Exon 28	3638-3639	CCCACACTACCA-GGACCCCCACAG	772.8 kb
		stop codon	*3892-3894 (Exon 28)*		
		Centromeric to *EGFR*			
454 ¬	22218-L31297	*GBAS*	7p11.2	CAACAGATCTCG-AGAAGACAGCTG	1.9 **M**b
291 ¬	12687-L31300	*POR*	7**q**11.23	GCCCTGGTGGTT-TTCTGCATGGCC	-

[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.

§ Mutation-specific probe. This probe will only generate a signal when the *EGFR* p.L858R or p.T790M mutation is present. It has been tested on artificial DNA **but not on positive human samples!**

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. Copy number alterations 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. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*Gene*	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
323	05272-L31851	*SPAST*	2p22	TTCTTTAATATA-AGTGCTGCAAGT	76.7 **M**b
414	12787-L20671	*EDAR*	2q13	TAATGACGCATG-TCTTTCAGGTGA	-
124	19616-L26275	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	12.4 **M**b
472	16938-L19881	*GSX2*	4q12	TACCTGTCTCGA-CTCCGGAGGATT	-
462	10685-L31301	*PKHD1*	6p12	TCTGGCATCTAT-ATCTGCAGTCCC	77.5 **M**b
192	14964-L16700	*LAMA2*	6q22	TCATTTGTCCAT-CTTTTTCAGGTT	-
169	09267-L31348	*DKK1*	10q21	CCGGATACAGAA-AGATCACCATCA	-
281	18668-L24022	*ANO5*	11p14	TACCGCCTGTCA-GTCTTTGCTACA	34.9 **M**b
239	08193-L31307	*SERPING1*	11q12	CACAATCTGAGT-TTGGTGATCCTG	-
130	20879-L29296	*NOS1*	12q24	ACTGCTGAACCT-TTCCTCTGGGAC	-
211	07019-L20663	*RPGRIP1*	14q11	TCTATTTGTCCA-CAGGCTGAGGAC	-
439	16538-L21570	*SPG11*	15q15	GTCAAAGGATTC-CCCGTTACTACT	-
367	15524-L25197	*SLC12A3*	16q13	CACCCGGAACCT-CAGCCTGATGAT	-
260	07592-L14699	*ADAMTS5*	21q21	GACCTACCACGA-AAGCAGATCCTG	-

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

Related SALSA MLPA probemixes

- **P105-Glioma 2**: This probemix contains probes for *EGFR*, *PDGFRA*, *CDKN2A*, *PTEN*, *TP53*, *CDK4-MIR26A2*, *MDM2* and *NFKBIA* genes.
- **P483-HER gene family**: This probemix contains nine probes for *EGFR*, eight probes for *ERBB2*, four probes for *ERBB3* and five probes for *ERBB4*.

References

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P315 product history	
Version	*Modification*
C2	Two probes have a small change in length, but no change in the sequence detected.
C1	Majority of reference probes replaced. New flanking probes for *EGFR* added.
B1	Content completely revised. Mutation-specific probes have been replaced and control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description

Version C2-01- 31 January 2023 (04P)
- Product description restructured and adapted to new template and a new C2 version.
- Various minor textual or layout changes.
- List of related probemixes updated on page 9.
- List of references updated.

Version C1-02 – 29 May 2020 (02P)
- NM sequence number corrected for EGFR (NM_005228.5).

Version C1-01 - 29 May 2019 (02P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *EGFR* gene updated according to new version of the NM_reference sequence.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

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