digitalMLPA: a new frontier in the simultaneous determination of copy number and methylation status across multiple imprinting-associated differentially methylated regions

MRC Holland RESEARCH

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INTRODUCTION

Multi-locus imprinting disturbances (MLIDs) involve aberrant methylation patterns at multiple loci, which complicates the diagnosis and management for a subset of imprinting disorder patients. To address the current difficulties in studying MLIDs, we developed SALSA® digitalMLPA™ Probemix DM009 Multi-locus Imprinting Disturbances (test version), a new methylation-specific SALSA® digitalMLPA™ (MS-digitalMLPA) test probemix for methylation and copy number (CN) detection in the regions outlined in ***Figure 1***.

The aim of this study is to compare the reliability of CN and methylation status detection of MS-digitalMLPA with a well-established technique, methylation-specific SALSA® MLPA® (MS-MLPA), across multiple loci. Additionally, the increased probe capacity of MS-digitalMLPA enables targeting more MLID regions per assay compared to MS-MLPA

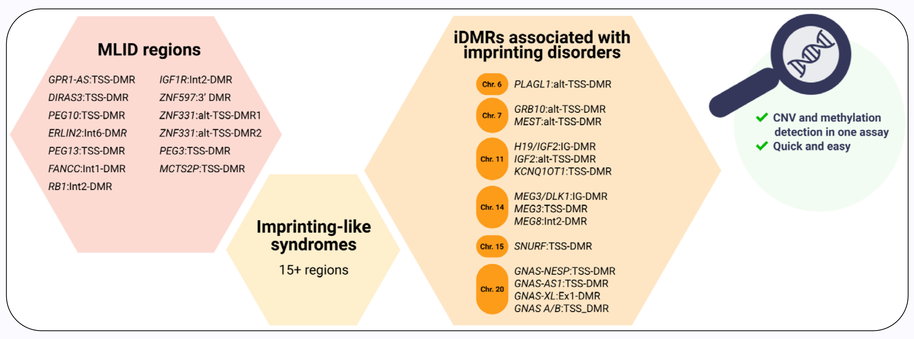

Figure 1. ***Regions targeted by the MS-digitalMLPA test probemix.***
This test probemix targets 27 iDMRs, of which some are associated with imprinting disorders. It also targets over 15 regions associated with imprinting-like disorders. Additionally, it contains probes that target stable regions across most autosomes.

MATERIALS AND METHODS

MS-digitalMLPA is a variant of SALSA® digitalMLPA™. Combining digitalMLPA with the methylation-sensitive endonuclease HhaI allows for the detection of both DNA CN and methylation status (***Figure 2***).

Several samples have been analysed with the DM009 Multi-locus Imprinting Disturbances test probemix:

- 126 control blood samples from healthy individuals
- Over 30 positive samples (blood and cell lines)
  - A subset was also tested with MS-MLPA probemixes (ME030 BWS/RSS and ME034 Multi-locus Imprinting).
- Part of the iDMRs were tested with over 1200 dried blood spots (DBS) card-derived DNA samples from healthy newborns.

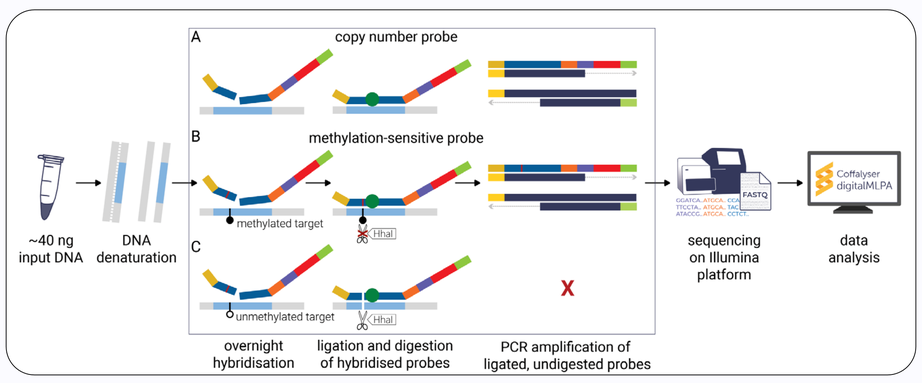

Figure 2. ***MS-digitalMLPA method.***
Copy number probes do not contain a HhaI restriction site (GCGC) in their target sequence and will not be digested ***(A)****. Methylation-specific probes have a HhaI restriction site in their target sequence. Depending on the presence* ***(B)*** *or absence* ***(C)*** *of methylation, the DNA-bound probe is protected from digestion or not, respectively. Undigested probes* ***(A & B)*** *are amplified by PCR, followed by sequencing of the PCR products using an Illumina platform.*

CONCLUSIONS

- SALSA® digitalMLPA™ Probemix DM009 Multi-locus Imprinting Disturbances (test version) can determine the methylation status of each targeted iDMR, as observed in the 126 control samples.
- Our results show that MS-digitalMLPA, like MS-MLPA, can accurately determine both copy number and methylation status for a positive sample with a known methylation aberration in the 11p15.5 region.
- This study underscores the potential of MS-digitalMLPA for detecting methylation aberrations across multiple loci in a single assay.
- Further testing, including additional positive DNA samples, is needed.

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RESULTS

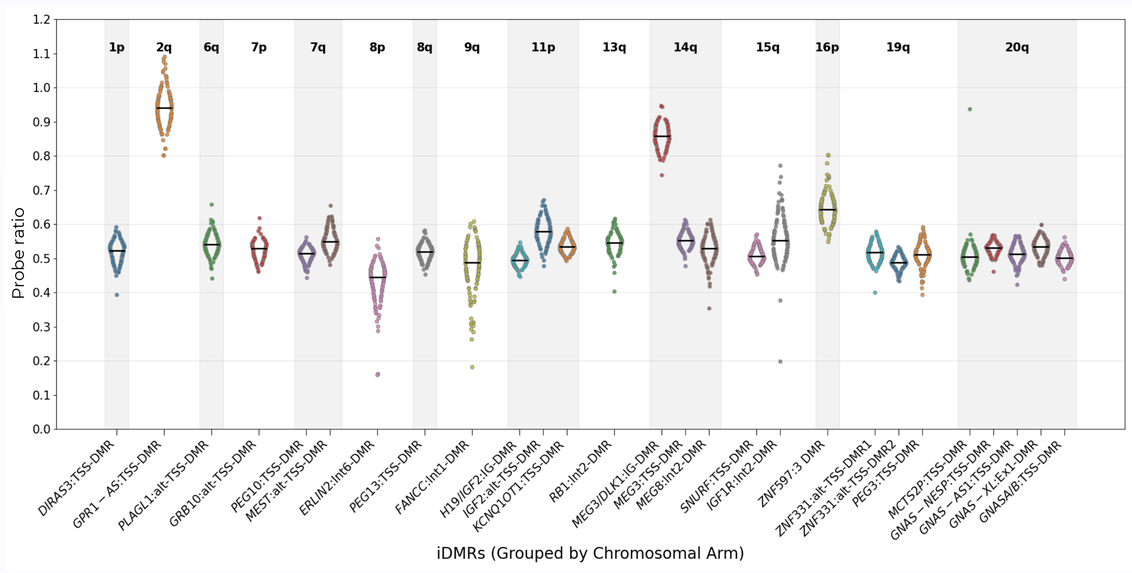

Figure 3. ***Median methylation ratios on 126 blood samples of healthy individuals.***
Ratio chart showing the median of all methylation-specific probe ratios for each iDMR in the 126 samples tested (black horizontal line). Each data point represents the median of the normalised ratios obtained for all methylation-specific probes targeting one iDMR in a HhaI digested sample.

First, the median methylation ratio variation per iDMR (27 iDMRs in total) in healthy individuals was investigated by analysing the ratios obtained for all methylation-specific probes per iDMR in 126 samples (***Figure 3***). Most iDMRs showed median methylation ratios between 0.45-0.64. Two iDMRs showed higher methylation ratios (0.86 and 0.94), concordant with what has been found in literature for those imprinting regions (Rosenski et al. 2024).

Stable data was observed when testing a few iDMRs with DBS cards, highlighting the versatility of MS-digitalMLPA for use with multiple blood-derived sample types.

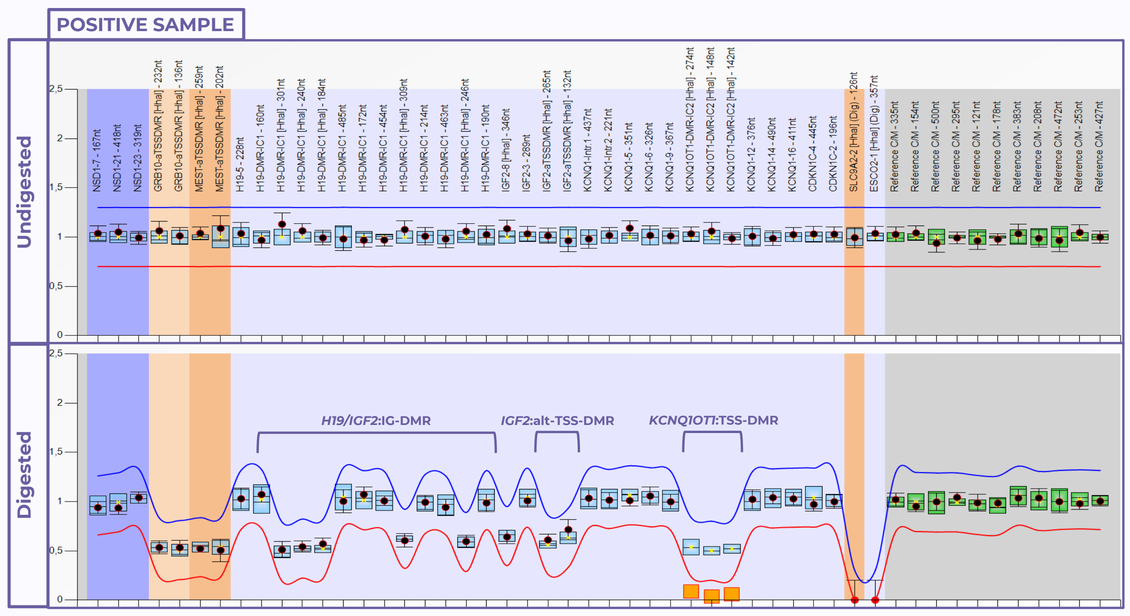

Figure 4. ***Results of the MS-MLPA analysis on the positive sample.***
Ratio chart indicating the copy number and methylation status of a sample with previously identified methylation aberrations at 11p15.5, as analysed with ME030 BWS/RSS. The ratios of each probe in the undigested reaction ***(top chart)*** *and digested reaction* ***(bottom chart)*** *are displayed.*

Next, CN and methylation status analysis was performed with both ME030 BWS/RSS (positive sample) (***Figure 4***) and the DM009 Multi-locus Imprinting Disturbances test probemix (control and positive sample) (***Figure 5***). Both experiments showed the same findings:

- *H19/IGF2*:IG-DMR shows normal methylation frequencies.
- *KCNQ1OT1*:TSS-DMR shows a clear loss of methylation (LOM).

These results were further corroborated with ME034 Multi-locus Imprinting (data not shown). Furthermore, the analysis with the DM009 Multi-locus Imprinting Disturbances test probemix revealed aberrations in an additional iDMR, namely the *FANCC*:Int1-DMR (***Figure 5***).

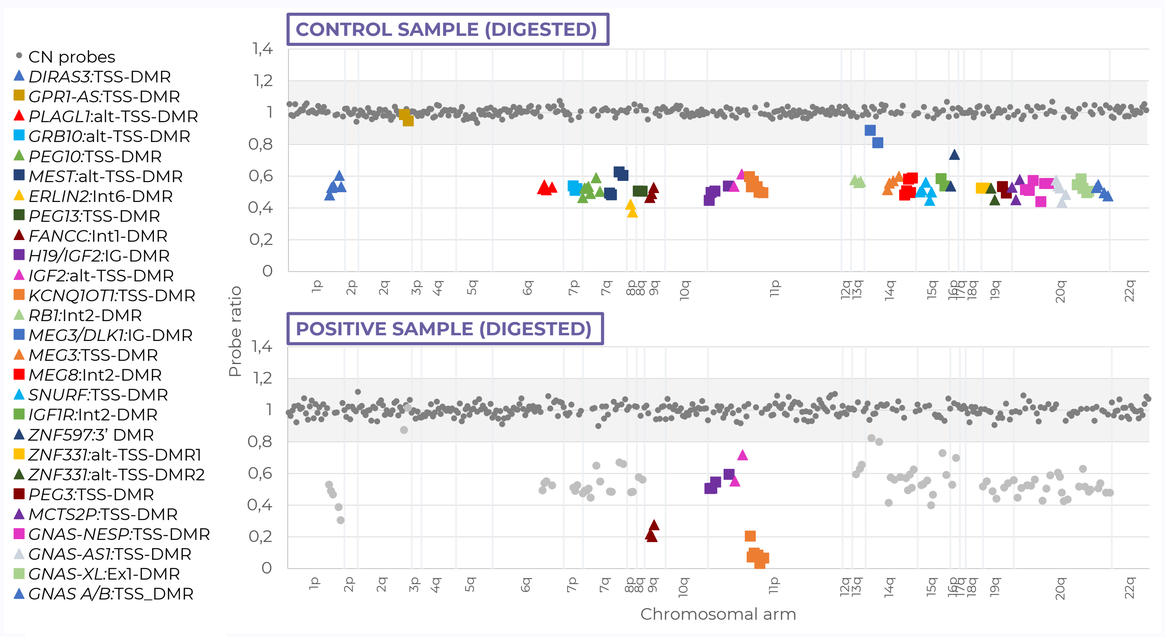

Figure 5. ***Results of the MS-digitalMLPA analysis on a control sample and a positive sample.***
Ratio chart showing the results obtained with the DM009 Multi-locus Imprinting Disturbances test probemix for one digested control sample ***(top)*** *and one digested positive sample with known methylation aberrations at 11p15.5* ***(bottom)****. The range of probe ratios that indicate a normal CN for CN probes, for this method, is shown in gray.*

References
1. Rosenski J et al. bioRxiv. 2024; https://doi.org/10.1101/2024.05.01.591988.

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