Product Description
SALSA® MLPA® Probemix P078-D3 Breast tumour

To be used with the MLPA General Protocol.

Version D3
As compared to version D2, one flanking probe has been added and one probe has a minor change in length but not in the sequence targeted. For complete product history see page 12.

Catalogue numbers
- **P078-025R:** SALSA® MLPA® Probemix P078 Breast tumour, 25 reactions
- **P078-050R:** SALSA® MLPA® Probemix P078 Breast tumour, 50 reactions
- **P078-100R:** SALSA® MLPA® Probemix P078 Breast tumour, 100 reactions

SALSA® MLPA® Probemix P078 Breast tumour (hereafter: P078) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P078-025R	**P078-050R**	**P078-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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
SALSA® MLPA® Probemix P078 Breast tumour is a **research use only (RUO)** assay for the detection of deletions or amplifications in genes/chromosomal regions of diagnostic/clinical importance in breast cancer, including 6q (*ESR1*), 7p (*EGFR*), 8p (*ZNF703*, *FGFR1*, *ADAM9*, *IKBKB*), 8q (*PRDM14*, *MTDH*, *MYC*), 11q (*CCND1*, *EMSY*), 16q (*CDH1*), 17q (*CPD*, *MED1*, *ERBB2*, *CDC6*, *TOP2A*, *MAPT*, *PPM1D*, *BIRC5*), 19q (*CCNE1*) and 20q (*AURKA*). See Table 1 and Table 2 for more detailed information about all included chromosomal regions and genes.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Exon numbering
The exon numbering used in this P078-D3 Breast tumour product description is the exon numbering from the MANE project based on MANE Select transcripts, as indicated in Table 2. Reference NM_ sequences and/or exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From product description version D2-02 onwards, we have adopted the MANE exon numbering. Please be aware that the MANE and previous exon numbering do not always correspond, and MANE exon numbering used here may differ from literature. 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
P078-D3 Breast tumour contains 56 MLPA probes with amplification products between 127 and 500 nucleotides (nt). This includes 42 probes for the following chromosomal regions/genes: 6q (ESR1), 7p (EGFR), 8p (*ZNF703*, *FGFR1*, *ADAM9*, *IKBKB*), 8q (*PRDM14*, *MTDH*, *MYC*), 11q (*CCND1*, *EMSY*), 16q (*CDH1*), 17q (*CPD*, *MED1*, *ERBB2*, *CDC6*, *TOP2A*, *MAPT*, *PPM1D*, *BIRC5*), 19q12 (*CCNE1*) and 20q (*AURKA*). In addition, 14 reference probes are included which target relatively copy number stable regions in various tumour types, including breast cancer. The identity of the genes targeted by the reference probes is available in Table 3. Partial probe sequences are available online at 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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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 formalin-fixed, paraffin-embedded (FFPE) tissues, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. For 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from healthy individuals who are from families without a history of breast 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 number NA07994, NA07081, NA02030, NA14485, NA03999, NA00959, NA12074, NA16445 and NA08123 from the Coriell Institute, and 8-MG-BA, CAL-148 and COLO-824 from Leibniz Institute DSMZ, have been tested with this P078-D3 probemix at MRC Holland and can be used as positive control samples to detect copy number alterations, as described in the table below. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Sample name	Chromosomal position of copy number alteration (hg18)*	Altered target genes in P078-D3	Expected copy number alteration
Germline samples from Coriell Institute			
NA07994	6q25.1	*ESR1*	Heterozygous duplication
NA07081	7p11.2	*EGFR*	Heterozygous duplication
NA14485	8p12-p11.21	*ZNF703, FGFR1, ADAM9, IKBKB*	Heterozygous duplication
NA02030	8p12-q24.1	*ZNF703, FGFR1, ADAM9, IKBKB, PRDM14, MTDH, MYC*	Heterozygous duplication
NA03999	8q24.21	*MYC*	Heterozygous deletion
NA00959	11q13.2-q13.5	*CCND1, EMSY*	Heterozygous duplication
NA12074	16q22.1	*CDH1*	Heterozygous deletion
NA16445	17q25.3	*BIRC5*	Heterozygous duplication
NA08123	20q13.2	*AURKA*	Heterozygous duplication
Cancer cell line samples from DSMZ			
8-MG-BA◊	7p11.2	*EGFR*	Gain
	8q13.3-q24.21	*PRDM14, MTDH, MYC*	Gain
	11q13.2-q13.5	*CCND1, EMSY*	Gain
	17q21.31-q25.3	*MAPT, PPM1D, BIRC5*	Gain
	19q12	*CCNE1*	Gain
	20q13.2	*AURKA*	Gain
CAL-148◊	7p11.2	*EGFR*	Gian
	16q22.1	*CDH1*	Heterozygous deletion
	20q13.2	*AURKA*	Gain
COLO-824◊	7p11.2	*EGFR*	Gain
	8q13.3-q22.1	*PRDM14, MTDH*	
	8q24.21	*MYC+*	
	20q13.2	*AURKA*	

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by P078-D3 Breast tumour.
◊ In these indicated cell lines, some of the reference probes are also affected by CNAs.
+ Ratios above 3.0 detected for *MYC*, indicating amplification.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 these 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region, or in or near the *CCNE* and *BIRC5* 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: https://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.

P078 Breast tumour 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 many tumour samples, genetic alterations in the chromosomal regions and genes included in this Probemix are small (point) mutations, most of which will not be detected by using P078 Breast tumour.
- 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 breast cancer samples 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 in sequence data 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 database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://hgvs-nomenclature.org.

Please report false positive results due to SNVs and unusual results (e.g., an amplification of *ERBB2* exons 3 and 15, but not exon 9) to MRC Holland: info@mrcholland.com.

Table 1. P078-D3 Breast tumour

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
127	Reference probe 15370-L19110	7q11		07-075,448
133	**EMSY probe** 09173-L21898		11q13.5	11-075,902
139	**ZNF703 probe** 17592-L28959		8p12	08-037,673
143 ¥	Reference probe 14199-L33288	2q13		02-108,894
148	**ERBB2 probe** 00675-L18842		17q12	17-035,118
154	**IKBKB probe** 11993-L22094		8p11.21	08-042,293
158	**MYC probe** 20780-L28945		8q24.21	08-128,822
166 «	**CCNE1 probe** 02881-L02348		19q12	19-035,005
172	**TOP2A probe** 11994-L12822		17q21.2	17-035,818
177 ±	**CDH1 probe** 16884-L21554		16q22.1	16-067,405
184	Reference probe 10710-L11292	6p12		06-052,016
190 «	**CCNE1 probe** 09170-L09344		19q12	19-035,000
196	**CDC6 probe** 08611-L13204		17q21.2	17-035,699
202	**ERBB2 probe** 17591-L12908		17q12	17-035,122
208	**PPM1D probe** 22339-L21557		17q23.2	17-056,056
214	**ESR1 probe** 11996-L12824		6q25.1	06-152,424
220	Reference probe 13789-L31486	10q23		10-095,547
226	**CPD probe** 09628-L21977		17q11.2	17-025,795
232	**ADAM9 probe** 11997-L21978		8p11.23	08-038,994
238	**AURKA probe** 17365-L21549		20q13.2	20-054,392
244	**ESR1 probe** 11998-L21550		6q25.1	06-152,457
250	**ERBB2 probe** 12048-L21551		17q12	17-035,136
256	**EMSY probe** 09175-L09349		11q13.5	11-075,927
262	**ZNF703 probe** 17595-L21581		8p12	08-037,675
268 ±	**EGFR probe** 05969-L20430		7p11.2	07-055,234
274	Reference probe 13796-L15290	3q25		03-157,716
280	**MYC probe** 14870-L26915		8q24.21	08-128,818
285 #	**MTDH probe** 04151-L21553		8q22.1	08-098,742
292	**CCND1 probe** 00583-L00148		11q13.2	11-069,175
298	Reference probe 18378-L31470	12p11		12-032,840
303 * ¬	**FLCN probe** 08601-L33231		17p11.2	17-017,058
310	Reference probe 09065-L09234	19p13		19-013,289
316	**ERBB2 probe** 00986-L28769		17q12	17-035,127
324 ¥	**BIRC5 probe** 03717-L33208		17q25.3	17-073,722
330	**TOP2A probe** 11999-L21541		17q21.2	17-035,813
337	**MTDH probe** 04152-L21907		8q22.1	08-098,788
346 #	Reference probe 03580-L02941	3p22		03-038,573
352	**MED1 probe** 09963-L21558		17q12	17-034,841
358	**CDH1 probe** 15622-L21559		16q22.1	16-067,329
365	**TOP2A probe** 12000-L28949		17q21.2	17-035,817
373	Reference probe 05953-L30687	2p22		02-032,222
380	**FGFR1 probe** 01046-L28764		8p12	08-038,434
385	Reference probe 09717-L28947	12q24	12q24.22	12-116,200
392 ±	**BIRC5 probe** 03025-L28946		17q25.3	17-073,724
400	**FGFR1 probe** 04440-L03826		8p12	08-038,392
409	Reference probe 01237-L27145	10p14		10-012,019
418	**MAPT probe** 20778-L28948		17q21.31	17-041,423
427	**EGFR probe** 02063-L03283		7p11.2	07-055,191
436 ¥	**BIRC5 probe** 03189-L02540		17q25.3	17-073,722
445	**PRDM14 probe** 12002-L12830		8q13.3	08-071,130
452	Reference probe 12459-L13460	14q24.3		14-076,832

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		Location (hg18) in kb
		Reference	Target region	
463	**IKBKB probe** 12003-L21560		8p11.21	08-042,303
472	**CCND1 probe** 05402-L21561		11q13.2	11-069,168
484 ¥	**AURKA probe** 17590-L33209		20q13.2	20-054,382
494	Reference probe 20779-L21727	4q22		04-090,869
500	Reference probe 06676-L21510	11p15		11-006,369

[a] See section Exon numbering on page 2 for more information.

* New in version D3.

¥ Changed in version D3. Minor alteration, no change in sequence detected

¬ 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.

± SNV rs187862045, rs767507216 and rs371975672 could influence probe signals at 177 nt, 268 nt and 392 nt, respectively. 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.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation 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. Target and flanking probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene/Exon[a]	Location / Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
ESR1 gene, 6q25.1. The estrogen receptor alpha (ER) encoded by *ESR1* gene is the nuclear transcription factor most commonly implicated in breast cancer, with approximately 70-75% of cases expressing ER (Waks AG et al. 2019). Gain or amplification of *ESR1* is a frequent event in breast cancer and suggested to be a clinical marker for response to hormone therapy (Albertson et al. 2012; Ooi et al. 2012). It is important to note that with FISH, up to 20% of breast cancer cases appear to have *ESR1* gene amplification; however, a study showed that FISH can also detect accumulated *ESR1* transcripts, leading to false positives results. MLPA, which measures copy number changes at DNA level, would provide more accurate *ESR1* copy number results (Ooi et al. 2012).					
214	11996-L12824	***ESR1***, ex 6	NM_000125.4; 1515-1516	TTCGACATGCTG-CTGGCTACATCA	33.4 kb
244	11998-L21550	***ESR1***, ex 7	NM_000125.4; 1628-1629	GTCCAGCACCCT-GAAGTCTCTGGA	-
EGFR gene, 7p11.2. Surface tyrosine kinase receptors belonging to the EGFR/ErbB family are often found upregulated in breast cancers (Hsu et al. 2016). *EGFR1* is over-expressed in up to 14% of breast tumours, caused by the amplification of the *EGFR* gene. *EGFR1* overexpression can also be caused by missense mutations, with a higher incidence in hereditary breast cancer compared to sporadic tumours (Park et al. 2007). Although both frequency and the prognostic value of *EGFR* amplification in breast cancer is still controversial, the role of EGFRs in cancer development has been elucidated in the recent years and many monoclonal antibodies and tyrosine kinase inhibitors are currently under investigation in preclinical studies (Maennling et al. 2019).					
427	02063-L03283	***EGFR***, ex 8	NM_005228.5; 1215-1216	AGCTATGAGATG-GAGGAAGACGGC	42.9 kb
268 ±	05969-L20430	***EGFR***, ex 23	NM_005228.5; 3037-3038	AGATCTCCTCCA-TCCTGGAGAAAG	20.2 **M**b to *POR* ref. gene
8p11-p12 amplifications Amplification of 8p11-p12 is detected in ~15% of breast cancer patients and it is associated with poor prognosis (Yang et al. 2010). ***ZNF703*** gene amplification at 8p11.23 and overexpression of this gene has been shown to have a strong impact on the pathogenesis of luminal B breast cancers (Holland et al. 2011; Sircoulomb et al. 2011). ***FGFR1*** gene amplification at 8p11.23 is suggested to be the best marker of poor prognosis in this chromosomal region. *FGFR1* copy number gains are largely found among luminal subtypes of breast cancer, particularly luminal B (HER2$^-$), and it is frequently accompanied by increased copy number of *ZNF703* (Bofin et al. 2022). Moreover, *FGFR1* is a putative therapeutic target, as it is a major contributor in endocrine therapy resistance (Turner et al. 2010). In breast cancer, the expression of ***ADAM9*** is up-regulated compared to normal tissue. *ADAM9* also contributes to disease progression and metastasis by promoting tumour extravasation and migration ability (Oria et al. 2018). ***IKBKB*** gene at 8p11.21, encodes for a kinase associated with IKK/NF-kB activation pathway, which makes it a potential therapeutic target within the 8p11-12 amplicon (Chin et al. 2006).					
139	17592-L28959	***ZNF703***, ex 1	NM_025069.3; 207-208	CAAATGAGCGAT-TCGCCCGCTGGA	2.5 kb
262	17595-L21581	***ZNF703***, ex 2	NM_025069.3; 1823-1824	CACTTTGGGCCT-AAGCCGGTACCA	716.4 kb
400	04440-L03826	***FGFR1***, ex 14	NM_023110.3; 2609-2610	TGCATACACCGA-GACCTGGCAGCC	42.6 kb
380	01046-L28764	***FGFR1***, ex 2	NM_023110.3; 732-733	CAACCTCTAACT-GCAGAACTGGGA	559.8 kb
232	11997-L21978	***ADAM9***	8p11.23	TGAGCACATCAT-TTATCGAATGGA	3.3 **M**b
154	11993-L22094	***IKBKB***, ex 10	NM_001556.3; 1029-1030	CAACTGATGCTG-ATGTGGCACCCC	9.8 kb
463	12003-L21560	***IKBKB***, ex 20	NM_001556.3; 2229-2230	GCCTCTCGACTT-AGCCAGCCTGGG	28.8 **M**b
8q amplifications ***MTDH*** gene activation by 8q22.1 genomic gain promotes chemoresistance and metastasis of breast cancer (Hu et al. 2009; Tokunaga et al. 2014). ***MYC*** gene amplification at 8q24.21 is detected in ~15% of breast cancer patients and is a marker of poor survival (Deming et al. 2000). In breast cancer, amplification and overexpression of *MYC* are frequent in high grade and invasive malignancies and are consistently correlated with poor outcome and early recurrence (Xu J et al. 2010).					
445	12002-L12830	***PRDM14***	8q13.3	CACTCTGGAGAC-AGACCATACCAG	27.6 **M**b
285 #	04151-L21553	***MTDH***, ex 2	NM_178812.4; 763-764	ACCTCAAAGTGT-AACAGCAAAGCA	45.6 kb
337	04152-L21907	***MTDH***, ex 8	NM_178812.4; 1553-1554	GAAGAAAGAGCT-TCACTTCTAAAG	30.0 **M**b
280	14870-L26915	***MYC***, ex 1	NM_002467.6; 242-243	CTGGAACTTACA-ACACCCGAGCAA	4.3 kb
158	20780-L28945	***MYC***, ex 3	NM_002467.6; 1520-1521	GAACGAGCTAAA-ACGGAGCTTTTT	-
11q13 amplifications ***CCND1*** gene amplifications at 11q13.3 are detected in ~15% of breast cancer patients and are associated with poor overall survival in ER+ patients (Holm et al. 2012). High expression levels of *CCND1* were shown to be associated with poor response to trastuzumab treatment in ER+ patients (Tanioka et al. 2014). ***EMSY*** is located on 11q13.5 in proximity to *CCND1* and its protein product transcriptionally silences *BRCA2* gene (Navazio et al. 2016). *EMSY* gene amplifications are detected in 7-13% of breast cancers and are suggested to associate with poor clinical outcome (Kirkegaard et al. 2008).					
472	05402-L21561	***CCND1***, ex 3	NM_053056.3; 598-599	CCTGGTGAACAA-GCTCAAGTGGAA	7.3 kb
292	00583-L00148	***CCND1***, ex 5	NM_053056.3; 927-928	CCCTGCTGGAGT-CAAGCCTGCGCC	6.7 **M**b
133	09173-L21898	***EMSY***, ex 11 (10)	NM_001300942.2; 1597-1598	AACCAAGTAAAA-TCTTACCCAAAC	24.5 kb
256	09175-L09349	***EMSY***, ex 17 (16)	NM_001300942.2; 2637-2638	ATGACCCAGGAA-AAGAGACATTCT	-

Length (nt)	MLPA probe	Gene/Exon[a]	Location / Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
CDH1 gene, 16q22.1. Loss of heterozygosity and deletions affecting the 16q arm are one of the most common genetic alterations in breast cancer, occurring in ~50% of all ductal carcinomas and even more frequently in lobular breast cancer. Loss of E-cadherin (encoded by *CDH1*) is thought to contribute to progression in breast cancer, especially in ductal and lobular breast carcinomas (Chalmers et al. 2001).					
358	15622-L21559	***CDH1***, ex 1	NM_004360.5; 44-45	TTGCGGAAGTCA-GTTCAGACTCCA	76.1 kb
177 ±	16884-L21554	***CDH1***, ex 9	NM_004360.5; 1422-1423	AGTGAACAACGA-TGGCATTTTGAA	-
17q amplifications ***ERBB2*** (HER-2/neu) gene at 17q12, is amplified in 15-30% of breast cancers. Amplification of *ERBB2* defines an aggressive subtype of breast cancer that can be treated with targeted therapy (Trastuzumab/Herceptin). Moreover, amplification of *ERBB2* has been shown to correlate with poor prognosis and resistance to conventional adjuvant chemotherapy and tamoxifen (Slamon et al. 1987; Borg et al. 1994) and is the most important predictive factor of response to HER2-targeted therapies (Singh et al. 2014). ***CPD*** and ***MED1*** genes centromeric and ***CDC6*** gene telomeric to *ERBB2* gene are frequently co-amplified with *ERBB2* (Ooi et al. 2019). ***TOP2A*** gene, at 17q21.2, is amplified in 25-40% of *ERBB2* amplified breast cancers. *TOP2A* is a direct molecular target of anthracycline drug action and several studies have shown that *TOP2A* amplification is a marker of sensitivity for anthracyclines (Nielsen et al. 2008). Moreover, it has reported that loss of *TOP2A* is a significant prognostic factor for poor survival in breast cancer (Bartlett et al. 2010). ***BIRC5*** gene amplification, at 17q25.3, is suggested to predict distant recurrence in breast carcinoma (Davis et al. 2007).					
303 ¬	08601-L33231	***FLCN***	17**p**11.2	GGACACACAGAA-GCTGCTGAGCAT	8.7 **M**b
226	09628-L21977	***CPD***	17q11.2	CCAGTGACTACT-TACAAAACTGGA	9.0 **M**b
352	09963-L21558	***MED1***	17q12	TATCTCACACCA-AGGAGTGGGGGT	277.2 kb
148	00675-L18842	***ERBB2***, ex 3 (8)	NM_004448.4; 423-424	GGTGCAGGGCTA-CGTGCTCATCGC	4.1 kb
202	17591-L12908	***ERBB2***, ex 9 (14)	NM_004448.4; 1286-1287	GCAAGAAGATCT-TTGGGAGCCTGG	5.0 kb
316	00986-L28769	***ERBB2***, ex 15 (20a)	NM_004448.4; 2021-2022	CCATCTGGAAGT-TTCCAGATGAGG	9.2 kb
250	12048-L21551	***ERBB2***, ex 24 (29)	NM_004448.4; 3079-3080	TGTCGGCCAAGA-TTCCGGGAGTTG	562.9 kb
196	08611-L13204	***CDC6***	17q21.2	GAACCAACAAAT-GTCCAAACCGTA	113.4 kb
330	11999-L21541	***TOP2A***, ex 20	NM_001067.4; 2482-2483	AGTTTGGTACCA-GGCTACATGGTG	4.0 kb
365	12000-L28949	***TOP2A***, ex 14	NM_001067.4; 1764-1765	AAAGGCTTGCTG-ATTAATTTTATC	1.6 kb
172	11994-L12822	***TOP2A***, ex 11	NM_001067.4; 1374-1375	CAAGTCCAGTTA-AACAAGAAGTGT	5.6 **M**b
418	20778-L28948	***MAPT***	17q21.31	TAAAACCTTGAA-AAATAGGCCTTG	14.6 **M**b
208	22339-L21557	***PPM1D***	17q23.2	TGTGGTCATCAT-TCGGGGCATGAA	17.7 **M**b
324	03717-L33208	***BIRC5***, ex 1	NM_001168.3; 132-133	CTCTACATTCAA-GAACTGGCCCTT	0.4 kb
436	03189-L02540	***BIRC5***, ex 2	NM_001168.3; 242-243	AGTGTTTCTTCT-GCTTCAAGGAGC	1.9 kb
392 ±	03025-L28946	***BIRC5***, ex 3 (4)	NM_001168.3; 312-313	GCATTCGTCCGG-TTGCGCTTTCCT	-
CCNE1 gene, 19q12. *CCNE1* is often amplified in breast cancer and *CCNE1* overexpression has been associated with an increased risk of breast cancer relapse (Keyomarsi et al. 2002) and with resistance to trastuzumab (Scaltriti et al. 2011). *CCNE1* amplification may confer resistance to chemotherapy and is associated with poor overall survival in triple-negative breast cancer (Zhao et al. 2019).					
190 «	09170-L09344	***CCNE1***, ex 6	NM_001238.4; 539-540	GGAAGTCTGGAA-AATCATGTTAAA	5.1 kb
166 «	02881-L02348	***CCNE1***, ex 11	NM_001238.4; 1187-1188	GATGGTTCCATT-TGCCATGGTTAT	-
AURKA gene, 20q13.2. *AURKA* gene amplification is a common genetic aberration in breast cancer, especially in tumours displaying basal-like phenotype (Staff et al. 2010). High-level 20q13 amplifications, including *AURKA* gene, have been suggested to be an indicator of poor clinical outcome in breast cancer (Tanner et al. 1995).					
484	17590-L33209	***AURKA***, ex 7 (9)	NM_198437.3; 905-906	GCTCCATCTTCC-AGGTATGTAACT	9.8 kb
238	17365-L21549	***AURKA***, ex 5 (7)	NM_198437.3; 435-436	CTAGGAGGCAGT-GGGCTTTGGAAG	-

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Partial 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.

± SNV rs187862045, rs767507216 and rs371975672 could influence probe signals at 177 nt, 268 nt and 392 nt, respectively. 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.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

SNVs located in the target sequence of a probe can influence probe hybridisation 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)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
373	05953-L30687	*SPAST*	2p22	GCAAGTTGTGCT-AGTTCTTTTTGG	02-032,222
143	14199-L33288	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108,894
346 #	03580-L02941	*SCN5A*	3p22	AAGATGATGAAA-ATGACAAAATAG	03-038,573
274	13796-L15290	*KCNAB1*	3q25	CTTTTCCAGAGA-GAGAAAGTGGAG	03-157,716
494	20779-L21727	*SNCA*	4q22	ACAGGAAGGAAT-TCTGGAAGATAT	04-090,869
184	10710-L11292	*PKHD1*	6p12	GGTTCCTGCTCT-TTCCAGTACCTC	06-052,016
127	15370-L19110	*POR*	7q11	GATGGGAAGTGA-GTGCCCACCCTG	07-075,448
409	01237-L27145	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
220	13789-L31486	*LGI1*	10q23	TAGAGCTGAGTT-TCAATGACTATA	10-095,547
500	06676-L21510	*SMPD1*	11p15	CTGCTGAAGATA-GCACCACCTGCC	11-006,369
298	18378-L31470	*PKP2*	12p11	AGCCAGGCCAGA-TCATCTGGTCAG	12-032,840
385	09717-L28947	*NOS1*	12q24	GCTTGCAGATAT-GCATACAGCAGG	12-116,200
452	12459-L13460	*POMT2*	14q24	ATCACTGTGAAG-AACCTCCGGATG	14-076,832
310	09065-L09234	*CACNA1A*	19p13	CTCAGGCCTTCT-ACTGGACTGTAC	19-013,289

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

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

Related products
For related products, see the product page https://www.mrcholland.com/product/P078 on our website.

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Selected publications using P078 Breast tumour

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P078 product history	
Version	*Modification*
D3	A new flanking probe of 17p has been added. Several probes have a change in length but not in the targeted sequence.
D2	Four reference probes were replaced and several probes have a small change in length, but no change in sequence detected.
D1	More than 40% of reference probes were replaced and/or added.
C2	Several probes have a small change in length, but no change in sequence detected. In addition, several reference probes were replaced and/or added.
C1	Two probes for *ZNF703* gene were included. In addition, several target, reference probes and 88 and 96 nt control fragments were replaced.
B1	Content completely revised. Target probes for *BIRC5* and *MTDH* added and several reference probes replaced.
A1	First release.

Implemented changes in the product description
Version D3-01 – 22 December 2025 (05P) - Product description restructured and adapted to a new template. - Various textual or layout changes. - Salt warnings removed for BIRC5 probes at 392 and 436 nt. - Added the 1nt difference remark for reference probe at 346 nt in Table 1 and 3. - Added the minor alteration remark for probes at 143, 324, 436 and 484 nt in Table 1. - List of references has been updated. - List of selected publications has been updated.

Version D2-02 – 18 October 2022 (04P)
- Product description restructured and adapted to a new template.
- Various minor textual or layout changes.
- Exon numbering of the *EMSY*, *ERBB2, EMSY, BIRC5* and *AURKA* genes has been changed in Table 2.
- Transcript number of the *EMSY* and *BIRC5* genes has been changed in Table 2 according to the MANE Select transcripts.
- Ligation sites of the probes targeting the *ESR1*, *FGFR1*, *MYC*, *CCND1* and *CDH1* genes updated according to new version of the NM_reference sequence.
- List of related probemixes updated on page 10.
- List of references has been updated.

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