Product Description SALSA® MLPA® Probemix P113-B1 FANCB

To be used with the MLPA General Protocol.

Version B1. For complete product history see page 5.

Catalogue numbers:
- **P113-025R:** SALSA MLPA Probemix P113 FANCB, 25 reactions.
- **P113-050R:** SALSA MLPA Probemix P113 FANCB, 50 reactions.
- **P113-100R:** SALSA MLPA Probemix P113 FANCB, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.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 P113 FANCB is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FANCB* gene, which is associated with Fanconi anemia.

Fanconi anemia is a rare genetically heterogeneous recessive disorder characterized by congenital malformations, progressive bone marrow failure, and predisposition to cancer due to cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. *FANCB* is the only gene known to cause X-linked Fanconi Anemia. Patients harboring the X-linked *FANCB* pathogenic variants usually present with severe congenital malformations resembling VACTERL syndrome with hydrocephalus.

The *FANCB* gene (10 exons), spans ~29.7 kb of genomic DNA and is located on chromosome Xp22.2, ~14.8 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/

Exon numbering: The *FANCB* exon numbering used in this P113-B1 FANCB product description is the exon numbering from the RefSeq transcript NM_001018113.3 (LRG_496), which is identical to the NG_007310.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 10/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P113-B1 FANCB contains 25 MLPA probes with amplification products between 142 and 346 nucleotides (nt). This includes one probe for each exon of the *FANCB* gene, with one additional probe for exon 3, 8 and 10. Furthermore, two probes located upstream of exon 1 and one probe downstream of exon 10 are included. In addition, nine reference probes are included

that detect locations on the X-chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

This probemix contains ten 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 two chromosome Y-specific fragments (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 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific) (*AMOT* gene)
105	Y-fragment (Y chromosome specific) (*UTY* gene)
121	Y-fragment (Y chromosome specific) (*ZFY* gene)

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

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals of the same sex is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA 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: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Fanconi anemia (FA). It is recommended to use samples of the same sex to facilitate interpretation. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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 each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe 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:

Copy number status: Male samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy number status: Female samples	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 (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *FANCB* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P113 FANCB.
- 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.

***FANCB* mutation database:** https://databases.lovd.nl/shared/genes/FANCB. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 *FANCB* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P113-B1 FANCB

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*FANCB*
64-121	Control fragments – see table in probemix content section for more information		
142	Reference probe 15550-L17405	Xq21	
151	**FANCB probe** 03642-L29329		**Exon 1**
160	**FANCB probe** 21139-L29421		**Exon 7**
166	Reference probe 13089-L14308	Xq21	
171	**FANCB probe** 21140-L29422		**Exon 5**
178	**FANCB probe** 21141-L29423		**Exon 2**
188	Reference probe 07653-L14244	Xp11	
197	**FANCB probe** 03644-L29330		**Upstream**
202	**FANCB probe** 21143-L29897		**Exon 8**
211	**FANCB probe** 21144-L29426		**Exon 10**
220	Reference probe 02975-L02406	Xp22	
226	**FANCB probe** 03645-L29898		**Upstream**
232	**FANCB probe** 21142-L29424		**Exon 9**
240	**FANCB probe** 21145-L29899		**Exon 10**
245	**FANCB probe** 03646-L28729		**Exon 3**
254	Reference probe 01366-L25396	Xp21	
265	Reference probe 16013-L17194	Xq22	
274	**FANCB probe** 21146-L29428		**Exon 8**
283	**FANCB probe** 03647-L29332		**Exon 4**
301	**FANCB probe** 21147-L29429		**Downstream**
310	**FANCB probe** 21148-L29430		**Exon 6**
319	Reference probe 16691-L19264	Xp11	
328	**FANCB probe** 21149-L29431		**Exon 3**
336	Reference probe 14801-L13868	Xq11	
346	Reference probe 19560-L26139	Xq26	

a) See above section on exon numbering for more information.

Table 2. *FANCB* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*FANCB* exon[a]	Ligation site NM_001018113.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*276-278 (exon 3)*		
226	03645-L29898	Upstream	520 nt before exon 1 reverse	GAAGCCGCCTCT-GAGGCCCGGGGA	0.2 kb
197	03644-L29330	Upstream	287 nt before exon 1 reverse	GGCGTCCGCATT-GGATTGGGCGGT	0.3 kb
151	03642-L29329	Exon 1	8-7 reverse	CGCCAGCGCGCT-GCATCCTGGGAG	4.1 kb
178	21141-L29423	Exon 2	145-144 reverse	CAAAGTAGTTTC-AGCTTCATCAGT	3.6 kb
245	03646-L28729	Exon 3	352-353	CCTTGTTTTCCA-GTTGTCTAAAGG	0.7 kb
328	21149-L29431	Exon 3	1065-1066	CTCGAAAGAATC-AGCTGATTTCAT	5.4 kb
283	03647-L29332	Exon 4	1255-1256	AAAACTTAGCTT-AGTACTGATAGA	1.5 kb
171	21140-L29422	Exon 5	77 nt after exon 5	AAAGAAAGGTCC-TTAAAGTATAAG	4.7 kb
310	21148-L29430	Exon 6	1601-intron 6	AGTGCAGAGGAG-GTAAAAGTAATC	2.5 kb
160	21139-L29421	Exon 7	1729-1728 reverse	CAACCAAGCTAT-CATCTATTACAC	5.3 kb
202	21143-L29897	Exon 8	1865-1864 reverse	GGATTTGTACTC-AACTTAATCACC	0.2 kb
274	21146-L29428	Exon 8	2005-2006	GTGTGTACAGAT-AATTACTGCTGT	0.5 kb
232	21142-L29424	Exon 9	2395-2396	ACTCTTCACTTG-GAAACAGAGAAC	0.7 kb
240	21145-L29899	Exon 10	2576-2575 reverse	CTAAGGGTGACT-AGTTCCTTCTCC	0.4 kb
211	21144-L29426	Exon 10	2970-2971	TTACTGTAGTAG-AAACTTTAGTTT	0.5 kb
301	21147-L29429	Downstream	489 nt after exon 10	CAAATTGACAAG-GGTTTCAAAACC	
		Stop Codon	*2853-2855 (exon 10)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P031/P032 FANCA: Fanconi anemia group A – genes included: *FANCA*.
- P057 FANCD2-PALB2: Fanconi anemia – genes included: *FANCD2*, *PALB2*.
- P260 PALB2-RAD50-RAD51C-RAD51D: Fanconi anemia – genes included: *PALB2*, *RAD50*, *RAD51C*, *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.

P113 Product history	
Version	*Modification*
B1	Six additional FANCB probes have been included and five replaced, eight reference probes have been replaced and two removed, all autosomal probes have been removed and six probe lengths have been adjusted.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

Implemented changes in the product description
Version B1-01 — 01 December 2020 (02P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *FANCB* gene updated according to new version of the NM_ reference sequence. *Version 13 – 07 June 2017 (55)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and 2, new picture included). - Ligation sites adjusted for the FANCB gene in Table 2 according to NM_001018113.2. - Changes of probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification products. - Various textual and layout changes throughout the document. *Version 12 – 10 January 2017 (55)* - Warning added in Table 1, 382 nt probe 01839-L10863. *Version 11 - 28 October 2015 (55)* - Product description adapted to 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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