Product Description
SALSA® MLPA® Probemixes P031-B4 / P032-B4 FANCA

To be used with the MLPA General Protocol.

P031-B4. As compared to version B3, two reference probes have been replaced.
P032-B4. As compared to version B3, one reference probe has been replaced.
For complete product history see page 9.

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 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.mrcholland.com).

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 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.

The *FANCA* gene (43 exons) spans ~79 kb of genomic DNA and is located on chromosome 16q24.3, ~88 Mb from the p-telomere (close to the q-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 *FANCA* exon numbering used in this P031-B4/P032-B4 FANCA product description is the exon numbering from the LRG_495 sequence. The exon numbering of the NM_ sequence that was used for determining a

probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P031-B4 FANCA mix 1 contains 31 MLPA probes with amplification products between 137 and 409 nucleotides (nt). This includes 22 probes for the *FANCA* gene. In addition, nine reference probes are included that detect autosomal chromosomal locations. The SALSA MLPA Probemix P032-B4 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 that detect autosomal chromosomal locations. Together, the mixes cover all exons of the *FANCA* gene. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

These probemixes contain 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, 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.mrcholland.com.

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

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

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals 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 different 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 (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID number NA09687 from the Coriell Institute has

been tested with these P031-B4/P032-B4 probemixes at MRC Holland and can be used as a positive control sample to detect a heterozygous duplication of the *FANCA* gene. 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.mrcholland.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 final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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 (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.

- 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: a lower injection voltage or a shorter injection time, 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 *FANCA* gene are small (point) mutations, none of which will 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

***FANCA* mutation database**

https://databases.lovd.nl/shared/genes/FANCA. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 SNVs and unusual results (e.g., a duplication of *FANCA* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P031-B4 FANCA mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*FANCA*
64-105	Control fragments – see table in probemix content section for more information		
137	Reference probe 16495-L18956	5q	
142	Reference probe 06595-L06153	8q	
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	14q	
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 21060-L29289	10q	
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	2q	
292	**FANCA probe** 01497-L01105		**Exon 25**
301	Reference probe 04750-L04098	9q	
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 21334-L29740	3p	
364	**FANCA probe** 01509-L01117		**Exon 37**
373	**FANCA probe** 15631-L17495		**Exon 39**
382	Reference probe 13329-L14755	18q	
391	**FANCA probe** 15632-L17496		**Exon 43**
400	**FANCA probe** 15633-L17497		**Exon 41**
409	Reference probe 12866-L19707	13q	

[a] See section Exon numbering on page 1 for more information.

* New in version B4.
« 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 1b. SALSA MLPA Probemix P032-B4 FANCA mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*FANCA*
64-105	Control fragments – see table in probemix content section for more information		
140	Reference probe 17132-L20324	1p	
148	Reference probe 17418-L22181	17p	
154 «	**FANCA probe** 16595-L19120		**Exon 4**
160 *	Reference probe 02310-L01801	19p	
166 «	**FANCA probe** 16594-L22023		**Exon 2**
172	Reference probe 07718-L07450	12q	
178	**FANCA probe** 16596-L19121		**Exon 6**
185	**FANCA probe** 16597-L22182		**Exon 8**
193	Reference probe 03217-L02642	10q	
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	9q	
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	13q	
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	7q	
383	**FANCA probe** 01707-L01275		**Exon 42**
391 ¬	GAS8 probe 01087-L00734		upstream
409	Reference probe 02718-L00732	14q	
418	Reference probe 10687-L11269	6p	

[a] See section Exon numbering on page 1 for more information.

* New in version B4.
« 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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

Length (nt) P031/P032	SALSA MLPA probe	*FANCA* exon[a]	Ligation site NM_000135.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
391 ¬	01087-L00734	*GAS8* gene		CTGGCACTAACT-TCATTGACACCT	227 kb
		start codon	*33-35 (Exon 1)*		
157 «	03222-L01222	Exon 1	17-18	GCCGCCGGGGCT-GTAGGCGCCAAG	0.7 kb
166 «	16594-L22023	Exon 2	215-214 *reverse*	CTTACCTCAAGC-AAAAGGGCATTC	1.3 kb
166 «	15624-L17488	Exon 3	281-282	TGTGACAGTTCT-GAGGCCTATGCT	3.5 kb
154 «	16595-L19120	Exon 4	382-383	AGCCGGGATGGT-TGCCTCTAGCGT	0.2 kb
178 «	01705-L01273	Exon 5	492-493	AGTTTGCTCAGT-ATTTATTGGCAC	2.4 kb
178	16596-L19121	Exon 6	601-602	CGTACAAGGCAT-TGTGAGCCTGCA	3.0 kb
184 «	01479-L12783	Exon 7	673-674	CTTCAGGAATCT-GTGCTGCCTTTG	1.9 kb
185	16597-L22182	Exon 8	51 nt before exon 8 *reverse*	GGTCTCCAAGCA-ACTGTGAATGGC	3.7 kb
202 «	15625-L17489	Exon 9	37 nt before exon 9 *reverse*	ATCTTGGTAATC-TTCTGTAATTTG	0.5 kb
202	16598-L19123	Exon 10	6 nt after exon 10 *reverse*	CCACCCTCAGGA-ACATACCAGCAC	3.3 kb
210	15626-L17490	Exon 11	1033-1034	CAGCCCTGTGCT-GAAAGGTAGGCC	3.4 kb
211	01484-L01092	Exon 12	1074-1075	GAGAGTGGAGCT-TTGCGCGGACAC	0.5 kb
220	15627-L17491	Exon 13	1147-1146 *reverse*	GCAAATGGCCAA-CCAACTCCTCTG	0.5 kb
220	01486-L01094	Exon 14	1289-1290	GCCCAGGCATTC-GAGAGCTGCCAG	6.6 kb
230	01487-L01095	Exon 15	1450-1451	CCTGGTCTTCCT-GTTTACGTTCTT	1.9 kb
229	01488-L09260	Exon 16	1557-1558	CCCTCCTCACAG-ACTACATCTCAT	0.1 kb
247	15628-L17492	Exon 17	1631-1630 *reverse*	GCTGATGACAAA-TCCTCGTAGAGT	3.0 kb
247	01490-L01098	Exon 18	1698-1699	AAAAGGCCATCA-TGGTGTTTGAGC	0.9 kb
256	01491-L01099	Exon 19	1771-1772	GCCTTACTACGT-GTCCCACTTCCT	0.1 kb
256	01492-L01100	Exon 20	1815-1816	CACAGCTCCCCA-AAGTCCCTGACT	3.1 kb
264	15629-L18639	Exon 21	19 nt after exon 21	CCTGGGCCGCTT-GTCCACTCTGGG	2.4 kb
265	01494-L01102	Exon 22	2004-2005	CAGCTGCACTGG-GAGAGCTGAGAG	1.5 kb
274	01495-L01103	Exon 23	2075-2076	GCAGTGATTTCT-GAAAGACTGAGG	1.2 kb
274	01496-L09261	Exon 24	2208-2209	TGCTGACGTCTT-TCTGTCAGAACC	0.4 kb
292	01497-L01105	Exon 25	2302-2303	GTGTGGACGTGT-GCTCCCTGCAGT	0.3 kb
295	01498-L09262	Exon 26	2488-2489	TGCGCTCTTTGA-CAGCCTCCTGAC	2.7 kb
310	01499-L18640	Exon 27	2573-2574	TCTCTCTGCAAG-TTTTCTTCCCAG	2.2 kb
304	01500-L09263	Exon 28	2735-2736	CTTCCTTCTGCA-GACTGGCAGAGA	3.0 kb
319	01501-L18641	Exon 29	2841-2842	TACACCTGGAGC-TGGAAATTCAAC	3.4 kb
310	16599-L22183	Exon 30	2984-2983 *reverse*	AGTGCGTTGACA-AGAATGGTACAC	6.5 kb
328	15630-L18642	Exon 31	3068-3069	GGTGGCCGCACA-GGAAATGAGGAT	2.3 kb
319	16600-L19125	Exon 32	3187-3186 *reverse*	GCCGTCTGCGGA-AAATCTCAAAGA	1.1 kb
341	01505-L22365	Exon 33	3327-3328	TCCAGGCAGAAC-AGCCCATCACTG	1.8 kb
337	01506-L01114	Exon 34	3402-3403	GCTCCCACGGAG-GTGCCCTGACAC	0.2 kb
350	01507-L22364	Exon 35	3496-3497	GGTCGACTTCAT-ACTGGCCAAGTG	1.6 kb
345	01508-L09321	Exon 36	3603-3604	GACACTGCCAGA-GCCCGCTGCCCC	2.2 kb
364	01509-L01117	Exon 37	3747-3748	AACAAGTCAGGG-AAGAAAACATCA	2.1 kb
355	16601-L19126	Exon 38	8 nt after exon 38	AAATGTAAGTCA-GACCTTACCAGC	0.7 kb
373	15631-L17495	Exon 39	5 nt before exon 39 *reverse*	TGGTGCTCTGTA-AACCGCAGGAGA	0.6 kb
364	01512-L09323	Exon 40	4038-4039	CTTTCGCTTTTT-ACAGGTACCTCC	0.3 kb
400	15633-L17497	Exon 41	4160-4161	ACAAGCACAGTT-TCACCTCCAGCT	0.2 kb
383	01707-L01275	Exon 42	4221-4222	TGGAACTGATAA-CAAAAGCTCGTC	0.9 kb
391	15632-L17496	Exon 43	4956-4957	AGCTCAGTCTCA-GCCTTGTGTTTG	
		stop codon	*3398-4400 (Exon 43)*		

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

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

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P031 product history	
Version	*Modification*
B4	Two reference probes have been replaced.
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*
B4	One reference probe has been replaced.
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 B4-01 – 4 August 2022 (04P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *FANCA* gene updated according to new version of the NM_ reference sequence.

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.

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