## METHODS

# Methods for discovering genomic loci exhibiting complex patterns of differential methylation; Supplementary Materials 

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## Simulation studies

Data are simulated using WGBSSuite version 0.4 , modified to include the effect of non-conversion rates by introducing a binomially distributed error in the number of methylated cytosines reported at each site. Plant-representative parameters were estimated by applying the analyse_WGBS.R script provided by WGBSSuite to the first million CpG cytosines on chromosome 1 of a single wild-type sample and the $d c l 2 / 3 / 4$ triple mutant in the Stroud et al[? ] dataset. Data were then simulated for one hundred thousand cytosines using the following command, where XXX and YYY are placeholders for the number of samples in each replicate group and the magnitude of difference in the proportion of methylation respectively.
Rscript simulate_WGBS.R 1000000.8517213581010340 .0158503393648432
+0.2 0.2 14.064221221 14.064221221 XXX 2 YYY 0.5
+0.0267845702643185,0.00339225464276114 plant_simulation binomial
+0.237414948538599,1e-15 0.0577772070946612,0.34
Version 0.4.4 of methylSig, 1.14 of methylKit, 1.10.0 of BSmooth and 3.4.2 of MethPipe were used to analyse these data using default settings for all libraries. Versions 2.9.5 of segmentSeq and 2.8.0 of baySeq were used to implement the approach described here.

## References

Stroud, H., Greenberg, M.C., Feng, S., Bernatavichute, Y., Jacobsen, S.: Comprehensive Analysis of Silencing Mutants Reveals Complex Regulation of the Arabidopsis Methylome. Cell 152(1), 352-364 (2013).
doi:10.1016/j.cell.2012.10.054


Figure S1 Boxplot of locus widths for each model/context.


Figure S2 Profiles of cytosine methylation in additional mutants for identified differentially methylated loci. Average methylation profiles in the ago4, cmt2, cmt3, ddm1, drm1/2, met1 cmt3, met1 het, nrpd1, and wild-type (WT) samples from Stroud et al, across the methylation loci (and the surrounding 4 Kb ) identified for each model/context with an FDR of $5 \%$. Profiles are shown for those model/context combinations in which at least 20 loci at this FDR could be identified.


Table S1 Numbers of loci associated with models of differential methylation.

| Model ID | Model definition | Estimated number of loci | Number of identified loci (FDR < 0.05) |
| :---: | :---: | :---: | :---: |
| A | \{WT, dcl2/3/4,dcl2/4,dcl3,dcl2,dcl4 \} | CpG: 67365 CHG: 53313 <br> CHH: 138593 | $\begin{aligned} & \text { CpG: } 17607 \\ & \text { CHG: } 9310 \\ & \text { CHH: } 30058 \end{aligned}$ |
| B | \{WT,dcl2/3/4,dcl2/4,dcl3,dcl4 $\gg\{\mathrm{dcl} 2\}$ | $\begin{aligned} & \text { CpG: } 10769 \\ & \text { CHG: } 5459 \\ & \text { CHH: } 4686 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 3587 \\ & \text { CHG: } 996 \\ & \text { CHH: } 137 \\ & \hline \end{aligned}$ |
| C | $\{\mathrm{WT}\}>\{\mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 2 / 4, \mathrm{dcl} 3, \mathrm{dcl} 2, \mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 8624 \\ & \text { CHG: } 692 \\ & \text { CHH: } 2290 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 119 \\ & \text { CHG: } 2 \\ & \text { CHH: } 4 \\ & \hline \end{aligned}$ |
| D | $\{\mathrm{WT}, \mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 2 / 4, \mathrm{dcl} 2, \mathrm{dcl} 4\}>\{\mathrm{dcl} 3\}$ | $\begin{aligned} & \text { CpG: } 6989 \\ & \text { CHG: } 644 \\ & \text { CHH: } 1383 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 1499 \\ & \text { CHG: } 27 \\ & \text { CHH: } 3 \end{aligned}$ |
| E | \{WT,dcl2/3/4,dcl2/4,dcl3,dcl2 $\gg\{\mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 6906 \\ & \text { CHG: } 631 \\ & \text { CHH: } 1306 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 877 \\ & \text { CHG: } 41 \\ & \text { CHH: } 9 \end{aligned}$ |
| F | $\{\mathrm{dcl} 3\}>\{\mathrm{WT}, \mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 2 / 4, \mathrm{dcl} 2, \mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 6131 \\ & \text { CHG: } 866 \\ & \text { CHH: } 1527 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 73 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \end{aligned}$ |
| G | \{WT, dcl2/3/4,dcl2/4,dcl4 $\gg\{\mathrm{dcl} 3, \mathrm{dcl} 2\}$ | $\begin{aligned} & \text { CpG: } 5167 \\ & \text { CHG: } 654 \\ & \text { CHH: } 1672 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 74 \\ & \text { CHG: } 24 \\ & \text { CHH: } 3 \\ & \hline \end{aligned}$ |
| H | $\{\mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 2 / 4, \mathrm{dcl3}, \mathrm{dcl2}, \mathrm{dcl} 4\}>\{\mathrm{WT}\}$ | $\begin{aligned} & \text { CpG: } 3665 \\ & \text { CHG: } 797 \\ & \text { CHH: } 614 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 1890 \\ & \text { CHG: } 43 \\ & \text { CHH: } 20 \\ & \hline \end{aligned}$ |
| I | \{WT,dcl2/3/4,dcl2/4,dcl3 $\gg\{\mathrm{dcl2,dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 2532 \\ & \text { CHG: } 738 \\ & \text { CHH: } 855 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 281 \\ & \text { CHG: } 28 \\ & \text { CHH: } 1 \end{aligned}$ |
| J | \{WT,dcl2/3/4,dcl2/4\}> ${ }^{\text {dcl3, dcl2,dcl4 }}$ | CpG: 2365 CHG: 331 CHH: 307 | $\begin{aligned} & \text { CpG: } 175 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \end{aligned}$ |
| K | \{WT, dcl2/4, dcl2,dcl4 $\gg\{\mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 3\}$ | $\begin{aligned} & \text { CpG: } 2208 \\ & \text { CHG: } 8384 \\ & \text { CHH: } 13657 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 62 \\ & \text { CHG: } 2314 \\ & \text { CHH: } 542 \end{aligned}$ |
| L | \{WT,dcl2/3/4,dcl2/4,dcl2 $\gg\{\mathrm{dcl} 3, \mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 1564 \\ & \text { CHG: } 229 \\ & \text { CHH: } 462 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 79 \\ & \text { CHG: } 7 \\ & \text { CHH: } 1 \end{aligned}$ |
| M | $\{\mathrm{WT}, \mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 2 / 4, \mathrm{dcl} 3\}>\{\mathrm{dcl} 4\}>\{\mathrm{dcl} 2\}$ | $\begin{aligned} & \text { CpG: } 968 \\ & \text { CHG: } 9 \\ & \text { CHH: } 15 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 218 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \\ & \hline \end{aligned}$ |
| N | $\{\mathrm{WT}, \mathrm{dcl} 2 / 4, \mathrm{dcl} 3, \mathrm{dcl} 2\}>\{\mathrm{dcl} 2 / 3 / 4\}>\{\mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 596 \\ & \text { CHG: } 7 \\ & \text { CHH: } 21 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 92 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \end{aligned}$ |
| O | $\{\mathrm{WT}, \mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 3, \mathrm{dcl} 2\}>\{\mathrm{dcl} 2 / 4\}>\{\mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 435 \\ & \text { CHG: } 2 \\ & \text { CHH: } 5 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 62 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \\ & \hline \end{aligned}$ |
| P | \{WT,dcl3,dcl2,dcl4 $\gg\{\mathrm{dcl} 2 / 4\}>\{\mathrm{dcl} 2 / 3 / 4\}$ | $\begin{aligned} & \text { CpG: } 306 \\ & \text { CHG: } 12 \\ & \text { CHH: } 42 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 74 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \end{aligned}$ |
| Q | $\{\mathrm{WT}, \mathrm{dcl} 3, \mathrm{dcl} 2, \mathrm{dcl} 4\}>\{\mathrm{dcl} 2 / 3 / 4\}>\{\mathrm{dcl} 2 / 4\}$ | $\begin{aligned} & \text { CpG: } 301 \\ & \text { CHG: } 5 \\ & \text { CHH: } 14 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 75 \\ & \text { CHG: } 1 \\ & \text { CHH: } 1 \\ & \hline \end{aligned}$ |
| R | $\{\mathrm{dcl} 2 / 4\}>\{\mathrm{WT}, \mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 3, \mathrm{dcl} 2, \mathrm{dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 2623 \\ & \text { CHG: } 3351 \\ & \text { CHH: } 4726 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 7 \\ & \text { CHG: } 153 \\ & \text { CHH: } 1 \end{aligned}$ |
| S | \{WT,dcl2/4,dcl3,dcl2,dcl4 $\gg\{\mathrm{dcl} 2 / 3 / 4\}$ | $\begin{aligned} & \text { CpG: } 2298 \\ & \text { CHG: } 3220 \\ & \text { CHH: } 6387 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 47 \\ & \text { CHG: } 360 \\ & \text { CHH: } 255 \\ & \hline \end{aligned}$ |
| T | \{dcl2/3/4,dcl2/4,dcl3,dcl4 $\gg\{\mathrm{WT}, \mathrm{dcl} 2\}$ | $\begin{aligned} & \text { CpG: } 1661 \\ & \text { CHG: } 2745 \\ & \text { CHH: } 1219 \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { CpG: } 16 \\ & \text { CHG: } 337 \\ & \text { CHH: } 3 \\ & \hline \end{aligned}$ |
| U | $\{\mathrm{WT}, \mathrm{dcl} 2 / 4, \mathrm{dcl} 2, \mathrm{dcl} 4\}>\{\mathrm{dcl} 3\}>\{\mathrm{dcl} 2 / 3 / 4\}$ | CpG: 207 CHG: 1746 CHH: 5435 | $\begin{aligned} & \text { CpG: } 10 \\ & \text { CHG: } 308 \\ & \text { CHH: } 307 \end{aligned}$ |
| V | \{WT, dcl2/4, dcl4 $\gg\{\mathrm{dcl} 2 / 3 / 4, \mathrm{dcl} 3, \mathrm{dcl} 2\}$ | CpG: 1719 CHG: 1315 CHH: 2831 | $\begin{aligned} & \text { CpG: } 12 \\ & \text { CHG: } 63 \\ & \text { CHH: } 37 \end{aligned}$ |
| W | \{dcl2/3/4,dcl2/4,dcl3 $\gg\{\mathrm{WT}, \mathrm{dcl2,dcl} 4\}$ | $\begin{aligned} & \text { CpG: } 2189 \\ & \text { CHG: } 1260 \\ & \text { CHH: } 606 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 35 \\ & \text { CHG: } 54 \\ & \text { CHH: } 1 \\ & \hline \end{aligned}$ |
| X | \{WT,dcl2/3/4,dcl3,dcl2,dcl4 $\gg\{\mathrm{dcl} 2 / 4\}$ | $\begin{aligned} & \text { CpG: } 1237 \\ & \text { CHG: } 757 \\ & \text { CHH: } 1886 \end{aligned}$ | $\begin{aligned} & \text { CpG: } 51 \\ & \text { CHG: } 79 \\ & \text { CHH: } 3 \\ & \hline \end{aligned}$ |

