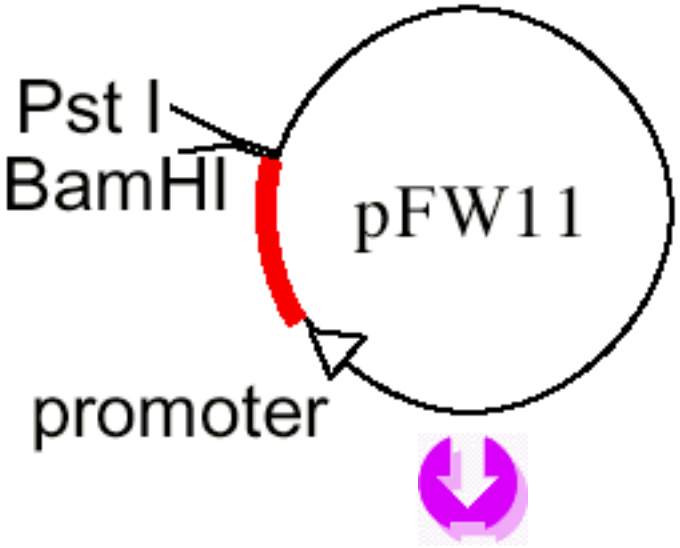
Incremental Truncation



Linearize vector by double digestion

Exo III nuclease treatment

Salt concentration (Nacl)

Exo III – helps stepwise removal of mononucleotides from 3´-hydroxyl termini of duplex DNA

Time-dependent sampling !!

Ostermeier, Nixon & Benkovic: Proc. Natl Acad. Sci :96, 3562-67, 1999

PstI

Recognition Sequence	Cut Site
5'CTGCAG 3' 3'GACGTC 5'	5'--CTGCA G--3' 3'--G ACGTC--5'

BamHI

Recognition	Cut site
5′-GGATTC-3′	5′-G GATTC-3′
3′-CCTAAG-5′	3′-CCTAA G-5′

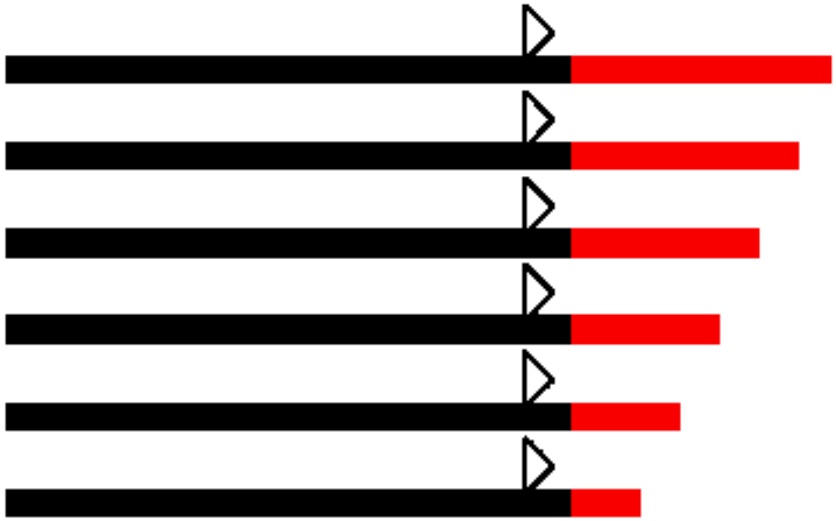
The 3’ overhangs are resistant to Exo III digestion whereas the 5’ overhangs are SUSCEPTIBLE.

resistant

susceptible

Removal of single-stranded extensions (3'and 5') to leave ligatable blunt ends

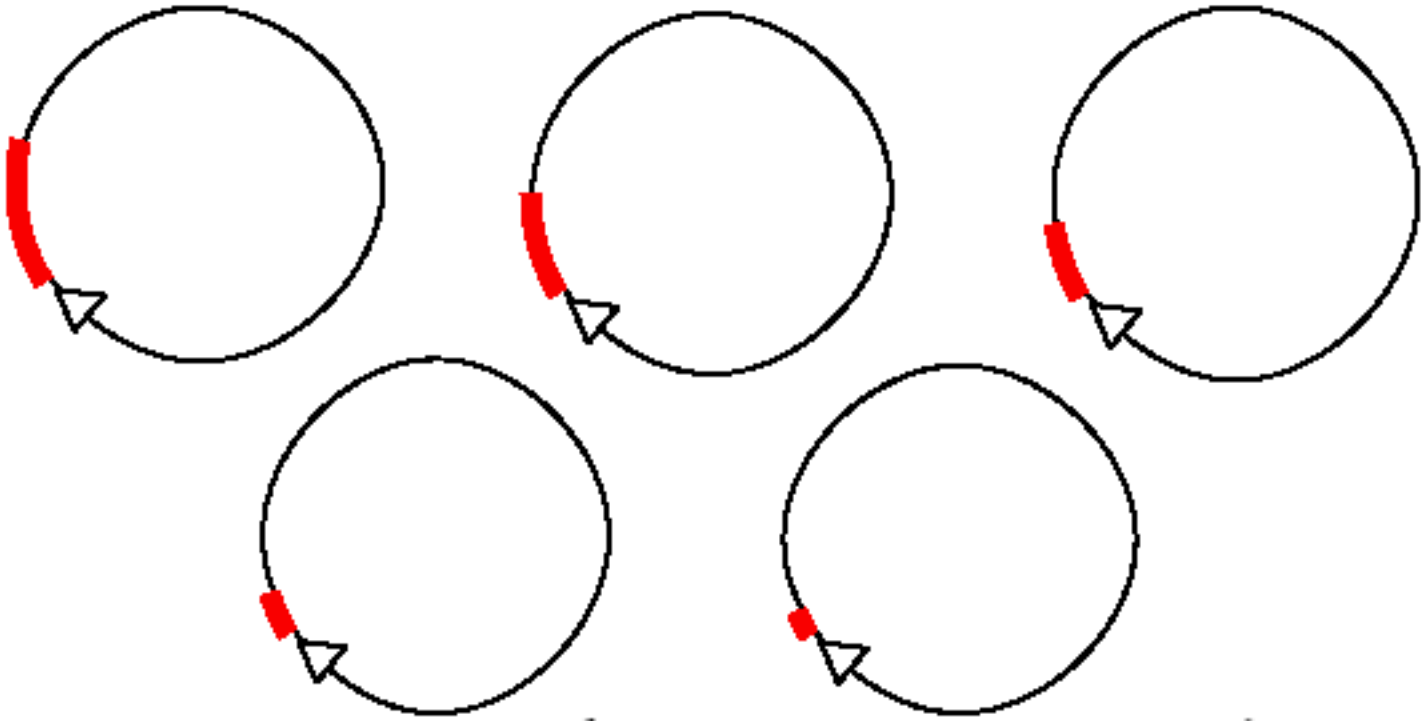
Mung bean nuclease treatment



Klenow treatment

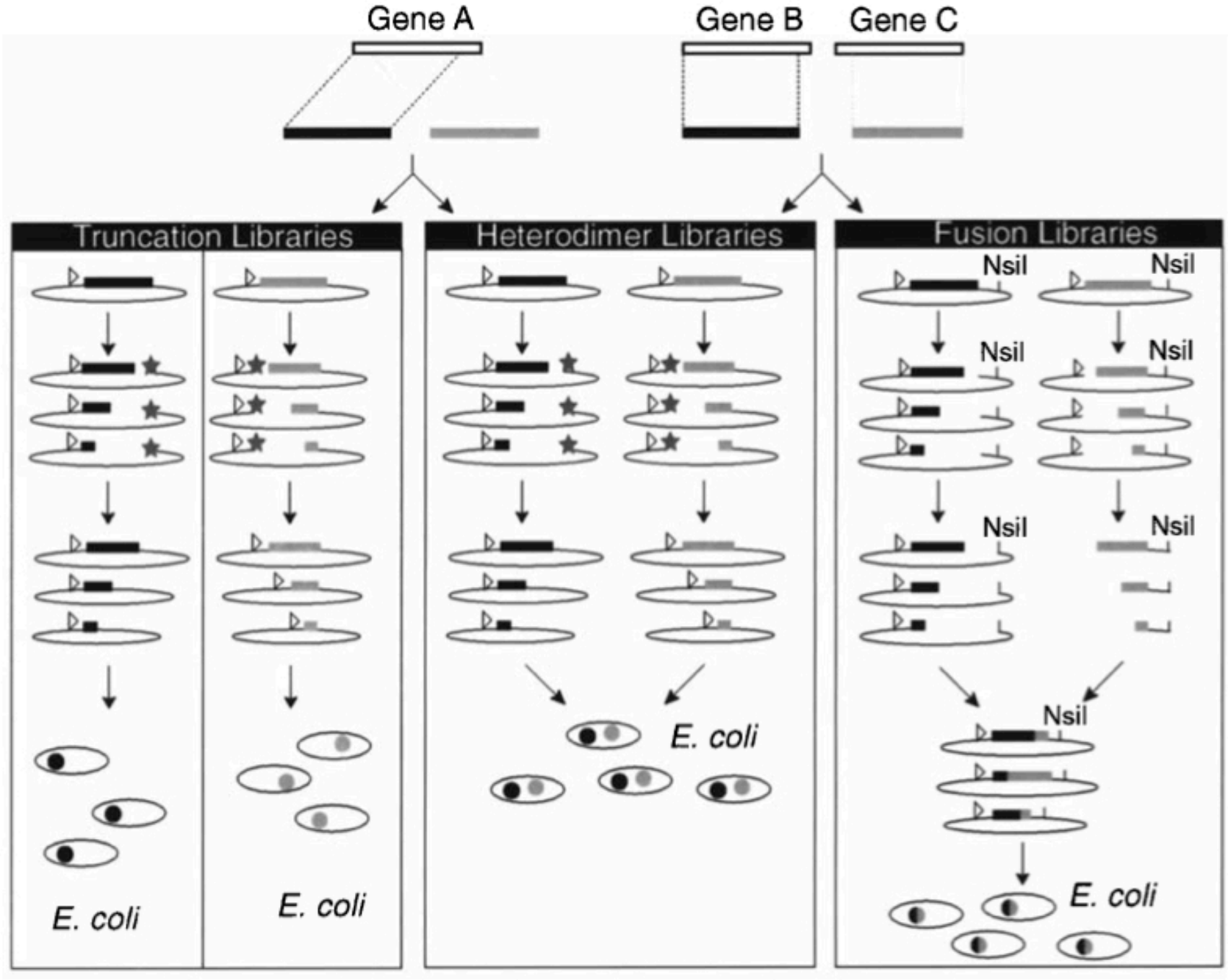
5’-3’ polymerase
3’-5’-exonuclease

NO 5’-3’ exonuclease

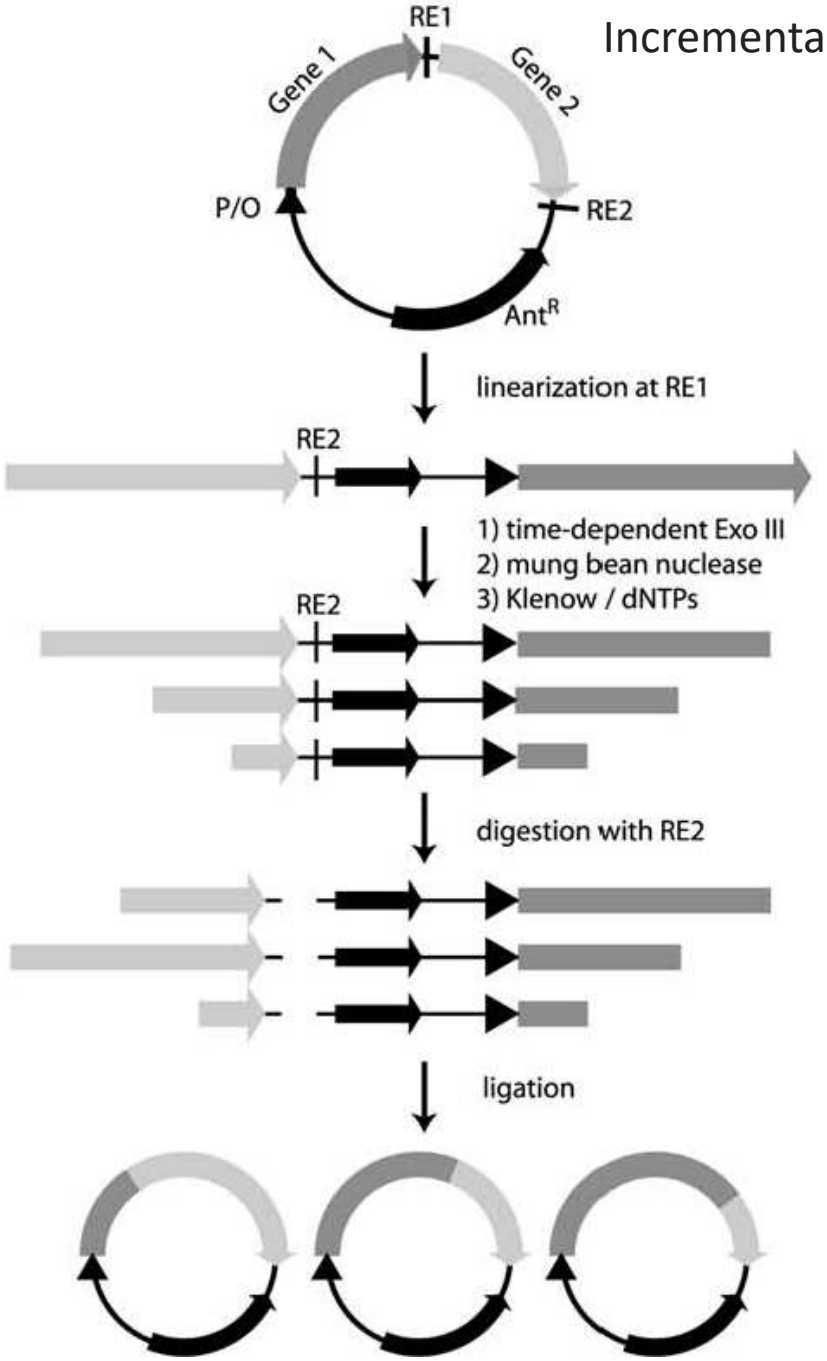


Diverse Library with every one base deletion

Combining Incremental Truncation Libraries



Incremental Truncation for the Creation of Hybrid enzYmes



ITCHY Hybrid Protein Library

The fusion of two incremental truncation libraries is called an ITCHY library.

An ITCHY library created from a single gene consists of genes with internal deletions and duplications.

An ITCHY library created between two different genes consists of gene fusions created in a DNA-homology independent fashion.

Case study

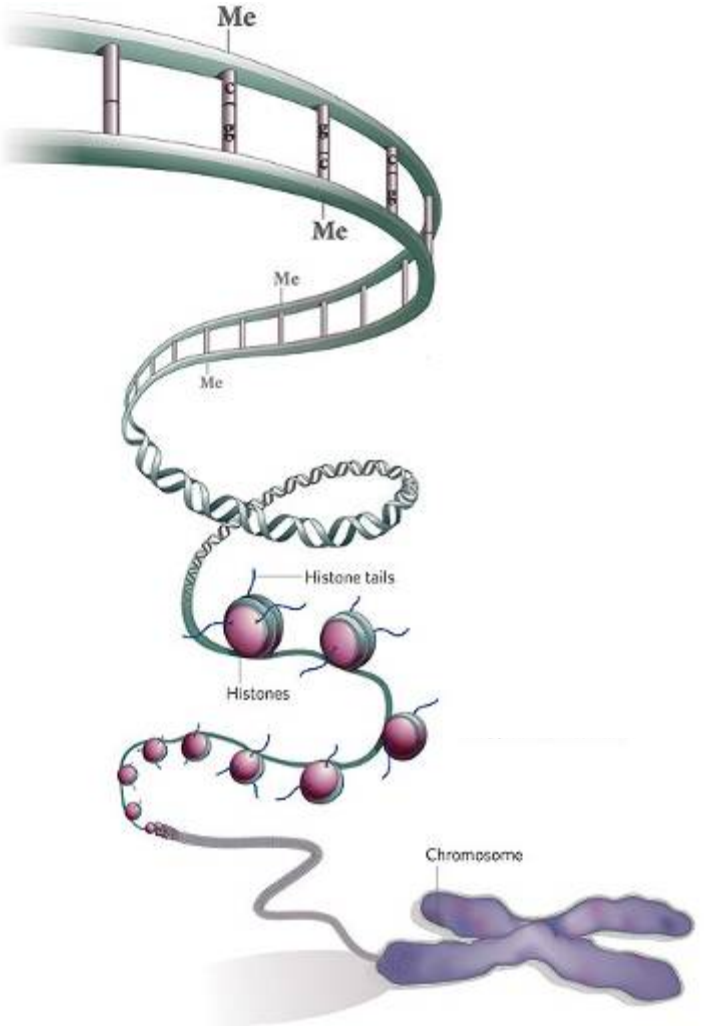
Non-Associating Heterodimeric DNA Methyltransferases as a Platform for Developing Site-Specific Methyltransferases

Outline

- DNA methylation background
- Site-biased and site-specific DNA methyltransferases
- Splitting monomeric methyltransferases
- Naturally split methyltransferases

Genome Versus the Epigenome

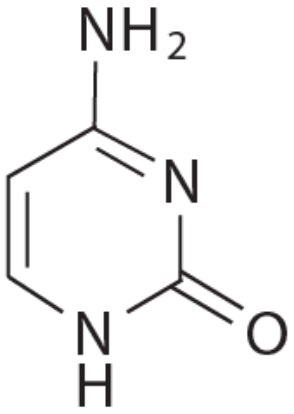
- Higher eukaryotic cells contain two layers of heritable information
  - Genome: Instructions encoded in DNA for making RNA and proteins
  - Epigenome: Instructions for how to utilize genetic information
    - DNA methylation
    - Histone modification



Nature **441**, 143-145(11 May 2006)

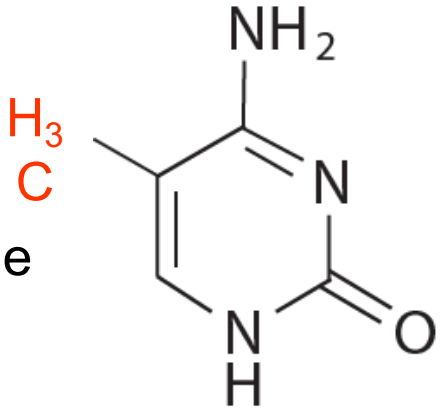
5-Methylcytosine in Mammalian and Plant Cells

cytosine



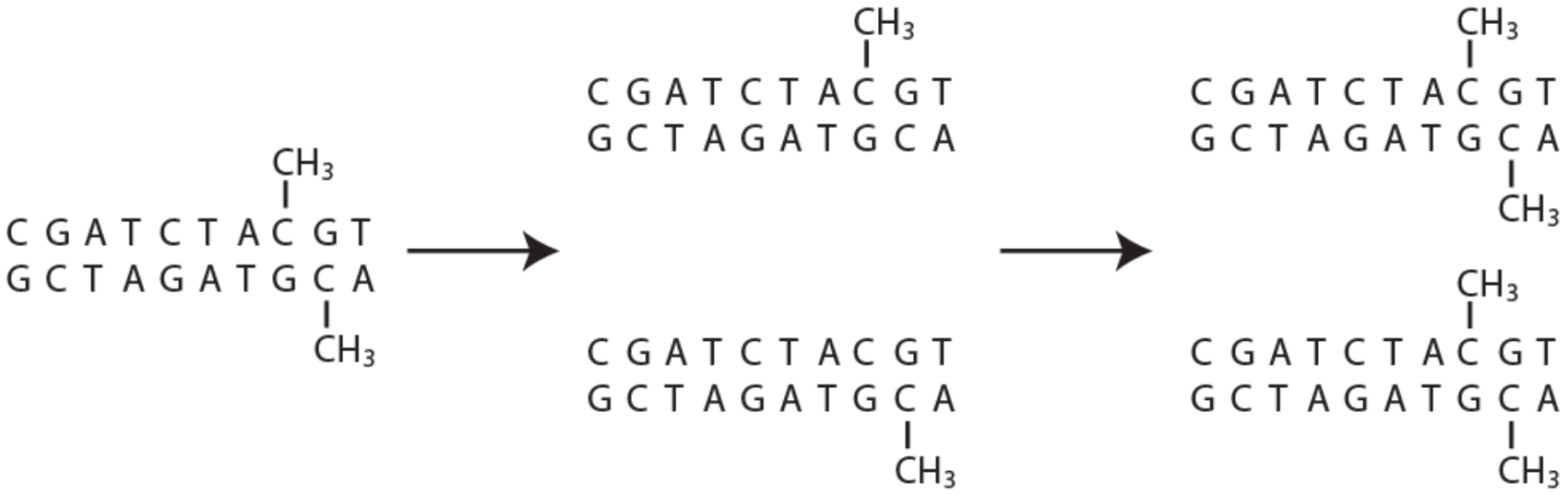
- In plants, 5-methylcytosine occurs at CpG, CpHpG and CpHpH sequences (where H = A, C or T).

5-methylcytosine (5mC)



- In fungi and animals, 5-methylcytosine predominantly occurs at CpG dinucleotides.

Heritable DNA Methylation



- Enzymes recognize and methylate DNA at hemi-methylated sites

Why is DNA Methylation Important?

- Methylation patterns are important in establishing correct gene expression
  - Embryonic development and cell differentiation
  - DNA imprinting

- Epigenetic diseases characterized by abnormal methylation patterns
  - Cancer
  - Diseases caused by deregulation of imprinted genes