Product Description SALSA® MLPA® Probemix P031-B3/B032-B3 FANCA

To be used with the MLPA General Protocol.

P031-B3. As compared to version B2, one reference probe has been removed.
P032-B3. As compared to version B2, one reference probe has been removed.
For complete product history see page 7.

Catalogue numbers:
- **P031-025R:** SALSA MLPA Probemix P031 FANCA mix 1, 25 reactions.
- **P031-050R:** SALSA MLPA Probemix P031 FANCA mix 1, 50 reactions.
- **P031-100R:** SALSA MLPA Probemix P031 FANCA mix 1, 100 reactions.

- **P032-025R:** SALSA MLPA Probemix P032 FANCA mix 2, 25 reactions.
- **P032-050R:** SALSA MLPA Probemix P032 FANCA mix 2, 50 reactions.
- **P032-100R:** SALSA MLPA Probemix P032 FANCA mix 2, 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: These SALSA MLPA Probemixes P031/P032 FANCA are **research use only (RUO)** assays for the detection of deletions or duplications in the *FANCA* gene, which is associated with Fanconi Anemia.

Fanconi Anemia (FA) is an autosomal recessive disorder characterised by physical abnormalities, bone marrow failure, and increased risk of malignancy. Mutations in several different genes can result in FA, however defects of the *FANCA* gene are the most frequent cause (60-70% of cases). Known defects of the *FANCA* gene include point mutations, small deletions/insertions and deletions of one or more complete exons.

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 P031-B3 FANCA mix 1 contains 31 MLPA probes with amplification products between 137 and 409 nt. This includes 22 probes for the *FANCA* gene. In addition, nine reference probes are included and detect nine different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

The SALSA MLPA Probemix P032-B3 FANCA mix 2 contains 32 MLPA probes with amplification products between 140 and 418 nt. This includes 21 probes for the *FANCA* gene and one flanking probe in the *GAS8* gene. In addition, ten reference probes are included and detect ten different autosomal chromosomal

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

These Probemixes contains nine quality control fragments generating amplification products between 64 and 121 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.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 121 nucleotides (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 121 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 or in or near the *FANCA* 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: 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:
- In most populations, the major cause of genetic defects in the *FANCA* gene are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemixes P031/P032 FANCA.
- 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: 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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *FANCA* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P031-B3 FANCA mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*FANCA*
64-105	Control fragments – see table in probemix content section for more information		
137	Reference probe 16495-L18956	5q14	
142	Reference probe 06595-L06153	8q24	
157 «	**FANCA probe** 03222-L01222		**Exon 1**
166 «	**FANCA probe** 15624-L17488		**Exon 3**
178 «	**FANCA probe** 01705-L01273		**Exon 5**
184	**FANCA probe** 01479-L12783		**Exon 7**
193	Reference probe 17916-L22221	14q23	
202	**FANCA probe** 15625-L17489		**Exon 9**
210	**FANCA probe** 15626-L17490		**Exon 11**
220	**FANCA probe** 15627-L17491		**Exon 13**
230	**FANCA probe** 01487-L01095		**Exon 15**
239	Reference probe 15064-L16822	4q31	
247	**FANCA probe** 15628-L17492		**Exon 17**
256	**FANCA probe** 01491-L01099		**Exon 19**
264	**FANCA probe** 15629-L18639		**Exon 21**
274	**FANCA probe** 01495-L01103		**Exon 23**
283	Reference probe 06005-L05430	2q36	
292	**FANCA probe** 01497-L01105		**Exon 25**
301	Reference probe 04750-L04098	9q33	
310	**FANCA probe** 01499-L18640		**Exon 27**
319	**FANCA probe** 01501-L18641		**Exon 29**
328	**FANCA probe** 15630-L18642		**Exon 31**
341	**FANCA probe** 01505-L22365		**Exon 33**
350	**FANCA probe** 01507-L22364		**Exon 35**
355	Reference probe 06521-L06079	3q27	
364	**FANCA probe** 01509-L01117		**Exon 37**
373	**FANCA probe** 15631-L17495		**Exon 39**
382	Reference probe 13329-L14755	18q21	
391	**FANCA probe** 15632-L17496		**Exon 43**
400	**FANCA probe** 15633-L17497		**Exon 41**
409	Reference probe 12866-L19707	13q14	

« 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.

Table 1b. SALSA MLPA Probemix P032-B3 FANCA mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*FANCA*
64-105	Control fragments – see table in probemix content section for more information		
140	Reference probe 17132-L20324	1p22	
148	Reference probe 17418-L22181	17p13	
154 «	**FANCA probe** 16595-L19120		**Exon 4**
160	Reference probe 11908-L12729	16p13	
166 «	**FANCA probe** 16594-L22023		**Exon 2**
172	Reference probe 07718-L07450	12q24	
178	**FANCA probe** 16596-L19121		**Exon 6**
185	**FANCA probe** 16597-L22182		**Exon 8**
193	Reference probe 03217-L02642	10q25	
202	**FANCA probe** 16598-L19123		**Exon 10**
211	**FANCA probe** 01484-L01092		**Exon 12**
220	**FANCA probe** 01486-L01094		**Exon 14**
229	**FANCA probe** 01488-L09260		**Exon 16**
238	Reference probe 12923-L14074	9q22	
247	**FANCA probe** 01490-L01098		**Exon 18**
256	**FANCA probe** 01492-L01100		**Exon 20**
265	**FANCA probe** 01494-L01102		**Exon 22**
274	**FANCA probe** 01496-L09261		**Exon 24**
283	Reference probe 16271-L18563	13q14	
295	**FANCA probe** 01498-L09262		**Exon 26**
304	**FANCA probe** 01500-L09263		**Exon 28**
310	**FANCA probe** 16599-L22183		**Exon 30**
319	**FANCA probe** 16600-L19125		**Exon 32**
337	**FANCA probe** 01506-L01114		**Exon 34**
345	**FANCA probe** 01508-L09321		**Exon 36**
355	**FANCA probe** 16601-L19126		**Exon 38**
364	**FANCA probe** 01512-L09323		**Exon 40**
373	Reference probe 14351-L16020	7q22	
383	**FANCA probe** 01707-L01275		**Exon 42**
391 ¬	GAS8 probe 01087-L00734		16q24
409	Reference probe 02718-L00732	14q11	
418	Reference probe 10687-L11269	6p12	

« 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.
¬ 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.

Note: The exon numbering used in this P031/P032-B3 FANCA product description is the exon numbering from the RefSeq transcript NM_000135.2, which is identical to the LRG_495 sequence. The exon numbering and NM sequence used is from 02/2019, 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. FANCA probes arranged according to chromosomal location

Length (nt) P031/P032	SALSA MLPA probe	FANCA Exon	Ligation site NM_000135.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
391 ¬	01087-L00734	GAS8		CTGGCACTAACT-TCATTGACACCT	227.1 kb
		start codon	*43-45 (exon 1)*		
157 «	03222-L01222	Exon 1	27-28	GCCGCCGGGGCT-GTAGGCGCCAAG	0.7 kb
166 «	16594-L22023	Exon 2	225-224 reverse	CTTACCTCAAGC-AAAAGGGCATTC	1.3 kb
166 «	15624-L17488	Exon 3	291-292	TGTGACAGTTCT-GAGGCCTATGCT	3.5 kb
154 «	16595-L19120	Exon 4	392-393	AGCCGGGATGGT-TGCCTCTAGCGT	0.2 kb
178 «	01705-L01273	Exon 5	502-503	AGTTTGCTCAGT-ATTTATTGGCAC	2.4 kb
178	16596-L19121	Exon 6	611-612	CGTACAAGGCAT-TGTGAGCCTGCA	3.0 kb
184	01479-L12783	Exon 7	683-684	CTTCAGGAATCT-GTGCTGCCTTTG	1.9 kb
185	16597-L22182	Exon 8	50 nt before exon 8 reverse	GGTCTCCAAGCA-ACTGTGAATGGC	3.7 kb
202	15625-L17489	Exon 9	36 nt before exon 9 reverse	ATCTTGGTAATC-TTCTGTAATTTG	0.5 kb
202	16598-L19123	Exon 10	7 nt after exon 10 reverse	CCACCCTCAGGA-ACATACCAGCAC	3.3 kb
210	15626-L17490	Exon 11 (12)	1043-1044	CAGCCCTGTGCT-GAAAGGTAGGCC	3.4 kb
211	01484-L01092	Exon 12 (13)	1084-1085	GAGAGTGGAGCT-TTGCGCGGACAC	0.5 kb
220	15627-L17491	Exon 13 (14)	1157-1156 reverse	GCAAATGGCCAA-CCAACTCCTCTG	0.5 kb
220	01486-L01094	Exon 14 (15)	1299-1300	GCCCAGGCATTC-GAGAGCTGCCAG	6.6 kb
230	01487-L01095	Exon 15 (16)	1460-1461	CCTGGTCTTCCT-GTTTACGTTCTT	1.9 kb
229	01488-L09260	Exon 16 (17)	1567-1568	CCCTCCTCACAG-ACTACATCTCAT	0.1 kb
247	15628-L17492	Exon 17 (18)	1641-1640 reverse	GCTGATGACAAA-TCCTCGTAGAGT	3.0 kb
247	01490-L01098	Exon 18 (18)	1708-1709	AAAAGGCCATCA-TGGTGTTTGAGC	0.9 kb
256	01491-L01099	Exon 18 (20)	1781-1782	GCCTTACTACGT-GTCCCACTTCCT	0.1 kb
256	01492-L01100	Exon 20 (21)	1825-1826	CACAGCTCCCCA-AAGTCCCTGACT	3.1 kb
264	15629-L18639	Exon 21 (22)	19 nt after exon 21	CCTGGGCCGCTT-GTCCACTCTGGG	2.4 kb
265	01494-L01102	Exon 22 (23)	2014-2015	CAGCTGCACTGG-GAGAGCTGAGAG	1.5 kb
274	01495-L01103	Exon 23 (24)	2085-2086	GCAGTGATTTCT-GAAAGACTGAGG	1.2 kb
274	01496-L09261	Exon 24 (25)	2218-2219	TGCTGACGTCTT-TCTGTCAGAACC	0.4 kb
292	01497-L01105	Exon 25 (26)	2312-2313	GTGTGGACGTGT-GCTCCCTGCAGT	0.3 kb
295	01498-L09262	Exon 26 (27)	2498-2499	TGCGCTCTTTGA-CAGCCTCCTGAC	2.7 kb
310	01499-L18640	Exon 27 (28)	2583-2584	TCTCTCTGCAAG-TTTTCTTCCCAG	2.2 kb
304	01500-L09263	Exon 28 (29)	2745-2746	CTTCCTTCTGCA-GACTGGCAGAGA	3.0 kb
319	01501-L18641	Exon 29 (30)	2851-2852	TACACCTGGAGC-TGGAAATTCAAC	3.4 kb
310	16599-L22183	Exon 30 (31)	2994-2993 reverse	AGTGCGTTGACA-AGAATGGTACAC	6.5 kb
328	15630-L18642	Exon 31 (32)	3078-3079	GGTGGCCGCACA-GGAAATGAGGAT	2.3 kb
319	16600-L19125	Exon 32 (33)	3197-3196 reverse	GCCGTCTGCGGA-AAATCTCAAAGA	1.1 kb
341	01505-L22365	Exon 33 (34)	3337-3338	TCCAGGCAGAAC-AGCCCATCACTG	1.8 kb
337	01506-L01114	Exon 34 (35)	3412-3413	GCTCCCACGGAG-GTGCCCTGACAC	0.2 kb
350	01507-L22364	Exon 35 (36)	3506-3507	GGTCGACTTCAT-ACTGGCCAAGTG	1.6 kb
345	01508-L09321	Exon 36 (37)	3613-3614	GACACTGCCAGA-GCCCGCTGCCCC	2.2 kb
364	01509-L01117	Exon 37 (38)	3757-3758	AACAAGTCAGGG-AAGAAAACATCA	2.1 kb
355	16601-L19126	Exon 38 (39)	8 nt after exon 38	AAATGTAAGTCA-GACCTTACCAGC	0.7 kb
373	15631-L17495	Exon 39 (40)	4 nt before exon 39 reverse	TGGTGCTCTGTA-AACCGCAGGAGA	0.6 kb
364	01512-L09323	Exon 40 (41)	4048-4049	CTTTCGCTTTTT-ACAGGTACCTCC	0.3 kb
400	15633-L17497	Exon 41 (42)	4170-4171	ACAAGCACAGTT-TCACCTCCAGCT	0.2 kb
383	01707-L01275	Exon 42 (43)	4231-4232	TGGAACTGATAA-CAAAAGCTCGTC	0.9 kb
391	15632-L17496	Exon 43 (44)	4966-4967	AGCTCAGTCTCA-GCCTTGTGTTTG	
		stop codon	*4408-4410 (exon 43)*		

« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

¬ 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.

Notes:

- **The FANCA exon numbering has changed.** From description version 16 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the FANCA gene. This exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2.
- The exon numbering used in this P031/P032-B3 FANCA product description is the exon numbering from the RefSeq transcript NM_000135.2, which is identical to the LRG_495 sequence. The exon numbering and NM sequence used is from 02/2019, 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 identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Related SALSA MLPA probemixes

- P057 FANCD2-PALB2: Fanconi Anemia. Genes included: *FANCD2*, *PALB2*.
- P113 FANCB: Fanconi Anemia group B. Gene included: *FANCB*.
- P260 PALB2-RAD50-RAD51C-RAD51D: Fanconi Anemia. Genes included: *PALB2*, *RAD50*, *RAD51C* and *RAD51D*.

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.
- 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.

P031 Product history	
Version	*Modification*
B3	One reference probe has been removed.
B2	One reference probe has been replaced.
B1	Ten target probes for FANCA and all reference probes have been replaced. Furthermore QDX2 fragments have been added.
A2	Four extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

P032 Product history	
Version	*Modification*
B3	One reference probe has been removed.
B2	One reference probe has been replaced.
B1	Eight target probes for FANCA and all reference probes have been replaced. Furthermore QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description

Version B3-01 – 05 March 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 17 – 10 January 2017 (55)
- Incorrect partial sequences of several probes corrected in Table 2.

Version 16 – 11 February 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1, new picture included).

- Various minor textual changes.
- Warning about non-specific peak in No DNA reaction removed.

Version 15 – 12 August 2015 (54)

- Various minor textual changes.
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.
- "Peak area" replaced with "peak height".

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200