INTRODUCTION

- Mutagencity:

  Refers to induction of permanent changes in the information content of genetic material.
- Mutation is replacement of nitrogen base with another in one or both the strands or adition or delation of a base pair in a DNA molecule.
- Substance (chemicals) which can induce mutations are collectively known as mutagens.

Classes of mutations

- **Spontaneous mutation**: They are mainly caused during DNA replication or by incorporation of incorrect nucleotide in the growing DNA chain. They occur by changes in DNA sequence.
- **Induced mutation**: They are caused by the changes in DNA brought by some environmental factors called mutagens.

EX: UV Light

Types of mutations

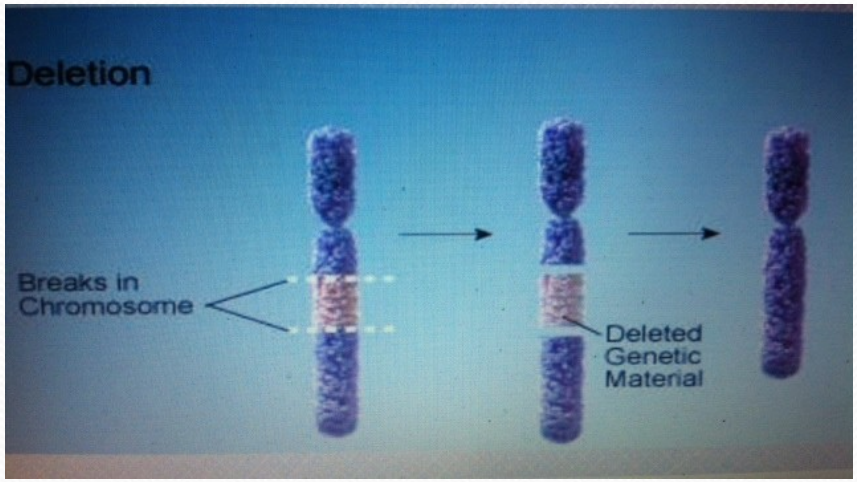
1. **Chromosome mutation**: changing the structure of a chromosome. Loss or gain of part of a chromosome.

Five types exist

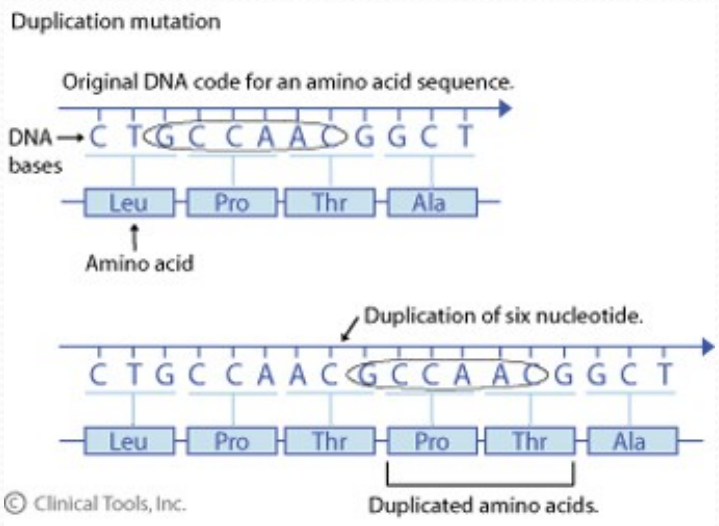
- Deletion
- Inversion
- Translocation
- Duplication
- nondisjunction

- Delection : Due to breakage a piece of chromosome is lost.
- Inversion : chromosome segment breaks off and reattches.
- Duplication : occurs when a gene sequence is repeated.
- Translocation :involves two chromosomes that are not homologous and a part of one is transferred to another chromosome.
- Nondisjunction : failure of chromosomes to separate during meisois.

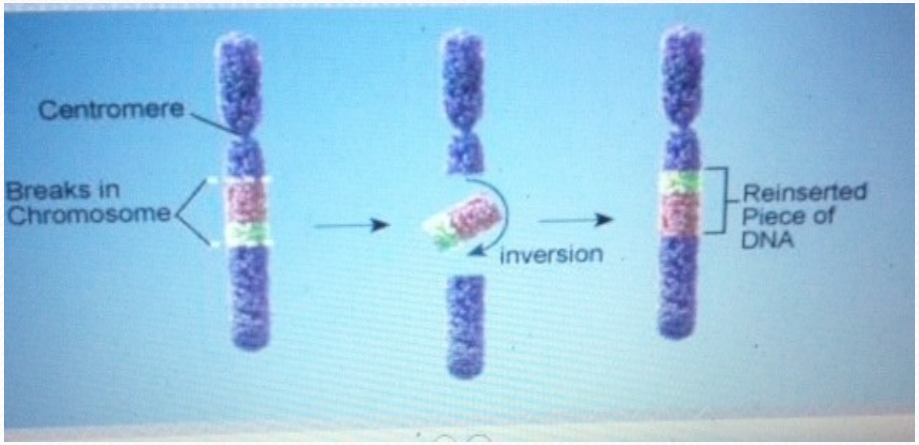
Deletion



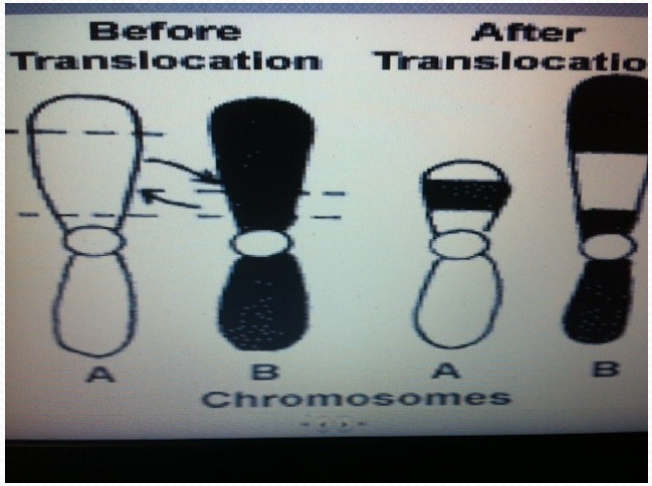
Duplication



Inversion



Translocation

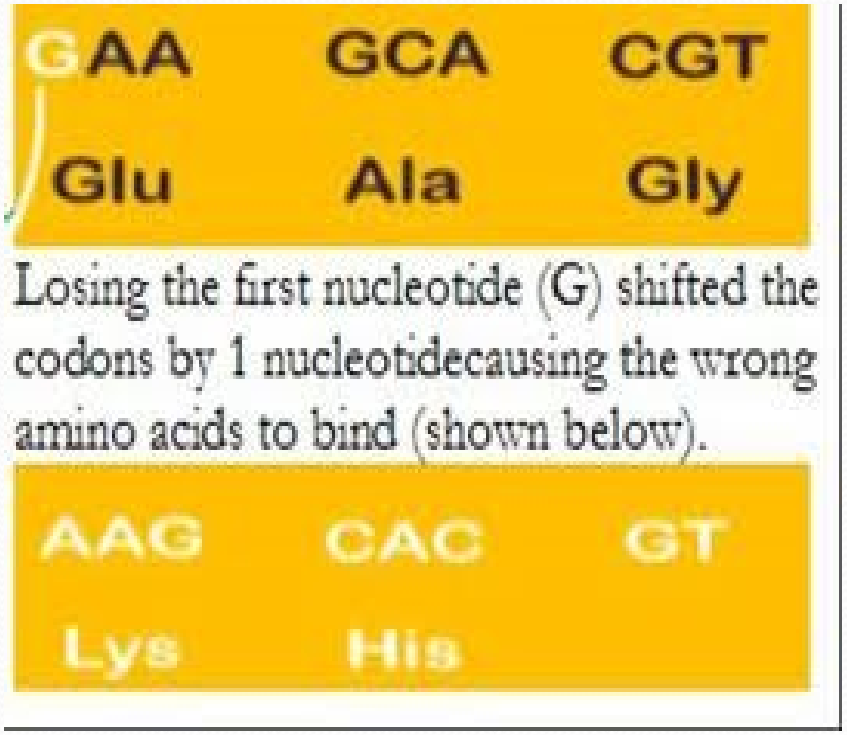


2) Point mutation

- Change in a single nucleotide.sickle cell disease is the result of one nucleotide substitution.

3) **Frame shift** : insertion or deleting one (or) more nucleotides.

changes the reading frame like changing a sentence.

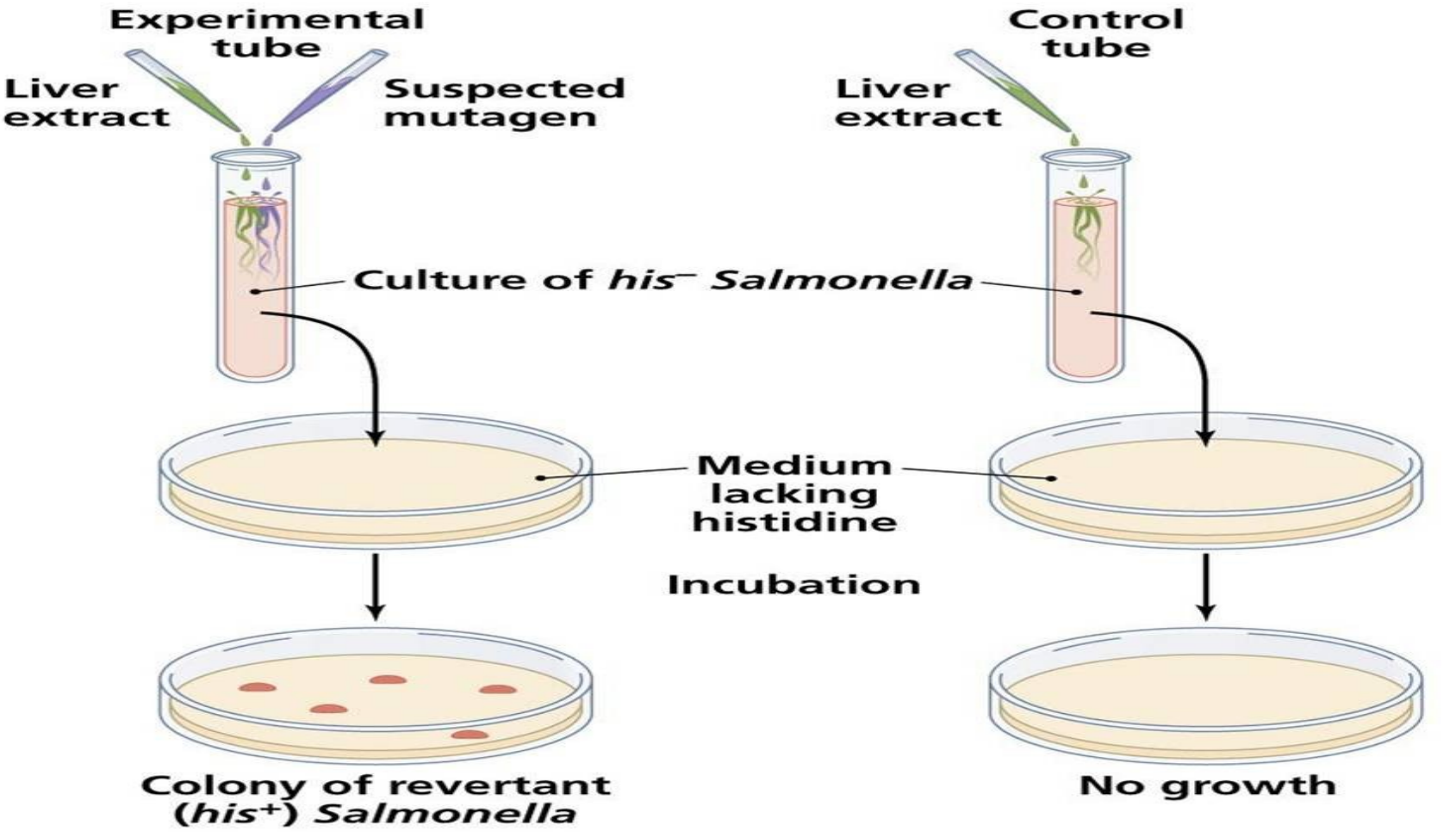


AMES TEST

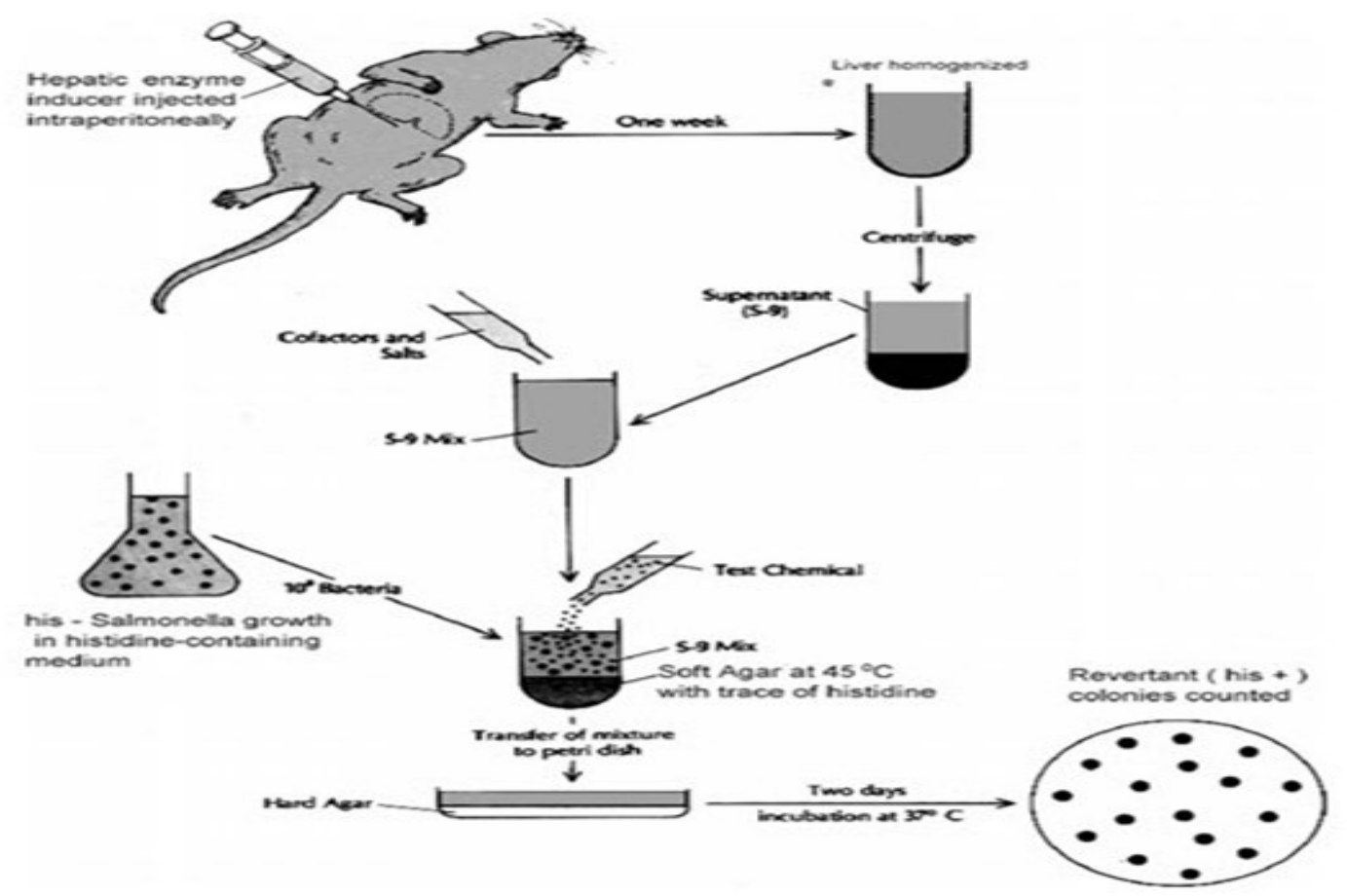
- A test for determining if a chemical is a mutagen.
- Named for its developer, Bruce Ames.
- The bacterium used in the test is a strain of *Salmonella typhimurium* that caries a defective (mutant) gene making it unable to synthesize the amino acid histidine (His) from the ingredients in its culture medium.
- Some types of mutations (including this one) can be reversed, a **back mutation**, with the gene regaining its function. These **revertants** are able to grow on a medium lacking histidine.

AMES TEST

- utilizes a histidine auxotroph of *Salmonella*
- determine if a chemical agent is a mutagen.
- Spontaneous back mutations (a reversion back to the strain of *Salmonella* that can synthesize histidine) is rare
- Appearance of many colonies of the microbe on the minimal plate after the addition of the test chemical is an indication that the chemical is a mutagen

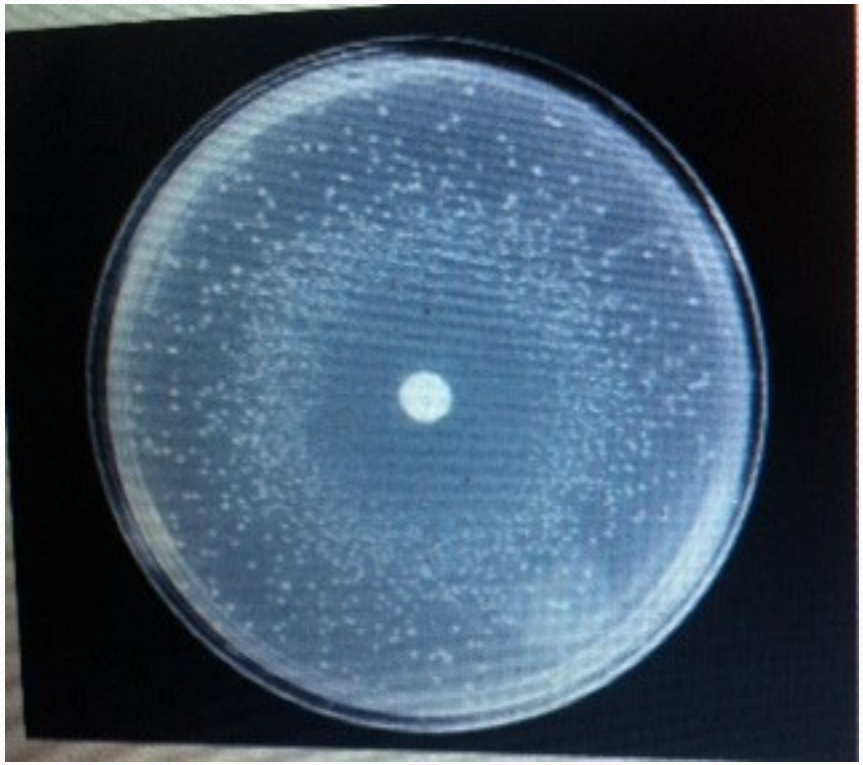


SALMONELLA ASSAY



SPOT TEST

- It consist of the incubation of a suitable tester strain of salmonella typhimurium+test agent place on the agar.
- Chemical is tested on one perti plate.
- The zone of inhibition is indicated of the toxicity for the bacterial growth.



HOST MEDIATED ASSAY

- Methodology:

✓ salmonella are injected intraperitonealy into rat or a hamster.

✓ The animal is treated with the test substance orally.

✓ Afterwards sample is withdrawn from peritoneal cavity and mutation in salmonella is measured.

COLIFORM ASSAY

- Coliform is a bacteria, it measure's the E.coli present in media.
- METHOD: Tester strain of E.coliPQ37+test substance incubation with or with out S-9 liver fraction, may show control for protein synthesis.

INVITRO METHODS

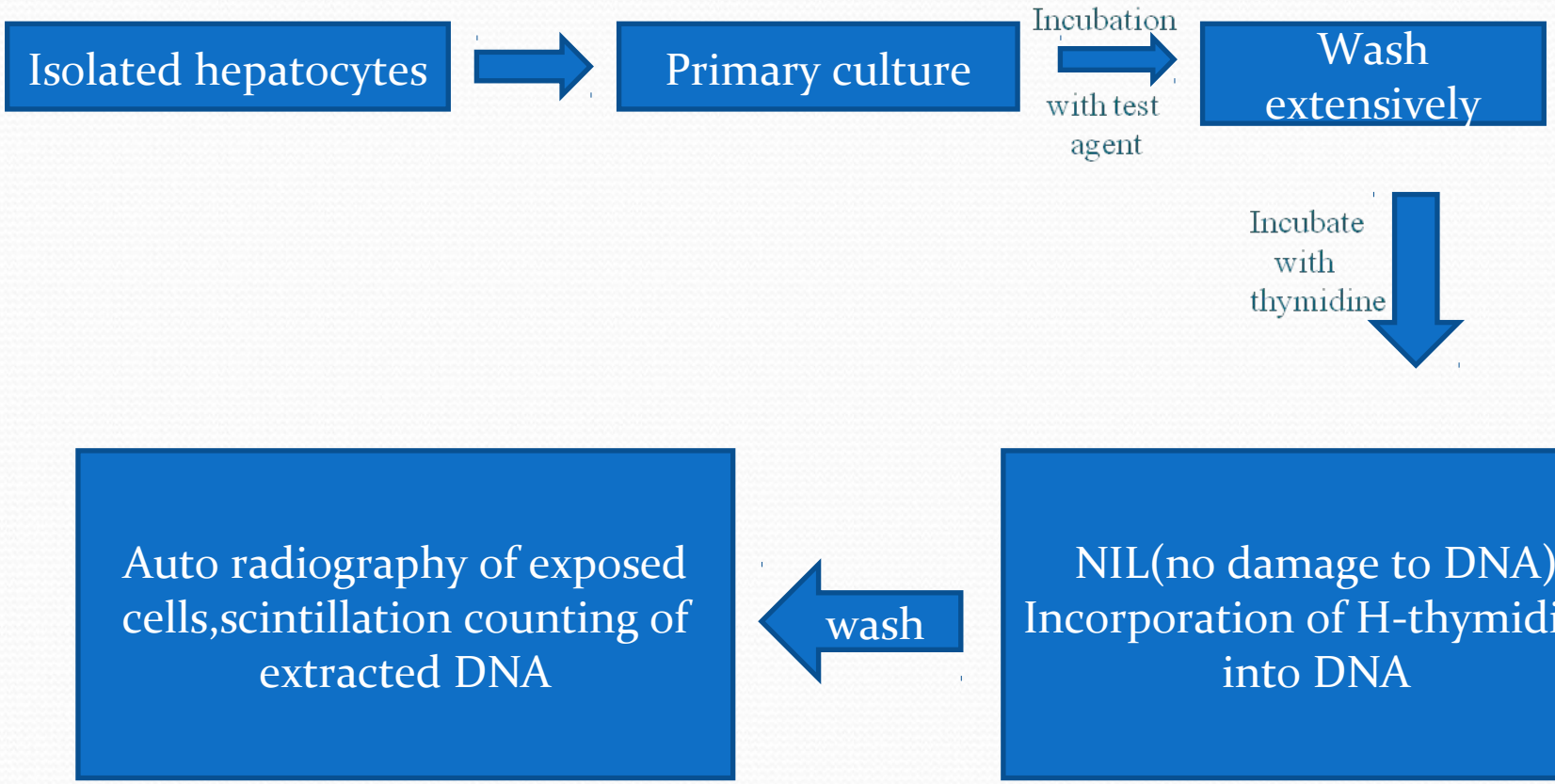
- Saccharomyces forward mutation assay
- Mammalian cell test systems

1) DNA damage/repair
2) Forward mutations in chinese hamster cells.
3) Mouse lymphoma cell assay
4) Chromosomal aberrations

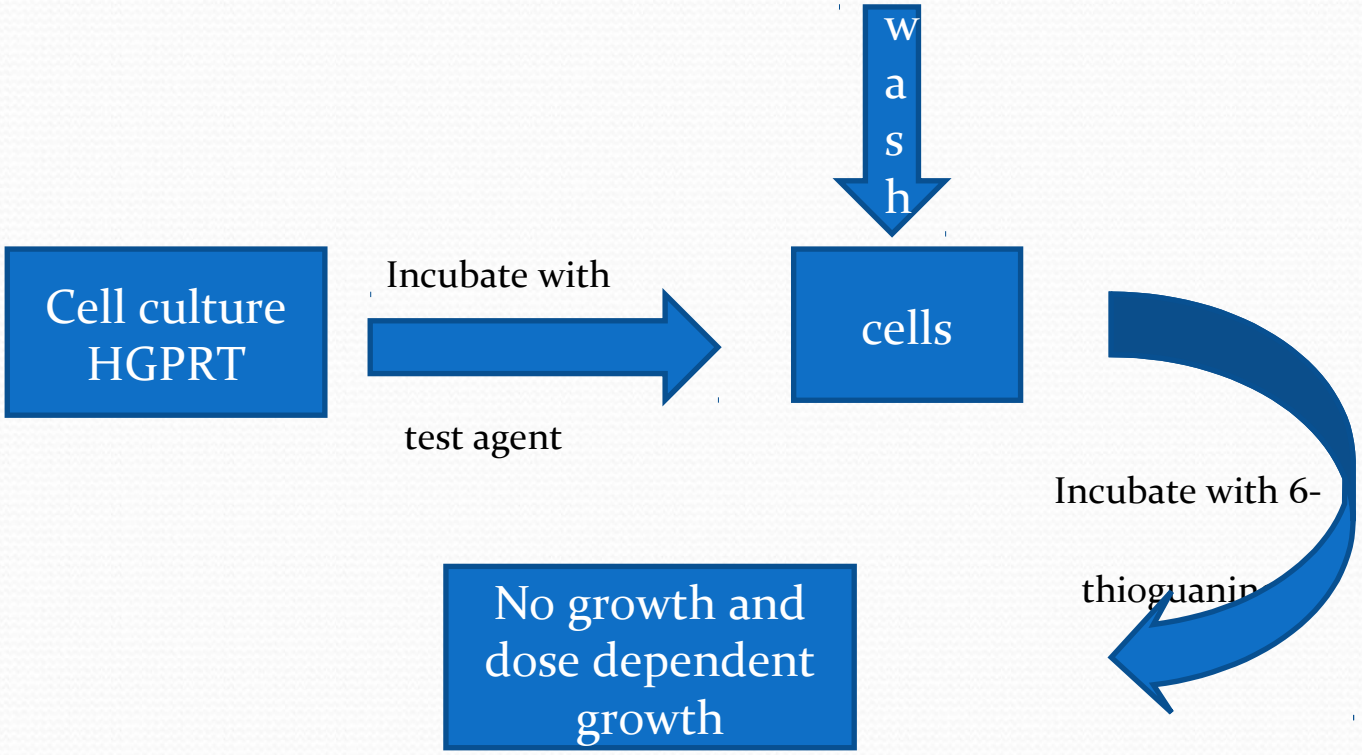
SACCHAROMYCES FORWARD-MUTATION ASSAY

- The test uses strain of S.cervisiae carring a deffetive mutation of the adenine-1 and 2 genes and growth in yeast culture results in production of red coloured colonies as a consequence of the acculmulation of intracellular pigment.
- Colonies that grow in culture being white when grow on a low adenine medium.

1) DNA DAMAGE/REPAIR



FORWARD MUTATIONS IN CHINESE HAMSTER CELLS



IN VIVO METHODS

- Micronuclei test
- Dominant lethal assay
- Comet assay

MICRO NUCLEI ASSAY

- The *in vitro* micronucleus assay is *a* mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments i.e. micronuclei in the cytoplasm of interphase cells.

- These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division.

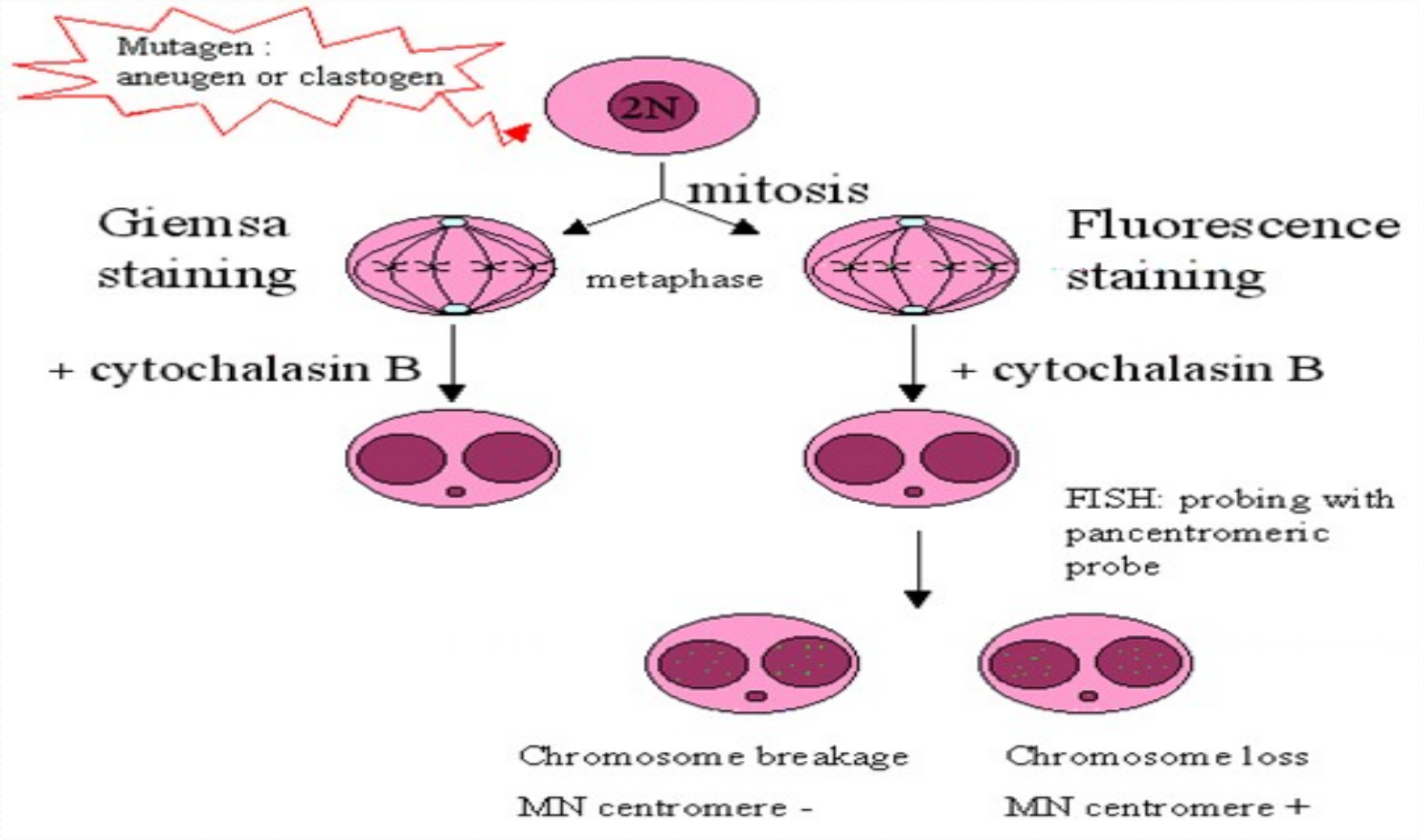
Contd..

- Micronuclei arise from chromosomal fragments that are not incorporated into daughter cell nuclei at mitosis because they lack a centromere and are not pulled to the oppropriate pole of the spindle.

MICRO NUCLEI ASSAY

- The purpose of the micronucleus assay is to detect those agents which modify chromosome structure and segregation is such a way as to lead to induction of micronuclei in interphase cells.

MICRONUCLEI ASSAY



DOMINANT LETHAL ASSAY

- Indicate that genetic damage has occurred in the form of structural or numerical chromosome aberrations.

METHODOLOGY

- ✓ Male mice or rats are treated with test agent
- ✓ Males mated with groups of untreated females
- ✓ Females are killed 14 days after mating,dissected and scored for corpora lutea, early fatal deaths and total implantations.

COMET ASSAY

- First introduced by OSTLING and JOHANSON in 1984.
- PRINCIPLE:
- Strand breakage of the supercoiled duplex DNA leads to the reduction of the size of the large molecule and these strands can be stretched out by electrophoresis.
- DNA migration is a function of both size and the number of broken ends of the DNA
- Tail length increases with damage initially and then reaches a maximum that is dependent on the electrophoretic conditions, not the size of fragments.

APPLICATIONS

- Major applications of the Comet assay are in the following areas:
- Genetic toxicology (DNA damage)

  In vivo & *in vitro* evaluation of genotoxic chemicals
- DNA damage:

  SSB's, DNA crosslinking, alkali labile sites
- DNA repair:

  Strand break repair

  Excision repair