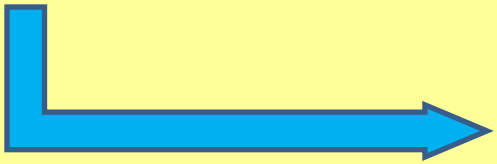
# Centrifugation – the method of separation of macromolecules

**And yet it moves!**

# *Orientation in the labyrinth of the methods for separation of macromolecules*

**Features used for separation of macromolecules (generally)**



**Molecular mass**

**Conformation and space**

**Charge**

**Density**

# *Orientation in the labyrinth of the methods for separation of macromolecules*

**Separation methods**

**Electro-migration**

**Chromatography**

**Centrifugation**

# *Orientation in the labyrinth of the methods for separation of macromolecules*

**Separation methods**

**Electro-migration**

**Separation of biological macromolecules by electrophoresis**

**Chromatography**

**Centrifugation**

**next time**

# *Orientation in the labyrinth of the methods for separation of macromolecules*

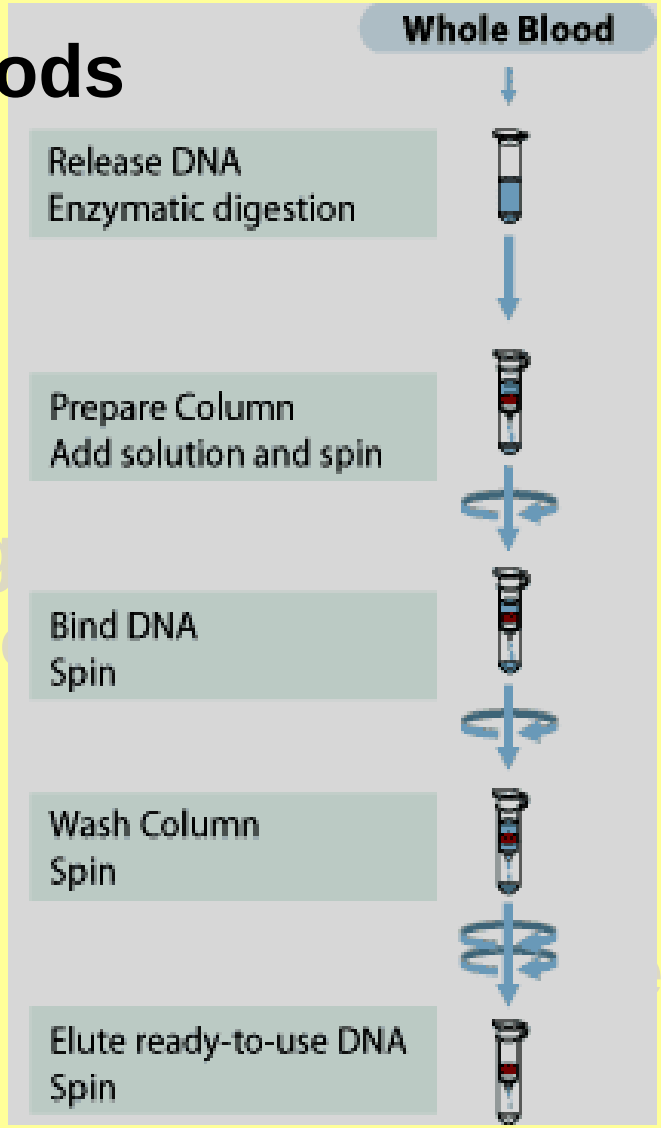
**Separation methods**

Electro-migration

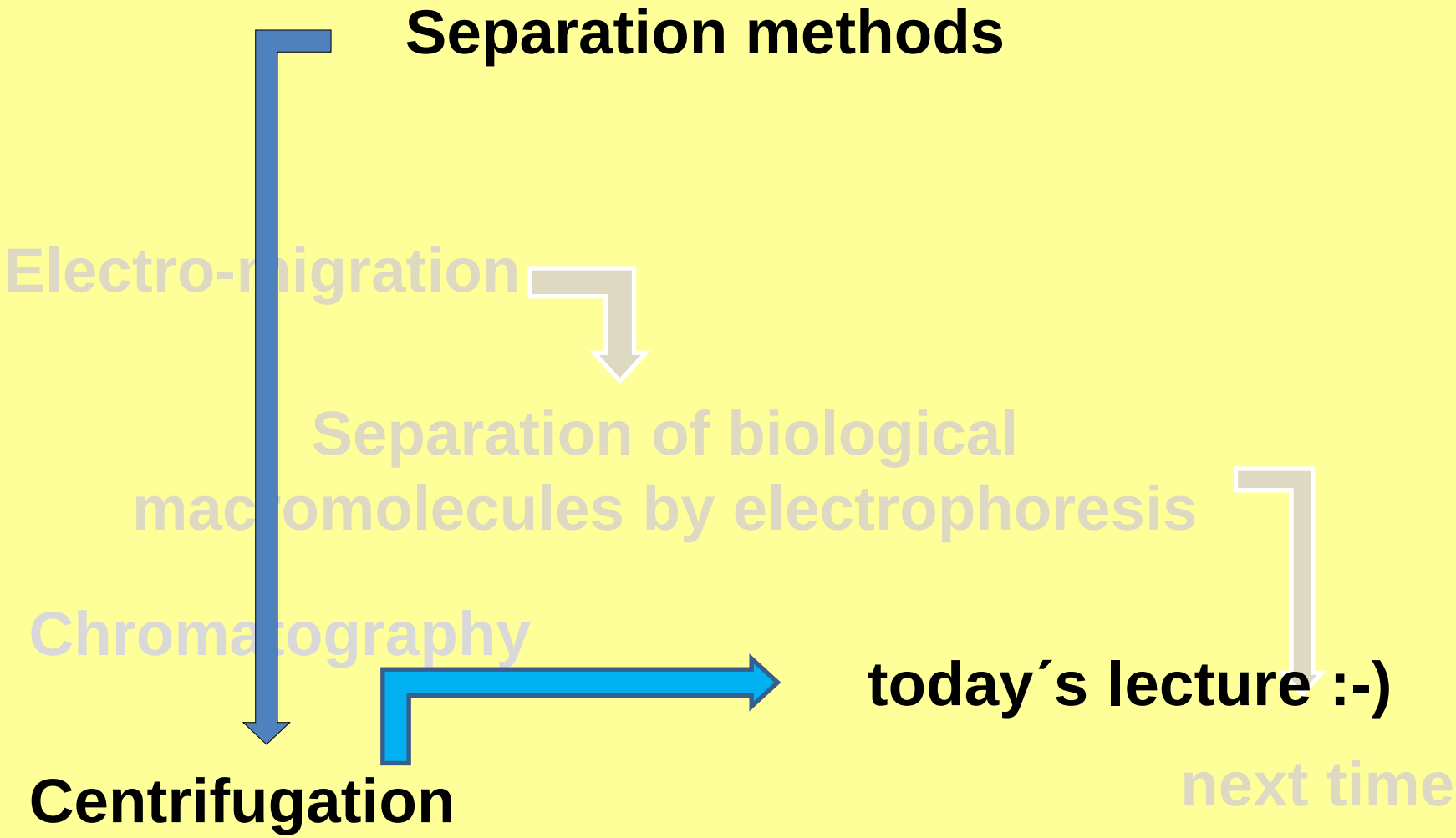
Separation of biolog
macromolecules by electr

**Chromatography**

Centrifugation



# *Orientation in the labyrinth of the methods for separation of macromolecules*



# *Centrifugation?*

**No !**

Centrifugation is a process that involves the use of the **centrifugal force** for the sedimentation of heterogeneous mixtures with a centrifuge

# *The methods of centrifugation*

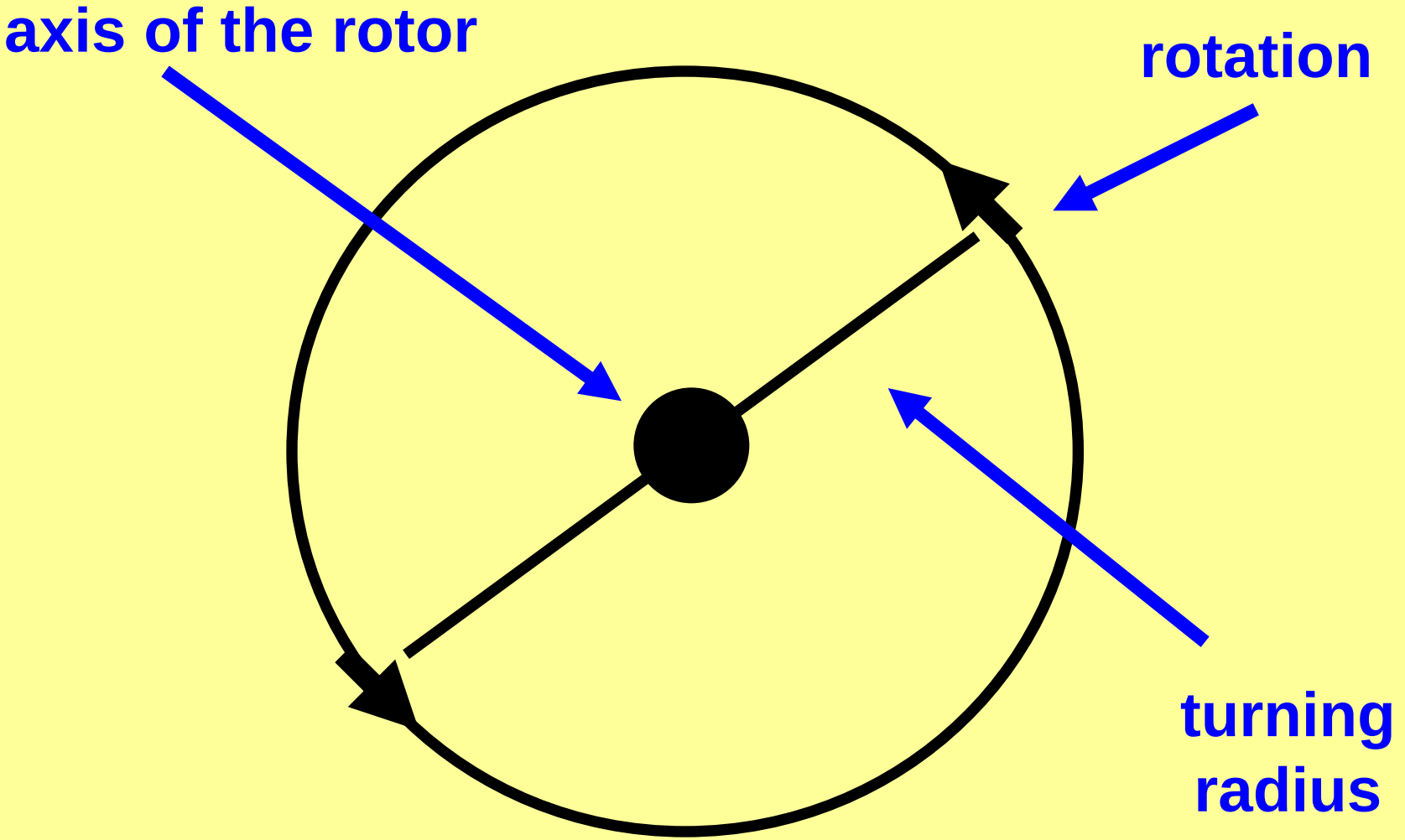
**Principle - correction**

**Movement of particles in liquid medium under gravitation force which arises under turning of rotor of the centrifuge**

**The movement of the particles dependents on:**

1. **Features of the particles**
2. **Features by environment**

# *The principles of centrifugation*



# The construction of centrifuges



# *The types of techniques of centrifugation*

**Differential centrifugation**

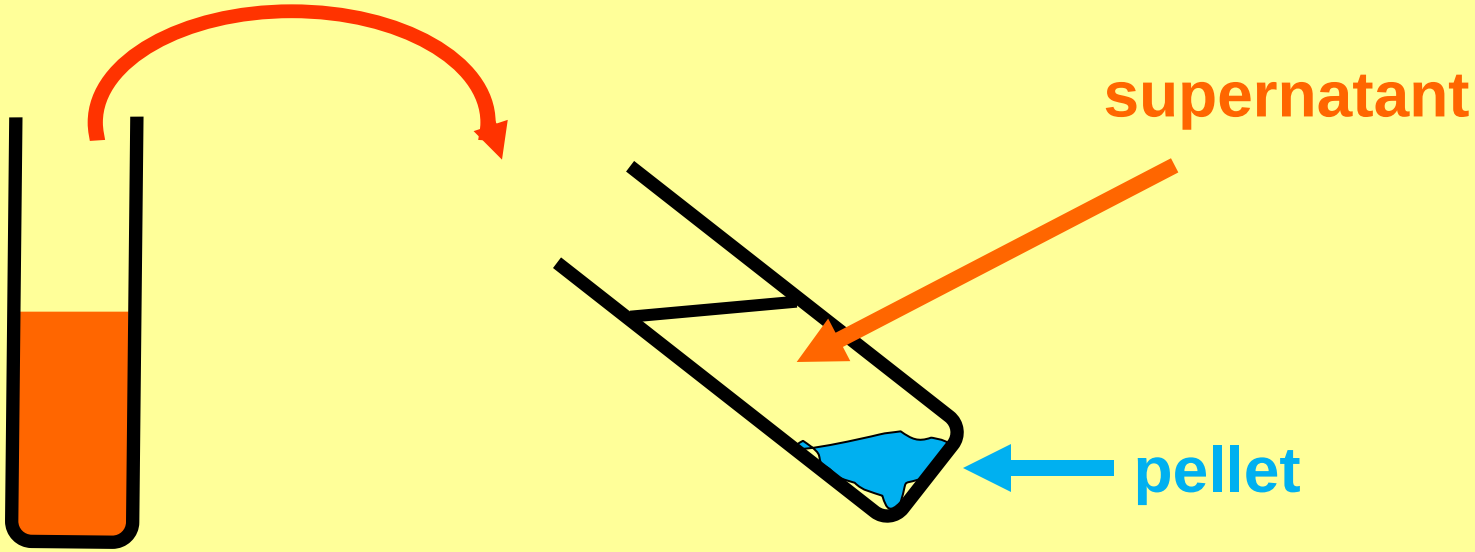
**Zonal centrifugation**

# *Differential centrifugation*

**Separation of mixture of heterogeneous particles in homogenous solution**

**A common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells**

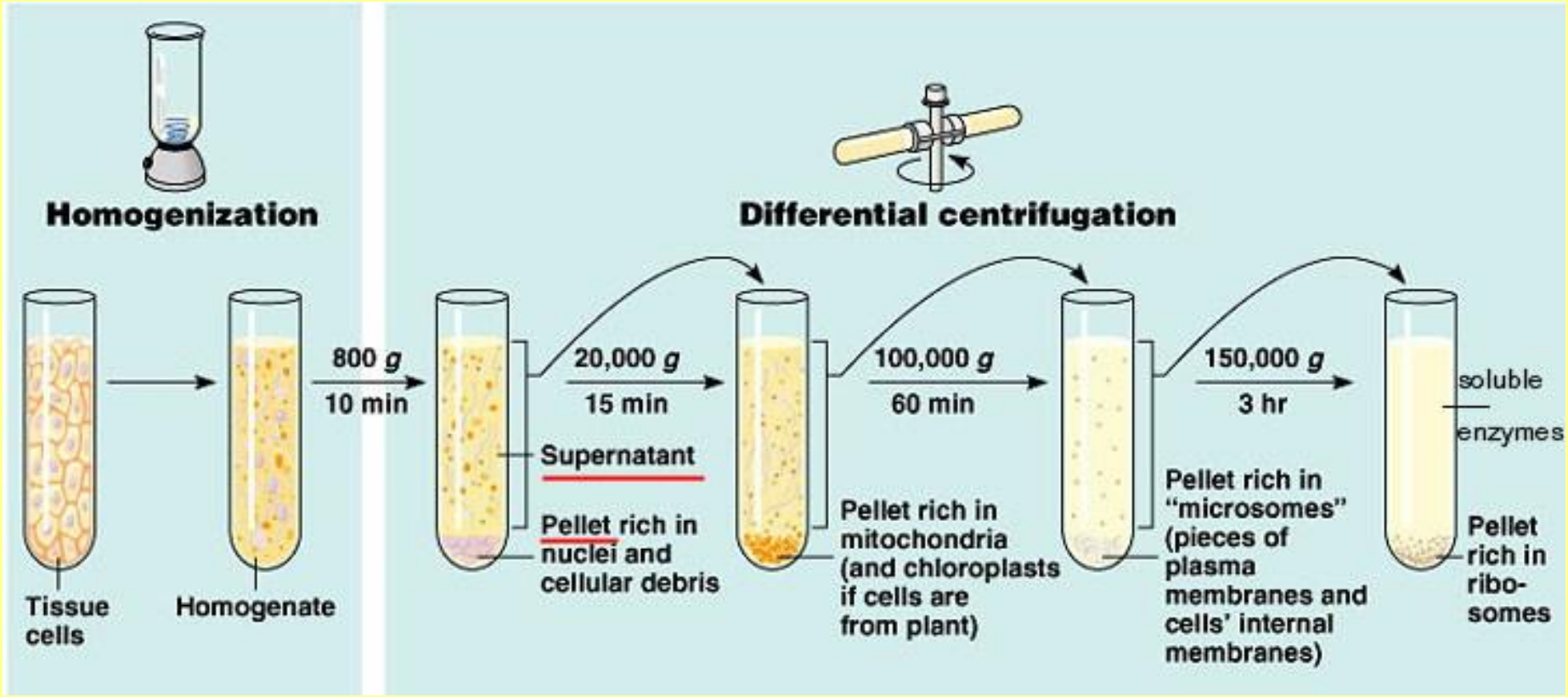
# *Differential centrifugation*




**Separation of nuclei, ribosomes, mitochondria, cell membranes, nucleic acids, proteins, …**

# *Differential centrifugation - praxis*

- **Particles differ by size, weigth or density = sedimentation by different speeds**
- **By repeating and accelerating of rpm the individual components can be separated as pellets**



Can I separate everything by differential centrifugation?

NO! You are not able to differentiate

- Different types of NAs
- Ribosomal subunits
- Other particles with the similar features

So, what can I do?

Use the zonal centrifugation

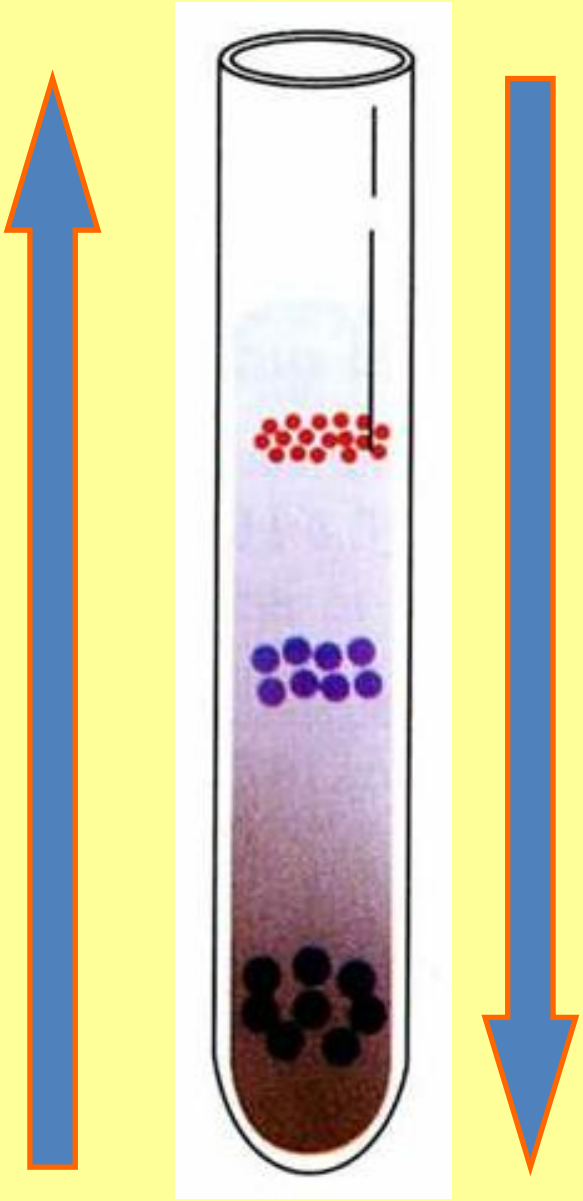
# *Zonal centrifugation*

**Separation of mixture of homogenous particles in gradient of solution**

**It is used as a purifying process for differential centrifugation**

# *Forces in zonal centrifugation*

**Buoyancy force**



**Centrifugation force**

# *Zonal centrifugation*

## Isokinetic centrifugation

- Separation according to speed of sedimentation of the particles
- Used to **determination of sedimentation coefficient S**

## Equilibrium (isopycnic) sedimentation

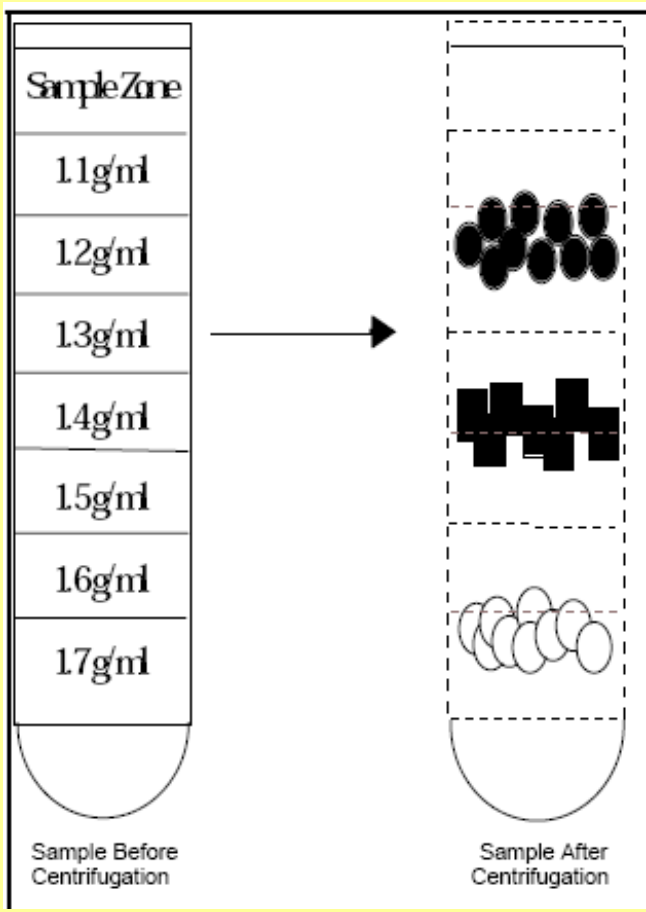
- Separation according to the particle density

# Zonal centrifugation

- Homogenous solution is replaced by a solution which concentration is growing from up to bottom of centrifugation tube (gradient solution)

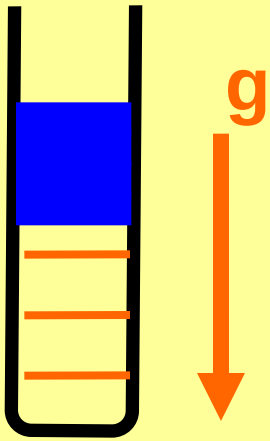
- The gradient solution is prepared from very good soluble and inert compounds – sucrose, glycerol

- Growing density and viscosity of gradient solution eliminate the effect of growing centrifugal acceleration (it is growing from the axis of the rotor) by which protect of growing speedy of particles sedimentation during centrifugation




www.coleparmer.com

# *How to perform the zonal centrifugation*



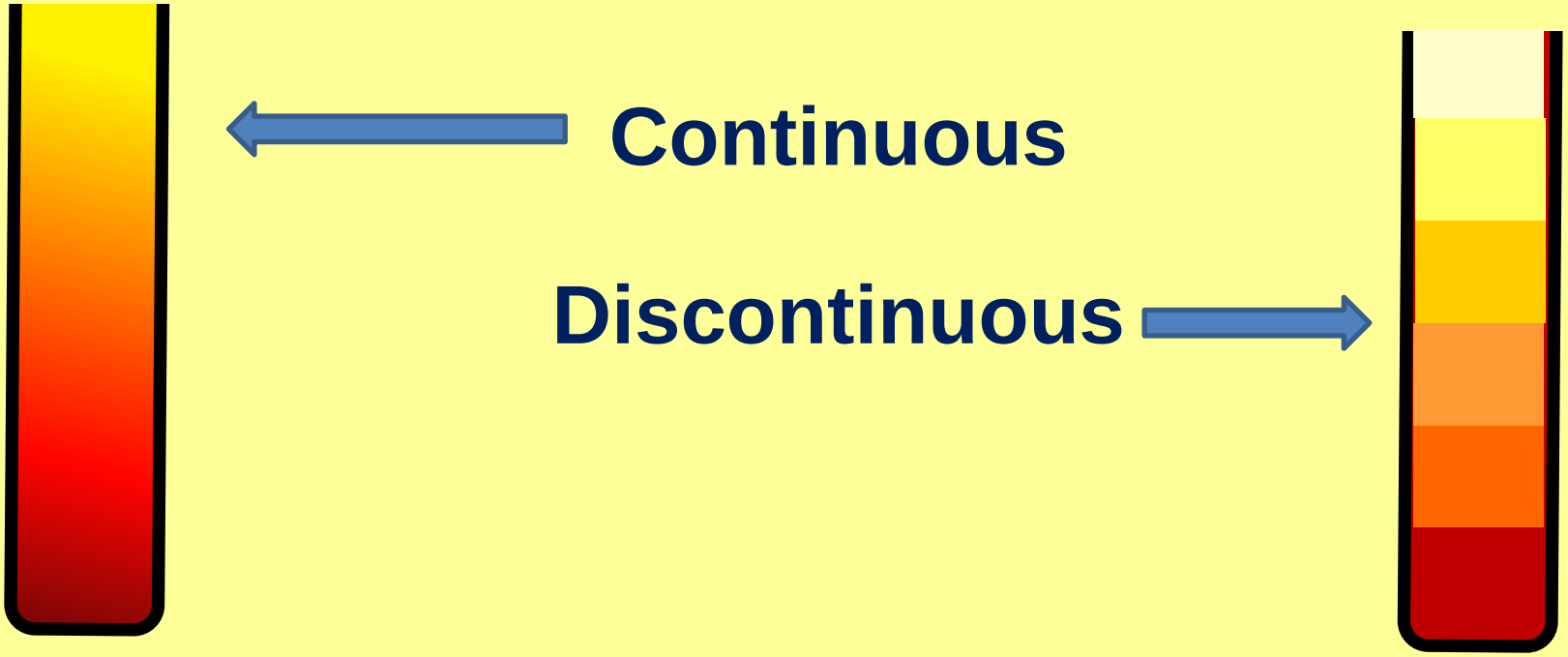

**growing density and viscosity**

**Gradient eliminates the effect of growing centrifugal acceleration**

**Particles are stratified according to**

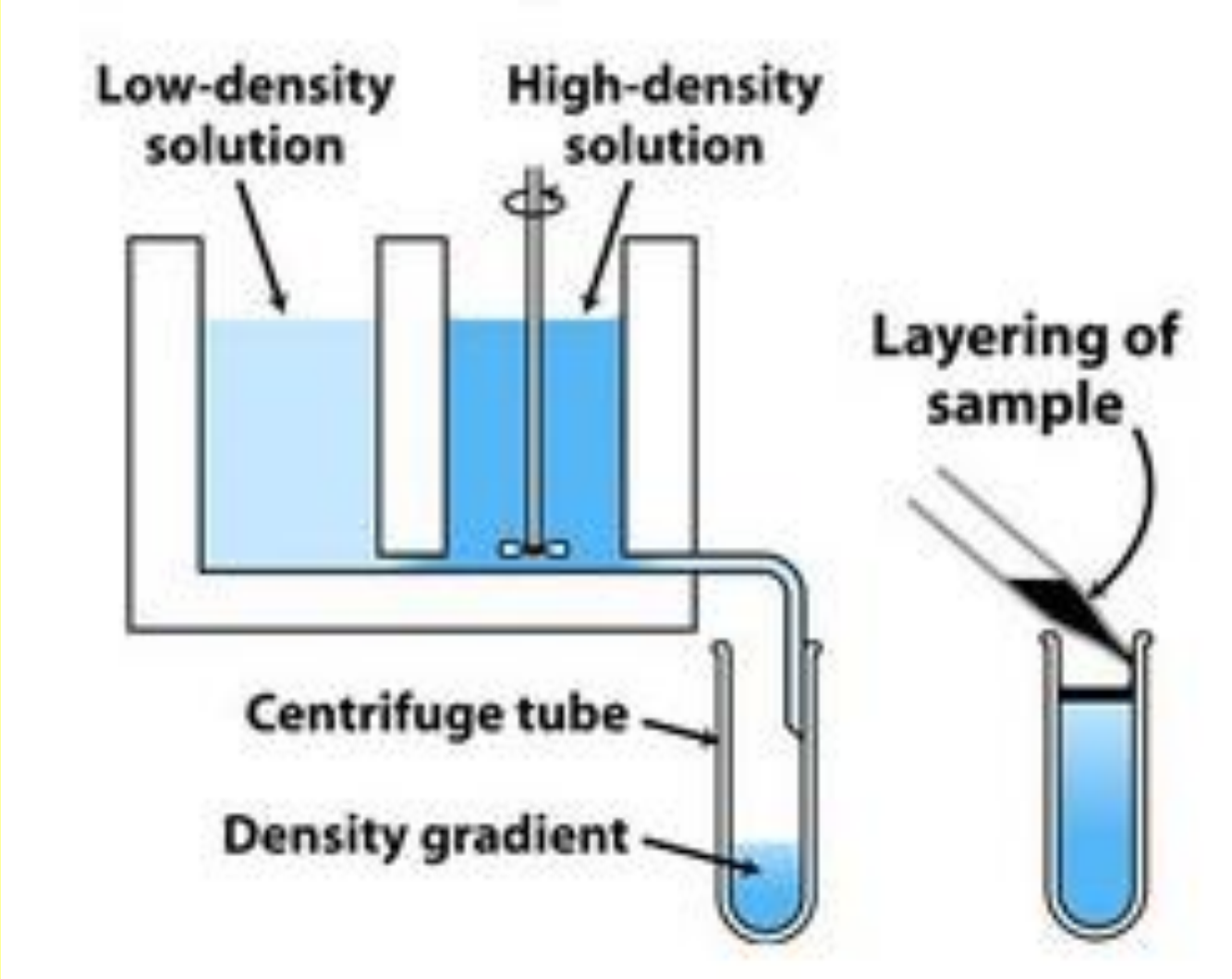
- **size**
- **shape**
- **density**

# *Density gradient*




**After centrifugation the separated particles of the same features are concentrated to narrow bands in the both compositions**

# Preparing of continuous gradient




http://quizlet.com/

# *Density gradient*

**Commonly used compounds to density gradient preparations are**

**Caesium chloride**

**Sucrose**

# *Density gradient*

**The differences in densities are about 1,0-1,3 g/ml for the sucrose, and 1,0-1,9 g/ml for CsCl, which enables to separate and isolate for example**

**Cell nuclei**
**Mitochondria**
**Nucleic acids**

**The purity of isolated compounds is extremely high**

# *Isokinetic centrifugation*

**This method of centrifugation is used to more detailed characterisation of particles**

**for example to exact determination of their size**

# *Isokinetic centrifugation*

**The 5-20% sucrose gradient is usually used in NA analysis. The concentration of sucrose changes linearly from up to bottom of tube**

***the speed of particle sedimentation is constant during the centrifugation***

# *Isokinetic centrifugation*

**The speed, in which any particle sediments depends on**

**Size of the particle**

**Shape of the particle**

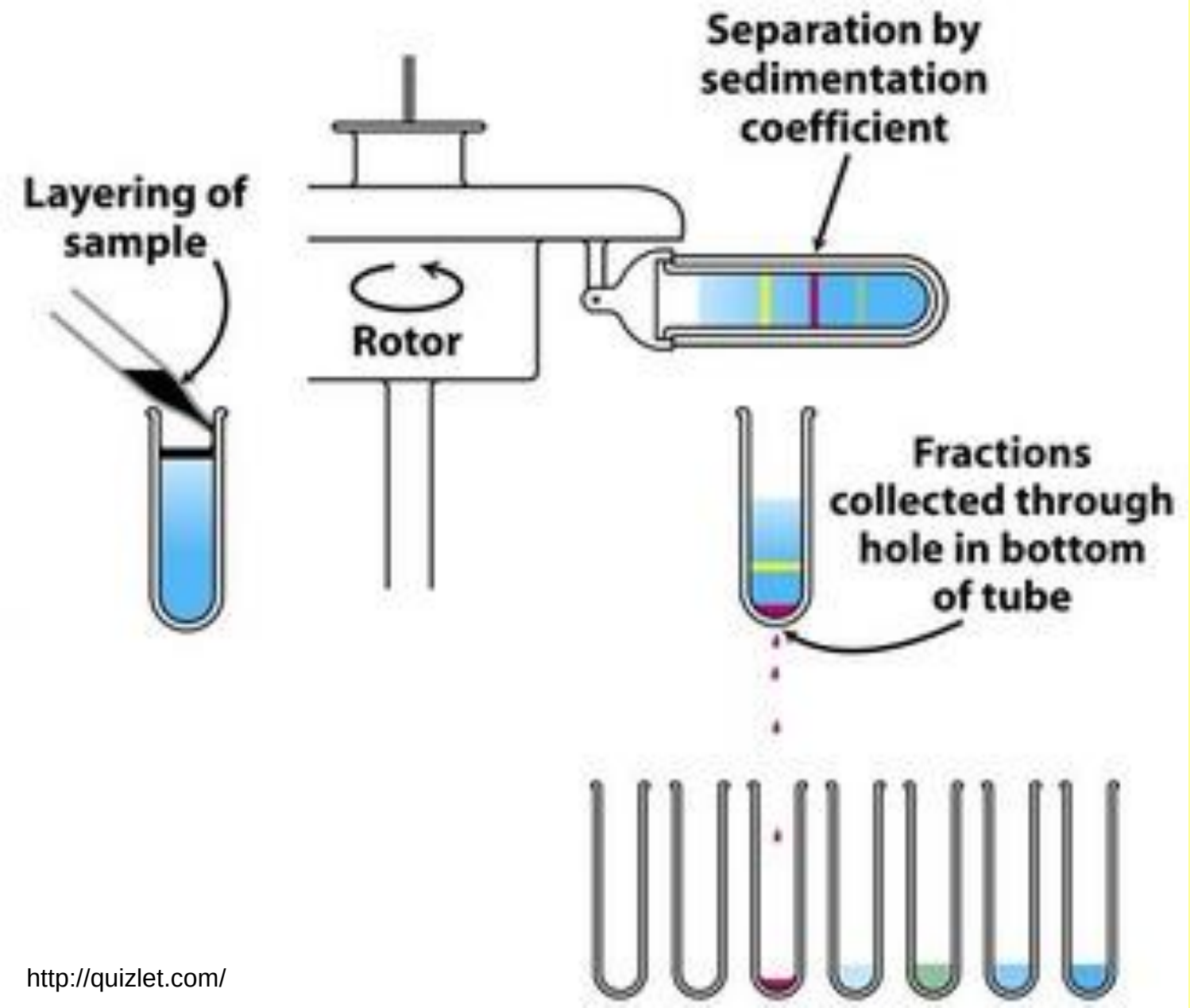
**Density of the particle**

**And is influenced by**

**Features of the environment**

**Conditions of the centrifugation**

# Isokinetic centrifugation




http://quizlet.com/

# *Sedimentation coefficient*

**It characterises the speed of particle moving during centrifugation => It is defined as the ratio of a particle's sedimentation velocity to the acceleration that is applied to it**

$$S = \frac{v_t}{r\omega^2} = \frac{m}{6\pi\eta r_0}$$

**S = sedimentation coefficient**

**$v_t$ = terminal velocity**

**r = distance from the axis of rotor**

**ω = rotational speed (angular velocity)**

**m = weight of particle**

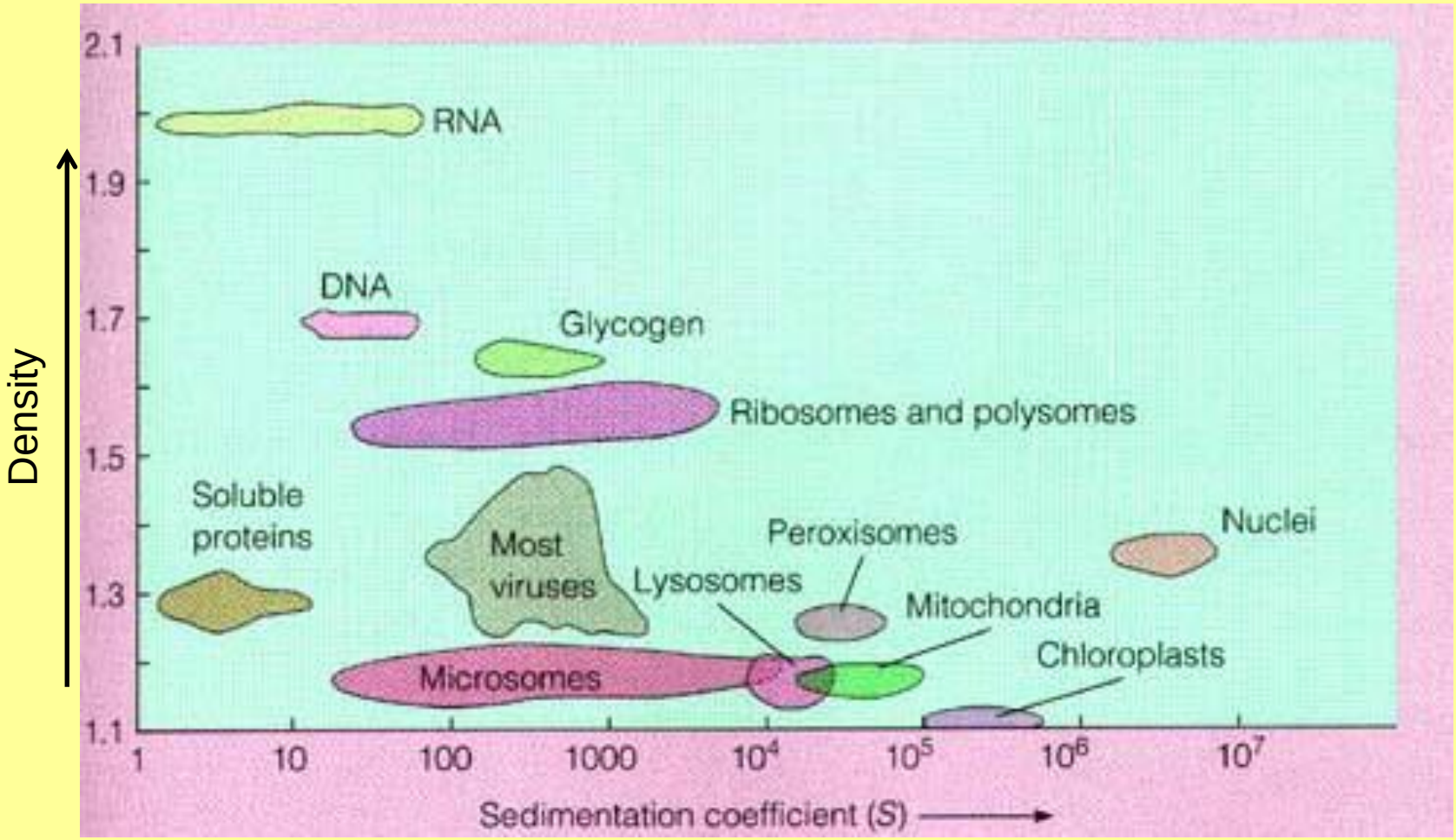
**η = viscosity of the medium**

**$r_0$ = radius of the particle**

**1 Svedberg = $10^{-13}$ s**

**23S-rRNA, 16S-rRNA, ribosomal units 30S, 50S**

# Examples of sedimentation coefficients $S^{o}_{20,w}$




http://jpkc.scu.edu.cn

# *Isopycnic centrifugation*

**Equilibrium sedimentation uses a gradient of a solution such as caesium chloride to separate particles based on their individual densities (mass/volume)**

***Centrifugation to equilibrium***

# *Isopycnic centrifugation*

**Density gradient is formed spontaneously during centrifugation the concentration gradient**

***Particles of lysed cells move by the both directions (up and down) so long***

***until they receive the position in which the density of the solution is the same as the density of the particles***

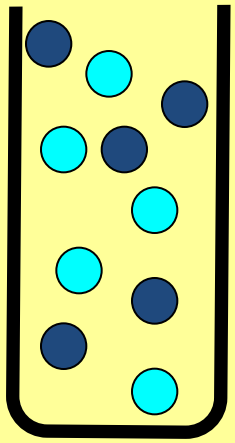
# *Isopycnic centrifugation*

**The density determined by this manner is named as floating density**

**The parameters of the floating density are influenced by interaction of the particles with ions in solution and they are usually higher than the density of the particles directly in cell**

# *Isopycnic centrifugation*

**CsCl solution which contains a mixture of particles**



**Centrifugation**

**lower density**

**higher density**

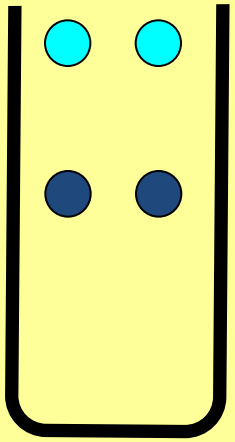
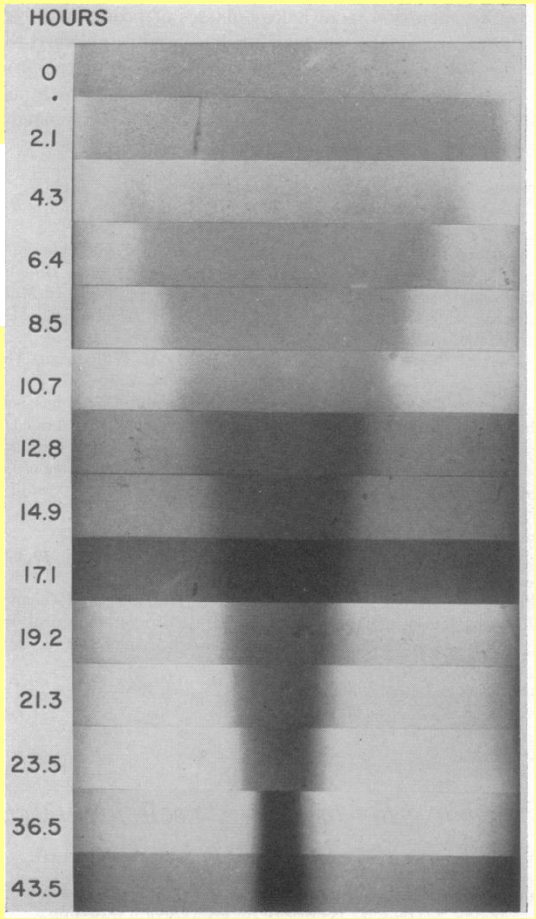
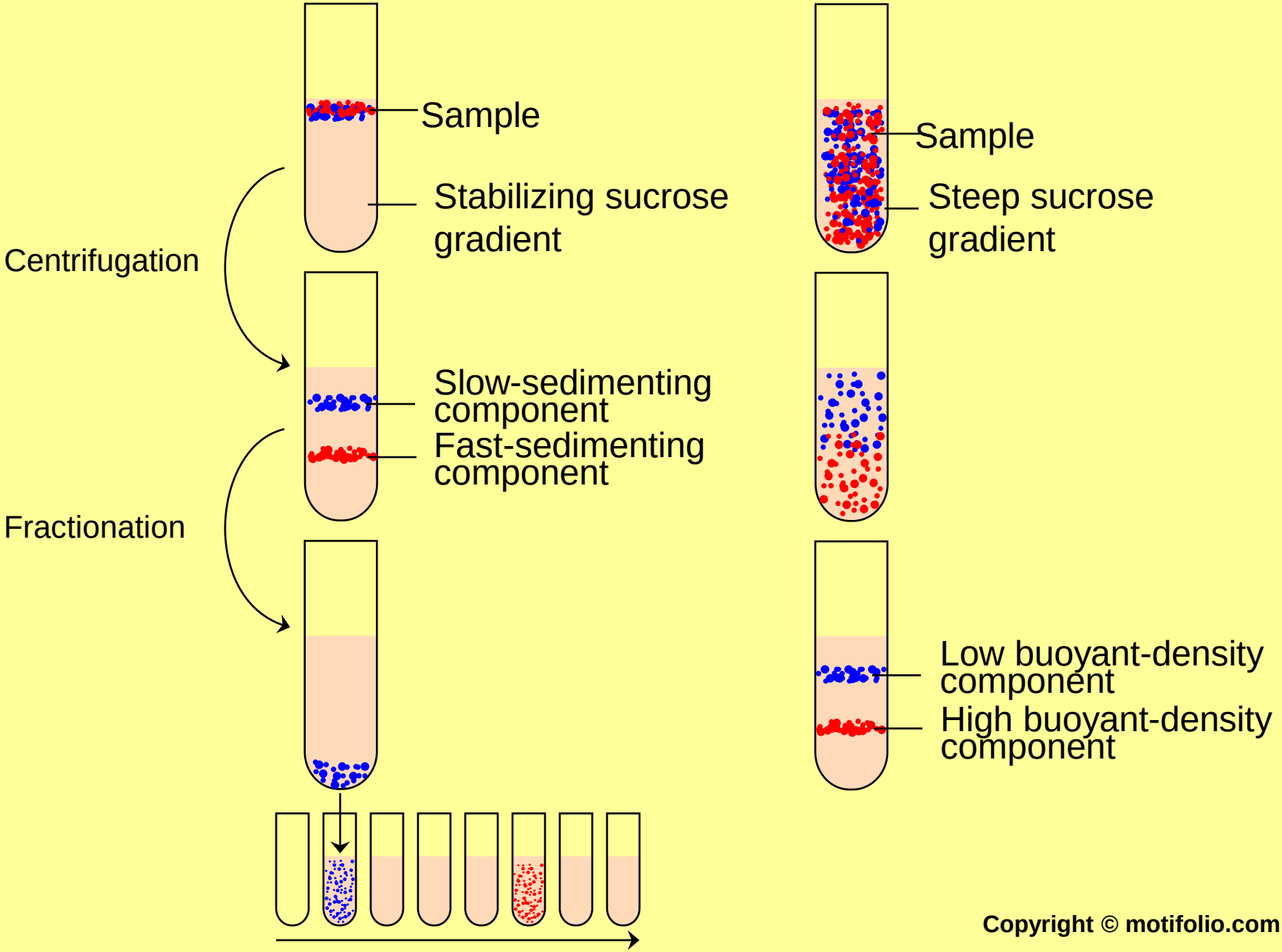


FIG. 1.—Ultraviolet absorption photographs showing successive stages in the banding of DNA from *E. coli*. An aliquot of bacterial lysate containing approximately $10^8$ lysed cells was centrifuged at 31,410 rpm in a CsCl solution as described in the text. Distance from the axis of rotation increases toward the right. The number beside each photograph gives the time elapsed after reaching 31,410 rpm.




MESELSON, M; STAHL, FW. The replication of DNA in E. coli.
*Proc. Natl Acad. Sci. USA*, 1958, vol. 44, pp. 671-682.

# Rate-zonal centrifugation versus equilibrium density gradient centrifugation



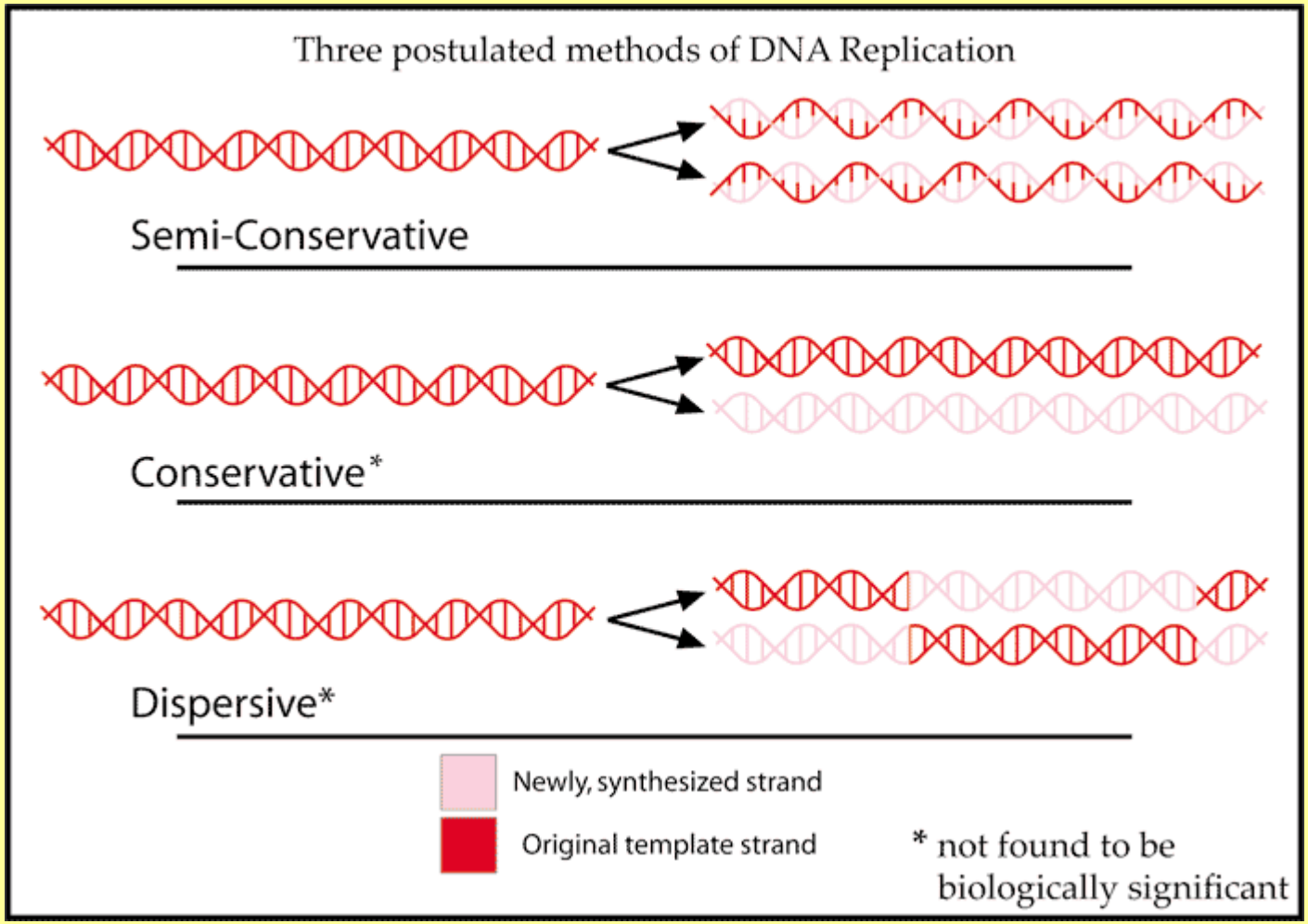
# *Determination of the floating density by isopycnic centrifugation*

$$\rho^{25\,^{\circ}C} = 10{,}8601 \times n^{D}_{25\,^{\circ}C} - 13{,}4974$$

$n^{D}_{25\,^{\circ}C}$ = refractive index of CsCl solution

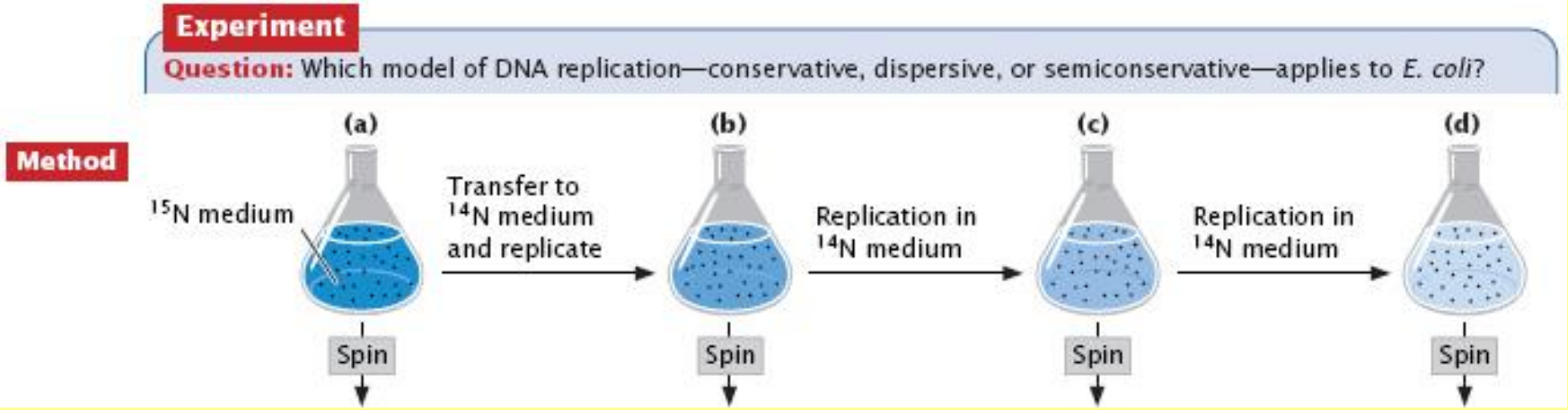
# *Isopycnic centrifugation - praxis*

## Stahl Meselson experiment 1958



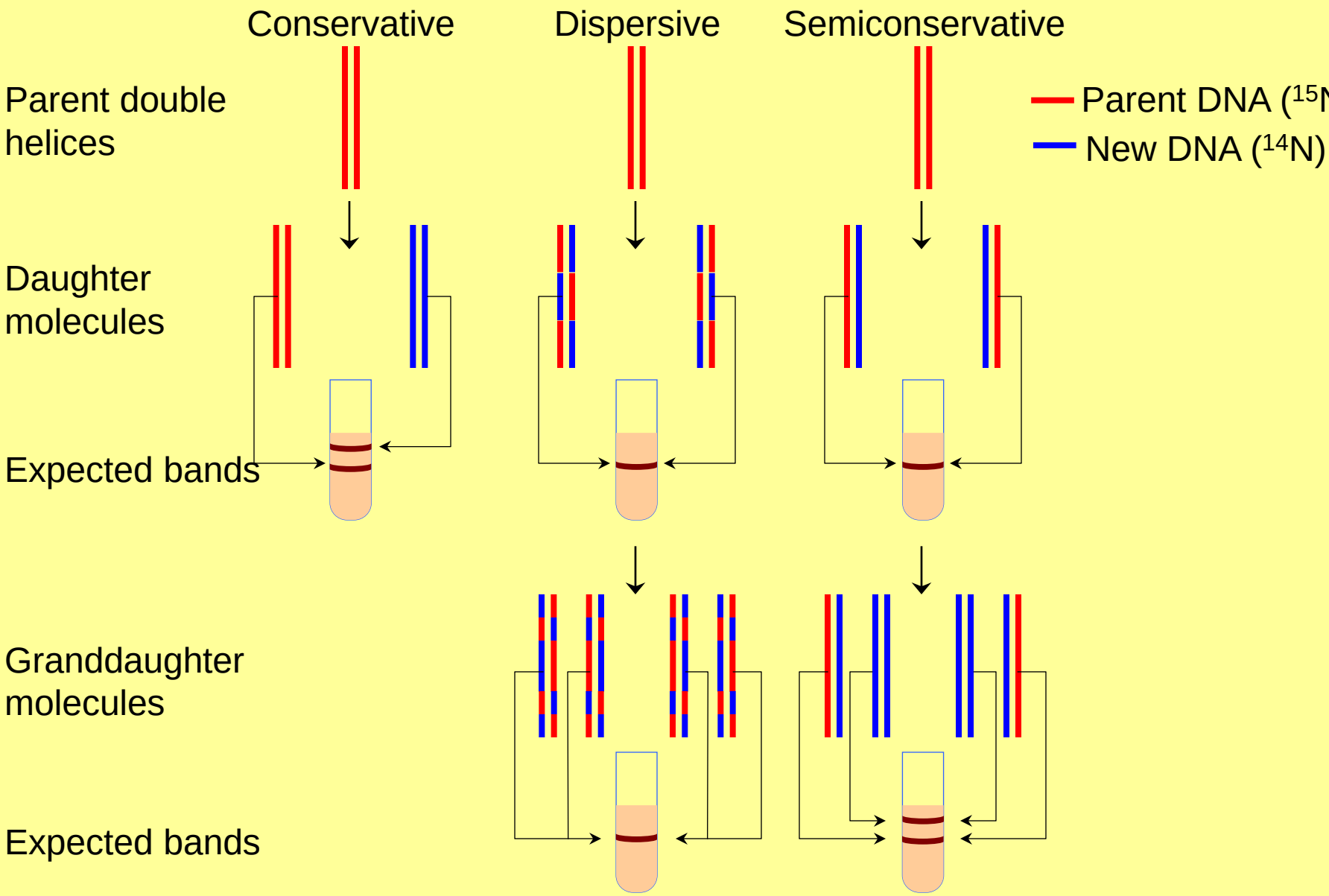

MESELSON, M; STAHL, FW. The replication of DNA in E. coli. *Proc. Natl Acad. Sci. USA*, 1958, vol. 44, pp. 671-682.

# Meselson-Stahl experiment - design

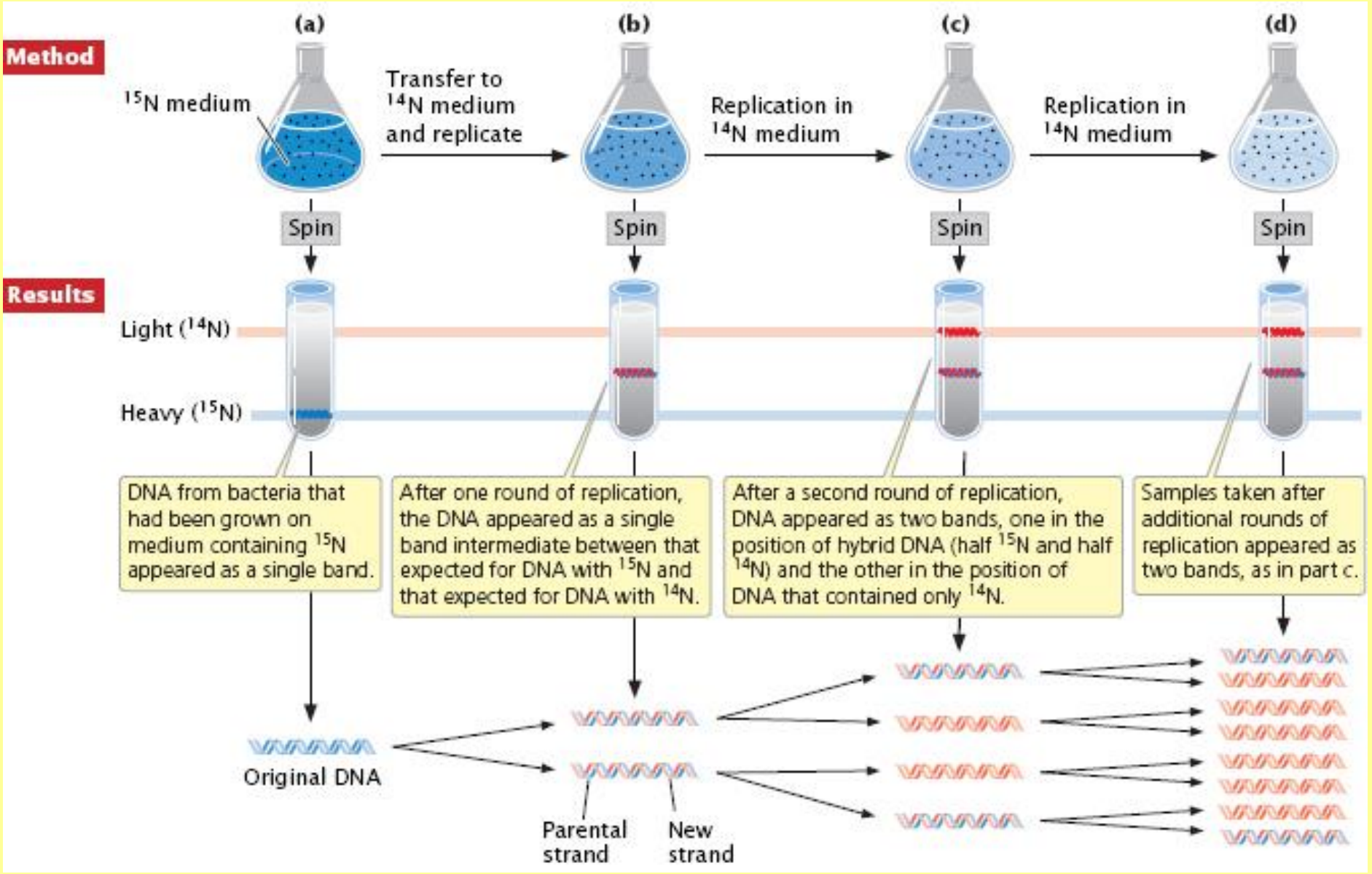



palyapbio.edu.glogster.com

# The Meselson-Stahl experiment – predicted results

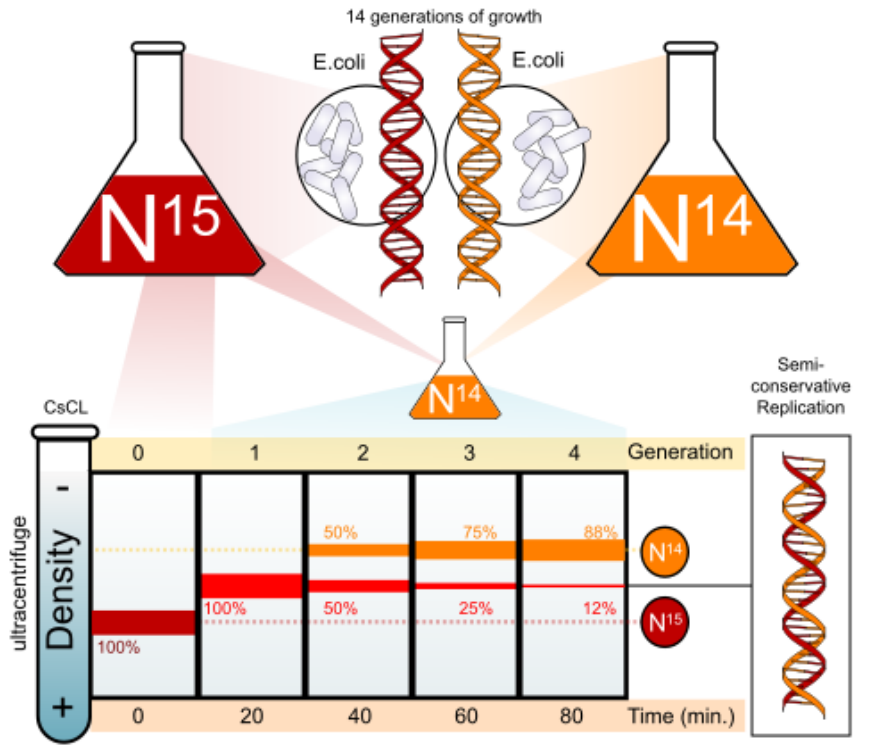


# Meselson-Stahl experiment - results



# Isopycnic centrifugation - praxis

## Stahl Meselson experiment 1958



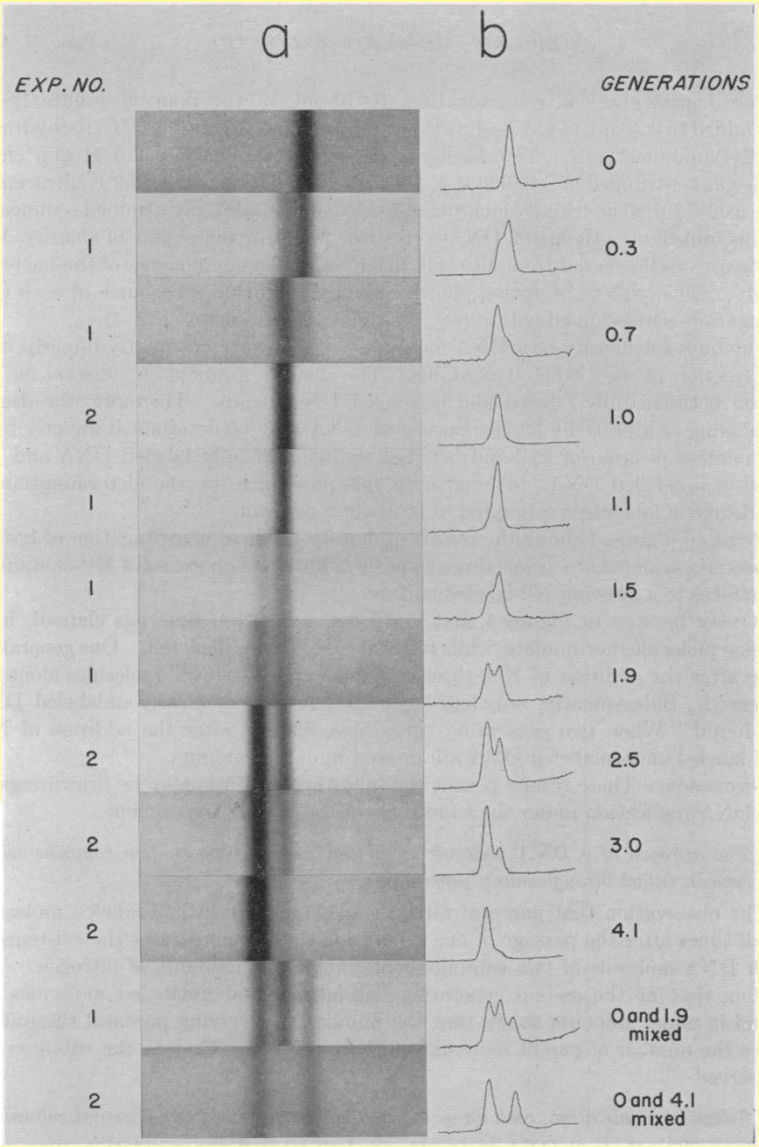




FIG. 4—*a:* Ultraviolet absorption photographs showing DNA bands resulting from density-gradient centrifugation of lysates of bacteria sampled at various times after the addition of an excess of $N^{14}$ substrates to a growing $N^{15}$-labeled culture. Each photograph was taken after 20 hours of centrifugation at 44,770 rpm under the conditions described in the text. The density of the CsCl solution increases to the right. Regions of equal density occupy the same horizontal position on each photograph. The time of sampling is measured from the time of the addition of $N^{14}$ in units of the generation time. The generation times for Experiments 1 and 2 were estimated from the measurements of bacterial growth presented in Fig. 3. *b:* Microdensitometer tracings of the DNA bands shown in the adjacent photographs. The microdensitometer pen displacement above the base line is directly proportional to the concentration of DNA. The degree of labeling of a species of DNA corresponds to the relative position of its band between the bands of fully labeled and unlabeled DNA shown in the lowermost frame, which serves as a density reference. A test of the conclusion that the DNA in the band of intermediate density is just half-labeled is provided by the frame showing the mixture of generations 0 and 1.9. When allowance is made for the relative amounts of DNA in the three peaks, the peak of intermediate density is found to be centered at 50 ± 2 per cent of the distance between the $N^{14}$ and $N^{15}$ peaks.

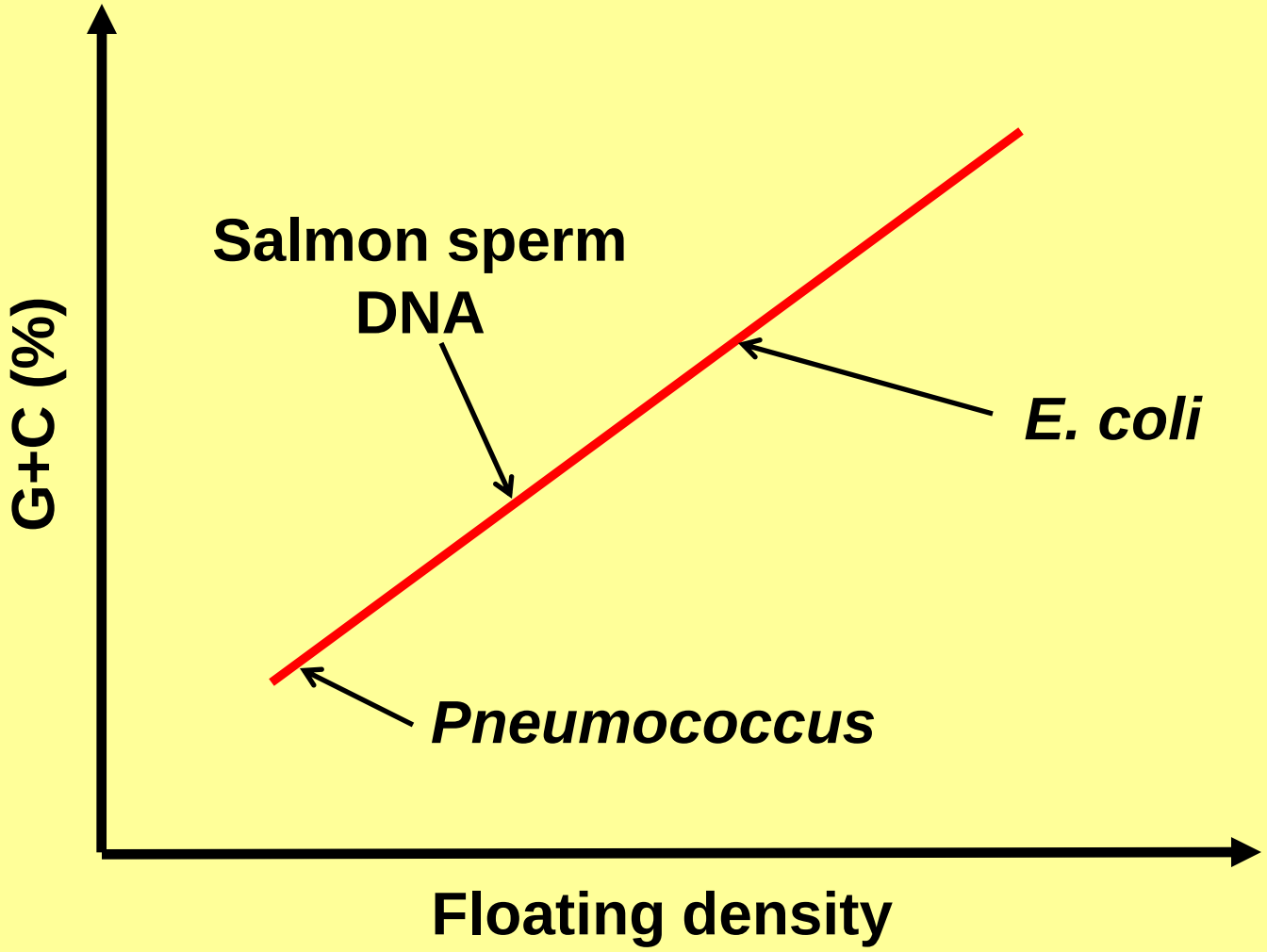
# *Calculation of (G+C) content*

- The composition of base pairs in dsDNA influence the floating density
- This is used for determination of the GC content in DNA samples according to the rule

$$\%\,(\mathbf{G}+\mathbf{C}) = \frac{\boldsymbol{\rho} - \mathbf{1.66}}{\mathbf{0.098}} \times \mathbf{100}$$

**ρ** = floating density of dsDNA

# *(G+C) content versus floating density*



# *Separation of different DNA forms*

- **A special example how to use the isopycnic centrifugation is separation of different structural forms of DNA in the gradient of CsCl in the presence of ethidium bromide (EtBr)**

- **After binding of EtBr to DNA the floating density of the DNA is significantly lowered. The amount of bonded EtBr and lowering the DNA density depends on the structural form of the DNA**

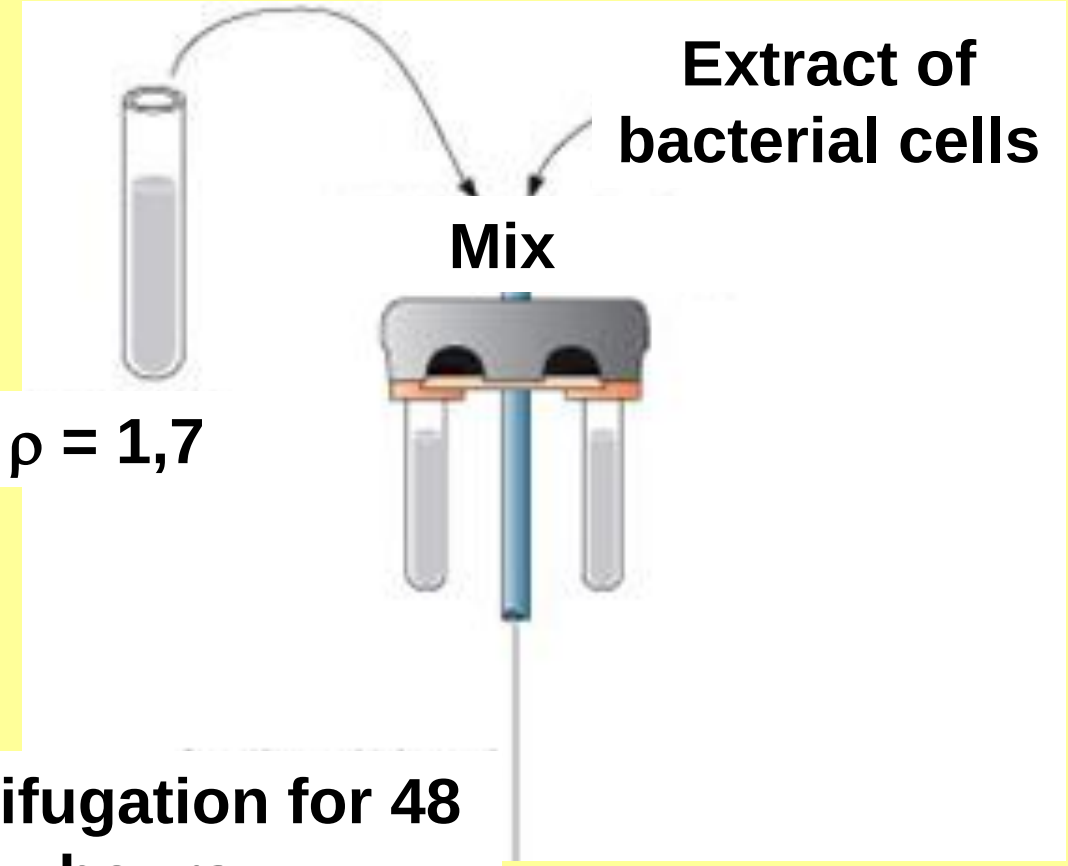
- **It enables to separate and to isolate different DNA forms, for example covalently closed circles of plasmid DNA from opened plasmid and linear chromosomal DNA molecules**

# *Isopycnic centrifugation - praxis*

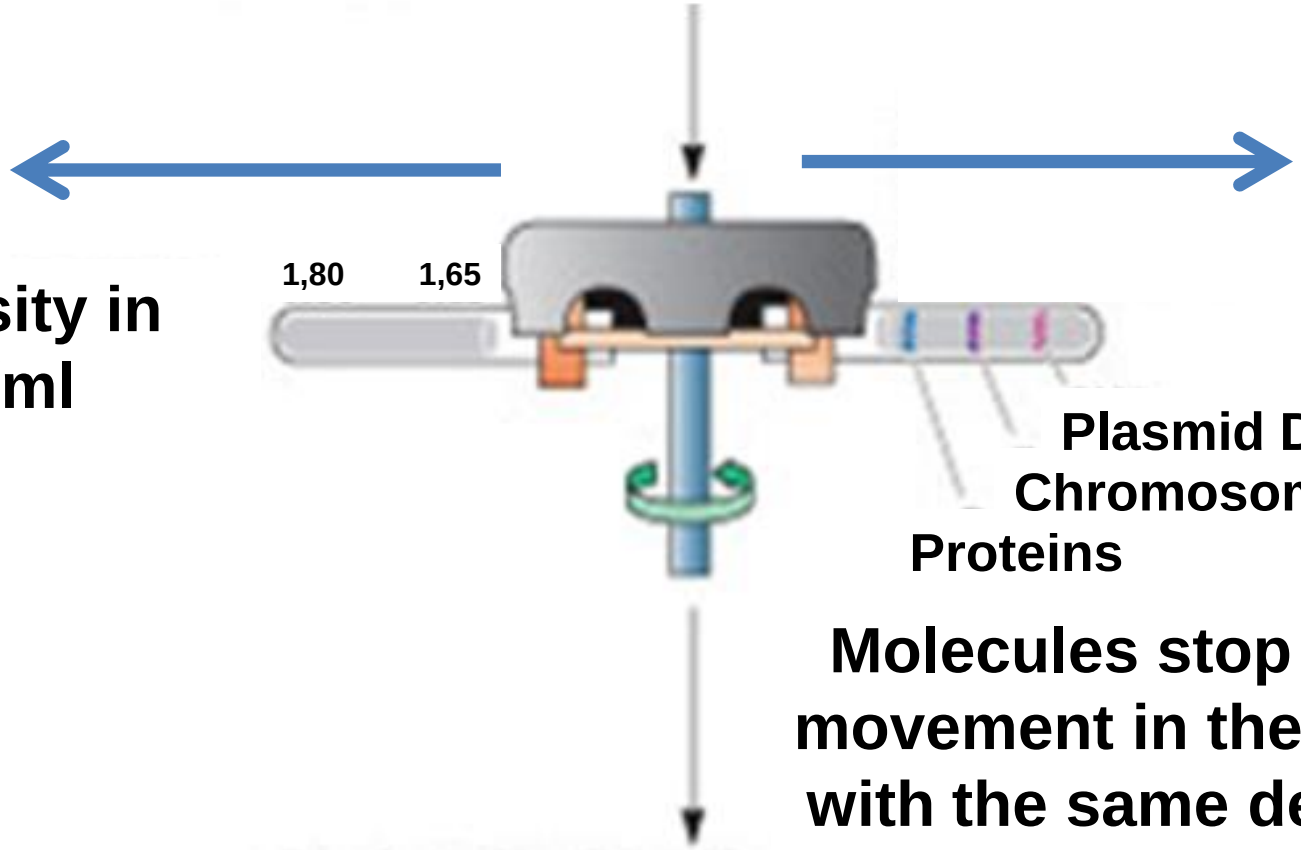
**I would like to separate linear nuclear DNA from circular mitochondrial DNA (or plasmids from bacterial chromosome) by centrifugation.**

**How to do it?**

# *Isopycnic centrifugation - praxis*

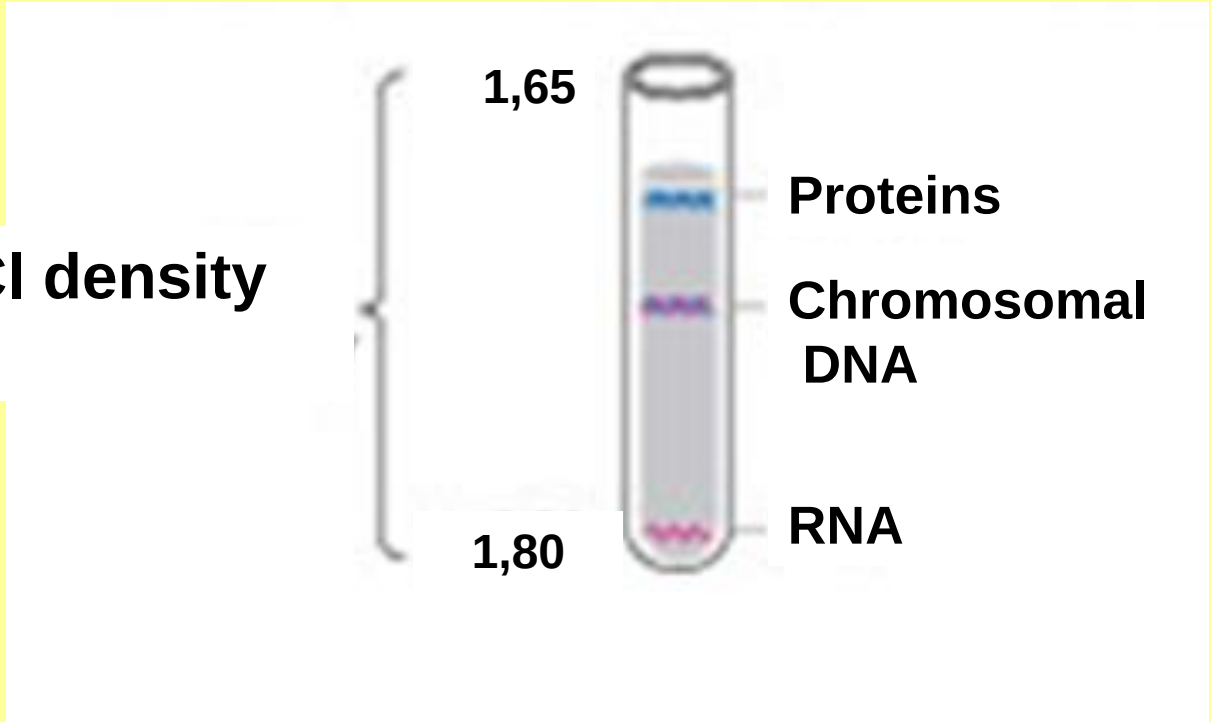


# Isopycnic centrifugation - praxis

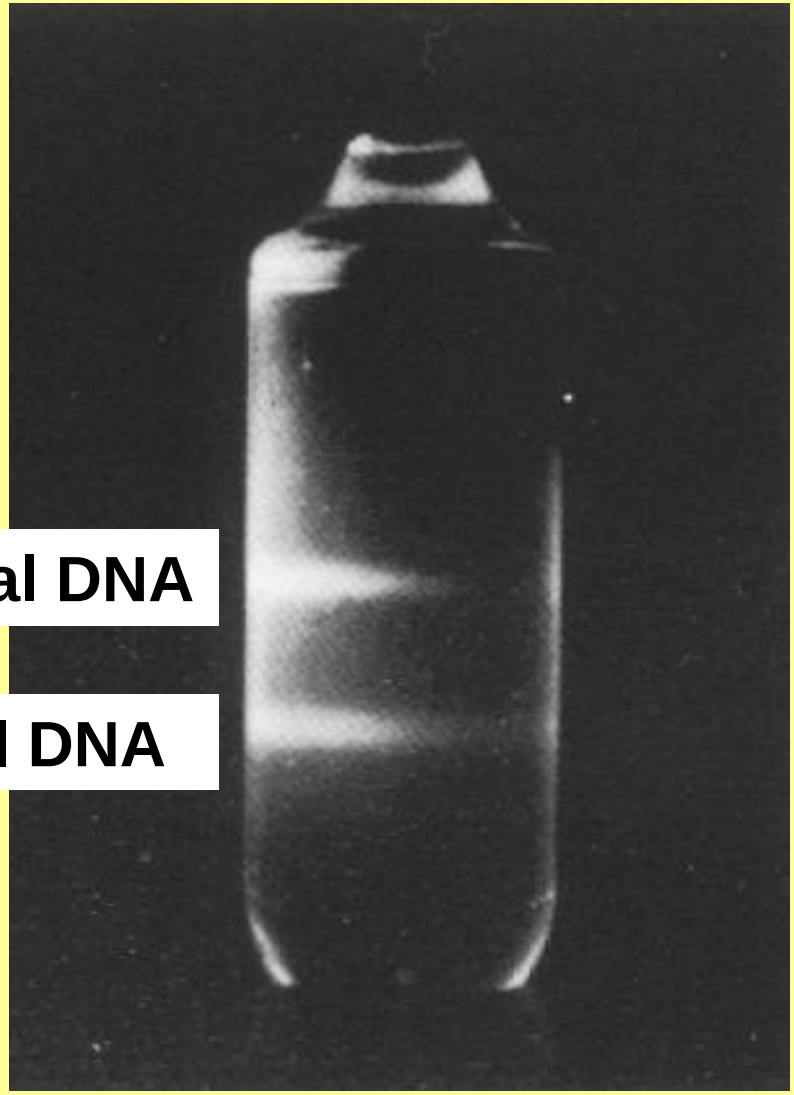



**Molecules stop their movement in the place with the same density as is the density of the solution**

# *Isopycnic centrifugation - practice*



# *Isopycnic centrifugation – real picture*



**Chromosomal DNA**

**Mitochondrial DNA**

centrifugation for 10 hrs at 100,000 rpm (450,000 x ***g***)

https://www.mun.ca

# Centrifugation – useful rule

$$RCF = 1{,}119 \times 10^{-5} \times rpm^2 \times r$$

RCF = relative centrifugal force (g)

rpm = repeats per minute

r = radius (cm)