A methyl-seq tool to capture genomic imprinted loci

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Comparison of 2 targeted capture-based methylation sequencing technologies

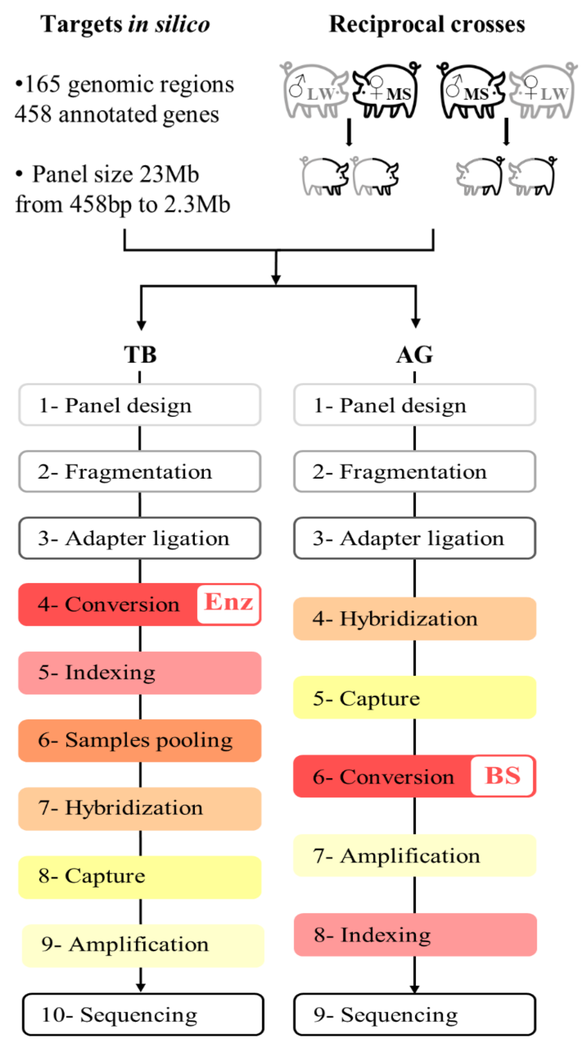
- **Standard bisulfite**-based method (Agilent®™ (AG)) *vs.* **novel enzymatic**-based method (Twist Bioscience ®™ (TB)) (Table 1)
- Evaluation of **165 candidate genomic imprinted regions in pigs** using reciprocal crosses (Figure 1)
- **Efficiency and homogeneity of panels comparable** between AG and TB reaching excellent levels (Figure 2a-b)
- **Specificity of panels more favorable in TB**, with much less off-target capture than in AG (Figure 2c)
- **Better capture of GC-rich regions with TB technology**, including CpG islands, independently of region size (Figure 2d)

	TB	AG
Input DNA	50 ng-200 ng	1 µg
Conversion	Enzymatic	Chemical
Methylation step	Pre-capture	Post-capture
Hybridization probes	dsDNA methylated	RNA
Librairies	Pooling of 8	Individual
Indexing	384	96
Probes number	170,792	314,240
Panel designed	20,488,283 bp (87%)	19,687,287 bp (84%)

Table 1: Main differences between the tested technologies
The AG technology and protocol correspond to the widely used AG SureSelect Custom DNA Target Enrichment Probes. The TB technology and protocols correspond to the novel Twist NGS Bioscience Methylation Detection System. TB=Twist Bioscience, AG=Agilent.

The strategy is applicable to any other species with its own custom capture

Figure 1: Schematic overview of the strategy, including the selection of 165 candidate regions for genomic imprinting in the pig based on knowledge from humans and mice, the use of a reciprocal cross (n=8) to ensure the determination of parental inheritance and the tested technologies, Twist Bioscience (TB) *vs.* Agilent (AG).



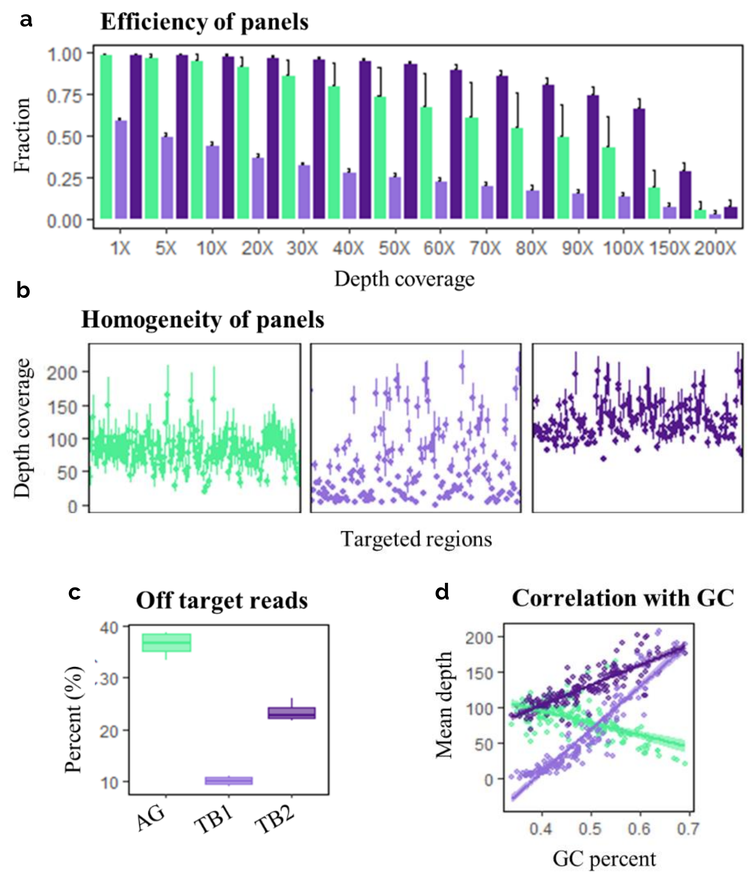


Figure 2: Strategy and performances of technologies
a, b, c. Panel performances by technology, including efficiency, that is represented as the mean +/- standard deviation of the fraction of targets covered at a specific depth (**a**), homogeneity, that is represented as the mean +/- standard deviation of depth coverage for the 165 targeted regions (**b**), specificity, that is represented as percentage of off-target reads (**c**), which mapped outside of the 165 targeted regions. **d.** Correlation of the mean coverage with the GC percentage of the 165 targeted regions.
AG and TB (classical TB1 and optimized TB2) protocols are in green and light or dark purple, respectively.

Identification of molecular signature of genomic imprinting

- Classification of **methylation status of CpGs** (Figure 3a)
- Identification of approximately **600 candidate DMRs** per sample (Figure 3a)
- Candidate **DMRs distributed in 123 of 165 genomic regions** (Figure 3b)

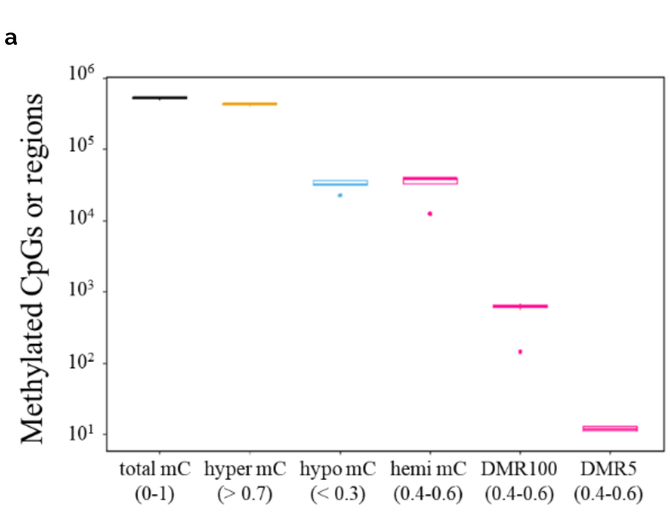
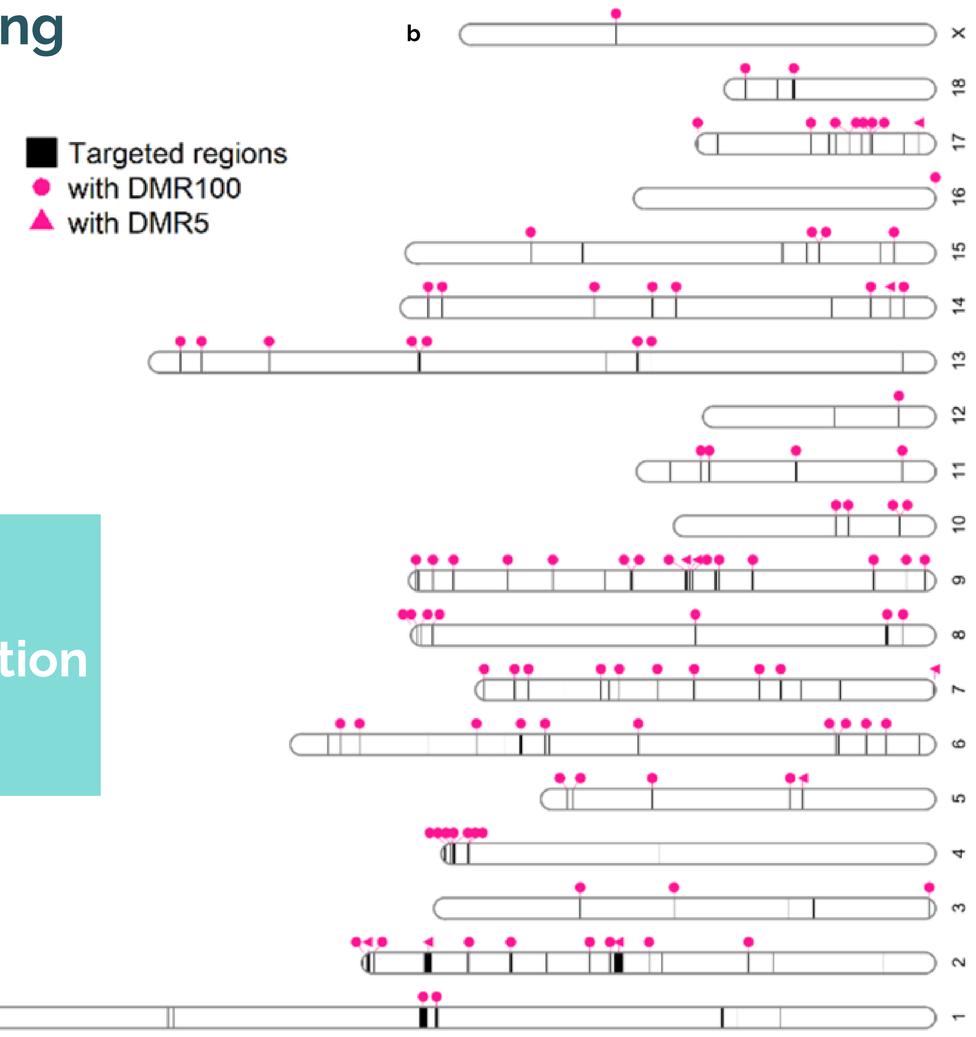


Figure 3: Detection, methylation and classification of CpGs
a. The methylation at CpGs was considered hyper/hypo/hemi when methylation was <70%, >30% and between 40% and 60%, respectively. DMR100 represents windows with at least 5 hemimethylated CpGs in 100 pb and DMR5 represents window with 5 consecutive hemimethylated CpGs. **b**, Location of the DMRs across the pig genome.



The molecular tool for a genome-wide evaluation of genomic imprinting

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