SALSA MLPA probemix P113-B1 FANCB

Lot B1-0417. As compared to version A2 (lots A2-0714 and A2-0511), six additional FANCB probes have been included and five have been replaced, eight reference probes have been replaced and two have been removed, all autosomal probes have been removed and six probe lengths have been adjusted.

The FANCB gene encodes a member of the Fanconi anemia complementation group B. This protein is assembled into a nucleoprotein complex that is involved in the repair of DNA lesions. Mutations in this gene can cause chromosome instability (https://www.ncbi.nlm.nih.gov/gene/2187).

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.

The P113-B1 probemix contains one probe for each exon of the FANCB gene, with one additional probe for exon 3, 8 and 10. Furthermore, two probes upstream of exon 1 and one probe downstream of exon 10 are included. In addition, 9 reference probes are included in this probemix, detecting several different locations on the X-chromosome.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene in a DNA sample. Deletions of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognisable by a 35-50% reduction in relative peak height. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in this gene is caused by deletions/duplications of complete exons. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents 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 Acids Research 30, e57 (2002).

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.

More information

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Data analysis

The P113-B1 FANCB probemix contains 25 MLPA probes with amplification products between 142 and 346 nt. In addition, it contains 10 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and two Y-fragments at 105 nt and 121 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can be normalised intra-sample by dividing the peak height of each amplification product by the total peak height of only the reference probes in the probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes that no changes occurred in the genomic regions targeted by the reference probes. It is recommended to use reference and patient samples of the same sex to minimize variation, but this is not strictly necessary. Sex determination can also be done by visual examination of the electropherogram.

Data normalisation should 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 blotting, long range PCR, qPCR, FISH.

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

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P113-B1 FANCB probemix

Length (nt)	SALSA MLPA probe	Chromosomal position reference	Chromosomal position FANCB
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		
100	X-fragment: Specific for the X chromosome		
105	Y-fragment: Specific for the Y chromosome		
121 ¥	Y-fragment: Specific for the Y chromosome		
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	

* New in version B1 (from lot B1-0417 onwards).
¥ Changed in version B1 (from lot B1-0417 onwards). Small change in length, no change in sequence detected.

Note: Exon numbering used here may differ from literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. FANCB probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	FANCB Exon	Ligation site NM_001018113.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*276-278 (exon 3)*		
226 ¥	03645-L29898	Upstream	527 nt before exon 1 reverse	GAAGCCGCCTCT-GAGGCCCGGGGA	0.2 kb
197 ¥	03644-L29330	Upstream	293 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)*		

* New in version B1 (from lot B1-0417 onwards).
¥ Changed in version B1 (from lot B1-0417 onwards). Small change in length, no change in sequence detected.

The NM_001018113.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P113-B1 FANCB sample pictures

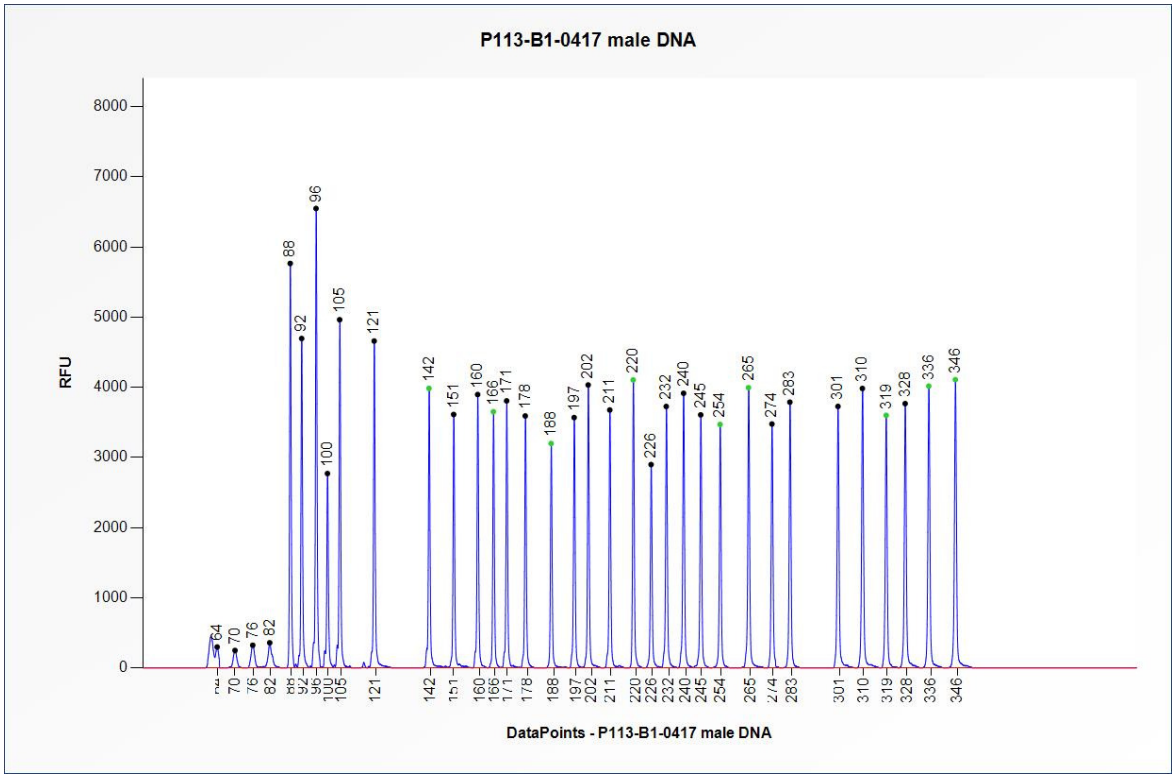

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P113-B1 FANCB (lot B1-0417).

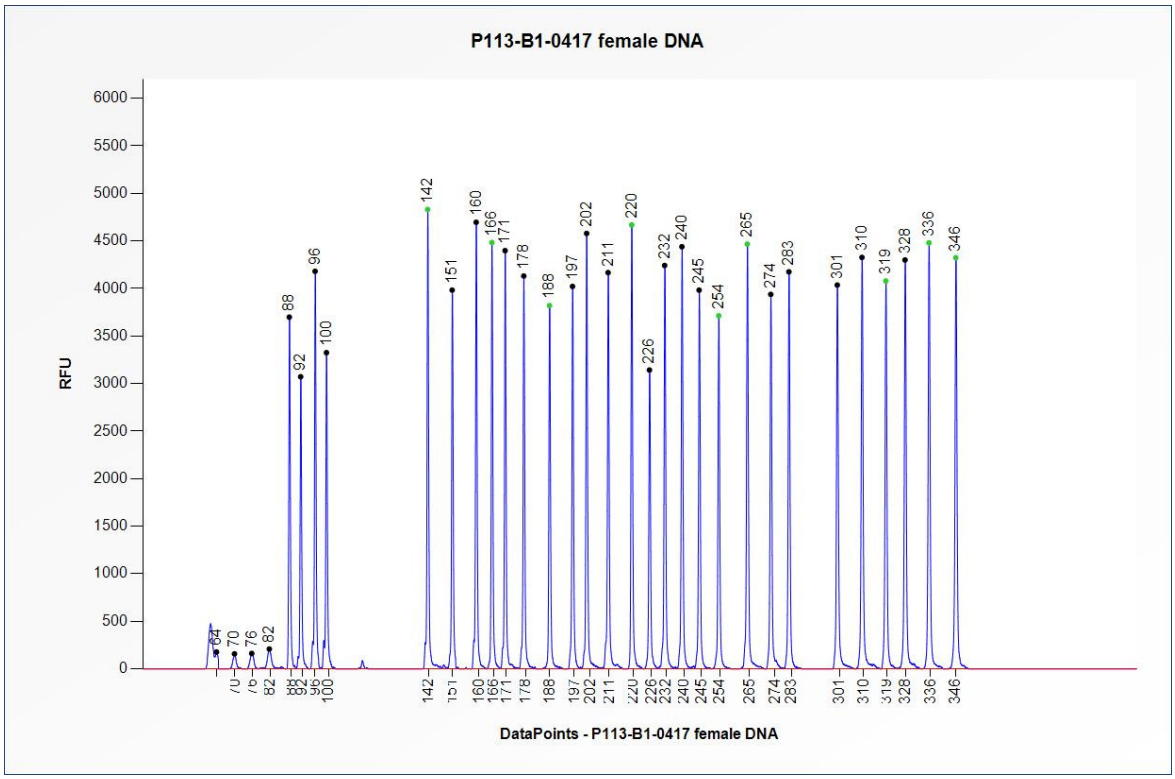

Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human female control DNA analysed with SALSA MLPA probemix P113-B1 FANCB (lot B1-0417).

Implemented Changes – compared to the previous product description versions.

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).

Version 10 (53)
- Warning added in Table 1, 148 nt probe 02935-L02326 and 256 nt 01769-L01333.

Version 09 (46)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 08 (46)
- Various minor layout changes.
- Ligation sites of the probes targeting the FANCB gene updated according to new version of the NM_reference sequence.
- Remark on transcript variant used and RefSeqGene standard added below Table 2.
- Warning added in Table 1, 174 nt probe 00508-L00075 and 193 nt probe 03514-L02291.
- Additional related SALSA MLPA kit added on p.1.

Version 07 (46)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1.
- Exon numbering of the FANCB gene has been changed in Tables 1 and 2.

Version 06 (46)
- Exon numbering of the FANCB gene has been changed in Tables 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Sentence "when only small numbers of samples are tested, visual comparison of peak profiles should be sufficient" removed from data analysis section.
- Various minor textual changes on page 1.
- Various minor layout changes.
- Tables have been numbered.
- Data analysis method has been modified.