Product Description
SALSA® MLPA® Probemix ME034-D1 Multi-locus Imprinting

To be used with the MS-MLPA General Protocol.

Version D1
As compared to version C1, two probes targeting a differentially methylated region in the *ZNF597* gene (16p13) have been added, three reference probes and one SNRPN probe have been replaced, and one GRB10 probe and one MEST probe have changed in length, not in sequence detected.
For complete product history see page 10.

Catalogue numbers:
- **ME034-025R:** SALSA MLPA Probemix ME034 Multi-locus Imprinting, 25 reactions.
- **ME034-050R:** SALSA MLPA Probemix ME034 Multi-locus Imprinting, 50 reactions.
- **ME034-100R:** SALSA MLPA Probemix ME034 Multi-locus Imprinting, 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 ME034-D1 probemix MRC Holland has observed non-specific peaks. 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. Furthermore, 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.

General information
The SALSA MLPA Probemix ME034 Multi-locus Imprinting is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences in 14 different differentially methylated regions (DMRs) on eight different chromosomes. Applications include the study of multi-locus imprinting disturbances and finding the parental origin of triploid pregnancies. This probemix can also be used to detect deletions/duplications in the aforementioned chromosomal regions.

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 disturbances originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al. 2013).

Multi-locus imprinting disturbances (MLIDs) have been identified in several patients. For example, a considerable number of patients with 11p15-associated imprinting disturbances have been reported to also carry methylation changes at other chromosomal locations (Eggermann et al. 2014).

Triploidy is one of the most common chromosomal abnormalities, occurring in 1–2% of all human conceptuses. Triploid pregnancies can have either the chromosomal constitution of two maternal and one

paternal set (digyny) or two paternal and one maternal set (diandry). The distinction between digyny and diandry is clinically important because the risk of the conceptus being molar and thereby inducing a risk for maternal complications (such as pre-eclampsia, postpartum haemorrhage and persistent gestational trophoblastic disease) is determined by the parental composition of the genome. As shown by Joergensen et al. (2013 and 2014), methylation-specific MLPA (MS-MLPA) analysis of imprinted regions is a reliable method to distinguish triploidies with a double paternal contribution from triploidies with a double maternal contribution.

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

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

Exon numbering
The *PLAGL1*, *GRB10*, *MEST*, *SNRPN*, *PEG3* and *ZNF597* exon numbering used in this ME034-D1 Multi-locus Imprinting product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcripts NM_001317162.2, NM_001350814.2, NM_002402.4, NM_003097.6, NM_006210.3 and NM_152457.3, respectively, which can be found in Table 2. The *SNRPN* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version D1-01 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select transcripts for this gene.

The *HYMAI*, *H19*, *KCQ1OT1*, *MEG3*, and *MEG8* exon numbering is derived from the NR_002768.3, NR_002196.3, NR_002728.4, NR_190993.1and NR_146000.1 sequences, respectively.

For the *GNAS* complex locus the exon numbering for *NESP55*, *GNAS-AS1*, *GNASXL* and *GNAS A/B* is derived from NM_016592.5, NR_002785.3, NM_080425.4 and NM_001309840.2 sequences, respectively.

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 ME034-D1 Multi-locus Imprinting contains 40 (methylation-specific) probes with amplification products between 122 and 465 nucleotides (nt). 27 of these probes are methylation-specific and contain an HhaI recognition site and provide information on the methylation status of different sequences in genes known to be methylated in either the paternal or the maternal allele. This includes three probes for each of the following genes: *H19* and *PEG3*, two probes for each of the following genes: *KCNQ1OT1*, *MEST*, *MEG3*, *MEG8, SNRPN, PLAGL1, GRB10*, and *ZNF597*, and five probes for the *GNAS* complex locus. All probes present will also give information on copy number changes in the analysed sample. In addition, eleven reference probes are included that are not affected by HhaI digestion and detect genes located outside the chromosomal regions targeted by this probemix. 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 signal 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 imprinting disturbances. 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.

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 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.

Interpretation of methylation signal on triploid samples

The following table gives an indication of the expected findings in digested triploid samples. Information on which regions are maternally imprinted and which are paternally imprinted can be found in Table 2 below.

Sample	% expected signal reduction of probes targeting paternally imprinted regions	% expected signal reduction of probes targeting maternally imprinted regions
Normal diploidy	50%	50%
Digynic triploidy	67%	33%
Diandric triploidy	33%	67%

- 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.

ME034 specific notes:
- Please note that several probes have multiple HhaI restriction sites. All of these sites need to be methylated in order to not be digested!
- *H19* locus: The three methylation-specific probes targeting the *H19* gene are located very close to each other. It is expected that all three probes provide similar results. We recommend using the median methylation signal of these *H19* probes to determine the methylation signal of the *H19* locus and to disregard aberrant methylation detected by a single *H19* methylation-specific probe.

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 that two different alleles of the sequence are present in the sample DNA and that a false positive MS-MLPA result was obtained.

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

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.
Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix ME034-D1 Multi-locus Imprinting

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	Chromosomal position (hg18)	
				Reference	Target[a]
64-105	Control fragments – see table in probemix content section for more information				
122	Reference probe 19616-L27299	-		4p	
130	**MEG3 probe** 19547-L25282	+	50%		*MEG3*:TSS-DMR
136 «	**GRB10 probe** 15742-L18941	+	50%		*GRB10*:alt-TSS-DMR
141 «	**PLAGL1 probe** 18458-L23635	+	50%		*PLAGL1*:alt-TSS-DMR
158	Reference probe 19051-L32326	-		21q	
164 «	**PLAGL1 probe** 18460-L32287	+	50%		*PLAGL1*:alt-TSS-DMR
171 ‡	**KCNQ1OT1 probe** 23000-L31883	+	50%		*KCNQ1OT1*:TSS-DMR
184	**H19 probe** 14063-L08764	+	50%		*H19/IGF2*:IG-DMR
190	Reference probe 06378-L05844	-		6p	
197 * ‡	**ZNF597 probe** 23415-L33135	+	60%		*ZNF597*:3' DMR
204 ¥	**MEST probe** 21198-L33153	+	50%		*MEST*:alt-TSS-DMR
214	Reference probe 15470-L26873	-		1p	
220	**MEG3 probe** 15754-L18942	+	50%		*MEG3*:TSS-DMR
232 ¥ «	**GRB10 probe** 18700-L24405	+	50%		*GRB10*:alt-TSS-DMR
238	**H19 probe** 14792-L16503	+	50%		*H19/IGF2*:IG-DMR
246	**PEG3 probe** 19531-L32288	+	50%		*PEG3*:TSS-DMR
256	Reference probe 19625-L26284	-		10p	
264 Ж	**NESP55 probe** 18126-SP0007-L22614	+	50%		*GNAS-NESP*:TSS-DMR
276	**KCNQ1OT1 probe** 07171-L06780	+	50%		*KCNQ1OT1*:TSS-DMR
283	**GNAS-AS1 probe** 22363-L22606	+	50%		*GNAS-AS1*:TSS-DMR
292 *	Reference probe 15724-L17704	-		12q	
301	**H19 probe** 06266-L05772	+	50%		*H19/IGF2*:IG-DMR
309	**MEST probe** 19546-L17775	+	50%		*MEST*:alt-TSS-DMR
317 Ð ‡	**GNASXL probe** 15645-L26874	+	50%		*GNASXL*:Ex1-DMR
322 ‡	**MEG8 probe** 22735-L32029	+	50%		*MEG8*:Int2-DMR
331 π	**Digestion control probe** 02734-L25117	+	0%	13q	
337	**SNRPN probe** 22586-L32289	+	50%		*SNURF*:TSS-DMR
355	Reference probe 21334-L29740	-		3p	
364 *	Reference probe 14059-L27828	-		5q	
372 π	**Digestion control probe** 21589-L32334	+	0%	2q	
380	**MEG8 probe** 23004-L32022	+	40%		*MEG8*:Int2-DMR
391 *	**ZNF597 probe** 23414-L33134	+	60%		*ZNF597*:3' DMR
402 « Ð ‡	**GNAS A/B probe** 03882-L22603	+	50%		*GNAS A/B*:TSS-DMR
410 *	Reference probe 10063-L33074	-		8q	
418 Ð	**PEG3 probe** 12861-L13981	+	50%		*PEG3*:TSS-DMR
427	Reference probe 22367-L16599	-		18q	
436	**PEG3 probe** 19532-L26047	+	50%		*PEG3*:TSS-DMR
443 *	**SNRPN probe** 22587-L31786	+	50%		*SNURF*:TSS-DMR
454	**GNASXL probe** 19545-L17516	+	50%		*GNASXL*:Ex1-DMR
465	Reference probe 19747-L26530	-		9q	

[a] 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 D1.

¥ Changed in version D1. Minor alteration, no change in sequence detected.

« 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

‡ 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.

Ð This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48˚C).

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. ME034-D1 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/DMR[b]	HhaI site	Ligation site/Exon[a]	Chromoso-mal position	Imprinted allele	Distance to next probe
		PLAGL1		**NM_001317162.2**			
164 «	18460-L32287	*PLAGL1*:alt-TSS-DMR	+	286 nt after exon 1; *HYMAI*: NR_002768.3; exon 1; 456-457	6q24.2	Maternal	0.4 kb
141 «	18458-L23635	*PLAGL1*:alt-TSS-DMR	+	Exon 1; 111-110, reverse; *HYMAI*: NR_002768.3; exon 1; 111-110, reverse	6q24.2	Maternal	
		GRB10		**NM_001350814.2**			
232 «	18700-L24405	*GRB10*:alt-TSS-DMR	+	Exon 1; 56-57	7p12.2	Tissue specific Maternal	0.2 kb
136 «	15742-L18941	*GRB10*:alt-TSS-DMR	+	175 nt before exon 1	7p12.2	Tissue specific Maternal	79.1 **M**b
		MEST		**NM_002402.4**			
309	19546-L17775	*MEST*:alt-TSS-DMR	+	550 nt before exon 1	7q32.2	Maternal	0.8 kb
204	21198-L33153	*MEST*:alt-TSS-DMR	+	Exon 1; 231-232	7q32.2	Maternal	
		H19		**NR_002196.3**			
301	06266-L05772	*H19/IGF2*:IG-DMR / IC1 (H19 DMR)	+	335 nt before exon 1	11p15.5	Paternal	0.1 kb
238	14792-L16503	*H19/IGF2*:IG-DMR / IC1 (H19 DMR)	+	480 nt before exon 1, reverse	11p15.5	Paternal	0.2 kb
184	14063-L08764	*H19/IGF2*:IG-DMR / IC1 (H19 DMR)	+	650 nt before exon 1	11p15.5	Paternal	700.8 kb
		KCNQ1OT1		**NR_002728.4**			
276	07171-L06780	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	Exon 1; 660-659, reverse	11p15.5	Maternal	0.5 kb
171 ‡	23000-L31883	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	Exon 1; 174-175	11p15.5	Maternal	
		MEG3		**NR_190993.1**			

220	15754-L18942	*MEG3*:TSS-DMR	+	377 nt before exon 1	14q32.2	Paternal	0.3 kb
130	19547-L25282	*MEG3*:TSS-DMR	+	93 nt before exon 1, reverse	14q32.2	Paternal	78.5 kb
		MEG8		**NR_146000.1**			
380	23004-L32022	*MEG8*:Int2-DMR	+	1.6 kb before exon 6, reverse	14q32.31	Maternal	0.1 kb
322 ‡	22735-L32029	*MEG8*:Int2-DMR	+	1.5 kb before exon 6	14q32.31	Maternal	
		SNRPN		**NM_003097.6**			
337	22586-L32289	*SNURF*:TSS-DMR (CpG island (PWS-SRO))	+	969 nt after exon 1 (exon 5)	15q11.2	Maternal	0.7 kb
443	22587-L31786	*SNURF*:TSS-DMR (CpG island (PWS-SRO))	+	1.6 kb after exon 1 (exon 5)	15q11.2	Maternal	
		ZNF597		**NM_152457.3**			
197 ‡	23415-L33135	*ZNF597*:3' DMR	+	499 nt after exon 4	16p13.3	Maternal	0.1 kb
391	23414-L33134	*ZNF597*:3' DMR	+	386 nt after exon 4, reverse	16p13.3	Maternal	
		PEG3		**NM_006210.3**			
436	19532-L26047	*PEG3*:TSS-DMR	+	2.0 kb after exon 1, reverse	19q13.43	Maternal	1.7 kb
246	19531-L32288	*PEG3*:TSS-DMR	+	228 nt after exon 1	19q13.43	Maternal	0.5 kb
418 Ð	12861-L13981	*PEG3*:TSS-DMR	+	146 nt before exon 1, reverse	19q13.43	Maternal	
		***GNAS* complex locus**					
264 Ж	18126-SP0007-L22614	*GNAS-NESP*:TSS-DMR	+	NM_016592.5; exon 1, 150-151, 179-180	20q13.32	Paternal	11.1 kb
283	22363-L22606	*GNAS-AS1*:TSS-DMR	+	NR_002785.3; 87 nt before exon 1	20q13.32	Maternal	4.1 kb
454	19545-L17516	*GNASXL*:Ex1-DMR	+	NM_080425.4; exon 1; 2370-2371	20q13.32	Maternal	0.1 kb
317 Ð ‡	15645-L26874	*GNASXL*:Ex1-DMR	+	NM_080425.4; exon 1; 2453-2454	20q13.32	Maternal	34.2 kb
402 « Ð ‡	03882-L22603	*GNAS A/B*:TSS-DMR	+	NM_001309840.2; exon 1; 155-156	20q13.32	Maternal	

[a] 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). DMR names used in previous versions of this product description can be found in between brackets.

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

« 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

‡ 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.

Ð This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48˚C).

Table 3. Sequences detected by the ME034-D1 probes

Length (nt)	SALSA methylation-specific probe	Partial sequence with HhaI site
130	19547-L25282	ATTCTGCTGCAATGCGCTCATTTCTCTAA-AAGTGATTGGCCGGAAAAATGAG
136	15742-L18941	CTTTCGGCATCGTCAGAGT-GGCCAGTGTGCGCGTCCTTGCCCA
141	18458-L23635	CTCGGGCGTGCCACCTCCGCGG-CCATGACGGCGACCCGGGGAAGCGCCCCG
164	18460-L32287	TGAGGTCCACAGACCCGATTCTTACAA-CCTGGCGCTCTAACCTCGCC
171	23000-L31883	CTCAGCGCGGTCCTCCTCGGTGCGTCA-GTCATCGTGGTTCTCCCCGGCGCGCCCC
184	14063-L08764	GTAGAGTGCGCCCGCGAGCCGTA-AGCACAGCCCGGCAACATGCGG
197	23415-L33135	CGCTTCCGGTAGCGCCTGCGCATTC-CTGGGGCAAGTGGACTGTGGT
204	21198-L33153	CGGCATGGGATAACGCGGCCAT-GGTGCGCCGAGATCGCCTCCGCAG
220	15754-L18942	CGATGGATGTTCCGAA-ACCGCCAGGTGTGGGATCTGCGCCCCGA
232	18700-L24405	TCGTCCTCCCGCTCCTCA-GGAGCGCCCAGTCCCTCGGAGG
238	14792-L16503	CAGAGGGAAGTGCCGCAA-ACCCCCTGGTGGGCGCGGTGCCAGCCCC
246	19531-L32288	GGATGGTTGGTGCCTTTTTGGGGCA-GGGGTGGGCGCAGCCCGGGCTGC
264	18126-SP0007-L22614	TTGCTCAGAGAGGCA-AGCAAGGCGCGGAGCTTTAGAAAGTTCTT-AAGTGGTCAGG
276	07171-L06780	CAGCTCACCTCAGCAA-CGCCAGTGATCACCCGTCCCGCGCCGTC
283	22363-L22606	CGACCCCAGCGCCGGGTCAGCCA-TTGGGCAGGGGTCATGCCAAT
301	06266-L05772	CAGCCATGTGCAAAGTA-TGTGCAGGGCGCTGGCAGGCAGGG
309	19546-L17775	TTGCTCGTGCCCTT-GGTGGTTACCGGTAGTTAAGCTTAGGGCGCATAGG
317	15645-L26874	CAAAGCCTCGCGCTCTCTCA-AGGTCAAGAAGGTACCCCTGGCGGAGAAGCGCAG
322	22735-L32029	AGTCAAGAGGGACCGGCGCTTTTGCT-CTGGCAGCGGCGCTTTTAACTGCGACAG
331	02734-L25117	CGCCCAAGGAGGGAGAGTGGCGCTC-CCGCCGAGGGTGCACTAGCCAGATAT
337	22586-L32289	TGCCTACTGTGGTGGTGGTGCTTTTTTA-TTAAAACTGCGCAATGCCTACACT
372	21589-L32334	TTTGGA-ATTCCATTGGCATTGGGGTGTCTTTGTTAAGTGCCTGGCCAGTAGCGCTG
380	23004-L32022	GGGGCGCCACAGACTCTTGGCTGGTGTCTGAAG-AATTCAGACTGTCTGATTC
391	23414-L33134	CTGTAGCGCAAGTCATCTCGTTGACACTTCCGCTGAC-CCGTGCAGTTGGTGGCCA
402	03882-L22603	GCAGGCGCTGCCTTGCGTGT-GAGTGCACCTCACTCACATGTAAGTCGGGGAGCGC
418	12861-L13981	CGGTTGCCACAGCA-ACAGTTTGGCGCGAAGGCTGGGGGCTGA
436	19532-L26047	CTGCCACCGTTAGCCAAA-AACACAGCGAGCGCGGTGATAACCC
443	22587-L31786	GCAGCTGTTCCTTTCCGGTTGTGG-CGCAGTAGAGGGGGGAG
454	19545-L17516	AGCCCCAGCGCAACTT-ACTCCGCAACTTTCTCGTGCA

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 several probes have 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.

Related products
For related products, see the product page on our website.

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Selected publications using SALSA MLPA Probemix ME034 Multi-locus Imprinting

- Bilo L et al. (2023). Molecular characterisation of 36 multilocus imprinting disturbance (MLID) patients: a comprehensive approach. *Clin Epigenetics*. 15:35.
- Eggermann T et al. (2020). Biallelic *PADI6* variants cause multilocus imprinting disturbances and miscarriages in the same family. *Eur J Hum Genet*. 29:587-580.
- Tayeh MK et al. (2022). Novel multilocus imprinting disturbances in a child with expressive language delay and intellectual disability. *Am J Med Genet*. 188:2209-2216.

ME034 product history	
Version	*Modification*
D1	Two probes targeting a differentially methylated region in the *ZNF597* gene (16p13) have been added, three reference probes and one SNRPN probe have been replaced, and one GRB10 probe and one MEST probe have changed in length, not in sequence detected.
C1	Five target probes have been removed, seven new target probes have been added, three reference probes and one digestion control probe have been replaced, three target probes and one digestion control probe have changed in length, no change in the sequence detected.
B1	Three target probes have been added. One target probe, one digestion control probe, and nine reference probes have been replaced. And one probe length has been adjusted.
A1	First release.

Implemented changes in the product description
Version D1-02 – 29 April 2025 (04M) - Gene structure and transcript variants section: link to MANE website added. - NR_sequence changed for *MEG3*. - Ligation site of the 130 nt and 220 nt probe in Table 2 adapted to new reference sequence (no change in actual target site). - Related SALSA MLPA products replaced with a reference to the product page. Version D1-01 – 09 October 2024 (04M) - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Various minor textual or layout changes. - Terms “Multi-locus imprinting disorder(s)” and “Multi-locus imprinting defect(s)” have been replaced by “Multi-locus imprinting disturbance(s)”. - Exon numbering of the *SNRPN* gene has been changed. - Ligation sites of the probes targeting the *GRB10, H19, KCNQ1OT1, SNRPN, GNAS-AS1* and *GNAS A/B* genes updated according to new version of the NM_ or NR_ reference sequence. - Small change of probe length in Table 1 in order to better reflect the true length of the amplification product. - DMR names were updated according to the nomenclature system as proposed in https://pubmed.ncbi.nlm.nih.gov/27911167/. DMR names used in previous versions of the product description can be found between brackets in Table 2. - Warning for overdigestion added to the probes at 317 nt, 402 nt and 418 nt in Table 1 and 2.

Version C1-04 – 05 December 2023 (04M) - Exon numbering of the *PLAGL1* gene has been changed. - Ligation sites of the probes targeting the *PLAGL1* and *HYMAI* genes updated according to new version of the NM_ and NR_ reference sequence.

More information: www.mrcholland.com; www.mrcholland.eu	
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