Product Description
SALSA® MLPA® Probemix ME033-B1 TNDM

To be used with the MS-MLPA General Protocol.

Version B1
As compared to version A1, all ten reference probes have been replaced, as well as seven target probes. Three probes have been changed in length, not in sequence detected, and five target probes have been added. For complete product history see page 11.

Catalogue numbers:
- **ME033-025R:** SALSA MLPA Probemix ME033 TNDM, 25 reactions.
- **ME033-050R:** SALSA MLPA Probemix ME033 TNDM, 50 reactions.
- **ME033-100R:** SALSA MLPA Probemix ME033 TNDM, 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.

Warning: In several no DNA reactions performed on this ME033-B1 probemix, MRC-Holland has observed that spinning down MLPA reactions in between the ligation and PCR reaction can cause **non-specific peaks** with predominant lengths at about 110, 114, 128 and 206 nt to appear. When insufficient sample DNA is used (as indicated by the Q-fragments) these peaks may also appear in between the probes. Always use at least 50 ng sample DNA in each reaction. The non-specific peaks are not expected to influence results when **not** spinning down your MLPA reactions in between the ligation and PCR reaction. Please notify us if you still regularly observe these peaks: info@mrcholland.com.

General information
The SALSA MLPA Probemix ME033 TNDM is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences of the *PLAGL1* gene on the 6q24 chromosomal region. This probemix can also be used to detect deletions/duplications in the *PLAGL1* gene; the chromosomal regions 6p22 and 6q24, as well as the chromosomal region 11p15.

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 like Transient Neonatal Diabetes Mellitus (TNDM) originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al. 2013).

TNDM is a form of diabetes that occurs in infants and is characterised by severe intra-uterine growth retardation, hyperglycemia, dehydration and absence of ketoacidosis.

Three different genetic mechanisms have been described as major causes of TNDM (Temple et al. 2005) (also see Figure 1): paternal uniparental disomy of chromosome 6 (pUPD6) (~40% of TNDM cases), duplication of the 6q24 paternal allele (~30% of TNDM cases), and hypomethylation of the maternal *PLAGL1* differentially methylated region (DMR) (*PLAGL1*:alt-TSS-DMR) (~30% of TNDM cases). In this last group, hypomethylation

can result from either an isolated imprinting variant (only affecting the *PLAGL1*:alt-TSS-DMR), or as part of multi-locus imprinting disturbances (MLIDs). Approximately half of the TNDM-MLID cases are due to a defect in the *ZFP57* gene.

In order to detect the majority of TNDM cases, several *PLAGL1*-specific probes, of which four methylation-specific probes targeting the *PLAGL1*:alt-TSS-DMR, as well as other probes targeting the 6q24 region, have been included in ME033-B1. Additionally, five *ZFP57*-specific MLPA probes detect copy number changes of *ZFP57*.

Copy number probes for two other genes are included in this probemix because of their involvement in TNDM: *INS* and *KCNJ11* (11p15). Recessive loss of function mutations in the *INS* gene have been reported in several patients with TNDM (Støy et al. 2021), whereas activating mutations in *KCNJ11* have been reported as a possible cause of TNDM (Gloyn et al. 2006). Additionally, probes for *ZC2HC1B* (6q24, downstream of *PLAGL1*), *SF3B5* (6q24, upstream of *PLAGL1*) and several other genes in the flanking region are included to determine copy numbers of the 6q24 chromosomal region.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1534/.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *INS*, *KCNJ11*, *PLAGL1*, *ZC2HC1B* and *ZFP57* exon numbering used in this ME033-B1 TNDM product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcripts NM_000207.3, NM_000525.4, NM_001317162.2, NM_001013623.3 and NM_001109809.5, respectively, which can be found in Tables 1 and 2. From description version A1-04 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select transcripts for these genes. The *HYMAI* exon numbering is derived from the NR_002768.3 sequence.

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. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix ME033-B1 TNDM contains 43 (methylation-specific) probes with amplification products between 124 and 445 nucleotides (nt). 21 probes target the 6q24 chromosomal region, of which ten target the *PLAGL1* gene. Four of the probes targeting *PLAGL1* are methylation-specific probes which contain an HhaI recognition site and provide information on the methylation status of the *PLAGL1*:alt-TSS-DMR. In addition, there are five probes targeting the *ZFP57* gene on 6p22 and five probes targeting *INS* and *KCNJ11* on 11p15. All probes present will also give information on copy number changes in the analysed sample. In addition, ten reference probes are included that are not affected by HhaI digestion and detect genes located outside the *PLAGL1* gene and the 6p22, 6q24 and 11p15 chromosomal 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. We recommend using SALSA HhaI enzyme (SMR50) as this restriction enzyme has been validated for use with MS-MLPA by MRC Holland.

Required specimens

Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MS-MLPA General Protocol.

The results of methylation-specific 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. Consequently, the use of this product on CVS samples should involve examining and validating the methylation status of each individual methylation-specific probe.

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 Transient Neonatal Diabetes Mellitus. 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. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis
Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Methylation analysis per DMR
The methylation-specific probes targeting the *PLAGL1*:alt-TSS-DMR are located close to each other. It is expected that all methylation-specific probes in the DMR provide similar results. We recommend using the median methylation status of these probes to determine the methylation status of the locus and to disregard aberrant methylation detected by a single methylation-specific probe.

Interpretation of copy number results
The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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 or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

NOTE: In case digestion control probes are not fully digested (>0.05[1]), please contact info@mrcholland.com for more information.

ME033 specific notes:

- Please note that one probe (*PLAGL1*; 18463-L23640) has multiple HhaI restriction sites. All of these sites need to be methylated in order to not be digested!
- Methylation-specific PLAGL1 probes 18458-L25628 (142 nt) and 15745-L24216 (154 nt) are more sensitive to certain experimental variations, as described in Table 1 and 2. Pay extra attention to the results of the digested reactions in case the undigested ratios of these two probes are different from those of the probes at 160 nt (*PLAGL1*; 18460-L33026) and 183 nt (*PLAGL1*; 18463-L23640).

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.
- A methylation-specific 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 a methylation-specific 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 ratio 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

[1] Signals ≤ 0.10 are displayed as intra ratio percentage by Coffalyser.Net. For more information see the Coffalyser.Net Reference Manual.

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://databases.lovd.nl/shared/genes. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *ZFP57* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix ME033-B1 TNDM

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	Chromosomal position (hg18)		
				Reference	*PLAGL1*[a,b]	Other region
64-105	Control fragments – see table in probemix content section for more information					
124 *	Reference probe S0645-L19362	-		3p		
130 * π	**Digestion control probe** S0750-L27753	+	0%	2q		
139 ¥	**ZC2HC1B probe** 18457-L32246	-				6q24
142 Δ « Đ	**PLAGL1 probe** 18458-L25628	+	50%		*PLAGL1*:alt-TSS-DMR	
148	**ZC2HC1B probe** 18459-L23636	-				6q24
154 * Δ «	**PLAGL1 probe** 15745-L24216	+	50%		*PLAGL1*:alt-TSS-DMR	
160 ¥ «	**PLAGL1 probe** 18460-L33026	+	50%		*PLAGL1*:alt-TSS-DMR	
166 *	Reference probe 16607-L19137	-		17q		
172 * π	**Digestion control probe** 22371-L29245	+	0%	8p		
178	**STXBP5-AS1 probe** 18462-L23639	-				6q24
183 * ‡ «	**PLAGL1 probe** 18463-L23640	+	50%		*PLAGL1*:alt-TSS-DMR	
191 *	**ZC2HC1B probe** 22802-L33025	-				6q24
196 *	Reference probe 05268-L30942	-		2p		
202	**ZC2HC1B probe** 18465-L23642	-				6q24
208	**INS probe** 19503-L17352	-				11p15
215	**PLAGL1 probe** 18466-L23643	-			Exon 4	
220 *	Reference probe 05939-L05368	-		1q		
232	**INS probe** 15500-L25657	-				11p15
240	**PLAGL1 probe** 18335-L25631	-			Exon 8	
247	**LINC01625 probe** 19506-L23654	-				6q24
253	**SASH1 probe** 19507-L23651	-				6q24
265	**HIVEP2 probe** 18468-L23645	-				6q24
274 ¥	**PLAGL1 probe** 22370-L25634	-			Exon 6	
283 *	Reference probe 21887-L31064	-		21q		
292 *	**ZFP57 probe** 22349-L31501	-				6p22
301 «	**KCNJ11 probe** 18470-L23647	-				11p15
306 *	**PLAGL1 probe** 22803-L32165	-			Exon 7	
312 *	Reference probe 12442-L26378	-		14q		
319 *	**ZFP57 probe** 22350-L31502	-				6p22
328 *	**SF3B5 probe** 22805-L32167	-				6q24
337 *	**ZFP57 probe** 22351-L31503	-				6p22
346 «	**KCNJ11 probe** 19505-L23644	-				11p15
353 *	**PLAGL1 probe** 22806-L32168	-			Upstream	
364 *	Reference probe 21636-L30252	-		22q		
373	**PLAGL1 probe** 18340-L23253	-			Exon 3	
382	**NMBR probe** 18476-L23653	-				6q24
391 «	**INS probe** 19504-L25656	-				11p15
400 *	Reference probe 17461-L21217	-		16p		
409	**ZFP57 probe** 18478-L23655	-				6p22
418	**PEX3 probe** 18479-L23656	-				6q24
426 *	**ZFP57 probe** 22807-L32169	-				6p22
436 *	Reference probe 20428-L27910	-		18q		
445 *	Reference probe 22487-L31637	-		10q		

[a] See section Exon numbering on page 2 for more information.

[b] 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).

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
Đ This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48˚C).
‡ 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. Single probe aberration(s) must be confirmed by another method.

Table 2. ME033-B1 target probes arranged according to chromosomal location

Table 2a. Chromosome 6

Length (nt)	SALSA MLPA probe	Gene/DMR [b] /Exon [a]	HhaI site	Ligation site	Distance to next probe
		ZFP57		**NM_001109809.5**	
319	22350-L31502	Exon 5	-	1632-1631, reverse	2.5 kb
292	22349-L31501	Exon 4	-	779-778, reverse	0.5 kb
337	22351-L31503	Exon 3	-	5 nt after exon 3	1.0 kb
409	18478-L23655	Exon 2	-	492-493	4.1 kb
426	22807-L32169	Exon 1	-	56-55, reverse	110.1 **Mb**
247	19506-L23654	*LINC01625*	-		2.6 **Mb**
382	18476-L23653	*NMBR*	-		698.8 kb
265	18468-L23645	*HIVEP2*	-		710.3 kb
418	18479-L23656	*PEX3*	-		379.3 kb
		ZC2HC1B		**NM_001013623.3**	
148	18459-L23636	Exon 1	-	39-40	21.6 kb
191	22802-L33025	Exon 3	-	232-233	17.0 kb
139	18457-L32246	Exon 6	-	632-633	35.1 kb
202	18465-L23642	Exon 8	-	805-806	3.0 kb
		PLAGL1		**NM_001317162.2**	
240	18335-L25631	Exon 8	-	2382-2381, reverse	7.0 kb
306	22803-L32165	Exon 7	-	706-707	12.3 kb
274	22370-L25634	Exon 6	-	366-365, reverse	5.7 kb
215	18466-L23643	Exon 4	-	24 nt before exon 4, reverse	2.7 kb
373	18340-L23253	Exon 3	-	241-240, reverse	38.9 kb
160 «	18460-L33026	*PLAGL1*:alt-TSS-DMR (Exon 1)	+	286 nt after exon 1; *HYMAI*: NR_002768.3; exon 1; 456-457	0.1 kb
154 Δ «	15745-L24216	*PLAGL1*:alt-TSS-DMR (Exon 1)	+	215 nt after exon 1; *HYMAI*: NR_002768.3; exon 1; 385-386	0.3 kb
142 Δ « Đ	18458-L25628	*PLAGL1*:alt-TSS-DMR (Exon 1)	+	111-110 exon 1, reverse; *HYMAI*: NR_002768.3; exon 1; 111-110, reverse	0.5 kb
183 ‡ «	18463-L23640	*PLAGL1*:alt-TSS-DMR (Exon 1)	+	350 nt before exon 1, reverse;	55.9 kb

				HYMAI: NR_002768.3; 350 nt before exon 1, reverse	
353	22806-L32168	up	-	56 kb before exon 1; NM_001080951.3; exon 1; 98-99	31.0 kb
328	22805-L32167	*SF3B5*	-		3.1 **Mb**
178	18462-L23639	*STXBP5-AS1*	-		1.4 **Mb**
253	19507-L23651	*SASH1*	-		

Table 2b. Chromosome 11

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	HhaI site	Ligation site	Distance to next probe
		INS		**NM_000207.3**	
232	15500-L25657	Exon 3	-	10 nt after exon 3	1.0 kb
208	19503-L17352	Exon 2	-	233-232, reverse	0.4 kb
391 «	19504-L25656	Exon 1	-	37-38	15.2 **Mb**
		KCNJ11		**NM_000525.4**	
346 «	19505-L23644	Exon 1	-	1623-1622, reverse	1.1 kb
301 «	18470-L23647	Exon 1	-	509-508, reverse	

[a] See section Exon numbering on page 2 for more information.
[b] 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).

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
Đ This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48˚C).
‡ 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.

Table 3. Sequences detected by the ME033-B1 probes

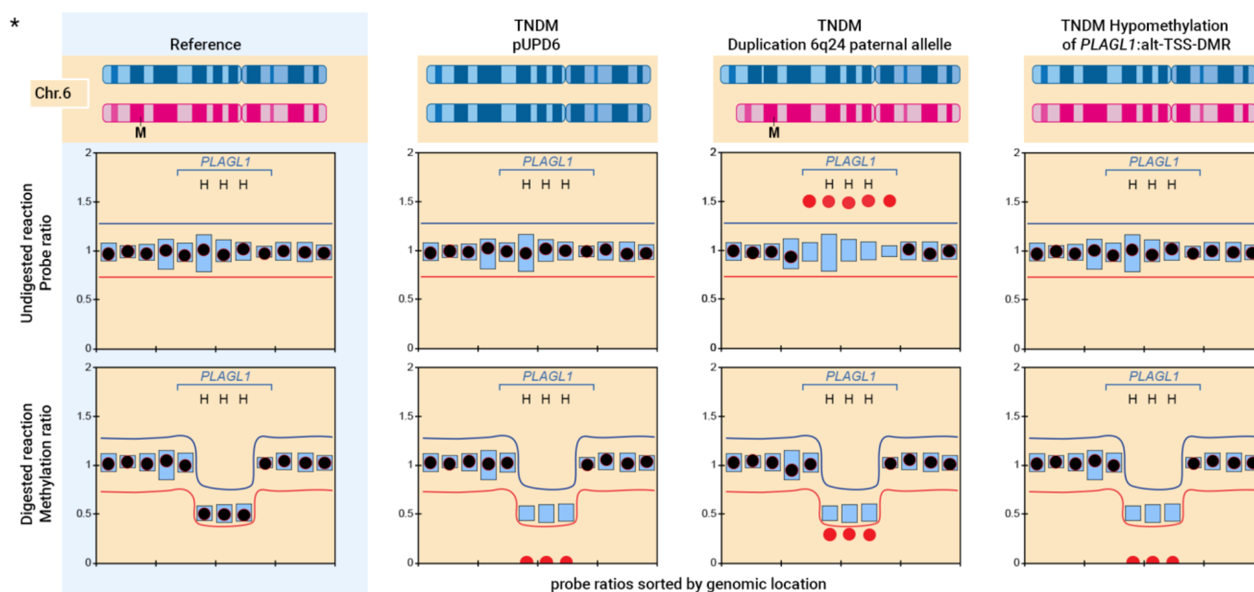
Length (nt)	SALSA methylation-specific probe	Partial sequence with HhaI site
142	**PLAGL1 probe** 18458-L25628	GCCACCTCCGCGG-CCATGACGGCGACCCGGGGAAGCGCCCCG
154	**PLAGL1 probe** 15745-L24216	TGCCGCCGG-ACCCCCTGGACGCCGCTGCCAGAGGCGTTCGCGCCTA
160	**PLAGL1 probe** 18460-L33026	AGACCCGATTCTTACAA-CCTGGCGCTCTAACCTCG
183	**PLAGL1 probe** 18463-L23640	TACAGCGCTGGCGCAGGTAGACCCGA-GCCGGCC

The HhaI sites are marked with grey. Ligation sites are marked with –. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Note: Please be aware that one probe (*PLAGL1*; 18463-L23640) has multiple HhaI restriction sites. All of these sites need to be methylated in order to not be digested.

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

Interpretation of copy number and methylation ratio results



* *Images are a simplified representation and do not show all probes.*
M *Targeted methylation site*
H *Target probe with an HhaI site*

Normal probe ratio distribution based on reference samples
Probe Ratio - not aberrant
Probe Ratio - aberrant

Figure 1. Simplified schematic representation of results that may be obtained with TNDM samples. Images do not show all probes. In TNDM, paternal uniparental disomy of chromosome 6 accounts for approximately ~40% of the 6q24-related TNDM cases, while duplication of the 6q24 paternal allele accounts for ~30% and hypomethylation of the maternal *PLAGL1*:alt-TSS-DMR another ~30% (as reported by https://www.ncbi.nlm.nih.gov/books/NBK1534/).

Related SALSA MLPA probemixes

ME032 UPD7-UPD14	Contains several probes for imprinted regions on chromosomes 7 and 14.
ME034 Multi-locus Imprinting	Contains probes for regions implicated in multi-locus imprinting disturbances on chromosomes 6, 7, 11, 14, 15, 16, 19 and 20.
P117 ABCC8	Contains probes for the *ABCC8* gene, associated with familial hyperinsulinemic hypoglycemia 1 (HHF1).
P241 MODY Mix 1	Contains probes for the *HNF1A*, *HNF1B*, *HNF4A* and *GCK* genes, associated with Maturity-Onset Diabetes of the Young (MODY).
P357 MODY Mix 2	Contains probes for the *KLF11*, *NEUROD1*, *PAX4*, *CEL*, *INS*, *PDX1* and *HNF1B* genes, associated with Maturity-Onset Diabetes of the Young (MODY).

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Selected publications using SALSA MLPA Probemix ME033 TNDM

- Fu JL et al. (2019). Relapsed 6q24-related transient neonatal diabetes mellitus successfully treated with sulfonylurea. *Chin Med J*. 132(7), 846.
- Mustafa M et al. (2021). Transient Neonatal Diabetes Mellitus with the Rare Association of Nonsuppurative Sialadenitis and Genetic Defects in 6q24. *Case Reports in Pediatrics*. 2021:5901898.

ME033 product history	
Version	*Modification*
B1	All ten reference probes have been replaced, as well as seven target probes. Three probes have been changed in length, not in sequence detected, and five target probes added.
A1	First release.

Implemented changes in the product description
Version B1-01 – 25 November 2024 (04M) - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - All probes that are in a DMR are now indicated as such in Table 1 and 2. - Various minor textual or layout changes. - Section on interpretation of copy number and methylation ratio results completely new. - DMR names were updated according to the nomenclature system as proposed in https://pubmed.ncbi.nlm.nih.gov/27911167/. Version A1-04 – 05 December 2023 (04M) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Exon numbering of the *NMBR* and *PLAGL1* genes has been changed. - Ligation sites of the probes targeting the *ZFP57*, *NMBR*, *HIVEP2*, *PLAGL1*, *HYMAI*, *INS and KCNJ11* genes updated according to new version of the NM_ reference sequences. Version A1-03 — 24 March 2020 (02M) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Exon numbering of the *ZFP57* gene has been changed. - Ligation sites of the probes targeting the *ZFP57, HIVEP2, PEX3, ZC2HC1B*, *PLAGL1*, and *SASH1* genes updated according to new version of the NM_ reference sequences. - Contact email addresses and website URL updated. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

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