MRC Holland

SALSA® MS-MLPA

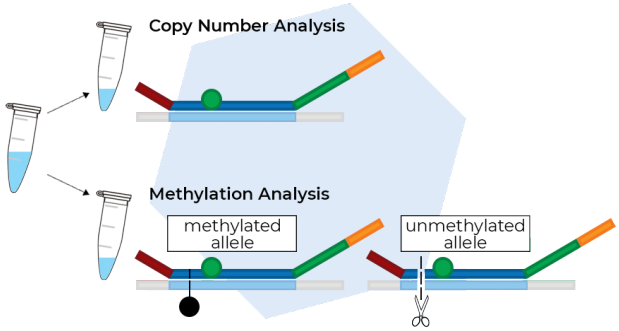
A Simple Solution for Copy Number Determination & Methylation Profiling

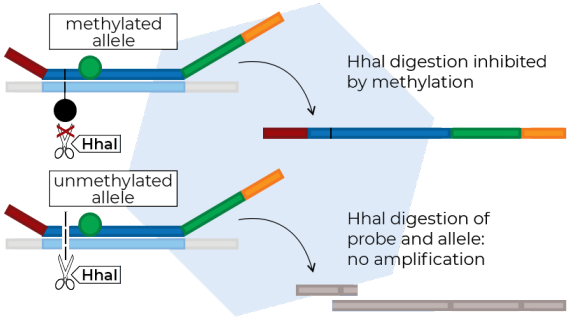
Methylation-Specific MLPA

Methylation-Specific MLPA (MS-MLPA) is a variant of the SALSA® MLPA® technique, the gold standard in copy number determination. By combining the MLPA technique with the use of the methylation-sensitive endonuclease HhaI, it is possible to detect both DNA copy number and methylation status without bisulfite treatment. With MS-MLPA, semi-quantitative methylation profiling of multiple targets is accomplished simultaneously and with ease.

The Principle of MS-MLPA

MLPA probes hybridise to their target sequences on the sample DNA. The MLPA reaction is split in two: an undigested reaction for copy number determination, and a digested reaction for methylation profiling. In the digested reaction, the HhaI enzyme is used to digest unmethylated double-stranded probe-DNA complexes.





Complexes with methylated sample DNA are not digested by HhaI. Digested probes cannot be amplified exponentially, and therefore produce no signal, while non-digested probes generate a normal signal. Methylation is displayed as the percentage of the targeted DNA sequence that was methylated in the sample.

Methylation-Specific Probemixes

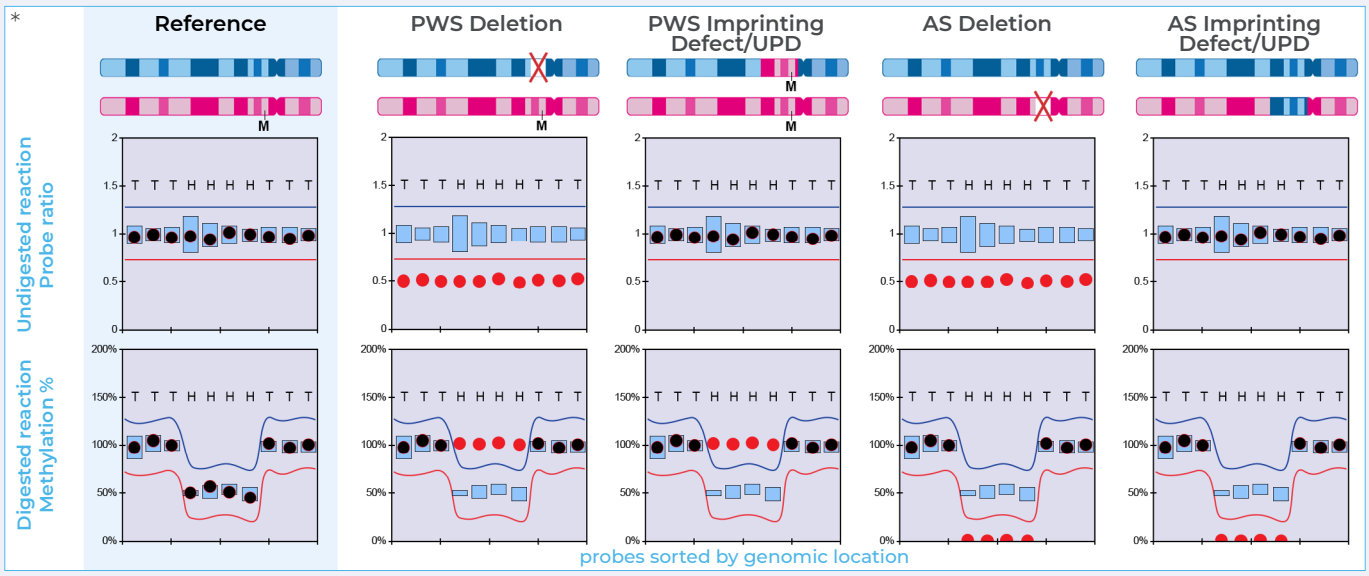
SALSA MLPA Probemix	Application
ME001 and ME002 Tumour suppressor mix 1 and 2	Methylation profiling for 25 tumour suppressor genes (TSG).
ME011 Mismatch Repair Genes	Mismatch repair genes methylation and associated Lynch syndrome genome changes profiling.
ME012 MGMT-IDH1-IDH2	Targeted glioma profiling including *MGMT* methylation and *IDH1/2* mutation detection.
ME024 9p21 CDKN2A/2B region	Cell cycle regulator profiling on 9p21 region associated with multiple tumour types including melanoma.
ME028 Prader-Willi/Angelman	Imprinting disorder profiling for Prader-Willi and Angelman syndromes.
ME029 FMR1/AFF2	Fragile X syndrome associated promoter methylation profiling for male samples.
ME030 BWS/RSS	Imprinting disorder profiling for Beckwith-Wiedemann and Russell-Silver syndromes.
ME031 GNAS	Imprinting disorder profiling for Albright hereditary osteodystrophy and pseudohypoparathyroidism.
ME032 UPD7-UPD14	Imprinting disorder profiling for UPD7, RSS, UPD14 (Temple & Kagami-Ogata syndromes).
ME033 TNDM	Imprinting disorder profiling for Transient Neonatal Diabetes Mellitus (TNDM).
ME034 Multi-locus Imprinting	Multi-locus imprinting disturbance profiling and to distinguish maternal and paternal triploidies.
ME042 CIMP	CpG Island Methylator Phenotype profiling (CIMP).

Application Highlight: Imprinting Disorders

MS-MLPA is frequently used to detect imprinting region methylation, like the Prader-Willi/Angelman (ME028 PWS/AS) and Beckwith-Wiedemann/Russell-Silver (ME030 BWS/RSS) syndromes critical regions. These regions are known to have imprinting defects, such as those caused by uniparental disomy (UPD). MS-MLPA gives reliable results for both copy number and methylation status in the regions of interest.

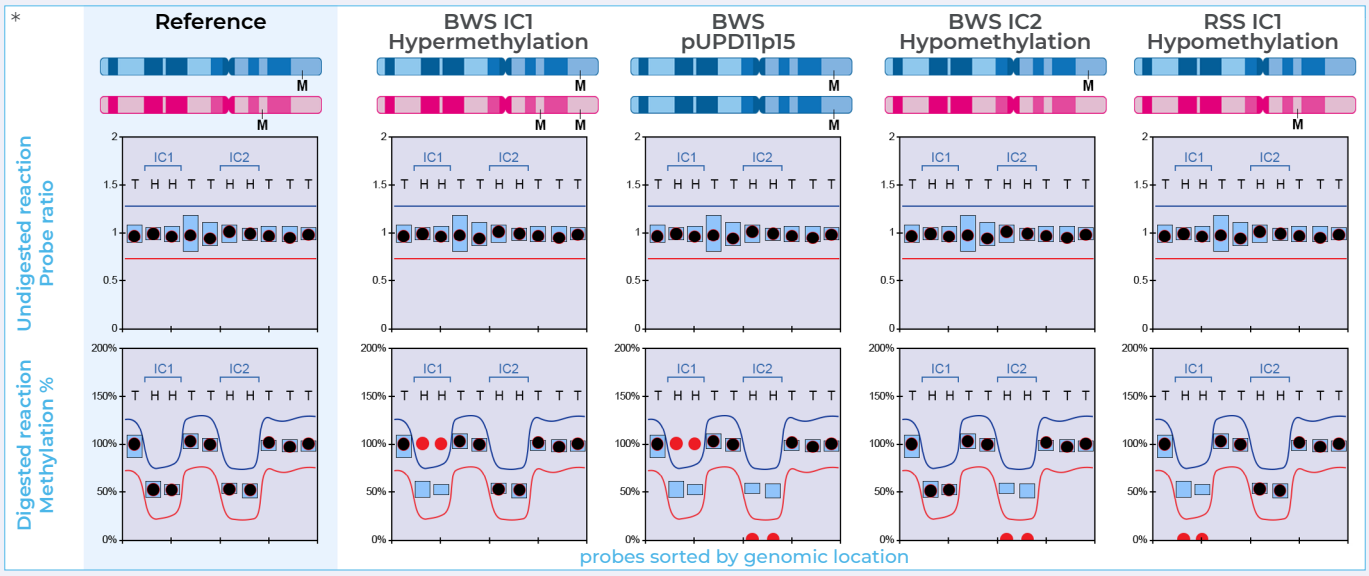
ME028 – PWS/AS Copy Number & Methylation Profiling

ME028 contains over 40 probes targeting the Prader-Willi/Angelman critical region, covering *SNRPN* and *MAGEL2* to establish DNA copy number. In addition, five dedicated methylation-sensitive probes enable methylation profiling of the region.+



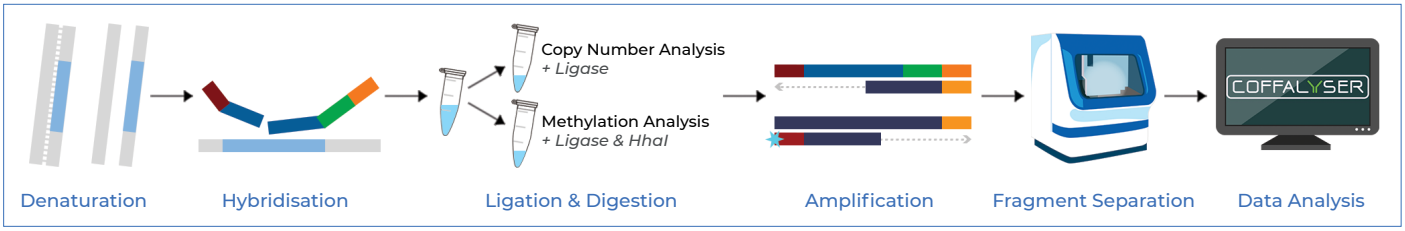
ME030 – BWS/RSS Copy Number & Methylation Profiling

ME030 contains over 40 probes that target the 11p15 Beckwith-Wiedemann/Russell-Silver critical region that are used for copy number determination. Methylation status of the region is determined by eight methylation-sensitive probes that detect sequences in the H19DMR/IC1 and KvDMR/IC2 domains.+



+ Sample results must be compared to results obtained on DNA samples from unaffected individuals.
** Images are an abridged representation of the target probe results for copy number and methylation profiling.*
***T**: target probe; **H**: target probe with an HhaI site; **M**: targeted methylation site*

SALSA® MS-MLPA Protocol



1. Sample DNA denaturation

   Sample DNA is heated to fully denature the DNA.

2. Hybridisation of probes to sample DNA

   SALSA MLPA Buffer and a SALSA MLPA Probemix consisting of up to 60 probes are added to the sample.

3. Ligation & digestion of hybridised probes

   At this step, your reaction is split in two. In both tubes, hybridised probes are ligated by adding SALSA Ligase-65 enzyme and SALSA Ligase Buffers to form fully amplifiable probes. HhaI enzyme is added to one tube. This results in the digestion of non-methylated probe/allele hybrids.

4. PCR amplification of ligated probes

   Ligated complete probes are amplified by adding SALSA Polymerase and a single fluorescently-labelled primer pair.

5. Fragment separation by capillary electrophoresis

   MLPA PCR products are loaded directly onto a capillary electrophoresis device.

6. Data analysis by Coffalyser.Net™

Coffalyser.Net™: SALSA® MS-MLPA Analysis

Coffalyser.Net™ is **free** MLPA/MS-MLPA data analysis software designed and supported by MRC Holland.

- User-friendly software for reliable MLPA and MS-MLPA data analysis.
- Extensive quality control developed specifically for MLPA and MS-MLPA data.
- Immediate access to the latest analysis panels (Coffalyser sheets).
- Server-client model that allows data sharing.

MRC Holland

Confidence in Copy Number Determination

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