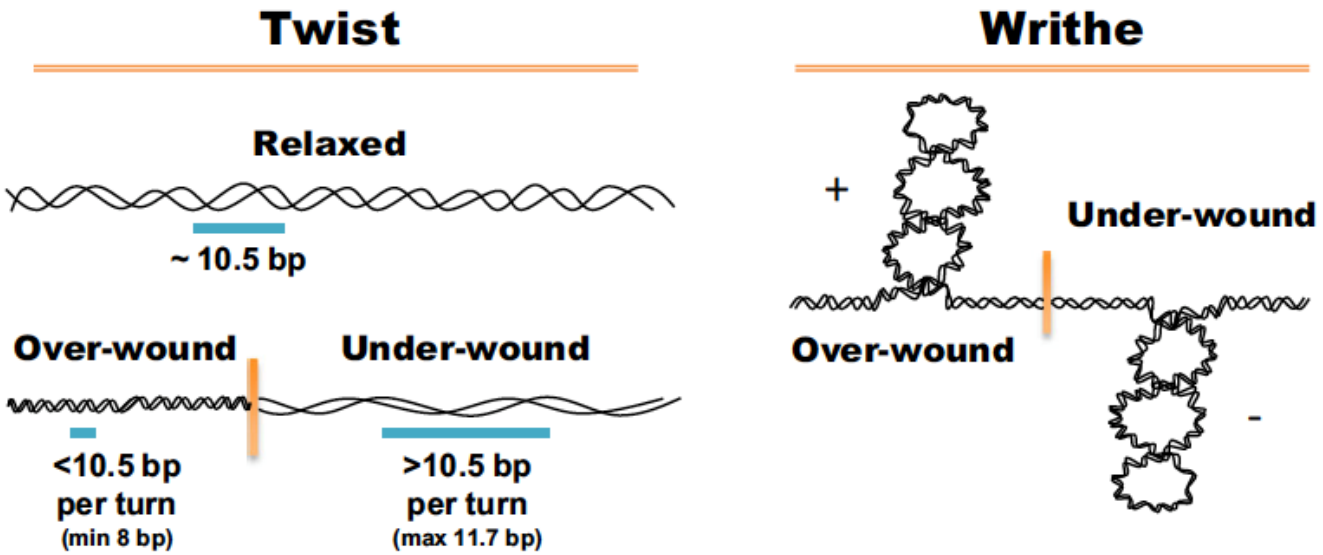
Transcription dependent propagation of DNA supercoiling

Catherine Naughton

Gilbert Lab

Biomedical Physics at Edinburgh
26th May 2025

DNA supercoiling



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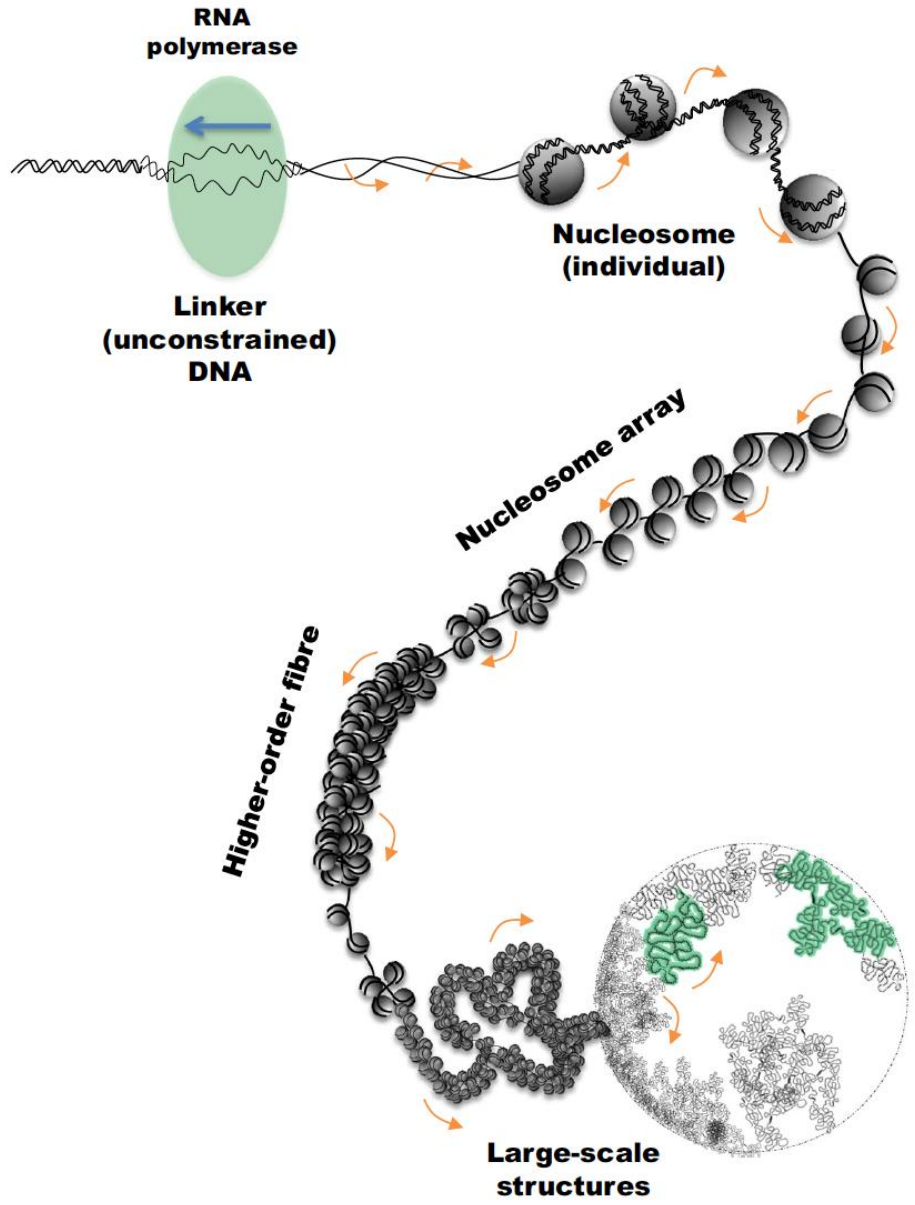

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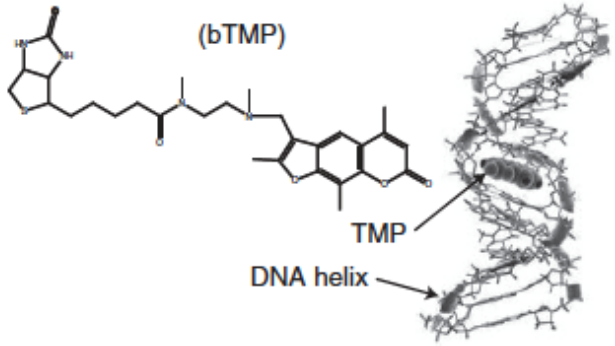

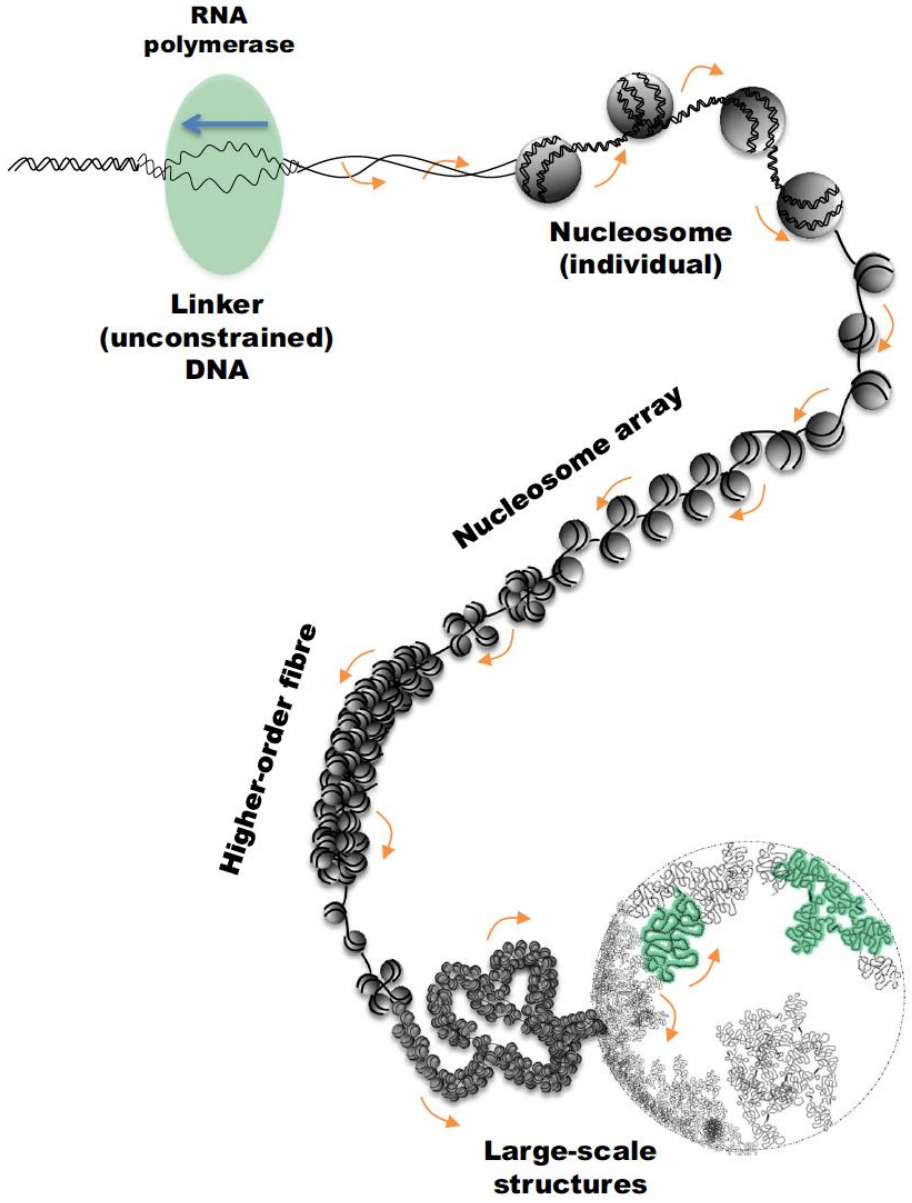

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Aim:

Use experimental data to determine the supercoiling flux between genes and relate this to gene orientation and local chromatin environment.

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Use experimental data to determine the supercoiling flux between genes and relate this to gene orientation and local chromatin environment.

Hypothesis:

DNA supercoiling can propagate from one gene to neighbouring genes and activate transcription

A Stochastic Model of Supercoiling-Dependent Transcription

Model incorporates two experimental observations:

1. Transcription by RNA polymerases creates supercoiling (positive in front and negative behind)

2. RNA polymerase binds more favorably to regions of the genome that are negatively supercoiled/unwound.

Brackley CA, Johnson J, Bentivoglio A, Corless S, **Gilbert N**, Gonnella G, **Marenduzzo D**. Phys Rev Lett. 2016

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Mapping DNA supercoiling genome wide – bTMP-seq

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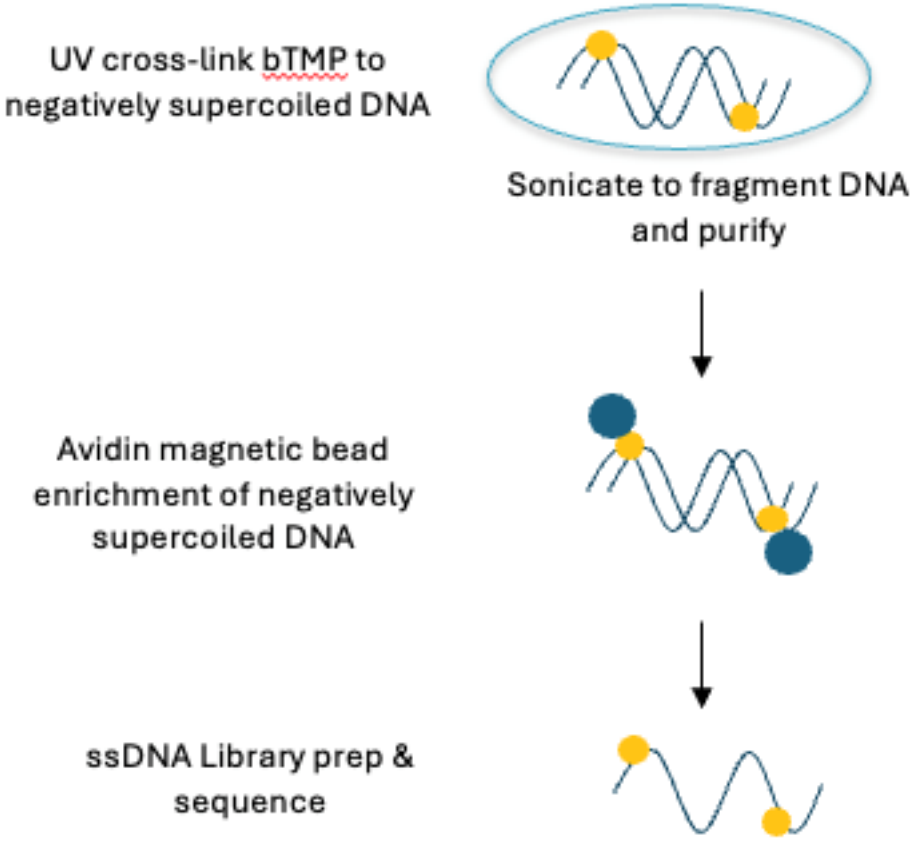
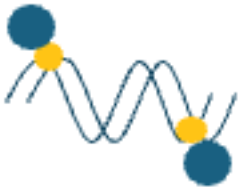


Mapping DNA supercoiling genome wide – bTMP-seq

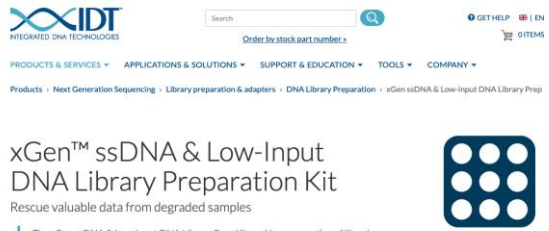
UV cross-link bTMP to negatively supercoiled DNA

Sonicate to fragment DNA and purify

Avidin magnetic bead enrichment of negatively supercoiled DNA



ssDNA Library prep & sequence

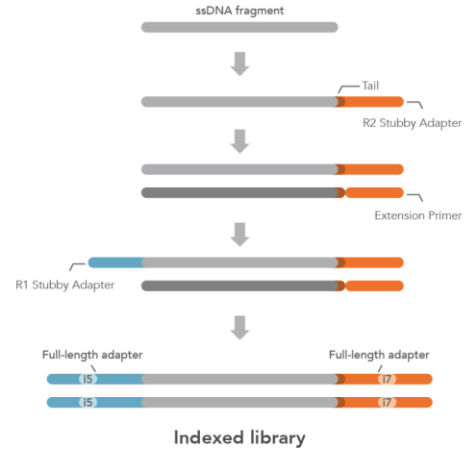


Adaptase technology
17 minutes

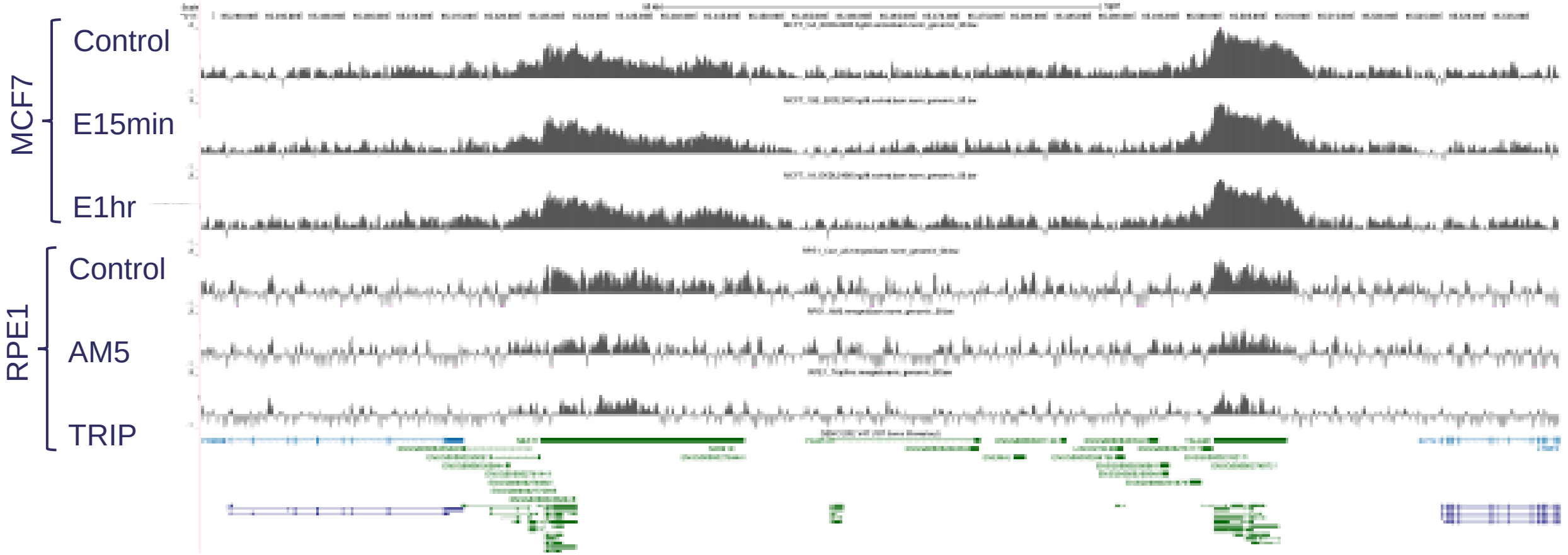
Extension
8 minutes

Ligation
15 minutes

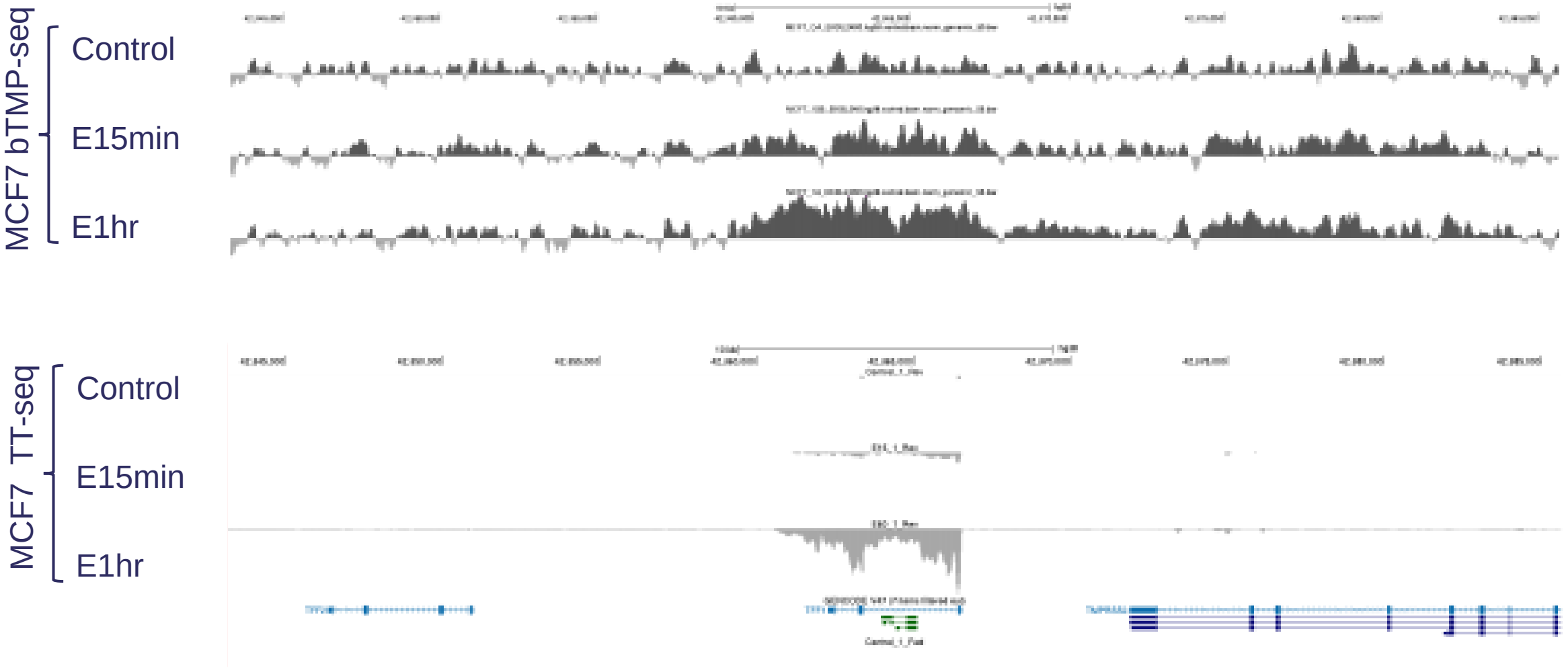
Indexing PCR
Time varies



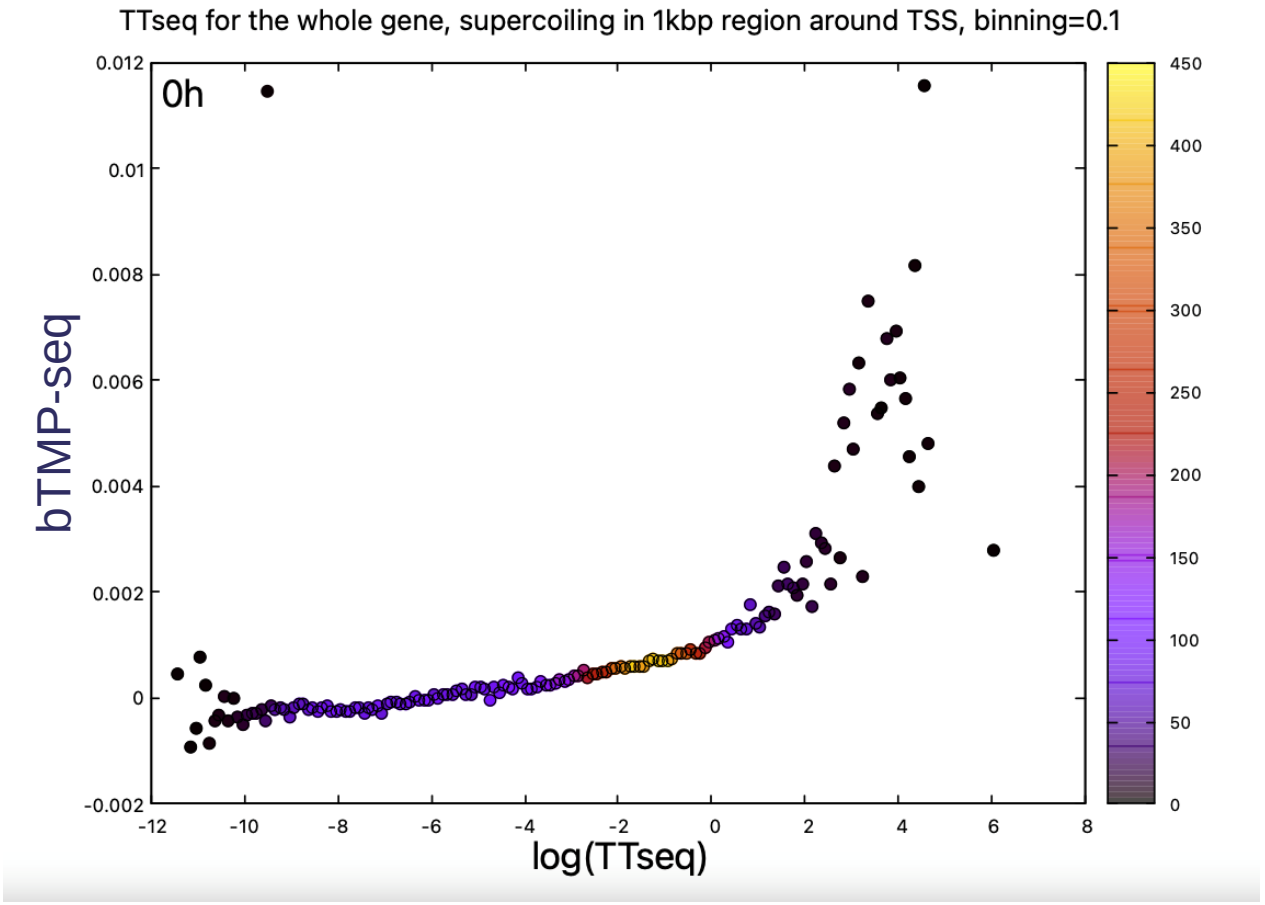
Mapping DNA supercoiling: Neat1 & MALAT1



Mapping DNA supercoiling: TFF1



Transcription dependent DNA supercoiling



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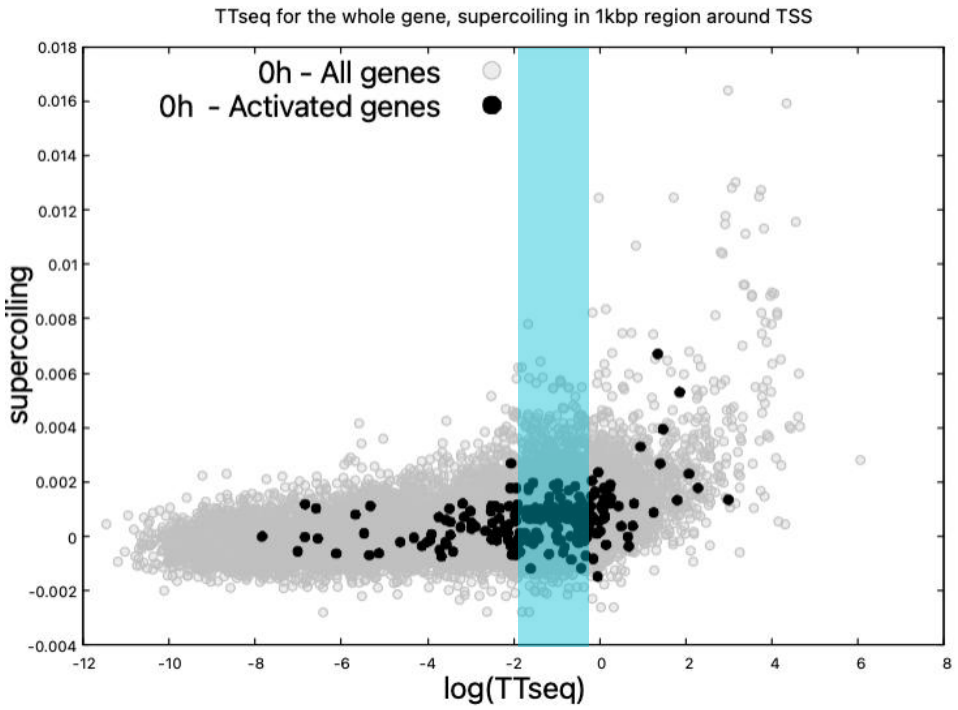
Transcription dependent DNA supercoiling

Supercoiling vs TTseq for estrogen activated genes



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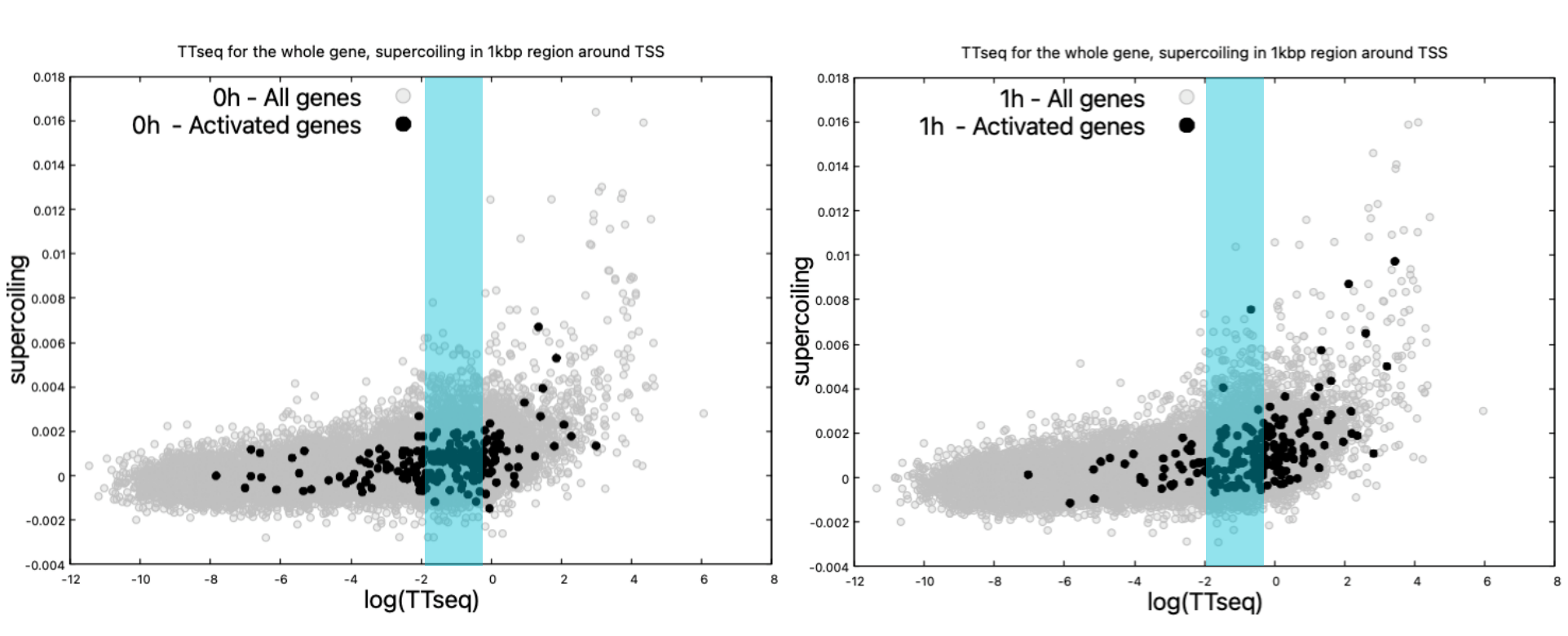

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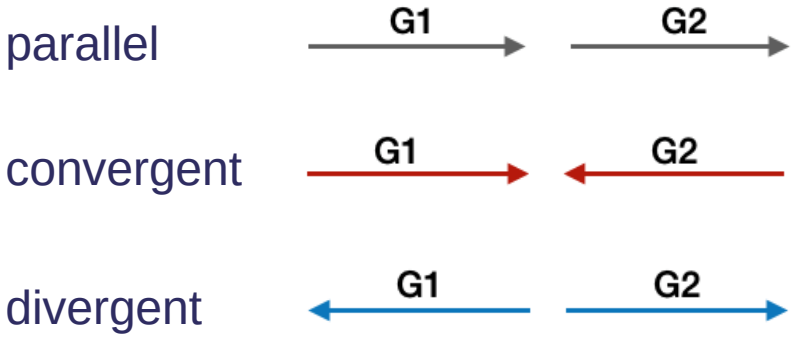

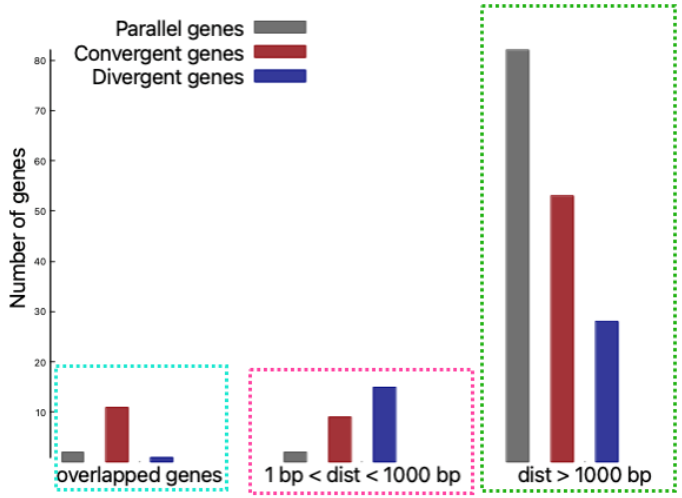

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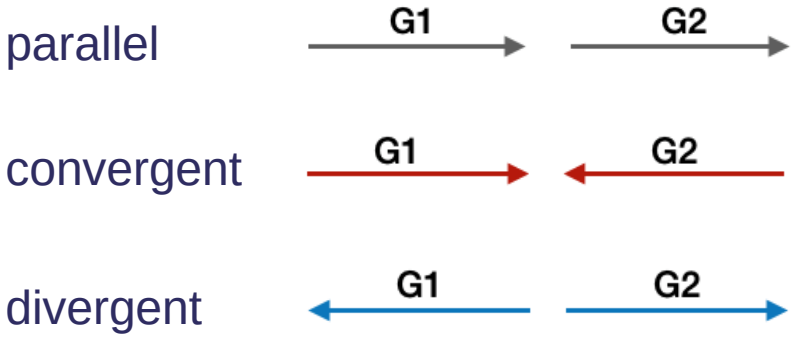

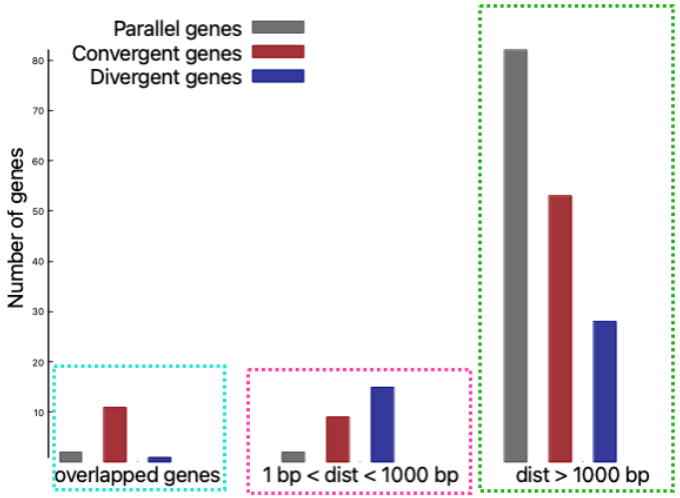

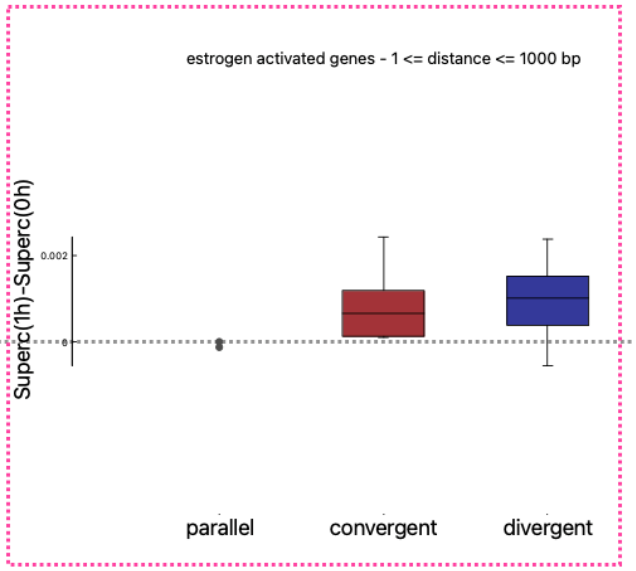

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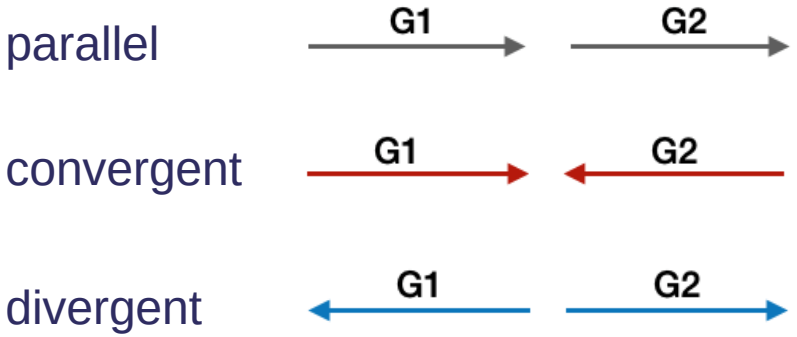

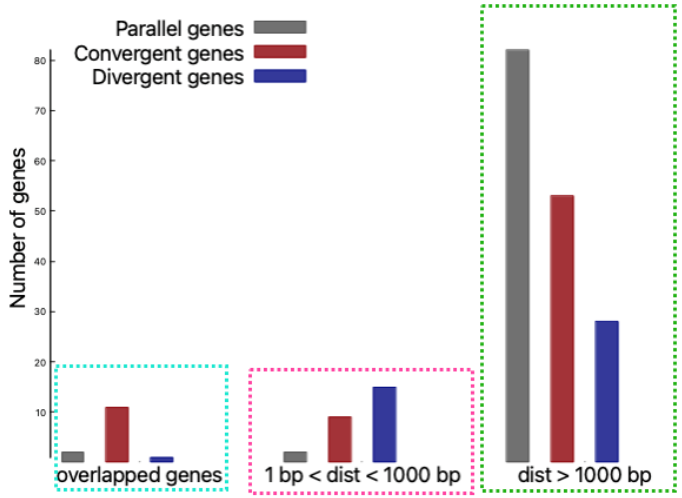

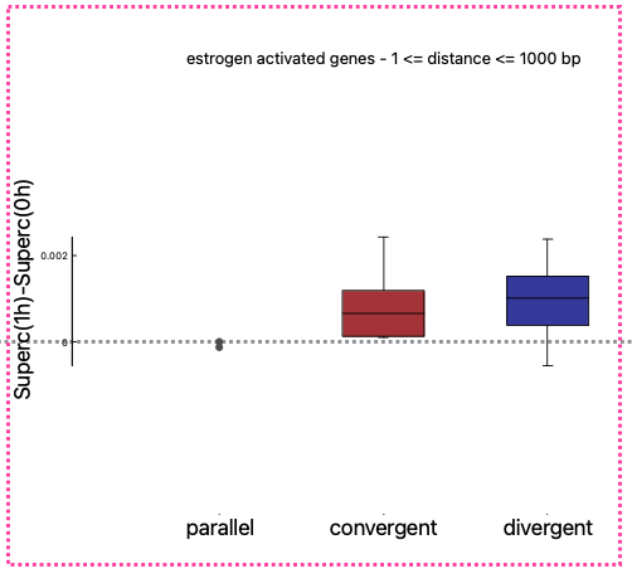

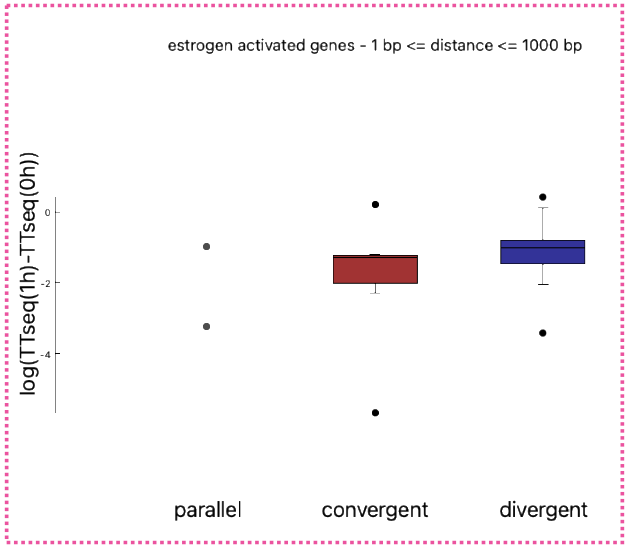

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DNA supercoiling at promoters

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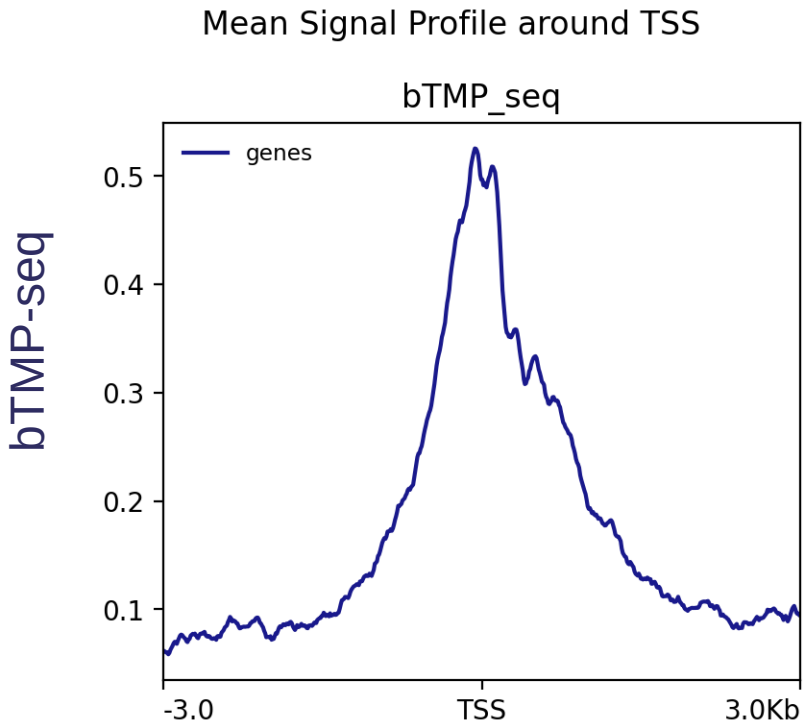
DNA supercoiling at all genes (1bp resolution)



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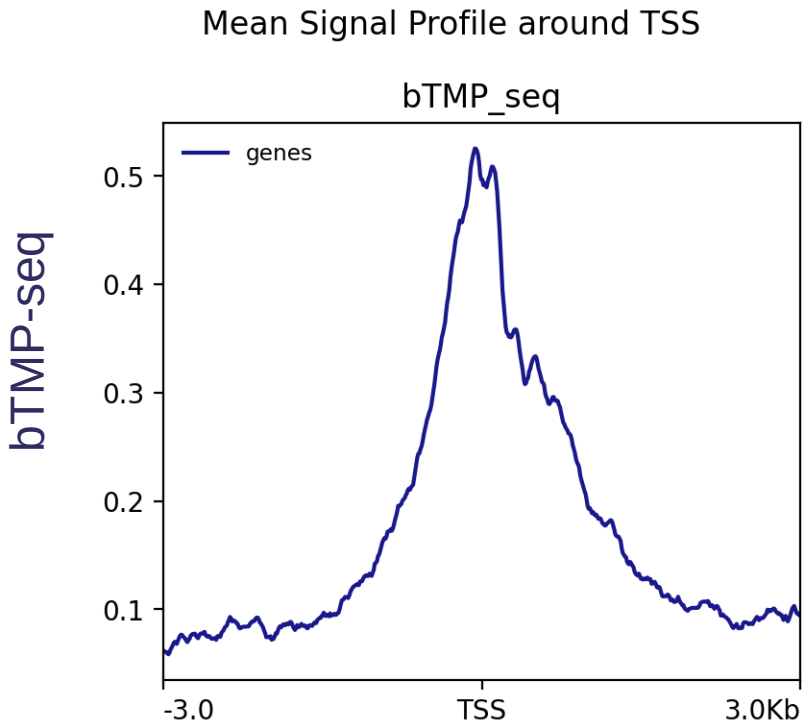
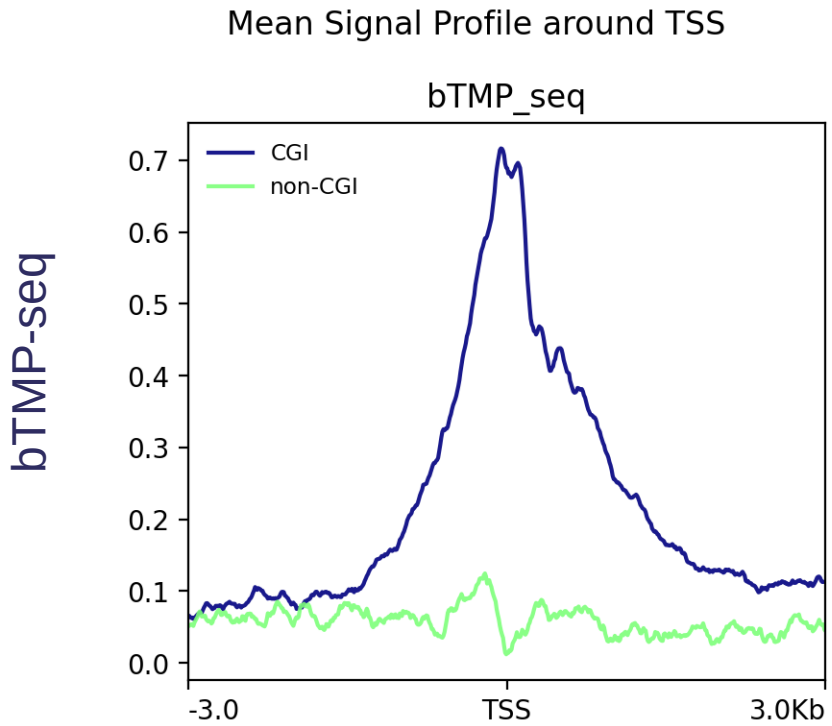


Under-wound DNA enriched at CpG island genes



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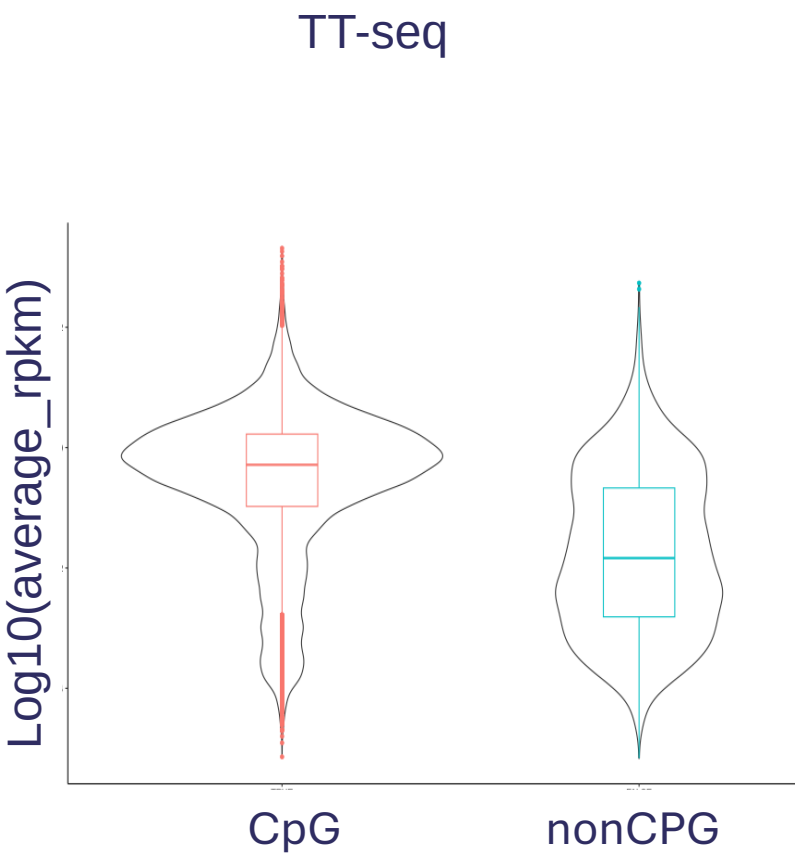
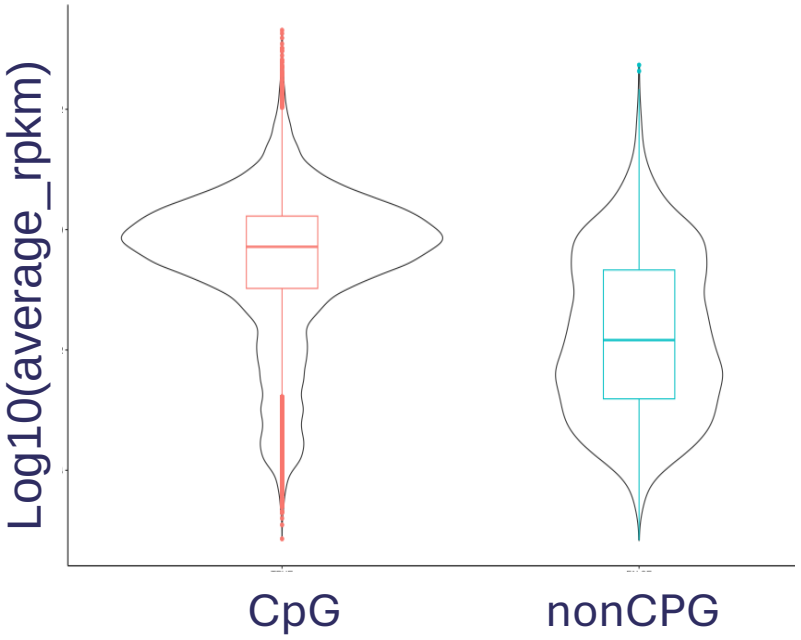
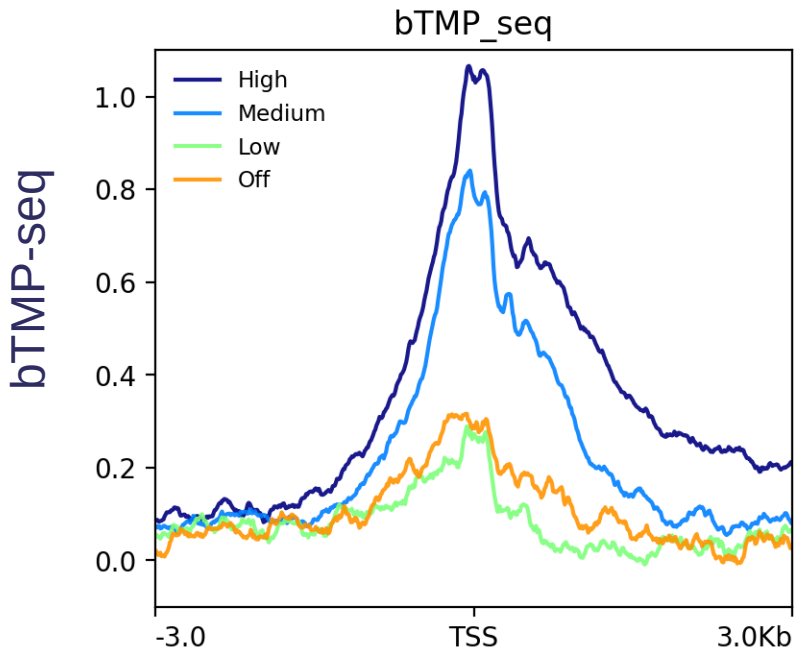


DNA supercoiling at promoters

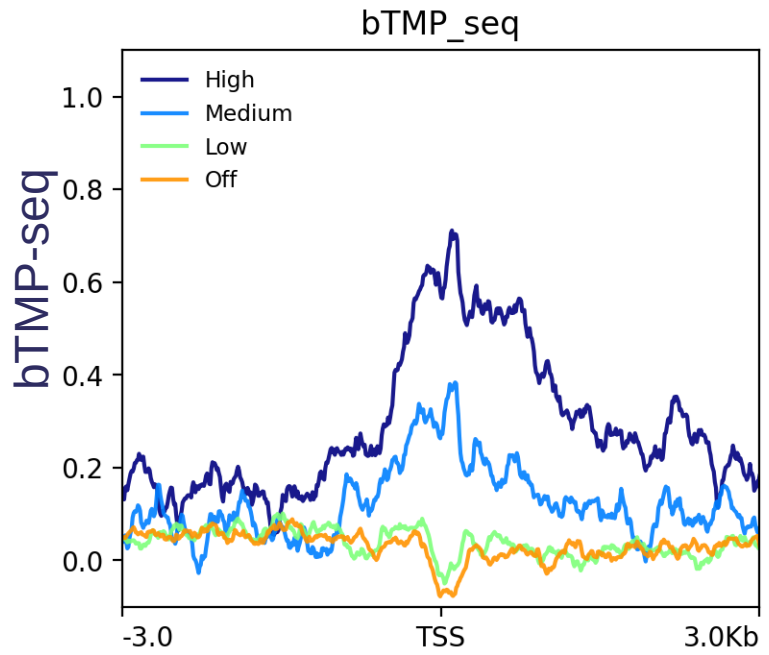
TT-seq



CpG island genes



Non CpG island genes



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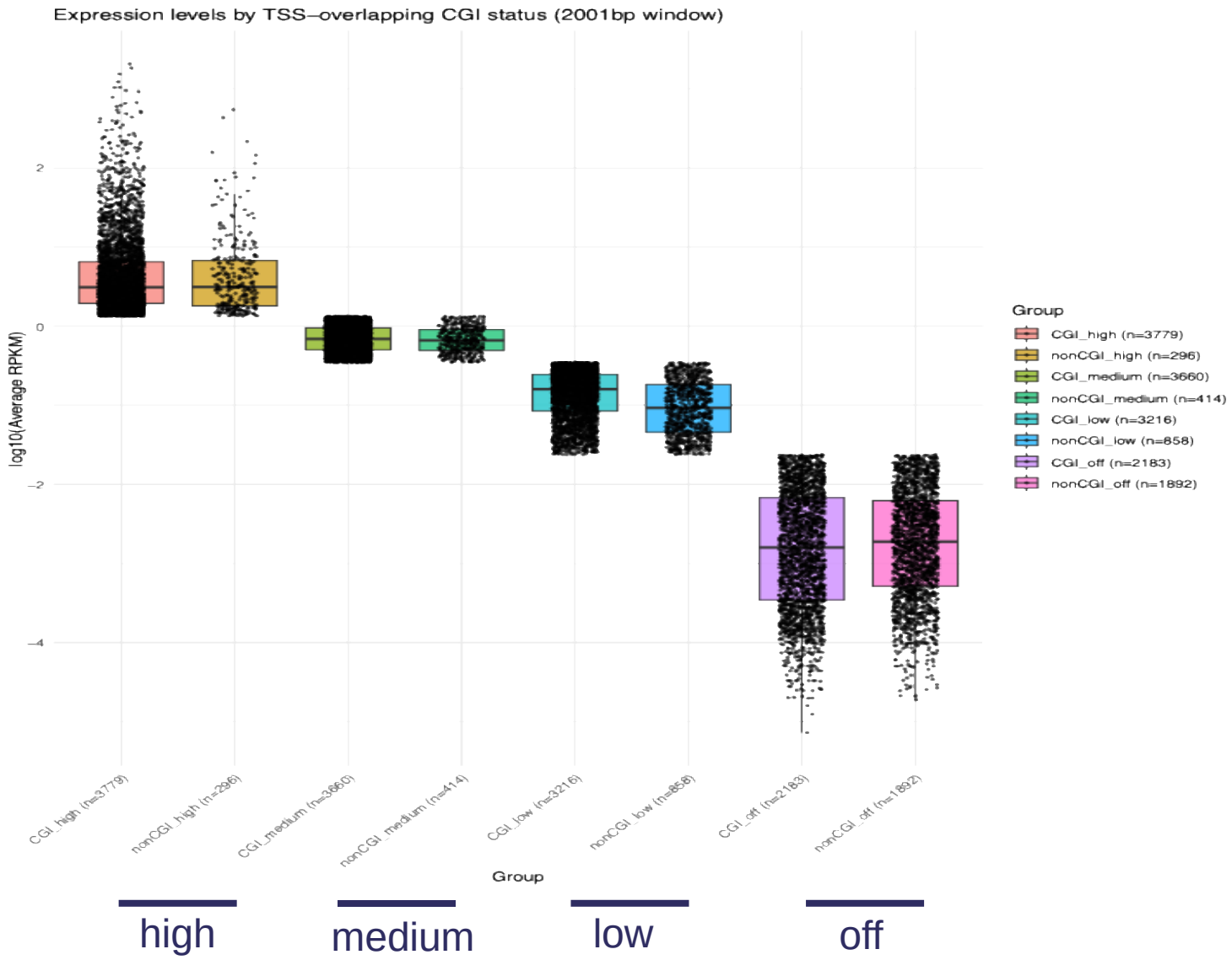

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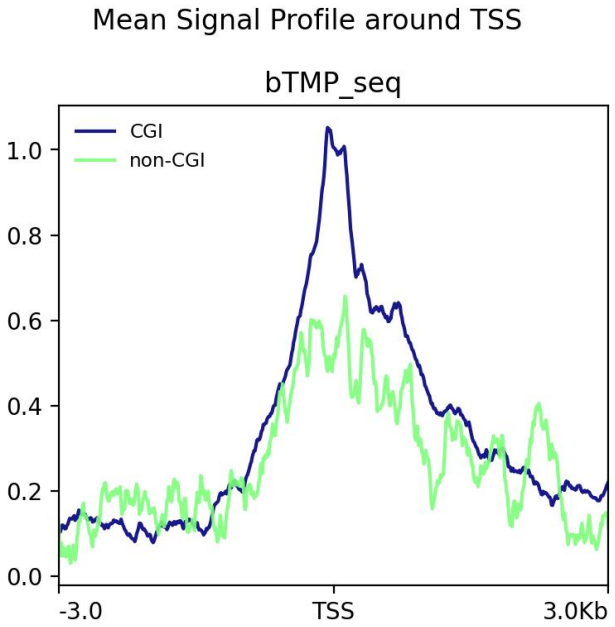

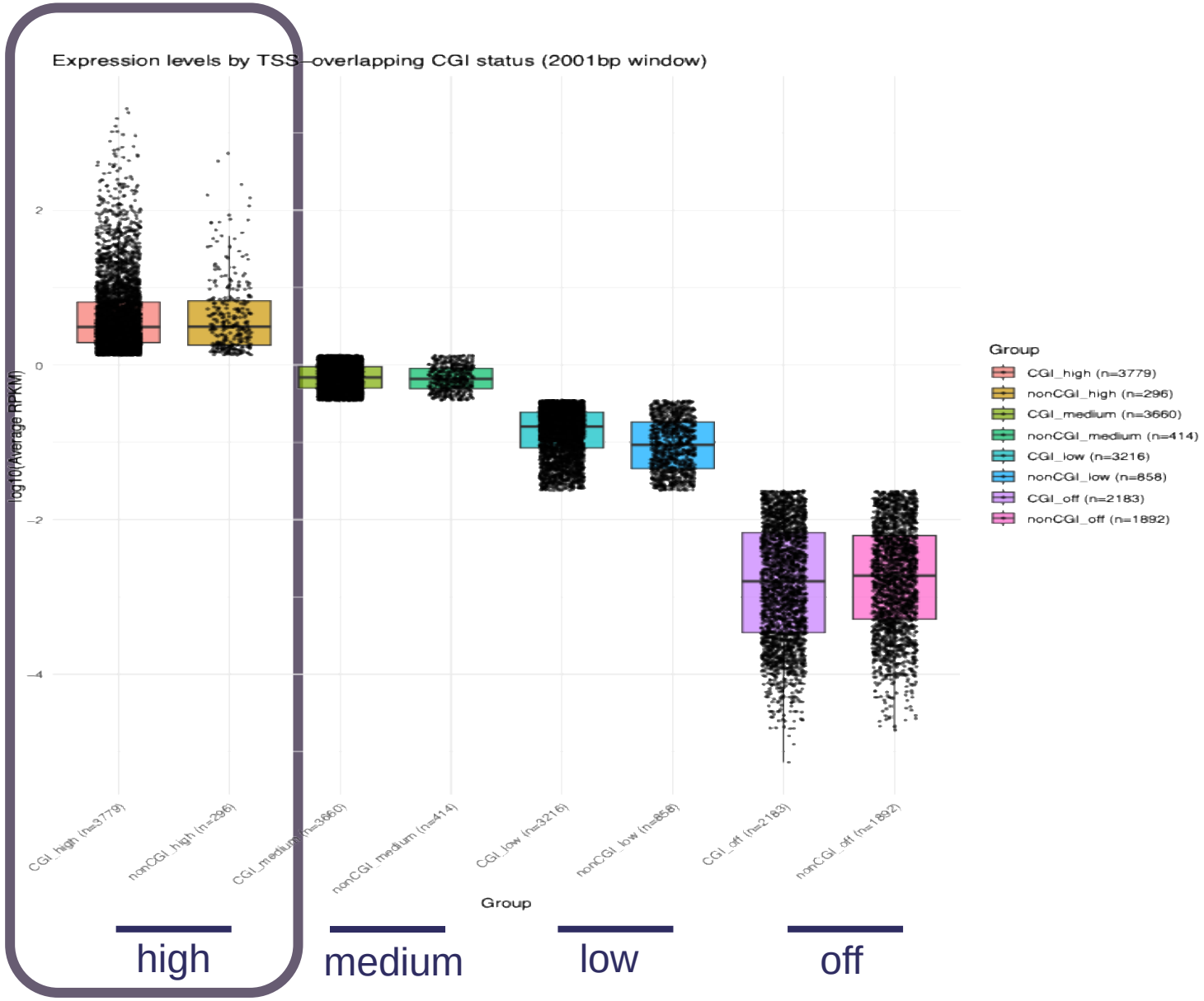

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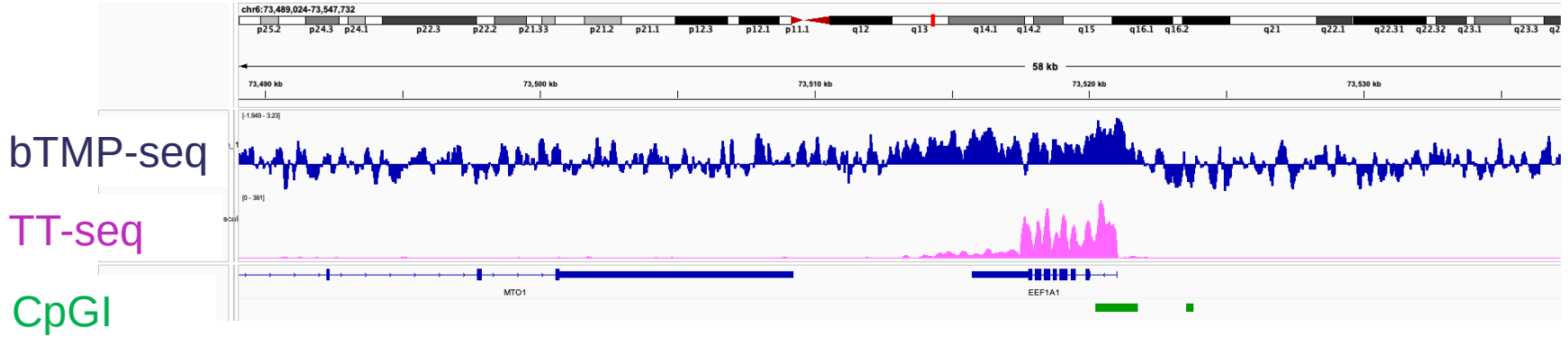

bTMP-seq

TT-seq

CpGI

+CGI

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+CGI

-CGI

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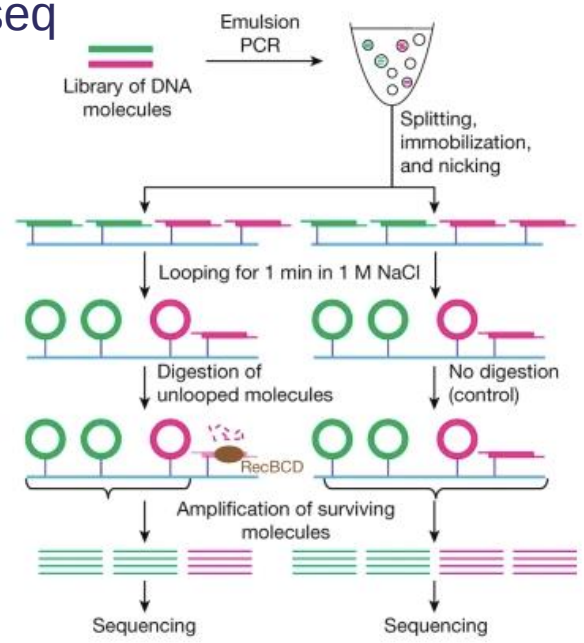
Loop-seq



Basu *et al.* 2022

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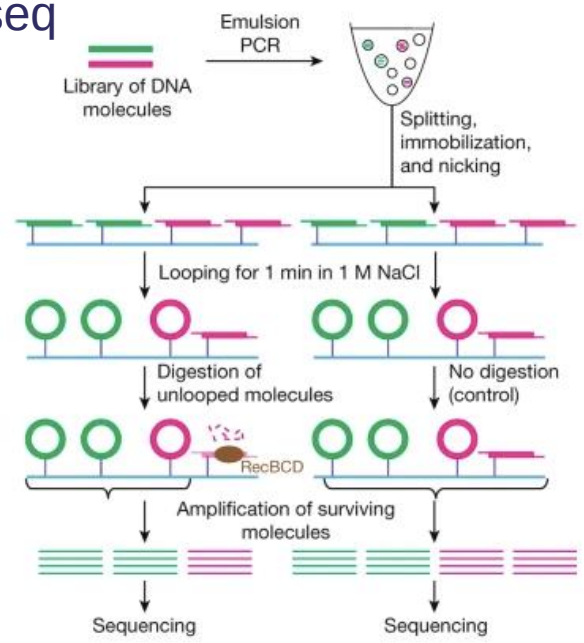

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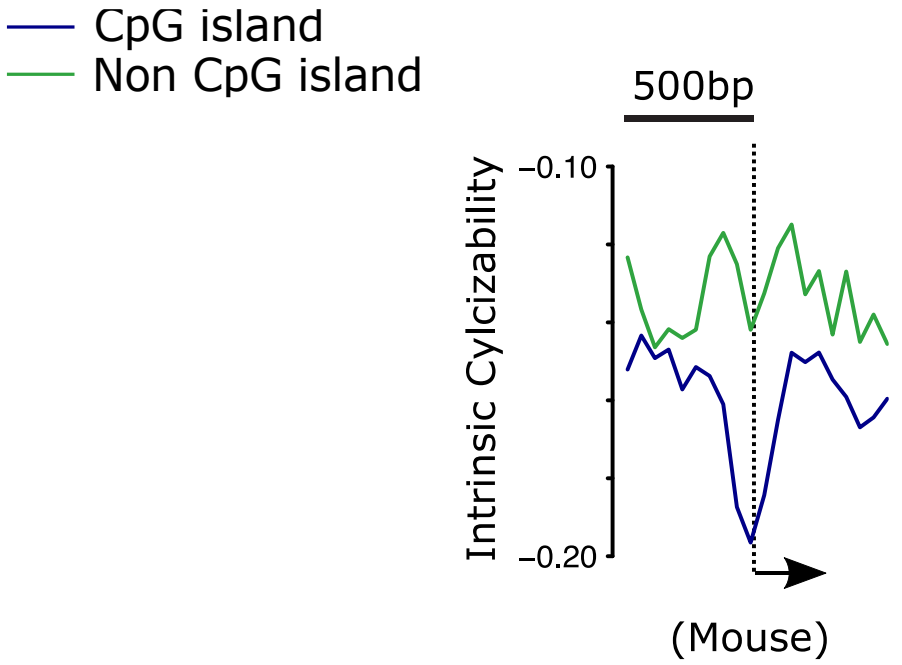

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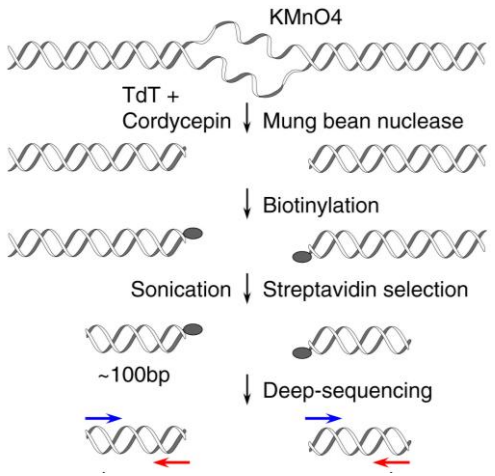

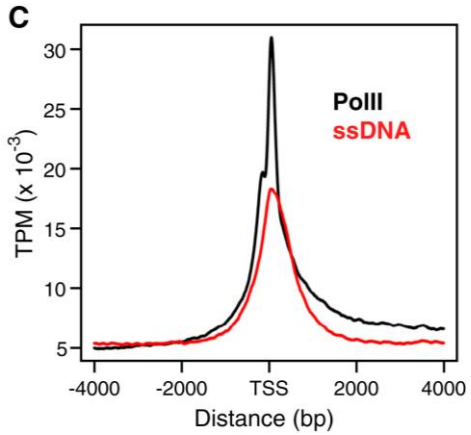

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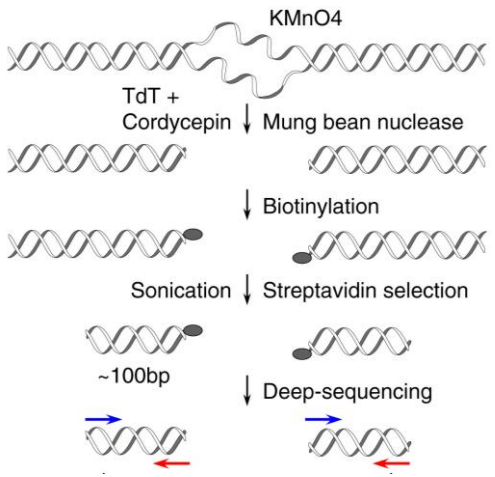
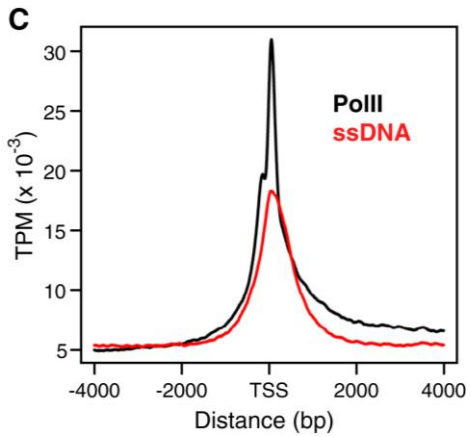
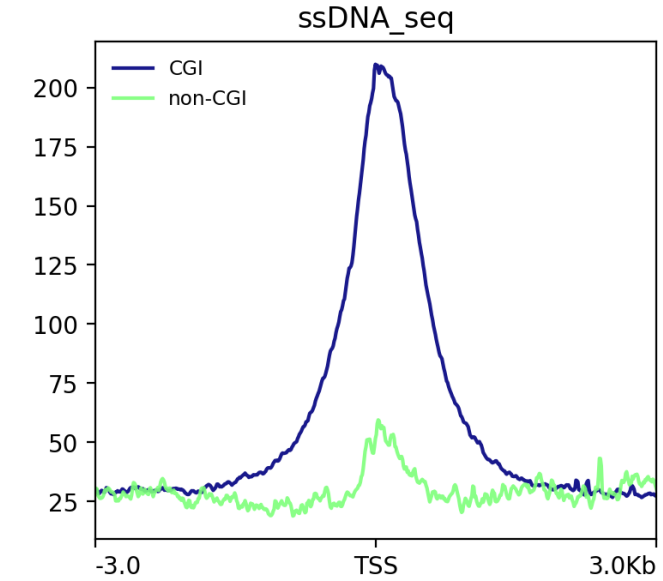
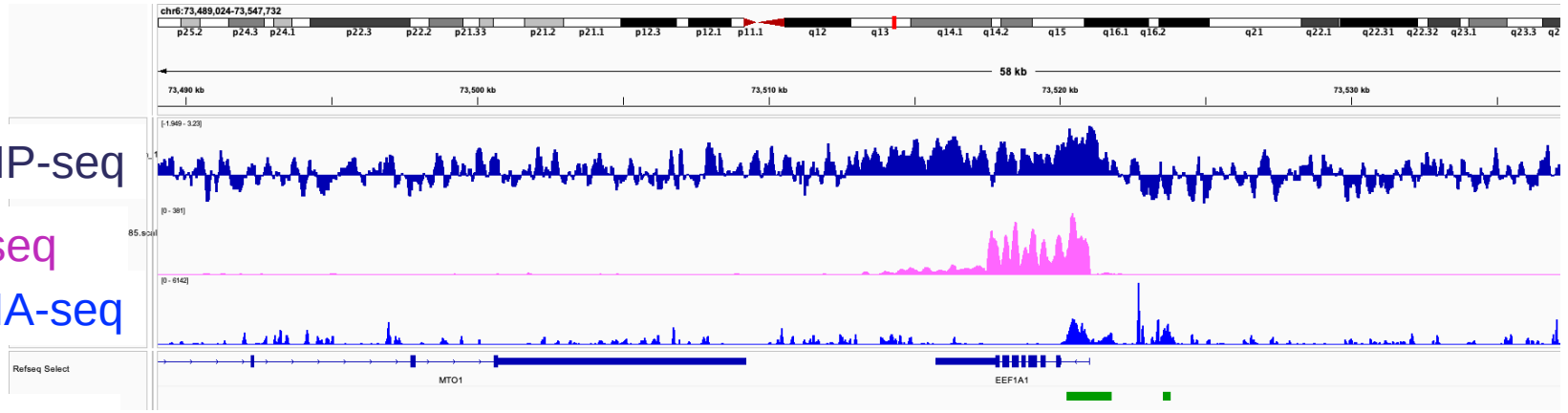
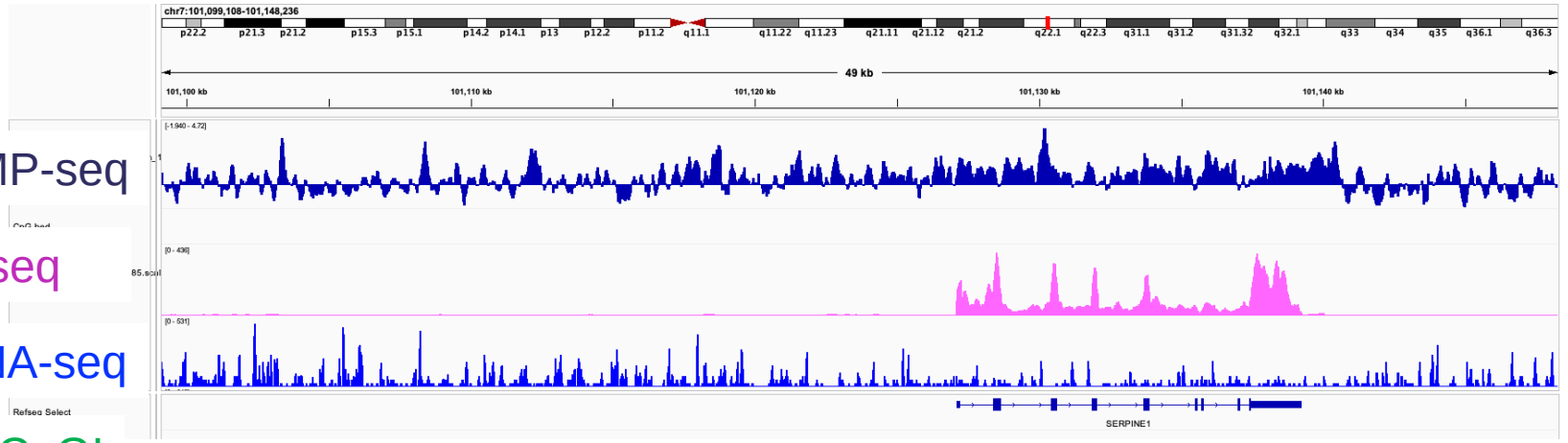






ssDNA enriched at CpGI





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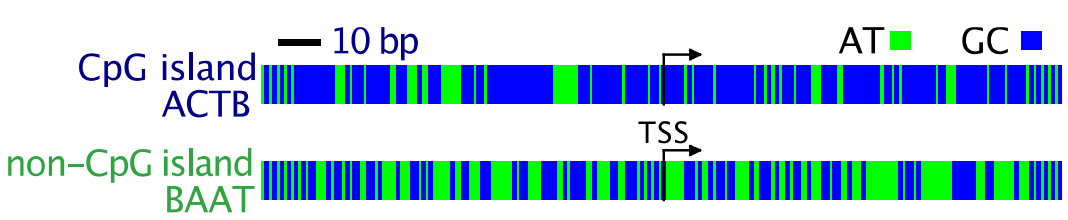
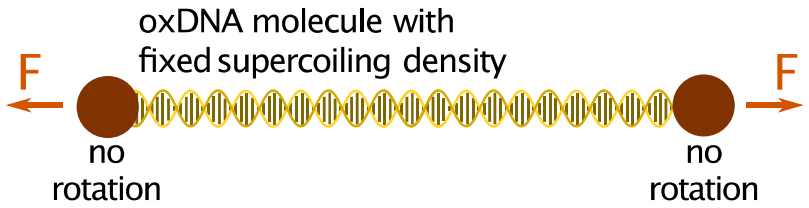
- OxDNA modelling of DNA melting at CpG islands

OxDNA modeling of DNA melting at CpG and non-CpG promoters





- 220bp of DNA
- σ= -0.06
- 3pN of tension to supress writhe (Matek et al 2015).
- 30 simulations

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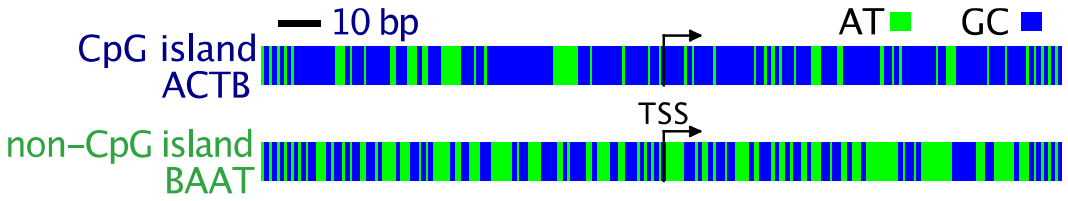
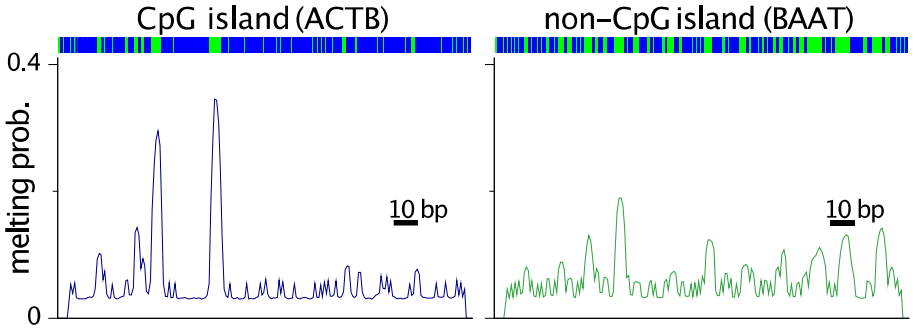
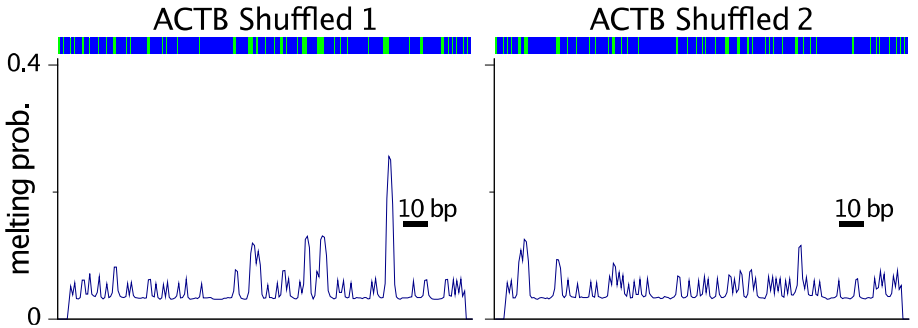
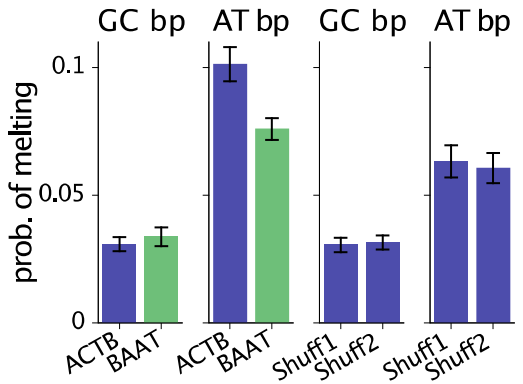
OxDNA modeling of DNA melting at CpG and non-CpG promoters









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Summary

Transcription dependent propagation of DNA supercoiling

CpG islands absorb DNA supercoils at highly expressed genes

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- Is there a DNA supercoiling transition threshold?

- Is this dependent on topoisomerase?
  - ChIP active topoisomerase
  - Topoisomerase degrons

- Diffusion of DNA supercoiling & relationship to local chromatin environment (timepoints)

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CpG islands absorb DNA supercoils at highly expressed genes

- OxDNA modelling
- Topoisomerase activity
- Alternative DNA structure…G4's

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Medical Research Council

THE UNIVERSITY of EDINBURGH

CANCER RESEARCH UK

Gilbert Lab

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