Instructions for Use

SALSA® MLPA® Probemix P163 GJB-WFS1-POU3F4

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P163 GJB-WFS1-POU3F4 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P163 GJB-WFS1-POU3F4**
Version	E1
Catalogue numbers	P163-025R (25 reactions) P163-050R (50 reactions) P163-100R (100 reactions)
Basic UDI-DI	872021148P1635Z
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797
RUO	ALL OTHER COUNTRIES

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

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

Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to the D1 version, one probe for *WFS1* has been replaced and one has been added, one new probe upstream of *POU3F4* and two new probes for *CRYL1* have been added, one probe for *ZMYM2* has been removed, and one probe for *GJB6* and five reference probes have been replaced. Some probes have also changed in length, but the actual target sites were not changed.

1. Intended Purpose

The SALSA MLPA Probemix P163 GJB-WFS1-POU3F4 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *GJB2, GJB6, WFS1,* and *POU3F4* genes, microdeletions upstream of *POU3F4*, and the wildtype sequence of six specific mutations in the *GJB2* gene in genomic DNA isolated from human peripheral whole blood specimens[3]. P163 GJB-WFS1-POU3F4 is intended to confirm a potential cause for hereditary hearing loss, and to confirm a potential cause for and clinical diagnosis of Wolfram syndrome type 1. This assay can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P163 GJB-WFS1-POU3F4 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *GJB2, POU3F4* and *WFS1* genes are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

[3] Probes targeting the *GJB3* gene may be used in a research setting only. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Target
GJB2, GJB6, WFS1 and *POU3F4*	*GJB3*

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of hereditary hearing loss or Wolfram syndrome type 1. • Should be from the same sex to facilitate interpretation.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*WFS1, GJB2, GJB6*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

Typical Results of *GJB2* Wildtype-Specific Probes

Final Ratio (FR)	Mutation Status
0	Specific homozygous *GJB2* mutation OR homozygous *GJB2* exon 2 deletion OR compound heterozygosity of these *GJB2* mutations
0.40 - 0.65	Specific heterozygous *GJB2* mutation OR heterozygous *GJB2* exon 2 deletion
0.80-1.20	*GJB2* mutation is not detected

Typical Results of X Chromosomal Probes (*POU3F4*; Compared to Reference Samples of the Same Gender)

Final Ratio (FR)	Copy Number Female	Copy Number Male	Description
0	0	0	**Female:** Homozygous deletion **Male**: Deletion
0.40 – 0.65	1	-	**Female:** Heterozygous deletion
0.80 – 1.20	**2**	**1**	**Normal**
1.30 – 1.65	3	-	**Female:** Heterozygous duplication
1.75 – 2.15	4	2	**Female**: Homozygous duplication or Heterozygous triplication **Male:** Duplication
All other values	-	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations, a study was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number tables above can be used. Cut-off values for copy number determination were verified with P163 GJB-WFS1-POU3F4 in 46 blood-derived samples from healthy individuals with normal copy numbers and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested. Artificial DNAs mimicking CNVs and a normal copy number were also tested and the expected FRs were found in 98% of measurements.
Expected values for SNV detection in normal and affected populations	The wildtype probes will only generate a signal when the corresponding wildtype sequence of the *GJB2* SNVs c.313del14 (312 nt probe), c.235delC (402 nt probe), c.167delT (396 nt probe), c.101T>C (274 nt probe), c.35delG (172 nt probe), and IVS1+1G>A (267 nt probe) is present. When no mutation is present, the FR will be between 0.80-1.20. If the specific heterozygous mutation or a deletion in *GJB2* is present, the FR will be between 0.40-0.65. When the specific homozygous mutation, a homozygous deletion in *GJB2,* or compound heterozygosity of these mutations is present, the FR will be zero. The expected value for mutation-specific probes was verified with P163 GJB-WFS1-POU3F4 using nine blood-derived mutation positive samples covering all six specific mutations, and the expected FR was found in all tested samples.
Limit of Detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P163 GJB-WFS1-POU3F4 on one blood-derived sample without a mutation and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td></td><td>A study using P163 GJB-WFS1-POU3F4 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on artificial DNA samples, two mimicking CNVs and one with normal copy number. Tested potential interferents, their potential source, testing concentrations and results are presented in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 213/234 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 228/234 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 230/234 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 222/234 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 77/234 measurements</td></tr>
</table>
* Results are summarised for all probes across all three samples tested.

EDTA, NaCl, $FeCl_3$ and heparin had a mild effect on copy number determination, with the majority of measurements falling within the expected FR range. Those falling outside the expected range were in the ambiguous range which, at most, would lead to delayed results. Hemoglobin had a large effect on the FRs, with more than two thirds of measurements falling outside the expected FR range and with both ambiguous and false results. Importantly, DNA extraction methods from blood remove hemoglobin, and during testing of 22 samples extracted from blood, the expected FRs were found.

To minimise variability across 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.</td></tr>
<tr><td>Cross-reactions</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. To determine whether probes are specific to their target sequence, three artificial DNA samples mimicking CNVs and normal copy number, 46 blood-derived samples from healthy individuals with normal copy number, and 13 samples with known CNVs and/or SNVs were tested using P163 GJB-WFS1-POU3F4. The expected FRs for the corresponding copy number were found in >99% of measurements across all samples tested.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies.

For trueness, seven previously genotyped samples were tested using P163 GJB-WFS1-POU3F4 and all measurements fell within the expected FR range. Assay precision was tested by repeatedly testing artificial DNA samples, two mimicking CNVs and one with normal copy number, over multiple days, and by multiple operators. Results showed a correct call in 1,154/1,170 data points, leading to a precision of >98%.</td></tr>
<tr><td>Clinical validity*</td><td>GJB2 and GJB6: Approximately 50% of autosomal recessive non-syndromic hearing loss can be attributed to the DFNB1 locus, caused by mutations in GJB2 and GJB6 (GeneReviews). The majority of individuals (99%) with DFNB1 are either homozygous or compound heterozygous for GJB2 pathogenic SNV variants. The remaining 1% are compound heterozygous for one GJB2 pathogenic SNV variant and a deletion in the GJB2 gene or one of three large deletions that includes sequences upstream of GJB2 such as CRYL1 and a portion of GJB6. The vast majority of pathogenic variants in GJB2 are single nucleotide variants (SNVs). Some of the most frequently occurring GJB2 SNVs are c.313del14, c.235delC, c.167delT, c.101T>C, c.35delG, and IVS1+1G>A (Hoefsloot et al. 2013).

POU3F4: Roughly 1% of hereditary hearing loss is caused by genes on the X-chromosome and, of these, 50% are caused by mutations in the POU3F4 gene and the upstream region of POU3F4 gene. Most mutations in the POU3F4 region are SNVs, though large deletions have been demonstrated in several publications (de Kok et al. 1996, Ozyilmaz et al. 2019, Song et al. 2010, Vore et al. 2005).

WFS1: Among hereditary hearing loss patients, mutations in WFS1 are rare. Furthermore, the majority of mutations in WFS1 are SNVs (95%; missense, nonsense, or splice-site changes) or small insertions and deletions. Large deletions are rare, however, they have been reported in two publications in a total of five patients (Resmerita et al. 2020, Riza et al. 2022)

*(Based on a 2007-2025 literature review)</td></tr>
</table>

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Mutation	Length (nt)	Probe number	Warnings
1p34.3	*GJB3*	Exon 1	0.8 kb		157	14206-L21338	
1p34.3	*GJB3*	Intron 1 (Exon 1)	2.8 kb		190	05367-L04758	Ø
1p34.3	*GJB3*	Exon 2	1.0 kb		241	05368-L31484	
1p34.3	*GJB3*	Exon 2			292	05369-L23138	
4p16.1	*WFS1*	Exon 1	7.7 kb		151	05375-L30787	
4p16.1	*WFS1*	Exon 2	9.6 kb		229	05376-L04767	
4p16.1	*WFS1*	Exon 3	1.9 kb		166	05377-L11414	
4p16.1	*WFS1*	Exon 4	2.3 kb		252	05378-L04769	
4p16.1	*WFS1*	Exon 5	0.6 kb		184	05379-L04770	
4p16.1	*WFS1*	Exon 6	3.2 kb		319	06702-L09985	
4p16.1	*WFS1*	Exon 7	5.6 kb		208	06699-L04772	
4p16.1	*WFS1*	Exon 8	1.0 kb		425	21749-L30414	
4p16.1	*WFS1*	Exon 8			132	21747-L30412	
13q12.11	*PSPC1*		221.2 kb		223	02399-L06768	¬
13q12.11	*ZMYM2*		194.3 kb		471	21765-L30428	¬
13q12.11	*GJB2*	Exon 2	1.3 kb		328	05365-L04756	
13q12.11	*GJB2*	Exon 2	0.1 kb	c.313del14 (wildtype)	312	09118-L30816	∞
13q12.11	*GJB2*	Exon 2	0.1 kb	c.235delC (wildtype)	402	13127-L15168	∞
13q12.11	*GJB2*	Exon 2	0.1 kb	c.167delT (wildtype)	396	13126-L30618	∞
13q12.11	*GJB2*	Exon 2	0.1 kb	c.101T>C (wildtype)	274	13150-L14407	∞
13q12.11	*GJB2*	Exon 2	3.2 kb	c.35delG (wildtype)	172	09862-L09256	∞
13q12.11	*GJB2*	Exon 1	0.1 kb	IVS1+1G>A (wildtype)	267	22413-L31695	∞
13q12.11	*GJB2*	Exon 1	0.1 kb		247	22362-L31524	
13q12.11	*GJB2*	Exon 1	29.0 kb		178	21979-L19379	+
13q12.11	*GJB6*	Exon 5	0.8 kb		337	05374-L04765	
13q12.11	*GJB6*	Exon 5	6.9 kb		259	22189-L31241	
13q12.11	*GJB6*	Exon 4	1.2 kb		282	06701-L04763	#, «
13q12.11	*GJB6*	Intron 2 (Exon 3)	0.3 kb		196	05371-L04762	Ø, #, «
13q12.11	*GJB6*	Intron 2 (Exon 2)	172.9 kb		162	05370-L04761	Ø, #, «
13q12.11	*CRYL1*	Exon 8	85.3 kb		493	21751-L30416	#, «, ¬
13q12.11	*CRYL1*	Exon 3	485.6 kb		445	21750-L30617	#, ¬
13q12.11	*LATS2*				355	06704-L03639	¬
Xq21.1	*POU3F4*	Upstream	0.1 kb		146	21762-L14398	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	52.0 kb		364	21763-L14400	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	0.3 kb		390	21764-L14397	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	137.3 kb		418	13139-L14396	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	0.2 kb		463	13142-L14399	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	613.5 kb		481	13144-L14401	ʃ, Ж
Xq21.1	*POU3F4*	Upstream	170.2 kb		202	21748-L30413	ʃ, Ж
Xq21.1	*POU3F4*	Exon 1	1.0 kb		436	12771-L13887	ʃ
Xq21.1	*POU3F4*	Exon 1			127	21761-L13889	ʃ
2p	Reference				373	08878-L08934	
3q	Reference				216	05707-L06963	
5q	Reference				121	19041-L31238	
6p	Reference				138	00662-L21335	
7q	Reference				500	10218-L14675	
8p	Reference				235	15108-L25275	
11q	Reference				409	02669-L02136	
12q	Reference				303	05697-L05139	
15q	Reference				346	02473-L01917	
20q	Reference				453	16287-L18579	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The exon numbers in this table are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with target at the edge of or slightly outside the coding region were altered. The exon numbering from the previous version of this product description is disclosed between brackets. Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

∞ Wildtype sequence detected. A lowered probe signal can be due to the specified mutation in the *GJB2* gene. Deletions in the *GJB2* gene or other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

¬ These probes are flanking probes, included to help determine the extent of a deletion. Copy number alterations of flanking probes alone are unlikely to be related to the condition tested.

Ø These probes target sequences outside of the known coding region.

#	These probes are expected to be affected by del(GJB6-D13S1830), a common deletion of 309 kb.
«	These probes are expected to be affected by del(GJB6-D13S1854), a common deletion of 232 kb.
Ж	These probes target the highly conserved non-coding DNA regions (HCNRs) upstream of the *POU3F4* gene (Naranjo et al. 2010). 81676 is covered by the 146 nt and 364 nt probes; 81728 is covered by the 390 nt and 418 nt probes; 81866 is covered by the 463 nt and 481 nt probes; 82478 is covered by the 202 nt probe.
+	The ligation site of this probe is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
∫	These probes target the X chromosome.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. No probes for *GJB6* exons 1, 2 and 3 are present.
2. Target probes for *GJB3* CNVs are included to be used for research purposes only and not for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. de Kok YJ et al. (1996). Identification of a hot spot for microdeletions in patients with X-linked deafness type 3 (DFN3) 900 kb proximal to the DFN3 gene POU3F4. *Hum Mol Genet.* 5:1229-1235.
2. Hoefsloot LH et al. (2013). EMQN Best Practice guidelines for diagnostic testing of mutations causing non-syndromic hearing impairment at the DFNB1 locus. *Eur J Hum Genet.* 21:1325-1329.
3. Naranjo S et al. (2010). Multiple enhancers located in a 1-Mb region upstream of POU3F4 promote expression during inner ear development and may be required for hearing. *Hum Genet.* 128:411-419.
4. Ozyilmaz B et al. (2019). First-Line Molecular Genetic Evaluation of Autosomal Recessive Non-Syndromic Hearing Loss. *Turk Arch Otorhinolaryngol.* 57:140-148.
5. Resmerita I et al. (2020). Genetics of Hearing Impairment in North-Eastern Romania-A Cost-Effective Improved Diagnosis and Literature Review. *Genes (Basel).* 11:1506.
6. Riza A-L et al. (2022). Non-Syndromic Hearing Loss in a Romanian Population: Carrier Status and Frequent Variants in the GJB2 Gene. *Genes.* 14:69.
7. Song MH et al. (2010). Clinical evaluation of DFN3 patients with deletions in the POU3F4 locus and detection of carrier female using MLPA. *Clin Genet.* 78:524-532.
8. Vore AP et al. (2005). Deletion of and novel missense mutation in POU3F4 in 2 families segregating X-linked nonsyndromic deafness. *Arch Otolaryngol Head Neck Surg.* 131:1057-1063.

Implemented changes in the product description

Version E1-07 – 9 January 2026 (03S)

- Product description was updated to a new template.
- Intended purpose updated, specifying the assay is manual, removing the *GJB3* gene for diagnostic use, limiting aberrations in *GJB2, GJB6, WFS1,* and *POU3F4* genes to deletions, and removing the clinical diagnosis of hereditary hearing loss and instead specifying it is intended to confirm a potential cause for hereditary hearing loss.
- In the interpretation of results section, a table for typical results of *GJB2* wildtype-specific probes was added.
- Exon numbering for targets of *GJB3* probe 05367-L04758 and *GJB6* probes 05370-L04761 and 05371-L04762 at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- SNV(s) rs542977017, rs147734431 and rs550975729 can affect the probe signal. However, the warning for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warning regarding flanking probes extended to include *CRYL1* probes 21751-L30416 and 21750-L30617.
- Warning that ligation site is >20nt from the nearest exon was added for *GJB2* probe 21979-L19379.
- Warnings for probes targeting sequences outside of the known coding region were added for probes *GJB3* probe 05367-L04758 and *GJB6* probes 05370-L04761 and 05371-L04762.
- Limitation added that no probes for *GJB6* exons 1, 2 and 3 are present in the probemix.
- Limitation added that target probes for *GJB3* CNVs are included to be used for research purposes only and not for diagnostic use.
- Probemix is now IVDR certified.