CHARACTERIZATION OF CELL LINE BY MORPHOLOGY CHROMOSOME ANALYSIS AND DNA CONTENT

INTRODUCTION

- **Cell line**: Once a primary culture is sub-cultured or passaged.
- **Normal cell line:** Divides a limited number of times.
- **Continuous cell line:** Cell line having the capacity for infinite survival (Immortal).
- ***Characterization*** *is the definition of the many traits of the cell line, some of which may be unique.*

Need for cell line characterization

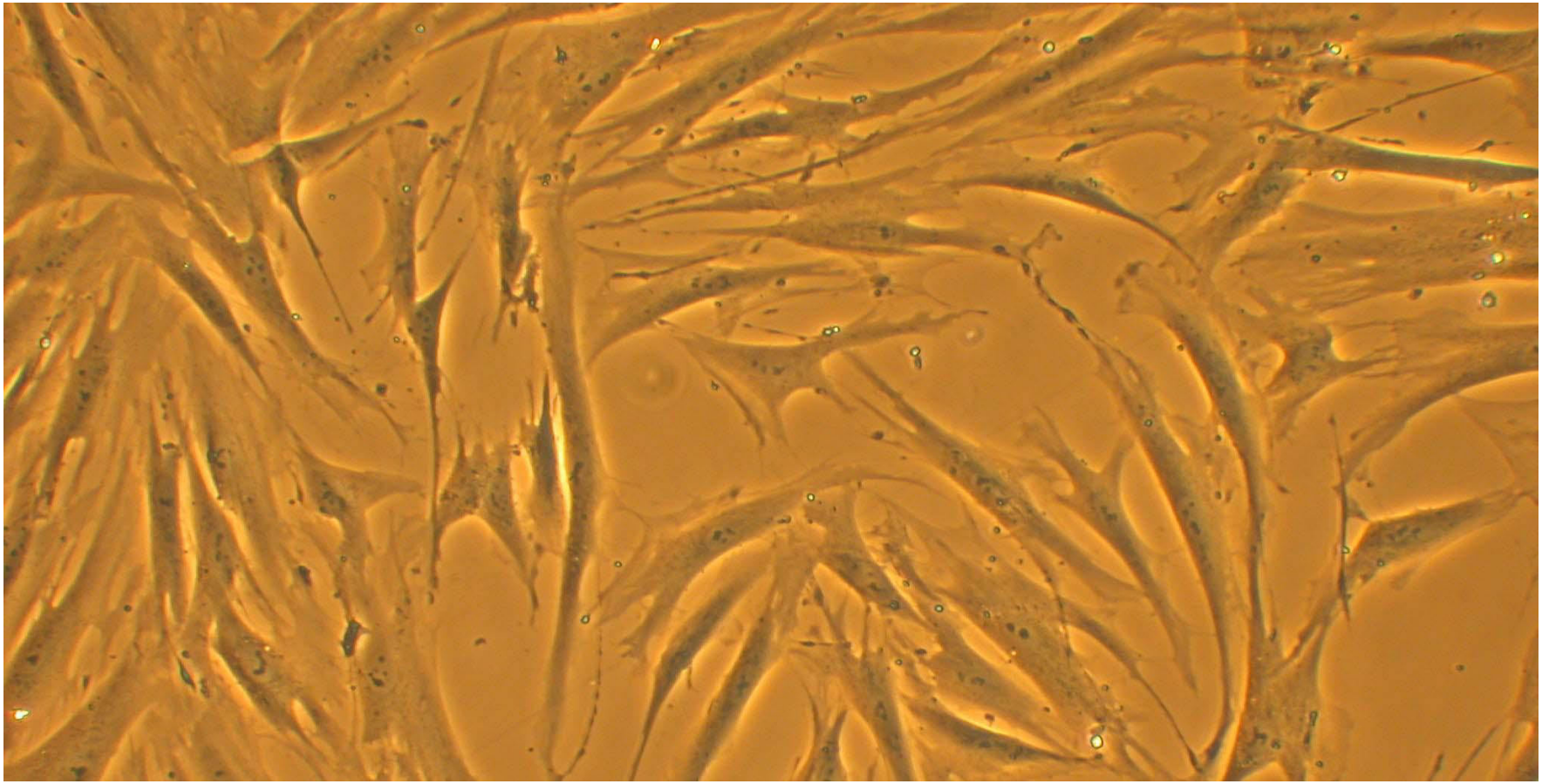
- Demonstration of absence of cross-contamination
- Confirmation of the species of origin
- Correlation with the tissue of origin
- To detect transformed cell line
- To see genetic instability

Methods of characterization

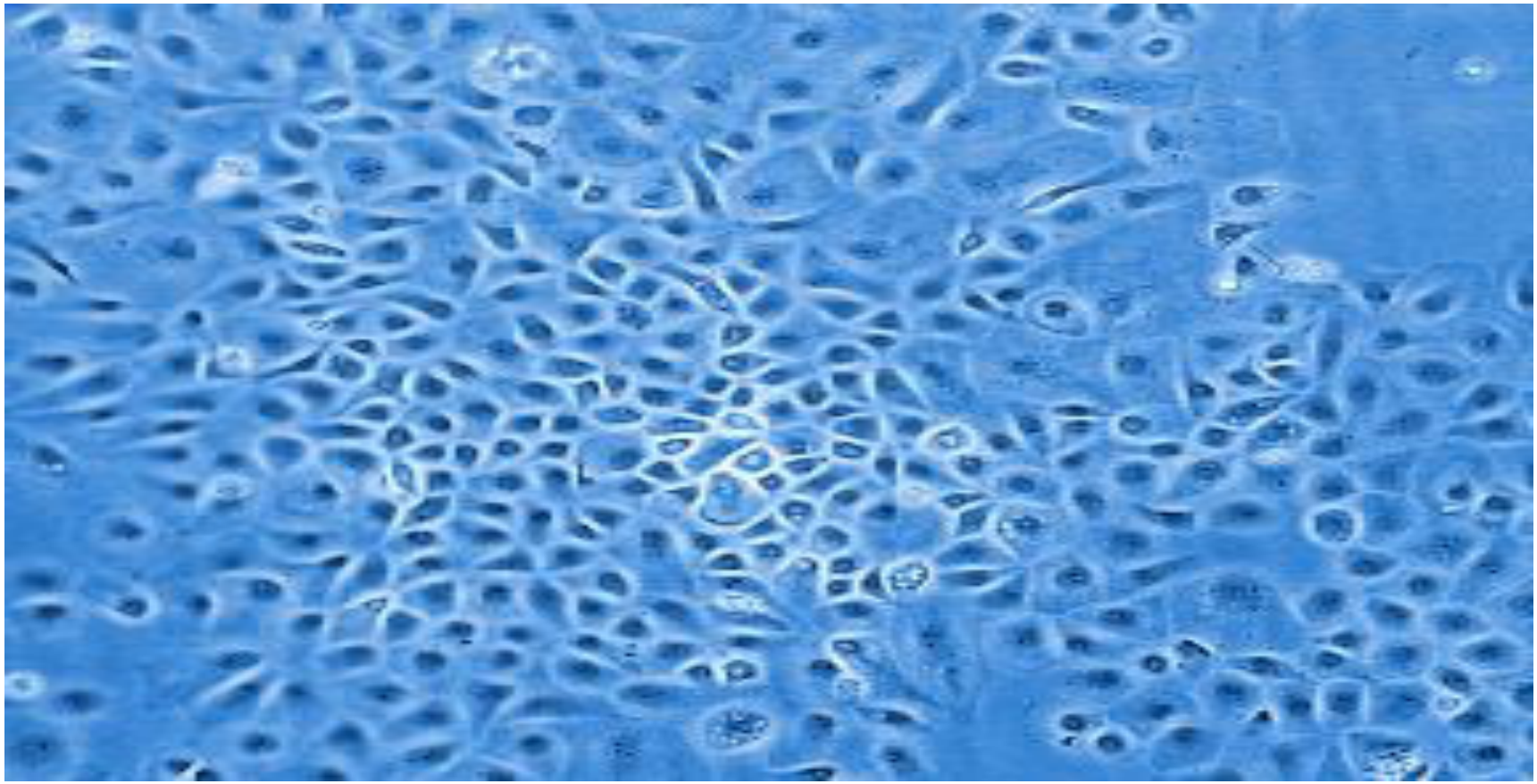
- Cell morphology
- Chromosome analysis
- DNA content
- RNA and protein expression
- Enzyme activity
- Antigenic markers
- Differentiation

MORPHOLOGY

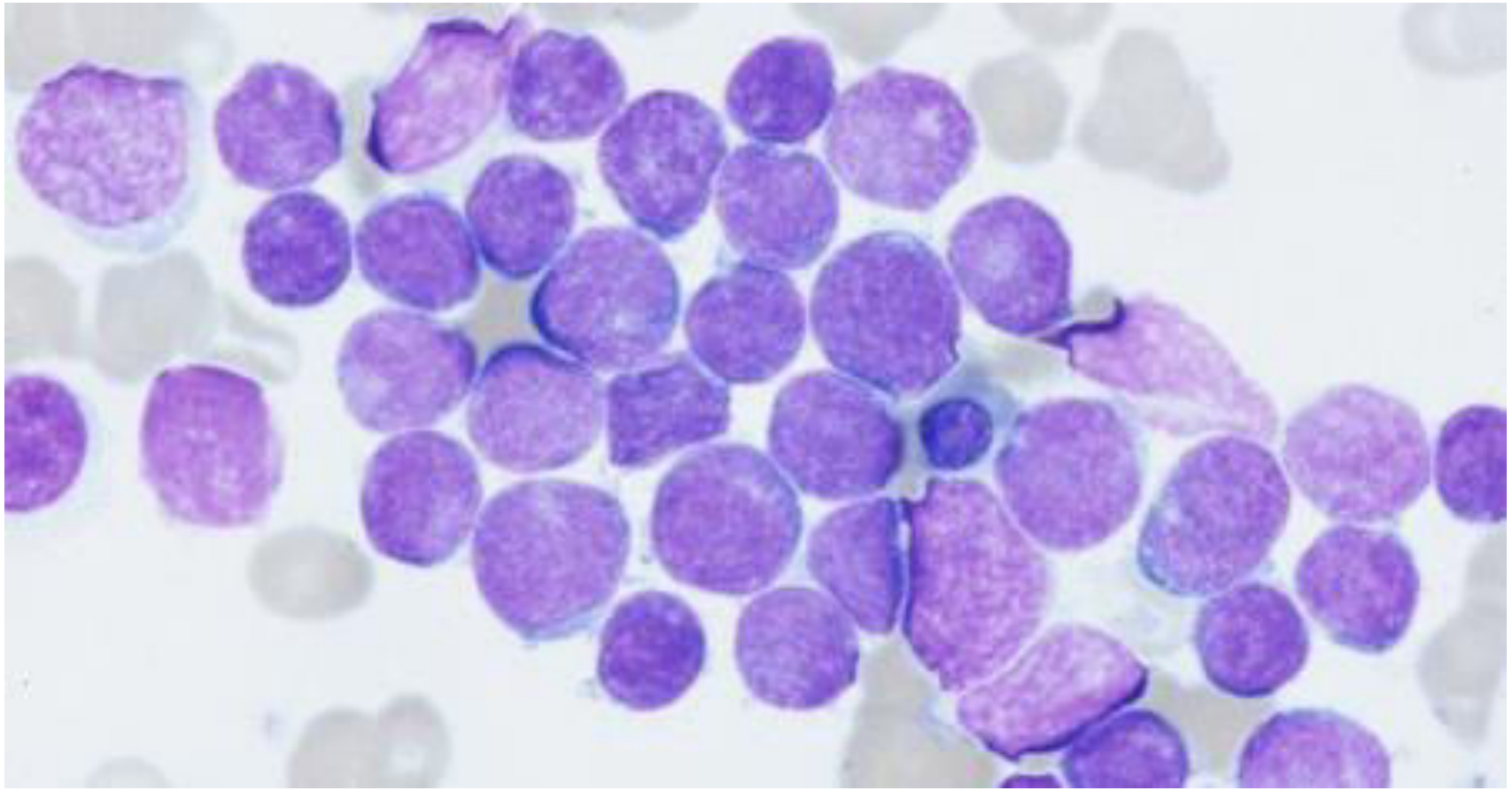
- Observation of morphology is the simplest and most direct technique to characterize cell lines.
- Study of the size, shape, and structure of cell.
- Most cells in culture can be divided in to five basic categories based on their morphology.
  - Fibroblastic
  - Epithelial-like
  - Lymphoblast-like
  - Endothelial
  - Neuronal



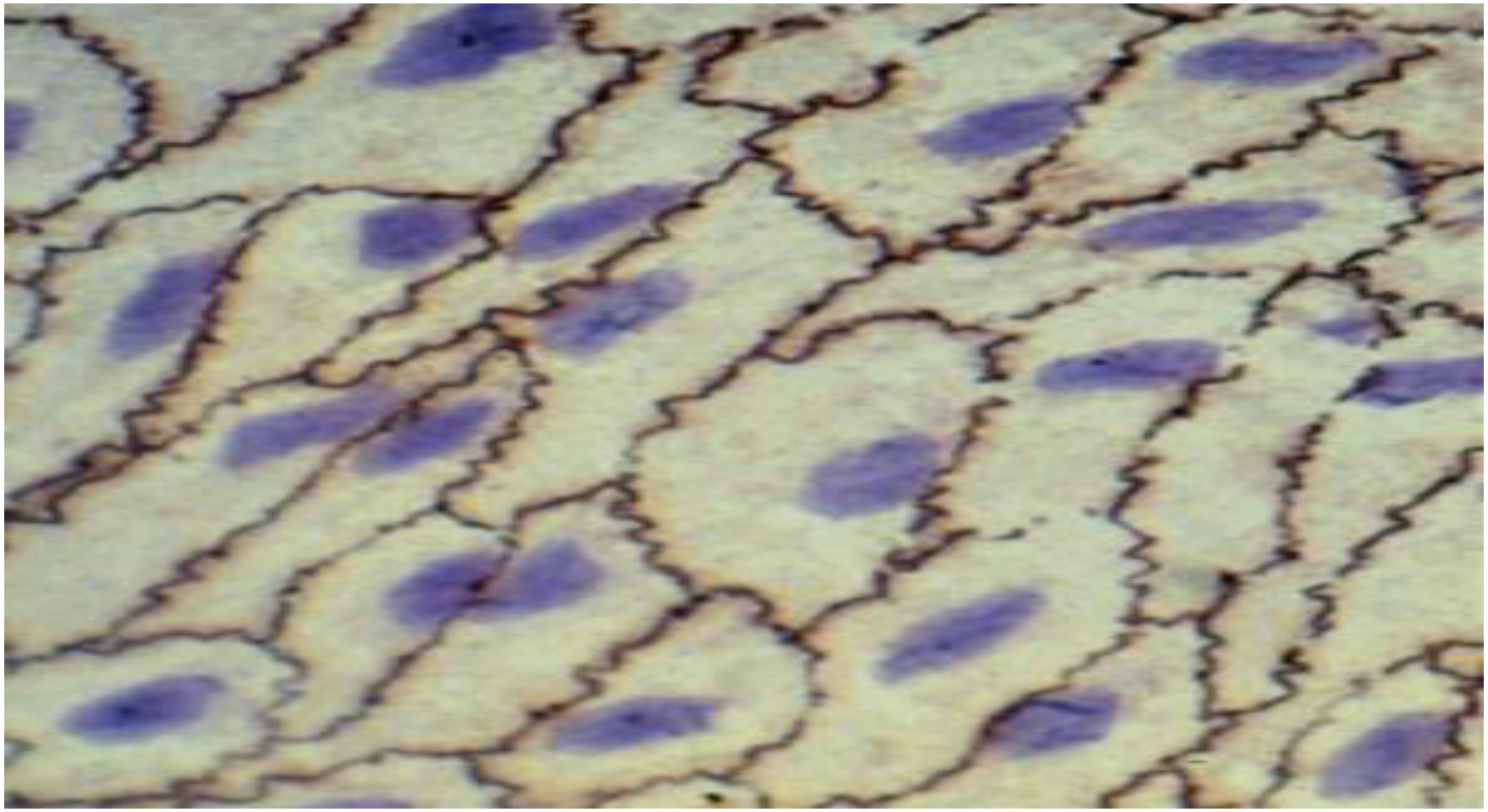
Fibroblastic (or fibroblast-like) **cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.**



Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.



Endothelial cells are very flat, have a central nucleus, are about 1-2 µm thick and some 10-20 µm in diameter

Neuronal cell line

Exist in different shapes and sizes, but they can roughly be divided into two basic morphological categories

- **Type I** with long axons used to move signals over long distances
- **Type II** without axons

Cell line	Organism	Origin tissue	Morphology
BEAS-2B	Human	Lung	Epithelial
BHK-21	Hamster	Kidney	Fibroblastic
HL-60	Human	Myeloblast	Blood cells
MDCK II	Dog	Kidney	Epithelium
CHO	Hamster	Ovary	Fibroblast

CHROMOSOME ANALYSIS

Karyotyping

- ***Chromosome banding***
- ***Chromosome painting***

KARYOTYPE

- A **karyotype** is the number and appearance of chromosomes in the nucleus of a eukaryotic cell.

- The chromosomes are depicted in a standard format known as a ***karyogram***: in pairs, ordered by size and position of centromere for chromosomes of the same size.

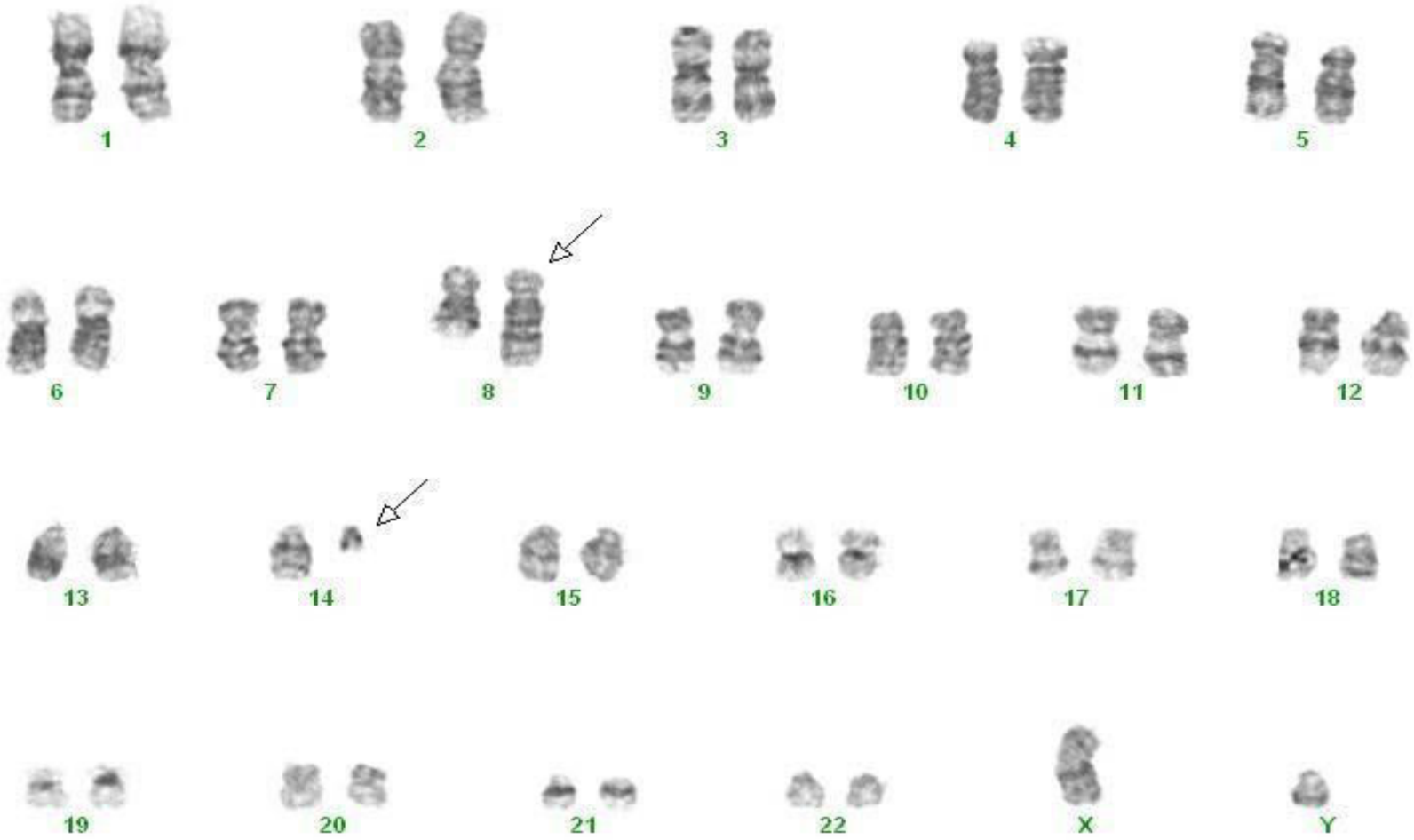
- **Karyotype analysis: a technique where chromosomes are visualized under a microscope.**

Cont...

- Karyotype analysis is best criteria for species identification.
- Genetic stability of ES and iPS cells are routinely monitored by karyotype analysis.
- Normal and transformed cells can be distinguished.
- Comparative phylogenetic studies of two species can be done.
- Confirmation or exclusion of a suspected cross-contamination.

OBSERVATIONS

- Differences in basic number of chromosomes
- Differences in absolute sizes of chromosomes
- Difference in the position of centromeres
- Differences in degree and distribution of heterochromatic region. Heterochromatin stains darker than euchromatin
- Difference in no. and position of satellite



The karyotype of COG-LL-317h T cell acute lymphoblastic leukemia cell line

(Wu_SQ *et al., 2003)*

CHROMOSOME BANDING

"***Treatment of chromosomes to reveal characteristic patterns of horizontal bands is called chromosome banding.***"

- The banding pattern lend each chromosome a distinctive appearance.
- Banding also permits recognition of chromosome deletions, duplications and other types of structural rearrangements of chromosomes.

DIFFERENT TYPES OF BANDING

G–Banding:

- Staining a metaphase chromosome with Giemsa stain is called G-Banding.
- Preferentially stains the regions that are rich in adenine and thymine and appear dark.

C-Banding:

To specifically stain the centromeric regions and other regions containing constitutive heterochromatin.

Q-Banding

- Quinacrine mustard (a fluorescent stain), an alkylating agent, was the first chemical to be used for chromosome banding.

- Quinacrine bright bands were composed primarily of DNA rich in bases adenine and thymine.

Used to identify

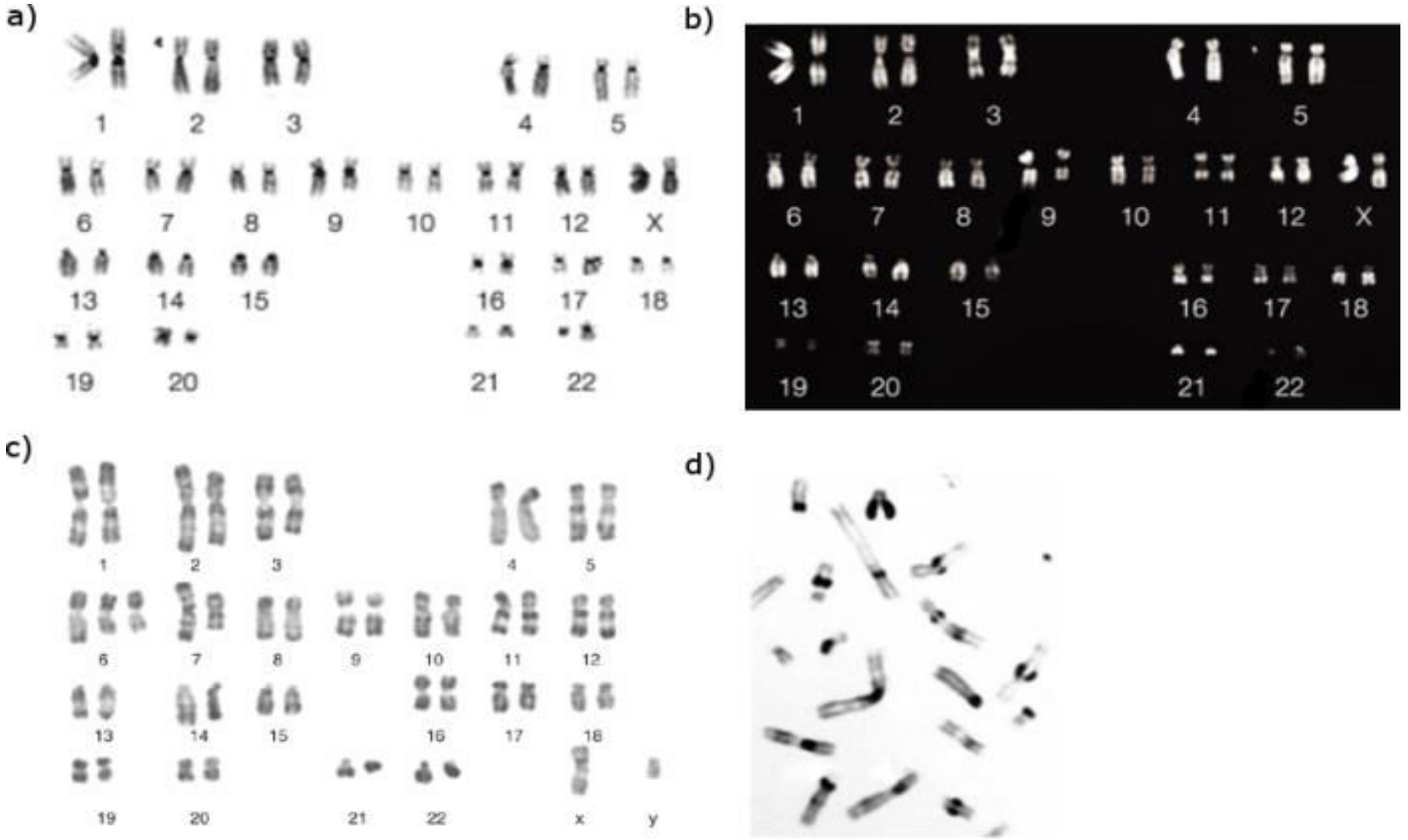
- Specific chromosomes and structural rearrangements.

- Various polymorphisms involving satellites and centromeres of specific chromosomes.

R (reverse banding)

- R-banding is the reverse of G-banding.
- The dark regions are euchromatic (guanine-cytosine rich regions) and the bright regions are heterochromatic (thymine-adenine rich regions).

T-banding: visualize telomeres.



a) C-banding, b) R-banding, c) Q-banding, d) G-banding

CHROMOSOME PAINTING

"Rendering a specific chromosome or chromosome segment distinguishable by DNA hybridization with a pool of many fluorescence-labeled DNA fragments derived from the full length of a chromosome or segment is called chromosome painting."

(McGraw-Hill Dictionary)

This technique employs *in situ* hybridization technology, also used for: extra chromosomal and cytoplasmic localization of specific nucleic acid sequences like specific mRNA species.

- **SKY**
- **M-FISH**

SPECTRAL KARYOTYPING (SKY)

- SKY is a powerful, whole-chromosome painting assay that allows the simultaneous visualization of each chromosome in different colors.

- Five spectrally distinct dyes are used in combination to create a cocktail of probes unique to each chromosome.

- The probe mixture is hybridized to metaphase chromosomes on a slide and then visualized with a spectral interferogram cube, which allows the measurement of the entire emission spectrum with a single exposure.

- The image is processed by computer software that can distinguish differences in color not discernible to the naked eye by assigning a numerical value.

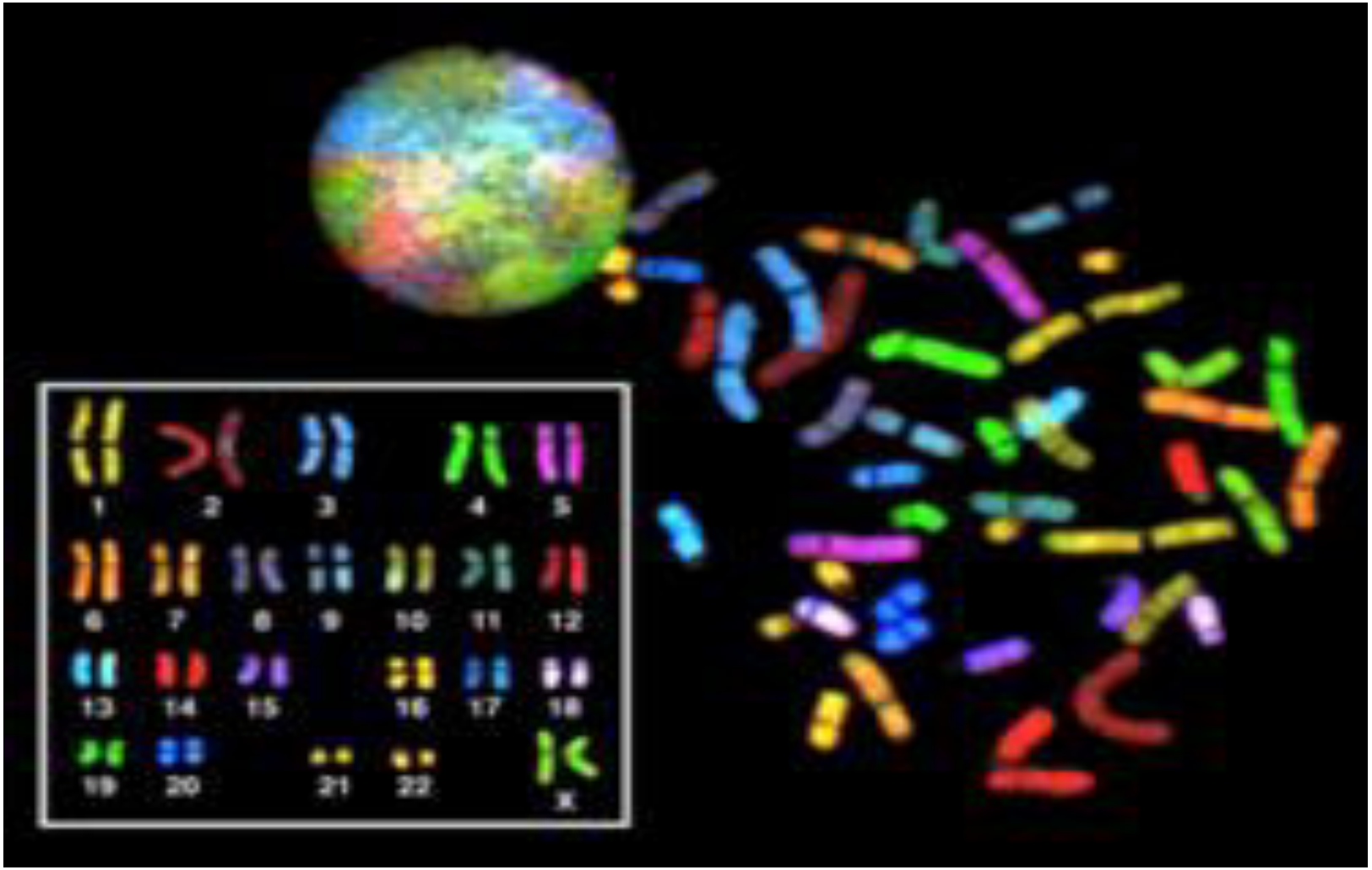


Fig. Spectral karyogram of a human female

(Schrock *et al.*, 1996)

SKY can detect

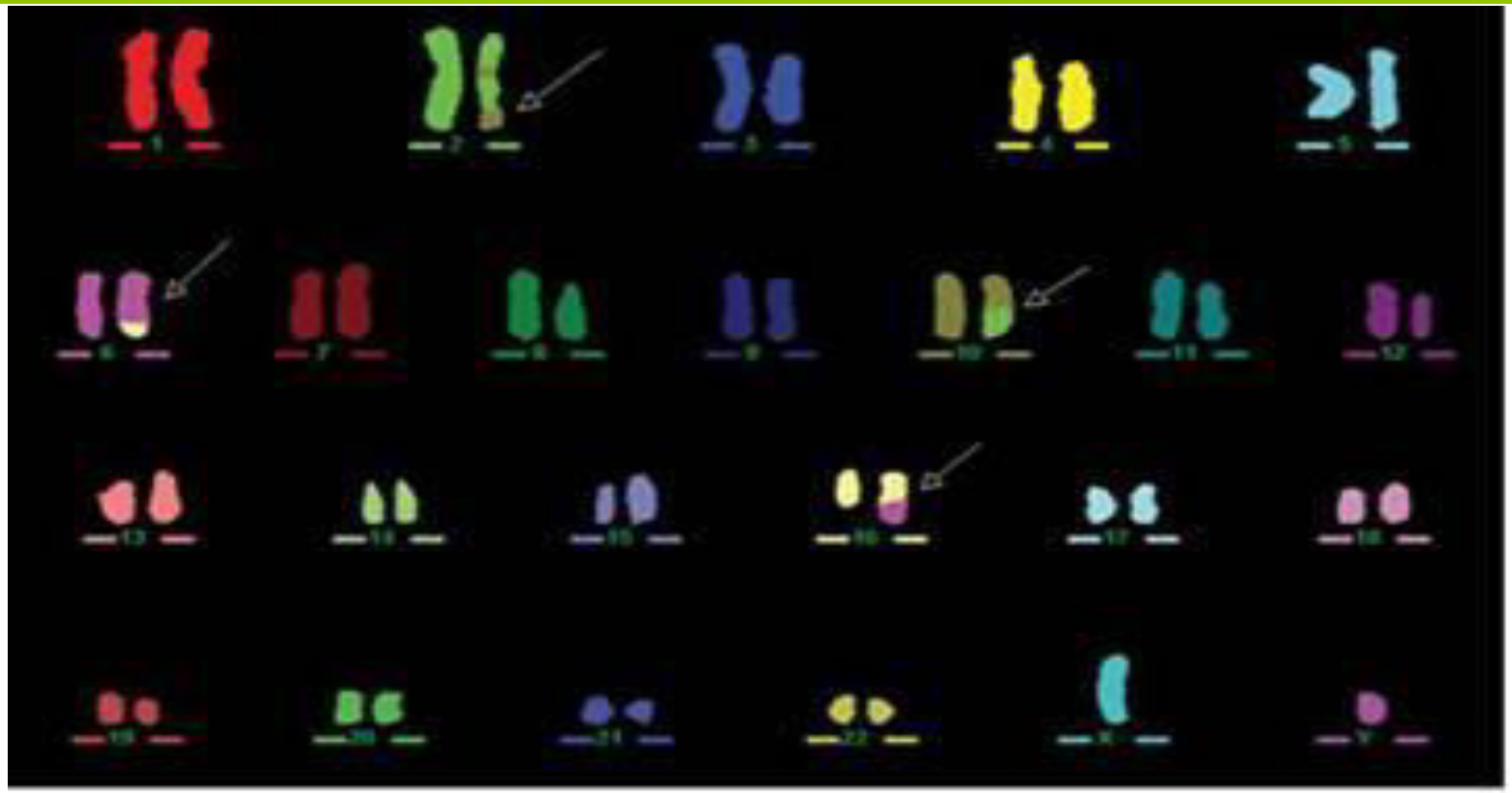
Chromosomal material of unknown origin, complex rearrangements, translocations, large deletions, duplications, aneuploidy.

Disadvantages

- Ineffective detection of micro deletions and inversions.
- It can only be performed on dividing cells.

Multicolor fluorescence *in situ* hybridization (M-FISH)

- It is based on chromosome painting.
- M-FISH identifies translocations and insertions.
- Reliable tool for diagnostic applications and Interphase nuclei are hybridized with the FISH probe.
- M-FISH is filter-based technology which does not rely on specialized instrumentation for its implementation as SKY.



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Source: Fauci AS, Kasper DL, Braunvald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J; *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Characterization of structural rearrangements: M-FISH (multicolor FISH) is used to detect a complex chromosome rearrangement involving a translocation between chromosome 6 and 16, as well as between chromosomes 2 and 10.

DNA Analysis

It involves three methods:

- **DNA hybridization**
- **DNA fingerprinting**
- **DNA profiling**

DNA CONTENT

DNA content can be measured by using DNA flourochromes, such as

- ***Propidium iodide***
- ***Hoechst 33258***
- ***DAPI***

Analysis of DNA content is particularly useful in the characterization of transformed cells that are often aneuploid and heteroploid.

DNA HYBRIDIZATION

"***DNA Hybridization is the process of establishing a non-covalent, sequence-specific interaction between two or more complementary strands of nucleic acids into a single hybrid, which in the case of two strands is referred to as a duplex***."

Provide information about :

- **Species-specific regions**
- **Amplified regions of the DNA**

e.g. ***amplification of DHFR gene, in cell lines selected for resistance to methotrexate*****.**

- **Altered base sequences that are characteristic to that cell line.**

e.g. ***Over expression of a specific oncogene in transformed cell lines.***

DNA FINGERPRINTING

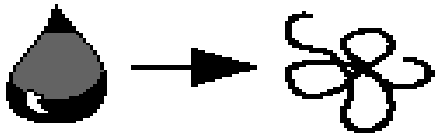
- Technology using *Variable number of tandem repeats* present in genome to identify individual cells.
- DNA contains regions known as satellite DNA that are apparently not transcribed.
- They give rise to regions of hyper variability.
- Cross contamination is confirmed by it.

The following techniques are used for DNA fingerprinting analysis :

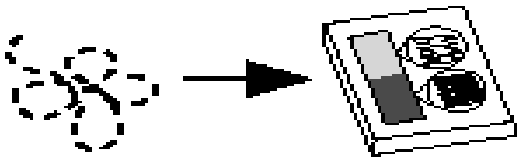
- **RFLP** ***(Restriction Fragment Length Polymorphism)***
- **AmpFLP** ***(Amplified Fragment Length Polymorphism)***
- **STR** ***(Short tandem repeats)***
- **SNP** ***(Single Nucleotide Polymorphism)***

THE PROCESS OF DNAFINGERPRINTING

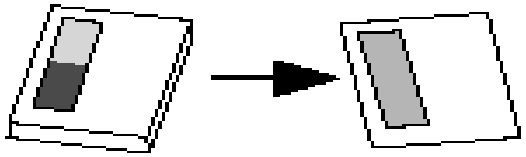
1. The process begins with a blood or cell sample from which the DNA is extracted.



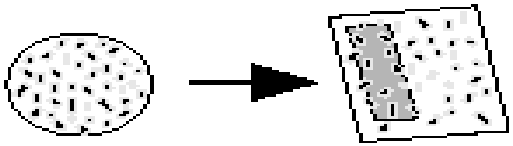
2. The DNA is cut into fragments using a restriction enzyme. The fragments are then separated into bands by electrophoresis through an agarose gel.



3. The DNA band pattern is transferred to a nylon membrane.



4. A radioactive DNA probe is introduced. The DNA probe binds to specific DNA sequences on the nylon membrane.



5. The excess probe material is washed away leaving the unique DNA band pattern.

6. The radioactive DNA pattern is transferred to X-ray film by direct exposure. When developed, the resultant visible pattern is the DNA FINGERPRINT.

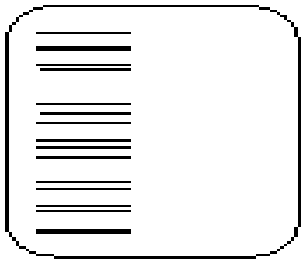
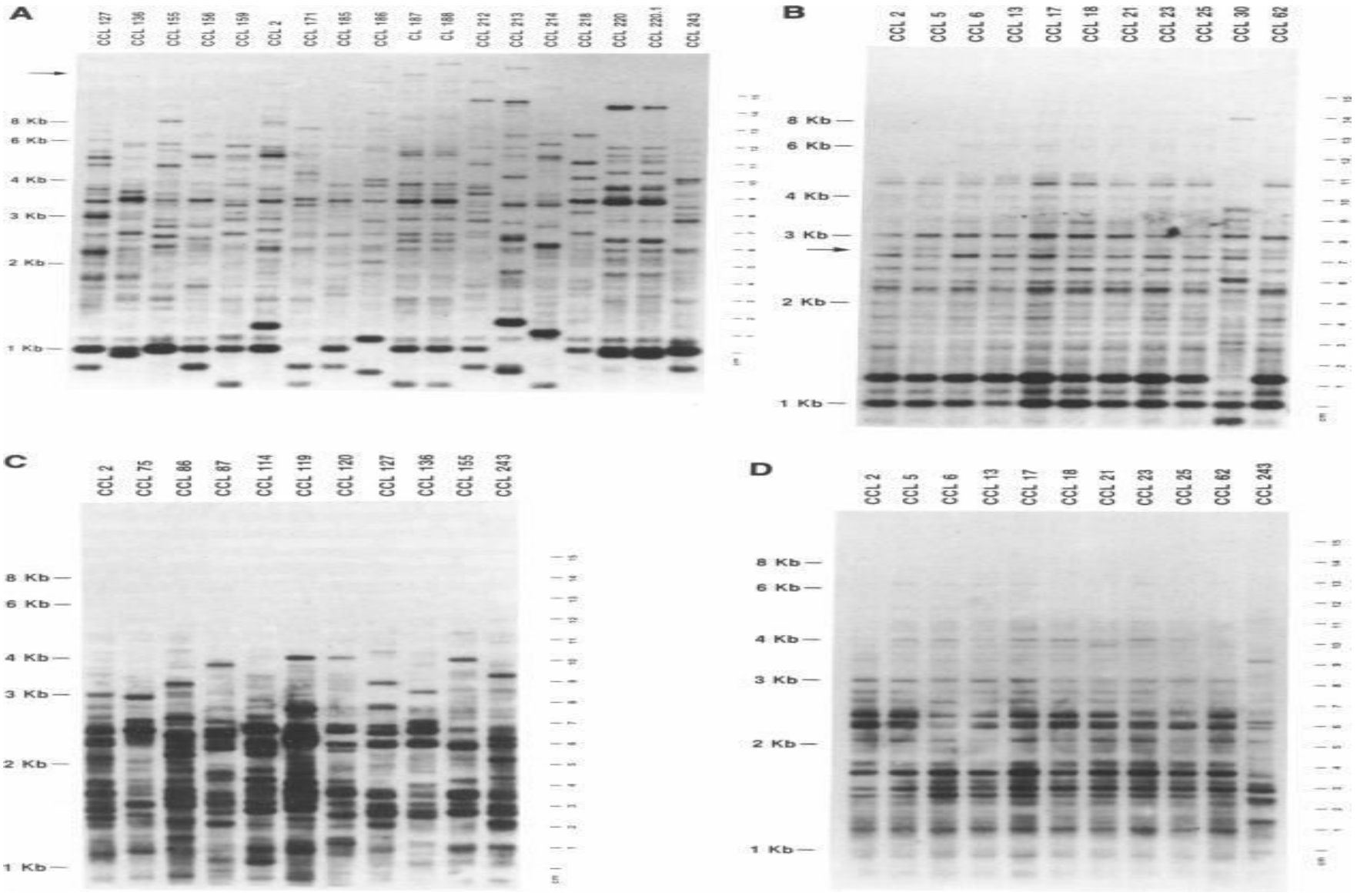


Figure 3.



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D A Gilbert et.al "Application of DNA fingerprints for cell-line individualization"Am J Hum Genet. 1990 September; 47(3): 499–514.

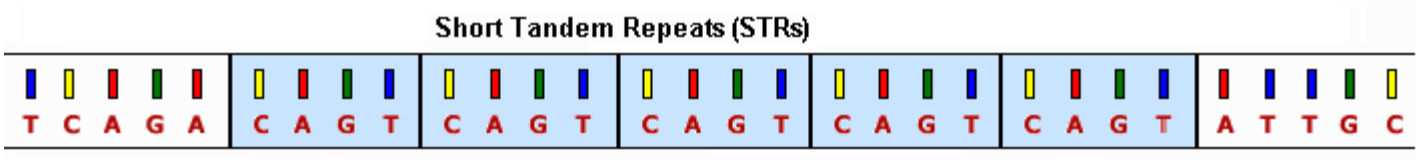
DNA PROFILING

- DNA profiling (also called DNA testing, DNA typing, or genetic fingerprinting) is a technique used to identify cell lines.

- DNA profiles are encrypted sets of numbers that reflect a cells DNA makeup, which can also be used as the cell line identifier.

- DNA profiling primarily examines "short tandem repeats," or STRs.

- STRs are repetitive DNA elements between two and six bases long that are repeated in tandem



- These STR loci are targeted with sequence-specific primers and amplified using PCR.

- Most extensively used with human cell lines.

Bovine genotype encompasses the following eighteen STR loci:

- ✓TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824 BM1818,SPS113, RM067, CSRM60, MGTG4B, CSSM66 and ILSTS006.

- ✓These are among the list of loci recommended by the *International Society for Animal Genetics (ISAG)* and the *Food and Agriculture Organization of the United Nations (FAO).*

CONCLUSION

Characterization of cell line is the first indispensible step after each cell line is generated for determining its functionality, authenticity, contamination, origin etc.

Morphology, Chromosome and DNA analysis have now became the major standard procedure for cell line identification.

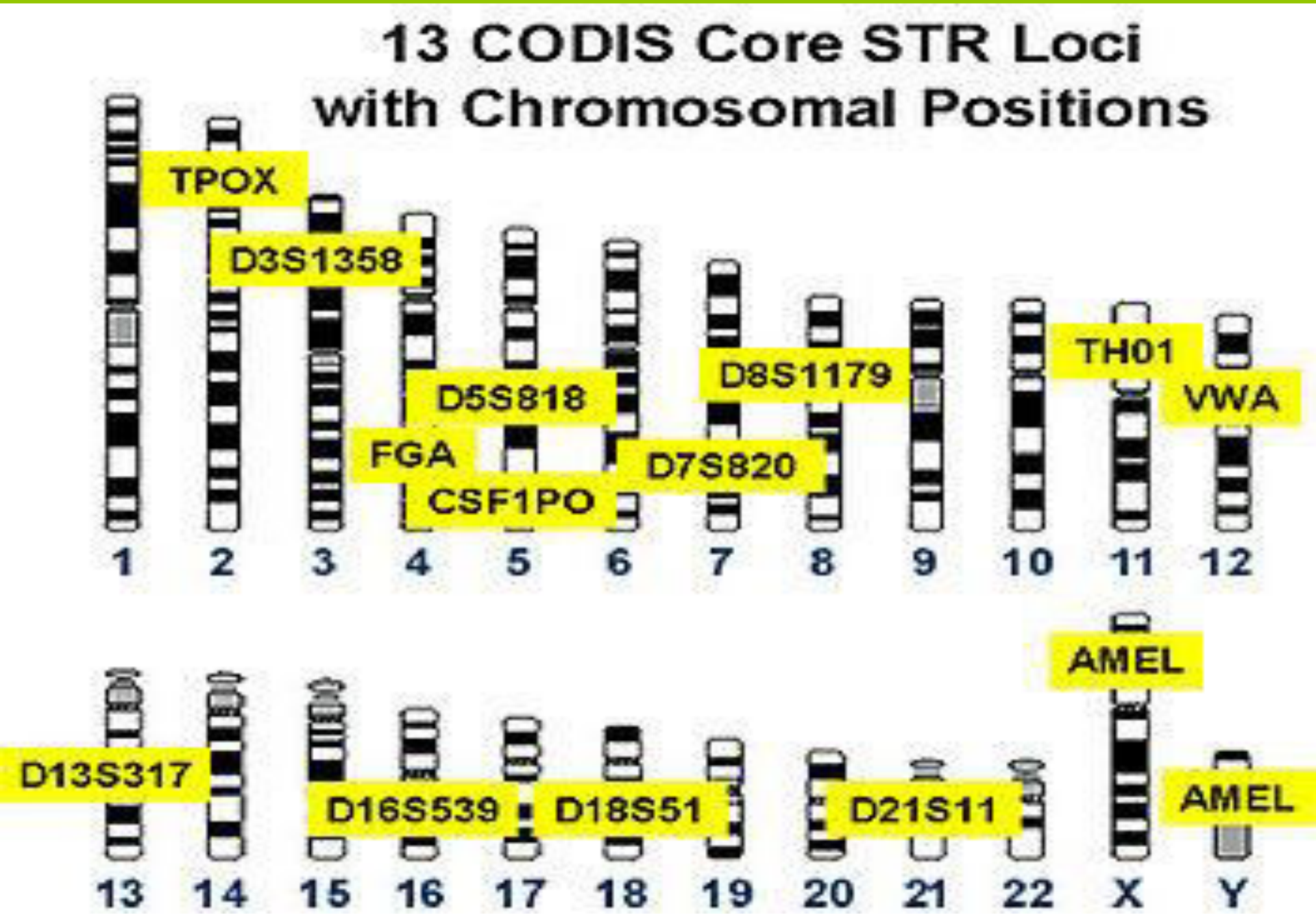
THANK YOU

- From country to country, different STR-based DNA-profiling systems are in use.

- In North America, systems which amplify the CODIS 13 core loci are almost universal.

- In the UK the SGM+ system is in use.

- SGM examines 7 (loci) different areas of the genome, where as SGM Plus™ examines 11.


13 CODIS Core STR Loci
with Chromosomal Positions
TPOX
D3S1358
D5S818
FGA
CSF1PO
D7S820
D8S1179
TH01
VWA
1 2 3 4 5 6 7 8 9 10 11 12
D13S317
D16S539
D18S51
D21S11
AMEL
AMEL
13 14 15 16 17 18 19 20 21 22 X Y