

3.5: Epigenetics Assay

Learning Objectives

- 1: Know different types of epigenetic assays.
- 2: Know how different epigenetic assays are used when cells are exposed to toxicants or mutagens.

5.1: DNA Methylation Assay

DNA methylation assays are important to know the epigenetic modification which is a heritable, enzyme-induced modification without alteration of the nucleotide base pairs. The transfer of a methyl-group to the 5-carbon on the cytosine in a CpG dinucleotide happens in the DNA methylation by DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B). The high level of promoter CpG island methylation results in gene silencing. The methylated DNA immunoprecipitation (MeDIP)-chip technique is used for DNA methylation assay.

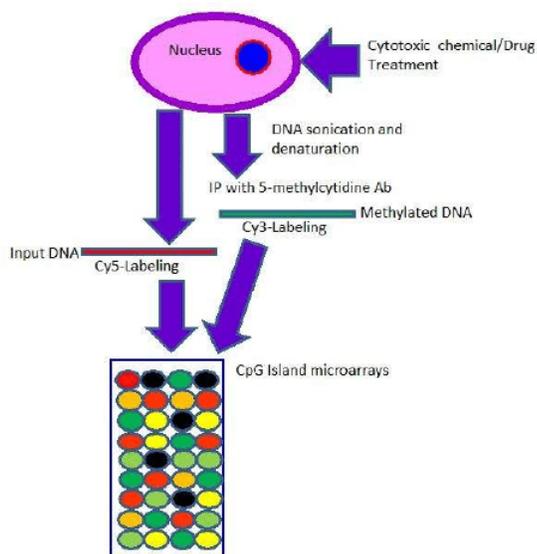


Figure 3.5.1: Schematic diagram of MeDIP protocol for DNA methylation assay.

In brief, the MeDIP-chip procedure is mentioned as follows. The genomic DNA is sheared to low molecular weight fragments (approximately 400 bp) by sonication. Then, the methylated DNAs are immunoprecipitated with the anti-methyl-cytosine antibody, and are amplified with PCR, if source material is less. Input and methylated DNA are labeled with fluorescent dyes Cy3 (green) and Cy5 (red), pooled, denatured, and are hybridized to a microarray slide containing all the annotated human CpG islands or other whole genome or promoter microarray designs. Then the slide is scanned using a scanner and each image is analyzed with the image analysis software (Figure 1).

5.2: Histone Modification Assay

Histone modification assays are useful to find the modification of histone proteins (e.g. lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, and lysine ubiquitination and sumoylation) which have important roles in epigenetic inheritance. The chromatin immunoprecipitation (ChIP) assay followed by hybridization to microarrays (ChIP-chip) (left) or by high-throughput sequencing (ChIP-seq) (right) are both powerful techniques to find histone modification.

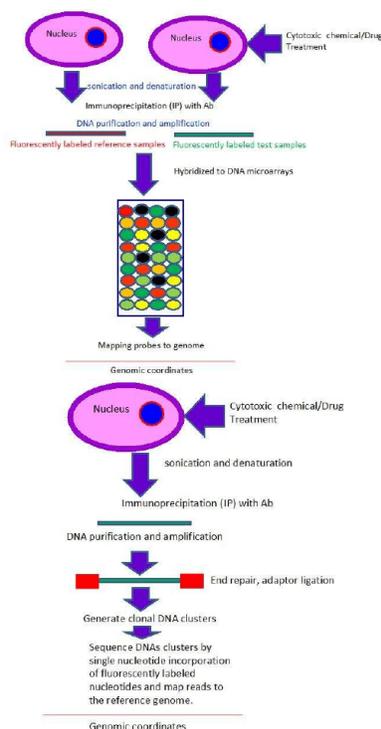


Figure 3.5.2: Schematic presentation of ChIP – chip microarray and sep of histone modification assay

5.3: MicroRNAs Assay

MicroRNAs assays are used to know the non-coding RNAs (17-25 nucleotides) which target messenger RNAs (mRNAs) and decayed the mRNAs or downregulated at the level of translation into protein. Almost, 60% of human protein coding genes are controlled by miRNAs and these miRNAs are epigenetically regulated. About 50% of miRNA genes are related with CpG islands, which may be repressed by epigenetic methylation. Other miRNAs are epigenetically controlled by either histone modifications or by DNA methylation. The expression of microRNAs are quantified by RT-PCR followed by quantitative PCR (qPCR). Then, miRNAs are hybridized to microarrays, slides or chips with probes to hundreds or thousands of miRNA targets. The microRNAs can be both invented and profiled by sequencing methods (microRNA sequencing).

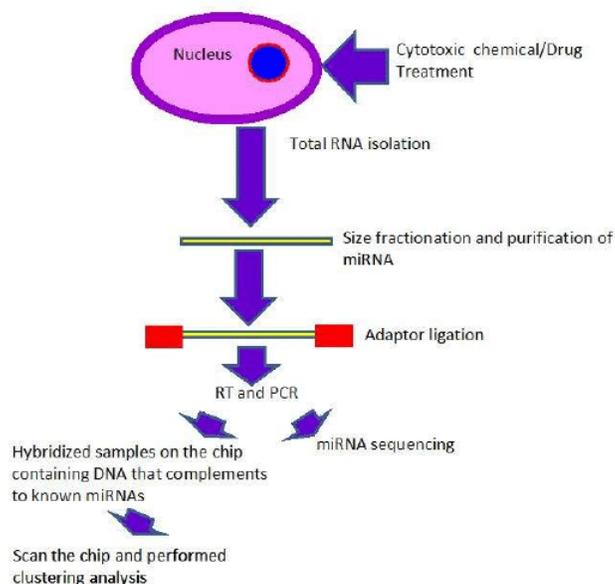


Figure 3.5.3: Schematic presentation of miRNA assay in epigenetic toxicology

Topic 5: Key Points

In this section, we explored the following main points:

- 1: Different types of Epigenetic assays.
- 2: How different Epigenetic assays namely DNA methylation assay, histone modification assay and MicroRNAs assay are used when cells are exposed to toxic chemicals or agents.

Knowledge Check

1. DNA methylation assays are important to know the non-epigenetic modification.

True

False

Answer

false

2. MicroRNAs assays are used to know the non-coding RNAs. These non-coding RNAs are :

17 to 25 nucleotides.

50 to 100 nucleotides.

200 to 400 nucleotides.

None of the above.

Answer

17 to 25 nucleotides.

3. Histone modification assays are useful to find the modification of histone proteins which have important roles in epigenetic inheritance:

True

False

Answer

true

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