Product Description
SALSA® MLPA® Probemix ME030-D1 BWS/RSS

To be used with the MS-MLPA General Protocol.

Version D1
As compared to version C3, eleven target probes have been replaced by 17 new target probes, ten reference probes have been replaced by eight new reference probes and one digestion control probe has been added. Additionally, five target probes, one reference probe and one digestion control probe have changed only in length, and not in the sequence that they detect. For complete product history see page 13.

Catalogue numbers:
- **ME030-025R:** SALSA MLPA Probemix ME030 BWS/RSS, 25 reactions.
- **ME030-050R:** SALSA MLPA Probemix ME030 BWS/RSS, 50 reactions.
- **ME030-100R:** SALSA MLPA Probemix ME030 BWS/RSS, 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 ME030-D1 probemix, MRC Holland has observed a series of non-specific peaks with predominant lengths at about 163, 172, 175, 181, 188, 209, 249 and 277 nt. These peaks appeared very sporadically and we have not yet been able to establish the cause. However, we found that the amount and height of these peaks is greatly reduced by not spinning down your MLPA reactions in between the ligation and PCR reaction. The non-specific peaks are not expected to influence results. Please notify us if you still regularly observe these peaks: info@mrcholland.com.

General information
The SALSA MLPA Probemix ME030 BWS/RSS is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences of the following differentially methylated regions (DMRs): *KCNQ1OT1*:TSS-DMR (also called IC2), *H19/IGF2*:IG-DMR (also called IC1) in the 11p15 chromosomal region associated with Beckwith-Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS). This probemix also includes probes for the *IGF2*:alt-TSS-DMR in the 11p15 chromosomal region. Additionally, this assay can be used for the detection of aberrant methylation of one or more sequences of the *MEST*:alt-TSS-DMR and *GRB10*:alt-TSS-DMR on chromosome 7 associated with RSS. This probemix can also be used to detect deletions/duplications in the aforementioned chromosomal regions. For better coverage of chromosome 7 and chromosome 14, we recommend to use SALSA MLPA Probemix ME032 UPD7-UPD14.

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).

BWS is a clinically heterogeneous overgrowth syndrome associated with an increased risk for embryonal tumour development. RSS is a genetically heterogeneous disorder involving both intrauterine and postnatal growth retardation. The incidence of both BWS and RSS is estimated to be approximately 1 in 10,000-15,000 newborns and around 85% of the cases are sporadic (Õunap 2016). These conditions are both caused by a

genetic or epigenetic alteration within two domains of imprinted growth regulatory genes on chromosomal region 11p15, leading to deregulated expression of the imprinted genes within this region. Approximately 60-70% of the patients have imprinting abnormalities at one of two imprinted domains IC1 or IC2, and these changes are frequently mosaic (see Figure 1 for a scheme of the imprinted gene cluster). Other known causes of BWS and RSS are uniparental disomy (UPD), trisomy 11p15, mutations in the *CDKN1C* gene, as well as small deletions and translocations. About 10% of RSS cases are caused by maternal UPD for chromosome 7 (Õunap 2016).

This SALSA MLPA Probemix ME030 BWS/RSS is capable of rapidly detecting most causes of BWS and RSS, as both copy numbers and methylation status of the 11p15 region can be determined. This MS-MLPA assay for BWS/RSS can also be useful for screening of childhood cancers, in particular Wilms' tumour. A strong linkage between hypermethylation of the IC1 locus, but not IC2, has been described in these patients resulting in biallelic expression of the *IGF2* gene (Maas et al. 2016).

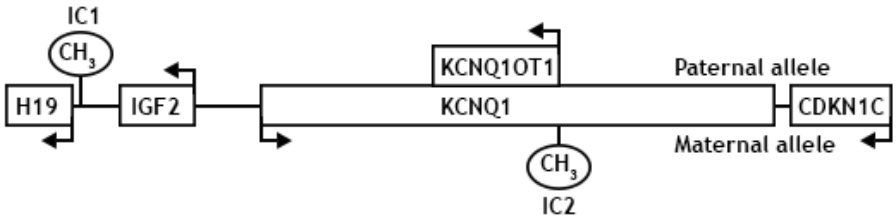

Figure 1. Scheme of the imprinted gene cluster on chromosome 11p15.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1394/ (BWS) and https://www.ncbi.nlm.nih.gov/books/NBK1324/ (RSS).

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/

Exon numbering
The *GRB10, MEST, IGF2, KCNQ1* and *CDKN1C* exon numbering used in this ME030-D1 BWS/RSS product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcripts NM_001350814.2, NM_002402.4, NM_000612.6, NM_000218.3 and NM_001122630.2, respectively, which can be found in Tables 1 and 2. The *IGF2*, *KCNQ1* and *CDKN1C* exon numbering have 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 these genes. The *H19* and *KCNQ1OT1* exon numbering is derived from the NG_016165.1 and NG_016178.2 sequences, respectively.

Probemix content
The SALSA MLPA Probemix ME030-D1 BWS/RSS contains 47 (methylation-specific) MLPA probes with amplification products between 121 and 500 nucleotides (nt). 30 probes are specific for the BWS/RSS 11p15 region, eleven of these are methylation-specific probes that contain an HhaI recognition site and provide information on the methylation status of the BWS/RSS 11p15 region. Furthermore, four methylation-specific probes contain an HhaI recognition site and provide information on the methylation status of the *MEST*:alt-TSS-DMR and *GRB10*:alt-TSS-DMR on chromosome 7. 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 BWS/RSS 11p15 region. 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.

Prenatal samples
MRC Holland has not validated the ME030 probemix for both copy number analysis and methylation analysis on prenatal samples. Therefore, it is not recommended to use such samples with this probemix. The results of methylation-specific probes tested on chorionic villi samples (CVS) and amniotic fluid samples might not reflect the actual epigenetic constitution of the fetus (Beygo et al. 2023, Eggerman et al. 2016 and 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. Paganini et al. also showed that methylation profiles in CVS can vary after culturing in a locus-specific manner. In addition, Gede LB et al. showed that methylation ratios in uncultured amniotic fluid are different and more variable than ratios obtained in blood samples. Furthermore, the uncertainty regarding timing of abnormal methylation and the mosaic nature of the syndromes complicates the interpretation of the results obtained on prenatal samples (Gede LB et al. 2016).

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 BWS/RSS. 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

Methylation-specific probes targeting the same DMR are located close to each other, and are expected to provide similar results in postnatal whole blood samples. We recommend using the median methylation status of these probes to determine the methylation status of each locus and to disregard aberrant methylation detected by a single methylation-specific probe. For the *H19/IGF2*:IG-DMR/ IC1 region, the 309 nt and 246 nt probes have been reported to be more variable and could give deviating results compared to the other three methylation-specific probes in this DMR. Therefore, we recommend to take the median of the 301 nt, 240 nt and 184 nt probes in case the results of these probes are deviating from each other.

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.

ME030 specific notes:

- Please note that one probe has multiple HhaI restriction sites. All of these sites need to be methylated in order to not be digested!
- Due to the high natural variability in methylation levels of the region it is essential to include both test and reference samples in the same experiment. Sample type and DNA extraction method for all samples should be the same.
- Internal data has shown that especially the methylation-specific probes in the *H19/IGF2*:IG-DMR and *IGF2*:alt-TSS-DMR are highly variable in unaffected prenatal samples. Also see section Prenatal samples on page 3.
- Interpretation of the results should only be done by a professional experienced in the field of imprinting disorders, as recommended by Brioude et al (2018).
- The majority of molecular disturbances in RSS and BWS are affected by mosaicism (Brioude et al (2018)), MS-MLPA is not suitable to detect low-level mosaicisms as it will be difficult to distinguish low-level mosaicism from the experimental variability inherent to the MS-MLPA technology. Therefore we cannot provide cut-off values for mosaicism.

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

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

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 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.

BWS and RSS mutation database

https://databases.lovd.nl/shared/diseases/00231 and https://databases.lovd.nl/shared/diseases/00232. 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 *KCNQ1* exons 12 and 16 but not exon 14) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix ME030-D1 BWS/RSS

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	Chromosomal position (hg18)	
				Reference	Target[a,b]
64-105	Control fragments – see table in probemix content section for more information				
121 *	Reference probe 19616-L27455	-		4p	
126 * π	**Digestion control probe** S0750-L21493	+	0%	2q	
131 *	**IGF2 probe** 21089-L29341	+	60%		*IGF2*:alt-TSS-DMR
136 * «	**GRB10 probe** 15742-L31339	+	50%		*GRB10*:alt-TSS-DMR
142 ¥	**KCNQ1OT1 probe** 07173-L29559	+	50%		KCNQ1OT1:TSS-DMR / IC2
148 * ‡	**KCNQ1OT1 probe** 22999-L31883	+	50%		KCNQ1OT1:TSS-DMR / IC2
154 *	Reference probe 14199-L27215	-		2q	
160	**H19 probe** 14731-L01713	-			*H19/IGF2*:IG-DMR / IC1
166 *	**KCNQ1OT1 probe** 23375-L33077	+	50%		*KCNQ1OT1*:TSS-DMR / IC2
172 *	**H19 probe** 21095-L29560	-			Upstream
178 *	Reference probe 18462-L23639	-		6q	
184	**H19 probe** 14063-L08764	+	50%		*H19/IGF2*:IG-DMR / IC1
190	**H19 probe** 16671-L19242	-			*H19/IGF2*:IG-DMR / IC1
196 «	**CDKN1C probe** 06262-L05768	-			Exon 2
202 *	**MEST probe** 21198-L17768	+	50%		*MEST*:alt-TSS-DMR
208	Reference probe 07404-L07051	-		12q	
214	**H19 probe** 10586-L11141	-			*H19/IGF2*:IG-DMR / IC1
221	**KCNQ1 probe** 14791-L16502	-			Intron 1
227	**H19 probe** 16670-L19241	-			Exon 5
232 * «	**GRB10 probe** 21197-L31461	+	50%		*GRB10*:alt-TSS-DMR
240 ¥	**H19 probe** 14792-L29339	+	50%		*H19/IGF2*:IG-DMR / IC1
246 * Δ	**H19 probe** 21933-L31326	+	60%		*H19/IGF2*:IG-DMR / IC1
253 *	Reference probe 16399-L28036	-		17q	
259 *	**MEST probe** 15756-L31316	+	50%		*MEST*:alt-TSS-DMR
265 *	**IGF2 probe** 21091-L31317	+	50%		*IGF2*:alt-TSS-DMR
274	**KCNQ1OT1 probe** 16654-L19204	+	50%		*KCNQ1OT1*:TSS-DMR / IC2
289 ¥	**IGF2 probe** 06272-L31873	-			Exon 3
295 ¥	Reference probe 03262-L31874	-		3q	
301	**H19 probe** 06266-L05772	+	50%		*H19/IGF2*:IG-DMR / IC1
309 * Δ	**H19 probe** 21092-L29344	+	65%		*H19/IGF2*:IG-DMR / IC1
326 ¥	**KCNQ1 probe** 22411-L31599	-			Exon 6
335 *	Reference probe 21110-L29672	-		1q	
351 *	**KCNQ1 probe** 22412-L32002	-			Exon 5
357 ¥ π	**Digestion control probe** 09153-L29245	+	0%	8p	
367 *	**KCNQ1 probe** 21096-L29348	-			Exon 9
376 *	**KCNQ1 probe** 20984-L31278	-			Exon 12
383	Reference probe 00973-L18737	-		10q	
411	**KCNQ1 probe** 03555-L21092	-			Exon 16
427 *	Reference probe 22367-L16599	-		18q	
437 ¥	**KCNQ1 probe** 14732-L31338	-			Intron 1
445 «	**CDKN1C probe** 14734-L20842	-			Exon 4
454	**H19 probe** 10588-L11143	-			*H19/IGF2*:IG-DMR / IC1
463 *	**H19 probe** 23001-L29562	-			*H19/IGF2*:IG-DMR / IC1
472 *	Reference probe 15127-L16898	-		13q	
485 *	**H19 probe** 23044-L32508	-			*H19/IGF2*:IG-DMR / IC1
490 *	**KCNQ1 probe** 21097-L32678	-			Exon 14
500 *	Reference probe 09682-L22509	-		3p	

[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 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.

‡ 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.

Δ More variable and could give deviating results compared to the other three methylation-specific probes in the *H19/IGF2*:IG-DMR.

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. ME030-D1 target probes arranged according to chromosomal location

Table 2a. Chromosome 7

Length (nt)	SALSA MLPA probe	Gene/Exon[a]/ DMR[b]	HhaI site	Ligation site [a]	MV location (Hg18)	Imprinted allele	Distance to next probe
		GRB10		**NM_001350814.2**			
232 «	21197-L31461	*GRB10*:alt-TSS-DMR	+	5 nt after exon 1	07-050.817578	Tissue specific	0.7 kb
136 «	15742-L31339	*GRB10*:alt-TSS-DMR	+	175 nt before exon 1	07-050.818232	Tissue specific	79.1 **M**b
		MEST		**NM_002402.4**			
259	15756-L31316	*MEST*:alt-TSS-DMR	+	550 nt before exon 1	07-129.918586	Maternal	0.8 kb
202	21198-L17768	*MEST*:alt-TSS-DMR	+	231-232 exon 1	07-129.919370	Maternal	

Table 2b. Chromosome 11

Length (nt)	SALSA MLPA probe	Gene/Exon[a]/ DMR[b]	HhaI site	Ligation site [a]	MV location (Hg18)	Imprinted allele	Distance to next probe
		H19		**NR_002196.2**			
227	16670-L19241	Exon 5	-	1879-1880	11-001.973411		2.4 kb
160	14731-L01713	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	138 nt before exon 1	11-001.975788		0.2 kb
301	06266-L05772	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	+	302 nt before exon 1	11-001.975956	Paternal	0.1 kb
240	14792-L29339	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	+	447 nt before exon 1 reverse	11-001.976099	Paternal	0.2 kb
184	14063-L08764	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	+	617 nt before exon 1	11-001.976269	Paternal	1.5 kb
485	23044-L32508	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	2.1 kb before exon 1, reverse	11-001.977799		0.9 kb
172	21095-L29560	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	3.0 kb before exon 1	11-001.978662		0.2 kb
454	10588-L11143	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	3.2 kb before exon 1	11-001.978896		0.1 kb
309 Δ	21092-L29344	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	+	3.4 kb before exon 1	11-001.979042	Paternal	0.4 kb

214	10586-L11141	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	3.8 kb before exon 1	11-001.979409		0.1 kb
463	23001-L29562	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	3.9 kb before exon 1, reverse	11-001.979556		1.3 kb
246 Δ	21933-L31326	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	+	5.2 kb before exon 1, reverse	11-001.980856	Paternal	1.5 kb
190	16671-L19242	*H19/IGF2*:IG-DMR / IC1 (H19DMR)	-	6.7 kb before exon 1, reverse	11-001.982389		129.0 kb
		IGF2		**NM_000612.6**			
289	06272-L31873	Exon 3 (8)	-	1378-1377, reverse	11-002.111396		14.0 kb
265	21091-L31317	*IGF2*:alt-TSS-DMR	+	8.3 kb before exon 1	11-002.125418	Paternal	0.8 kb
131	21089-L29341	*IGF2*:alt-TSS-DMR	+	9.1 kb before exon 1	11-002.126250	Paternal	313.2 kb
		KCNQ1		**NM_000218.3**			
437	14732-L31338	Intron 1 (Exon 2)	-	16.2 kb after exon 1 (NM_181798.2; 212-213)	11-002.439436		66.1 kb
221	14791-L16502	Intron 1 (Intron 2)	-	137 nt before exon 2	11-002.505564		44.2 kb
351	22412-L32002	Exon 5	-	800-801	11-002.549808		0.9 kb
326	22411-L31599	Exon 6 (7)	-	914-915	11-002.550659		14.8 kb
367	21096-L29348	Exon 9	-	1302-1301, reverse	11-002.565428		111.6 kb
		KCNQ1OT1		**NR_002728.4**			
166	23375-L33077	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	748-747 exon 1, reverse	11-002.677031	Maternal	0.1 kb
274	16654-L19204	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	660-659 exon 1, reverse	11-002.677116	Maternal	0.5 kb
148 ‡	22999-L31883	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	174-175 exon 1	11-002.677596	Maternal	0.4 kb
142	07173-L29559	*KCNQ1OT1*:TSS-DMR / IC2 (KvDMR)	+	195 nt before exon 1, reverse	11-002.677971	Maternal	68.7 kb
		KCNQ1		**NM_000218.3**			
376	20984-L31278	Exon 12	-	1652-1653	11-002.746662		8.1 kb
490	21097-L32678	Exon 14	-	5 nt after exon 14	11-002.754809		71.8 kb
411	03555-L21092	Exon 16 (17)	-	2908-2909	11-002.826573		35.0 kb
		CDKN1C		**NM_001122630.2**			
445 «	14734-L20842	Exon 4 (3)	-	1159-1160	11-002.861603		1.6 kb
196 «	06262-L05768	Exon 2 (1)	-	154-155	11-002.863233		

[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 and could give deviating results compared to the other three methylation-specific probes in the *H19/IGF2*:IG-DMR.

« 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.

‡ 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 ME030-D1 probes

Length (nt)	Methylation-specific probe	Partial sequence with HhaI site
126	S0750-L21493	GGACACTTTGGA-ATTCCATTGGCATTGGGGTGTCTTTGTTAAGTGCCTGGCCAGTAGCGCTG
131	21089-L29341	CCTAGGTTTAGCTTCTCTCCTCCTGATGGCG-CACCTGGTCCCCCTTGCTGCTCTCCCAGCCT
136	15742-L31339	CGGAGGGCTTTCGGCATCGTCAGAGT-GGCCAGTGTGCGCGTCCTTGCCCATCAGGCG
142	07173-L29559	AGAGTCTCGTTTTGATGCCACCCGGGCTCAGAT-TGGCCCAGCGGGTCCAGCGCCGCATGAG
148 ‡	22999-L31883	CCCTCAGCGCGGTCCTCCTCGGTGCGTCA-GTCATCGTGGTTCTCCCCGGCGCGCCCCTCG
166	23375-L33077	GTCGACCCTGGCCACGCTGTCCATAAGGT-GCAGATGGGAGCGCACTGCCCAGGCCAGG
184	14063-L08764	GTAGAGTGCGCCCGCGAGCCGTA-AGCACAGCCCGGCAACATGCGGTCTTCAGAGT
202	21198-L17768	GCGGCATGGGATAACGCGGCCAT-GGTGCGCCGAGATCGCCTCCGCAGGTGAGTGT
232	21197-L31461	TCCAGTGCTGCCGCCCGCTCTCCAGGTACT-CAGGTGGGCTCCGCCGCGGGCGCTGGGCGGT
240	14792-L29339	GTGCTGAGGGGCAGAGGGAAGTGCCGCAA-ACCCCCTGGTGGGCGCGGTGCCAGCCCCCCA
246	21933-L31326	CACGTACAGCCGATTCTGCGCCATCAGGGCAG-TGAGACGGGCCTCCCTTGGTGAGGTCAGC
259	15756-L31316	TCCCTAGCCGCTTGCTCGTGCCCTT-GGTGGTTACCGGTAGTTAAGCTTAGGGCGCATAGGGC
265	21091-L31317	CCAGTCCTGAGGTGAGCTGCTGTGGCCTGT-GGCCCAGGCGACCCCAGCGCTCCCAGAACTG
274	16654-L19204	GCGGGGCACACAGCTCACCTCAGCAA-CGCCAGTGATCACCCGTCCCGCGCCGTCCGC
301	06266-L05772	CGGCCCCCAGCCATGTGCAAAGTA-TGTGCAGGGCGCTGGCAGGCAGGGAGCA
309	21092-L29344	AGGGATCCTGGGAGCCTGCCAAGCAG-AGCGCACAGTGTTCCTGGAGTCTCGCTGCCCAGA
357	09153-L29245	CCTCCTCCTAGCCTGGCGCGCGATT-ATTTGAAGACGCTCACGGAGCGGCTGGCTAGGCTGA

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.

‡ 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.

Interpretation of copy number and methylation ratio results

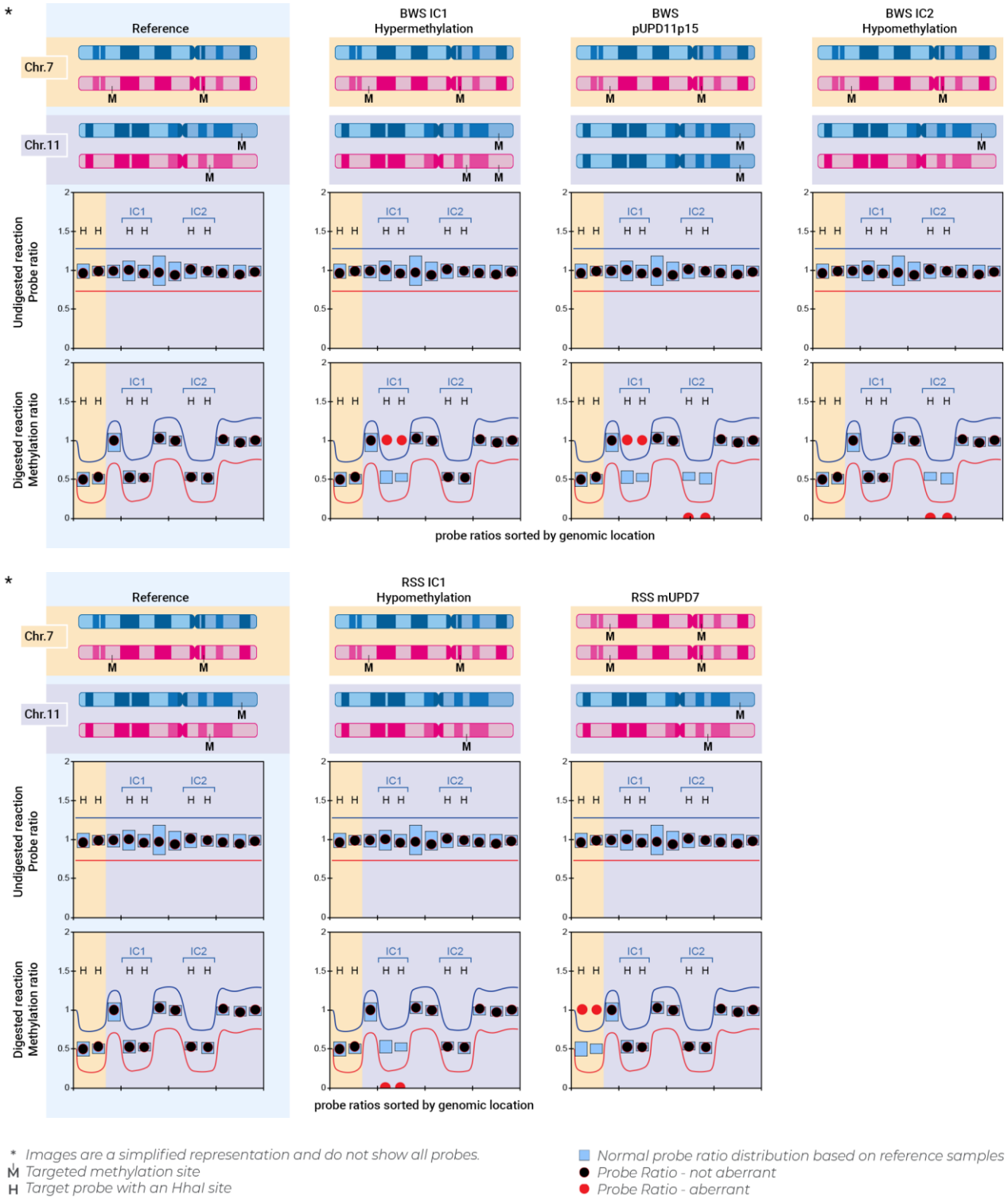

Figure 2. Simplified schematic representation of results that may be obtained with BWS/RSS samples. Images do not show all probes. For simplification gain of methylation (GOM) and loss of methylation (LOM) are represented as ratios of approximately 1 and 0, in practice the majority of molecular disturbances in RSS and BWS are affected by mosaicism. In BWS IC1 hypermethylation causes approximately 5% of cases, IC2 hypomethylation 50% and paternal UPD (pUPD) 20% (as reported by www.ncbi.nlm.nih.gov/books/NBK1394/). In RSS IC1 hypomethylation causes ~35-50% of cases and maternal UPD (mUPD) ~7-10% (as reported by www.ncbi.nlm.nih.gov/books/NBK1324/). N.B. ME030 cannot distinguish between BWS or RSS caused by UPD or imprinting defects.

Related SALSA MLPA probemixes

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

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ME030 product history	
Version	*Modification*
D1	Eleven target probes replaced by 17 new target probes, ten reference probes replaced by eight new reference probes, one digestion control added, five target probes, one reference probe and one digestion control probes changed in length, not in sequence detected.
C3	All probes are identical to lot C1-0711. However, the formation of a secondary structure within the probe oligonucleotides has been reduced.
C2	*KCNQ1OT1* probe at 138 nt has been elongated by 2 nt.
C1	Three probes for *H19* and two for *KCNQ1*, several reference probes, the digestion control probe and the 88 and 96 nt D-fragments have been replaced. One *H19* probe has been removed and one *CDKN1C* probe has been added. For the *NSD1* gene, two probes have been included.
B2	Two extra control fragments at 100 and 105 nt added.
B1	One *H19* probe and one reference probe have been replaced.
A1	First release.

Implemented changes in the product description

Version D1-06 – 18 February 2026 (04M)
- Exon information updated in Table 1 and 2 for KCNQ1 probes at 221 and 437 nt.

Version D1-05 – 08 December 2025 (04M)
- Removed section 'Selected Publications using SALSA MLPA Probemix ME030 BWS/RSS' as it is present in the Product Page.
- Adjusted reference Eggerman et al. 2016 in text to correct year of publication.

Version D1-04 – 22 May 2025 (04M)
- Probe warnings for probes at 246 and 309 nt below Tables 1 and 2 added.
- Rephrased General Information.
- Updated section on: Prenatal samples, Detection of mosaicism and Methylation analysis of the H19/IGF2:IG-DMR.
- Related SALSA MLPA products section replaced with reference to product page on website.
- Small textual changes.

Version D1-03 – 03 December 2024 (04M)
- Small change of two probe lengths in Table 1, 2 and 3 in order to better reflect the true lengths of the amplification product, for the probes at 131 and 227 nt.

Version D1-02 – 18 November 2024 (04M)
- Small change of one probe length in Table 1 and 2 in order to better reflect the true length of the amplification product, for the probe at 437 nt.
- Additional information regarding testing of prenatal samples added on page 3.
- ME030 specific note regarding methylation-specific probes in the *H19/IGF2*:IG-DMR and *IGF2*:alt-TSS-DMR in prenatal samples added on page 5.
- Information on DMR nomenclature added below Table 1 and 2.
- Probemixes P026 Sotos and ME034 Multi-locus Imprinting added to related products.
- List of References updated.

Version D1-01 – 07 May 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.
- Exon numbering of the *IGF2, KCNQ1* and *CDKN1C* genes has been changed, old exon numbering is between brackets in Table 2. All probes that are in a DMR are now indicated as such in Table 1 and 2.
- 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.

- Ligation sites of the probes targeting the *KCNQ1OT1* and *CDKN1C* genes updated according to new version of the NM_ reference sequence.
- Section on interpretation of copy number and methylation ratio results completely rewritten.
- Related SALSA MLPA probemixes updated.

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