PHASE 2 SUMMARY

DNA METHYLATION AND ITS BASIC FUNCTION

DNA methylation is a mechanism which transfers methyl groups onto cytosine to form 5-methylcytosine. One of its most important roles is to surpress gene expression. It is catalyzed by DNA methyltransferases- Dnmts

Majority of methylation is done on CpG sites- parts of genome where cytosine precedes guanine

Stretches of DNA which are averagely long 1000 bases, with a higher concentration of CpG sites, form CpG islands. Majority of gene promoters reside in CpG islands.

CpG islands are not as methylated as lonely CpG sites.

CpG shores- regions bordering CpG islands, which can be at an distance of even 2kb

Methylation can occur on non CpG sites, but is rare and not yet understood.

There are 3 classes of enzymes which take part in methylation: writers, erasers, readers

Writers add methyl groups, erasers modify and as the name suggests, erase methyl groups, and readers recognize and bind to methyl groups.

Dnmts are important writers: Dnmt1, Dnmt3a, Dnmt3b, Dnmt3L

Dnmt1- prefers hemimethylated DNA, has the ability to repair methylation, because of this it is called maintenance Dnmt

Dnmt3a and Dnmt3b- can introduce methylation to naked DNA, without preferences- de novo Dnmt

Dna demethylation- cannot be done directly, but is done by transforming 5mC to cytosine with multiple steps

Methylation and Demethylation and their coordination is extremely important during CNS development, but it can have an effect in adult years, causing diseases and disorders.

AN INTRODUCTION TO WGBS

Bisulfite sequencing-use of bisulfite on DNA before sequencing to determine the pattern of methylation. Converts unaffected cytosine to uracil, but leaves 5mC intact.

Direct sequencing- subtype of bisulfite sequencing, uses bisulfite treated DNA, displays uracil as tymine

Methylation specific PCR(MSP)- alternative method of methylation analysis, uses bisulfite treated DNA but doesn't use sequencing. Displays unaffected cytosines as tymines, and uses primers to achieve amplification- bigger the amplification, bigger the methylation

Limitations:

1. 5-Hydroxymethylcytosine- bisulfite treatment cant differentiate it from 5-methylcytosine
2. Incomplete conversion
3. Degradation of DNA during bisulfite treatment

Applications: possibility of applying on large scale, probable advancement in cloning technology

Oxidative bisulfite technology: method used to differentiate between 5-methylcytosine and 5-hydroxymethylcytosine

DNA methylation-calling tools

Nanopore sequencing- doesn't modify sequence, finds methylation by comparing difference in electrical current between methylated and unmethylated bases

12 tools for detecting methylation

Steps for methylation detection:

1.Basecalling and quality control using Guppy and NanoPack

2.Genome assembly and polishing

3.Methylation calling and evaluation

QUESTIONS

1. How important is the number of methylated cytosines in a gene? For example, will the methylation effects be the same on a gene 1000 bases long which is methylated on 1 cytosine, compared to one methylated on 20 cytosines.
2. It is said in the first article that 45% of mammalian genome consists of transponsable and viral elements that are silenced by methylation, and that they are harmful if demethylated. Why do they exist, what is their purpose?
3. Why are CpG islands less methylated than sole CpG sites?
4. And finally, why not display Uracil in bisulfite sequencing, instead of displaying it as thymine?