BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024, Tamil Nadu,
India

Programme: M.Sc., Biomedical Science

Course Title : Drug Discovery and Assay Development
Course Code : 18BMS48ES

Unit-IV

Genotoxicity

Dr. P.S.Dhivya
Guest Lecturer
Department of Biomedical Science

GENETIC TOXICITY

Genotoxicity assays

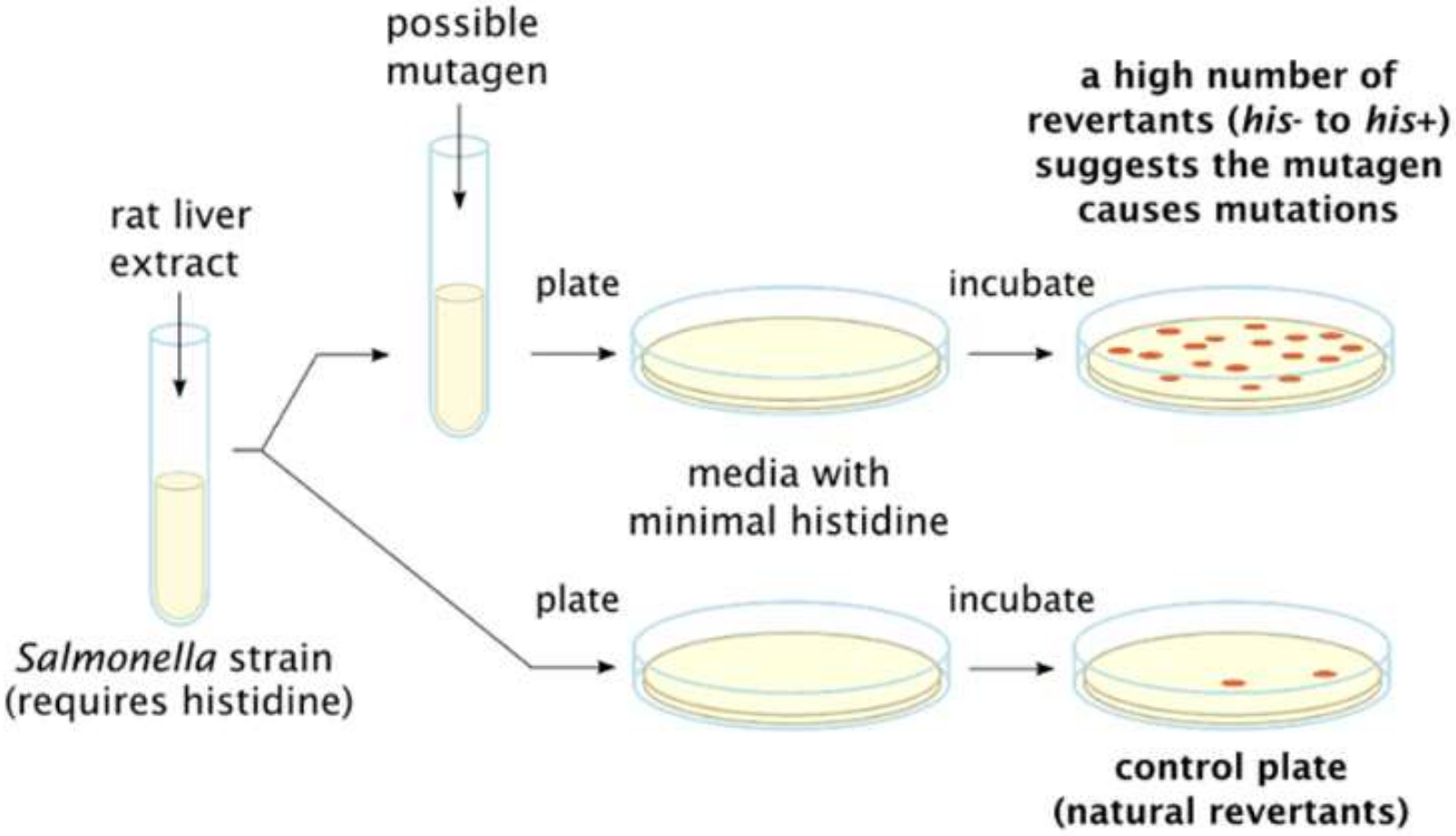
- Genotoxicity assays have different endpoints, such as single- and double-strand breaks, point mutations, deletions, chromosomal aberrations, micronuclei formation, DNA repair and cell-cycle interactions
- These endpoints are related to genotoxicity in vitro (human and mammalian cells) as well as in vivo (population biomonitoring and animal studies)

- The Ames and mouse lymphoma (MLA) assays or analyzing of HPRT mutations in different cells and using transgenic rodents - Mutagenicity.
- DNA adduct or strand break analysis, chromosome aberration (CA), micronucleus (MN), sister chromatid exchange (SCE), and unscheduled DNA synthesis (UDS) - **Genotoxicity but not mutagenicity.**

Ames test

- Bacterial reverse mutation assay
- 1970s by Bruce Ames
- in vitro mutagenesis test.
- Salmonella typhimurium (TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538)
- Escherichia coli (WP2 and WP2urvA) strains
- Carrying a defective (mutant) gene, which inhibits expression of the essential amino acid.

- In order to incorporate the aspect of mammalian host metabolism, liver extracts used to be added in the original Ames test, thereby simulating the action of mammalian liver enzymes that are known to play a role in metabolite generation.



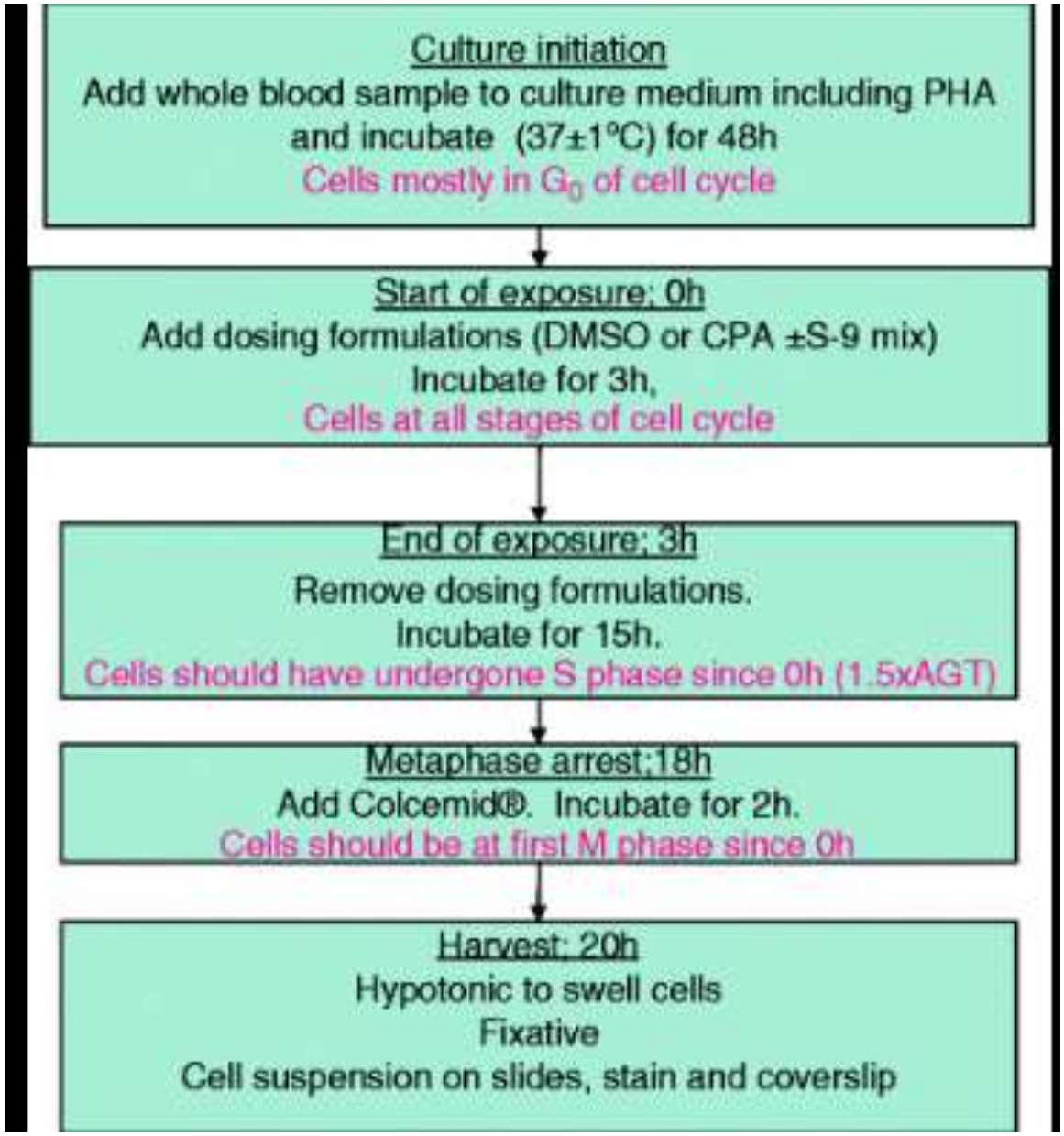
It utilizes bacteria to test whether a given chemical can cause mutations in the DNA of the test organism

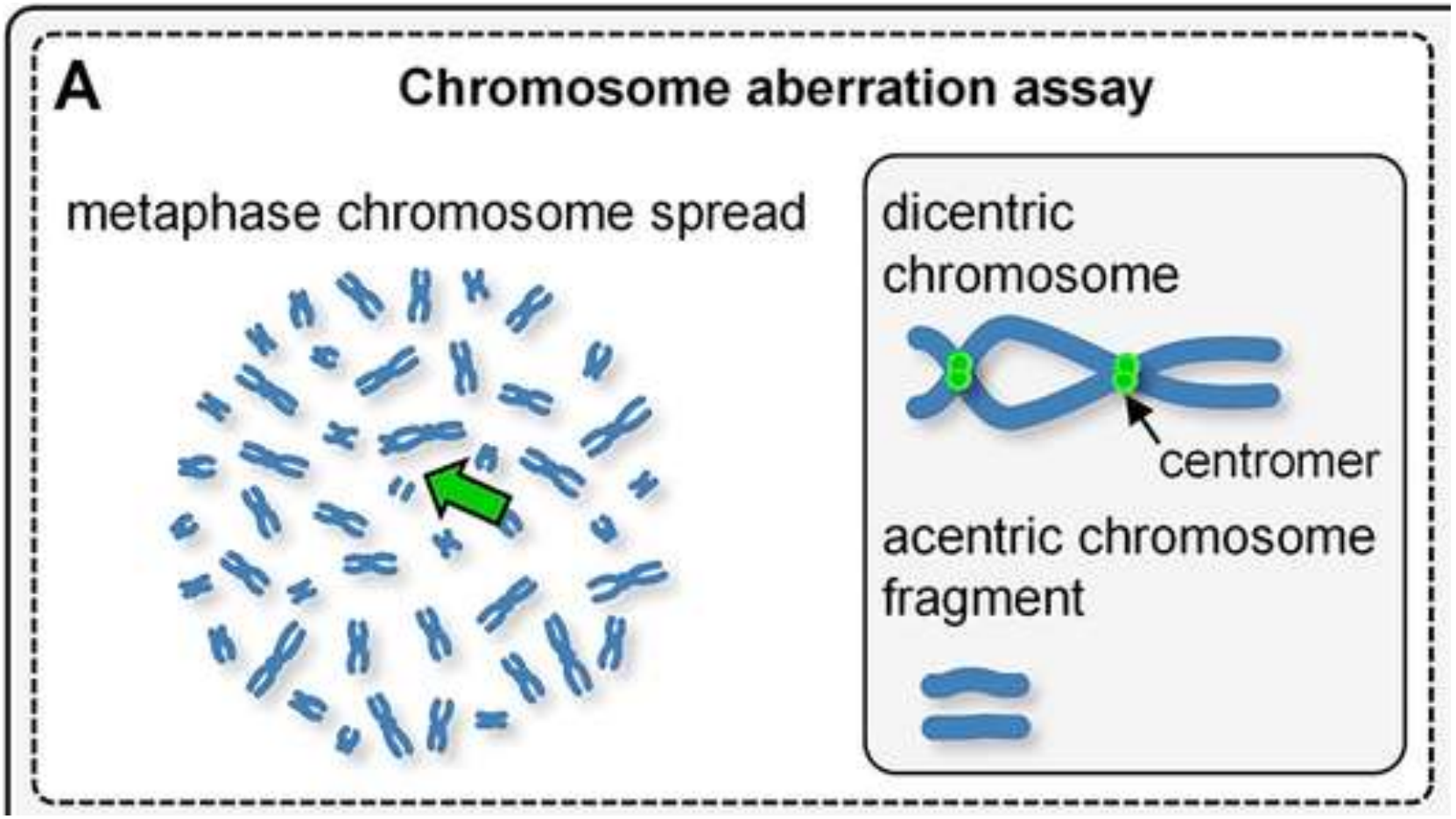
- It utilizes bacteria to test whether a given chemical can cause mutations in the DNA of the test organism
- These mutations result in his- or trp- organisms that cannot grow unless histidine or tryptophan is supplied.

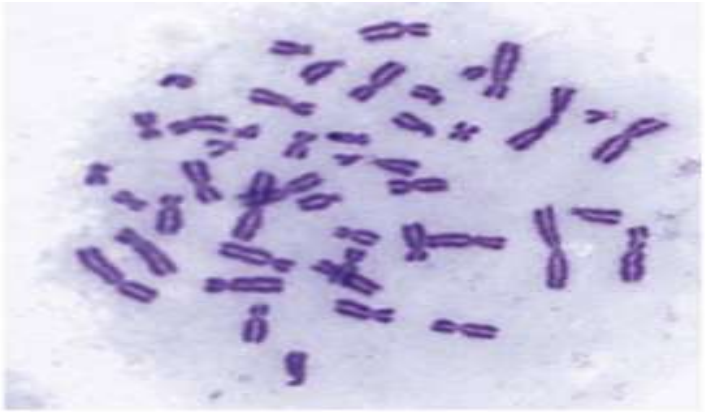
- But culturing His- *Salmonella* is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine **(His+**)
- This is to say that when a mutagenic event occurs, **base substitutions or frameshifts within the gene can cause a reversion to amino acid prototrophy.** This is the reverse mutation

Chromosomal aberration test

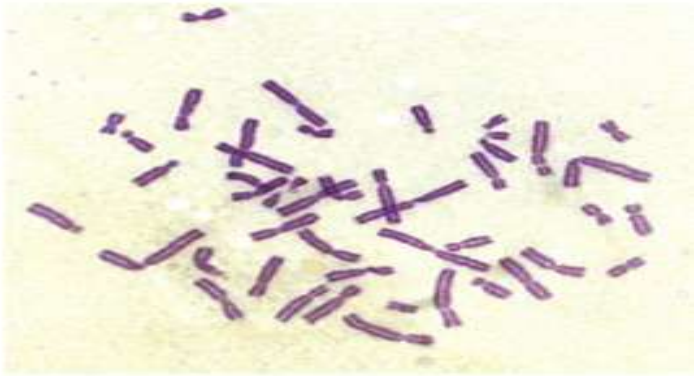
- Induce abnormal chromosomes, breaks of chromosome or chromatid and translocations
- The effects of agents can be studied either in whole animals (in vivo) or in cultured cells (in vitro)
- In both situations, the cell or animal is treated with the test substance and incubated with a metaphase inhibitor (commonly colcemide or colchicine)
- Presence of aberration is analyzed using microscope following appropriate staining procedure.


Culture initiation
Add whole blood sample to culture medium including PHA and incubate (37±1°C) for 48h
Cells mostly in G_0 of cell cycle
Start of exposure; 0h
Add dosing formulations (DMSO or CPA ±S-9 mix)
Incubate for 3h,
Cells at all stages of cell cycle
End of exposure; 3h
Remove dosing formulations.
Incubate for 15h.
Cells should have undergone S phase since 0h (1.5xAGT)
Metaphase arrest;18h
Add Colcemid®. Incubate for 2h.
Cells should be at first M phase since 0h
Harvest; 20h
Hypotonic to swell cells
Fixative
Cell suspension on slides, stain and coverslip

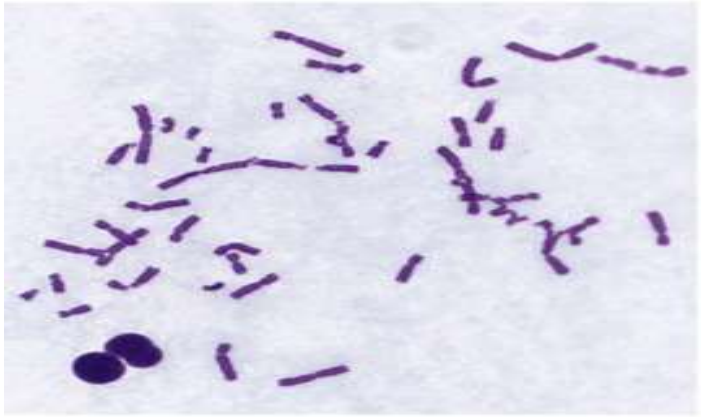

A
Chromosome aberration assay
metaphase chromosome spread
dicentric chromosome
centromer
acentric chromosome fragment



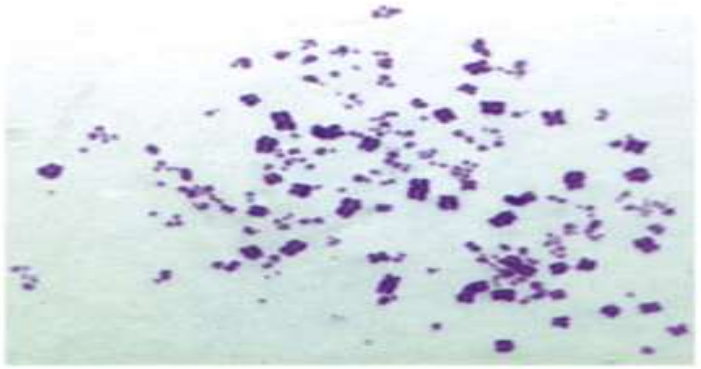
Chromatid gap



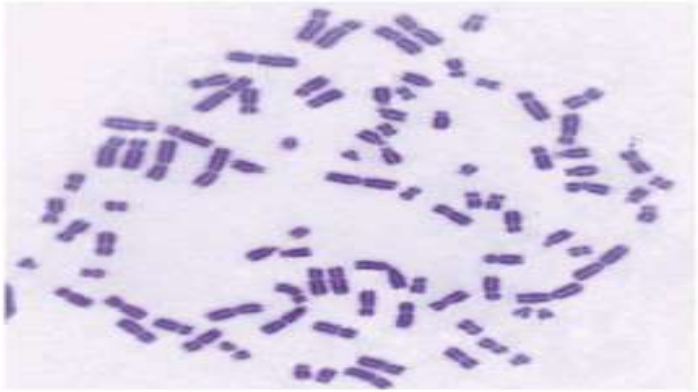
Chromosome gap



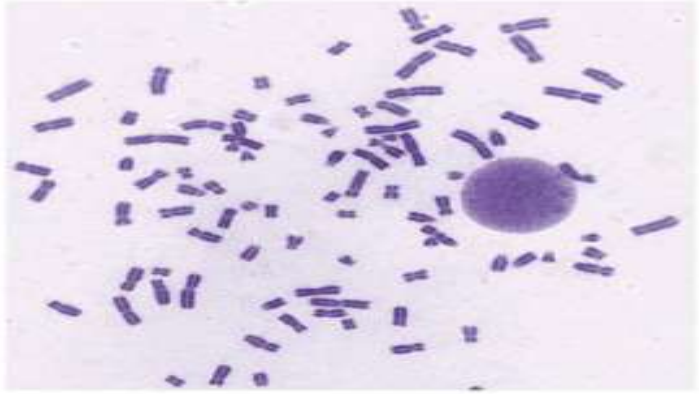
Multiple aberrations



Pulverized



Polyploid



Polyploid

Fluorescence in situ hybridization (FISH)

- Fluorescent painting of specific chromosomes or chromosomal regions through the use of DNA sequence or chromosome specific labeled probes.
- Chromosomal inversions and reciprocal translocations

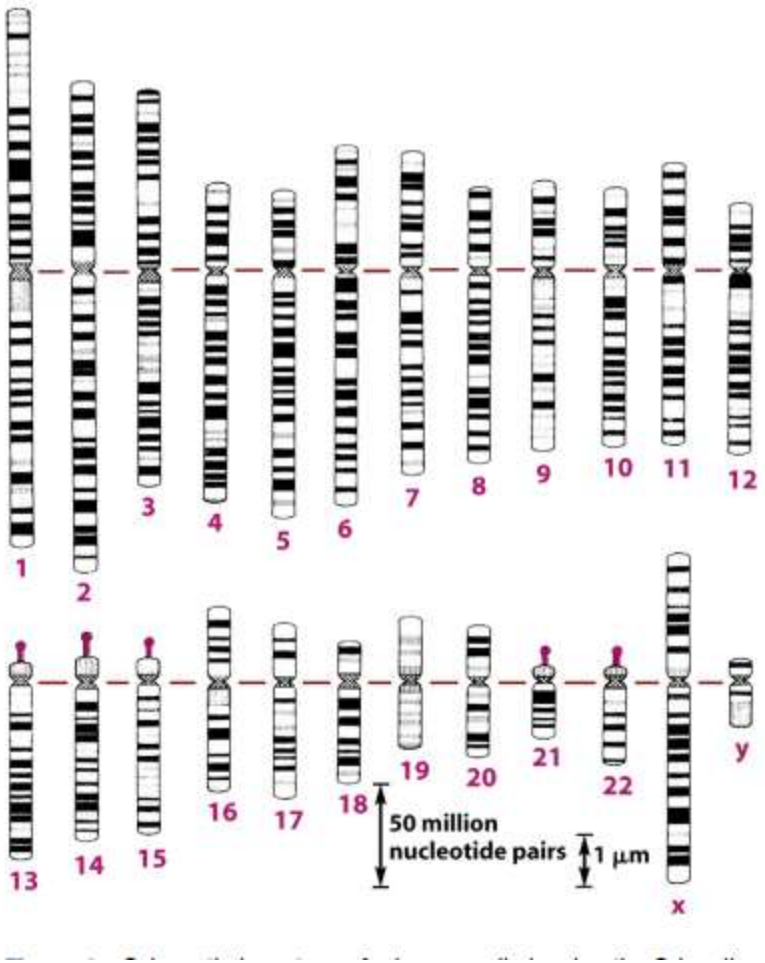


Figure 3 Human breast cancer cell showing aneuploidy for some chromosomes and translocations. The Biology of Cancer, ed. By Robert A. Weinberg, 2006 by Garland Science.

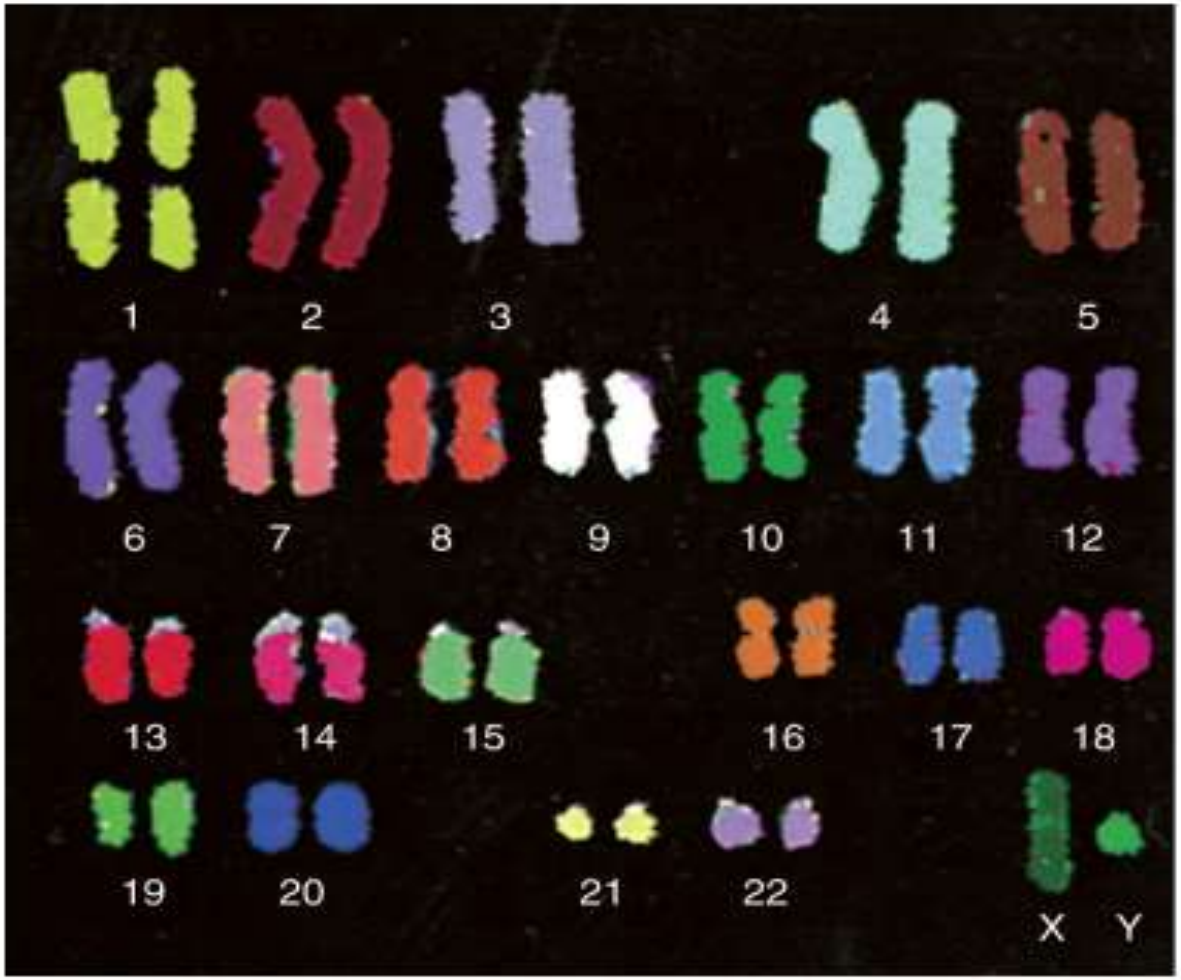


Figure 2 Karyotype of a human cell using FISH; appropriate chromosome probe sets allow for each chromosome pair to be computer colorized so that it has a specific color for analysis. The Biology of Cancer, ed. By Robert A. Weinberg, 2006 by Garland Science.

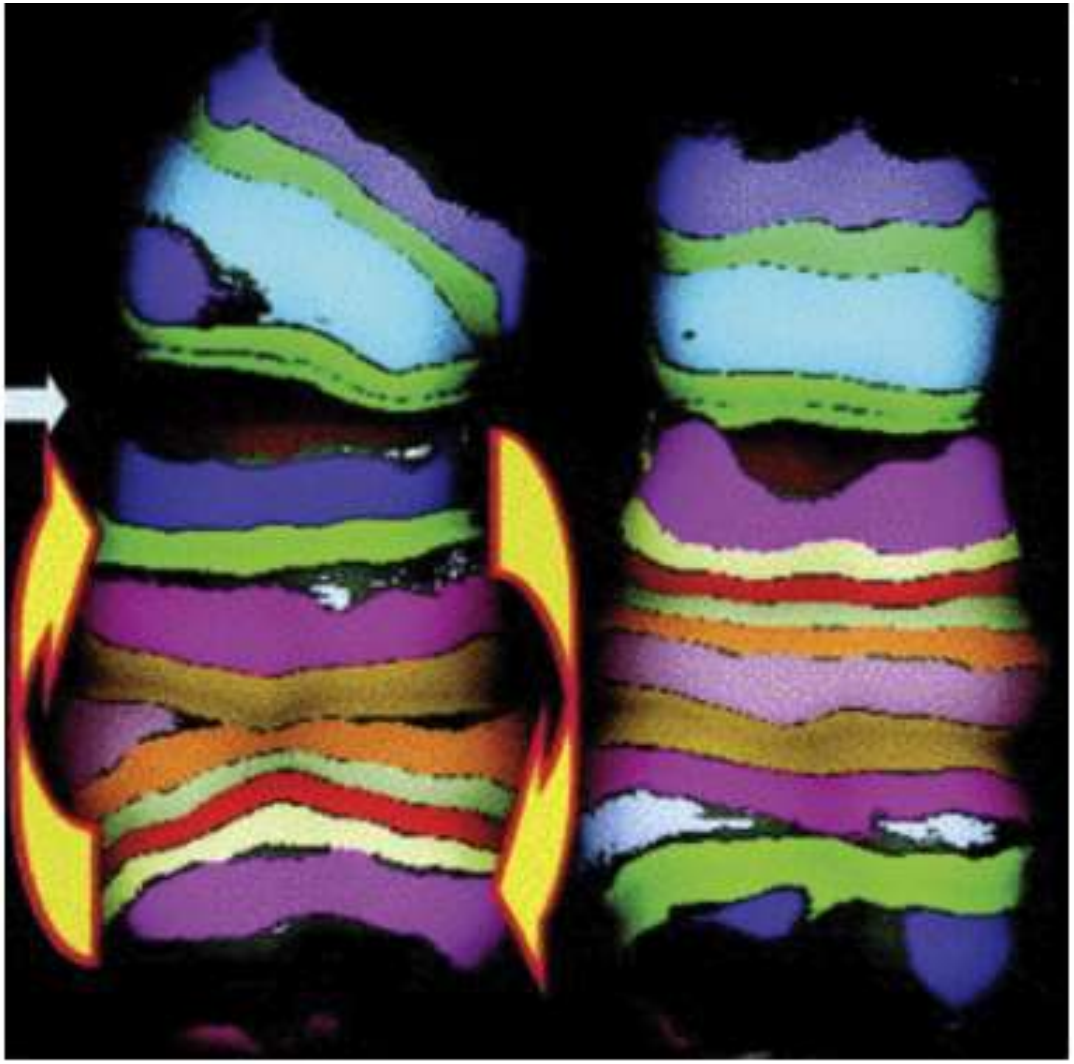


Figure 4 Region of human chromosome 5 stained by M-FISH; the left-hand segment has been inverted as can be seen when compared with the right-hand normal segment. The Biology of Cancer, ed. By Robert A. Weinberg, 2006 by Garland Science.

Comet assay

- Single cell gel electrophoresis (SCGE) or micro-gel electrophoresis (MGE)
- Ostling and Johanson
- Detect DNA damage
- Cells are covered in agarose on a glass slide and lysed in high salt and detergent to eliminate soluble cell components, membranes, and histones.
- DNA is left as nucleoids, consists of supercoiled DNA loops bound to a matrix.

- DNA breaks relax supercoiling, and relaxed loops of DNA are capable of extending during electrophoresis (normally at high pH), forming a ’comet tail,’
- Observed by fluorescence microscopy.
- Intensity of comet tail refers the break frequency.

Comet Assay Overview

Bacterial Culture

Blood/Serum Sample

Tissue Sample

DNA Damage (Chemicals or radiation)

Analysis of a DNA Comet

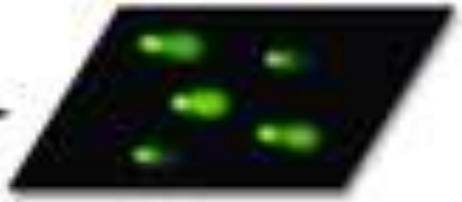
"Head" = intact DNA

"Tail" = damaged DNA

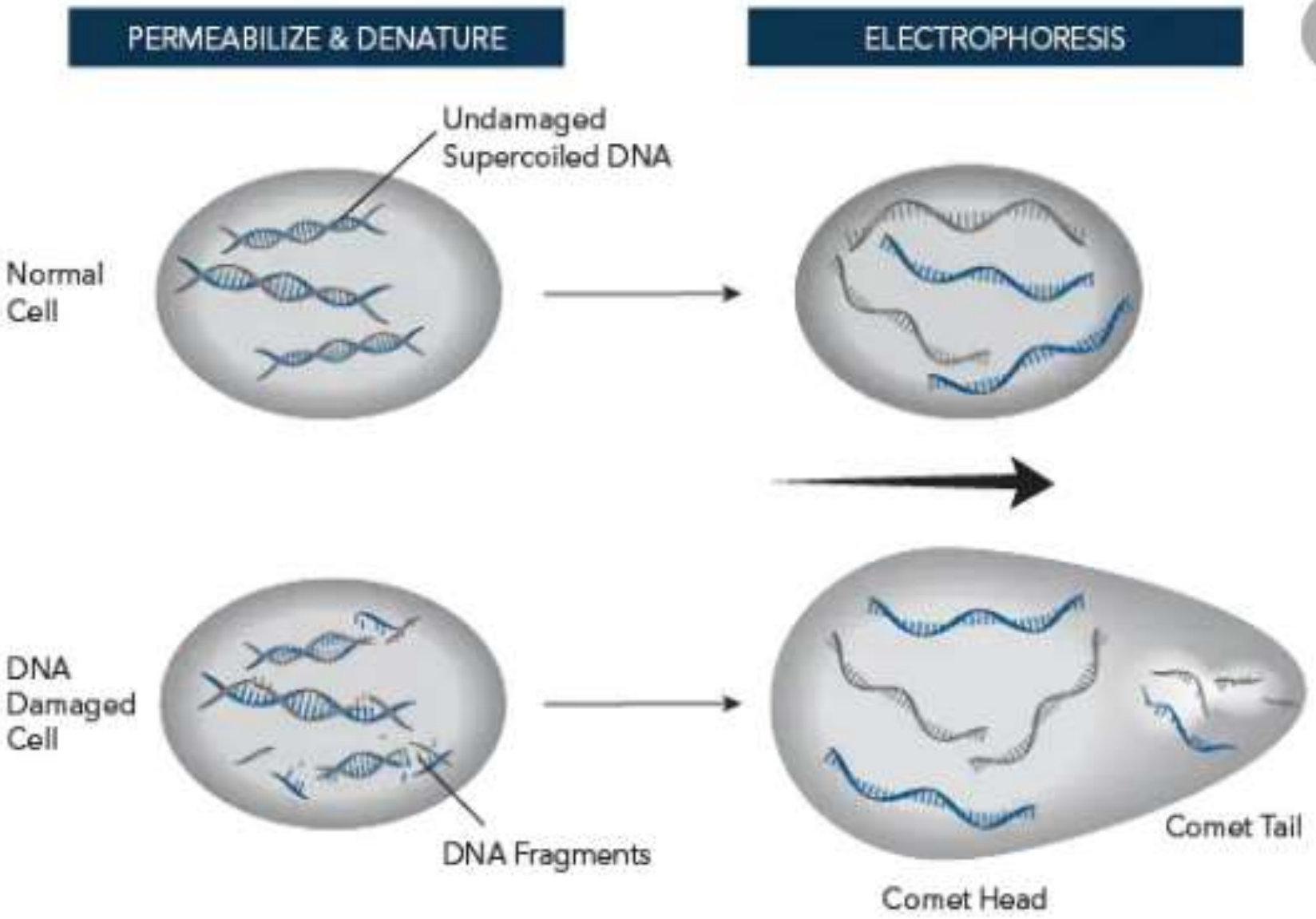
$^{Fluor}Tail$; $^{Fluor}Head$ $\propto$ extent of DNA damage

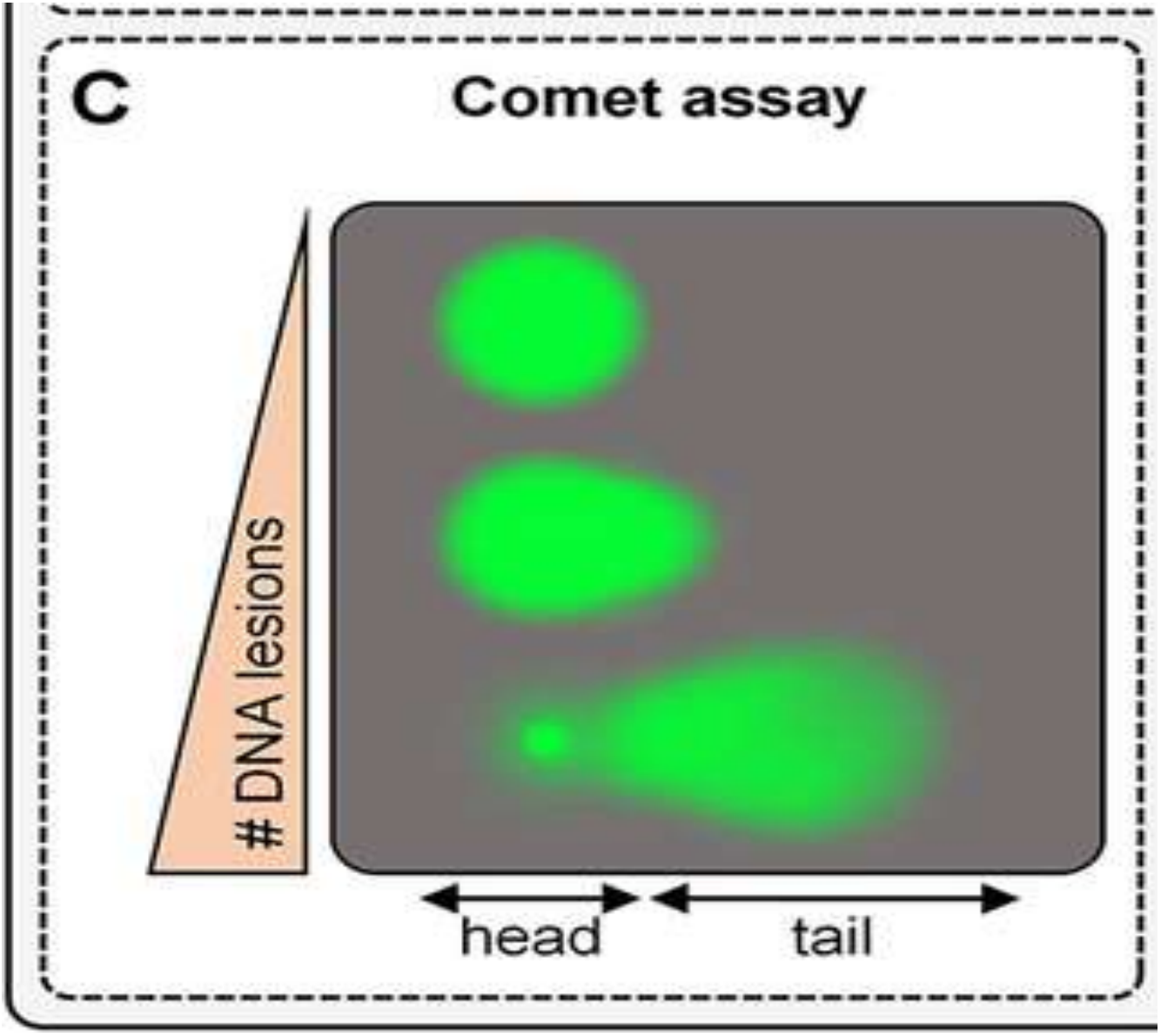
Damaged (relaxed) DNA in cells occurs from single-strand and double-strand breaks

Single cells are embedded on agarose-coated slides and lysed



Electrophoresis and fluorescent staining generate DNA "comets"


PERMEABILIZE & DENATURE
ELECTROPHORESIS
Undamaged
Supercoiled DNA
Normal
Cell
DNA
Damaged
Cell
DNA Fragments
Comet Tail
Comet Head


C
Comet assay
DNA lesions
head
tail

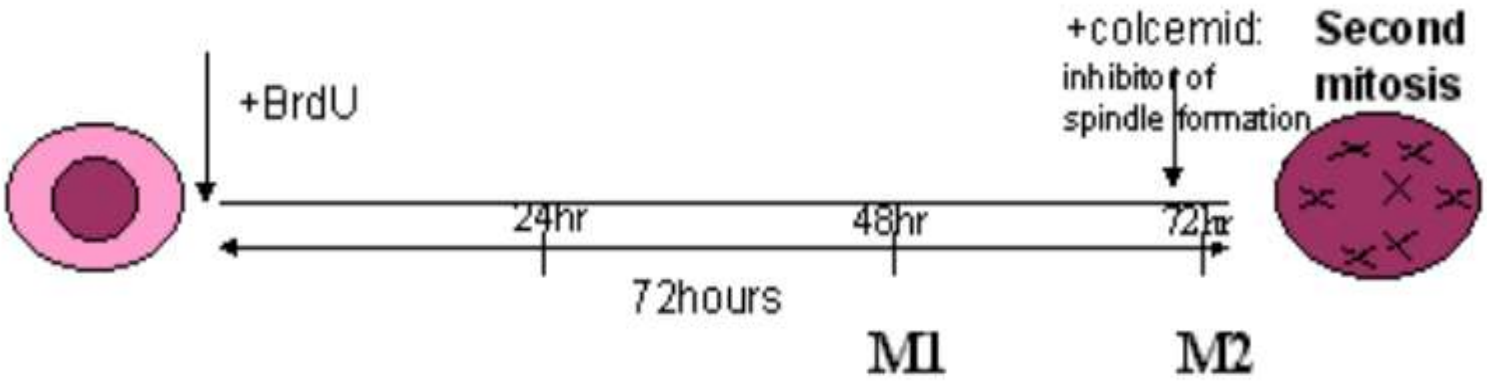
Alkaline comet assay

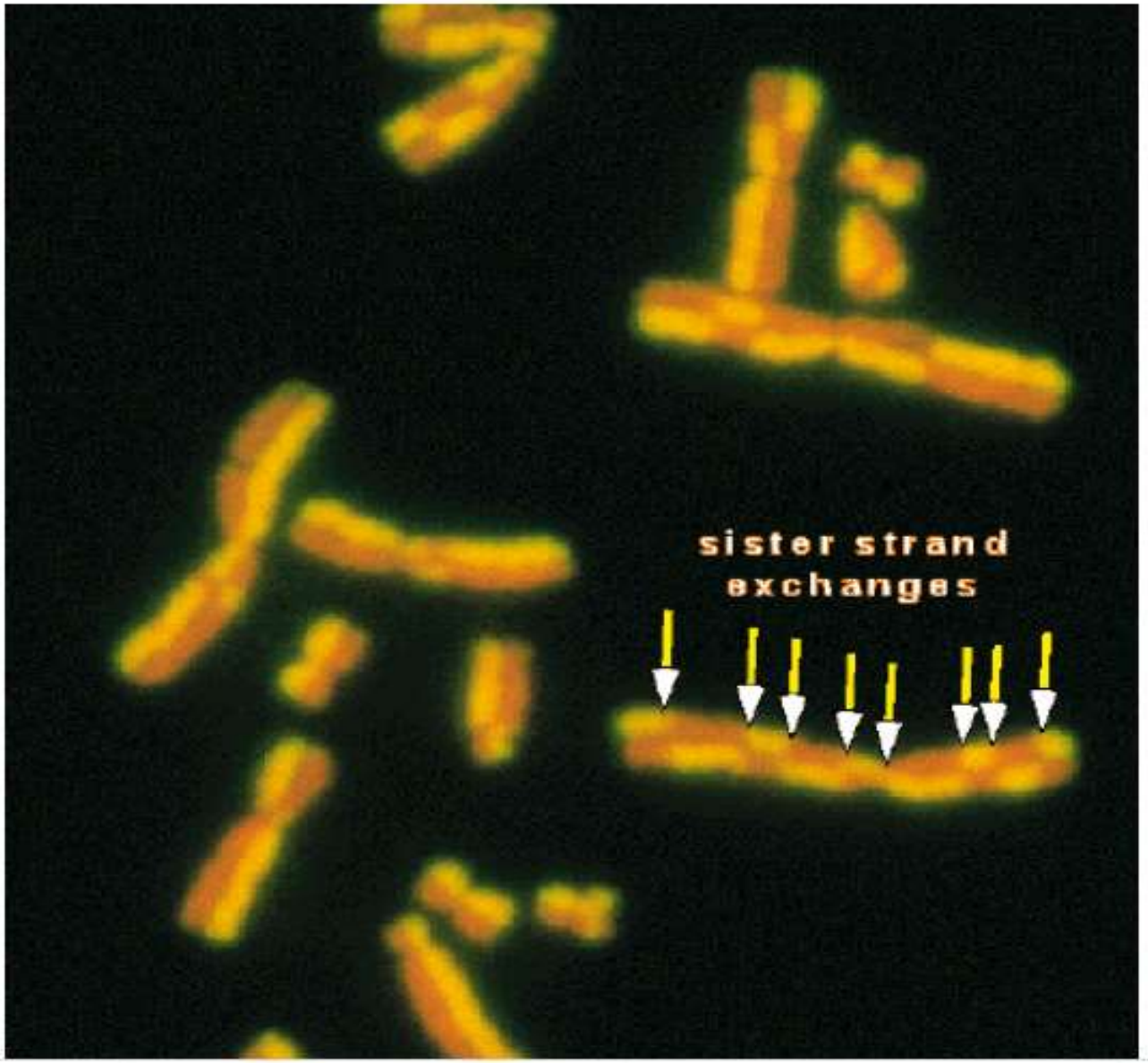
- Base damages,
- DNA single-strand breaks
- Double-strand breaks.

SCE test

- SCE involves the breaking down of two sister chromatids during DNA replication and rejoining each other, where the parts of the parental strands in the duplicated chromosomes are physically exchanged.

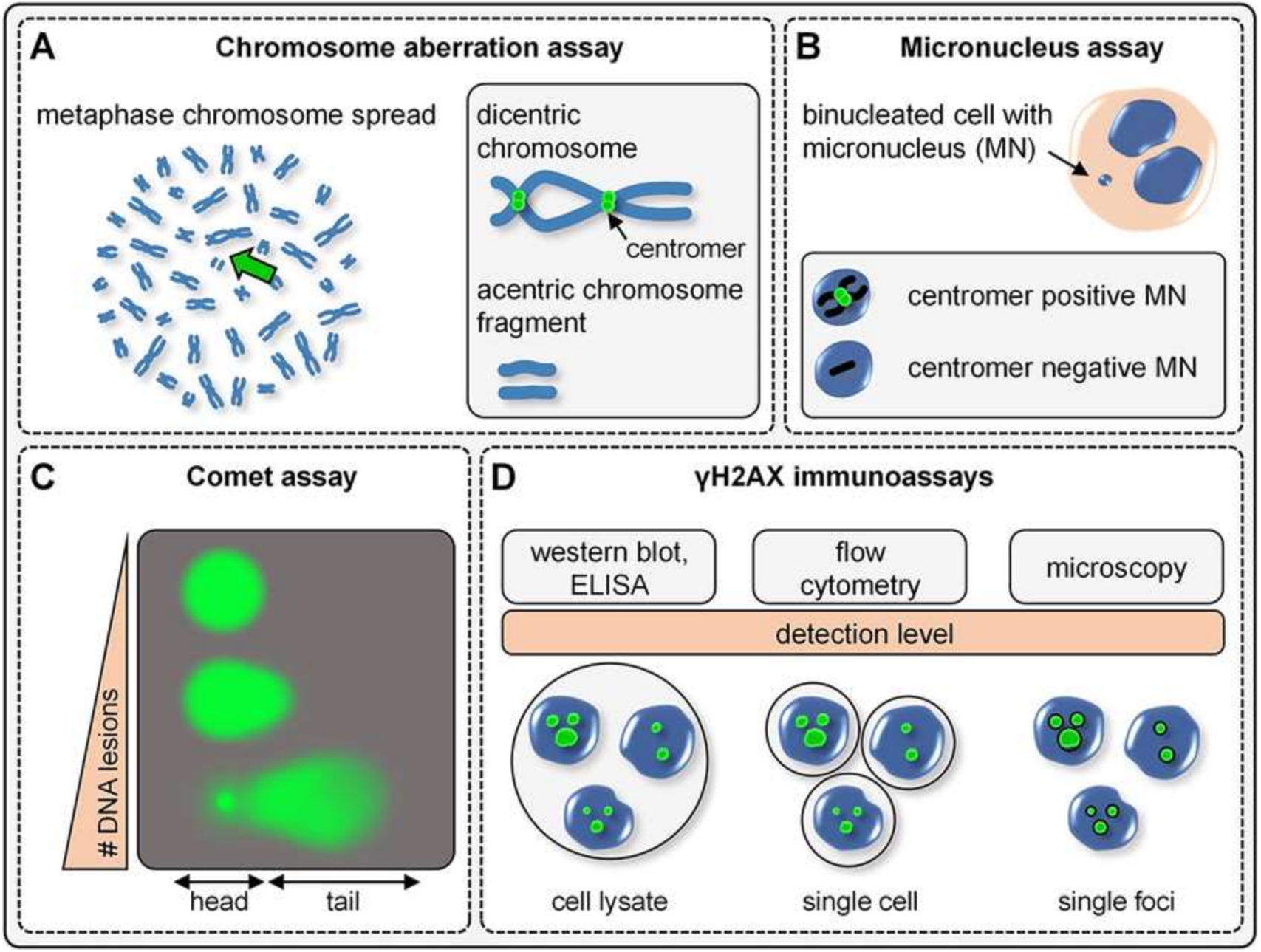
- In SCE assay, mammalian cells are treated with the agent and growth for two cycles of replication in 5-Bromo-2-deoxyuridine (5-BrdU)-containing media.
- Following treatment with a spindle inhibitor agent such as colchicine to arrest cells in a metaphase stage of mitosis (c-metaphase)
- Cells are collected and chromosomes are Prepared for observation with fluorescence plus Giemsa (FPG) procedure


+colcemid:
inhibitor of
spindle formation
Second
mitosis
+BrdU
24hr
48hr
72hr
72hours
M1
M2


sister strand
exchanges

- 3D culture systems, flow cytometric analysis, and highthroughput methods of gene expression analysis
- Interpretation of genotoxicity test outcomes, to better understand the mechanism of action of chemicals and to improve extrapolation to possible effects in human and environmental health.

- Limitations of current genotoxicity testing procedures include false positive results which effect the reliability of the methods.
- There is also the difficulty of comparing in vivo experimental models used in genotoxicity studies to human system in addition to the difficulty in classifying genotoxic and non-genotoxic carcinogens according to their specific pathogenic mechanism


A
Chromosome aberration assay
metaphase chromosome spread
dicentric chromosome
centromer
acentric chromosome fragment
B
Micronucleus assay
binucleated cell with micronucleus (MN)
centromer positive MN
centromer negative MN
C
Comet assay
DNA lesions
head
tail
D
γH2AX immunoassays
western blot, ELISA
flow cytometry
microscopy
detection level
cell lysate
single cell
single foci

Acknowledgement

- The Presentation is being used for educational and non commercial Purposes
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.