Simultaneous copy number and methylation analysis using methylation-specific digitalMLPA in congenital abnormalities and intellectual disability

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Introduction

In the general population, the prevalence of intellectual disability (ID) is estimated at 1-3% and that of congenital abnormalities (CA) at 2-4%, which can be caused by many genetic and epigenetic defects (***Figure 1***). When no distinct syndromic features are displayed, finding the (epi)genetic cause often proves to be challenging.

Nowadays the copy number (CN) detection and methylation analysis are often performed separately or only on limited regions. Testing a broad range of regions and imprinting-associated differentially methylated regions (iDMRs) would therefore be a useful contribution to elucidate the genetic causes of CA and ID. **SALSA® digitalMLPA™ Probemix DM025 Congenital Anomalies** is an assay in development that can simultaneously determine CN and methylation status of these regions (***Figure 1***).

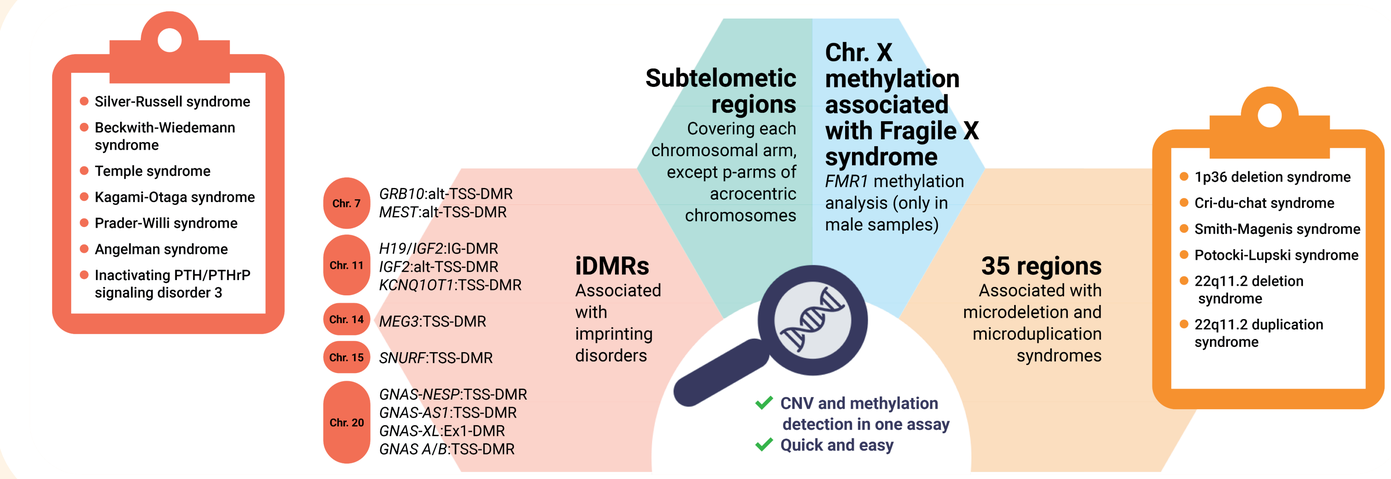


Figure 1. **DM025 Congenital Anomalies content.** The iDMRs targeted by this probemix are listed per chromosome, as well as the imprinting disorders associated with these regions (left). Examples of microdeletion and microduplication syndromes associated with the regions targeted by this probemix are also provided (right).

Materials and Methods

SALSA® digitalMLPA™ Probemix DM025 Congenital Anomalies is a methylation-specific digitalMLPA probemix currently in development by MRC Holland. Methylation-Specific digitalMLPA (MS-digitalMLPA) is a variant of SALSA® digitalMLPA™. Combining digitalMLPA with the methylation-sensitive endonuclease HhaI allows for the detection of both DNA copy number and methylation status (***Figure 2***).

This assay was tested with 136 control samples and 10 positive samples. Data normalisation was performed using at least three undigested reference samples.

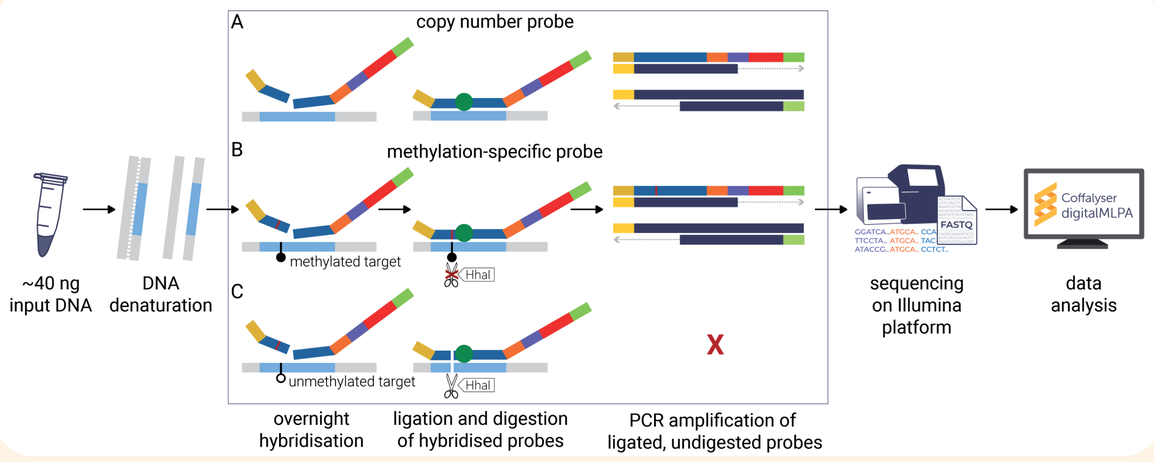


Figure 2. **MS-digitalMLPA steps. A**) CN probes do not contain a HhaI restriction site (GCGC) in their target sequence. **B** and **C**) Methylation-specific probes have a *HhaI* restriction site in their target sequence. MS probes are digested by *HhaI* when their target is not methylated.

Methylation detection in samples of healthy individuals

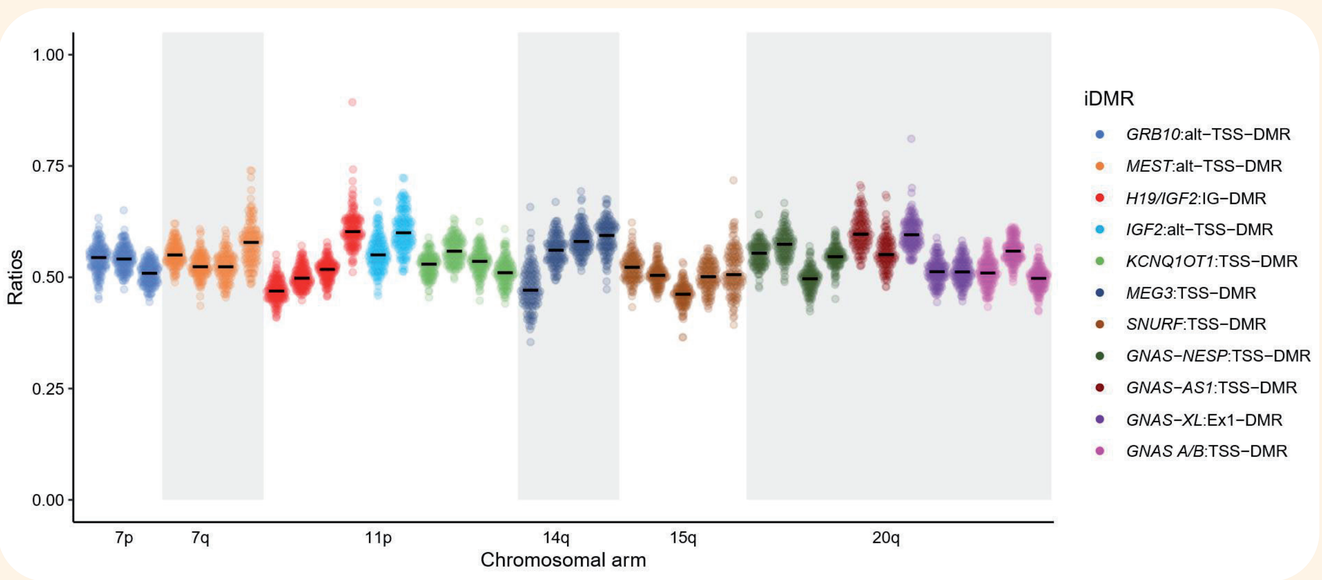


Figure 3. **Methylation results on 136 samples of healthy individuals.** Ratio chart showing the digested probe ratios for all methylation-specific probes in the 136 samples tested. Each data point represents the normalised ratio obtained for a probe in a sample. Results per probe are shown, with colours indicating the different iDMRs. The summary of the data is shown as a horizontal line indicating the median.

Methylation-specific probes targeting imprinting regions were analysed in 136 samples to investigate the **methylation level variation in healthy individuals** (***Figure 3***).

Probes showed a median methylation status of 46-60% per probe. The expected CN was found (data not shown).

Positive samples

Six Coriell samples with known deletions and duplications in the regions of interest were analysed and the expected CNVs in all samples were found (***Figure 4***).

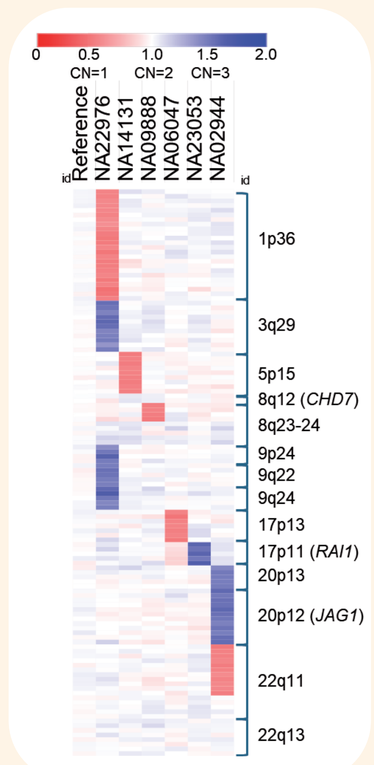
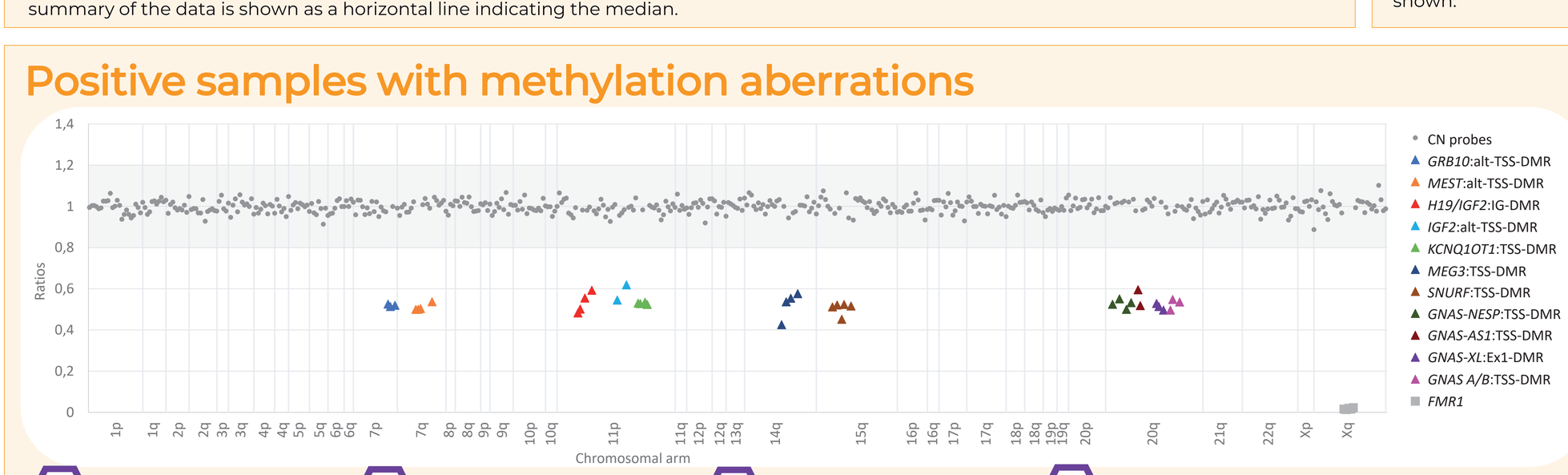


Figure 4. **Copy number variations in selected regions.** Heat map displaying inter ratios obtained using Coffalyser digitalMLPA™ indicating CN status of 6 Coriell samples with known genotypes. A subset of the regions targeted with DM025 Congenital Anomalies is shown.

Positive samples with methylation aberrations



CN and methylation status were accurately identified for four positive samples with methylation aberrations (***Figure 5***):

1. CN=2. *H19/IGF2*:IG-DMR and *IGF2*:alt-TSS-DMR show loss of methylation (LOM) while *KCNQ1OT1*:TSS-DMR methylation is ~50%.
2. CN=2. *H19/IGF2*:IG-DMR and *IGF2*:alt-TSS-DMR show gain of methylation (GOM) while *KCNQ1OT1*:TSS-DMR shows LOM.
3. CN=2. *SNURF*:TSS-DMR methylation is ~0%.
4. CN=2. *SNURF*:TSS-DMR methylation is ~100%.

Figure 5. **Samples positive for imprinting disorders.** Ratio charts showing the results of one digested control sample (top) and four samples positive for imprinting disorders (bottom). The range of accepted ratios for CN probes is shown in gray. Results of all samples are normalised over undigested control samples. Results were confirmed with methylation-specific SALSA® MLPA® probemixes (ME030 BWS/RSS and ME028 Prader-Willi/Angelman).

For further information, please contact
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digitalMLPA is for research use only. Not for use in diagnostic procedures.

The product described concerns a test version that is not available for general purchase.

Conclusions

- SALSA® digitalMLPA™ Probemix DM025 Congenital Anomalies provides a quick and easy solution to reliably detect CNVs and methylation status in a single assay.
- This assay identified the methylation status in control and positive samples and confirmed deletions and duplications in positive samples with known aberrations (Coriell).
- Further testing including additional positive DNA samples is needed.

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