

# Cell-free Synthetic Biology

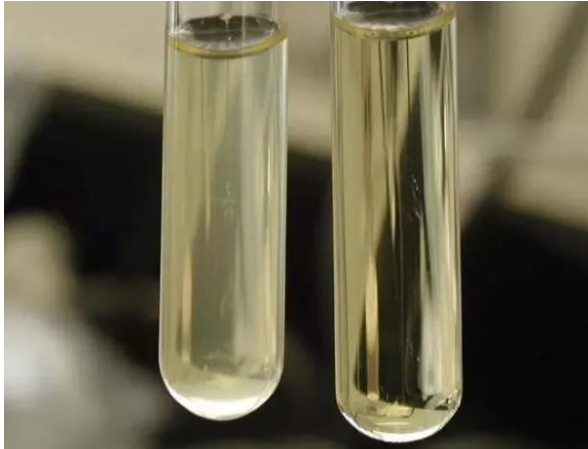
EE-490J

26.09.2023

# Objectives

- How to grow bacteria and measure them?
- What are the stages of bacterial growth curve?
- What is plasmid?
- How to isolate plasmid from bacteria?

# BACTERIAL GROWTH IN LIQUID MEDIUM



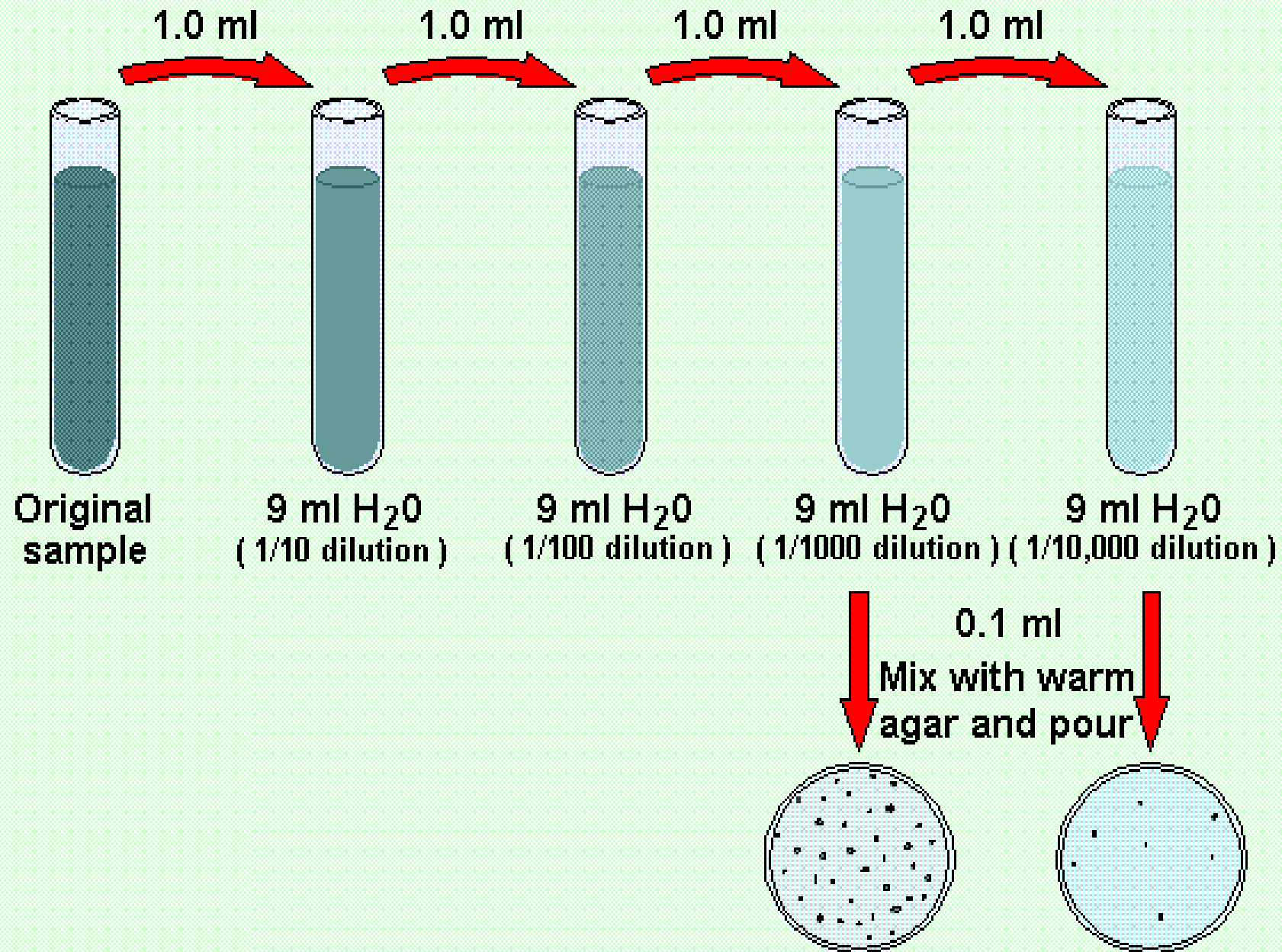
# BACTERIAL GROWTH ON SOLID MEDIUM



## ➤ Solid media advantage:

- Cultures can be easily checked for the presence of contamination (purity of culture)
- A viable cell count can be carried out using spread plate or pour plate techniques .

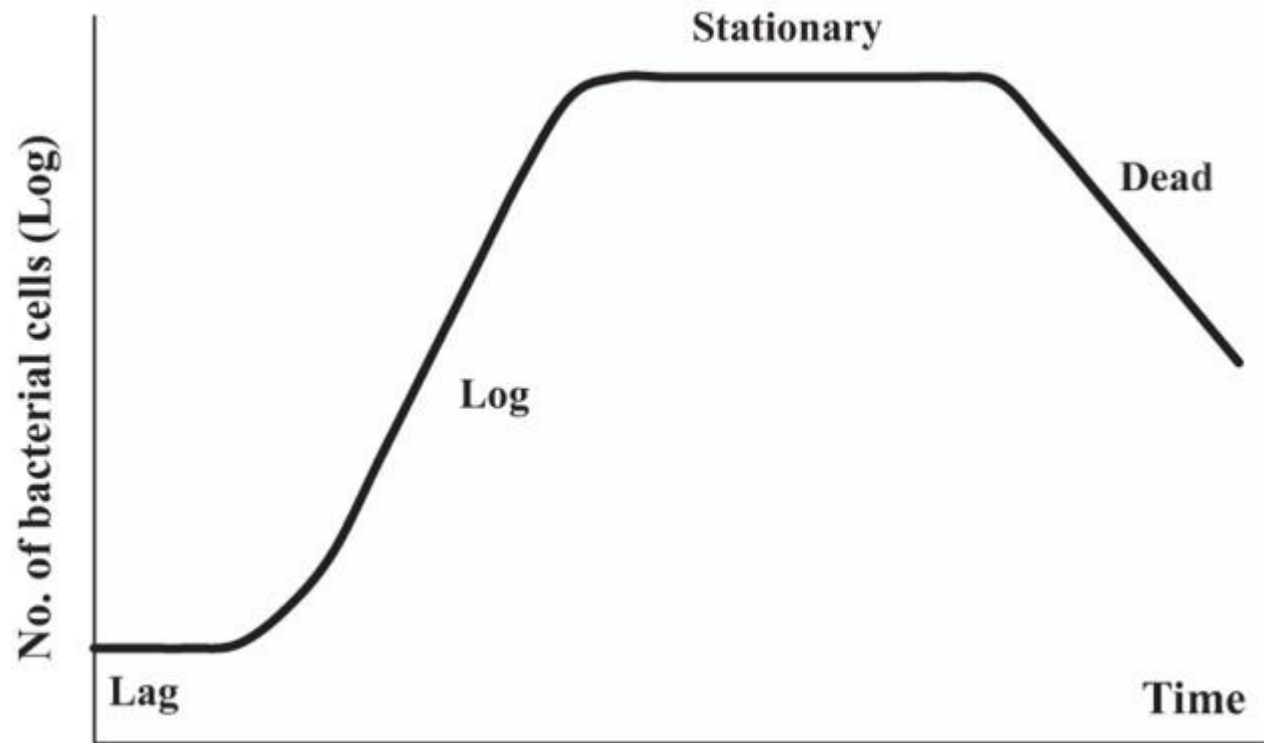
# Serial dilution



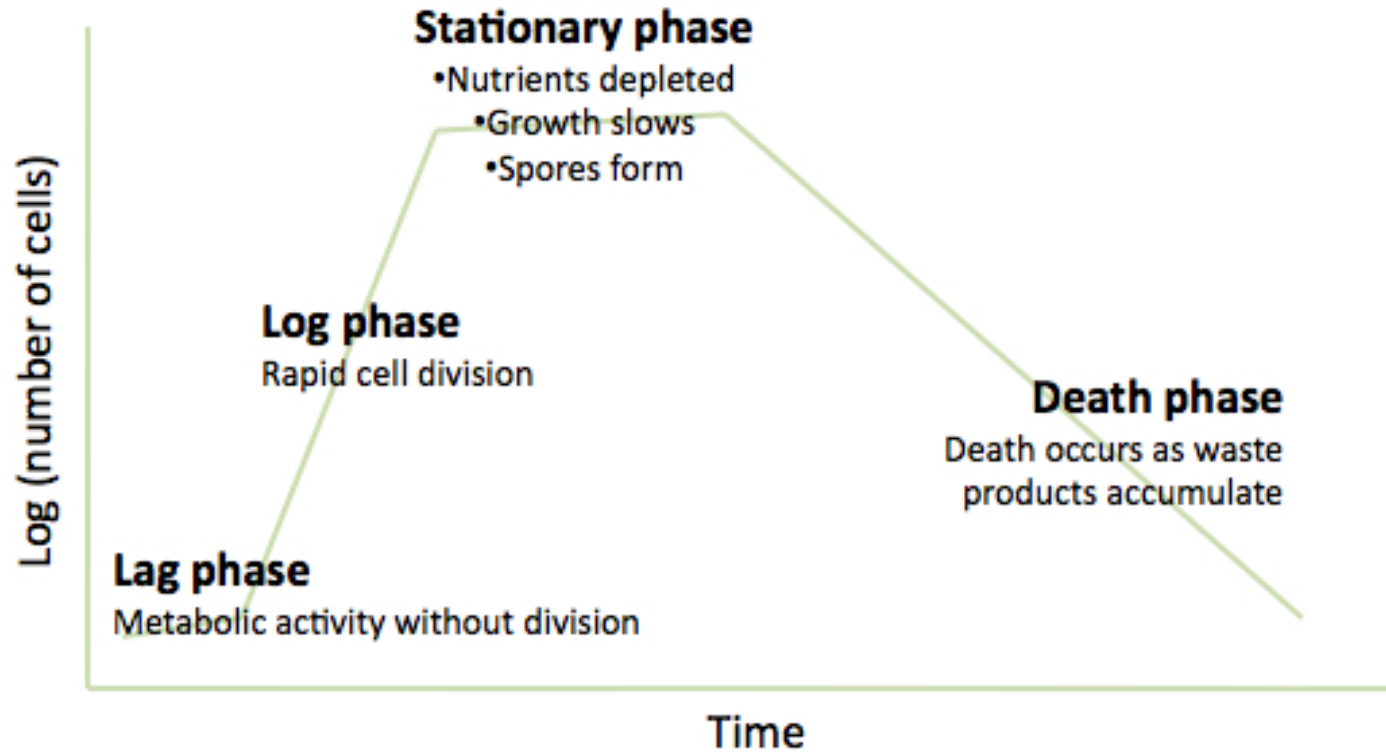
# BACTERIAL GROWTH

- The mathematics of bacterial growth is fairly simple, since each original cell divides to form two new cells, with the loss of the original parent.
- the mathematical series describing growth is: 1, 2, 4, 8, 16, ... .
- This can be written as  $2^0, 2^1, 2^2, 2^3, 2^4, 2^5, ..$

# Bacterial growth curve



# Bacterial growth curve



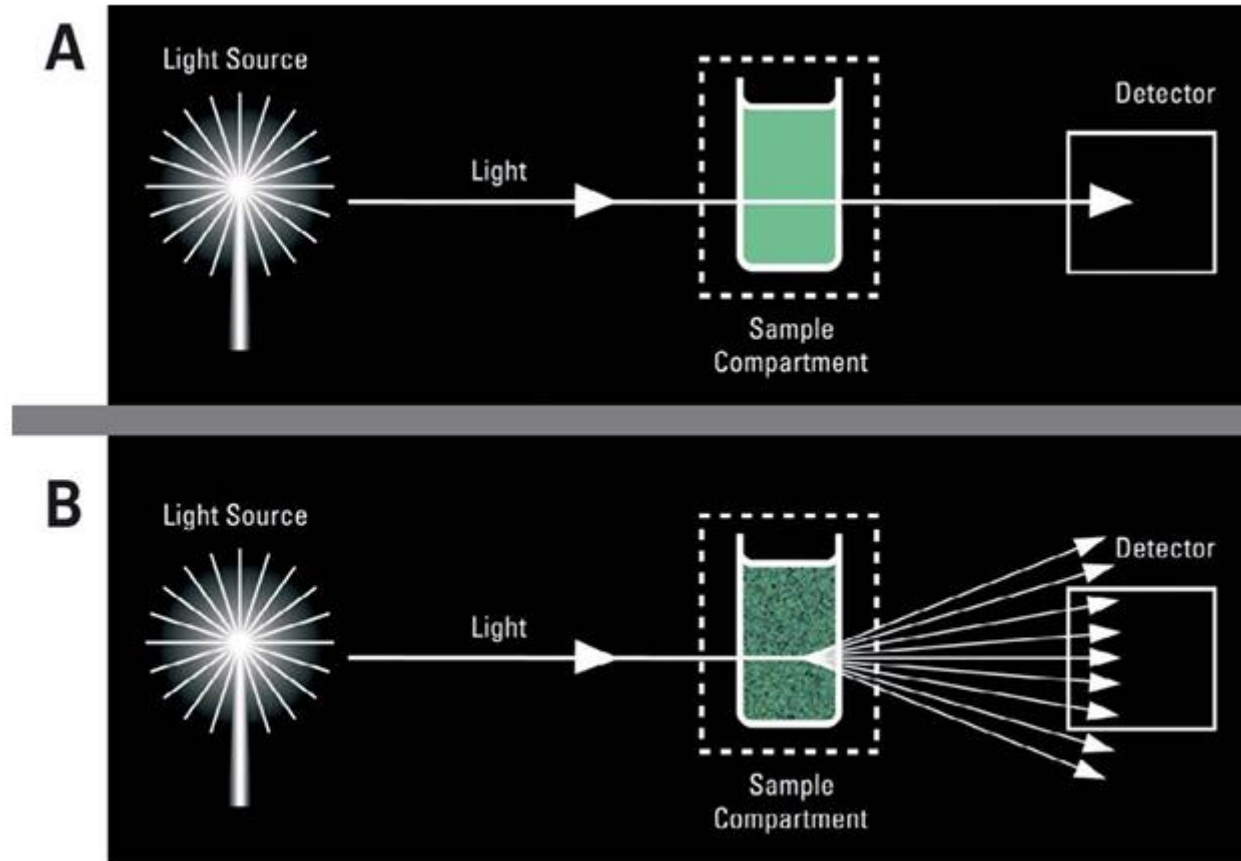
- Doubling time
- Generation time
- Growth rate

$$G = t/n$$

$$b = B \times 2^n$$

$$G = \frac{t}{3.3 \log b/B}$$

# Spectrophotometry measurement



# Optical density

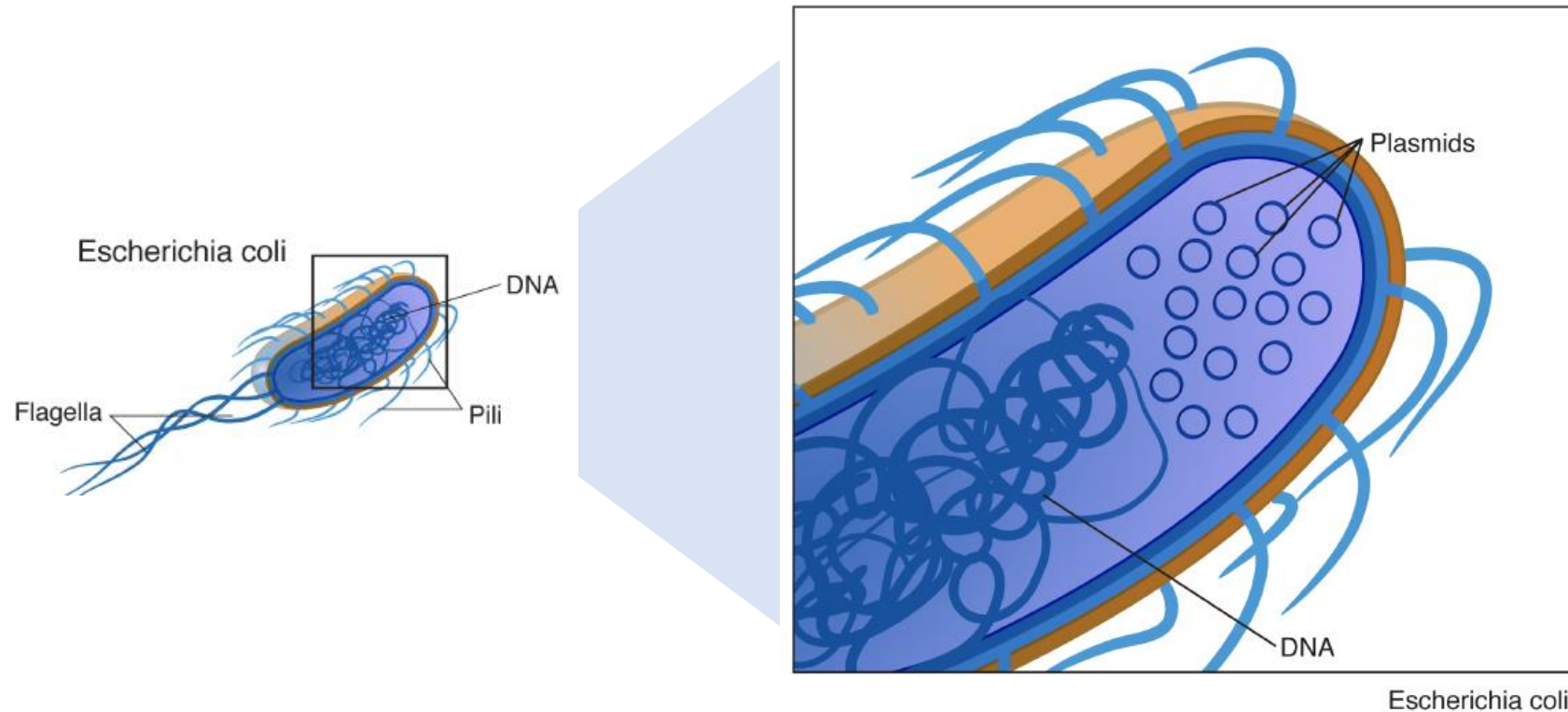
- OD 600 nm
  - Less harmful to cells
  - Not absorbed by the LB (Lysogeny broth) media
- Beer-lambert's law

$$A = \epsilon cl$$

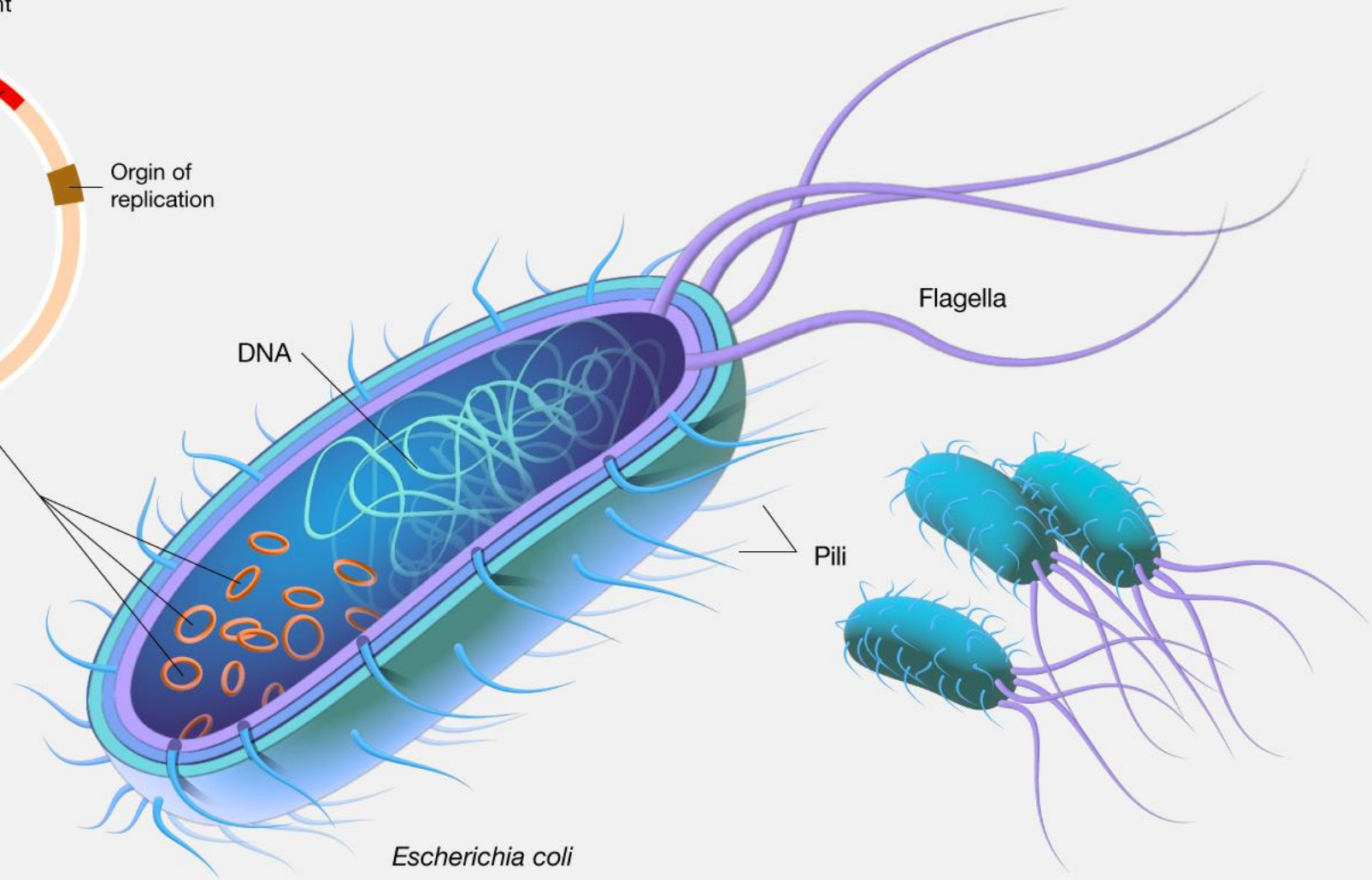
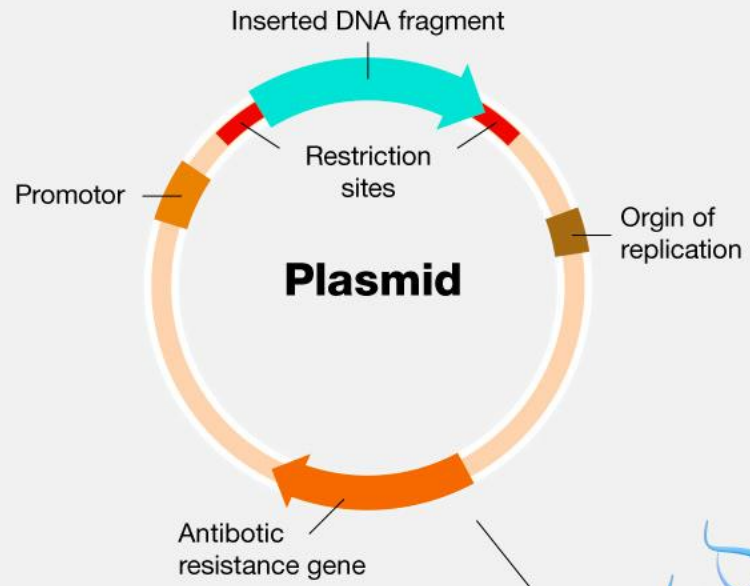
|            |                              |                 |
|------------|------------------------------|-----------------|
| $A$        | Absorbance                   |                 |
| $\epsilon$ | Molar absorption coefficient | $M^{-1}cm^{-1}$ |
| $c$        | Molar concentration          | $M$             |
| $l$        | optical path length          | $cm$            |

# Plasmids

Small circular DNA molecules that replicate independently of the host chromosomes



Indispensable tools that allow molecular biologists to obtain essentially unlimited amounts of a DNA sequence



# How to isolate plasmid from bacteria?

Plasmid purification is based on their distinctive physical properties

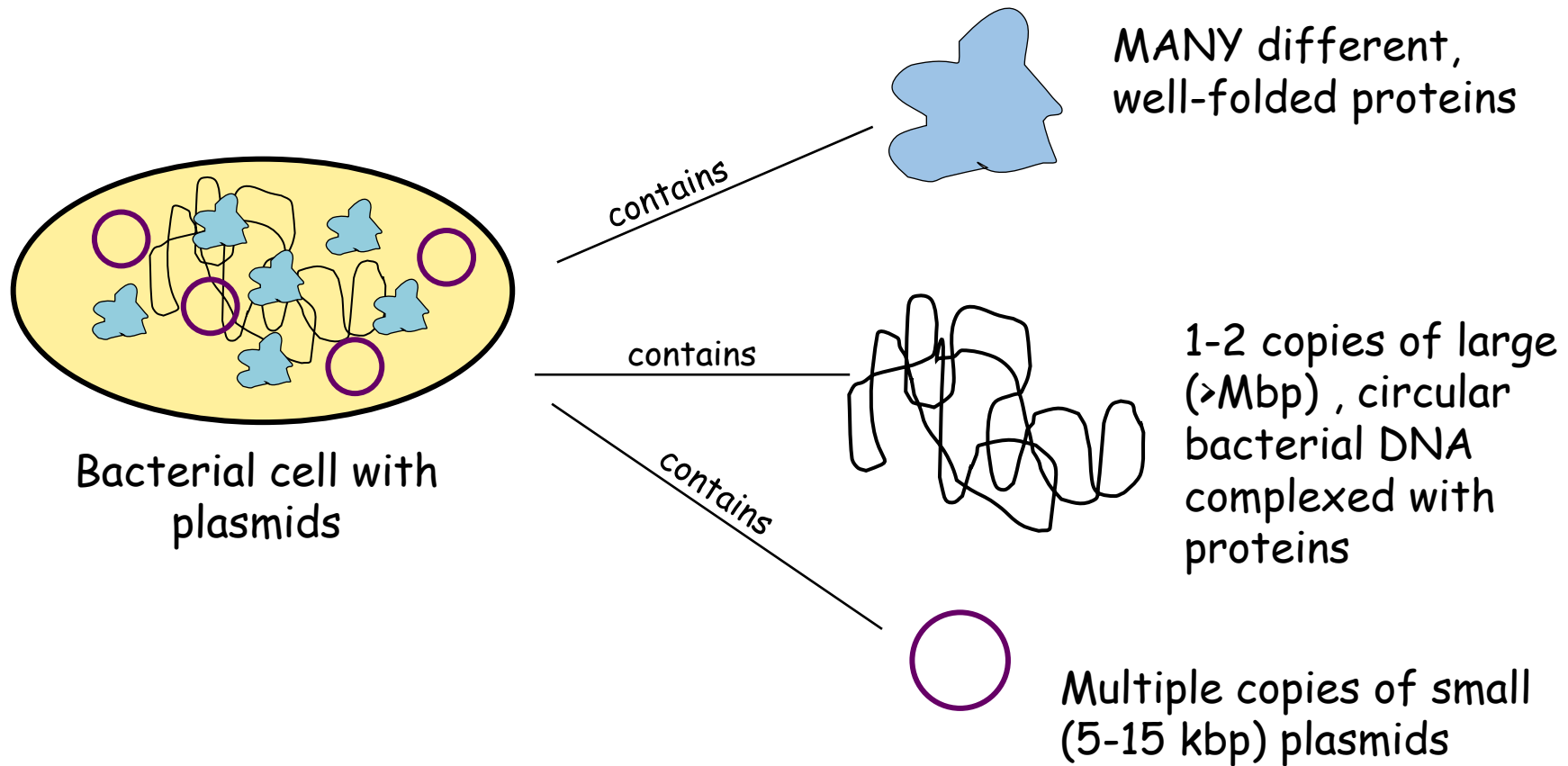
Plasmids are much smaller than bacterial chromosomes

Plasmids are supercoiled in their native form

Supercoiling allows plasmids to renature quickly after they are denatured

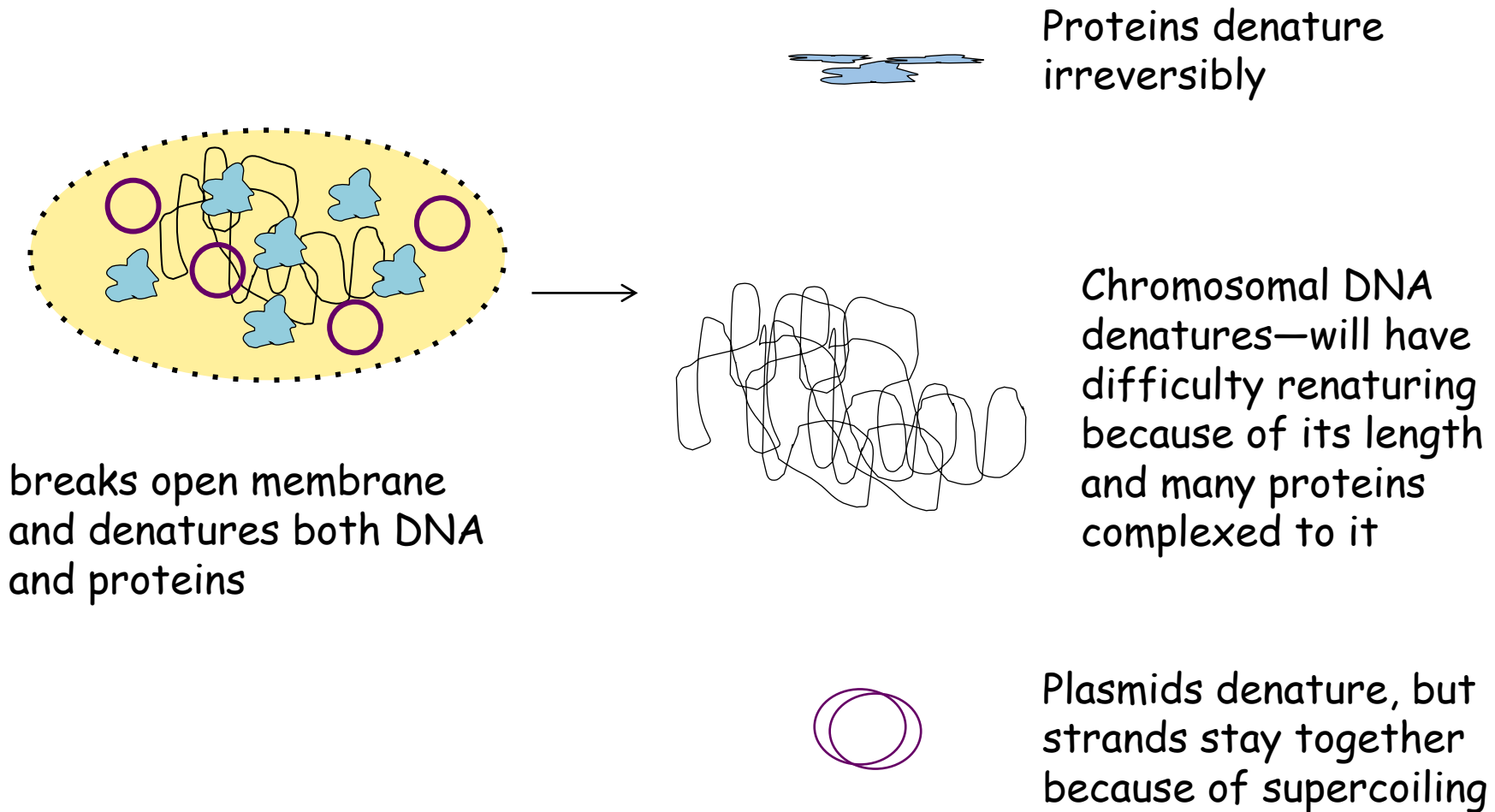


## Plasmid purification from bacteria relies on their unique physical properties

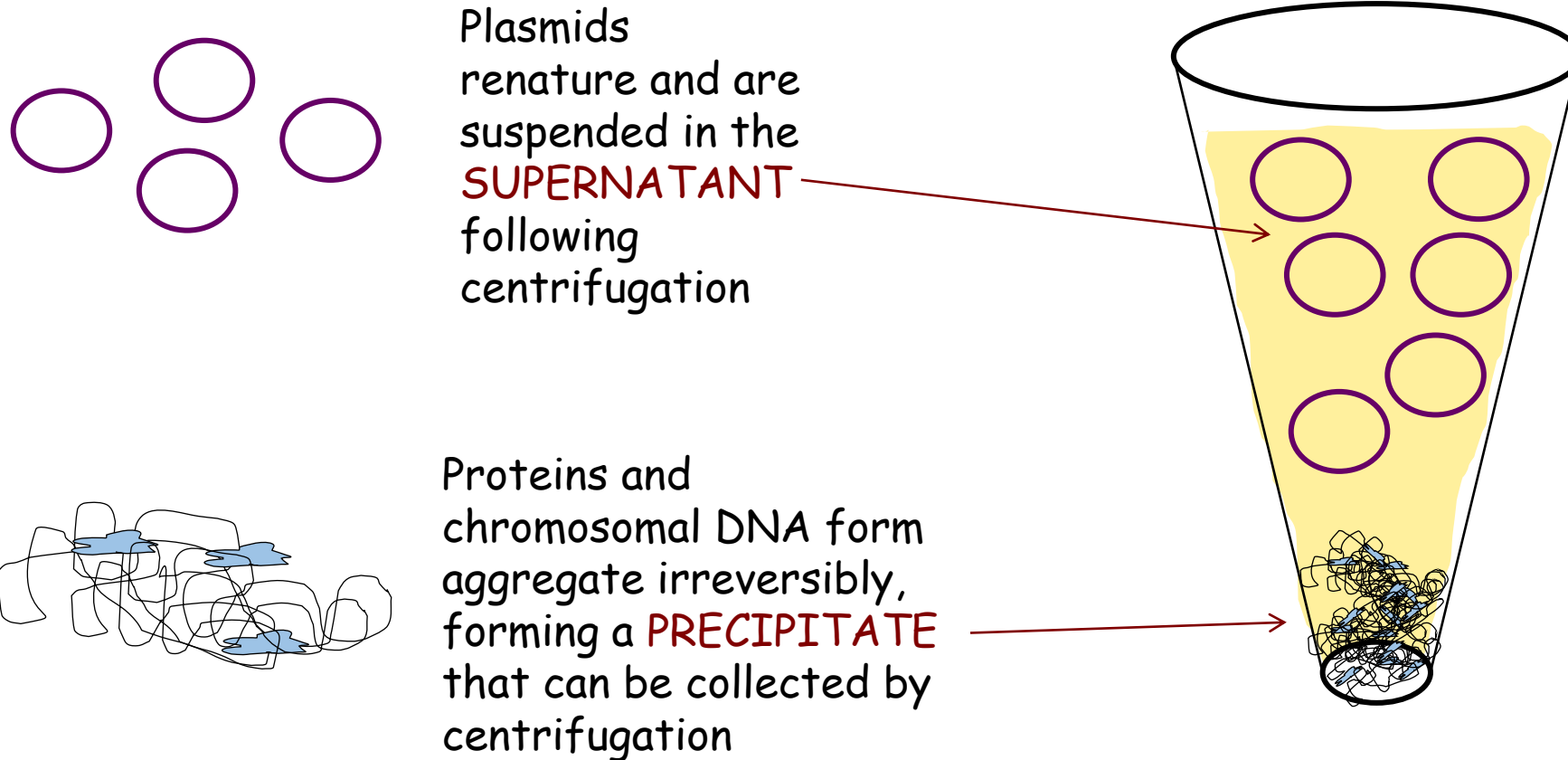


Purification involves sequential denaturation and renaturation steps

## Cells are first treated with base and a detergent



Extract is neutralized to allow DNA molecules to renature



When purifying plasmids, use a micropipette to remove the supernatant for further processing steps