# Comparative Genomics

# The conserved functional role of non-CpG methylation in mammalian and avian brain

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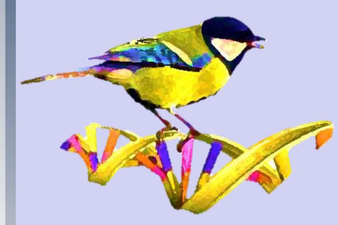
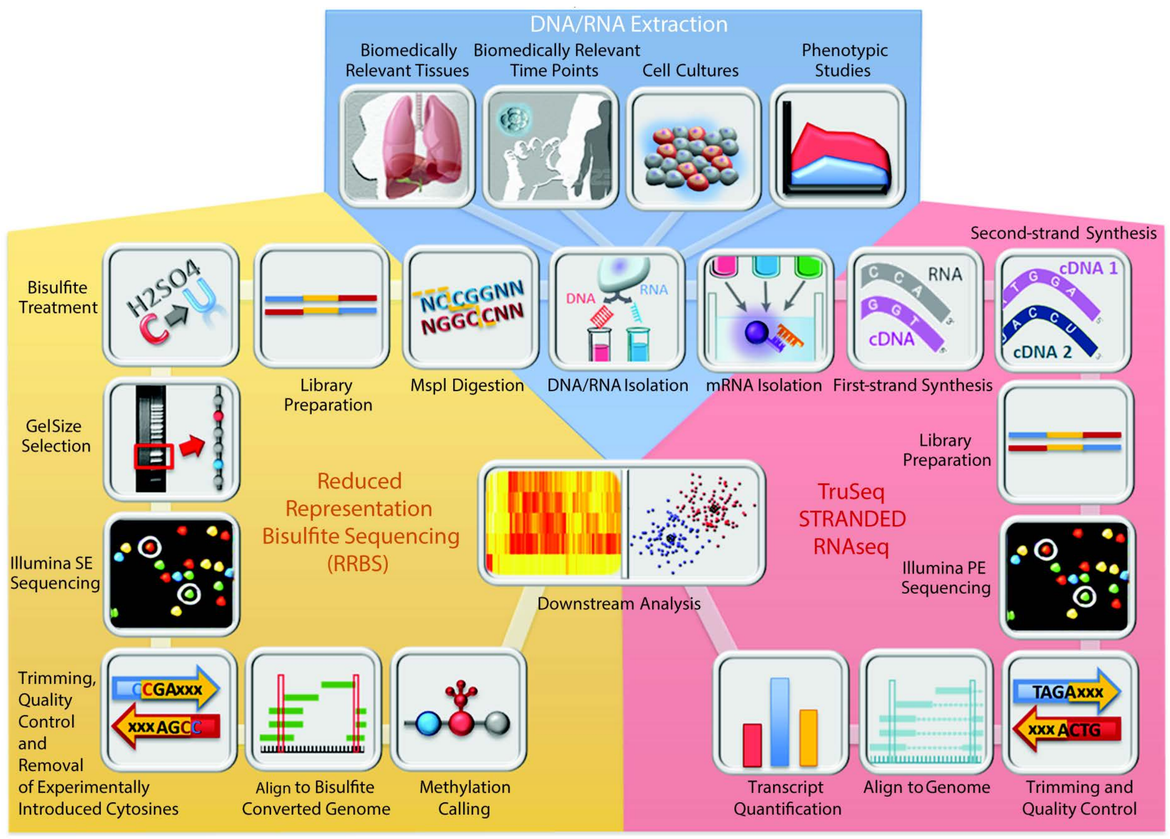


## Introduction:

- DNA methylation is an epigenetic regulator of gene expression that plays a role in many cellular processes affecting a variety of traits.
- DNA methylation mainly occurs at CpG sites in animals.
- Non-CpG methylation is observed in embryonic stem cells, oocytes, and neurons of humans and mice.
- Both CpG and non-CpG methylation are negatively correlated with gene expression at transcription start sites (TSS) and gene bodies in human and mouse neurons.
- DNA methylation also suppresses transposable element (TE) activity, and TEs are highly active in neuronal tissue.
- Little is known about the regulatory role of non-CpG methylation in porcine and great tit neurons.

## Materials and Methods:




- Frontal lobe DNA methylation patterns were assessed in three pigs using reduced representation bisulfite sequencing (RRBS)
  - Alignment and methylation calling using BSseeker2
  - 1.5 million CpG and 5.5 million non-CpG sites covered
- Whole brain DNA methylation patterns were assessed in one great tit using whole genome bisulfite sequencing (WGBS)
  - Alignment and methylation calling using BSseeker2
  - 10.2 million CpG and 167.4 million non-CpG sites covered
- Gene transcription patterns were profiled using RNA-seq
  - Alignments performed using Tophat2
  - Expression quantification performed using Cufflinks
- Whole brain TE transcription patterns were profiled using RNA-seq in the great tit
  - Alignments performed using Tophat2
  - Expression quantification performed using Cufflinks

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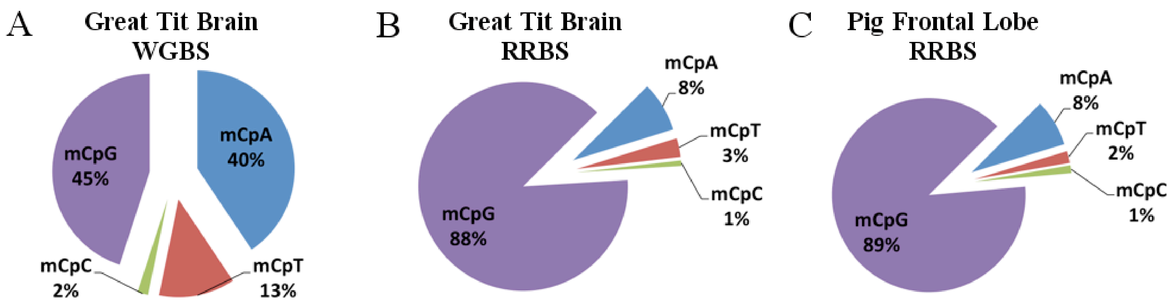
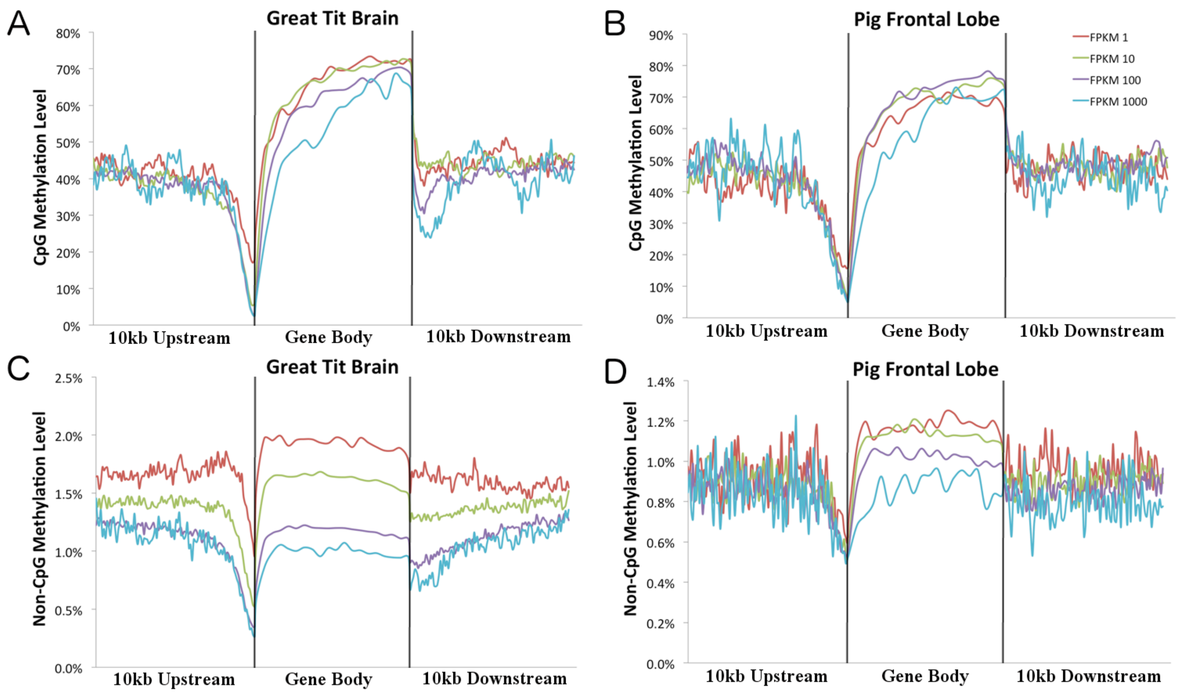
## Results:

Figure 1: Neuronal DNA methylation distribution amongst dinucleotides



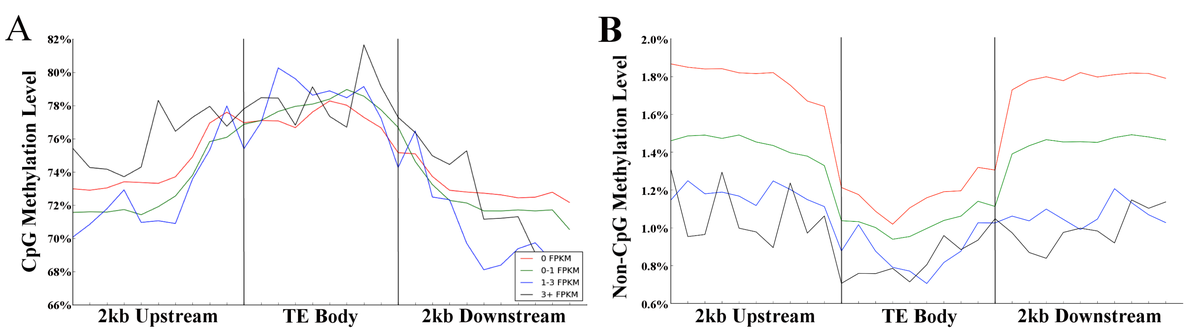

Distribution of methylated (>10%) cytosines in (a) great tit brain and (c) pig frontal lobe. Differences between (a) great tit and (b) pig neuronal patterns are due to differences between (a) WGBS and (b) RRBS techniques.

Figure 2: DNA methylation is negatively correlated with gene expression




(a) Great tit brain and (b) pig frontal lobe CpG methylation is negatively correlated with gene expression at TSS (Spearman's Rho = -0.30 and -0.16, respectively, $P < 1x10^{-15}$) and gene bodies (Spearman's Rho = -0.32 and -0.08, respectively, $P < 1x10^{-15}$). (c) Great tit brain and (d) pig frontal lobe non-CpG methylation is negatively correlated with gene expression at TSS (Spearman's Rho = -0.24 and -0.05, respectively, $P < 1x10^{-14}$) and gene bodies (Spearman's Rho = -0.46 and -0.11, respectively, $P < 1x10^{-15}$).

Figure 3: Non-CpG methylation is associated with TE activity in great tit brain




(a) TE CpG methylation is not correlated with TE activity in great tit brain (Spearman's Rho TE body = 0.033, upstream = -0.006, downstream = -0.002). (b) TE non-CpG methylation is negatively correlated with TE activity in great tit brain (Spearman's Rho TE body = -0.11, upstream = -0.20, downstream = -0.19, $P < 1x10^{-15}$).

## Conclusions:

- Both CpG and non-CpG methylation are negatively correlated with gene expression in great tit and pig neurons.
- Non-CpG methyation is negatively correlated with TE activity in great tit neurons.
- These findings provide the first evidence for conservation of non-CpG methylation between mammalian and avian neurons, and suggest a functional role for non-CpG methylation in avian neurons.