Product Description

SALSA® MLPA® Probemix P483-A1 HER gene family

To be used with the MLPA General Protocol.

Version A1. For complete product history see page 10.

Catalogue numbers:
- **P483-025R:** SALSA MLPA Probemix P483 HER gene family, 25 reactions.
- **P483-050R:** SALSA MLPA Probemix P483 HER gene family, 50 reactions.
- **P483-100R:** SALSA MLPA Probemix P483 HER gene family, 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 P483 HER gene family is a **research use only (RUO)** assay for the detection of deletions or duplications in the HER family of receptor tyrosine kinases. This gene family is comprised of four members promoting cell proliferation, motility and invasion via signalling through homo- or heterodimerization: *EGFR* (*ERBB1/HER1*), *ERBB2* (*HER2*), *ERBB3* (*HER3*), and *ERBB4* (*HER4*) (Sergina and Moasser, 2007).

Amplifications in the Human Epidermal Growth Factor Receptor (HER) gene family are associated with a high invasiveness of human cancers, most notably in breast cancer (Gan et al, 2013; Sergina and Moasser, 2007). As transmembrane proteins, the products of these genes are suitable targets for anticancer drugs. For example, breast cancer patients with tumours overexpressing Her2 (20-30% of breast tumours; Mitri et al. 2012) are treated with trastuzumab (Herceptin), which impairs receptor signalling by binding to its extracellular domain.

EGFR amplifications have been observed in a variety of cancers, including glioblastomas and colorectal carcinoma (Ayati et al. 2020). These amplifications are sometimes paired with rearrangements, such as EGFRvIII (deletion of exons 2-7; Gan et al. 2013) rendering the receptor constitutively active.

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 *EGFR* exon numbering used in this P483-A1 HER gene family product description is the exon numbering from the LRG_304 sequence, the *ERBB2* exon numbering is from LRG_724, and the *ERBB3* exon numbering is from LRG_996. The *ERBB4* exon numbering used is the exon numbering from the NG_011805.2 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 P483-A1 HER gene family contains 54 MLPA probes with amplification products between 132 and 508 nucleotides (nt). This includes nine probes for *EGFR*, eight probes for *ERBB2*, four probes for *ERBB3*, and five probes for *ERBB4*. Furthermore, this probemix contains 14 probes targeting the regions flanking these four genes, as well as regions on the other arm of their respective chromosomes. This includes two probes for *WSB1*, in the CEP17 region, which is used as an internal control for HER2 amplification calling in other techniques (Allison, 2017).

In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types including breast cancer, glioblastoma and colorectal carcinoma. 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 probes over the experiment.

Required specimens

Extracted DNA, derived from peripheral blood for germline testing, or from tumour tissue and corresponding healthy tissue. The latter includes DNA derived from fresh frozen or 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 for germline analysis should be derived from different healthy individuals without a history of hereditary cancers, and for tumour analysis 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. Samples mentioned in the table below have been tested with this P483-A1 HER gene family probemix at MRC Holland and can be used as positive control samples to detect copy number alterations as specified. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P483-A1	Expected copy number alteration
NA10401	Coriell Institute	2p22.3-q34	*SPAST*, *CPS1*, ***ERBB4***, *IKZF2*	Heterozygous duplication
NA01229	Coriell Institute	2q34	*CPS1*, ***ERBB4***, *IKZF2*	Heterozygous duplication
NA10918	Coriell Institute	2q34	***ERBB4***, *IKZF2*	Heterozygous deletion
NA07081	Coriell Institute	7p11.2	*VSTM2A*, ***EGFR***, *LANCL2*	Heterozygous duplication
ACC-410 (MFE-280) ◊	DSMZ	2p22.3	*SPAST*	Heterozygous duplication
		7p11.2	*VSTM2A*, ***EGFR***, *LANCL2*	Heterozygous duplication
		7q22.3	*SLC26A4*	Heterozygous deletion
		12q13.2	*RPS26*, ***ERBB3***, *PA2G4*	Amplification
		17p11.2-q12	*FLCN*, *WSB1*	Amplification
		17q12	*PGAP3*, ***ERBB2***, *MIEN1*	Heterozygous duplication
ACC-589 (JIMT-1) ◊	DSMZ	17q11.1	*WSB1*	Homozygous duplication
		17q12	*PGAP3*, ***ERBB2***, *MIEN1*	Amplification

* 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 P483-A1 HER gene family probemix.

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

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 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. 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.

Limitations of the procedure

- Small (point) mutations in *EGFR*, *ERBB2*, *ERBB3*, and *ERBB4* will generally not be detected by using SALSA MLPA Probemix P483 HER gene family.
- 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 (i.e. in breast tumours).

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.

LOVD and COSMIC mutation databases

http://cancer.sanger.ac.uk/cosmic; http://www.lovd.nl. We strongly encourage users to deposit germline positive results in the Leiden Open Variant Database (LOVD) database or for somatic mutations 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 duplication of *ERBB4* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P483 HER gene family

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		EGFR 7p11.2	*ERBB2* 17q12	*ERBB3* 12q13.2	*ERBB4* 2q34	Reference
64-105	Control fragments – see table in probemix content section for more information					
132	Reference probe 22310-L31738					4q22
137	Reference probe 22309-L11248					6p12
142 ¬	SLC26A4 probe 09253-L31440	7q22.3				
148 ¬	RPS26 probe 18804-L31441			Upstream		
154	**ERBB3 probe** 22311-L31431			**Exon 25**		
160	**ERBB2 probe** 12049-L12914		**Exon 32**			
166	Reference probe 16253-L31432					19p13
173	**EGFR probe** 05956-L20559	**Exon 10**				
178	**ERBB4 probe** 03172-L29132				**Exon 1**	
184	**EGFR probe** 22327-L31449	**Exon 20**				
190	**ERBB2 probe** 20106-L29064		**Exon 13**			
196	Reference probe 22312-L31739					10q22
202	**EGFR probe** 15736-L29210	**Exon 11**				
208	**ERBB3 probe** 22328-L32522			**Exon 10**		
214 ¬	LANCL2 probe 22329-L32523	Downstream				
220	Reference probe 22313-L31433					1p31
226	**ERBB4 probe** 04196-L29066				**Exon 2**	
232 ¬	SPAST probe 20173-L29067				2p22.3	
238	**ERBB4 probe** 22369-L31532				**Exon 12**	
244	**ERBB2 probe** 20068-L31445		**Exon 23**			
250	Reference probe 22314-L04215					5p13
256	**ERBB2 probe** 22330-L32524		**Exon 14**			

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		EGFR 7p11.2	*ERBB2* 17q12	*ERBB3* 12q13.2	*ERBB4* 2q34	Reference
263	**ERBB3 probe** 04268-L31741			**Exon 3a**		
269 ¬	MIEN1 probe 22332-L31454		Downstream			
275	Reference probe 22316-L32525					21q21
280	**EGFR probe** 22333-L31455	**Exon 3**				
287 ¬	VSTM2A probe 22331-L31797	Upstream				
292	**ERBB2 probe** 08088-L31742		**Exon 24**			
301	Reference probe 20124-L32194					3p12
306	**EGFR probe** 22317-L32193	**Exon 21**				
313 ¬	WSB1 probe 22784-L32195		Upstream			
319 ¬	GRIN2B probe 17454-L21210			12p13.1		
326 ¬	WSB1 probe 22785-L32394		Upstream			
333 ¬	IKZF2 probe 17110-L32132				Upstream	
340	Reference probe 20129-L32131					9q21
346	**EGFR probe** 22334-L31456	**Exon 5**				
355	**ERBB3 probe** 22335-L31457			**Exon 21**		
364	**ERBB2 probe** 12042-L12906		**Exon 8**			
372	Reference probe 22318-L31436					14q22
382	**EGFR probe** 12901-L29185	**Exon 28**				
391	**EGFR probe** 05436-L04852	**Exon 3**				
400 ¬	PA2G4 probe 22337-L31459			Downstream		
409 ക	**ERBB2 probe** 12040-L29143		**Exon 1**			
418	Reference probe 22319-L31437					11p11
427	**ERBB2 probe** 12045-L12909		**Exon 17**			
436 ¬	PGAP3 probe 22338-L31460		Upstream			
445 ¬	CPS1 probe 22368-L31458				Downstream	
454	Reference probe 22320-L31438					15q24
462	**EGFR probe** 22323-L20672	**Exon 2**				
475	**ERBB4 probe** 22324-L31446				**Exon 20**	
483 ¬	FLCN probe 22325-L31744		17p11.2			
494	**ERBB4 probe** 22326-L31448				**Exon 3**	
500	Reference probe 21783-L27807					10p11
508	Reference probe 22321-L31439					5q31

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

¬ 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.

ക The significance of *ERBB2* exon 1 deletions is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

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. P483-A1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
ERBB4, located on 2q34**.** Indicated ligation sites are according to the NM_005235.3 sequence. Exon numbering is according to NG_011805.2.						
232 ¬	20173-L29067	*SPAST*	2**p**22.3	AGAGTACTTGTA-ATGGGTGCAACT	178.9 **M**b	02-032,216
445 ¬	22368-L31458	*CPS1*	2q34	CCACAAAAGTGG-TAGCTGTAGACT	1.0 **M**b	02-211,161
		stop codon	*4197-4199 (exon 28)*			
475	22324-L31446	***ERBB4***, exon 20	2616-2617	CACACCTAGTCC-GGTTGCTGGGTG	140.1 kb	02-212,135
238	22369-L31532	***ERBB4***, exon 12	1601-1602	CAGGGCATCACC-TCTCTACAGTTC	245.4 kb	02-212,275
494	22326-L31448	***ERBB4***, exon 3	569-570	TACCTGCCTCTG-GAGAATTTACGC	177.3 kb	02-212,520
226	04196-L29066	***ERBB4***, exon 2	405-406	ACCTGGAACAGC-AGTACCGAGCCT	413.7 kb	02-212,698
178	03172-L29132	***ERBB4***, exon 1	274-275	CTTCCAAAAAAT-GAAGCCGGCGAC	469.5 kb	02-213,111
		start codon	*273-275 (exon 1)*			
333 ¬	17110-L32132	*IKZF2*	2q34	GCGATTCAGCTA-CCCAGATATTCA	-	02-213,581
EGFR, located on 7p11.2**.** Indicated ligation sites are according to the NM_005228.5 sequence. Exon numbering is according to LRG_304.						
287 ¬	22331-L31797	*VSTM2A*	7**p**11.2	AGCAAAGAGCAA-ATCGCCTGTAAA	573.2 kb	07-054,604
		start codon	*262-264 (exon 1)*			
462	22323-L20672	***EGFR***, exon 2	440-441	TAACTGTGAGGT-GGTCCTTGGGAA	1.0 kb	07-055,178
391	05436-L04852	***EGFR***, exon 3	533-534	TTATGTCCTCAT-TGCCCTCAACAC	0.1 kb	07-055,178
280	22333-L31455	***EGFR***, exon 3	625-626	CCTTAGCAGTCT-TATCTAACTATG	7.9 kb	07-055,179
346	22334-L31456	***EGFR***, exon 5	823-824	ACATTTCAGGCC-AAAAGTGTGATC	5.5 kb	07-055,186
173	05956-L20559	***EGFR***, exon 10	1434-1435	GATCCACAGGAA-CTGGATATTCTG	0.9 kb	07-055,192
202	15736-L29210	***EGFR***, exon 11	1496-1497	GGCTTGGCCTGA-AAACAGGACGGA	23.8 kb	07-055,193
184	22327-L31449	***EGFR***, exon 20	2699-2700	TGGCTCCCAGTA-CCTGCTCAACTG	10.3 kb	07-055,217
306	22317-L32193	***EGFR***, exon 21	2733-2732 reverse	AAGTAGTTCATG-CCCTGAAACAGA	13.7 kb	07-055,227
382	12901-L29185	***EGFR***, exon 28	3638-3639	CCCACACTACCA-GGACCCCCACAG	225.9 kb	07-055,241
		stop codon	*3892-3894 (exon 28)*			
214 ¬	22329-L32523	*LANCL2*	7**p**11.2	TCCAGCATTTGA-ACTTGACTCTTC	51.7 **M**b	07-055,466
142 ¬	09253-L31440	*SLC26A4*	7q22.3	TGCGATTGTGAT-GATCGCCATTCT	-	07-107,122
ERBB3, located on 12q13.2**.** Indicated ligation sites are according to the NM_001982.4 sequence. Exon numbering is according to LRG_996.						
319 ¬	17454-L21210	*GRIN2B*	12**p**13.1	CCTGAGCCCAAA-AGCAGTTGTTAC	40.9 **M**b	12-013,798
148 ¬	18804-L31441	*RPS26*	12q13.2	CCCATCCTGTCG-CAGACAAAGAAA	42.6 kb	12-054,722
		start codon	*137-139 (exon 1a)*			
263	04268-L31741	***ERBB3***, exon 3a	412-413	GTGGCCATGAAT-GAATTCTCTACT	7.7 kb	12-054,765
208	22328-L32522	***ERBB3***, exon 10	1252-1253	TTCAGAGACCCC-TGGCACAAGATC	5.0 kb	12-054,773
355	22335-L31457	***ERBB3***, exon 21	2606-2607	AGGGAATGTACT-ACCTTGAGGAAC	2.1 kb	12-054,778
154	22311-L31431	***ERBB3***, exon 25	3161-3162	TGGAGCCAGAAC-TAGACCTAGACC	7.1 kb	12-054,780
		stop codon	*4163-4165 (exon 28)*			

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
400 ¬	22337-L31459	*PA2G4*	12q13.2	TTACAGGTATTG-CTTTTCCCACCA	-	12-054,787
ERBB2, located on 17q12. Indicated ligation sites are according to the NM_004448.4 sequence (unless indicated otherwise). Exon numbering is according to LRG_724.						
483 ¬	22325-L31744	*FLCN*	17**p**11.2	TGGCATTCAGAT-GAACAGTCGGAT	5.6 **M**b	17-017,072
313 ¬	22784-L32195	*WSB1*	17q11.1	ATTGCCAAGACA-AAATAGTGATGG	8.1 kb	17-022,655
326 ¬	22785-L32394	*WSB1*	17q11.1	ATTGATGAGGAT-TATCCAGTGCAA	12.4 **M**b	17-022,663
436 ¬	22338-L31460	*PGAP3*	17q12	AAAATGGACTAC-TTCTGTGCCTCC	14.2 kb	17-035,084
409 ൭	12040-L29143	***ERBB2***, exon 1	NM_001005862.3; 105-106	GTGTCCATATAT-CGAGGCGATAGG	20.3 kb	17-035,098
		start codon	*176-178 (exon 6)*			
364	12042-L12906	***ERBB2***, exon 8	530-531	CCGTGCTAGACA-ATGGAGACCCGC	3.5 kb	17-035,118
190	20106-L29064	***ERBB2***, exon 13	1148-1149	AGGTGACAGCAG-AGGATGGAACAC	0.4 kb	17-035,122
256	22330-L32524	***ERBB2***, exon 14	1297-1298	TTTGGGAGCCTG-GCATTTCTGCCG	3.4 kb	17-035,122
427	12045-L12909	***ERBB2***, exon 17	1604-1605	ACCTCTGCTTCG-TGCACACGGTGC	7.8 kb	17-035,126
244	20068-L31445	***ERBB2***, exon 23	2356-2357	AAGGTGCTTGGA-TCTGGCGCTTTT	0.3 kb	17-035,133
292	08088-L31742	***ERBB2***, exon 24	2390-2391	CCCAGGGCATCT-GGATCCCTGATG	4.0 kb	17-035,134
160	12049-L12914	***ERBB2***, exon 32	3764-3765	ACCCCGAGTACT-TGACACCCCAGG	1.9 kb	17-035,138
		stop codon	*3941-3943 (exon 32)*			
269 ¬	22332-L31454	*MIEN1*	17q12	TTTGAGATAGAG-ATAAATGGACAG	-	17-035,139

[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.

¬ 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.

൭ The significance of *ERBB2* exon 1 deletions is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

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
220	22313-L31433	*RPE65*	1p31	CGTACGGGCAAT-GACTGAGAAAAG	01-068,683
301	20124-L32194	*GBE1*	3p12	ACCGAGTTGGAA-CAGCATTGCCAG	03-081,667
132	22310-L31738	*PKD2*	4q22	CGCATTCACAAA-CTACACTATTTC	04-089,187
250	22314-L04215	*NIPBL*	5p13	CAAGTGCCTGTT-TTACAACAGAAC	05-037,012
508	22321-L31439	*SLC22A5*	5q31	GATCTGCTTCGA-ACCTGGAATATC	05-131,753
137	22309-L11248	*PKHD1*	6p12	GGAAGATTGGAA-ACTTTTGATTTT	06-052,046
340	20129-L32131	*PCSK5*	9q21	GACTATGAAGAA-TGTGTCCCTTGT	09-078,045
500	21783-L27807	*PARD3*	10p11	GATCAGCCTTCC-CACTCTCTGGAG	10-034,646
196	22312-L31739	*NODAL*	10q22	AGAGCGGTTTCA-GATGGACCTATT	10-071,865
418	22319-L31437	*MYBPC3*	11p11	CATCGGTGCCAA-GCGTACCCTGAC	11-047,321
372	22318-L31436	*GCH1*	14q22	AATGTTGGGTGT-GTTCCGGGAGGA	14-054,381
454	22320-L31438	*SEMA7A*	15q24	GGGACCTGGCTT-CAATGTTTCTAC	15-072,498
166	16253-L31432	*RNASEH2A*	19p13	AGGACACGGACT-TTGTCGGCTGGG	19-012,779
275	22316-L32525	*BACH1*	21q21	ATGCACAAGCTT-ACTCCAGAACAG	21-029,624

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

Related SALSA MLPA probemixes

- P004 ERBB2: Contains probes for *ERBB2* and several flanking genes in 17p and 17q. Suitable also for detecting CEP17.
- P078 Breast tumour: Contains probes for *EGFR* and *ERBB2*.
- P105 Glioma-2: Contains probes for *EGFR*.
- P175 Tumour Gain: Contains probes for *EGFR* and *ERBB2*.
- P315 EGFR: Contains 30 probes for the *EGFR* gene, including probes specific for the L858R and the T790M point mutations.

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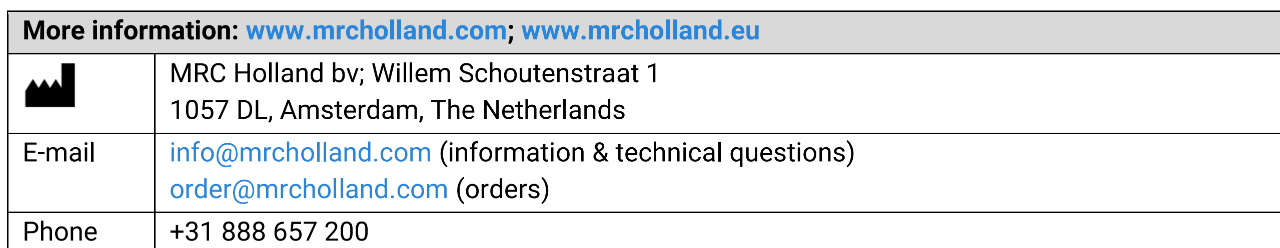
- Laurito S et al. (2020). Working together for the family: determination of HER oncogene co-amplifications in breast cancer. *Oncotarget* 11: 2774-92.
- Soosanabadi M et al. (2019). Application of Multiplex Ligation-Dependent Probe Amplification in Determining the Copy Number Alterations of HER Gene Family Members in Invasive Ductal Breast Carcinoma. *Rep Biochem Mol Biol.* 8:91-101.

P483 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 06 October 2021 (04P) - The altered target genes updated for sample ACC-410 in section 'Positive control DNA samples'.
Version A1-01 – 22 September 2021 (04P) - Not applicable, new document.



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