A genome-wide survey of DNA methylation in hexaploid wheat

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Epigenetics

“heritable changes in gene function that cannot be explained by changes in DNA sequence”

Epigenetic marks:

- DNA methylation
- Histone modifications

Methylation of promoters is linked to gene silencing whereas gene body methylation is associated with actively transcribed genes

In plants at CpG, CHG and CHH sites (H=A,T or C)
Methyl-seq in wheat

- We study DNA methylation in Chinese Spring wheat using a combination of gene capture & bisulfite sequencing

Gene capture from genomic DNA

HiSEQ
Sequence capture

- 6 Mbp capture probe set
- RNA based probes 120 bp long
- Biotinylated probes in solution allow streptavidin capture
- Probes bind in the presence of SNPs and therefore one probe captures 3 homoeologs
Background

Sequence capture design

- Probes distributed across gene space of wheat
- Redundancy collapsed (A, B and D genomes)
- Chloroplast, mitochondrial and repetitive sequence removed
- Select probes with:
  - surrounding sequence for mapping
  - gene annotation
  - SNPs

Select "best" probe per contig
Using paired-end reads we can capture ~4X the probe space

- 6 Mbp => 27 Mbp analyzed per genome (27 * 3) => 81 Mbp
Mapping: Bismark
Reference: probe
design space
3 genomes collapsed to
allow methylation
comparison between
homoeologs

Sequencing data

Remove duplicate/unmapped reads

SNP call

Conserved positions

Associate sequencing reads with SNPs

Sub-genome discrimination

For each sub-genome cytosine position:
calculate % methylation

Homoeologous SNPs
Questions

Wheat DNA methylation

- Can differential methylation be seen between the sub-genomes of Chinese Spring hexaploid wheat?

- Can methylation be linked to gene expression changes?

- Using different growth temperatures (12°C and 27°C) can temperature alter methylation state?

- How is methylation conserved over time? (comparison with *Ae. tauschii*)
**Findings**

**Tri-, bi- and uni-methylation**

- Majority of methylation is conserved across the sub-genomes
- Sub-genome specific methylation observed in approximately equal proportions

![Diagram showing methylation distribution across sub-genomes](image)
Findings

Methylation distribution is uniform

Tri-genome methylation

Uni-/Bi-genome methylation
Genome A:BD

Uni-/Bi-genome methylation
Genome B:AD

Uni-/Bi-genome methylation
Genome D:AB
Findings

Average methylation levels

- Methylated cytosine sites:
  - 60.0% of CpG
  - 4.58% of CHG
  - 1.42% of CHH
Findings

Genome specific methylation profile differs

- Non-transcribed regions: less CpG and more CHH/CHG methylation as observed in other plants

  - Uni-genome
  - Bi-genome
  - Tri-genome

- Transcribed regions: expect more CpG methylation, however, for uni- and bi-genome methylation CHG/CHH methylation is significant

  - Uni-genome
  - Bi-genome
  - Tri-genome

- CHG/CHH uni- and bi-genome methylation in transcribed regions …pseudo genes in wheat?
Methylation links to gene expression

- Tri-genome methylation correlates with:
  - equal sub-genome expression
  - loss of high expression in promoters

- Uni-genome methylation correlates with:
  - loss of high expression in promoters
  - lower average expression of methylated genome \( (t = 2.4916, p = 0.0129) \)
Findings

Temperature has a small effect on methylation

- Using growth temperatures 12°C and 27°C:
  - differences seen in 0.1% sites

- 77% of annotated sites could be directly associated with either temperature/stress sensitivity or methyltransferase activity
Findings

Tri-genome methylation is more stable than genome specific methylation

- Uni-genome methylation conservation with *Ae. tauschii*:
  - A specific 25.51%
  - B specific 26.13%
  - D specific 86.43%

- Tri-genome methylation conservation with *Ae. tauschii*:
  - 95.2%
Conclusions

- Coming soon to Ensembl
- Majority of methylation is tri-genome; sub-genome specific observed
- Methylation links to gene expression
- Temperature has a small effect on methylation
- Tri-genome methylation is more stable than genome specific methylation
Current Work

- 12Mb capture probe set designed as per the 6Mb
- Increased tiling over NBS-LRR genes (Brande Wulff & Burkhard Steuernagel JIC)
  Used on Watkins bread wheat landrace cultivar collection (826 lines): core set of 119 represent >96% of the diversity of the full panel
- Quantify methylation variation across the Watkins core set
  In general
  In NBS-LRR gene set
Thank you

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