Chromosome I

Dr.G.MATHAN
Assistant Professor
Department of Biomedical Science
Bharathidasan University
Tiruchirappalli, Tamil Nadu

What is a chromosome?

Chromosomes are the rod-shaped, filamentous bodies present in the nucleus, which become visible during cell division.

They are the carriers of the gene or unit of heredity.

- Chromosomes were first described by Strausberger in 1875.
- The term “Chromosome”, however was first used by Waldeyer in 1888.
- (Chromo = colour; Soma = body) due to affinity for basic dyes.
- Their number can be counted easily only during mitotic metaphase.

Prokaryotic chromosomes

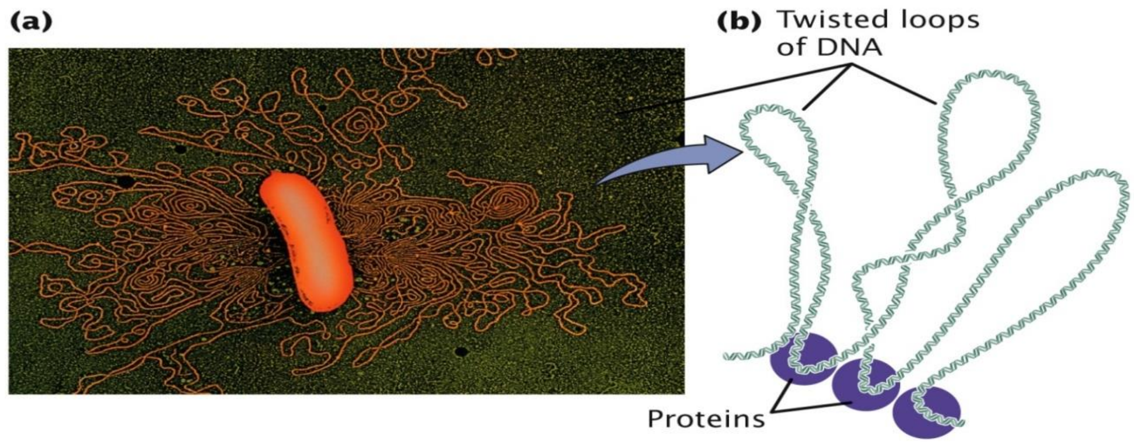


Fig_11-03 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

- Single and Circular, The chromosome exists as a highly folded and coiled structure dispersed throughout the cell.

- Complexed with protein in a structure termed the nucleoid
  There are about 50 loops in the chromosome of E.coli.

- loops are highly **twisted** or **supercoiled** structure with about four million nucleotide pairs.

- Continuous replication (no cell cycle)

Eukaryotic Chromosomes

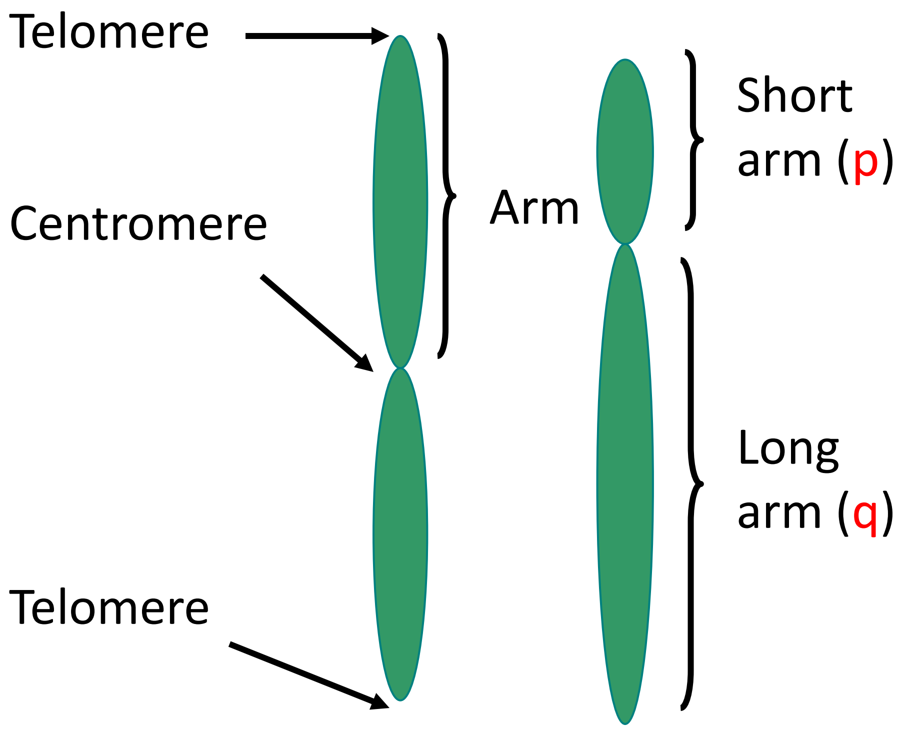
- Located in the nucleus

- Each chromosome consists of a single molecule of DNA and its associated proteins
  - The DNA and protein complex found in eukaryotic chromosomes is called chromatin
  - 1/3 DNA and 2/3 protein

- Complex interactions regulate gene and chromosomal function

Chromosome Morphology

- Telomere: chromosome ends
- Centromere: site of spindle attachment
  - Constriction of the metaphase chromosome at the centromere defines two arms
- Nucleosome: DNA double helix wrapped around histone proteins



Centromeres and Telomeres

- Centromeres and telomeres are two essential features of all eukaryotic chromosomes.
- Each provide a unique function i.e., absolutely necessary for the stability of the chromosome.
- Centromeres are required for the segregation of the centromere during meiosis and mitosis.
- Teleomeres provide terminal stability to the chromosome and ensure its survival

The centromere

- The region where two chromatids are joined.
- The sites of attachment to the mitotic spindle via kinetochore.
- During **mitosis**, the centromere that is shared by the sister chromatids
- first meiotic division the centromere of sister chromatids must remain intact. Whereas during meiosis II they must act as they do during mitosis.
- **Therefore the centromere is an important component of chromosome**

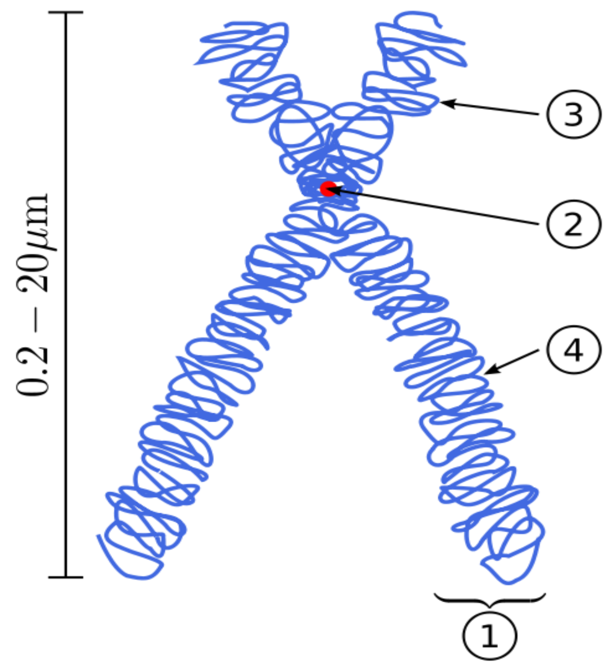
Chromosomal components:

① **Chromatid**

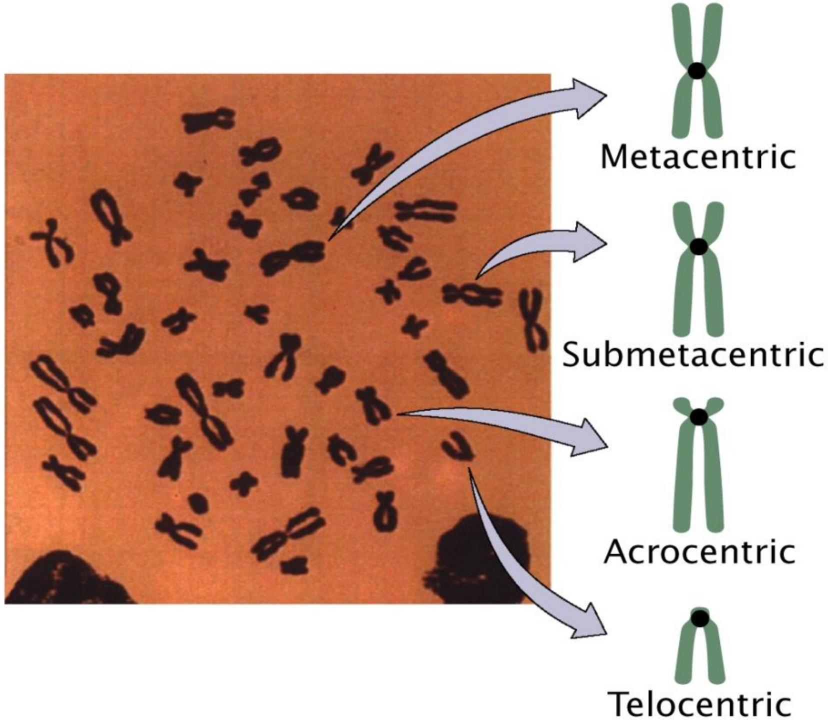
② **Centromere/Primary Constriction**

③ **Short arm**

④ **Long arm**



- In general, if the centromere is near the middle, the chromosome is **metacentric**
- If the centromere is toward one end, the chromosome is **acrocentric** or **submetacentric**
- If the centromere is very near the end, the chromosome is **telocentric**.



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Kinetochore

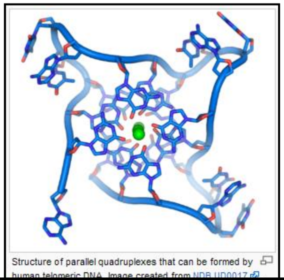
- The actual location where the attachment occurs is called the **kinetochore** and is composed of both DNA and protein.
- The DNA sequence within these regions is called ***CEN* DNA**.

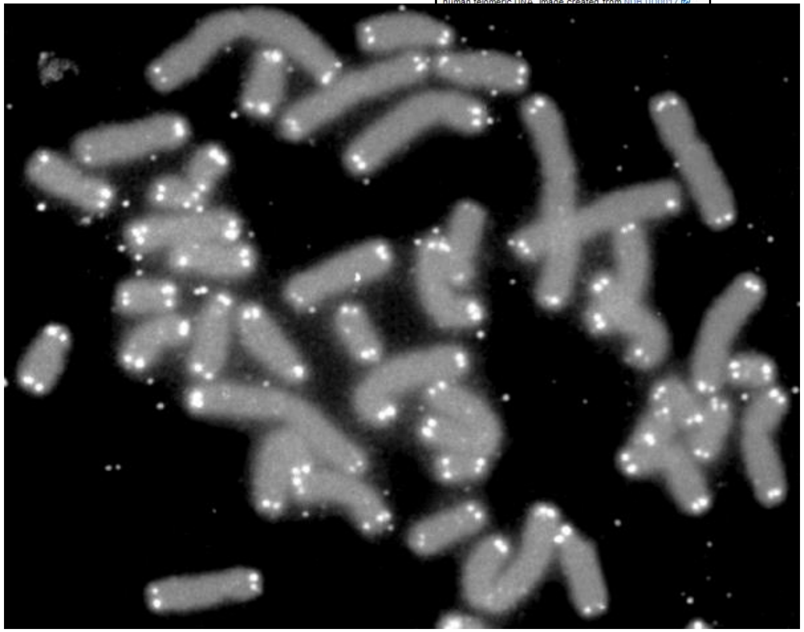
120 base pairs long → **3 sub domains-CDE-I, CDE-II and CDE-III.**

- A complex of three proteins called **Cbf-III** binds to normal CDE-III regions but can not bind to a CDE-III region with a point mutation that prevents mitotic segregation.

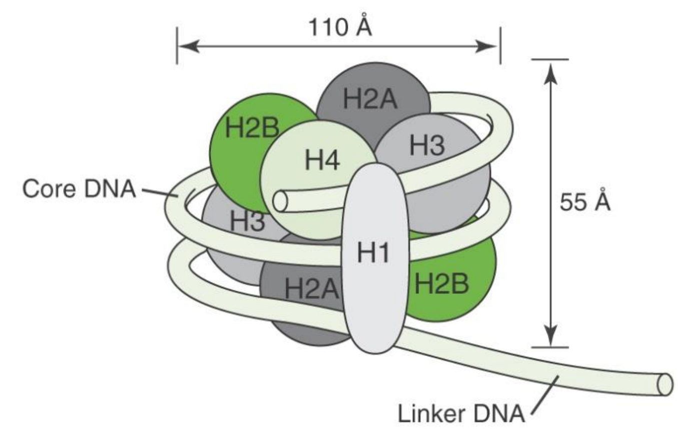
Telomeres

- Specialized DNA sequences which form the ends of the linear DNA of the eukaryotic chromosome.
- Contains up to hundreds copies of a short repeated sequence (5’-TTAGGG-3’ in human).
- Synthesized by the enzyme telomerase (a ribonucleoprotein) independent of normal DNA replication.
- The telomeric DNA forms a special secondary structure to protect the chromosomal ends from degradation.





- Detailed analysis of these nucleosome core particles has shown that they contain **146 base pairs of DNA wrapped 1.75 times around a histone** core consisting of two molecules each of H2A, H2B, H3, and H4 (the core histones).



- One molecule of the fifth **histone H1,** is bound to the DNA as it enters and exists each nucleosome core particle. stabilizes the DNA between the nucleosome cores.

- This forms a chromatin subunit known as **chromatosome**, which consist of 166 base pairs of DNA wrapped around histone core and held in place by H1 (a linker histone)

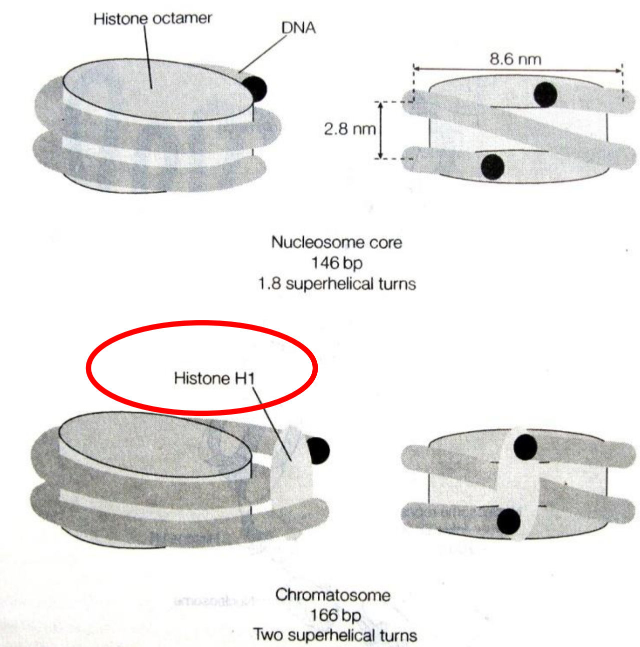
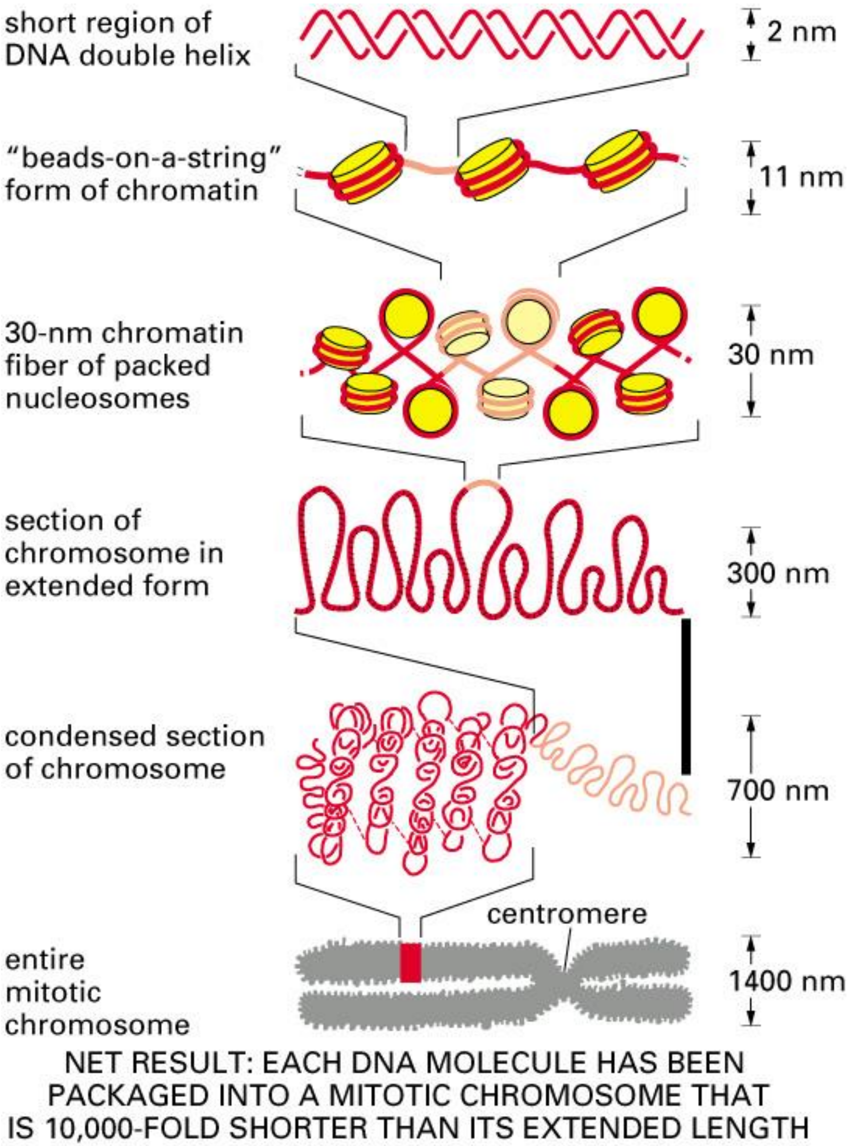


Fig. 1. A schematic view of the structure of a nucleosome core and a chromatosome.

Levels of chromatin structure



Larger DNA structures mediate more compaction.

Euchromatin: transcribed and less condensed

"Loops" of 30-nm fibers seen at interphase

Heterochromatin: more condensed, genes silenced, replicated later in S phase.

Number of chromosomes

- Normally, all the individuals of a **species have the same number** of chromosomes.
- Closely related species usually have similar chromosome numbers.
- Presence of a whole sets of chromosomes is called **euploidy**.
- It includes haploids, diploids, triploids, tetraploids etc.
- Gametes normally contain only one set of chromosome – this number is called **Haploid**
- Somatic cells usually contain two sets of chromosome - **2n : Diploid**

3n – triploid

4n – tetraploid

The condition in which the chromosomes sets are present in a multiples of “n” is **Polyploidy**

When a change in the chromosome number does not involve entire sets of chromosomes, but only a few of the chromosomes - is **Aneuploidy.**

- Monosomics (2n-1)
- Trisomics (2n+1)
- Nullisomics (2n-2)
- Tetrasomics (2n+2)

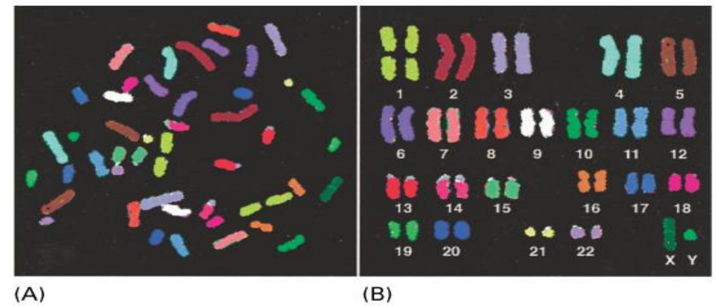
Organism	No. chromosomes
• Human	46
• Chimpanzee	48
• Dog	78
• Horse	64
• Chicken	78
• Goldfish	94
• Fruit fly	8
• Mosquito	6
• Round worm	2
Rice	24
Maize	20
Haploppus gracilis	4
Crepis capillaris	6

Chromosome Size

- In contrast to other cell organelles, the size of chromosomes shows a remarkable variation depending upon the stages of cell division.
- **Interphase:** chromosome are longest & thinnest
- **Prophase:** there is a progressive decrease in their length accompanied with an increase in thickness
- **Metaphase:** Chromosomes are the most easily observed and studied during metaphase when they are very thick, quite short and well spread in the cell.
- **Anaphase:** chromosomes are smallest.

- **Therefore, chromosomes measurements are generally taken during mitotic metaphase.**

<u>Karyotype</u>



- International System fc

Figure 5-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

  Human Cytogenetic Nomenclature (ISCN)
  - 46, XX – normal female
  - 46, XY – normal male
  - In a species , a pictorial or photographic representation of all the different chromosomes in a cell of an individual, chromosomes are usually ordered by size and numbered from largest to smallest.

Staining and Banding chromosome

Staining procedures have been developed in the past two decades and these techniques help to study the karyotype in plants and animals.

1. **Feulgen Staining**:

   Cells are subjected to a mild hydrolysis in 1N HCl at 60^0C for 10 minutes.

   This treatment produces a free aldehyde group in deoxyribose molecules.

   When Schiff's reagent (basic fuschin bleached with sulfurous acid) to give a deep pink colour.

   Ribose of RNA will not form an aldehyde under these conditions, and the reaction is thus specific for DNA

2. Q banding:

The Q bands are the **fluorescent bands** observed after **quinacrine mustard staining** and observation with UV light.

The distal ends of each chromatid are not stained by this technique.

The Y chromosome become brightly fluorescent both in the interphase and in metaphase.

3. R banding:

The R bands (from **reverse**) are those located in the zones that do not fluoresce with the quinacrine mustard, that is they are between the Q bands and can be visualized as green.

4. G banding:

The G bands (from **Giemsa)** have the same location as Q bands and do not require fluorescent microscopy.

Many techniques are available, each involving some pretreatment of the chromosomes.

In **ASG (Acid-Saline-Giemsa**) cells are incubated in citric acid and NaCl for one hour at 60^0C and are then treated with the Giemsa stain.

5. C banding:

The C bands correspond to **constitutive heterochromatin**.

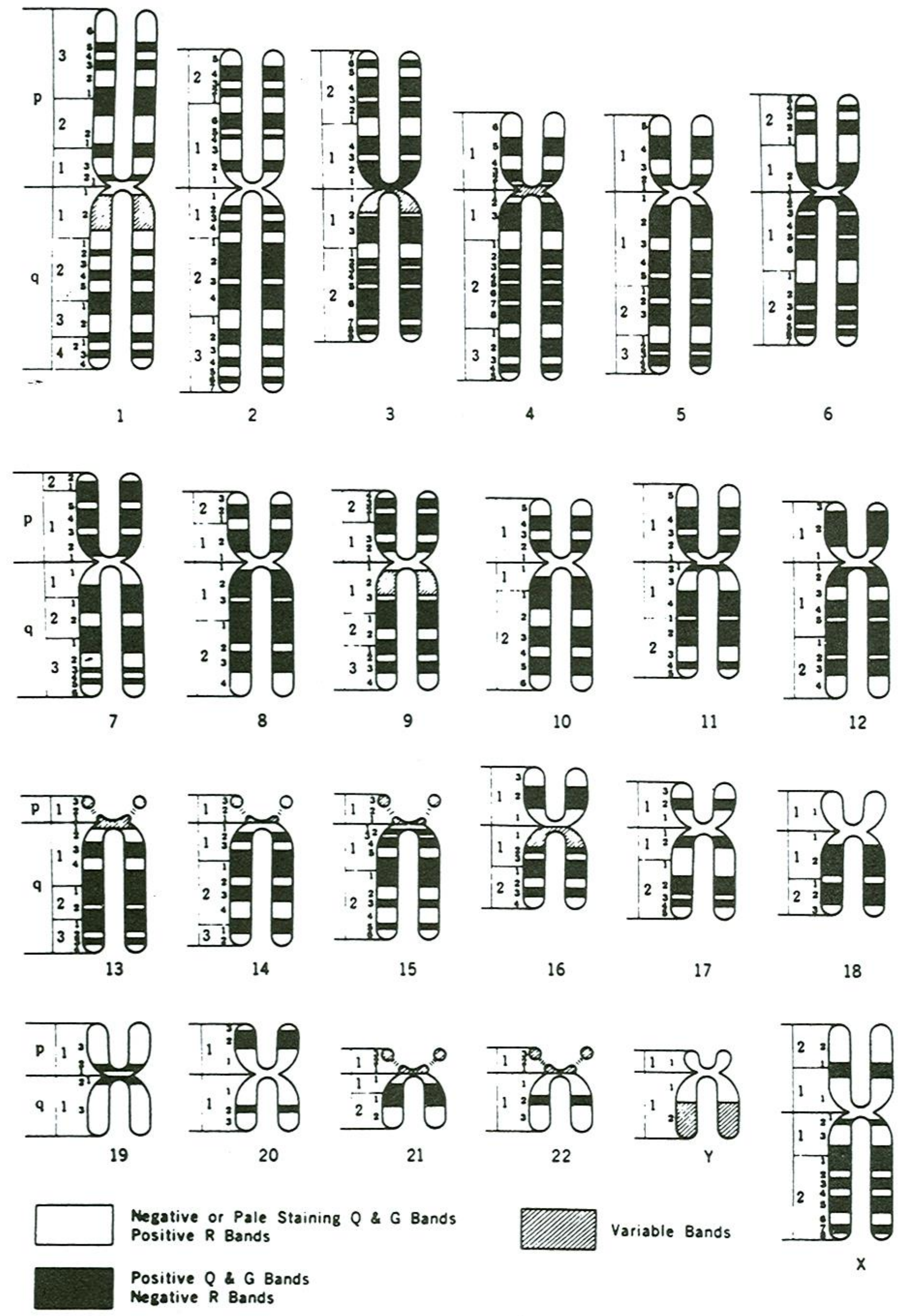
The heterochromatin regions in a chromosome distinctly differ in their stainability from euchromatic region.

Ideogram

- Diagramatic representation of a karyotype

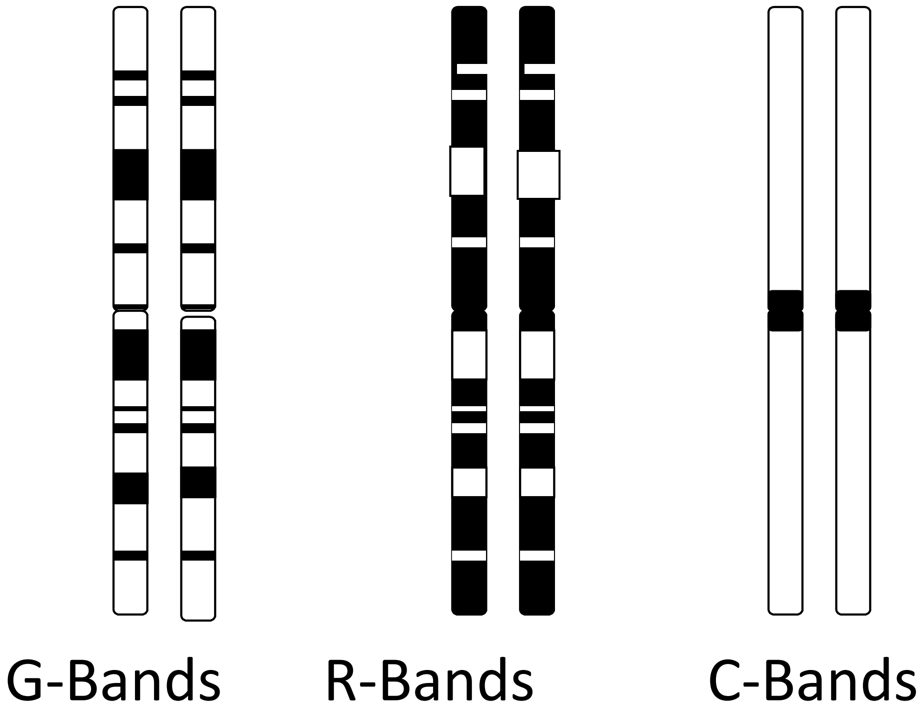
- Individual chromsomes are recognized by
  - -arm lengths
    - p, short
    - q, long
  - -centromere position
    - metacentric
    - sub-metacentric
    - acrocentric
    - telocentric
  - -staining (banding) patterns

From Miller & Therman (2001) Human Chromosomes, Springer

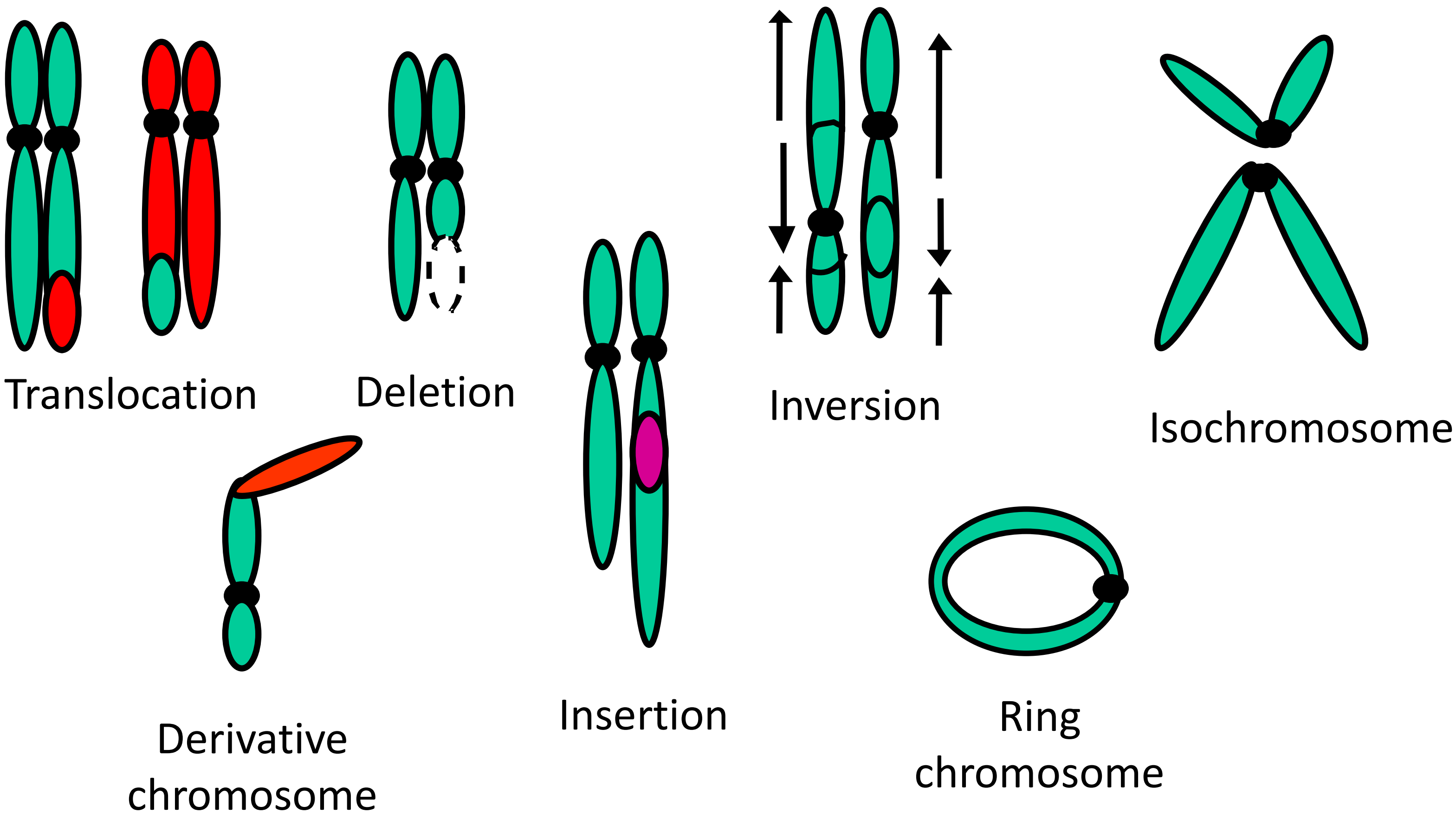


Visualizing Metaphase Chromosomes (Banding)

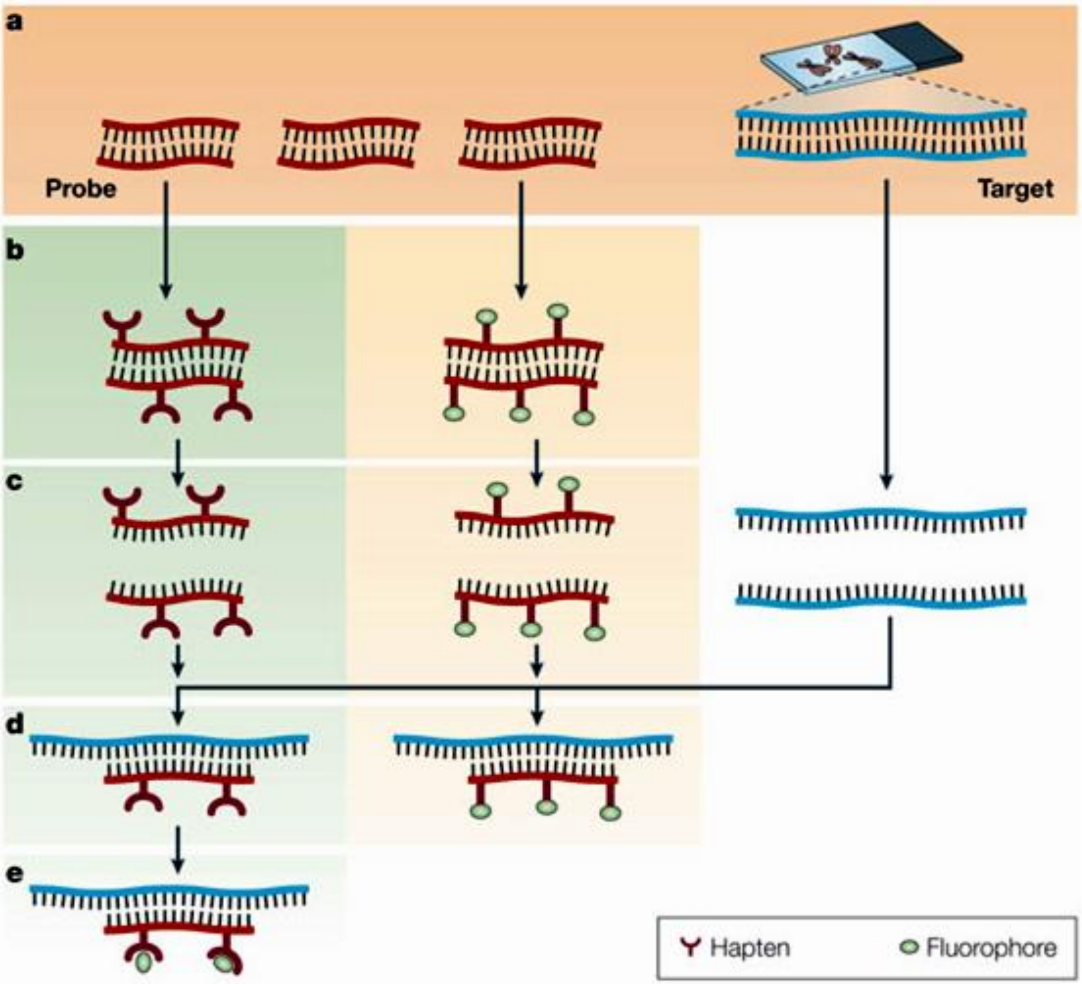
- Q (quinicrine) & G (Giemsa) banding preferentially stain AT rich regions
- R (reverse banding) preferentially stains GC-rich regions
- C-banding (denaturation & staining) preferentially stains constitutive heterochromatin, found in the centromere regions and distal Yq



Chromosome Structure Abnormalities



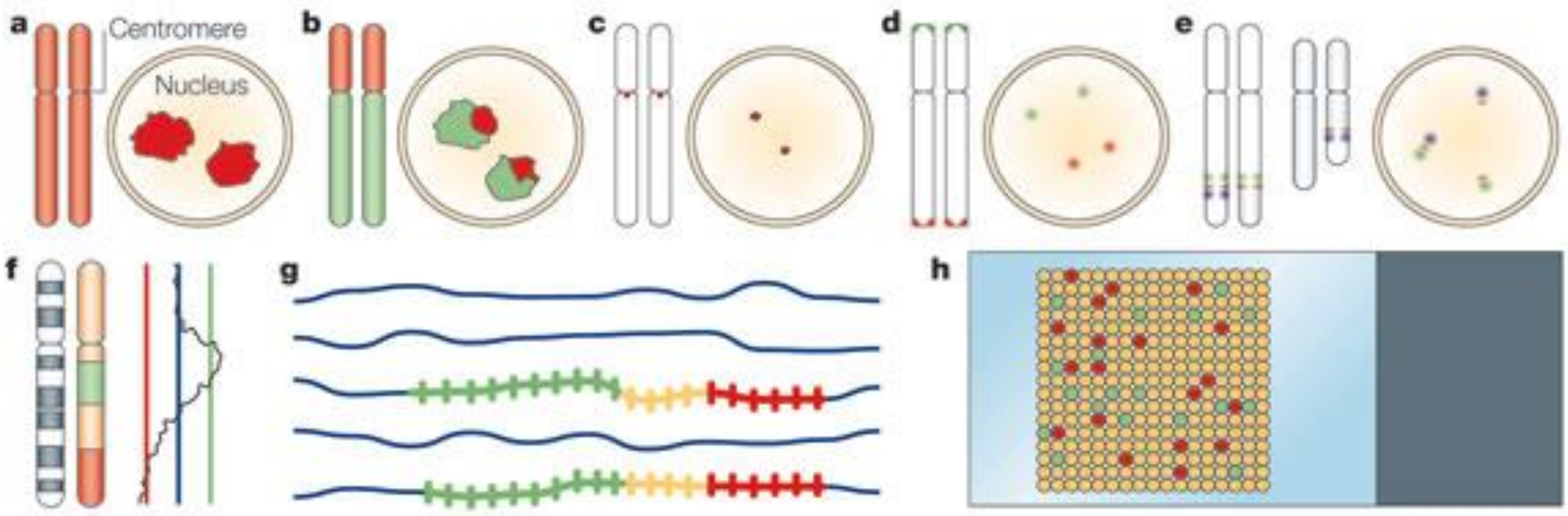
Fluorescence in situ hybridization (FISH)



Nature Reviews | Genetics

a | The basic elements of fluorescence in situ hybridization are a DNA probe and a target sequence. b | Before hybridization, the DNA probe is labelled. Two labelling strategies are commonly used — indirect labelling (left panel) and direct labelling (right panel). For indirect labelling, probes are labelled with modified nucleotides that contain a HAPTEN, whereas direct labelling uses the incorporation of nucleotides that have been directly modified to contain a fluorophore. c | The labelled probe and the target DNA are denatured to yield ssDNA. d | They are then combined, which allows the annealing of complementary DNA sequences. e | If the probe has been labelled indirectly, an extra step is required for visualization of the non-fluorescent hapten that uses an enzymatic or immunological detection system. Whereas FISH is faster with directly labelled probes, indirect labelling offers the advantage of signal amplification by using several layers of antibodies, and might therefore produce a signal that is brighter compared with background levels. Finally, the signals are evaluated by fluorescence microscopy (not shown). [From Speicher & Carter (2005) Nature Rev Genet 6:782]

Fluorescence in situ hybridization (FISH)



[From Speicher & Carter (2005) Nature Rev Genet 6:782]

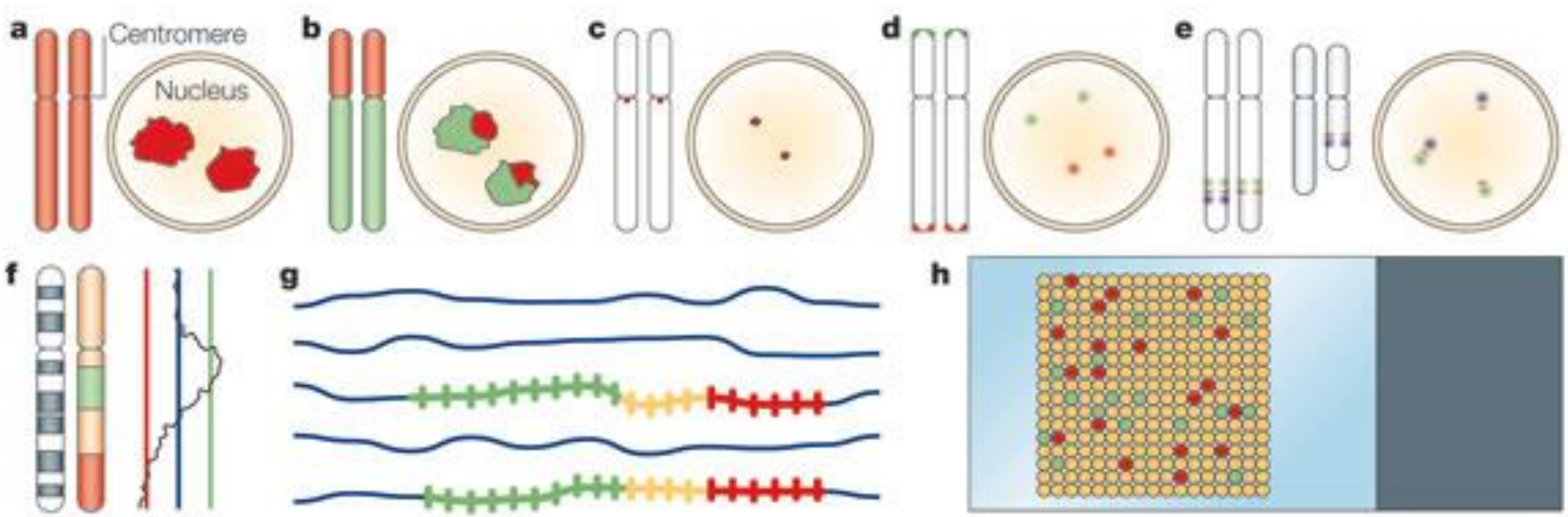
a | Painting probes stain entire chromosomes.

b | Regional painting probes

c | Centromeric-repeat probes are available for almost all human chromosomes.

d | Subtelomeric probes, which are often used to screen for cryptic translocations that are not usually visible in conventional chromosome-banding analyses, are shown in this example e | Special probe sets can be designed to facilitate diagnosis of known structural rearrangements. In this example, the probe set includes a breakpoint-spanning probe (red) and two breakpoint-flanking probes (green and blue).

f | Genomic DNA is used as the probe in comparative genomic hybridization (CGH) to establish copy number. An analysis of chromosome 8 is shown as an example. Simultaneous visualization of both test DNA (green region) and normal reference DNA (red region) fluorochromes shows balanced regions in orange (equal amounts of green and red fluorochromes



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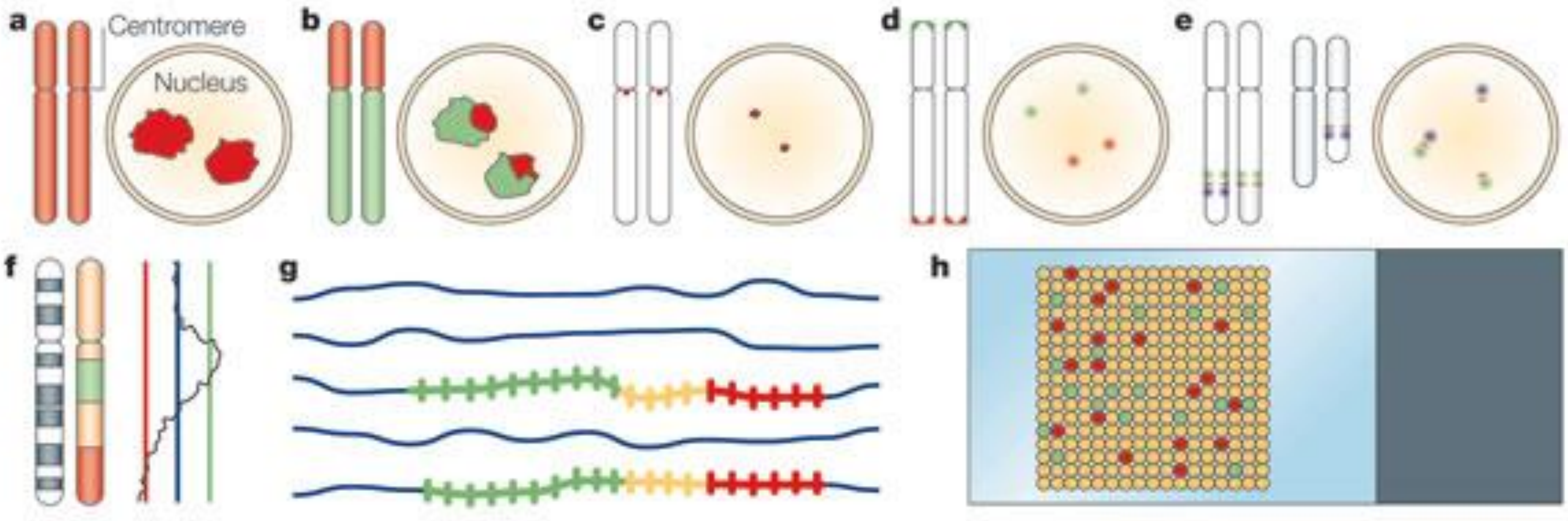

g | For high-resolution analysis, DNA fibres can be used as the target for probe hybridization. The simultaneous hybridization of two different probes is shown, labelled green and red.

h | Microarrays can be used as targets for hybridization to provide resolutions down to the single-nucleotide level. A BAC array is shown, to which test DNA and reference DNA are hybridized. Individual clones show different colours after hybridization depending on whether the corresponding DNA in the test sample is lost (red on the array), gained (green on the array) or neither (yellow on the array).

Acknowledgement

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- Thanks are due to all those original contributors and entities whose pictures used for making this presentation.