Product Description SALSA® MLPA® Probemix P057-B2 FANCD2-PALB2

To be used with the MLPA General Protocol.

Version B2. For complete product history see page 7.

Catalogue numbers:
- **P057-025R:** SALSA MLPA Probemix P057 FANCD2-PALB2, 25 reactions.
- **P057-050R:** SALSA MLPA Probemix P057 FANCD2-PALB2, 50 reactions.
- **P057-100R:** SALSA MLPA Probemix P057 FANCD2-PALB2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P057 FANCD2-PALB2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FANCD2* and *PALB2* genes, which are associated with Fanconi Anemia (FA).

FA is an autosomal recessive disorder affecting all bone marrow elements and associated with cardiac, renal, and limb malformations as well as with dermal pigmentary changes. Several FA-associated genes have been identified so far, the products of which function in the FA/BRCA pathway. A key event in the pathway is the monoubiquitination of the FANCD2 protein, which depends on a multiprotein FA core complex. Defects in the *FANCD2* gene are one of the possible causes of FA. The *FANCD2* gene (44 exons) spans ~73 kb of genomic DNA and is located on chromosome 3p25.3, about 10 Mb from the p-telomere.

FA has also been linked to defects in the *PALB2* gene. It was shown that mutations in *PALB2* result in an increased susceptibility to breast cancer and that biallelic mutations cause Fanconi anemia subtype FA-N and predispose to childhood cancers. *PALB2* mutations have also been detected in approximately 3% of familial pancreatic cancer families, especially those families in which also breast cancer cases occur (Slater et al. 2010). The *PALB2* gene spans ~38 kb of genomic DNA and is located on chromosome 16p12.2, about 24 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1401/.

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/

Probemix content: The SALSA MLPA Probemix P057-B2 FANCD2-PALB2 contains 39 MLPA probes with amplification products between 130 and 431 nt. This includes 15 probes targeting 14 out of 44 exons of the *FANCD2* gene. Furthermore, it also contains 13 probes for the *PALB2* gene, one probe for every exon. In addition, 11 reference probes are included and detect 11 different autosomal chromosomal locations.

Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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, 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.mlpa.com.

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

No DNA controls results in only five major peaks shorter than 105 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

Required specimens: Extracted DNA 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.

Reference samples: 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 unrelated individuals who are from families without a history of Fanconi Anemia. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change, therefore samples should be validated before use.

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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

Confirmation of results: The P260 PALB2-RAD50-RAD51C-RAD51D can be used for confirmation of results for seven PALB2 probes of the P057 (see Table 2b). The other six PALB2 probes cannot be confirmed by probemix P260 because their ligation site is similar or close to the probes in the P057.

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.

Fanconi Anemia mutation database: http://www2.rockefeller.edu/fanconi/. We strongly encourage users to deposit positive results in the Fanconi Anemia mutation database of the Rockefeller University. 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 SNPs and unusual results (e.g., a duplication of *PALB2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P057-B2 FANCD2-PALB2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	*FANCD2*	*PALB2*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q31		
142	**PALB2 probe** 07492-L09488			**Exon 1**
148	Reference probe 00798-L00316	13q32		
154	**PALB2 probe** 07933-L07714			**Exon 9**
161	**PALB2 probe** 16145-L18314			**Exon 12**
166	**PALB2 probe** 07501-L07163			**Exon 10**
171	**FANCD2 probe** 02129-L07570		**Exon 2**	
177	**FANCD2 probe** 02130-L07571		**Exon 4**	
184	Reference probe 10973-L11644	14q31		
193	**FANCD2 probe** 02131-L01624		**Exon 9**	
200	Reference probe 16990-L19999	17q24		
208	**PALB2 probe** 16146-L18315			**Exon 7**
214	**FANCD2 probe** 02132-L28606		**Exon 12**	
220	**FANCD2 probe** 16147-L18316		**Exon 30**	
226	Reference probe 10240-L04097	9q33		
232	**FANCD2 probe** 16148-L18317		**Exon 6**	
240	Reference probe 17009-L20056	1q32		
249	**FANCD2 probe** 16149-L28607		**Exon 10**	
255	**FANCD2 probe** 16150-L18319		**Exon 29**	
265	**FANCD2 probe** 01649-L01226		**Exon 1**	
274	**FANCD2 probe** 02137-L01630		**Exon 32**	
283	**FANCD2 probe** 02138-L01631		**Exon 35**	
292	**PALB2 probe** 07502-L07164			**Exon 11**
301	**FANCD2 probe** 02139-L19933		**Exon 41**	
310	**FANCD2 probe** 02140-L01633		**Exon 43**	
319	Reference probe 01042-L10915	8q24		
330	**FANCD2 probe** 01650-L19934		**Exon 1**	
337	**PALB2 probe** 07504-L07166			**Exon 13**
346	Reference probe 16492-L18948	12q23		
355	**PALB2 probe** 07495-L28608			**Exon 4**
364	**FANCD2 probe** 16151-L18320		**Exon 38**	
373	**PALB2 probe** 07497-L07159			**Exon 6**
382	**PALB2 probe** 16152-L18321			**Exon 8**
391	Reference probe 12522-L13572	18q21		
400	**PALB2 probe** 07494-L07156			**Exon 3**
409	**PALB2 probe** 07496-L06744			**Exon 5**
418	Reference probe 07594-L22945	15q26		
427	**PALB2 probe** 07493-L28609			**Exon 2**
431	Reference probe 15541-L25346	2q23		

Note: The exon numberings used in this P057-B2 FANCD2-PALB2 product description are the exon numberings from the RefSeq transcripts NM_001018115.1 (*FANCD2*) and NM_024675.3 (*PALB2*), which are identical to the LRG_306t1 and LRG_308 sequences respectively. The exon numberings and NM sequences used are from 07/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. P057-B2 probes arranged according to chromosomal location

Table 2a. *FANCD2* gene

Length (nt)	SALSA MLPA probe	*FANCD2* exon	Ligation site NM_001018115.1	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*79-81 (Exon 2)*		
265 #	01649-L01226	Exon 1	190 nt before exon 1	AGCTTCTCTTCA-CCGGGGCGCAGT	0.1 kb
330	01650-L19934	Exon 1	49 nt before exon 1	CTTCCGGCGCGG-AAGTTGGCGTCA	2.3 kb
171	02129-L07570	Exon 2	103-104	GAAGACTGTCAA-AATCTGAGGATA	5.8 kb
177	02130-L07571	Exon 4	306-307	ATAGCTTTCCAA-AAGAAGCTCTTT	0.7 kb
232	16148-L18317	Exon 6	495-494 reverse	TCAATCCCCAGA-AGCAGTTTGATG	4.6 kb
193	02131-L01624	Exon 9	710-711	GCAGCATGACAT-CATCACCAGCCT	1.9 kb
249	16149-L28607	Exon 10	802-801 reverse	GATTGGGACAGT-GAGTGAAGTATT	1.4 kb
214 #	02132-L28606	Exon 12	996-997	GAGAAGTTGGAT-CTGCAGCATTGT	31.5 kb
255	16150-L18319	Exon 29	2852-2851 reverse	CCAGCTCTCGGA-AAAAAGCATGGG	3.6 kb
220	16147-L18316	Exon 30	3029-3030	GACACCTCCTAT-TGCCAGGAGAGT	3.3 kb
274	02137-L01630	Exon 32	3288-3289	CAGATTTTTCAT-GGGCTTTTTGCT	7.1 kb
283	02138-L01631	Exon 35	3596-3597	TGGGGATAAAGA-GAAGAGCAACAT	3.7 kb
364	16151-L18320	Exon 38	3864-3863 reverse	AGGAGTTTCTCT-TCATGAATCTGG	3.1 kb
301	02139-L19933	Exon 41	4070-4071	ACTGGAAACCTT-CCAGTTGGACAC	3.6 kb
310	02140-L01633	Exon 43a	12 nt after exon 43a	TATCTCTACAAA-ACCCACCAGAGT	
		stop codon	*4432-4434 (Exon 44)*		

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Table 2b. *PALB2* gene

Length (nt)	SALSA MLPA probe	*PALB2* exon	Ligation site NM_024675.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*201-203 (Exon 1)*		
142 »	07492-L09488	Exon 1	180-181	ACGGCTGCTCTT-TTCGTTCTGTCG	3.1 kb
427 »	07493-L28609	Exon 2	289-288 reverse	GGGCTAGTGTCT-TGCTGTATTCCC	0.2 kb
400	07494-L07156	Exon 3	388-387 reverse	GCTGCGGTGAGA-GATCCTGCTGAG	1.7 kb
355	07495-L28608	Exon 4	546-547	GCCCAGGAGGAT-TACCTATACAAA	6.1 kb
409	07496-L06744	Exon 5	2208-2209	CAGAAATGGAGG-ACTTAGAAGAGG	0.9 kb
373 »	07497-L07159	Exon 6	2741-2742	CTTCCTGCTTCT-GATAGCATAAAC	3.0 kb
208	16146-L18315	Exon 7	2 nt after exon 7	CTTCGCAGAGGT-AAGTGGGAATCT	2.1 kb
382	16152-L18321	Exon 8	1 nt before exon 8 reverse	AATACTGGAACC-TAAATAAAACAA	1.1 kb
154	07933-L07714	Exon 9	3143-3144	GTTAGTAGCAGT-GGGACCCTTTCT	1.6 kb
166 »	07501-L07163	Exon 10	3247-3248	TATACTAACTTT-TGCTGAGGTCCA	7.4 kb
292 »	07502-L07164	Exon 11	3368-3367 reverse	CAGACTGAAGCT-TGGTAAGAATCA	6.2 kb
161	16145-L18314	Exon 12	3529-3530	GCTGTACTGTCT-TCCTCCAGGGCA	4.3 kb
337 »	07504-L07166	Exon 13	3590-3591	TGTGCAGCAGCA-ATCTTGACTTCT	
		stop codon	*3759-3761 (Exon 13)*		

Note: The exon numberings used in this P057-B2 FANCD2-PALB2 product description are the exon numberings from the RefSeq transcripts NM_001018115.1 (*FANCD2*) and NM_024675.3 (*PALB2*), which are identical to the LRG_306t1 and LRG_308 sequences respectively. The exon numberings and NM sequences used are from 07/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

» The ligation sites of these probes are the same, or in close proximity to probes present in SALSA MLPA P260 probemix.

Related SALSA MLPA probemixes

P031 FANCA mix 1	Fanconi Anemia group A. Probes for the *FANCA* gene included.
P032 FANCA mix 2	Fanconi Anemia group A. Probes for the *FANCA* gene included.
P113 FANCB	Fanconi Anemia group B. Probes for the *FANCB* gene included.
P260 PALB2-RAD50-RAD51C-RAD51D	Probes for the *PALB2*, *RAD50*, *RAD51C* and *RAD51D* genes included.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Slater EP et al. (2010). PALB2 mutations in European familial pancreatic cancer families. *Clin Genet*. 78:490-4.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P057 FANCD2-PALB2

- Blanco A et al. (2012). Detection of a large rearrangement in PALB2 in Spanish breast cancer families with male breast cancer. *Breast Cancer Res Treat.* 132:307-15.
- Francies FZ et al. (2018). Diagnosis of Fanconi Anaemia by ionising radiation-or mitomycin C-induced micronuclei. *DNA Repair (Amst)*. 61:17-24.
- Ghiorzo P et al. (2012). Contribution of germline mutations in the BRCA and PALB2 genes to pancreatic cancer in Italy. *Fam Cancer.* 11:41-7.
- Guenard F et al. (2010). Evaluation of the Contribution of the Three Breast Cancer Susceptibility Genes CHEK2, STK11, and PALB2 in Non-BRCA1/2 French Canadian Families with High Risk of Breast Cancer. *Genet Test Mol Biomarkers.* 14:515-26.
- Harinck F et al. (2012). Routine testing for PALB2 mutations in familial pancreatic cancer families and breast cancer families with pancreatic cancer is not indicated. *Eur J Hum Genet.* 20:577-9.

P057 Product history	
Version	*Modification*
B2	Four reference probes have been replaced and one added.
B1	Probes for *FANCD2* exons 17, 23 and 28 has been removed (pseudo region). New probes added for *FANCD2* exons 10, 29, 30 and 38 and one probe (exon 6) redesigned. Three probes for *PALB2* have been redesigned. Six Reference probes have been replaced and one reference probe was added.
A2	Control fragments at 88-96-100-105 nt have been added.
A1	First release.

Implemented changes in the product description
Version B2-01 – 10 October 2018 (01P) - Product description restructured and adapted to a new template. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - A remark was added for probes with ligation sites similar or close to the probes in probemix P260. *Version 18 – 24 June 2015 (54)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - Small textual changes on page 1 and 2. *Version 17 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 16 (48)* - This product description has been changed to incorporate a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Minor textual changes. *Version 15 (45)* - This product description has been changed to incorporate a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Small textual changes page 1. Data analysis section has been modified. *Version 14 (43)* - This product description has been changed to incorporate a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

More information: www.mlpa.com; www.mlpa.eu	
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