

SALSA MLPA KIT P045-B1 BRCA2/CHEK2

Lot 0108, 1007

BREAST CARCINOMA is the most common malignancy among women in developed countries and family history remains the strongest single predictor of breast cancer risk. Mutations in the BRCA1 and BRCA2 genes are linked to a high risk of young-onset breast cancer, and appear to be responsible for approximately 10% of total breast cancer cases. Women with these mutations have a cumulative risk of developing breast cancer (up to age 70) of 55-85%. The BRCA1 and BRCA2 proteins are associated with the activation of double-strand break repair and/or homologous recombination. Unlike BRCA1, BRCA2 has not been linked to ovarian cancer. BRCA2 mutations are less frequent than BRCA1 mutations but in families with male breast cancer cases, BRCA2 mutations may be more frequent.

The P045-B1 probemix contains probes for all exons of the BRCA2 gene. Two probes are present for exons 1, 3 and 27, and for the large exon 11. For a reference, 8 probes for other human genes located on different chromosomes are included. In addition, two probes are present for sequences just before and after the BRCA2 gene. In addition, three probes for the CHEK2 gene on 22q12.1 are included. One of these probes will only result in an amplification product in case the DNA sample contains the CHEK2 1100delC mutation. The 1100delC allele appears to result in a two fold increase of breast cancer risk in woman and a 10-fold increase of risk in men. The 1100delC allele has been found in the Netherlands in 1.1% of healthy individuals and in 5.1% of individuals with breast cancer, including 13.5% of individuals from families with male breast cancer.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the BRCA2 and CHEK2 genes. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test. BRCA2 deletions might be more frequent in families with male breast cancer.

SALSA® MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.

The use of this MLPA kit requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

Related SALSA MLPA kits

- P090 BRCA2: Identical to P045 BRCA2/CHEK2, but does not contain probes for CHEK2.
- P002 BRCA1: Hereditary breast cancer, primary screening BRCA1
- P087 BRCA1: Hereditary breast cancer, confirmation BRCA1
- P239 BRCA1 region: Characterization of BRCA1 deletions/duplications.
- P190 CHEK2: Breast cancer susceptibility, genes included: CHEK2, ATM, BRCA1&2, PTEN, TP53
- P057 FANCD2/PALB2: Mutations in PALB2 have been linked to a higher risk on breast cancer.
- P240 BRIP1/CHEK1: Mutations in BRIP1 has been linked to a higher risk on breast cancer.
- P041/P042 ATM: Mutations in ATM has been linked to a higher risk on breast cancer.

More information

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References of SALSA MLPA kit P045

- Lim Y. et al. (2007). Identification of novel BRCA large genomic rearrangements in Singapore Asian breast and ovarian patients with cancer. *Clin Genet.* 2007 Apr;71(4):331-42.
- Karhu R. et al. (2006). Large genomic BRCA2 rearrangements and male breast cancer. *Cancer Detect Prev.* 2006;30(6):530-4.
- Preisler S. et al. (2006). Gross rearrangements in BRCA1 but not BRCA2 play a notable role in predisposition to breast and ovarian cancer in high-risk families of German origin. *Cancer Genet Cytogenet.* 2006 Jul 1;168(1):44-9.
- Thomassen M. et al. (2006). Low frequency of large genomic rearrangements of BRCA1 and BRCA2 in western Denmark. *Cancer Genet Cytogenet.* 2006 Jul 15;168(2):168-71.
- Agata S et al (2005). Large genomic deletions inactivate the BRCA2 gene in breast cancer families. *J Med Genet.* 2005 Oct;42(10):e64
- Woodward AM, et al. (2005). Large genomic rearrangements of both BRCA2 and BRCA1 are a feature of the inherited breast/ovarian cancer phenotype in selected families. *J Med Genet.* 42(5):e31.

Data analysis

This P045-B1 BRCA2 probemix contains 43 different MLPA probes with amplification products between 130 and 481 nt. In addition, it contains 7 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt and three DNA denaturation control fragments (D-fragments) at 88-92-96 nt. Only in samples with the CHEK2 1100delC mutation, an extra fragment should appear at 490 nt.

Data generated by this probemix can be intra-normalized by dividing the peak area of each probe's amplification product by the total area of only the reference probes in this probemix (block normalization). Secondly, normalisation can be achieved by dividing this intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference samples. Please note that this type of normalization assumes that no changes occurred in the genomic regions targeted by the reference probes.

When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to easily identify exon deletions. Comparison of results should preferably be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blots or long range PCR.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

CHEK2del1100 probe

We have received reports of experiments at three different laboratories in which the CHEK2del1100 peak spuriously appeared in known normal controls as well as all samples. At MRC-Holland we have noticed this same phenomenon once with a similar CHEK2 probe in the P056 TP53 probemix. This did not seem to be a contamination problem, as it appeared in some experiments but not in others in which the same vials of probemix and reagents were used. Despite many attempts with variations in the protocol, we have not been able to reproduce this. Results obtained with this CHEK2 probe should therefore be treated with caution. Please note that the presence of this allele increases the risk on breast cancer in woman only two-fold.

This probemix was developed by at MRC-Holland. Info/remarks/suggestions for improvement: info@mlpa.com.

SALSA MLPA P045-B1 BRCA2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position		
		Reference	CHEK2	BRCA2
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA			
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation			
130	Reference probe 0797-L00463	5q31		
137	BRCA2 probe 2283-L01774		Exon 1	
148	BRCA2 probe 2285-L01776		Exon 1	
154‡	BRCA2 probe 9297-L08066		Exon 14	
161	FRY probe 2143-L09586		20 Kb before BRCA2	
166	BRCA2 probe 2486-L01985		Exon 2	
170‡†	BRCA2 probe 8898-L09587		Exon 3	
177	BRCA2 probe 1599-L10642		Exon 3	
184	Reference probe 1217-L00694	4q		
191‡	BRCA2 probe 9812-L10643		Exon 23	
197	BRCA2 probe 1600-L04671		Exon 4	
202‡	BRCA2 probe 8265-L08128		Exon 7	
211	Reference probe 1344-L00555	9q		
220	BRCA2 probe 1602-L01184		Exon 8	
229	BRCA2 probe 1603-L01185		Exon 9	
238	Reference probe 0517-L00097	2q		
247	BRCA2 probe 1604-L01186		Exon 10	
256	BRCA2 probe 2279-L01770		Exon 11 start	
265	HCS20 probe 6800-L02040	In CHEK2 promotor region		
274	BRCA2 probe 1606-L01188		Exon 11 end	
283	BRCA2 probe 1607-L01189		Exon 12	
292	Reference probe 0990-L00567	11q		
301	BRCA2 probe 2280-L01771		Exon 13	
310‡	BRCA2 probe 9809-L10257		Exon 5	
319‡	BRCA2 probe 9296-L11090		Exon 27	
328	BRCA2 probe 1610-L01192		Exon 15	
337	BRCA2 probe 1611-L01193		Exon 16	
346	BRCA2 probe 4585-L03983		Exon 6	
355	BRCA2 probe 2281-L01772		Exon 17	
364	BRCA2 probe 1613-L01195		Exon 18	
373	Reference probe 2667-L04984	11q		
382	BRCA2 probe 1614-L01196		Exon 19	
391‡	BRCA2 probe 8266-L08129		Exon 20	
400	CHEK2 probe 2579-L02041	CHEK2 Exon 9		
409	BRCA2 probe 2069-L01970		Exon 21	
418	BRCA2 probe 1617-L01199		Exon 22	
427	Reference probe 1108-L00679	8q		
436‡	BRCA2 probe 8267-L08130		Exon 24	
445‡	BRCA2 probe 8268-L08131		Exon 25	
454	CG018 probe 2144-L01619		9 Kb after BRCA2	
463	BRCA2 probe 4586-L03984		Exon 26	
472‡	BRCA2 probe 9293-L09584		Exon 27	
481	Reference probe 1060-L00628	17q		
490†	CHEK2 probe 1772-L01336	CHEK2 exon 11, Mutation 1100delC specific!		

‡ New from P045-B lot 1007 onwards.

† Mutation 1100delC specific! This peak will only appear if the point mutation is present. Please read the note on this probe on page 2.

‡ The 170 nt exon 3 probe is a (wildtype) probe whose ligation should be disrupted by the relatively frequent ex3, 504del 5068inCCAT mutation. A 50% decrease in probe signal is expected when such a heterozygous mutation is present. The 177 nt exon 3 probe does not detect this mutation.

Note: Exon numbering might be different as compared to literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

BRCA2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	BRCA2 Exon	Ligation site NM_000059.3	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
161	2143-L09586	FRY gene		CCCAGAGTTA-CCGAGTCCTC	20 Kb
	<i>Start codon</i>		<i>228-230</i>		
137	2283-L01774	Exon 1	Promoter region	GCGCGGGCTT-GTGGCGCGAG	0.2 Kb
148	2285-L01776	Exon 1	Promoter region	GTAGTGGGTT-GGGACGAGCG	0.7 Kb
166	2486-L01985	Exon 2	271-270 reverse	CGTGTCTTAA-AAATTTCAAA	2.7 Kb
177	1599-L10642	Exon 3	472-473	TAATATTCAA-AGAGCAAGGG	0.2 Kb
170‡	8898-L09587	Exon 3	Intron 3, 108 nt after exon 3	GGGCAAATCA-GTCTCTCTGG	5.7 Kb
197	1600-L04671	Exon 4	569-570	TAGTAGACAT-AAAAGTCTTC	1.0 Kb
310†	9809-L10257	Exon 5	688-689	TAACACCACA-AAGAGATAAG	0.1 Kb
346	4585-L03983	Exon 6	728-727 reverse	AAACTTTGGT-GTATGAAACA	0.2 Kb
202	8265-L08128	Exon 7	812-813	GTCTTGGTCA-AGTTCCTTAG	2.6 Kb
220	1602-L01184	Exon 8	Intron 8, 264 nt after exon 8	TGACTTTCCA-ACTCATTGTG	1.8 Kb
229	1603-L01185	Exon 9	1001-1002	CACAAATCAA-AGAGAAGCTG	1.6 Kb
247	1604-L01186	Exon 10	1374-1375	AGTGACAAAA-TCTCCAAGGA	3.7 Kb
256‡	2279-L01770	Exon 11	2192-2193	TGAAGAACCA-ACTTTGTCTT	4.8 Kb
274	1606-L01188	Exon 11	6992-6993	TCTTTTTACA-TGTCCGAAA	3.2 Kb
283	1607-L01189	Exon 12	Intron 12, 183 nt after exon 12	ACAGAACAAA-AATGTAATTG	2.5 Kb
301	2280-L01771	Exon 13	7216-7215 reverse	ACACAGGTAA-TCGGCTCTAA	8.1 Kb
154	9297-L08066	Exon 14	7394-7395	TGCTACAAGA-AATGAAAAAA	1.5 Kb
328	1610-L01192	Exon 15	7762-7763	GTCTGTATCT-TGCAAAAACA	1.3 Kb
337	1611-L01193	Exon 16	7975-7976	AGTTGGCTGA-TGGTGGATGG	4.7 Kb
355	2281-L01772	Exon 17	8158-8157 reverse	AGGCATCTAT-TAGCAAATTC	0.8 Kb
364	1613-L01195	Exon 18	8482-8483	AGAAGATTAT-TCTTCATGGAG	7.0 Kb
382	1614-L01196	Exon 19	8602-8603	ATACCAAAC-TGGATTCTTT	0.5 Kb
391	8266-L08129	Exon 20	8743-8744	CTGGATTATA-CATATTTCCG	5.7 Kb
409	2069-L01970	Exon 21	8909-8910	AAGACAGCAA-GTTCGTGCTT	2.7 Kb
418	1617-L01199	Exon 22	9100-9101	CTGAACAAAA-GGAACAAGGT	0.3 Kb
191	9812-L10643	Exon 23	9214-9215	CATCAGATTT-ATATTCTCTG	0.3 Kb
436	8267-L08130	Exon 24	9455-9454 reverse	AACGACAAAT-CCTATTAGGT	14.8 Kb
445	8268-L08131	Exon 25	9706-9707	AGACATTCAA-CAAAATGAAA	2.0 Kb
463	4586-L03984	Exon 26	9786-9787	CTGCATGCAA-ATGATCCCAA	1.3 Kb
472†	9293-L09584	Exon 27	9988-9989	AGTCTTGTA-AGGGGAGAAA	0.6 Kb
319†	9296-L11090	Exon 27	10638-10639	CGGGCAAAAA-TCGTTTTGCC	8.3 Kb
	<i>Stop codon</i>		<i>10482-10484</i>		
454	2144-L01619	CG018 gene		TTATTATTGA-TAATACCAAC	

‡ The 170 nt exon 3 probe is a (wildtype) probe whose ligation should be disrupted by the relatively frequent ex3, 504del 5068inCCAT mutation. A 50% decrease in probe signal is expected when such a heterozygous mutation is present. The 177 nt exon 3 probe does not detect this mutation.

‡ The benign BRCA2 polymorphism, 2192C>G (P655R), can result in a lower probe signal of the 256 nt exon 11 probe (false positive signal).

† The two exon 27 probes each have a higher than average standard variation. Several other exon 27 probes prepared at MRC-Holland also showed high variability. Apparent deletions of only one of the exon 27 probes could well be a false positive. Similarly, a copy number change of the more variable exon 5 probe is unlikely when the exon 6 probe, which is at very close distance, has no copy number change. Distance on chromosomal 22q12 DNA between the 265 and 400 nt CHEK2 probes is 44 Kb. 13CDNA73 is renamed FRY.

Note: Exon numbering might be different as compared to literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA kit P045-B1 BRCA2 sample pictures

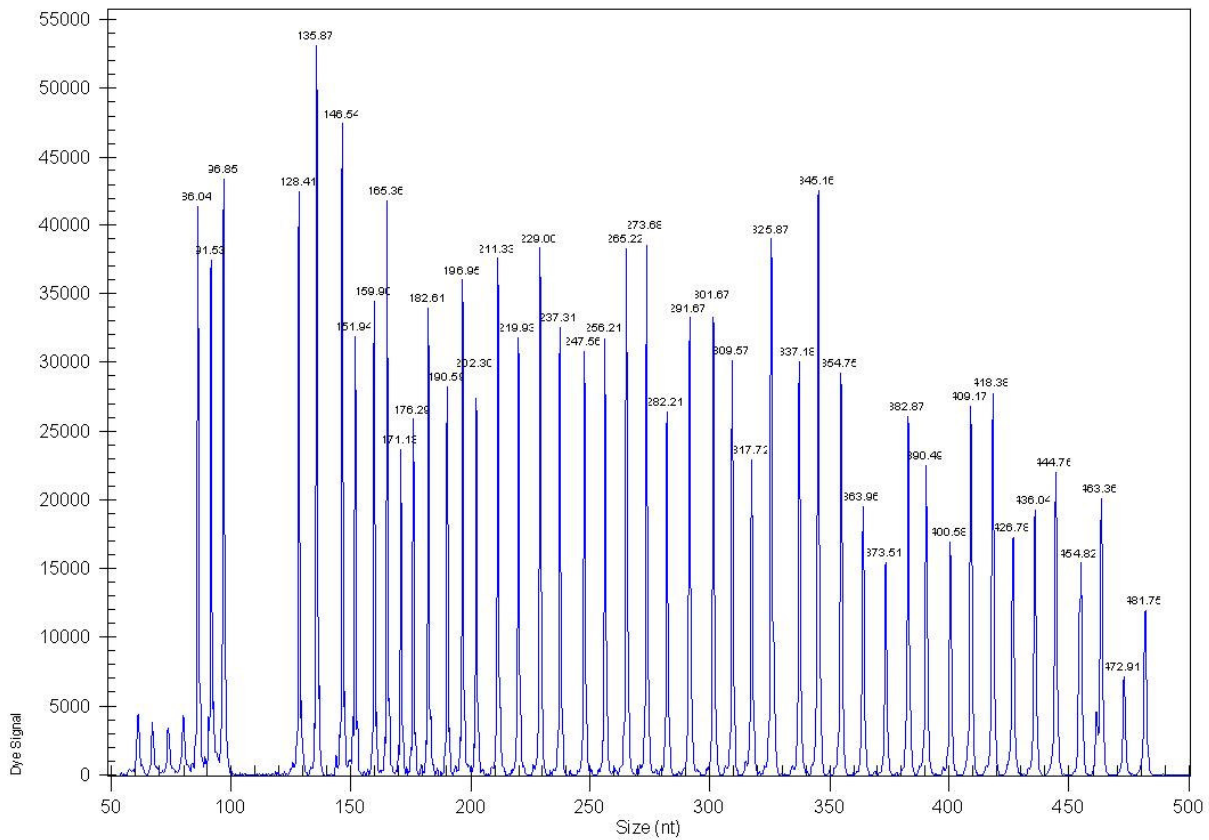


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P045-B1 BRCA2 (lot 0108).

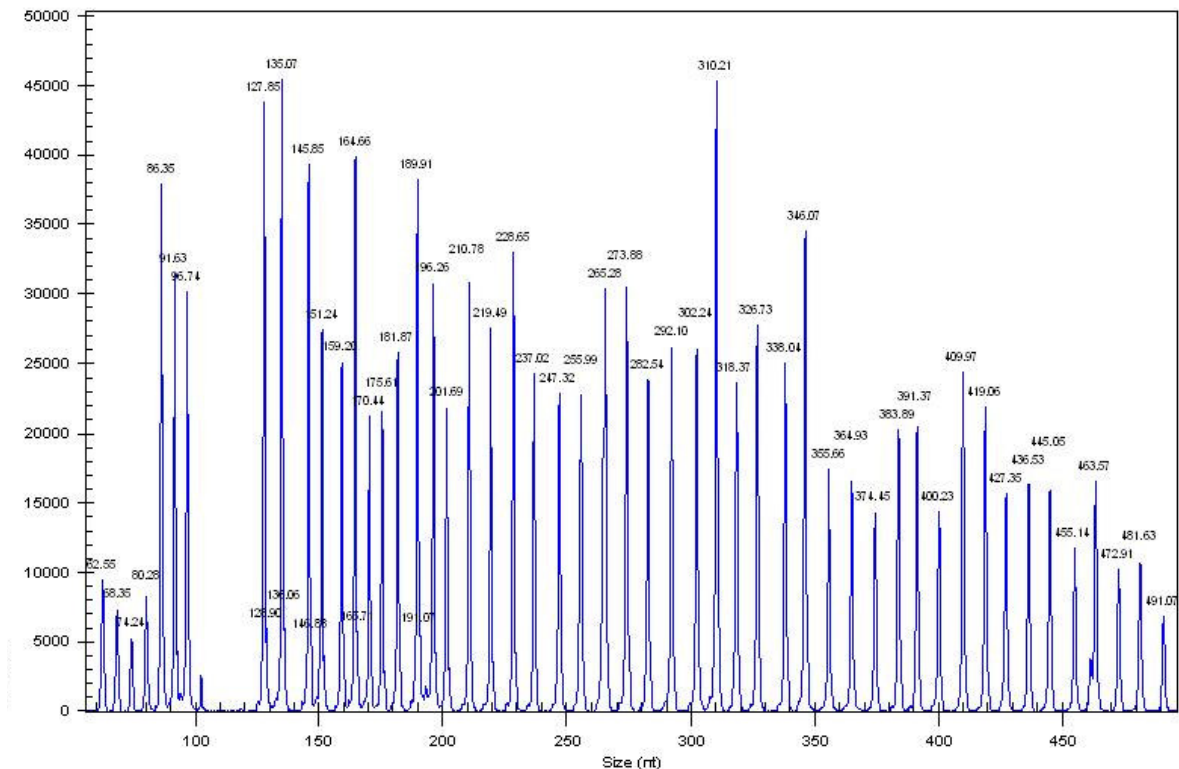


Figure 2. Capillary electrophoresis pattern from a sample of a CHEK2 exon 11, 1100delC mutation carrying patient, approximately 50 ng human DNA analyzed with SALSA MLPA kit P045-B BRCA2 (lot 1007).