3.3: Different Genetic-Toxicology Assays

Learning Objectives

- 1: Know different types of genetic-toxicology assays
- 2: Know how different genetic-toxicology assays are used in toxicology when cells are exposed to mutagens.

The goal of genetic toxicology assay is to determine whether any chemical or mutagen will do any adverse effect on genetic material or may cause different diseases including cancer. The assays can be performed using bacterial, yeast, or mammalian cells. One can early control and save vulnerable organisms from genotoxic chemicals by preforming genetic toxicology assay.

The following different types of genetic toxicology assays are used now a days:

- Bacterial Reverse Mutation Assay (Ames Assay)
- Genetic mutation assay
  - Allele-Specific PCR
  - Sanger Dideoxy Sequencing
- Chromosome aberration study
- Micronucleus assay

3.1: Bacterial Reverse Mutation Assay (Ames Assay)

This assay was discovered by Bruce Ames in 1970. This assay is widely used to test for gene mutation. The technique uses several strains of the bacterium Salmonella typhimurium which carry mutations in genes involved in histidine synthesis. These strains are auxotrophic mutants and they require histidine for growth and they cannot produce it. This assay examine the ability of the chemical or mutagen in creating mutations or a "prototrophic" state of strains, when the strains can grow on a histidine-free medium.

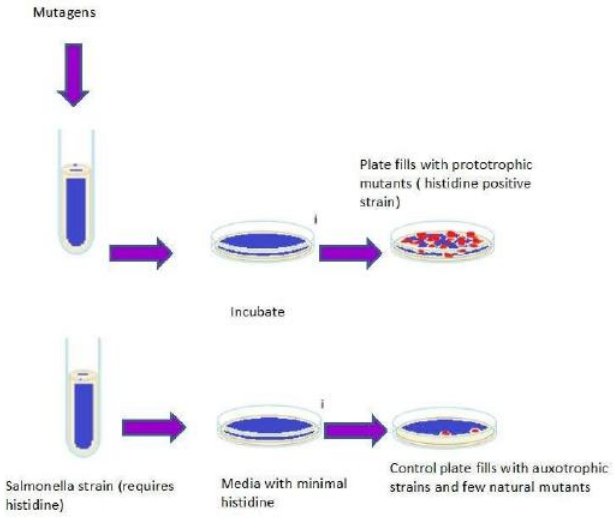


Figure 3.3.1: Ames test

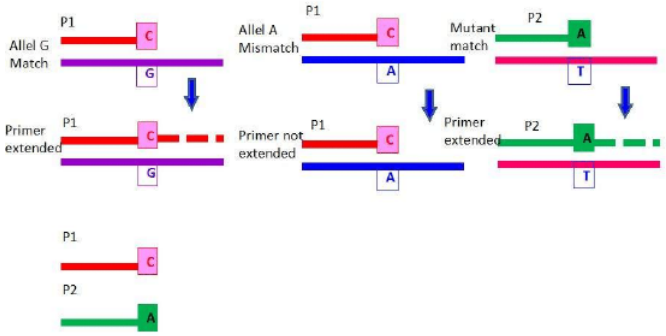
3.2: Genetic Mutation Assay

Following are the different molecular assay to study nucleotide variants or alternation of genetic material caused by mutagens:

Allele-Specific PCR

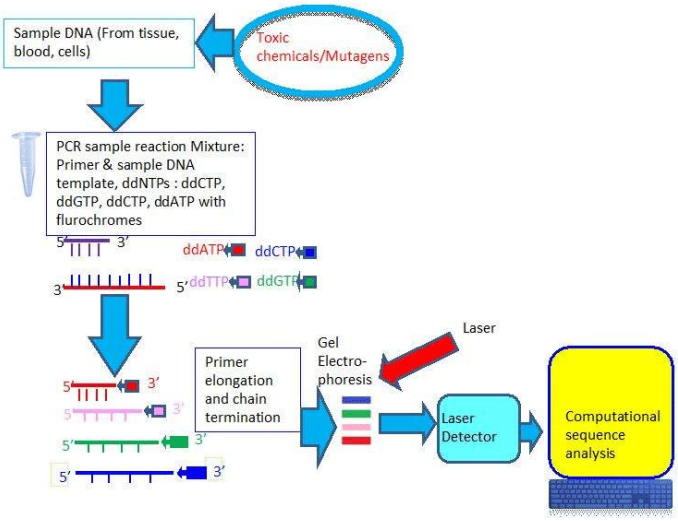
Single nucleotide polymorphism (SNP) resulted from base substitution mutation can be analyzed by this method. In this real-time PCR, fluorescent reporter probes are added to the reaction mixture and one fluorescent reporter probe is selected for wild type and other fluorescent probe is used for mutant. The PCR primers with fluorescent probe will match or mismatch one of the alleles at 3’

end of the primer. DNA polymerase extends the probes in a complementary fashion and releasing the reporter fluorescent molecules for detection. The PCR cycles with the reporter probes show the amplified signals and allow for precise measurement of one or both alleles of interest. Similarly, the 3' end of the mutant-specific primer is extended only in the presence of DNA with that mutation.



Sanger Dideoxy Sequencing

The goal of this method is to detect unknown mutations including single nucleotide variants (SNVs) and small duplications, insertions, deletions, and indels of interest caused by mutagens. In this method, sequencing primers hybridized to the PCR product and are extended using the four deoxynucleotides (dNTPs), a mixture of fluorescently labeled dideoxynucleotides (ddNTPs) and DNA polymerase. Four ddNTPs are marked with a different fluorescent dye. Random incorporation of the marked ddNTPs shows in termination of strands at each location along the sequence. The gel electrophoresis separates the strands by size. Fluorescence spectroscopy measured the terminating nucleotides.



3.3: Chromosome Aberration Study

Cytogenetic assays of mammalian cells are performed to detect different types of structural and numerical chromosomal aberrations caused by genotoxic chemicals. The clastogenic or aneugenic effects from the genotoxic chemicals will result in an increase in frequency of structural (premature centric separation, chromosome breaks, dicentric chromosomes, ring) complex rearrangements (Figure 4) or numerical aberrations of the genetic material in mammalian cells.

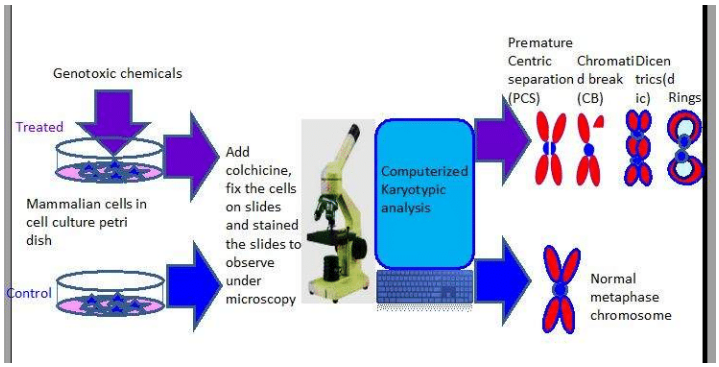


Figure 3.3.2: Chromosome aberration study

3.4: Micronucleus Assay

Micronucleus assay is used as a tool to evaluate genetic damage caused by genotoxic chemicals. The number of micronuclei (Figure 5) generated directly relates to the amount of DNA damage in the cells.

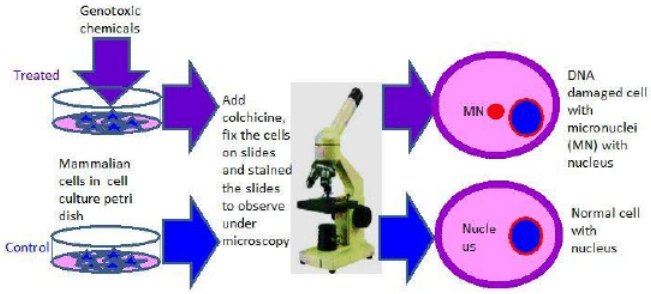


Figure 3.3.3: Micronucleus assay

Topic 3: Key Points

In this section, we explored the following main points:

- 1: Definition of genetic toxicology assay
- 2: Different types of genetic-toxicology assays.
- 3: How genetic mutation assays are performed by Allele-Specific PCR and Sanger Dideoxy Sequencing techniques.
- 4: What are the different types of chromosomal aberrations observed under microscope by chromosome aberration study?
- 5: Changes of micronuclei observed under microscope by Micronucleus assay.

Knowledge Check

1. Cytogenetic assays of mammalian cells are performed to detect different types of structural and numerical chromosomal aberrations caused by a genotoxic chemicals. The structural chromosomal aberrations are:

premature centric separation

ring

chromosome breaks

dicentric chromosomes

All of the above

Answer

All of the above

2. In Allele-Specific PCR, fluorescent reporter probes are added to the reaction mixture and one fluorescent reporter probe is selected for wild type and other fluorescent probe is used for mutant.

True

False

Answer

True

3. Which instrument is used to measure the terminating nucleotides in Sanger Dideoxy Sequencing?

Fluorescence spectroscopy

Spectrophotometer

Fluorescence microscopy

None of the above

Answer

Fluorescence spectroscopy

4. The Ames technique uses several strains of the bacterium Salmonella typhimurium which carry mutations in genes involved in:

Arginine Synthesis.

Histidine synthesis.

Lysine Synthesis.

None of the above.

Answer

Histidine synthesis.
