Motivation for integration

DNA methylation occurs in a variety of forms. 5-methylcytosine (mc) is the most widely studied, and there is ample evidence for its importance in development and gene regulation. 5-hydroxymethylcytosine (hmC) is a less abundant, but appears to be a good marker for epigenetic changes correlating with changes in gene expression.

The problem: Widely-used assays measuring methylation (e.g. BSSeq & RRBS) do not distinguish mc and hmc. This may confuse biological interpretation because it is unclear which mark is playing a role relative to a phenotype.

Integrating assays measuring mc+hmc (e.g. BSSeq & RRBS) with others measuring mc (e.g. oxBSeq & MeDiPSeq) or hmc (e.g. TASeq & MeDiPSeq), helps differentiate the mc and hmc marks from one another.

The solution: We developed the mint pipeline to
• be a complete pipeline from raw reads to interpretation
• do sample methylation quantification
• do differential methylation analysis between groups
• differentiate between regions of mc vs hmc
• automatically generate UCSC genome browser tracks
• provide summaries of genomic annotations to facilitate biological interpretation.

Supported designs

Hybrid
Bisulfite-based assay and pulldown assay.

Pulldown
Pulldown assays for mc and hmc.

Sample-wise
Integration on a per-sample basis.

Comparison-wise
Integration on a per-group basis.

Setting up and running mint

On a Mac or Linux system:
1. Get mint at github.com/sartorlab/mint
2. Setup dependencies and reference genomes.
3. Annotate the experimental design.
4. Initialize the project with:
   Rscript init.R --project name --genome g --datapath path
5. Customize the config, m file with desired parameters.
6. Use make to run the analysis modules as per the design:
   make bisulfite_align
   make pulldown_align
   make bisulfite_compare
   make pulldown_compare
   make bisulfite_classification
   make pulldown_classification

Overall mint workflow

1. QC
   FastQC + MultiQC
   Adapter / Quality Trimming
   Trim Galore
2. Alignment
   bismark / bowtie2
3. Sample Meth. Quantification
   bismark / macs2
4. Differential Meth.
   methylSig (1) / PePr (2)
5. Classification
6. Annotation and Visualization
   annotatr (3)

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Annotation and Visualization

Quality control

bismark methylation extractor

methylSig methylSig DM status by annotation

PePr Bead changes by annotation

Classifications

Simple classification

Comparison classification

Bis. Simple classification by annotation

UCSC Genome Browser Track Hub

References

1. Park Y, et al. (2014) "MethylSig: a whole genome DNA methylation analysis pipeline" Bioinformatics
2. Zhang Y, et al. (2014) "PePr: A peak-calling prioritization pipeline to identify consistent or differential peaks from replicated CHIP-seq data" Bioinformatics

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