Instructions for Use

# SALSA® MLPA® Probemix P018 SHOX

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 P018 SHOX product page on our website to find Certificates of Analysis and a list of related products.

| **Product Name** | **SALSA® MLPA® Probemix P018 SHOX** |
|---|---|
| **Version** | G2 |
| **Catalogue numbers** | P018-025R (25 reactions)<br>P018-050R (50 reactions)<br>P018-100R (100 reactions) |
| **Basic UDI-DI** | 872021148P0185P |
| **Ingredients** | Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA |

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

**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.

| **Regulatory Status** | |
|---|---|
| IVD | EUROPE CE 2797<br>COLOMBIA<br>ISRAEL |
| RUO | ALL OTHER COUNTRIES |

| **Label Symbols** | | | |
|---|---|---|---|
| IVD | In Vitro Diagnostic | RUO | Research Use Only |

| **More Information:** www.mrcholland.com | |
|---|---|
| | MRC Holland BV; Willem Schoutenstraat 1<br>1057 DL, Amsterdam, the Netherlands |
| E-mail | info@mrcholland.com (information & technical questions);<br>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 version G1, one SHOX probe and one SHOX area probe have been adjusted. Minor alteration, no change in sequence detected.

## 1. Intended Purpose

The SALSA MLPA Probemix P018 SHOX is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the human short stature homeobox (*SHOX*) gene and its regulatory regions on Xp22.33/Yp11.32 in genomic DNA isolated from human peripheral whole blood specimens. In the context of disorders associated with short stature, P018 SHOX is intended to confirm a potential cause for Idiopathic short stature (ISS), Leri-Weill dyschondrosteosis (LWD), and Langer mesomelic dysplasia (LMD). The device can further be used to confirm a clinical diagnosis of LWD (detection of monoallelic pathogenic variants) and LMD (detection of biallelic pathogenic variants)[3].

Copy number variations (CNVs) detected with P018 SHOX should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. In the majority of patients, defects in the *SHOX* gene region are deletions. However, point mutations can occur 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] Certain probes targeting additional genes included in P018 SHOX may only be used in a research setting. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

| IVD Targets | RUO Targets |
|---|---|
| *SHOX; SHOX Area;* Xp22-PAR1 | *PPP2R3B; CRLF2; CSF2RA; IL3RA; ASMT; ZBED1; ARSF PRKX; NLGN4X; ANOS1; FANCB; AIFM1; VAMP7* |

## 2. Sample Requirements

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

| Sample Types | | |
|---|---|---|
| Test Sample | • Provided by user | |
| Reference Samples (Required) | • Provided by user<br>• Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples.<br>• Have a normal copy number and ≤0.10 standard deviation for all probes.<br>• At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of disorders related to short stature.<br>• Should all be from the same sex for correct data analysis.<br>• Should be of the same sex as the test sample to facilitate interpretation. | |
| No-DNA Control (Preferably) | • Provided by user<br>• $TE_{0.1}$ buffer instead of DNA<br>• 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. |
| Validation Samples (Required) | • In the validation experiments of this probemix, DNA samples from healthy individuals of the same sex should be used.<br>• This probemix contains target probes that target sequences with natural variation. The validation experiments of this probemix should result in a standard deviation ≤0.10 for all reference probes. | |

*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[1]-118[2] | Chromosome Y presence control fragments |

[1]Targets the *UTY* gene, located ~11 Mb from PAR1.
[2]Targets the *ZFY* gene, located just outside PAR1 at ~470 kb distance from the 456 nt ZBED1 probe. A small signal for the 118 nt fragment may be observed in some female samples.

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 **Pseudoautosomal Sequences** (PAR1 and PAR2) in Male and Female Samples

| 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 **X Probes** (Outside of PAR1 and PAR2, Compared to Same Sex)

| Final Ratio (FR) | Copy Number Female | Copy Number Male | Description |
|---|---|---|---|
| 0 | 0 | 0 | **Female:** Homozygous deletion<br>**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<br>**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.

For a detailed interpretation guide, see the **Appendix**.

## 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 1500 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 table in the product description can be used. Cut-off values for copy number determination were verified with P018 SHOX in 61 samples from healthy individuals with normal copy number and ten samples with known CNVs. The expected FRs for the corresponding copy number were found in 99% (2050/2054) of measurements. |
| 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 P018 SHOX on two samples with known |

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td></td><td>CNVs and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>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.<br><br>A study using P018 SHOX was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.<br><br>
<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>Expected FR for 231/234 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 234/234 measurements</td></tr>
<tr><td>$Fe^{3+}$ ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 234/234 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 234/234 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 120/234 measurements</td></tr>
</table>
* Results are summarised for all probes across all three samples tested.<br><br>NaCl, $FeCl_3$, and heparin did not interfere with copy number status determination, while an effect on the final ratios (FRs) was observed for a low number of probes with EDTA. Hemoglobin had the largest effect on the FRs.<br><br>Coffalyser.Net issued warnings for the samples in which the interferents showed an effect, as well as lowered quality scores, which in practice would lead to samples needing a re-test.<br><br>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-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, eleven previously genotyped samples were tested using P018 SHOX and found to have the expected results in 99% (490/493) of measurements. Assay precision was tested by repeatedly testing samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 1167/1170 data points, leading to a precision of 99%.</td></tr>
<tr><td>Clinical validity*</td><td>LWD: ~43-52% of cases are caused by CNVs in SHOX region.<br>ISS: ~4-13% of cases are caused by CNVs in SHOX region.<br>LMD: cannot be estimated due to the rarity of the disease.<br><br>(Benito-Sanz et al. 2006, Benito-Sanz et al. 2012a, Chen et al. 2009, GeneReviews).<br><br>*(Based on a 2006-2026 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 | Length (nt) | Probe number | Warnings |
|---|---|---|---|---|---|---|
| **Start of PAR1** | | | | | | |
| Xp22.33/Yp11.32 | *PPP2R3B* | | 91.6 kb | 211 | 09333-L10292 | ¬ |
| ***SHOX* region** | | | | | | |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE-5 | 61.7 kb | 364 | 18889-L25087 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE-3 | 56.3 kb | 130 | 18885-L24430 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE-2 | 63.5 kb | 427 | 18891-L25088 | Ø |
| Xp22.33/Yp11.32 | *SHOX* (LOC159015) | Upstream | 4.7 kb | 266 | 01341-L20651 | Ø |
| Xp22.33/Yp11.32 | *SHOX* | Upstream (Exon 1) | 6.7 kb | 166 | 01145-L00702 | Ø |
| Xp22.33/Yp11.32 | *SHOX* | Exon 1 (Exon 2) | 3.6 kb | 204 | 01146-L06220 | |
| Xp22.33/Yp11.32 | *SHOX* | Exon 2 (Exon 3) | 6.2 kb | 245 | 01147-L00802 | |
| Xp22.33/Yp11.32 | *SHOX* | Exon 3 (Exon 4) | 0.2 kb | 300 | 01148-L15501 | ∫ |
| Xp22.33/Yp11.32 | *SHOX* | Exon 4 (Exon 5) | 3.6 kb | 337 | 21538-L30066 | |
| Xp22.33/Yp11.32 | *SHOX* | Exon 5 (Exon 6) | 6.4 kb | 231 | 09337-L00911 | |
| Xp22.33/Yp11.32 | *SHOX* | Exon 5 (Exon 6) | 6.4 kb | 226 | 09336-L20178 | |
| Xp22.33/Yp11.32 | *SHOX* | Downstream (Intron 6) | 56.9 kb | 395 | 09338-L24247 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE2/3 | 19.8 kb | 136 | 05642-L05096 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE3 | 19.4 kb | 154 | 13821-L14642 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE4 | 37.4 kb | 172 | 18886-L24431 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE5 | 29.6 kb | 199 | 13296-L20175 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE7 | 28.1 kb | 481 | 18893-L25091 | ^ Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE7/8 | 8.4 kb | 318 | 05645-L05099 | ^ Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE8/9 | 10.7 kb | 439 | 05646-L24249 | ^ Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE8/9 | 6.3 kb | 466 | 13297-L24253 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE9 | 0.4 kb | 290 | 06291-L06222 | Ø |
| Xp22.33/Yp11.32 | *SHOX* Area | CNE9 | 15.4 kb | 185 | 06293-L20177 | Ø |
| Xp22.33/Yp11.32 | Xp22-PAR1 | Downstream | 48.7 kb | 148 | 05648-L06218 | Ø |
| Xp22.33/Yp11.32 | Xp22-PAR1 | Downstream | 64.4 kb | 178 | 05649-L20176 | Ø |
| Xp22.33/Yp11.32 | Xp22-PAR1 | Downstream | 66.0 kb | 445 | 09335-L30792 | Ø |
| Xp22.33/Yp11.32 | Xp22-PAR1 | Downstream | 338.0 kb | 379 | 14697-L24245 | Ø |
| Xp22.33/Yp11.32 | *CRLF2* | | 73.9 kb | 403 | 13911-L19678 | ¬ |
| Xp22.33/Yp11.32 | *CSF2RA* | | 69.8 kb | 389 | 10251-L24246 | ¬ |
| Xp22.33/Yp11.32 | *IL3RA* | | 280.7 kb | 142 | 13597-L15055 | ¬ « |
| Xp22.33/Yp11.31 | *ASMT* | | 706.4 kb | 310 | 01153-L00712 | ¬ |
| Xp22.33/Yp11.31 | *ZBED1* | | 593.8 kb | 456 | 16858-L25227 | ¬ |
| **End of PAR1** | | | | | | |
| Xp22.33 | *ARSF* | | 536.9 kb | 254 | 16846-L20647 | ¬ x |
| Xp22.33 | *PRKX* | | 2.6 **M**b | 328 | 16898-L19768 | ¬ x |
| Xp22.31 | *NLGN4X* | | 2.3 **M**b | 283 | 05587-L04577 | ¬ # x |
| Xp22.31 | *ANOS1* | | 6.3 **M**b | 238 | 06402-L09795 | ¬ # x |
| Xp22.2 | *FANCB* | | 114.3 **M**b | 274 | 03906-L03066 | ¬ x |
| Xq25 | *AIFM1* | | 25.7 **M**b | 420 | 00820-L25090 | ¬ x |
| **Start of PAR2** | | | | | | |
| Xq28/Yq12 | *VAMP7* | | | 355 | 01156-L00659 | ¬ Δ |
| **Reference probes in autosomal regions** | | | | | | |
| 1p | Reference | | | 160 | 04966-L04696 | |
| 1q | Reference | | | 346 | 06560-L06118 | |
| 2p | Reference | | | 504 | 09870-L19465 | |
| 4p | Reference | | | 191 | 06057-L05512 | |
| 7q | Reference | | | 124 | 15370-L13762 | |
| 13q | Reference | | | 219 | 03247-L02684 | |
| 15q | Reference | | | 412 | 09793-L12593 | |
| 16p | Reference | | | 476 | 09888-L10301 | |
| 18q | Reference | | | 261 | 00587-L20649 | |

CNE = Conserved Non-coding DNA Element.

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 *SHOX* exon numbers are derived from the MANE project and are based on the MANE Select transcript.
Locations of the upstream regulatory regions (CNE-2, CNE-3 and CNE-5) are based on Durand et al. (2010) and Benito-Sanz et al. (2012a).
Locations of the downstream regulatory regions (CNE2 through CNE9) are based on Benito-Sanz et al. (2012a) and Fukami et al. (2006).
For more information, see the probe sequences document available on the product page at www.mrcholland.com. 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

| | |
|---|---|
| ¬ | These probes are flanking probes, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested. However, several genes detected by the flanking probes have been associated with other disorders (Mullighan et al. 2009, Nguyen et al. 2020, Russell et al. 2009). |
| x | These probes target the X chromosome outside PAR1 and PAR2. A 50% reduced signal in male samples will be observed if female reference samples are used. |
| ^ | These probes target sequences within the recurrent ~47.5 kb deletion region described by (Benito-Sanz et al. 2012b). |
| Δ | This probe is located very close to the X and Y q-telomeres in PAR2. |
| « | This probe is 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. |
| Ø | These probes targets sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance. |
| # | The specificity of these probes relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene. |
| ∫ | A founder *SHOX* point mutation in the Spanish Gypsy population, c.508G>C (p.A170P), results in a strongly reduced probe signal of the 300 nt exon 3 probe (Barca-Tierno et al. 2011). |

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.
5. Large deletions are often detected with this probemix. **The slope correction algorithm in Coffalyser.Net may confuse a large deletion for sloping, leading to an incorrectly applied slope correction and a false warning or ambiguous results for multiple probes.** Contact MRC Holland if you suspect that this happened.
6. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.
7. Copy number variation in healthy individuals has been reported for several genes outside of the *SHOX* region.
8. Complete or partial duplications found within *SHOX* or its surrounding regulatory regions have been found in LWD and ISS patients (Benito-Sanz et al. 2011b).
9. Breakpoints of partial *SHOX* deletions have been reported to frequently occur in intron 2 (Benito-Sanz et al. 2017).
10. A partial *SHOX* deletion encompassing the last 20 nt of *SHOX* exon 2 and first part of intron 2 has been described (Funari et al. 2019). This deletion will not be detected by P018 SHOX as there is no probe present that targets this region.
11. Deletion of the *SHOX* exon 5 and downstream probes, which are located downstream of the stop codon of transcript variant 1 (*SHOXa*), may not affect *SHOX* gene function.
12. Please note that single exon deletions have a considerable chance of being a false positive result, either due to non-pathogenic copy number variants (Benito-Sanz et al. 2011a), mutations within the probe binding site (Barca-Tierno et al. 2011) or due to impurities in the DNA.
13. Not all copy number changes detected by SHOX Area probes will affect *SHOX* gene function. Analysis of family members may be required for correct interpretation of results.
14. Large deletions and duplications interrupted by one or more probe(s) with a normal copy number have been reported and might be the result of an inversion followed by a deletion/duplication or may indicate compound heterozygosity (Dupont et al. 2007). In these cases, parental evaluation can assist data interpretation.

Technique-specific precautions

See the MLPA General Protocol.

## 8. Limitations

Probemix-specific limitations

1. Target probes for *PPP2R3B, CRLF2, CSF2RA, IL3RA, ASMT, ZBED1, ARSF, PRKX, NLGN4X, ANOS1, FANCB, AIFM1*, and *VAMP7* 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. Barca-Tierno V et al. (2011). Identification of a Gypsy SHOX mutation (p.A170P) in Leri-Weill dyschondrosteosis and Langer mesomelic dysplasia. Eur J Hum Genet. 19:1218-1225.
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3. Benito-Sanz S et al. (2011a). A non-pathogenic pseudoautosomal region 1 copy number variant downstream of SHOX. Am J Med Genet A. 155A:935-937.
4. Benito-Sanz S et al. (2011b). Clinical and molecular evaluation of SHOX/PAR1 duplications in Leri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). J Clin Endocrinol Metab. 96:E404-412.
5. Benito-Sanz S et al. (2012a). Identification of the first PAR1 deletion encompassing upstream SHOX enhancers in a family with idiopathic short stature. Eur J Hum Genet. 20:125-127.
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7. Benito-Sanz S et al. (2017). Identification of 15 novel partial SHOX deletions and 13 partial duplications, and a review of the literature reveals intron 3 to be a hotspot region. J Hum Genet. 62:229-234.
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10. Durand C et al. (2010). Enhancer elements upstream of the SHOX gene are active in the developing limb. Eur J Hum Genet. 18:527-532.
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13. Mullighan CG et al. (2009). Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 41:1243-1246.
14. Nguyen TA et al. (2020). Neuroligins and Neurodevelopmental Disorders: X-Linked Genetics. Front Synaptic Neurosci. 12:33.
15. Russell LJ et al. (2009). Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 114:2688-2698.


**Implemented changes in the product description**

*Version G2-09 – 18 June 2026 (03S)*

- Product description updated to a new template.
- The intended purpose was updated by specifying that the assay is manual. Buccal swabs have been removed as a specimen type. A footnote describing which probes are RUO and which are IVD was added.
- Exon numbering updated for SHOX probes 01145-L00702, 01146-L06220, 01147-L00802, 01148-L15501, 21538-L30066, 09337-L00911, 09336-L20178, and 09338-L24247.
- Warning for probes targeting sequences outside of the known coding region added for SHOX and SHOX Area probes 18889-L25087, 18885-L24430, 18891-L25088, 01341-L20651, 01145-L00702, 09338-L24247, 05642-L05096, 13821-L14642, 18886-L24431, 13296-L20175, 18893-L25091, 05645-L05099, 05646-L24249, 13297-L24253, 06291-L06222, 06293-L20177, 05648-L06218, 05649-L20176, 09335-L30792, and 14697-L24245.
- Warning related to LRG exon numbering removed for probe 09336-L20178.
- Warnings for probes changed in version G2 removed for probes 21538-L30066 and 09335-L30792.
- Probemix-specific precaution regarding pseudoautosomal dominant disorders removed.
- Performance characteristics updated with data from analytical performance experiments.
- A limitation regarding the presence of RUO probes was added.
- The format of the probemix-specific precaution related to sloping was changed to bold.
- Reference to SSP added.
- Notified body number added to CE-mark.
- Basic UDI-DI added.
- Various textual clarifications carried out.
- Information concerning test sample examples moved to Appendix (new).
- Probemix is now IVDR certified.

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## 10. Appendix

Table depicting examples of test samples with typical results per probe type and per reference sample sex used. Explanations on the obtained FRs are also provided.

| Type of test sample | Probes | Typical FR *when compared to* | | Explanation |
|---|---|---|---|---|
| | | Male reference samples | Female reference samples | |
| Healthy **Female** DNA | Probes Targeting **Pseudoautosomal Sequences** (PAR1 and PAR2) | 1 | 1 | PAR1 and PAR2 are located on X **and** Y:<br>• healthy females: 2 copies;<br>• healthy males: 2 copies. |
| | **X Probes** (outside PAR1 and PAR2) | **2** | 1 | Targeted sequences present **only** on X:<br>• healthy females: 2 copies;<br>• healthy males: 1 copy. |
| | 105 & 118 nt **Chromosome Y Presence Control** Fragments | **0** | **-** | Targeted sequences present **only** on Y:<br>• healthy females: 0 copies;<br>• healthy males: 1 copy. |
| Healthy **Male** DNA | Probes Targeting **Pseudoautosomal Sequences** (PAR1 and PAR2) | 1 | 1 | PAR1 and PAR2 are located on X **and** Y:<br>• healthy females: 2 copies;<br>• healthy males: 2 copies. |
| | **X Probes** (outside PAR1 and PAR2) | 1 | **0.5** | Targeted sequences present **only** on X:<br>• healthy females: 2 copies;<br>• healthy males: 1 copy. |
| | 105 & 118 nt **Chromosome Y Presence Control** Fragments | 1 | **∞** | Targeted sequences present **only** on Y:<br>• healthy females: 0 copies;<br>• healthy males: 1 copy. |
| **Female** DNA with a heterozygous deletion that includes one or more exons of the *SHOX* gene | Deletion shown in Probes Targeting **Pseudoautosomal Sequences** (in PAR1 including SHOX/SHOX area) | **0.5** | **0.5** | Heterozygous deletion in essential *SHOX* area in PAR1.<br>PAR1 is located on X **and** Y.<br>**This typically causes LWD or ISS.** |
| | Deletion shown in **X Probes** (outside PAR1 and PAR2) | **1** | **0.5** | Heterozygous deletion outside PAR1.<br>Sequences present **only** on X. |
| **Male** DNA with a heterozygous deletion that includes one or more exons of the *SHOX* gene | Deletion shown in Probes Targeting **Pseudoautosomal Sequences** (in PAR1 including SHOX/SHOX area) | **0.5** | **0.5** | Heterozygous deletion in essential *SHOX* area in PAR1.<br>PAR1 region is located on X **and** Y.<br>**This typically causes LWD or ISS.** |
| | Deletion shown in **X Probes** (outside PAR1 and PAR2) | **0** | **0** | Heterozygous deletion outside PAR1.<br>Sequences present **only** on X. |
| **Female** or **Male** DNA with a heterozygous deletion in the area upstream or downstream of *SHOX*, but not including *SHOX* gene probes | Deletion shown in Probes Targeting **Pseudoautosomal Sequences** (in PAR1 but **not** including SHOX, only upstream/downstream/SHOX area) | **0.5** | **0.5** | Heterozygous deletion.<br>PAR1 region is located on X **and** Y.<br>**Deletions in this region have been associated with LWD & ISS** ((Benito-Sanz et al. 2005, Benito-Sanz et al. 2012a, Benito-Sanz et al. 2012b, Chen et al. 2009)).<br>**Not all deletions detected by these probes will result in LWD or ISS.** |
| **Female** sample with Turner syndrome (45,X) | Probes Targeting **Pseudoautosomal Sequences** (PAR1 and PAR2) | **0.5** | **0.5** | PAR1 & PAR2 are located on X **and** Y.<br>Only one X chromosome is present in this case. |
| | **X Probes** (outside PAR1 and PAR2) | 1 | **0.5** | Targeted sequences located **only** on X.<br>Only one X chromosome is present in this case. |
| | 105 & 118 nt **Chromosome Y Presence Control** Fragments | **0** | - | Targeted sequences located **only** on Y.<br>No Y chromosome present in this case. |
| **Male** sample with Klinefelter syndrome (47,XXY) | Probes Targeting **Pseudoautosomal Sequences** (PAR1 and PAR2) | **1.5** | **1.5** | PAR1 & PAR2 are located on X **and** Y.<br>Three copies of these regions present. |
| | **X Probes** (outside PAR1 and PAR2) | **2** | 1 | Probe sequences present **only** on X.<br>Two X chromosomes are present in this case. |
| | 105 & 118 nt **Chromosome Y Presence Control** Fragments | 1 | **∞** | Probe sequences present **only** on Y.<br>One Y chromosome present in this case. |
| **Female** sample with Triple X-syndrome (47,XXX). | Probes Targeting **Pseudoautosomal Sequences** (PAR1 and PAR2) | **1.5** | **1.5** | PAR1 & PAR2 are located on X **and** Y.<br>Three copies of these regions present. |
| | **X Probes** (outside PAR1 and PAR2) | **3** | **1.5** | Probe sequences present **only** on X.<br>Three X chromosomes are present in this case. |
| | 105 & 118 nt **Chromosome Y Presence Control** Fragments | **0** | - | Probe sequences located **only** on Y.<br>No Y chromosome present in this case. |