Whole genome DNA methylation profile of the jewel wasp (*Nasonia vitripennis*)

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Keywords: DNA methylation, *Nasonia*, epigenetics
Abstract

The epigenetic mark of DNA methylation, the addition of a methyl group to a cytosine residue, has been extensively studied in many mammalian genomes and while it is commonly found at the promoter regions of genes, it is also involved in a number of different biological functions. In other complex animals, such as social insects, DNA methylation has been determined to be involved in caste differentiation and to occur primarily in gene bodies. The role of methylation in non-social insects, however, has not yet been explored thoroughly. Here we present the whole genome DNA methylation profile of the non-social hymenopteran, the jewel wasp, *Nasonia vitripennis*. From high-throughput sequencing of bisulfite converted gDNA extracted from male *Nasonia* thoraces we are able to determine which cytosine residues are methylated in the entire genome. We find that an overwhelming majority of methylated sites (99.7%) occur at cytosines followed by a guanine in the 3’ direction (CpG sites). Additionally, we find that a majority of methylation in *Nasonia* occurs within exonic regions of the genome (over 62%). Overall, methylation is sparse in *Nasonia*, occurring only at 0.18% of all sites and at 0.63% of CpGs. Our analysis of the *Nasonia* methylome reveals that in contrast to the methylation profile typically seen in mammals, methylation is sparse and constrained primarily to exons. This methylation profile is more similar to that of the social hymenopteran species, the honey bee (*Apis mellifera*). In presenting the *Nasonia* methylome, we hope to promote future investigation of the regulatory function of DNA methylation in both social and non-social hymenoptera.
Article Summary

The epigenetic mark of DNA methylation has been studied in many genomes in mammals. It is known to be involved in a number of different biological functions, including gene regulation. In other complex animals, such as social insects, DNA methylation has been determined to be involved in caste differentiation and occurs in the body of genes. In this study we use high throughput sequencing to investigate the whole genome DNA methylation profile of the non-social jewel wasp, *Nasonia vitripennis*. We discover that the wasp genome methylation profile is much more similar to social insects than mammals.
**Introduction**

DNA methylation is the process by which methyl (CH$_3$) groups are added to cytosine residues in genomic DNA (Li 2002). The addition of a methyl group occurs predominately at cytosines that are followed by a guanine in the 3’ direction (known as CpG sites) and allows the genome to hold more information than the sequence of the four bases alone. Thus, methylation patterns are of great interest. DNA methylation is particularly prevalent in mammals, where 60-90% of CpGs are methylated across the entire genome (Lister et al. 2009) and methylation is found at both promoters and intergenic regions (Elango and Yi 2008). In mammals, DNA methylation has several well-characterized regulatory functions including; X chromosome inactivation, genomic imprinting, alternative splicing, and cellular differentiation (Schwartz and Ast 2010). However, the role of methylation in other complex animals, including insects, is not as well understood (Drewell et al. 2012).

In insects, patterns of DNA methylation differ strongly from those observed in mammals (Glastad et al. 2011; Lyko and Maleszka 2011), suggesting that the regulatory function of methylation within these organisms also differs (Drewell et al. 2012; Patalano et al. 2012). First, some insect species, such as *Drosophila melanogaster*, have unmethylated genomes, and lack both the maintenance (Dnmt1) and de novo (Dnmt3) DNA methyltransferases (Lyko and Maleszka 2011). Second, those insects that have the enzymatic machinery to carry out DNA methylation, such as the honey bee *Apis mellifera*, show much lower overall levels of methylation (approximately 0.69% of all CpGs are methylated) than mammals, and methylation occurs primarily in exonic regions (Glastad et al. 2011; Lyko et al. 2010). In recent years, analyses of a number of additional insect methylomes have been performed, including the silkworm (Xiang et al. 2010), fire ant (Wurm et al. 2011) and desert locust (Falckenhayn et al. 2013). The role of DNA methylation in insects is particularly interesting since its functional activity is not entirely conserved across all the different species analyzed to date (Flores and Amdam 2011). The majority of functional studies of DNA methylation in Hymenoptera have focused on the eusocial honey bee *Apis mellifera* and it is now clear that methylation is central to caste polyphenism (Herb et al. 2012; Li-Byarlay et al. 2013). For example, over 550 genes show different patterns of methylation between queens and workers (Lyko et al. 2010; Shi et al. 2012). The functional role of DNA methylation in non-social hymenoptera is especially intriguing, since methylation does not play a role in caste differentiation as is the case in their social relatives.
The parasitic jewel wasp, *Nasonia vitripennis*, provides a suitable species for studying the function of DNA methylation in non-social hymenoptera since its genome has recently been sequenced (WERREN et al. 2010). One significant discovery from this sequencing project was that *Nasonia* has both Dnmt1 and Dnmt3 (PARK et al. 2011), the two enzymes that are essential for DNA methylation. As an emerging model species, *Nasonia*, therefore potentially represents a new methylome for studies aimed at generating a better understanding of the functional role of methylation in hymenopterans. Indeed, some functions of DNA methylation within *Nasonia* are already understood. DNA methylation is essential for development in *Nasonia*, as a knockdown of maternally-provided *Dmnt1a* is lethal to embryos (ZWIER et al. 2012). DNA methylation is involved in alternative splicing of genes related to sex determination (PARK et al. 2011). Park et al. found for the small number of genes they examined, DNA methylation is generally sparse, occurring primarily within exonic regions (PARK et al. 2011); a result consistent with the honey bee methylome (LYKO et al. 2010). Here, we present the whole genome DNA methylation profile of *Nasonia vitripennis*. Our goal is to provide an additional methylome to those already available from insects, so that the role of methylation in social and non-social Hymenoptera may be better understood in future comparative studies.
Results and Discussion

Sequencing of bisulfite-converted DNA from male *Nasonia vitripennis* thoraces (along with whole lambda bacteriophage DNA as a control), allowed us to obtain a dataset of 65 million reads after quality control (see Methods for full details). To estimate the overall rate of bisulfite conversion in non-methylated bases in our experiments, we measured the C to U deamination rate in the unmethylated bacteriophage lambda DNA as a spike in control. We found that only 99.28% of cytosines were converted in the lambda DNA (see Methods), indicating that the false negative rate is less than 1%. Median coverage of CpG sites in the *Nasonia* genome assembly was 8 reads, with 86.4% of sites covered by 2 or more reads (Figure 1). The complete dataset of sequence reads is available at: drewell.sites.hmc.edu/restricted/Nasonia_methylome

Overall, a relatively small proportion of cytosines are methylated in the *Nasonia* genome: approximately 176,000 sites, or 0.18% of all sites and 0.63% of all CpGs. Methylation occurs predominately at CpG dinucleotides, accounting for 99.7% of methylated sites (Table 1). At 68,071 individual CpGs methylation occurs on both DNA strands, accounting for 136,142 (77.4%) of all the methylated CpGs in the genome. The majority of methylation occurs within exonic regions, accounting for over 62% of all methylated sites (Table 2). Amongst these significantly methylated sites there are many examples of individual sites that are methylated in 100% of the reads, although in some cases this may be a reflection of the overall low number of reads at some of these sites. In addition, we could not detect any evidence of significant methylation at the annotated transposable elements in the *Nasonia* genome (WERREN et al. 2010). This methylome profile mirrors previous observations in both the adult honey bee methylome (LYKO et al. 2010) and in the small sub-set of *Nasonia* genes previously analyzed (PARK et al. 2011) indicating that DNA methylation outside of gene bodies and at non-CpG residues is rare within Hymenoptera. These results are drastically different from the methylation profile seen in mammals, where 60-90% of CpGs are methylated across the entire genome (LISTER et al. 2009) and widespread methylation at transposons is involved in transcriptional suppression (JONES and TAKAI 2001).

In an effort to investigate the highly methylated genes in the *Nasonia* genome we analyzed the 20 most methylated genes by both the proportion (number of methylated sites over number of base pairs in each annotated gene) (Table 3) and by the total number of methylated sites (Table S1). It is important to note that determining the most methylated genes by proportion is potentially biased toward shorter
genes, while determining most methylated genes by number of sites is biased toward longer genes. Eight of the top methylated genes by proportion consist of only a single exon, lacking any intronic regions. All but one of the genes with more than one exon have at least one mCpG in an intron (Table 3 and Figure 2). In general, the most-methylated genes mirror the overall methylation profile of the genome, where a majority of methylation occurs in exonic regions. The 13th ranked NV12600-RA is the only gene to deviate from this pattern, showing extensive methylation within its first intronic region (Figure 2). Additionally, in a number of the most frequently methylated genes (including NV12600-RA), there are methylated sites in close proximity to the exon-intron boundaries (Figure 2), a pattern that may be consistent with a possible functional role for methylation in regulating alternative splicing (Flores et al. 2012). Indeed, the overall pattern of high levels of methylation in exons in Nasonia is consistent with a potential role in the regulation of splicing, as has been previously found in honeybees (Flores et al. 2012), where exons that are included during splicing often have higher levels of methylation at the start and end of the exon when compared to skipped exons.

We used the predicted protein sequence of the 20 most methylated genes from NasoniaBase (Munoz-Torres et al. 2011) (both by proportion and number of sites) to assign Gene Ontology (GO) terms in Blast2Go based on homology to the top BLAST hit and the corresponding GO annotations (Götz et al. 2008). GO analysis revealed an enrichment of genes involved in metabolic and cellular processes and biological regulation in the top methylated genes by proportion (Figure 3 and Table S2). Intriguingly, the 7th ranked gene by proportion, NV15491-RA, is a putative methyltransferase (Table S2). The top methylated genes by number of sites were also enriched for cellular and metabolic processes (Figure S1 and Table S3). Analysis of the top 1% of methylated genes in the genome, by either proportion or total number of sites, revealed very similar GO term distributions to the top 20 genes (data not shown).
Conclusions

Many aspects of the DNA methylation profile of *Nasonia vitripennis* are similar to the patterns of methylation in *Nasonia*'s social relative, the honey bee. First, methylation occurs overwhelmingly only at CpG sites (99.7% of sites). Additionally, while a majority of the methylation appears on promoters within mammalian methylomes, *Nasonia* primarily has methylation within gene bodies. Overall, methylation within the *Nasonia* genome is sparse (0.18% of all sites, and 0.63% of CpGs). The Gene Ontology (GO) terms for the most-methylated genes in *Nasonia* indicate enrichment of genes involved in cellular and metabolic processes and biological regulation. Given the recent indication that DNA methylation patterns in insects can be tissue- and/or cell-type specific (FORET et al. 2012), it is important to note that the methylome in our study is from a heterogeneous mix of cells in the wasp thorax. However, the *Nasonia* methylome can be used for comparative studies of methylomes within the order Hymenoptera. Previous studies of just a handful of genes determined that epigenetic marks, specifically DNA methylation, could be playing a role in alternative splicing of genes involved in sex determination (PARK et al. 2011; VERHULST et al. 2010). Several aspects of the DNA methylation profile of the *Nasonia* genome are consistent with DNA methylation having a role in the regulation of alternative splicing (FLORES et al. 2012). For example, a number of the top methylated genes have methylation in exons and/or associated with exon-intron boundaries. By presenting the whole *Nasonia* methylome, we hope to catalyze further analysis of the regulatory function of DNA methylation in both *Nasonia* specifically and Hymenoptera in general.
Methods

DNA Sources

*Nasonia vitripennis* were reared under standard laboratory conditions (WERREN and LOEHLIN 2009) and the thoraces from 150 adult males were collected. Extraction of genomic DNA was performed using the DNeasy Blood and Tissue Kit (Qiagen) following the protocol for purification of total DNA from insects. A total of 10.7µg gDNA was obtained. As a control for bisulfite conversion, 107ng of lambda phage DNA (New England Biolabs, N3011S) purified with PureLing Quick PCR Purification Kit (Invitrogen) was added to the sample.

Sequencing of Bisulfite Converted DNA Libraries

Library construction, bisulfite conversion and sequencing were performed at the Beijing Genomics Institute. Briefly, DNA was fragmented into 100-300 bp fragments by sonication (Covaris S-2, Woburn USA). The fragmentation parameters were: Duty cycle 10%; Intensity: 5; Cycles/burst: 200; Cycles: 16; Total fragmentation time: 960 sec. Fragmentation was confirmed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Fragments were end repaired (Illumina) as recommended by the manufacturer. Repaired fragments were ligated with methylated sequencing adaptors using a paired end adaptor oligo kit and oligo mix 5 (Illumina). Ligated fragments were selected by gel electrophoresis and fragments of size 360 bp extracted using a QIAquick gel extraction kit (Qiagen).

Size-selected fragments were bisulfite treated using an EZ-DNA methylation kit (Zymo Research, Irvine USA) and enriched using a MethyMiner methylated DNA enrichment kit (Invitrogen). It should be noted that this kit uses the DNA binding domain from human methyl-binding domain 2 protein to enrich for methylated DNA and therefore, when compared to experimental approaches that do not use this step, it will likely introduce selection for methylated fragments. Libraries were amplified using T4 polymerase (Enzymatics), and sequenced on Illumina’s HiSeq platform.

Sequence Analysis and Mapping DNA Methylation
Data were filtered to remove adaptor sequences, duplicate sequences, contamination and low quality reads using BGI software. For methylation analysis we followed Lyko et al. (LYKO et al. 2010). We mapped our reads onto the *Nasonia vitripennis* genome assembly 1.0 (MUNOZ-TORRES et al. 2011) using BSMAP version 2.6 (XI and LI 2009) with seed size 12 and maximum allowed mismatches 5. Similarly, we mapped our reads onto the complete lambda phage genome (GenBank: J02459.1). 73% of our reads mapped onto the Nasonia genome and 22% mapped onto the lambda genome. We considered only reads that mapped uniquely, and bases within reads that had a quality score of 20 or more, and that were next to 3 matches with quality scores of at least 15 (ALTSHULER et al. 2000). From these data we determined the number of converted and unconverted reads at each C position in the *Nasonia* and lambda genomic assemblies, accounting for the fact that each read comes from a bisulfite reaction on one strand or the other.

To estimate the overall rate of bisulfite conversion in non-methylated bases in our experiments, we used the C to T conversion rate in the lambda phage DNA, in which all cytosines should have been converted. We found that 99.28% of cytosines were converted in the lambda DNA. We also determined the conversion rate by examining the rate of C to T conversion at cytosines that were not in a CpG context since we have found these sites to be virtually unmethylated in *Nasonia* (<0.3%). For our data, 99.71% of these were converted to T. Both methods of determining the bisulfite conversion rate indicate that our false negative rate is less than 1%.

To identify individual cytosines that were significantly methylated in the *Nasonia* genome, we compared the number of converted and non-converted reads at each site. We used only sites that had coverage of more than 1 and less than 31 reads. We asked how likely these counts were under a binomial distribution where the probability of success is one minus the conversion rate, and corrected this probability value for multiple testing (BENJAMINI and HOCHBERG 1995). From this, we were able to determine the top methylated genes both by the number of methylated sites and the proportion of methylated sites.
Figures and Tables

Figure 1. Sequencing coverage over all cytosines in male *Nasonia* thorax. The left panel shows cumulative coverage, e.g. approximately 20% of sites are covered by 11 or more reads. The right panel gives the proportion of sites that have a certain level of coverage, e.g. approximately 2% of sites have a coverage of 14 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.

Figure 2. Annotations of methylation patterns for the top 20 most methylated genes by proportion. Black bars represent exonic regions and red circles represent methylated sites. Genes presented in order of ranking from left-to-right then top-to-bottom. The three isoforms of NV12835 are presented as a single annotation.

Figure 3. Gene Ontology (GO) categories associated with top 20 methylated genes by proportion of gene.

Table 1. Methylated cytosines in CG, CHG and CHH genomic contexts (*H* = A, T or C). Methylation occurs predominantly as CG sites.

Table 2. Mapping DNA methylation to genomic regions. The values represent the percentage of CGs that are methylated within a particular genomic region and the percentage of all methylation sites of a given type that fall in a particular region. For example 4.17% of CGs are methylated in exons and 62.07% of all mCpGs fall in exons.

Table 3. List of top 20 methylated genes by proportion of gene. Three genes marked with an asterisk are isoforms of the same gene. The percentages of methylated sites that occur in intronic regions are provided for genes that have more than one exon.
Supporting Information

Table S1. List of top 20 methylated genes by number of sites.

Figure S1. Gene Ontology (GO) categories associated with top 20 methylated genes by number of sites.

Table S2. Gene Ontology of top 20 methylated genes by proportion of gene. Sequence description and Gene Ontology (GO) terms were assigned by Blast2Go.

Table S3. Gene Ontology of top 20 methylated genes by number of sites. Sequence description and Gene Ontology (GO) terms were assigned by Blast2Go.
Availability of Supporting Data

The data set supporting the results of this article is available at: drewell.sites.hmc.edu/projects/restricted/Nasonia_methylome

Competing Interests

The author(s) declare that they have no competing interests

Author Contributions

SMB performed the Nasonia dissections, gDNA preparation, participated in the design of the study and sequence analysis and drafted the manuscript. GTW established the genomics pipeline and carried out the sequence analysis. JMZ performed the Nasonia dissections and gDNA preparation. ECB established the genomics pipeline and participated in the design and coordination of the study. EJR and BPO conceived of the study and helped to draft the manuscript. RAD conceived of the study, and participated in its design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Patrick Feree for his advice on Nasonia rearing and dissection. This work was funded by National Institutes of Health (GM090167) and National Science Foundation (IOS-0845103) grants to RAD, a National Science Foundation (MCB-0918335) grant to EB, an Australian Research Council Discovery grant to BPO, and Howard Hughes Medical Institute Undergraduate Science Education Program grants (52006301 and 52007544) to the Biology department at Harvey Mudd College.
References

Altshuler, D., V. J. Pollara, C. R. Cowles, W. J. Van Etten, J. Baldwin et al., 2000 An SNP map of the human genome generated by reduced representation shotgun sequencing. Nature 407: 513-516.

Benjamini, Y., and Y. Hochberg, 1995 Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B 57: 289-300.

Drewell, R. A., N. Lo, P. R. Oxley and B. P. Oldroyd, 2012 Kin conflict in insect societies: a new epigenetic perspective. Trends in Ecology and Evolution 27: 367-373.

Elango, N., and S. V. Yi, 2008 DNA methylation and structural and functional bimodality of vertebrate promoters. Molecular Biology and Evolution 25: 1601-1608.

Falckenhayn, C., B. Boerjan, G. Raddatz, M. Frohme, L. Schoofs et al., 2013 Characterization of genome methylation patterns in the desert locust Schistocerca gregaria. J Exp Biol 216: 1423-1429.

Flores, K., F. Wolschin, J. J. Corneveaux, A. N. Allen, M. J. Huentelman et al., 2012 Genome-wide association between DNA methylation and alternative splicing in an invertebrate. BMC Genomics 13: 480.

Flores, K. B., and G. V. Amdam, 2011 Deciphering a methylome: what can we read into patterns of DNA methylation? J Exp Biol 214: 3155-3163.

Foret, S., R. Kucharski, M. Pellegrini, S. Feng, S. E. Jacobsen et al., 2012 DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. Proc Natl Acad Sci USA 109: 4968-4973.

Glastad, K. M., B. G. Hunt, S. V. Yi and M. A. Goodisman, 2011 DNA methylation in insects: on the brink of the epigenomic era. Insect Molecular Biology 20: 553-565.

Götz, S., J. M. García-Gómez, J. Terol, T. D. Williams, S. H. Nagaraj et al., 2008 High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 36: 3420-3435.

Herb, B. R., F. Wolschin, K. D. Hansen, M. J. Aryee, B. Langmead et al., 2012 Reversible switching between epigenetic states in honeybee behavioral subcastes. Nature Neuroscience 15: 1371-1373.

Jones, P. A., and D. Takai, 2001 The role of DNA methylation in mammalian epigenetics. Science 293: 1068-1070.

Li-Byarlay, H., Y. Li, H. Stroud, S. Feng, T. C. Newman et al., 2013 RNA interference knockdown of DNA methyltransferase 3 affects gene alternative splicing in the honey bee. Proc Natl Acad Sci USA 110: 12750-12755.

Li, E., 2002 Chromatin modification and epigenetic reprogramming in mammalian development. Nature Reviews Genetics 3: 662-673.

Lister, R., M. Pelizzola, R. H. Dowen, R. D. Hawkins, G. Hon et al., 2009 Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462: 315-322.

Lyko, F., S. Foret, R. Kucharski, S. Wolf, C. Falckenhayn et al., 2010 The honey bee epigenomes: differential methylation of brain DNA in queens and workers. PLoS Biology 8: e1000506.
LYKO, F., and R. MALESZKA, 2011 Insects as innovative models for functional studies of DNA methylation. Trends in Genetics 27: 127-131.

MUNOZ-TORRES, M. C., J. T. REESE, C. P. CHILDE, A. K. BENNETT, J. P. SUNDARAM et al., 2011 Hymenoptera Genome Database: integrated community resources for insect species of the order Hymenoptera. Nucleic Acids Res. 39: D658-662.

PARK, J., Z. PENG, J. ZENG, N. ELANGO, T. PARK et al., 2011 Comparative analyses of DNA methylation and sequence evolution using Nasonia genomes. Molecular Biology and Evolution 28: 3345-3354.

PATALANO, S., T. A. HORE, W. REIK and S. SUMNER, 2012 Shifting behaviour: epigenetic reprogramming in eusocial insects. Curr. Opin. Cell Biol. 24: 367-373.

SCHWARTZ, S., and G. AST, 2010 Chromatin density and splicing destiny: on the cross-talk between chromatin structure and splicing. EMBO J. 29: 1629-1636.

SHI, Y. Y., W. Y. YAN, Z. Y. HUANG, Z. L. WANG, X. B. WU et al., 2012 Genomewide analysis indicates that queen larvae have lower methylation levels in the honey bee (Apis mellifera). Naturwissenschaften 100: 193-197.

VERHULST, E. C., L. W. BEUKEBOOM and L. VAN DE ZANDE, 2010 Maternal control of haplodiploid sex determination in the wasp Nasonia. Science 328: 620-623.

WERREN, J. H., and D. W. LOEHLIN, 2009 Strain maintenance of Nasonia vitripennis (parasitoid wasp). Cold Spring Harbor Protocols 2009: pdb.prot5307.

WERREN, J. H., S. RICHARDS, C. A. DESJARDINS, O. NIEHUIS, J. GADAU et al., 2010 Functional and evolutionary insights from the genomes of three parasitoid Nasonia species. Science 327: 343-348.

WURM, Y., J. WANG, O. RIBA-GROGNUZ, M. CORONA, S. NYGAARD et al., 2011 The genome of the fire ant Solenopsis invicta. Proc Natl Acad Sci USA 108: 5679-5684.

XI, Y., and W. LI, 2009 BSMAP: whole genome bisulfite sequence MAPping program. BMC Bioinformatics 27: 10:232.

XIAO, H., J. ZHU, Q. CHEN, F. DAI, X. LI et al., 2010 Single base-resolution methylome of the silkworm reveals a sparse epigenomic map. Nature Biotechnology 28: 516-520.

ZWIER, M. V., E. C. VERHULST, R. D. ZWAHLEN, L. W. BEUKEBOOM and L. VAN DE ZANDE, 2012 DNA methylation plays a crucial role during early Nasonia development. Insect Molecular Biology 21: 129-138.
|        | Sites in Genome | Methylated Sites | % of all mCGs |
|--------|-----------------|------------------|---------------|
| CG     | 28,048,814      | 175,884          | 99.7          |
| CHG    | 16,637,411      | 97               | .055          |
| CHH    | 54,832,489      | 431              | .244          |
| Unclassified | 19,320    | 1                | 5.67 × 10⁻⁴  |
| Total  | 99,538,034      | 176,413          |               |
| Genomic Location | CGs      | mCGs     | % mCGs | % of all mCG |
|------------------|----------|----------|--------|--------------|
| Exons            | 2,625,400| 109,496  | 4.17   | 62.07        |
| Introns          | 8,935,722| 31,386   | 0.351  | 17.79        |
| Intergenic Regions | 16,487,692| 35,531 | 0.216  | 20.14        |
| Gene Name     | Scaffold Number | Proportion Methylated | Sites Methylated | % of sites in introns |
|--------------|----------------|-----------------------|------------------|----------------------|
| NV21674-RA   | 1              | 0.08811               | 20               | n/a                  |
| NV12835-RC*  | 9              | 0.08025               | 52               | 9.26                 |
| NV12835-RB*  | 9              | 0.08025               | 52               | 9.26                 |
| NV12835-RA*  | 9              | 0.08025               | 52               | 9.26                 |
| NV10326-RA   | 1              | 0.07347               | 18               | n/a                  |
| NV14078-RA   | 16             | 0.07023               | 21               | n/a                  |
| NV15491-RA   | 27             | 0.06983               | 28               | n/a                  |
| NV12080-RA   | 7              | 0.06852               | 42               | 28.6                 |
| NV18118-RA   | 136            | 0.06739               | 31               | 19.4                 |
| NV23881-RA   | 1270           | 0.06485               | 31               | 19.4                 |
| NV15716-RA   | 28             | 0.06237               | 30               | 13.3                 |
| NV14867-RA   | 21             | 0.06080               | 29               | 17.2                 |
| NV12600-RA   | 9              | 0.05908               | 68               | 73.5                 |
| NV17630-RA   | 94             | 0.05902               | 18               | n/a                  |
| NV30486-RA   | 18             | 0.05727               | 13               | n/a                  |
| NV14145-RA   | 16             | 0.05719               | 37               | n/a                  |
| NV12795-RA   | 9              | 0.05534               | 70               | n/a                  |
| NV11037-RA   | 3              | 0.05495               | 20               | 10.0                 |
| NV10438-RA   | 1              | 0.05491               | 33               | 3.03                 |
| NV15750-RA   | 29             | 0.05487               | 40               | 0.00                 |
