Product Description
SALSA® MS-MLPA® Probemix ME034-C1 Multi-locus Imprinting

To be used with the MS-MLPA General Protocol.

Version C1
As compared to version B1, 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. For complete product history see page 10.

Catalogue numbers:
- **ME034-025R:** SALSA MS-MLPA Probemix ME034 Multi-locus Imprinting, 25 reactions.
- **ME034-050R:** SALSA MS-MLPA Probemix ME034 Multi-locus Imprinting, 50 reactions.
- **ME034-100R:** SALSA MS-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-C1 probemix MRC-Holland has observed non-specific peaks at 95, 143 and 203 nt. 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 (mainly for the 143 and 203 nt peaks). 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 MS-MLPA Probemix ME034 Multi-locus Imprinting is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences of the in 11 different imprinted locations in seven different chromosomal regions. Applications include the study of multi-locus imprinting disorders 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 disorders originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al. 2013).

Multi-locus imprinting defects (MLIDs) have recently been identified in several patients. For example, a considerable number of patients with 11p15-associated imprinting disorders 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), 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 MS-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/

Exon numbering
The exon numbering used in this ME034-C1 Multi-locus Imprinting product description is the exon numbering from the following NG-sequences or NM/NR RefSeq transcripts:

- NM_001317162.2 (*PLAGL1*)
- NR_002768.3 (*HYMAI*)
- NG_012305.2 (*GRB10*)
- NG_009226.1 (*MEST*)
- NG_16165.1 (*H19*)
- NG_008935.1 (*KCQ1OT1*)
- NG_016853.2 (*MEG3*)
- NG_045000.6 (*MEG8*)
- NG_012958.1 (*SNRPN*)
- NG_012989.1 (*PEG3*)
- NM_016592.5 (*NESP55*)
- NG_021433.1 (*GNAS-AS1*)
- NM_080425.4 (*GNASXL*)
- NG_016194.2 (*GNAS A/B*)

The exon numbering of the NM/NR_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG 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.

The *PLAGL1* 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 C1-04 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select transcripts for this gene.

Probemix content
The SALSA MS-MLPA Probemix ME034-C1 Multi-locus Imprinting contains 38 (MS-)MLPA probes with amplification products between 122 and 466 nucleotides (nt). 25 MS-MLPA probes 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 *GNASXL*, and one probe for each of the following genes: *NESP55*, *GNAS-AS1* and *GNAS A/B*. All probes present will also give information on copy number changes in the analysed sample. In addition, 11 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 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. Consequently, the use of this product on CVS samples should involve examining and validating the methylation status of each individual MS-MLPA 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 disorders. 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 results 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), 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 status of these *H19* probes to determine the methylation status of the *H19* locus and to disregard aberrant methylation detected by a single *H19* MS-MLPA 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.
- 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.

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 MS-MLPA Probemix ME034-C1 Multi-locus Imprinting

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	% expected signal reduction	Chromosomal position (hg18)	
					Reference	Target
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%	50%		MEG3_TSS_DMR
135 * «	**GRB10 probe** 15742-L18941	+	50%	50%		GRB10:alt-TSS-DMR
141 * «	**PLAGL1 probe** 18458-L23635	+	50%	50%		PLAGL1_TSS_alt-DMR
158 ¥	Reference probe 19051-L32326	-			21q	
164 ¥ «	**PLAGL1 probe** 18460-L32287	+	50%	50%		PLAGL1_TSS_alt-DMR
171 * ‡	**KCNQ1OT1 probe** 23000-L31883	+	50%	50%		KvDMR/IC2
178	**SNRPN probe** 04106-L13905	+	50%	50%		CpG island (PWS-SRO)
184	**H19 probe** 14063-L08764	+	50%	50%		H19 DMR/IC1
190	Reference probe 06378-L05844	-			6p	
202 «	**MEST probe** 21198-L17768	+	50%	50%		MEST_alt-TSS_DMR
214	Reference probe 15470-L26873	-			1p	
220	**MEG3 probe** 15754-L18942	+	50%	50%		MEG3_TSS_DMR
228 ¥ «	**GRB10 probe** 18700-L18819	+	50%	50%		GRB10:alt-TSS-DMR
238 *	**H19 probe** 14792-L16503	+	50%	50%		H19 DMR/IC1
246 ¥	**PEG3 probe** 19531-L32288	+	50%	50%		PEG3:TSS-DMR
256 *	Reference probe 19625-L26284	-			10p	
264 Ж	**NESP55 probe** 18126-SP0007-L22614	+	50%	50%		GNAS-NESP:TSS-DMR
276	**KCNQ1OT1 probe** 07171-L06780	+	50%	50%		KvDMR/IC2
283	**GNAS-AS1 probe** 22363-L22606	+	50%	50%		GNAS-AS1:TSS-DMR
292	Reference probe 18670-L24024	-			11p	
301 +	**H19 probe** 06266-L05772	+	50%	50%		H19 DMR/IC1
309 «	**MEST probe** 19546-L17775	+	50%	50%		MEST_alt-TSS_DMR
317	**GNASXL probe** 15645-L26874	+	50%	50%		GNASXL:Ex1-DMR
322 * ‡	**MEG8 probe** 22735-L32029	+	50%	50%		MEG8:Int2-DMR
331 * π	Digestion control probe 02734-L25117	+	0%	100%	13q	
337 *	**SNRPN probe** 22586-L32289	+	50%	50%		CpG island (PWS-SRO)
355	Reference probe 21334-L29740	-			3p	
364	Reference probe 19252-L25357	-			16q	

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	% expected signal reduction	Chromosomal position (hg18)	
					Reference	Target
372 ¥ π	Digestion control probe 21589-L32334	+	0%	100%	2q	
380 *	**MEG8 probe** 23004-L32022	+	50%	50%		MEG8:Int2-DMR
402 « ‡	**GNAS A/B probe** 03882-L22603	+	50%	50%		GNAS A/B:TSS-DMR
418	**PEG3 probe** 12861-L13981	+	50%	50%		PEG3:TSS-DMR
427	Reference probe 22367-L16599	-			18q	
436	**PEG3 probe** 19532-L26047	+	50%	50%		PEG3:TSS-DMR
445	Reference probe 22366-L09098	-			5q	
454	**GNASXL probe** 19545-L17516	+	50%	50%		GNASXL:Ex1-DMR
466	Reference probe 19747-L26530	-			9q	

* New in version C1.

¥ Changed in version C1. Minor alteration, no change in sequence detected.

\+ More variable. Take extra care when interpreting the results of 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.

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.

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-C1 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]/DMR	HhaI site	Ligation site	Chromosomal position	Imprinted allele	Distance to next probe
		PLAGL1					
164 «	18460-L32287	PLAGL1_TSS_alt-DMR	+	286 nt after exon 1 (2) (NM_001317162.2) *HYMAI*: 456-457 (exon 1) (NR_002768.3)	6q24.2	Maternal	0,4 kb
141 «	18458-L23635	PLAGL1_TSS_alt-DMR	+	111-110 reverse (exon 1 (2)) (NM_001317162.2) *HYMAI*: 111-110 reverse (exon 1) (NR_002768.3)	6q24.2	Maternal	
		GRB10					
228 «	18700-L18819	GRB10:alt-TSS-DMR	+	10.4 kb after exon 1 (NM_001001555.3) 56-57 (exon 2) (NM_001350814.2)	7p12.2	Tissue specific Maternal	0,2 kb
135 «	15742-L18941	GRB10:alt-TSS-DMR	+	10.2 kb after exon 1 (NM_001001555.3) 175 nt before exon 2 (NM_001350814.2)	7p12.2	Tissue specific Maternal	79 **M**b
		MEST		**NM_002402.4**			

Length (nt)	SALSA MLPA probe	Gene/Exon[a]/DMR	HhaI site	Ligation site	Chromosomal position	Imprinted allele	Distance to next probe
309 «	19546-L17775	MEST:alt-TSS-DMR	+	550 nt before exon 1	7q32.2	Maternal	0,8 kb
202 «	21198-L17768	MEST:alt-TSS-DMR	+	231-232 (exon 1)	7q32.2	Maternal	
		H19		**NR_002196.2**			
301 +	06266-L05772	H19 DMR/IC1	+	302nt before exon 1	11p15.5	Paternal	0,1 kb
238	14792-L16503	H19 DMR/IC1	+	447 nt before exon 1 reverse	11p15.5	Paternal	0,2 kb
184	14063-L08764	H19 DMR/IC1	+	617 nt before exon 1	11p15.5	Paternal	700,8 kb
		KCNQ1OT1		**NR_002728.3**			
276	07171-L06780	KvDMR/IC2	+	664-663 reverse (exon1)	11p15.5	Maternal	0,5 kb
171 ‡	23000-L31883	KvDMR/IC2	+	178-179 (exon 1)	11p15.5	Maternal	
		MEG3		**NR_003530.2**			
220	15754-L18942	MEG3_TSS_DMR	+	367 nt before exon 1	14q32.2	Paternal	0,3 kb
130	19547-L25282	MEG3_TSS_DMR	+	83 nt before exon 1	14q32.2	Paternal	78,5 kb
		MEG8		**NR_146000.1**			
380	23004-L32022	MEG8:Int2-DMR	+	1.6 kb before exon 6 reverse	14q32.2	Maternal	0,1 kb
322 ‡	22735-L32029	MEG8:Int2-DMR	+	1.5 kb before exon 6	14q32.2	Maternal	
		SNRPN		**NM_022807.5**			
178	04106-L13905	CpG island (PWS-SRO)	+	7.1 kb before exon 5	15q11.2	Maternal	1,0 kb
337	22586-L32289	CpG island (PWS-SRO)	+	6.1 kb before exon 5	15q11.2	Maternal	
		PEG3		**NM_006210.3**			
436	19532-L26047	PEG3:TSS-DMR	+	2 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	12861-L13981	PEG3:TSS-DMR	+	146 nt before exon 1 reverse	19q13.43	Maternal	
		20q13					
264 Ж	18126-SP0007-L22614	GNAS-NESP:TSS-DMR	+	NM_016592.5; 150-151, 179-180 (exon 1)	20q13.32	Paternal	11,1 kb
283	22363-L22606	GNAS-AS1:TSS-DMR	+	NR_002785.2; 86 nt before exon 1	20q13.32	Maternal	4,1 kb
454	19545-L17516	GNASXL:Ex1-DMR	+	NM_080425.4; 2370-2371 (exon 1)	20q13.32	Maternal	0,1 kb
317	15645-L26874	GNASXL:Ex1-DMR 1	+	NM_080425.4; 2453-2454 (exon 1)	20q13.32	Maternal	34,2 kb
402 « ‡	03882-L22603	GNAS A/B:TSS-DMR	+	2.0 kb before exon 1 (NM_001077488.5) 155-156 (exon 1) (NM_001309840.2)	20q13.32	Maternal	

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

+ More variable. Take extra care when interpreting the results of 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.

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.

Table 3. Sequences detected by the ME034-C1 probes

Length (nt)	SALSA MS-MLPA probe	Partial sequence with HhaI site
130	19547-L25282	TTCTGCTGCAATGCGCTCATTTCTCTAA-AAGTGATTGGCCGGAAAAATGAGCCCCC
135	15742-L18941	GGAGGGCTTTCGGCATCGTCAGAGT-GGCCAGTGTGCGCGTCCTTGCCCATCAG
141	18458-L23635	TCCGCTCGGGCGTGCCACCTCCGCGG-CCATGACGGCGACCCGGGGAAGCGCCCC
164	18460-L32287	ATGAGGTCCACAGACCCGATTCTTACAA-CCTGGCGCTCTAACCTCGCCAACGGGCC
171	23000-L31883	CCTCAGCGCGGTCCTCCTCGGTGCGTCA-GTCATCGTGGTTCTCCCCGGCGCGCCCC
178	04106-L13905	CGCTGCTGCAGCGAGTCTGGCGCAGAGT-GGAGCGGCCGCCGGAGATGCCTGACGCA
184	14063-L08764	GTAGAGTGCGCCCGCGAGCCGTA-AGCACAGCCCGGCAACATGCGGTCTTCA
202	21198-L17768	GCGGCATGGGATAACGCGGCCAT-GGTGCGCCGAGATCGCCTCCGCAGGTGA
220	15754-L18942	GCCCGCATCCTCCGATGGATGTTCCGAA-ACCGCCAGGTGTGGGATCTGCGCCCCGA
228	18700-L18819	CCTCCGTCGTCCTCCCGCTCCTCA-GGAGCGCCCAGTCCCTCGGAGGCTGAGT
238	14792-L16503	TGCTGAGGGGCAGAGGGAAGTGCCGCAA-ACCCCCTGGTGGGCGCGGTGCCAGCCCC
246	19531-L32288	TGTGGATGGTTGGTGCCTTTTTGGGGCA-GGGGTGGGCGCAGCCCGGGCTGCTGCGT
264	18126-SP0007-L22614	CATCCCTTCTTCTTGCTCAGAGAGGCA-AGCAAGGCGCGGAGCTTTAGAAAGTTCTT-AAGTGGTCAGGAAGGTAGGTGCTTCCCT
276	07171-L06780	GGGCACACAGCTCACCTCAGCAA-CGCCAGTGATCACCCGTCCCGCGCCGTC
283	22363-L22606	CTCCGACCCCAGCGCCGGGTCAGCCA-TTGGGCAGGGGTCATGCCAATCAAGGCT
301	06266-L05772	CGGCCCCCAGCCATGTGCAAAGTA-TGTGCAGGGCGCTGGCAGGCAGGGAGCA
309	19546-L17775	CTCCCTAGCCGCTTGCTCGTGCCCTT-GGTGGTTACCGGTAGTTAAGCTTAGGGCGCAT
317	15645-L26874	CCCCAGCCCAAAGCCTCGCGCTCTCTCA-AGGTCAAGAAGGTACCCCTGGCGGAGAAGCGCAG
322	22735-L32029	ACAGTCAAGAGGGACCGGCGCTTTTGCT-CTGGCAGCGGCGCTTTTAACTGCGACAG
331	02734-L25117	TCTCGCCCAAGGAGGGAGAGTGGCGCTC-CCGCCGAGGGTGCACTAGCCAGATATTC
337	22586-L32289	TGCCTACTGTGGTGGTGGTGCTTTTTTA-TTAAAACTGCGCAATGCCTACACTGCCG
372	21589-L32334	TGGTATGCTGTGGTAGGGACACTTTGGA-ATTCCATTGGCATTGGGGTGTCTTTGTTAAGTGCCTGGCCAGTAGCGCTG
380	23004-L32022	GGGCGCCACAGACTCTTGGCTGGTGTCTGAAG-AATTCAGACTGTCTGATTCCACCGCACC
402	03882-L22603	CCGGCCGGCAGGCGCTGCCTTGCGTGT-GAGTGCACCTCACTCACATGTAAGTCGGGGAGCGC
418	12861-L13981	CCAATCAGGCTGCGGTTGCCACAGCA-ACAGTTTGGCGCGAAGGCTGGGGGCTGA
436	19532-L26047	GGTGAGCCTCTGCCACCGTTAGCCAAA-AACACAGCGAGCGCGGTGATAACCCGTC
454	19545-L17516	CCGCCGAAAGCCCCAGCGCAACTT-ACTCCGCAACTTTCTCGTGCAAGCCTTC

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 SALSA MLPA probemixes

- ME028 PWS: Probes for the 15q11 imprinted region implicated in Prader-Willi and Angelman syndromes.
- ME030 BWS: Probes for the 11p15 imprinted region implicated in Beckwith-Wiedemann & Russel-Silver syndromes.
- ME031 GNAS: Probes for the 20q13 imprinted *GNAS* region.
- ME032 UPD7-UPD14: Probes for the 6q24, 7p12.1, 7q32.2 and 14q32.2 imprinted regions.
- ME033 TNDM: Probes for the 6q24 imprinted region implicated in transient neonatal diabetes mellitus.

References

- Eggermann T et al. (2014). Additional molecular findings in 11p15-associated imprinting disorders: an urgent need for multi-locus testing. *J Mol Med (Berl)*. 92:769-77.
- Ishida M et al. (2013). The role of imprinted genes in humans. *Mol Aspects Med*. 34:826-840.
- Joergensen MW et al. (2013). Methylation-specific multiplex ligation-dependent probe amplification: utility for prenatal diagnosis of parental origin in human triploidy. *Prenat Diagn*. 33:1131-6.

- Joergensen MW et al. (2014). Triploid pregnancies: genetic and clinical features of 158 cases. *Am J Obstet Gynecol*. Epub. PMID: 24657790.
- Nygren AO et al. (2005). Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences. *Nucleic Acids Res*. 33:e128.
- Paganini L et al. (2015). Beckwith-Wiedemann syndrome prenatal diagnosis by methylation analysis in chorionic villi. *Epigenetics*. 10:643-649.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem*. 421:799-801.

Selected publications using SALSA MS-MLPA Probemix ME034 Multi-locus Imprinting

- Eggermann T et al. (2014). Additional molecular findings in 11p15-associated imprinting disorders: an urgent need for multi-locus testing. *J Mol Med (Berl)*. 92:769-77.
- Joergensen MW et al. (2013). Methylation-specific multiplex ligation-dependent probe amplification: utility for prenatal diagnosis of parental origin in human triploidy. *Prenat Diagn*. 33:1131-6.
- Joergensen MW et al. (2014). Triploid pregnancies: genetic and clinical features of 158 cases. *Am J Obstet Gynecol*. Epub. PMID: 24657790.

ME034 product history	
Version	*Modification*
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 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.

Version C1-03 – 26 August 2021 (04M)
- *GRB10* was added to the probemix content section on page 2.

Version C1-02 – 31 March 2021 (04M)
- SALSA MLPA probe name in Table 1 and gene name in the sections Exon numbering and Probemix content adjusted for: the NESPAS probe 22363-L22606 (at 283 nt) and GNAS probe 03882-L22603 (at 402 nt), into GNAS-AS1 (probe) and GNAS A/B (probe), respectively.
- Gene name of the probes at 317 nt and 454 nt changed in Table 1 and 2 (from GNAS-XL to GNASXL).

Version C1-01– 27 January 2021 (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).
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the *PLAGL1, GRB10, MEST, SNRPN, PEG3, NESP55, NESPAS, GNASXL* and *GNAS* genes updated according to new version of the NM_ reference sequence.

Version B1-04 – 06 September 2019 (01M)
- 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.
- Catalogue number SALSA HhaI adjusted.

Version B1-03 – 07 June 2019 (01M)
- Percentage methylated and expected signal reduction in normal blood-derived DNA for the 208 nt digestion control probe (19490-L25113) corrected in Table 1.
- Various minor textual changes.

Version B1-02 – 09 May 2019 (01M)
- Version number in Implemented changes corrected.
- Updated NR-sequence for KCNQ1OT1 in Table 2.

Version B1-01 - 07 March 2019 (01M)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Updated the NM sequences for *MEG3* and *SNRPN*.
- Added a warning for potential non-specific peaks in no DNA reactions at 120, 131 and 143 nt.

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