Supplementary Figures and Tables

Supplementary Figure 1. Sequencing coverage over all cytosines (left panels) and CpGs (right panels) in egg (blue), sperm (green) and drone (red) genomes. The top panels show cumulative coverage, e.g. approximately 20% of sites are covered by 19 or more reads. The bottom panels give the proportion of sites that have a certain level of coverage, e.g. approximately 2% of sites have a coverage of 15 reads. Note that every read can trace its origin to a bisulfite conversion event that happened on one strand or the other. Here we only count reads that were on the correct strand to be informative at a particular cytosine.

Supplementary Figure 2. Histograms of the number of significantly methylated exonic CpGs. The values for the egg (top) and sperm (bottom) genomes are calculated independently. For comparison the histograms on the right show the distribution that would be expected if the same number of methylated CpGs in each sample were distributed randomly among exons, weighted by length.

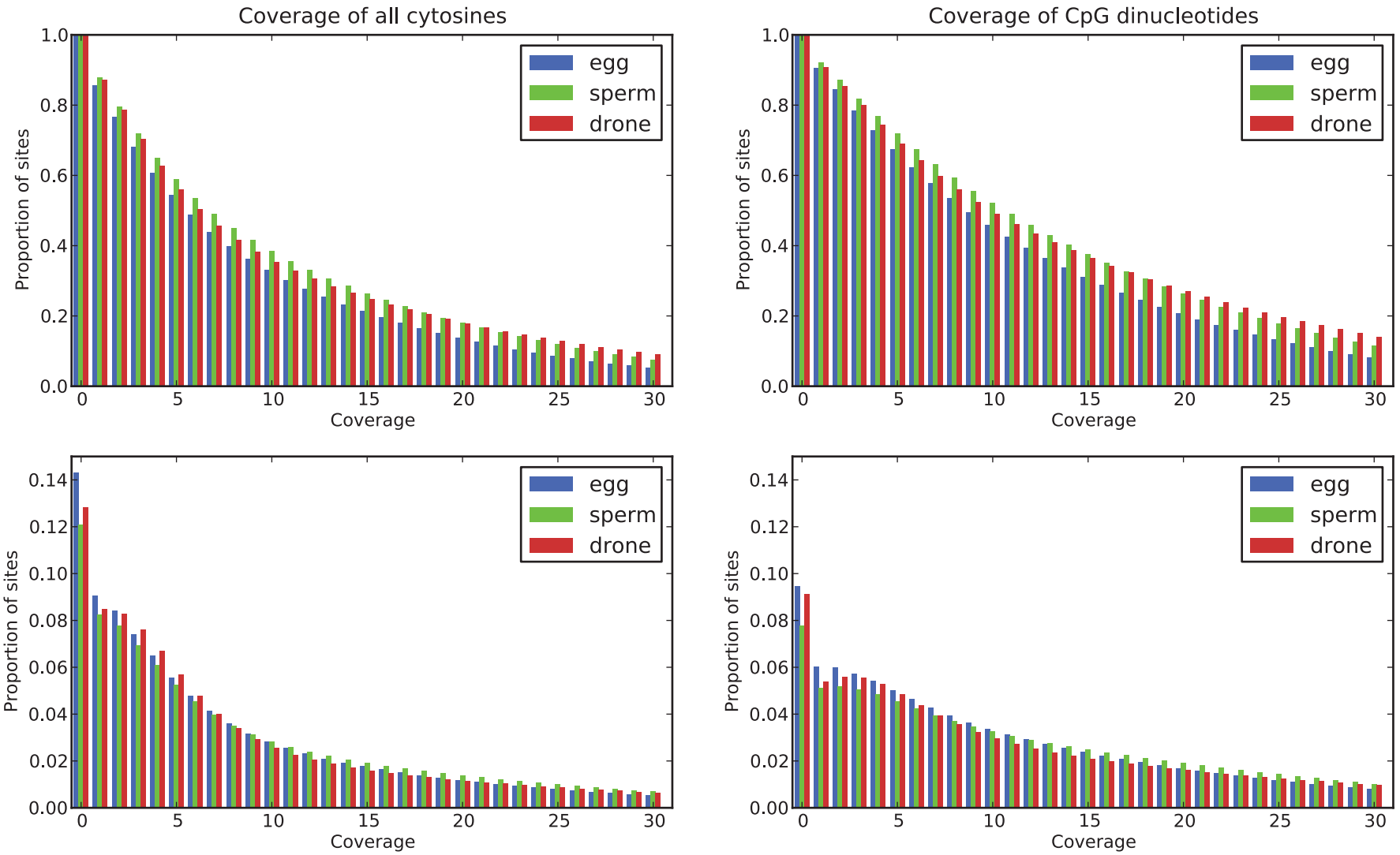
Supplementary Figure 3. The number of methylated CpG sites per gene for egg versus sperm DMGs and non-DMGs (left panel). This counts only sites occurring in exons. A site was considered methylated if it was significantly methylated in either eggs or sperm. The right pane shows the coding length of genes, which is the length of all the exons of a gene concatenated.

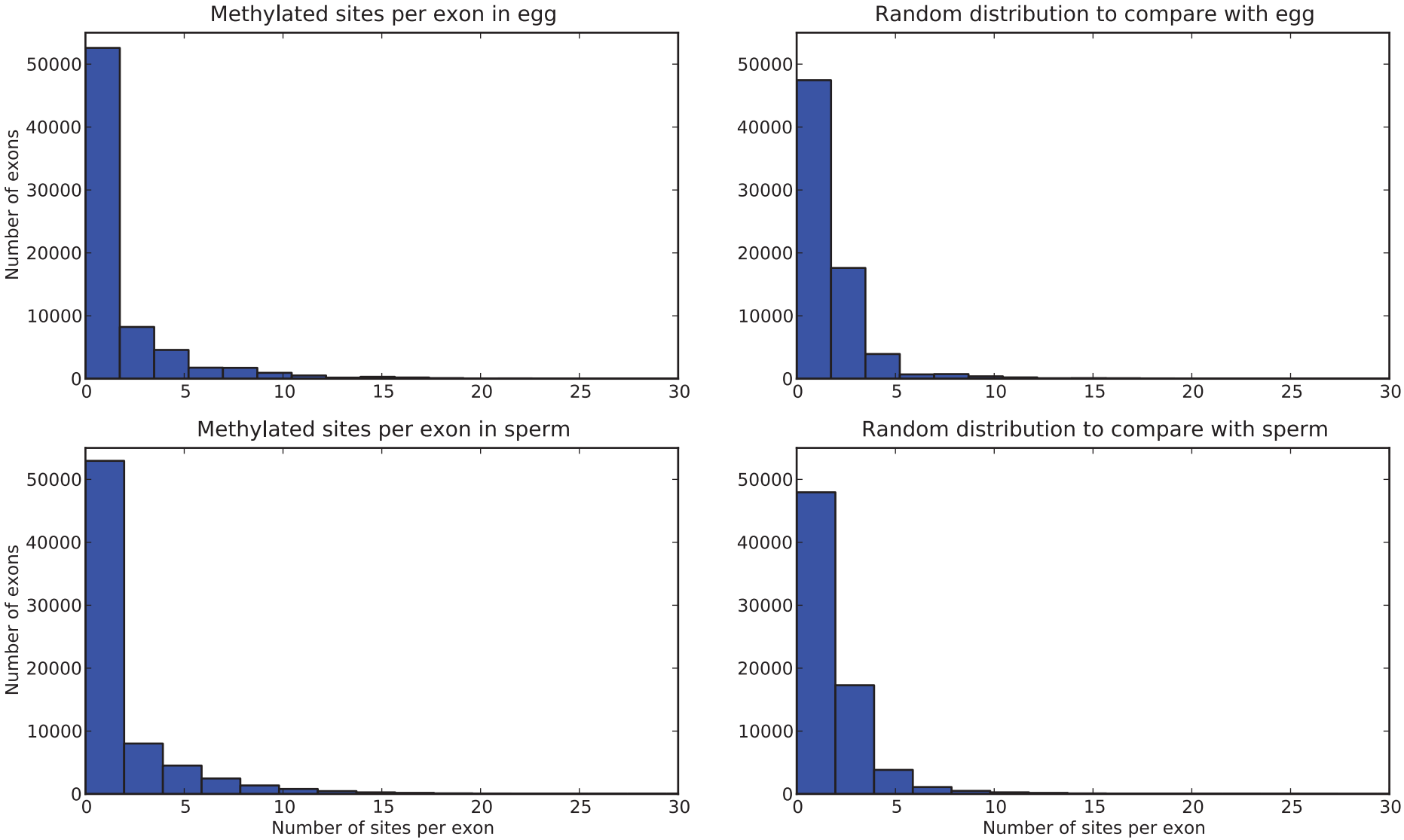
Supplementary Figure 4. CpG methylation profile of the top DMG in eggs when compared to sperm, *Stoned B* (GB17185). Comparison of the frequency of methylation at CpGs in egg, sperm and drone (blue, green and red) from (A) whole genome bisulfite sequencing with the frequency of methylation detected in (B) bisulfite PCR from two independently collected egg, drone and sperm samples. The individual reads for the bisulfite PCR data is shown in Figure 7. Exons are indicated in grey.

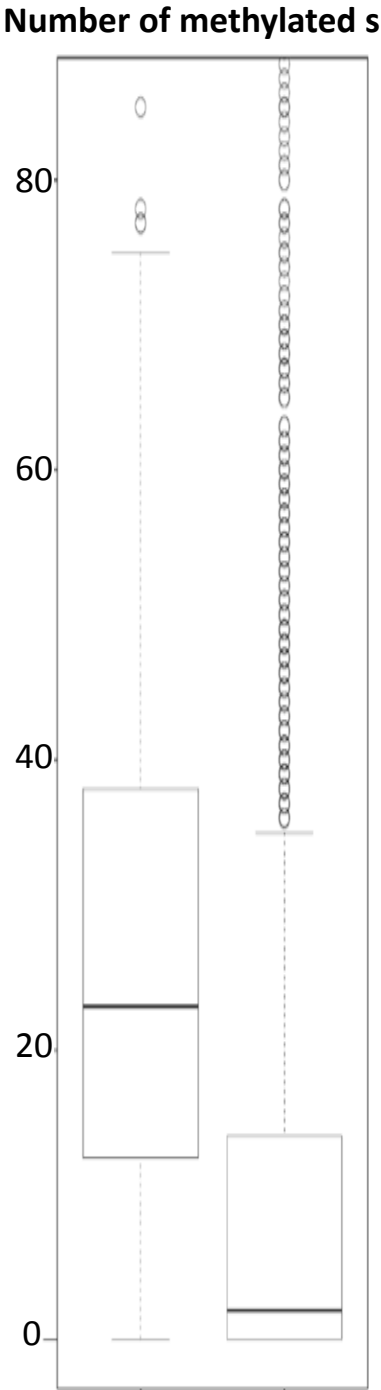
Supplementary Figure 5. CpG methylation profile of the top DMG over in eggs and drone compared to sperm, *n-acetyltransferase 15-like* (GB19408). Comparison of the frequency of methylation at CpGs in egg, sperm and drone (blue, green and red) from (A) whole genome bisulfite sequencing with the frequency of methylation detected in (B) bisulfite PCR from two independently collected egg, drone and sperm samples. The individual reads for the bisulfite PCR data is shown in Figure 8. Exons are indicated in grey.

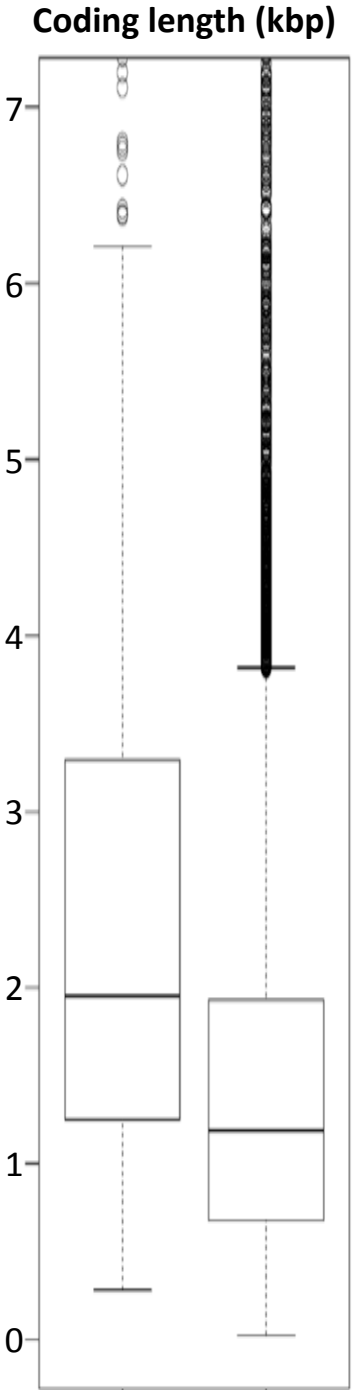
Supplementary Table 1. DMG three way comparisons. For example, in eggs, three genes are significantly under-methylated than drone thorax and sperm (EggUnder) and 289 are over-methylated (EggOver). Gene Ontology (GO) terms were assigned by Blast2Go and GB numbers assigned from BeeBase. Two-way rankings are indicated**.**

Supplementary Table 2. 381 differentially methylated genes (DMGs) from two way comparison of eggs (320 more highly methylated) and sperm (61). Gene Ontology (GO) terms were assigned by Blast2Go and GB numbers assigned from BeeBase. Queen vs worker refers to differentially methylated genes (DMGs) between queen and worker.


Coverage of all cytosines
Coverage of CpG dinucleotides
Proportion of sites
Coverage
egg
sperm
drone
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0
5
10
15
20
25
30

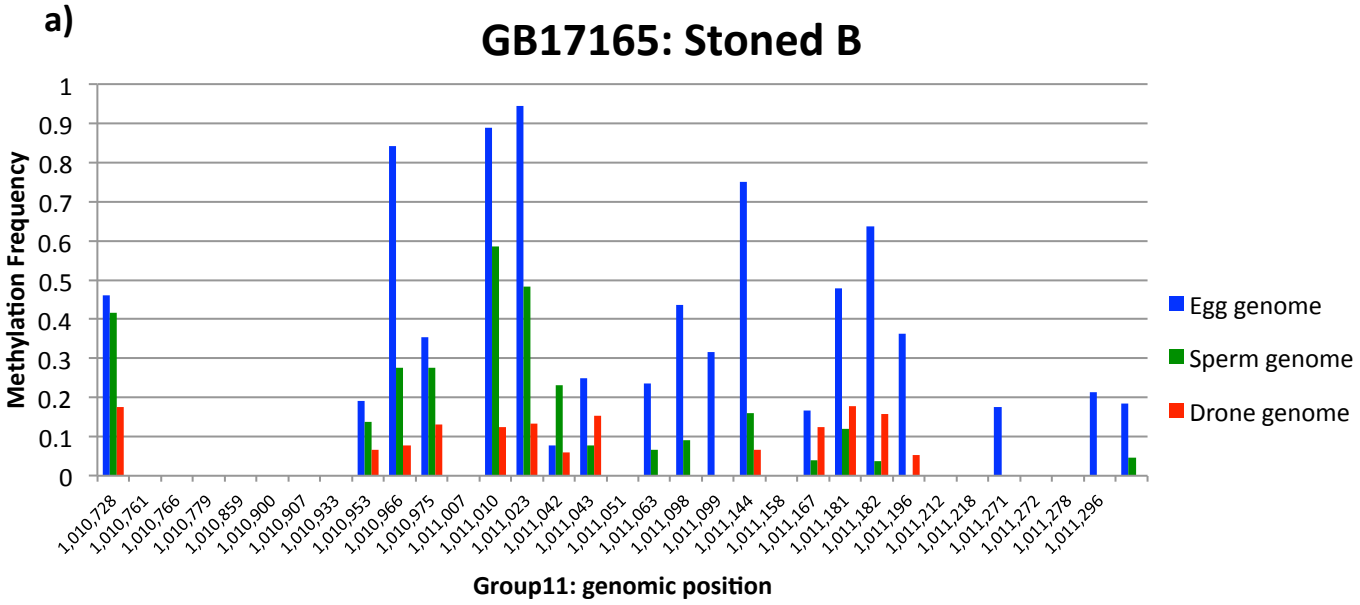

Methylated sites per exon in egg
Random distribution to compare with egg
Methylated sites per exon in sperm
Random distribution to compare with sperm
Number of exons
Number of sites per exon
0
10000
20000
30000
40000
50000
0
5
10
15
20
25
30


Number of methylated sites
80
60
40
20
0

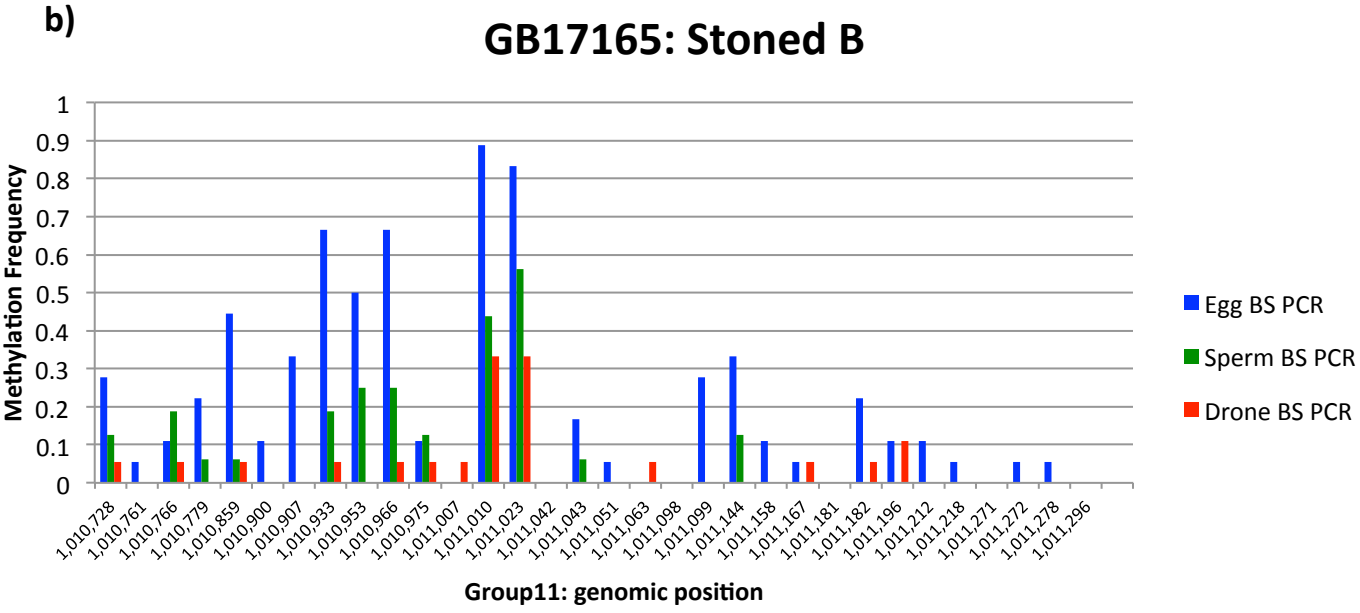

Coding length (kbp)
7
6
5
4
3
2
1
0

Exon 5
Exon 4

a)


GB17165: Stoned B
Methylation Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1,010,728
1,010,761
1,010,766
1,010,779
1,010,859
1,010,900
1,010,907
1,010,933
1,010,953
1,010,966
1,010,975
1,011,007
1,011,010
1,011,023
1,011,042
1,011,043
1,011,051
1,011,063
1,011,098
1,011,099
1,011,144
1,011,158
1,011,167
1,011,181
1,011,182
1,011,196
1,011,212
1,011,218
1,011,271
1,011,272
1,011,278
1,011,296
Group11: genomic position
Egg genome
Sperm genome
Drone genome

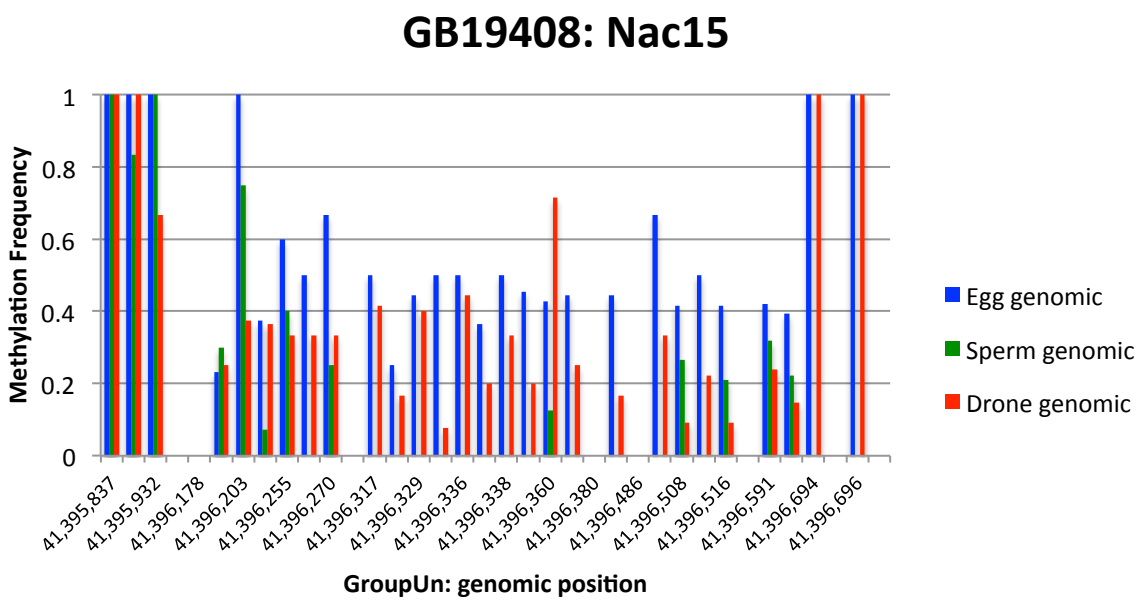
b)


GB17165: Stoned B
Methylation Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1,010,728
1,010,761
1,010,766
1,010,779
1,010,859
1,010,900
1,010,907
1,010,933
1,010,953
1,010,966
1,010,975
1,011,007
1,011,010
1,011,023
1,011,042
1,011,043
1,011,051
1,011,063
1,011,098
1,011,099
1,011,144
1,011,158
1,011,167
1,011,181
1,011,182
1,011,196
1,011,212
1,011,218
1,011,271
1,011,272
1,011,278
1,011,296
Group11: genomic position
Egg BS PCR
Sperm BS PCR
Drone BS PCR

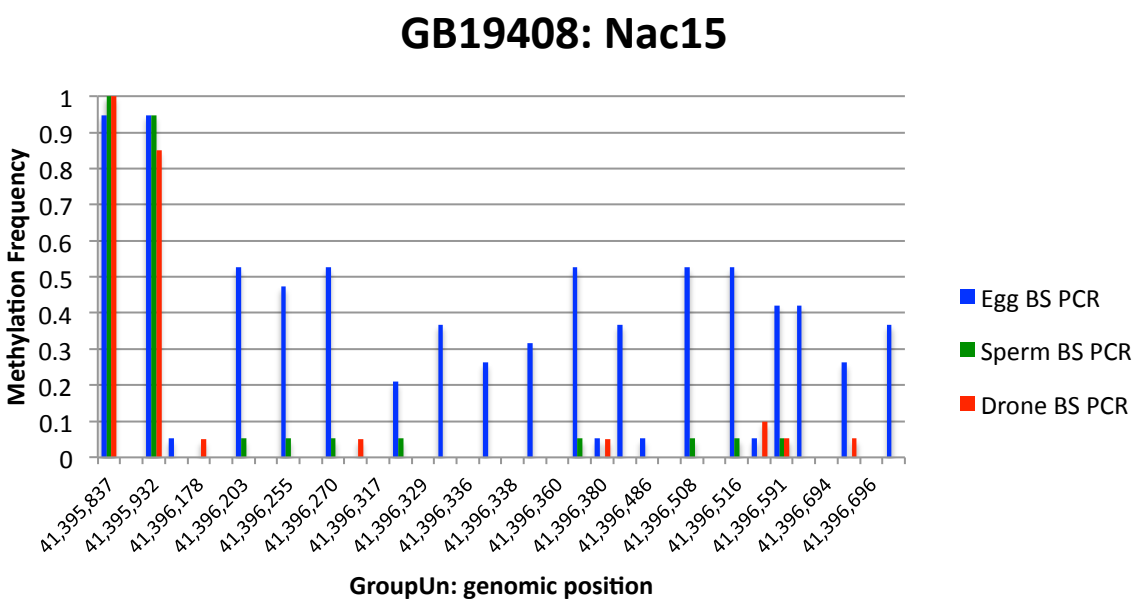
GB19408 *n-acetyltransferase-like 15*

Exon 2

a)



b)



Download Table S1

Download Table S2