Product Description

SALSA® MLPA® Probemix ME032-B1 UPD7-UPD14

To be used with the MS-MLPA General Protocol.

Version B1
As compared to version A1, six PLAGL1 target probes have been removed, nine target probes have been replaced and nine target probes have been added; two digestion control probes and 11 reference probes have been replaced, one reference probe has been removed; two target probes have changed in length, not in sequence detected. For complete product history see page 12.

Catalogue numbers:
- **ME032-025R:** SALSA MLPA Probemix ME032 UPD7-UPD14, 25 reactions.
- **ME032-050R:** SALSA MLPA Probemix ME032 UPD7-UPD14, 50 reactions.
- **ME032-100R:** SALSA MLPA Probemix ME032 UPD7-UPD14, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SALSA HhaI (SMR50) 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 MS-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.

In several No DNA reactions performed on this probemix, MRC Holland has observed a series of non-specific peaks. These peaks appeared very sporadically and we have not yet been able to establish the cause. However, we found that the amount and height of these peaks is greatly reduced by not spinning down your MLPA reactions in between the ligation and PCR reaction. The non-specific peaks are not expected to influence results. Please notify us if you still regularly observe these peaks: info@mrcholland.com.

Intended purpose
The SALSA MLPA Probemix ME032 UPD7-UPD14 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of copy number variations (CNVs) and methylation status of the imprinted 7q32, 7p12 and 14q32 regions, including the *GRB10, MEST, DLK1, MEG3* and *RTL1* genes in genomic DNA isolated from human peripheral blood, buccal swab and saliva. ME032 UPD7-UPD14 is intended to confirm a potential cause for and clinical diagnosis of Temple syndrome (TS), Kagami-Ogata syndrome (KOS) and Russel-Silver syndrome (RSS) and for molecular genetic testing of at-risk family members.

CNVs and methylation changes detected with ME032 UPD7-UPD14 should be confirmed with a different technique. In particular, CNVs and methylation changes detected by only a single probe always require confirmation by another method.

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, parental evaluation, 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 in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

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

Clinical background

Genomic imprinting is the monoallelic expression of genes, dependent on the parental origin of the chromosome. It plays a role in growth and development. Imprinting disorders originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al., 2013). A frequent cause of imprinting disorders is uniparental disomy (UPD), which occurs when both alleles are inherited from a single parent instead of one copy from each parent. This aberrant genetic inheritance causes disturbed genomic imprinting and results in either the overexpression or complete silencing of genes that are expressed parent-specifically. Differentially methylated regions (DMRs) act as imprinting control regions to regulate the imprinted expression of the genes. Most frequently, UPD does not result in any phenotypical anomalies but UPD can cause unmasking of an autosomal-recessive disease or can present itself as a syndromic imprinting disorder.

Russell-Silver syndrome (RSS; Silver-Russell syndrome (SRS)) is characterised as a clinically heterogeneous condition with a variable phenotype (Wakeling et al. 2017). Intrauterine and postnatal growth retardation, however, are consistently observed in all RSS patients, who are at significant risk for developmental delay and learning disabilities. Although rare, RSS can be inherited in an autosomal dominant or recessive manner. The prevalence is estimated between 1 and 9 in 1.000.000 (Orphanet, https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=813).

Temple syndrome (TS; TS14; maternal uniparental disomy 14 (UPD14mat)) is a short stature disorder of imprinting, characterised among other features by low birth weight and length and premature puberty (Hoffmann and Heller, 2011). In TS patients, the proportion of children with learning difficulties or mental retardation is increased compared to the general population. Intrauterine growth retardation occurs in 90% of the TS patients. TS shows an age-dependent overlap with the well-known maternal UPD15 Prader-Willi syndrome. TS is a very rare disease with a prevalence < 1 in 1.000.000 (Orphanet, https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=254516).

Kagami-Ogata syndrome (KOS; KOS14; paternal uniparental disomy 14 (UPD14pat)) is a foetal malformation syndrome, characterised by polyhydramnios and a bell-shaped chest with angulated ribs (“coat-hanger configurations”), which leads to pulmonary hypoplasia and abdominal wall defects (Hoffmann and Heller 2011). The clinical course of KOS is marked by the severe and often lethal respiratory insufficiency, feeding difficulties with postnatal growth failure, and moderate to severe mental retardation. The prognosis in most cases is very poor. However, there has been no report of death in patients ≥4 years of age (Ogata and Kagami 2016). After intense medical treatment in infancy, these patients become free of mechanical ventilation, tracheostomy and feeding tube. Despite developmental delay, they manage to get on their daily lives from childhood. KOS is a very rare disease with a prevalence < 1 in 1.000.000 (Orphanet, https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=254519).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1324/ (RSS); https://www.omim.org/entry/616222 (TS); and https://www.omim.org/entry/608149 (KOS).

Gene structure and exon numbering

The exon numbering used in this ME032-B1 UPD7-UPD14 product description is the exon numbering from the LRG sequences referred to below, which are available at www.lrg-sequence.org.

The *GRB10* gene is located on chromosome 7p12.2 (reverse strand), spans ~203 kilobases (kb) and contains 20 exons. The *GRB10* LRG_1032 is identical to GenBank NG_012305.2. Expression of the *GRB10* gene is mono-allelic or bi-allelic, depending on the tissue-type.

The *MEST* gene spans ~20 kb on chromosome 7q32.2 and contains 12 exons. The *MEST* LRG_1033 is identical to GenBank NG_009226.1. The *MEST* gene is imprinted, exhibiting preferential expression from the paternal allele in foetal tissues.

The *DLK1* gene spans ~11 kb on chromosome 14q32.2 and contains 5 exons. The *DLK1* LRG_1044 is identical to GenBank NG_016863.3. *DLK1* is a paternally expressed imprinted gene.

The *MEG3* gene spans ~35 kb on chromosome 14q32.2 and contains 13 exons. The *MEG3* LRG_1098 is identical to GenBank NG_016853.2. *MEG3* is a maternally expressed imprinted gene.

The *RTL1* gene is located on chromosome 14q32.31 (reverse strand), spans ~24 kb and contains 4 exons. The *RTL1* LRG_1059 is identical to GenBank NG_045001.2. The *RTL1* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. The *RTL1* gene is paternally expressed imprinted gene that is highly expressed at the late foetal stage in both the foetus and the placenta.

MicroRNA-380 (*MIR380)* spans 61 base pairs (bp) on chromosome 14q32.31 and contains 1 exon. The *MIR380* GenBank NR_029872.1 sequence is available at www.ncbi.nlm.nih.gov.

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 ME032 UPD7-UPD14 contains 48 (MS-)MLPA probes with amplification products between 115 and 511 nt. Nine probes are specific for the 7p12.2 region, eight for the 7q32.2 region, 12 for the 14q32.2 region and three for the 14q32.31 region. Ten of these probes contain an HhaI recognition site and provide information about the methylation status of the target sequence. All probes in the probemix will also give information on copy number changes in the analysed sample. In addition, 14 reference probes are included which are not affected by HhaI digestion and detect genes located outside the 7p12, 7q32 and 14q32 regions. Also, two digestion control probes are included in this probemix indicating whether or not restriction endonuclease digestion in the MS-MLPA reaction was complete. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains 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 MS-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)

MS-MLPA technique

The principles of the MS-MLPA technique (Nygren et al. 2005, Schouten et al. 2002) are described in the MS-MLPA General Protocol (www.mrcholland.com).

MS-MLPA technique validation
Internal validation of the MS-MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MS-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.

Results of MS-MLPA are highly dependent on the HhaI enzyme used. HhaI enzymes that are resistant to heat inactivation are NOT compatible with the MS-MLPA technique and will give aberrant results. These include, but may not be limited to, Thermo Fisher Scientific enzymes HhaI, ANZA 59 HhaI, and FastDigest HhaI. SALSA HhaI enzyme (SMR50) must be used in combination with this probemix as this restriction enzyme has been validated for use with MS-MLPA by MRC Holland.

Required specimens
Extracted DNA from peripheral blood, buccal swab and saliva, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MS-MLPA General Protocol.

The results of methylation-specific MLPA probes tested on chorionic villi samples (CVS) might not reflect the actual epigenetic constitution of the foetus. This is because the locus of interest might not have reached its final imprinting status in CVS (Paganini et al. 2015). Furthermore, Paganini et al. demonstrated that the rate at which the imprinting is set may differ between, and even within, loci. Additionally, since this product has not been validated on prenatal samples, DNA extracted from CVS samples should not be used in a diagnostic setting.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MS-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. When selecting reference samples, please note that methylation patterns may vary between tissue types and even age groups! Reference samples should be derived from different unrelated individuals who are from families without a history of RSS, TS or KOS. More information regarding the selection and use of reference samples can be found in the MS-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 numbers NA10925, NA07081, NA13410 and NA12519 from the Coriell Institute have been tested with this ME032-B1 probemix at MRC Holland and can be used as a positive control samples for only copy number variations (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Altered targets in ME032-B1	Expected copy number alteration
NA10925	*GRB10*	Heterozygous deletion of *GRB10*
NA07081	*GRB10*	Heterozygous duplication of *GRB10*
NA13410	*DLK1; MEG3; RTL1; MIR380*	Heterozygous duplication of *DLK1*, *MEG3*, *RTL1* and *MIR380*
NA12519	*MEST*	Homozygous duplication of *MEST*

Performance characteristics
In approximately 5-10% of RSS cases, RSS is caused by UPD7mat (Cerrato et al. 2020). Microdeletions/-duplications and epimutations on chromosome 7 have been reported in only a few cases (<1%) (Hoffmann and Heller 2011). The diagnostic sensitivity of ME032 for RSS is therefore estimated to be 6-11%. TS and KOS are caused by UPD14, microdeletions/-duplications or epimutations on chromosome 14 (Cerrato et al. 2020). As probes targeting all possible causes of the two syndromes are included in ME032, the diagnostic sensitivity for TS and KOS is estimated to be >99%. To date, only a few individual cases have been described in which a

microdeletion, a microduplication or hypomethylation was inherited from a parent without an apparent phenotype (based on a 2014-2021 literature review). This indicates that ME032 also detects aberrations in individuals who are phenotypically normal. According to screening guidelines, however, diagnosis should be based on a combination of clinical and molecular findings. In the presence of indicative clinical features and associated molecular aberrations, the diagnostic specificity for RSS, TS and KOS can therefore be assumed to be >99%. The analytical sensitivity and specificity for the detection of deletions, duplications and methylation in the targeted genes is very high and can be considered >99% (based on a 2014-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MS-MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the 7q32, 7p12 and 14q32 regions of MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication). Allele copy numbers of 0 (homozygous deletion) and 4 (homozygous duplication / heterozygous triplication) are very rare. 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.

Please note that these above mentioned final ratios are affected in mosaic samples.

Methylation cut-off values

In healthy individuals, the DNA sequences that are detected by methylation-specific probes are imprinted and expected to be maternally or paternally methylated, for all probes except for the MIR380 probe, which is expected to be fully methylated.

Methylation status in healthy individuals	Final ratio (FR)
Fully methylated (*MIR380*)	FR ≥ 0.85
Normal imprinted methylation (*GRB10, MEST, MEG3*)	0.40 ≤ FR ≤ 0.65

In RSS, TS and KOS patients, hypomethylation or hypermethylation is expected. Cut-off values for hypomethylation and hypermethylation could not be determined at MRC Holland, as clinical patient samples are rare, due to the low prevalence of these syndromes.

For each of the imprinted genes *GRB10, MEST* and *MEG3*, three methylation-specific probes, located very close to each other, are included in this probemix. It is expected that all three probes provide similar results for each gene. We recommend using the median of the three methylation-specific probes per gene to determine the methylation status of the *GRB10, MEST* and *MEG3* loci and to disregard aberrant methylation detected by a single MS-MLPA probe.

- 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. 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 MS-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.
- Digestion control probes: The target sequences of the digestion control probes are unmethylated in most blood-derived DNA samples. The signals of the digestion control probes should be gone upon complete digestion by HhaI.
- mRNA levels: We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels.
- Copy number changes detected by reference 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.

NOTE: In case digestion control probes are not fully digested (>0.05), please contact info@mrcholland.com for more information.

ME032 specific notes:

- This probemix contains one methylation-specific probe (247 nt) in *MIR380* that is located in an CpG island which is always fully methylated.
- The molecular aetiology of RSS is unknown in about 30%-40% of patients. Molecular causes of RSS other than UPD7mat include hypomethylation of the paternal imprinting center 1 (IC1) on chromosome 11p15.5 (40-60%) and pathogenic variants of the *CDKN1C* and *IGF2* genes (<1%, Cerrato et al. 2020). These

molecular aberrations will not be detected by this probemix. SALSA MLPA Probemix ME030 BWS/RSS can be used to determine copy number and methylation status in the 11p15.5 region.

Limitations of the procedure

- MS-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 MS-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.
- An MS-MLPA probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated!
- Rare cases are known in which apparent methylation as detected by an MS-MLPA probe proved to be due to a sequence change in or very near the HhaI site.
- No discrimination between uniparental disomy and imprinting defects can be made with this probemix. For this, it is necessary to perform microsatellite analysis in the patient and parents.

Confirmation of results

Confirmation of methylation status can be performed with another technique, such as MSP (methylation-specific PCR), pyrosequencing, digestion-based PCR assays, etc. 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 MS-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.

LOVD mutation database

https://www.lovd.nl/. We strongly encourage users to deposit positive results in the LOVD 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., aberrant methylation of the MEG3 220 nt and MEG3 292 nt probes, but not the MEG3 119 nt probe) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix ME032-B1 UPD7-UPD14

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	Chromosomal position (hg18)	
				Reference	Target
64-105	Control fragments – see table in probemix content section for more information				
115 *	Reference probe S0973-L26704			4p	
118 * ±	**MEG3 probe** S0910-L25280	+	50%		*MEG3*:TSS-DMR
124 *	**MEG3 probe** 22725-L32749				*MEG3*:TSS-DMR
132 * π	Digestion control probe S0750-L25688	+	0%	2q	
136 «	**GRB10 probe** 15742-L18941	+	50%		*GRB10*:alt-TSS-DMR
142 *	Reference probe 16305-L18830			2p	
148 *	**MEG3 probe** 22726-L32020				*MEG3/DLK1*:IG-DMR
152 «	**GRB10 probe** 15744-L24405	+	50%		*GRB10*:alt-TSS-DMR
158 *	**MEST probe** 22727-L32021				**Exon 12**
161 «	**GRB10 probe** 15746-L24414	+	50%		*GRB10*:alt-TSS-DMR
168	Reference probe 08222-L24899			10q	
172 * «	**MEST probe** 23111-L32734				*MEST*:alt-TSS-DMR
184 «	**MEST probe** 15749-L17768	+	50%		*MEST*:alt-TSS-DMR
190 «	**MEST probe** 15750-L17769	+	50%		*MEST*:alt-TSS-DMR
196	Reference probe 16425-L18878			18q	
202 * «	**GRB10 probe** 22729-L32023				*GRB10*:alt-TSS-DMR
214	Reference probe 07733-L07423			20q	
220	**MEG3 probe** 15754-L18942	+	50%		*MEG3*:TSS-DMR
226 * «	**GRB10 probe** 22731-L32025				*GRB10*:alt-TSS-DMR
232 ¥ «	**MEST probe** 22661-L17775	+	50%		*MEST*:alt-TSS-DMR
238 *	Reference probe 18213-L04552			13q	
247	**MIR380 probe** 18481-L18864	+	100%		**Exon 1**
256 *	**GRB10 probe** 22732-L32026				**Exon 14**
266 *	Reference probe 22711-L31966			16p	
274	**RTL1 probe** 18336-L23249				**Exon 1**
283 *	**DLK1 probe** 22733-L32027				**Exon 3**
292 ‡ *	**MEG3 probe** 22668-L32796	+	50%		*MEG3*:TSS-DMR
300 *	Reference probe 00348-L00174			19q	
317 ¥	**GRB10 probe** 22660-L32466				**Exon 7**
332 *	Reference probe 17414-L21123			3p	
340	**RTL1 probe** 15763-L17782				**Exon 1**
347	**DLK1 probe** 15762-L18818				**Exon 4**
355 * π	Digestion control probe 19043-L29021	+	0%	8p	
364 *	Reference probe 18676-L24030			11p	
373 *	**MEG3 probe** 22736-L32030				*MEG3/DLK1*:IG-DMR
391 *	**DLK1 probe** 22738-L32032				**Exon 1**
400 *	Reference probe 14839-L16547			1p	
409 *	**GRB10 probe** 22739-L32033				**Exon 20**
418 «	**MEST probe** 18343-L23256				**Exon 5**
427	**GRB10 probe** 18381-L23252				**Exon 11**
436 *	Reference probe 22240-L31355			22q	
445 *	**MEG3 probe** 22740-L32034				*MEG3/DLK1*:IG-DMR
453 * «	**MEST probe** 23112-L32648				*MEST*:alt-TSS-DMR
463 *	**MEG3 probe** 22742-L32036				*MEG3*:TSS-DMR
472 *	Reference probe 12029-L12891			6p	
490 *	**MEG3 probe** 22744-L32038				*MEG3*:TSS-DMR
500 * «	**MEST probe** 22745-L32467				*MEST*:alt-TSS-DMR
511 *	Reference probe 18539-L32066			17q	

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.
± SNPs rs577606722 and rs944662889 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
π Digestion control: warns for insufficient digestion. Upon digestion, this probe should not give a signal.
‡ This probe contains two GCGC motifs for HhaI. Loss of probe peak signal (indicative of absence of methylation) will occur not only if both sites are unmethylated but also if one of the sites is unmethylated and the other methylated.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

The DMR nomenclature used in this product description has been developed by the imprinted loci task force of the European Network for Human Congenital Imprinting Disorders (Monk et al. 2018).
Alt-TSS-DMR: alternative transcription start site differentially methylated region.

Table 2. ME032-B1 probes arranged according to chromosomal location

Table 2a. Chromosome 7

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	HhaI site	Ligation site	Chromoso-mal position (hg18)	Imprinted allele	Distance to next probe
		GRB10		**NM_001001555.3**			
409	22739-L32033	Exon 20	-	2037-2038	7p12.2		19.8 kb
256	22732-L32026	Exon 14	-	1481-1482	7p12.2		5.3 kb
427	18381-L23252	Exon 11	-	1167-1166 reverse	7p12.2		56.5 kb
317	22660-L32466	Exon 7	-	577-576 reverse	7p12.2		107.8 kb
161 «	15746-L24414	*GRB10*:alt-TSS-DMR	+	10.8 kb after exon 1	7p12.2	Tissue specific	0.4 kb
152 «	15744-L24405	*GRB10*:alt-TSS-DMR	+	10.4 kb after exon 1	7p12.2	Tissue specific	0.1 kb
202 «	22729-L32023	*GRB10*:alt-TSS-DMR	-	10.2 kb after exon 1	7p12.2		0.1 kb
136 «	15742-L18941	*GRB10*:alt-TSS-DMR	+	10.1 kb after exon 1	7p12.2	Tissue specific	0.1 kb
226 «	22731-L32025	*GRB10*:alt-TSS-DMR	-	10 kb after exon 1	7p12.2		79.1 **Mb**
		MEST		**NM_002402.4**			
500 «	22745-L32467	*MEST*:alt-TSS-DMR	-	999 nt before exon 1 reverse	7q32.2		0.5 kb
232 «	22661-L17775	*MEST*:alt-TSS-DMR	+	550 nt before exon 1	7q32.2	Maternal	0.1 kb
190 «	15750-L17769	*MEST*:alt-TSS-DMR	+	472 nt before exon 1	7q32.2	Maternal	0.7 kb
184 «	15749-L17768	*MEST*:alt-TSS-DMR	+	231-232 exon 1	7q32.2	Maternal	0.2 kb
453 «	23112-L32648	*MEST*:alt-TSS-DMR	-	221 nt after exon 1 reverse	7q32.2		0.5 kb
172 «	23111-L32734	*MEST*:alt-TSS-DMR	-	696 nt after exon 1 reverse	7q32.2		5.2 kb
418 «	18343-L23256	Exon 5	-	2 nt after exon 5	7q32.2		6.7 kb
158	22727-L32021	Exon 12	-	3 nt before exon 12	7q32.2		

Table 2b. Chromosome 14

Length (nt)	SALSA MLPA probe	Gene/Exon/DMR[a]	HhaI site	Ligation site	Chromoso-mal position (hg18)	Imprinted allele	Distance to next probe
		DLK1		**NM_003836.7**			
391	22738-L32032	Exon 1	-	239-240	14q32.2		1.9 kb
283	22733-L32027	Exon 3	-	430-431	14q32.2		3.1 kb
347	15762-L18818	Exon 4	-	555-556	14q32.2		77.1 kb
		MEG3		**NR_003530.2**			
373	22736-L32030	*MEG3/DLK1*:IG-DMR	-	16.8 kb before exon 1 reverse	14q32.2		0.8 kb
445	22740-L32034	*MEG3/DLK1*:IG-DMR	-	15.9 kb before exon 1 reverse	14q32.2		1.0 kb
148	22726-L32020	*MEG3/DLK1*:IG-DMR	-	14.9 kb before exon 1 reverse	14q32.2		14.5 kb
490	22744-L32038	*MEG3*:TSS-DMR	-	456 nt before exon 1	14q32.2		0.1 kb
220	15754-L18942	*MEG3*:TSS-DMR	+	367 nt before exon 1	14q32.2	Paternal	0.2 kb
124	22725-L32749	*MEG3*:TSS-DMR	-	211 nt before exon 1	14q32.2		0.2 kb
292 ‡	22668-L32796	*MEG3*:TSS-DMR	+	16 nt before exon 1 reverse	14q32.2	Paternal	0.1 kb
118 ±	S0910-L25280	*MEG3*:TSS-DMR	+	63-64 exon 1	14q32.2	Paternal	0.1 kb
463	22742-L32036	*MEG3*:TSS-DMR	-	58 nt after exon 1 reverse	14q32.2		55.7 kb
		RTL1		**NM_001134888.3**			
274	18336-L23249	Exon 4 (Exon 1)	-	3162-3161 reverse	14q32.31		2.4 kb
340	15763-L17782	Exon 4 (Exon 1)	-	701-702	14q32.31		140.6 kb
		MIR380		**NR_029872.1**			
247	18481-L18864	Exon 1	+	47-46 reverse	14q32.31	n.a.	

[a] See section Gene structure and exon numbering on page 2 for more information.

± SNPs rs577606722 and rs944662889 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
π Digestion control: warns for insufficient digestion. Upon digestion, this probe should not give a signal.
‡ This probe contains two GCGC motifs for HhaI. Loss of probe peak signal (indicative of absence of methylation) will occur not only if both sites are unmethylated but also if one of the sites is unmethylated and the other methylated.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

The DMR nomenclature used in this product description has been developed by the imprinted loci task force of the European Network for Human Congenital Imprinting Disorders (Monk et al. 2018).
Alt-TSS-DMR: alternative transcription start site differentially methylated region.

Related SALSA MLPA probemixes

ME028 Prader-Willi/Angelman	Primary screening for Prader-Willi syndrome and Angelman syndrome.
ME030 BWS/RSS	Primary screening for Beckwith-Wiedemann syndrome and Russell-Silver syndrome.
ME031 GNAS	Probes for the 20q13.32 imprinted *GNAS* region.
ME033 TNDM	Contains probes for *PLAGL1* and other genes related to transient neonatal diabetes mellitus.
ME034 Multi-locus Imprinting	Contains probes for regions implicated in multi-locus imprinting disturbances on chromosomes 6, 7, 11, 14, 15, 19 and 20.

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ME032 product history	
Version	*Modification*
B1	Six PLAGL1 target probes have been removed, nine target probes have been replaced, and nine target probes have been added; two digestion control and 11 reference probes have been replaced, one reference probe has been removed; two target probes have changed in length, not in sequence detected.
A1	First release.

Implemented changes in the product description

Version B1-03 – 28 June 2022 (04M)
- Exon numbering of two GRB10 probes (22660-L32466 and 18381-L23252) in Table 1 corrected to be based on the LRG and in line with Table 2.
- Link to DMR information replaced by literature reference Monk et al. (2018).
- Imprinted allele information added for the MIR380 probe (18481-L18864) in Table 2b.
- Reference Monk et al. (2018) added to reference section.
- Various minor textual or layout changes.

Version B1-02 – 22 March 2022 (04M)
- Version for internal use only.

Version B1-01 – 20 January 2022 (04M)
- 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).
- ME032-B1 is now CE marked.
- Ligation sites of the probes targeting the *GRB10*, *MEST*, *DLK1 and RTL1* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version A1-03 – 12 January 2021 (02M)
- Chromosomal location of target probe at 418 nt updated in Table 1.

Version A1-02 – 19 September 2019 (02M)
- Product description adapted to a new template.
- Advice on not spinning down your MLPA reactions in between the ligation and PCR reaction was added to the warning about non-specific peaks on page 1.
- Use of SALSA HhaI (SMR50) with ME032 added.
- Small change of probe length in Table 1 in order to better reflect the true length of the amplification product.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Adjustment of exon number in Table 1 for the 383 nt MEG3 probe.

Version A1-01 – 16 February 2018 (01M)
- Product description restructured and adapted to a new template.

- The probemix introduction was updated - Additional references regarding the Kagami-Ogata and Temple syndrome were added. - Table 1, 2 and 3 were updated.

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