# LAB EXPERIMENT 2

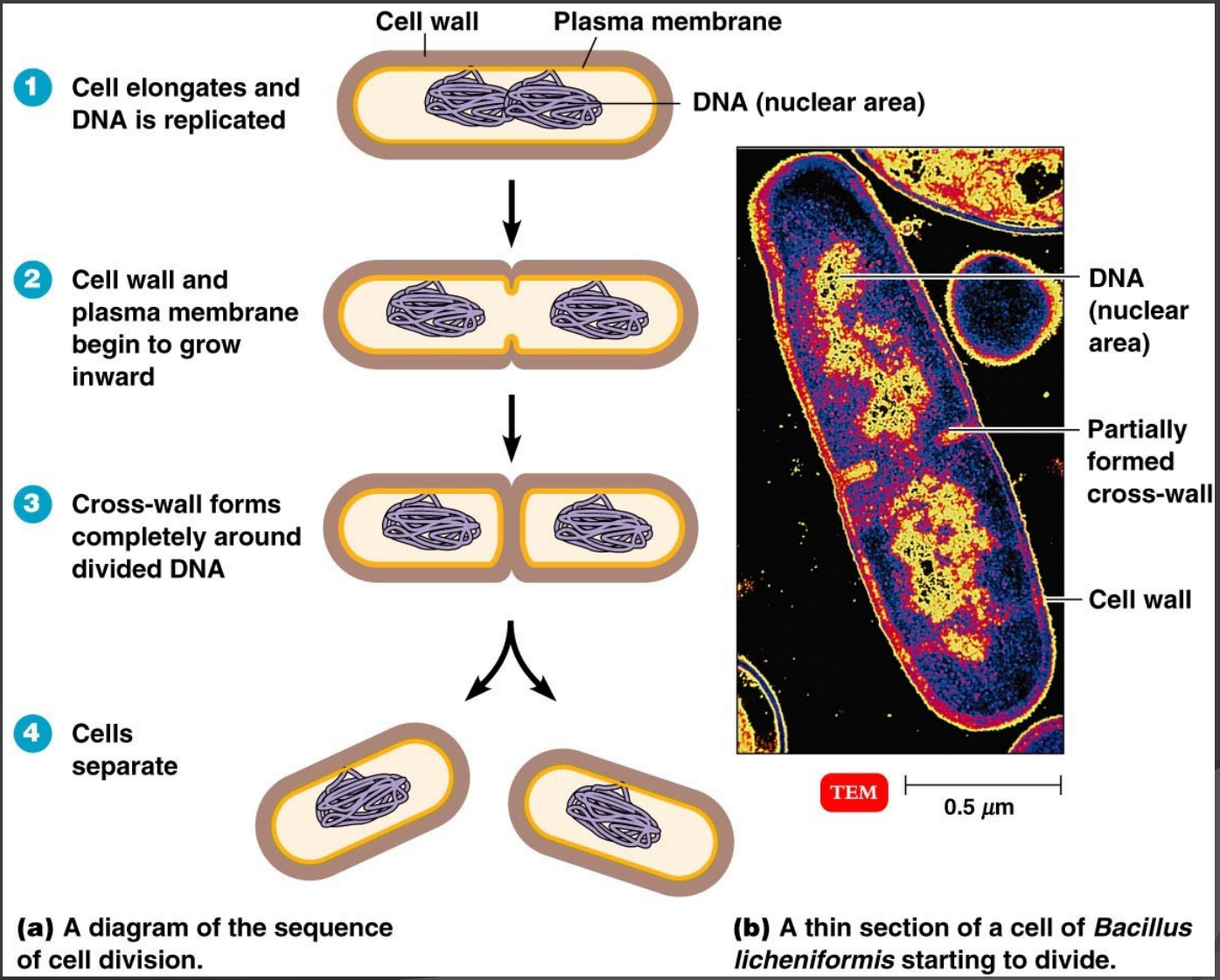
Lecturer: Ta Van Quang

# INTRODUCTION

# Reproduction in Prokaryotes

- Binary fission
- Budding
- Conidiospores (actinomycetes)
- Fragmentation of filaments

# Binary Fission



# Log Phase

| Generation Number | Number of Cells | $\log_{10}$ of Number of Cells |
|---|---|---|
| 0 | $2^0 = 1$ | 0 |
| 5 | $2^5 = 32$ | 1.51 |
| 10 | $2^{10} = 1{,}024$ | 3.01 |
| 15 | $2^{15} = 32{,}768$ | 4.52 |
| 16 | $2^{16} = 65{,}536$ | 4.82 |
| 17 | $2^{17} = 131{,}072$ | 5.12 |
| 18 | $2^{18} = 262{,}144$ | 5.42 |
| 19 | $2^{19} = 524{,}288$ | 5.72 |
| 20 | $2^{20} = 1{,}048{,}576$ | 6.02 |

# Log Phase with Calculations

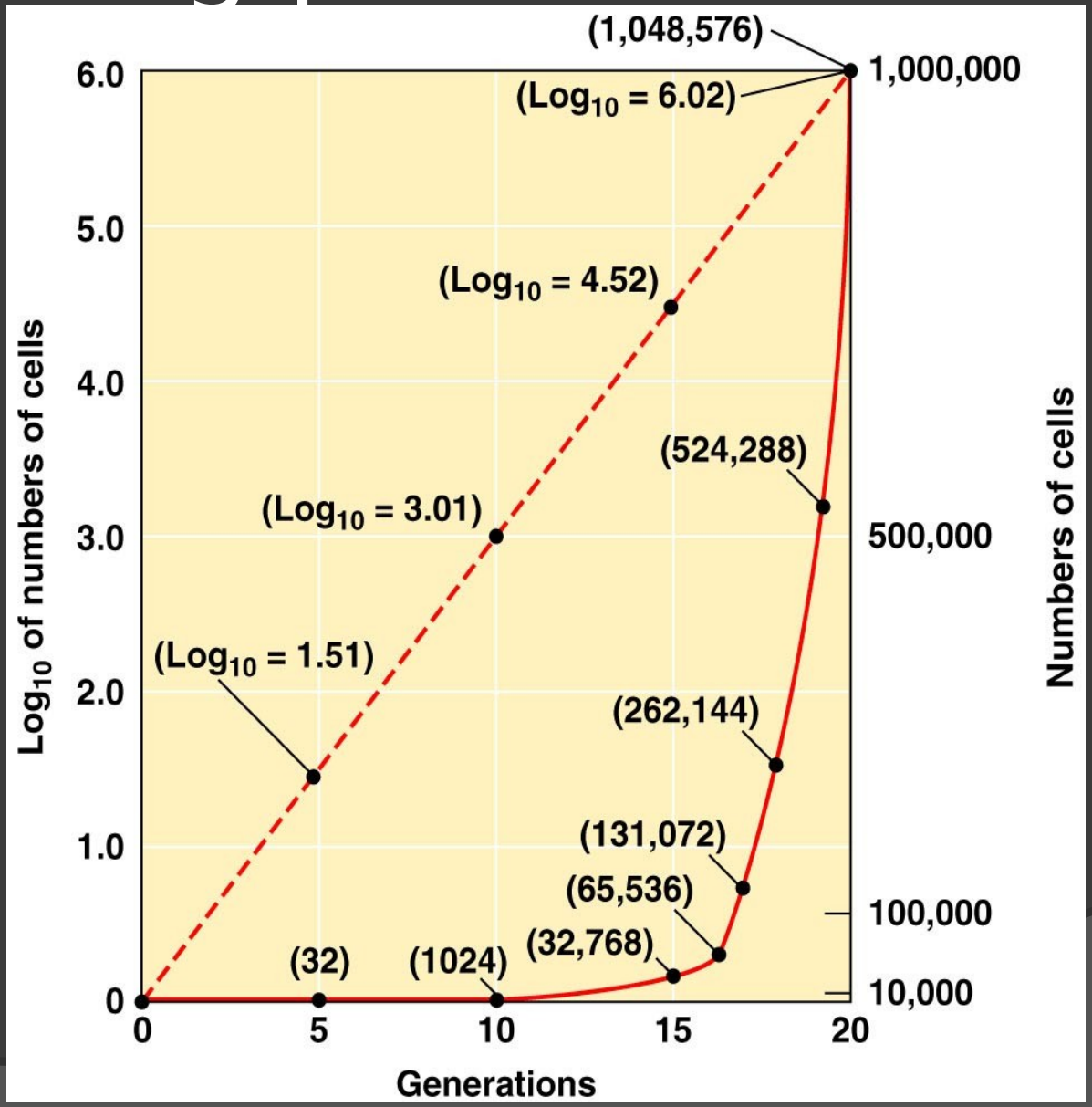
$$\text{Number of generations} = \frac{\text{Log number of cells (end)} - \text{Log number of cells (beginning)}}{0.301}$$

$$\text{Generation time} = \frac{60 \text{ min} \times \text{hours}}{\text{Number of generations}} = 21 \text{ minutes/generation}$$

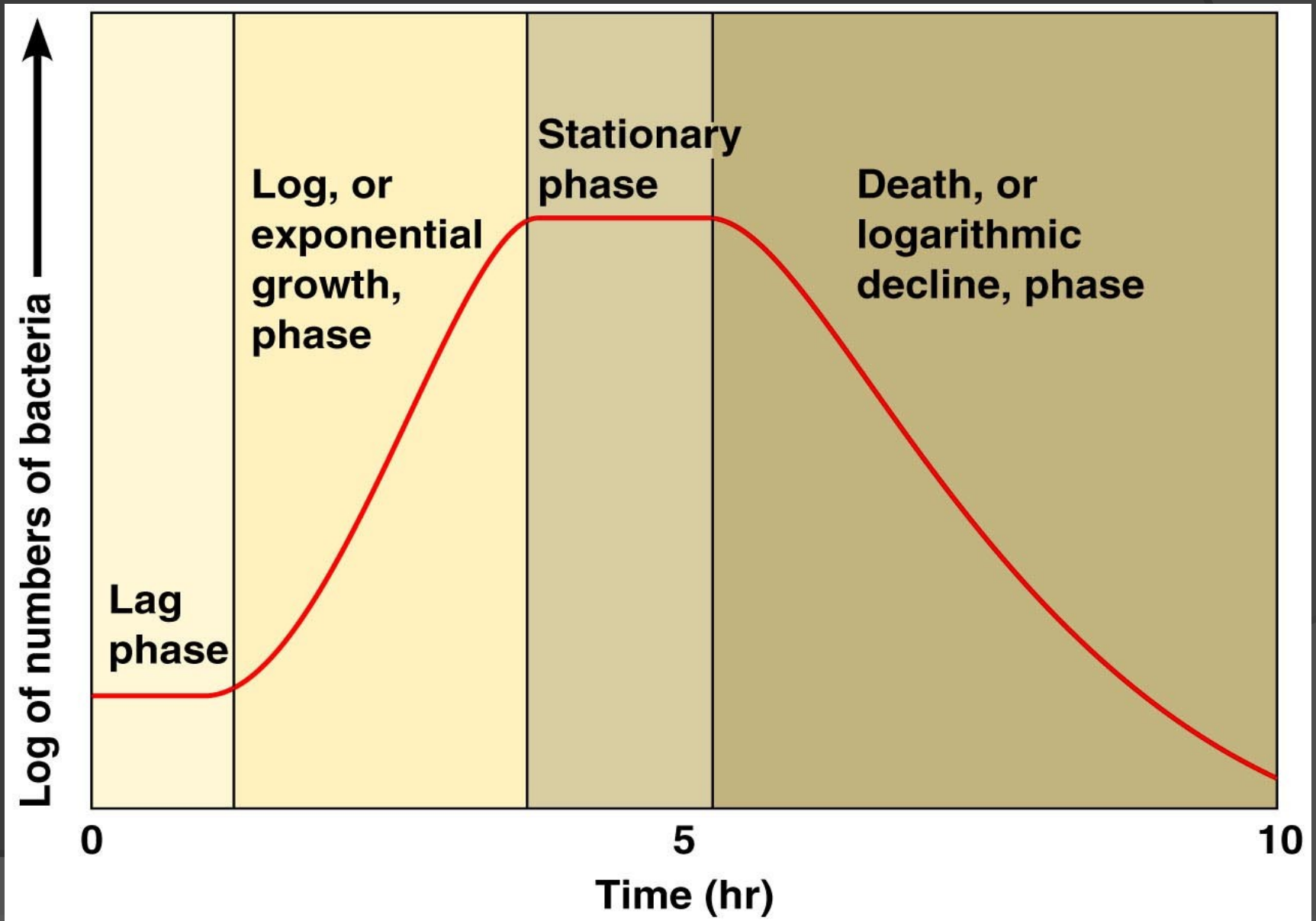
# Phases of Growth

- 4 Phases:
- 1) Lag phase-
- 2) Log (logarithmic) phase
- 3) Stationary phase
- 4) Decline phase or death phase

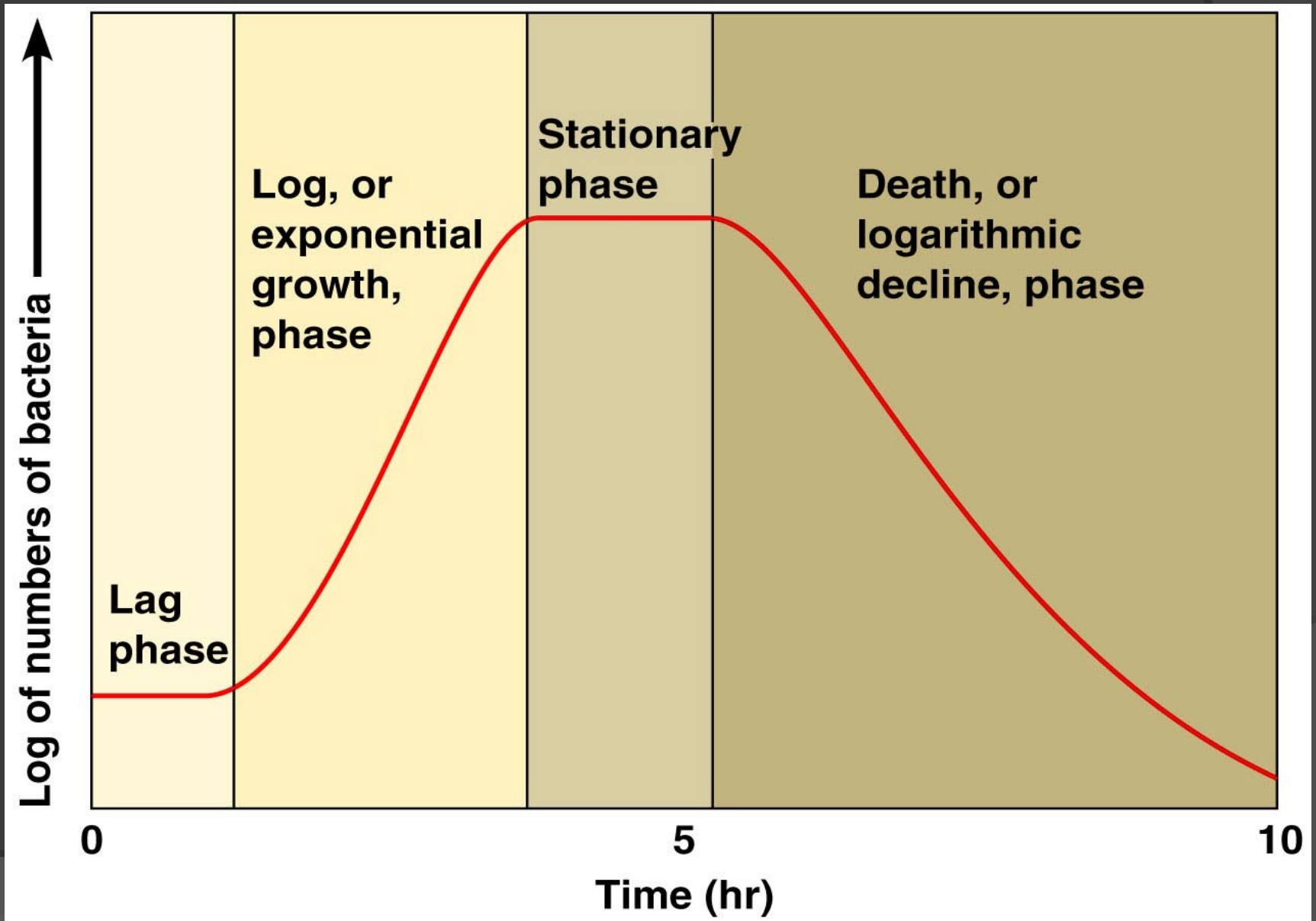
# Log phase


(1,048,576)
1,000,000
6.0
(Log10 = 6.02)
5.0
(Log10 = 4.52)
4.0
(524,288)
(Log10 = 3.01)
3.0
500,000
(Log10 = 1.51)
2.0
(262,144)
1.0
(131,072)
(65,536)
100,000
(32)
(1024)
(32,768)
0
10,000
0
5
10
15
20
Generations
Log10 of numbers of cells
Numbers of cells

# 4 Phases of Microbial Growth



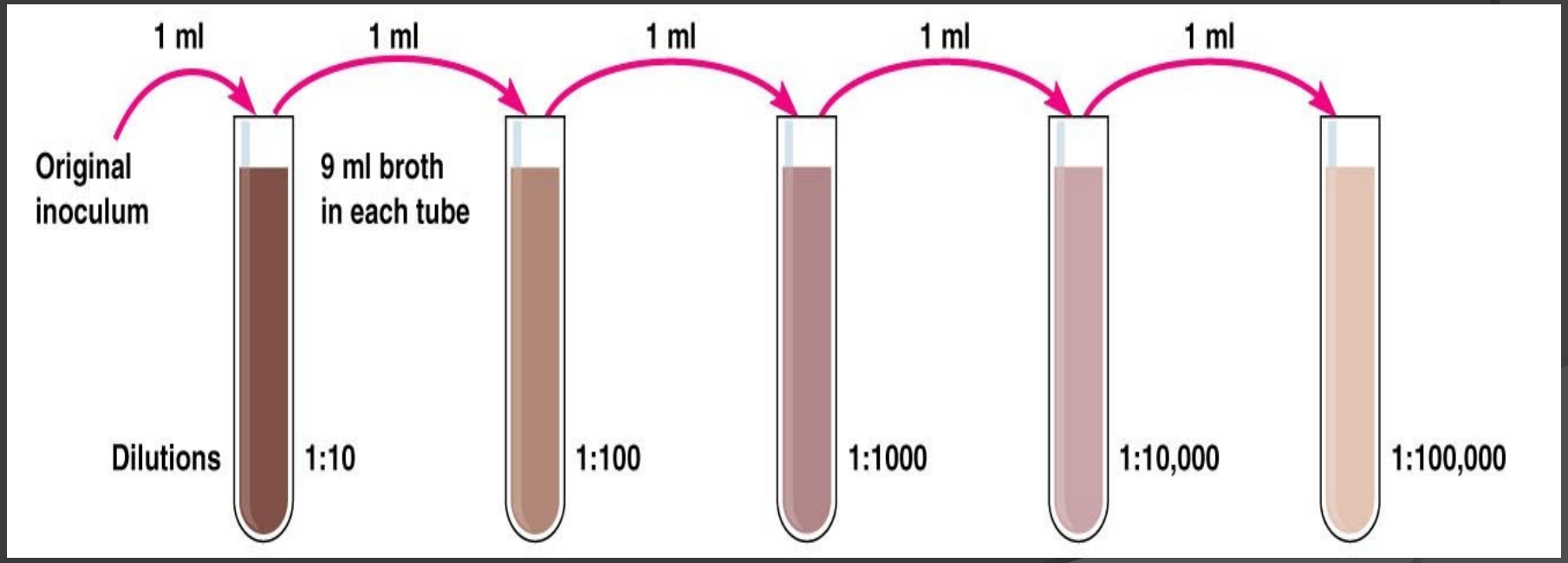
# 4 Phases of Microbial Growth



# Measuring Bacterial Growth

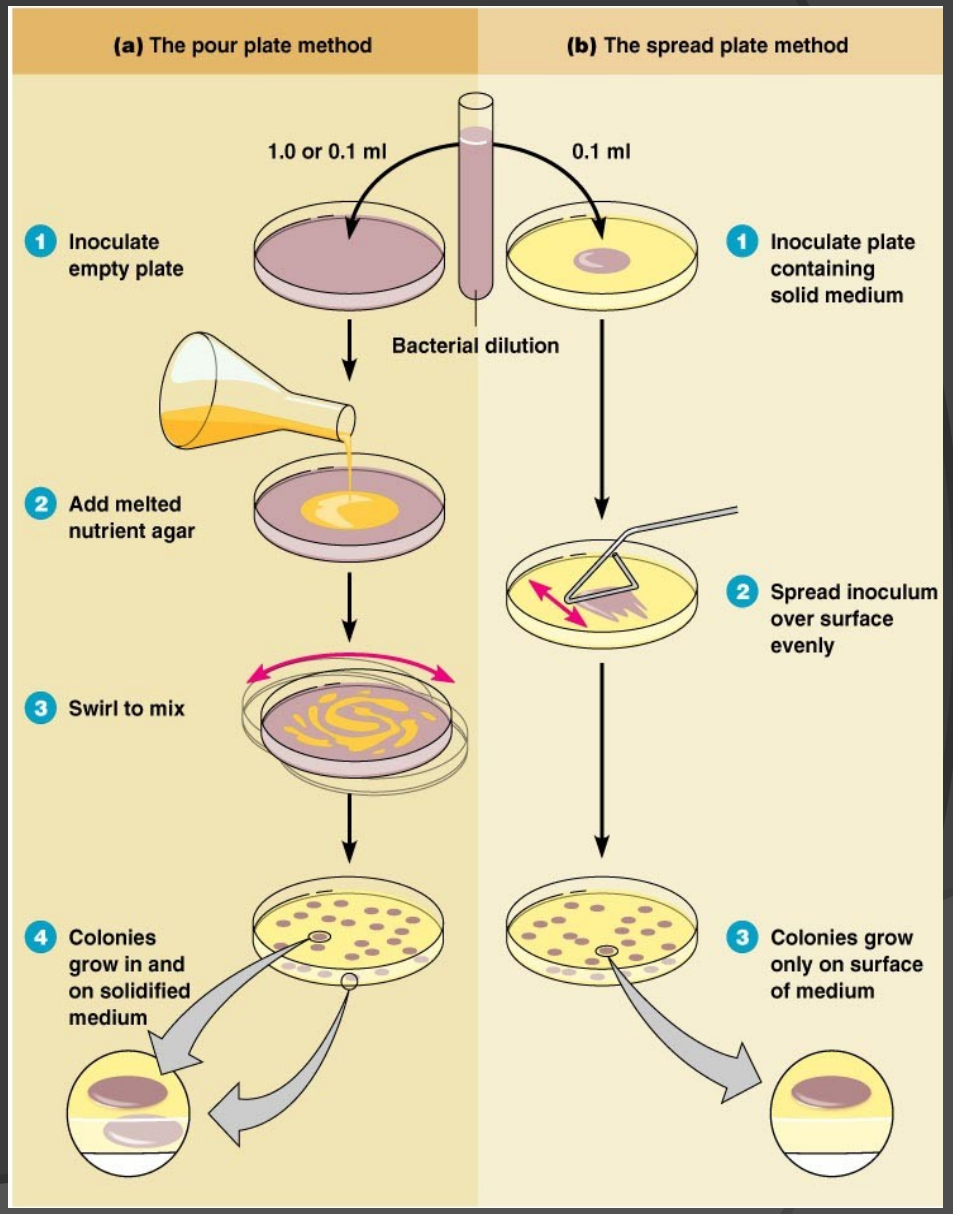
## Serial Dilutions

- Direct Measurements of Microbial Growth
- Plate counts: Perform serial dilutions of a sample



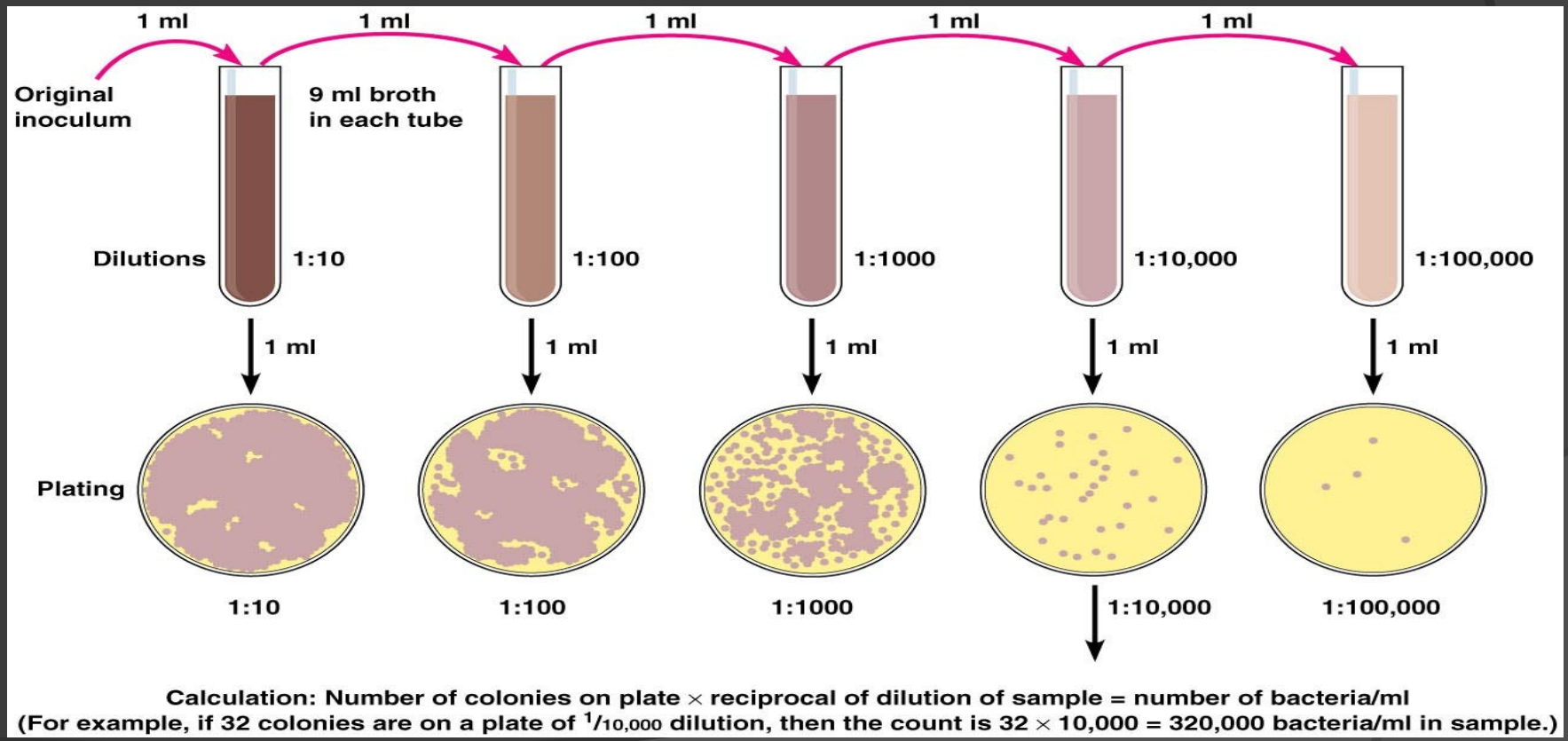
# Standard Plate Count

Inoculate Petri plates from serial dilutions
2 methods:
Pour Plate
Spread Plate



# Plate Count

After incubation, count colonies on plates that have 25-250 colonies (CFUs)



# Measuring Microbial Growth

Direct methods

- Plate counts
- Filtration
- MPN
- Direct microscopic count
- Dry weight

Indirect methods

- Turbidity
- Metabolic activity
- Dry weight

# Types of Media

- There are generalized media, like (Nutrient ager)that will grow many different types of microbes.
- This media is the type most often used to culture bacteria

# Selective Media

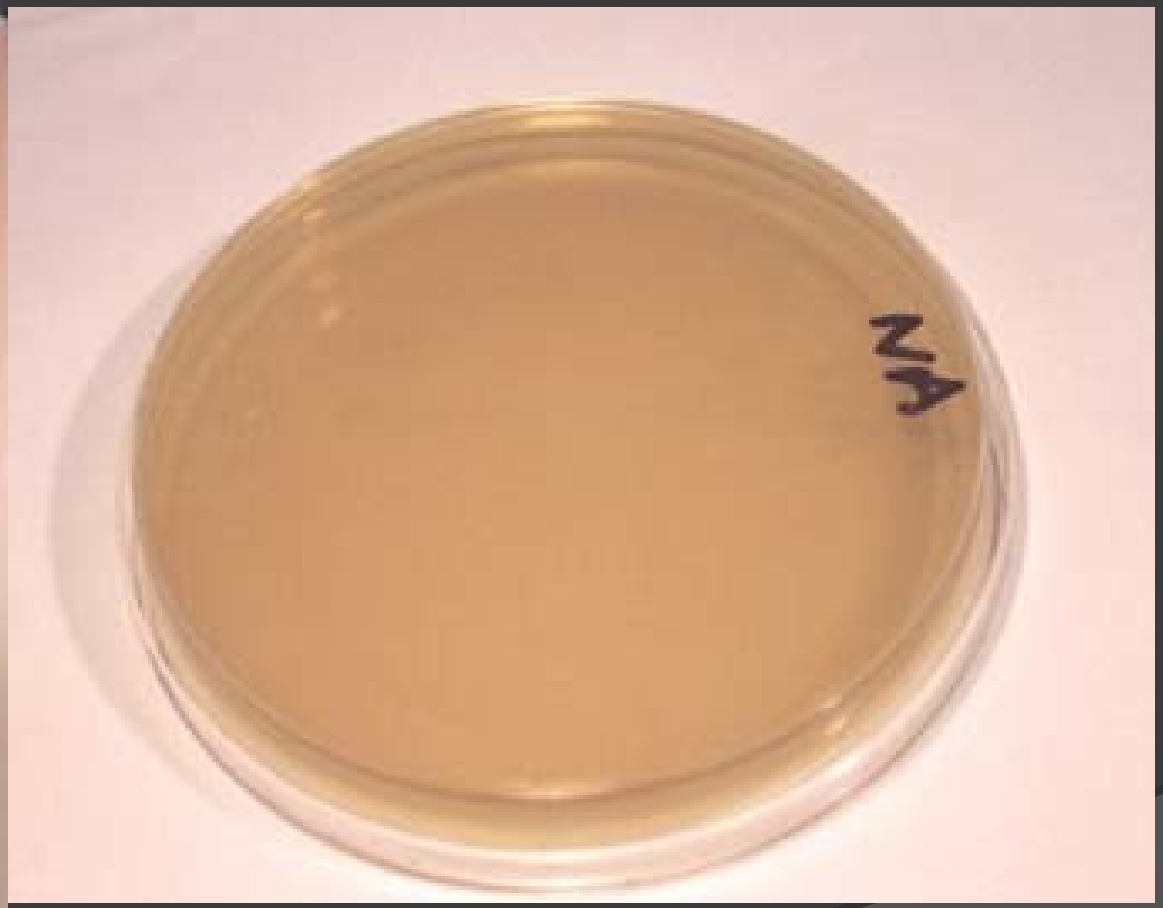
- culture medium that allows the growth types of organisms, while inhibiting the growth of other organisms

Example:

EMB (Eosin Methylene Blue)
dyes inhibit Gram (+) bacteria
selects for Gram (-) bacteria

# General Media:

## Nutrient Broth and Nutrient Agar




OXOID
CM0337
MUELLER-HINTON
AGAR

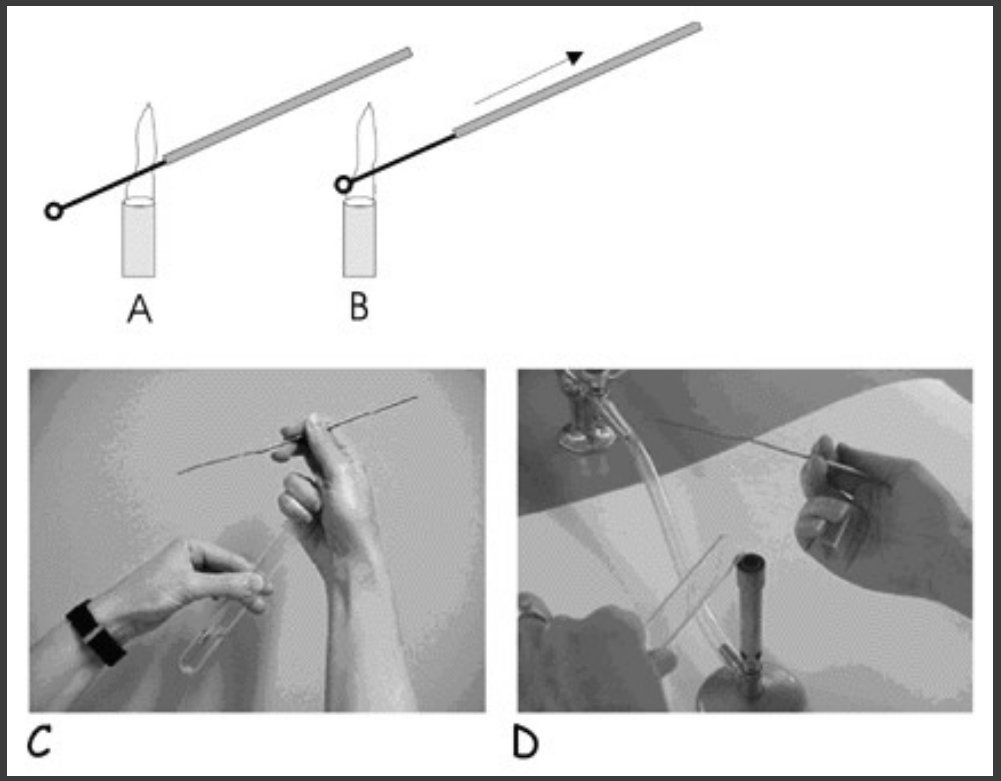

200mL
±5%
150
100
50

# How is media made?

- When lab personnel make media they measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH(7)**.
- They dispense the media into bottles (flask, tube), cap it and **autoclave**. The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is **autoclaved** it is sterile

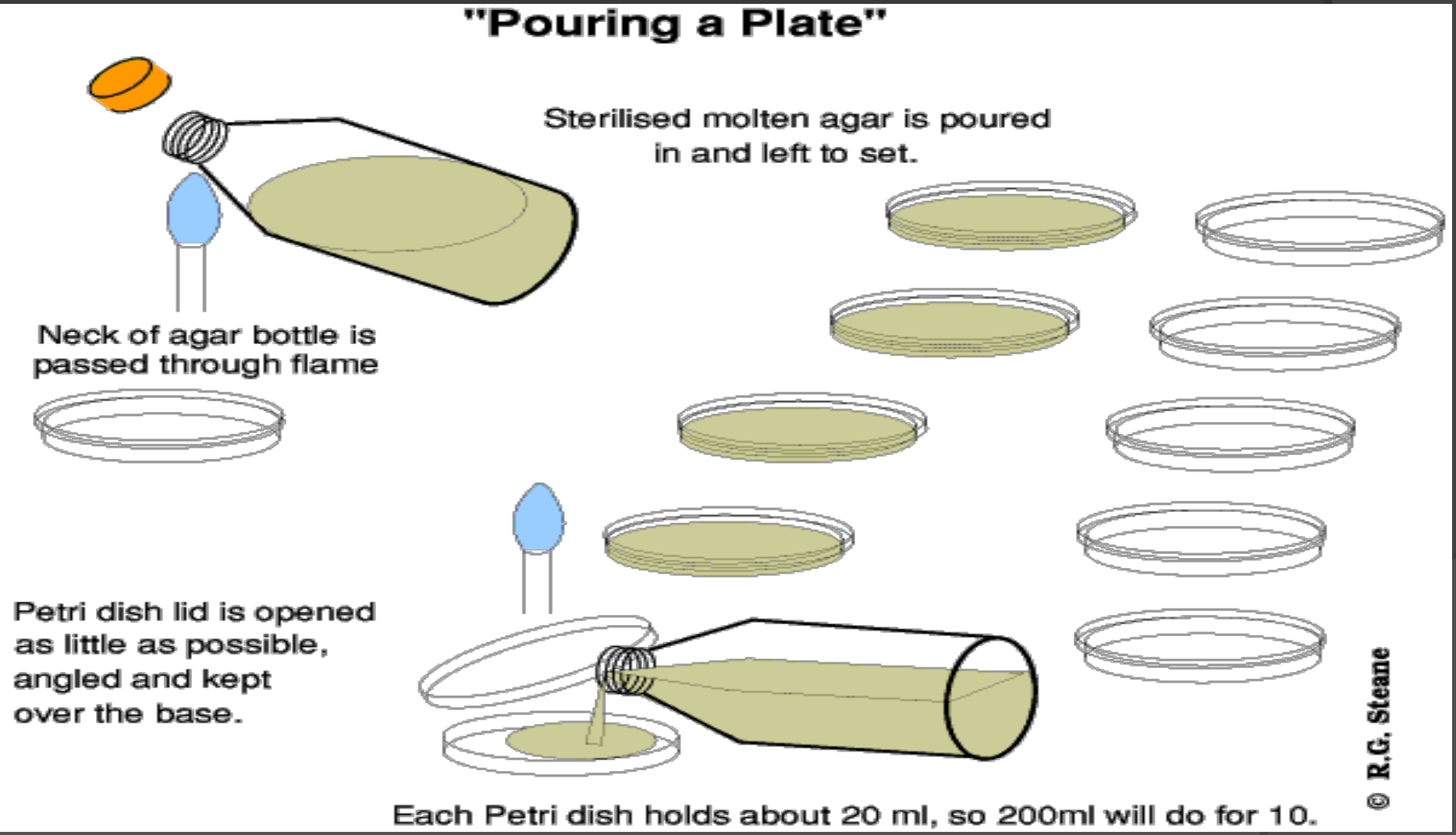
*(all microoranism forms killed)*

- Innoculation: Producing a pure culture
- Introduce bacteria into a growth medium using “aseptic technique” to prevent contamination.  Tools:  Bunsen burner, loop. Needle, etc.




# Aseptically pouring agar plates

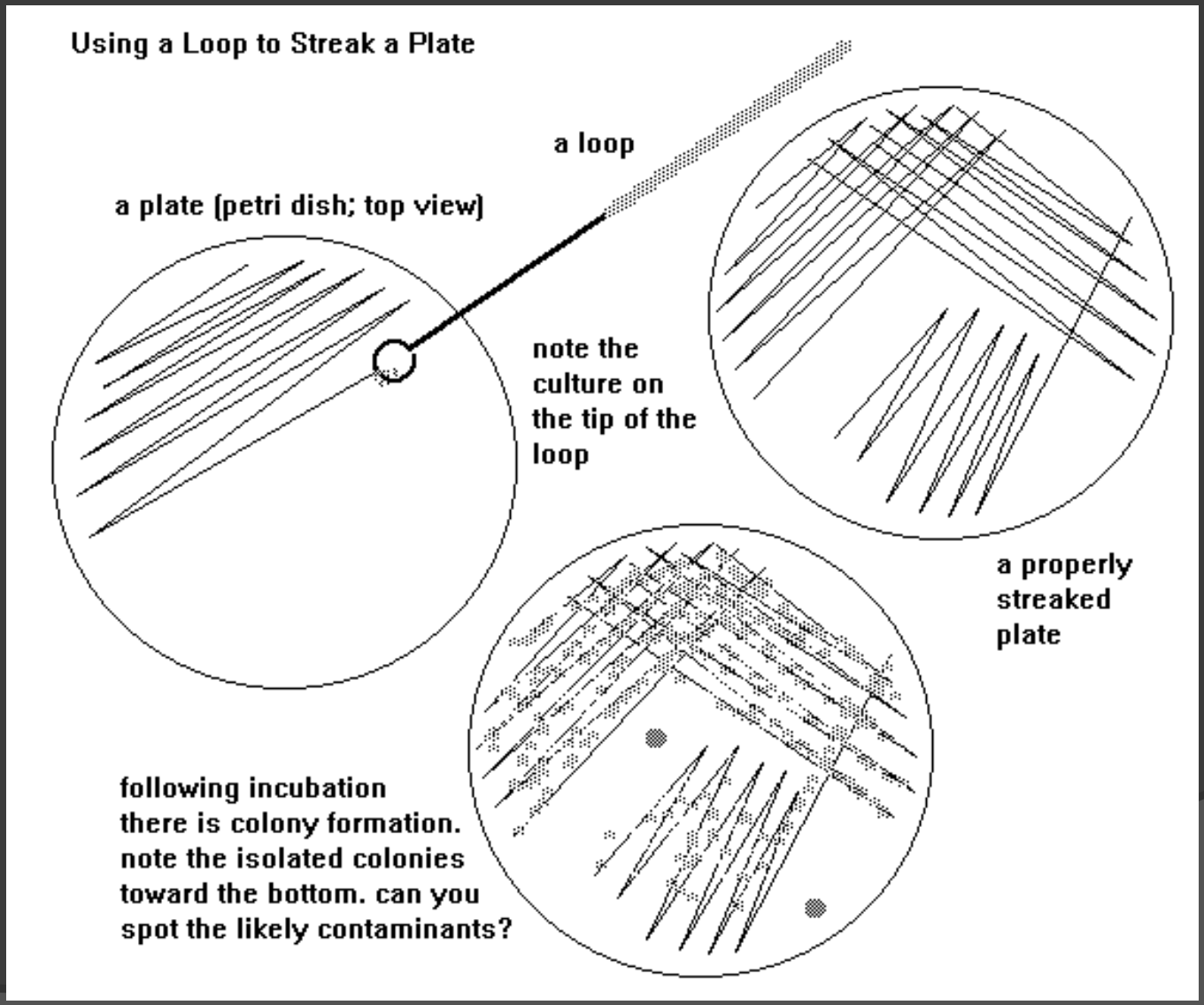
- # Innoculation: Producing a pure culture
- Introduce bacteria into a growth medium using “aseptic technique” to prevent contamination.  Tools:  Bunsen burner, loop. Needle, etc.



- All labeling is done on the **bottom** of the agar plate

1. Initials
2. Date (mm/dd/yy)
3. Code # or letter

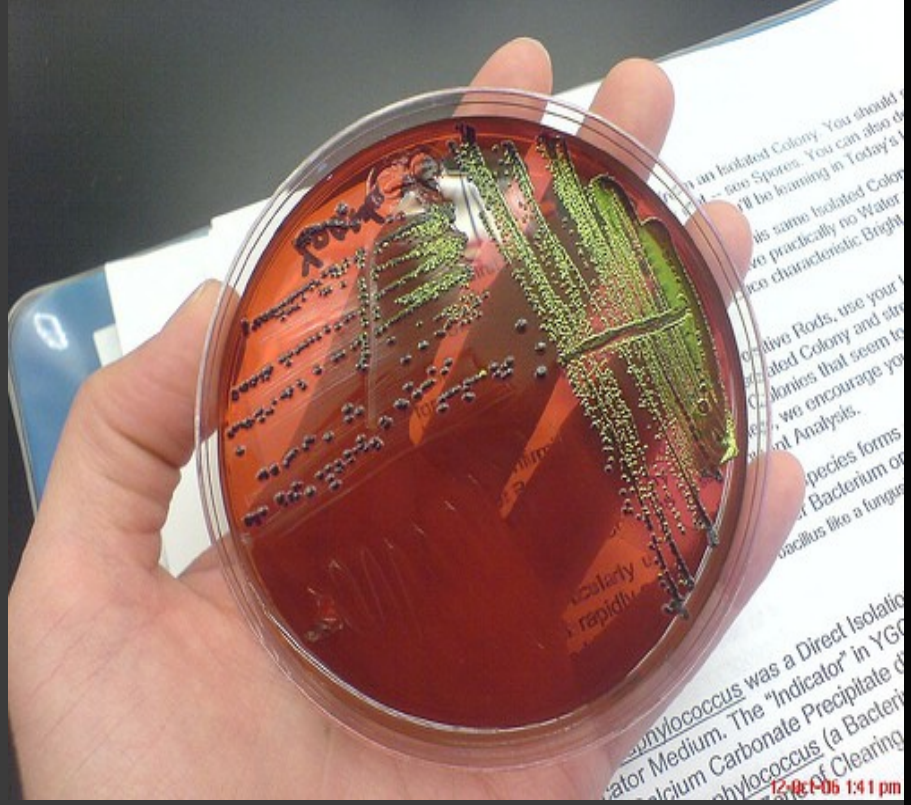
- Isolation:  Colony on media, one kind of microbe, pure culture – Streak Plates

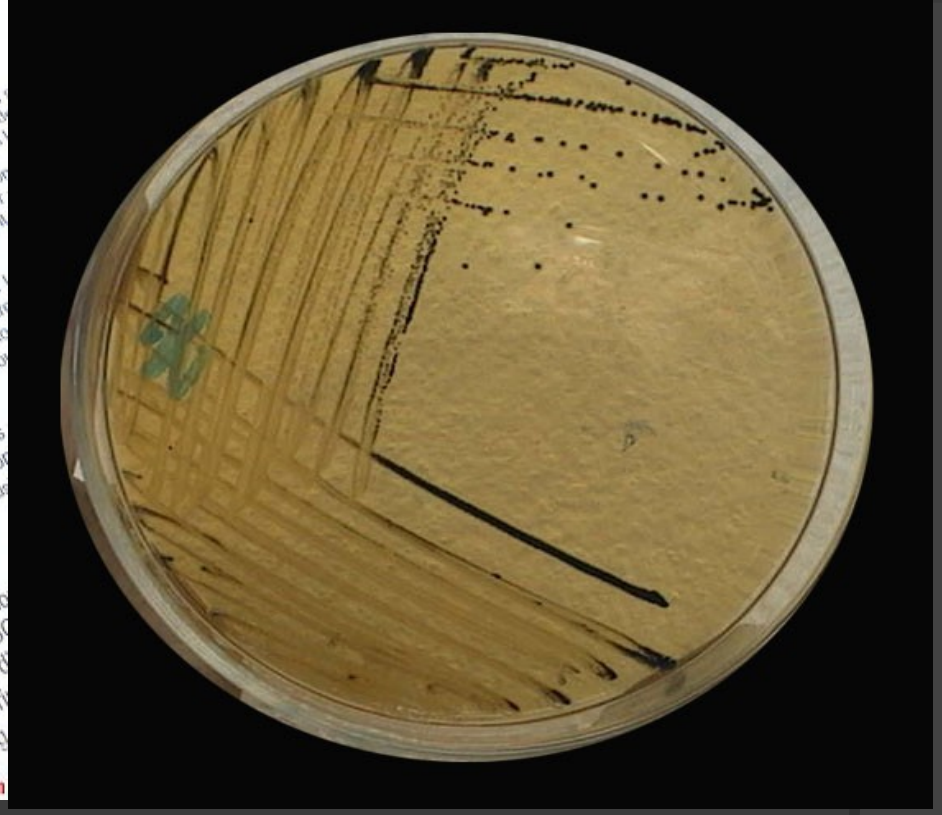


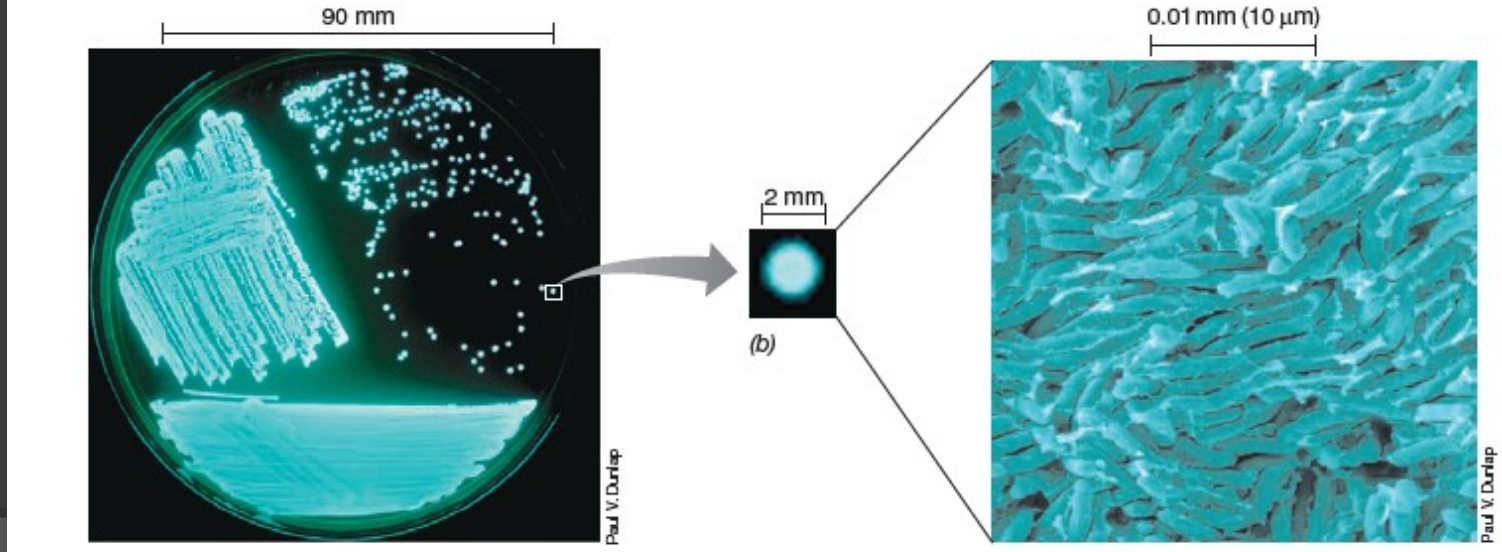
- Place all inoculated material in **incubator Culture tubes** should be stored **upright in plastic beakers**, while **Petri plates** should be **incubated upside-down** (lid on the bottom )

Agar plates are stored upside down to prevent condensation.

- These plates will be incubated at 37°C for 24 hours and then stored at refrigerater until next week when you will observe for results.






90 mm
0.01 mm (10 μm)
2 mm
(b)
Paul V. Dunlap
Paul V. Dunlap
(a)
(c)

# Cloning Vectors

- The molecular analysis of DNA has been made possible by the cloning of DNA. The two molecules that are required for cloning are the DNA to be cloned and a cloning vector.
- **Cloning vector** - a DNA molecule that carries foreign DNA into a host cell, replicates inside a bacterial (or yeast) cell and produces many copies of itself and the foreign DNA
- **Three features of all cloning vectors**
  - sequences that permit the propagation of itself in bacteria (or in yeast for YACs)
  - a cloning site to insert foreign DNA; the most versatile vectors contain a site that can be cut by many restriction enzymes
  - a method of selecting for bacteria (or yeast for YACs) containing a vector with foreign DNA; uually accomplished by selectable markers for drug resistance

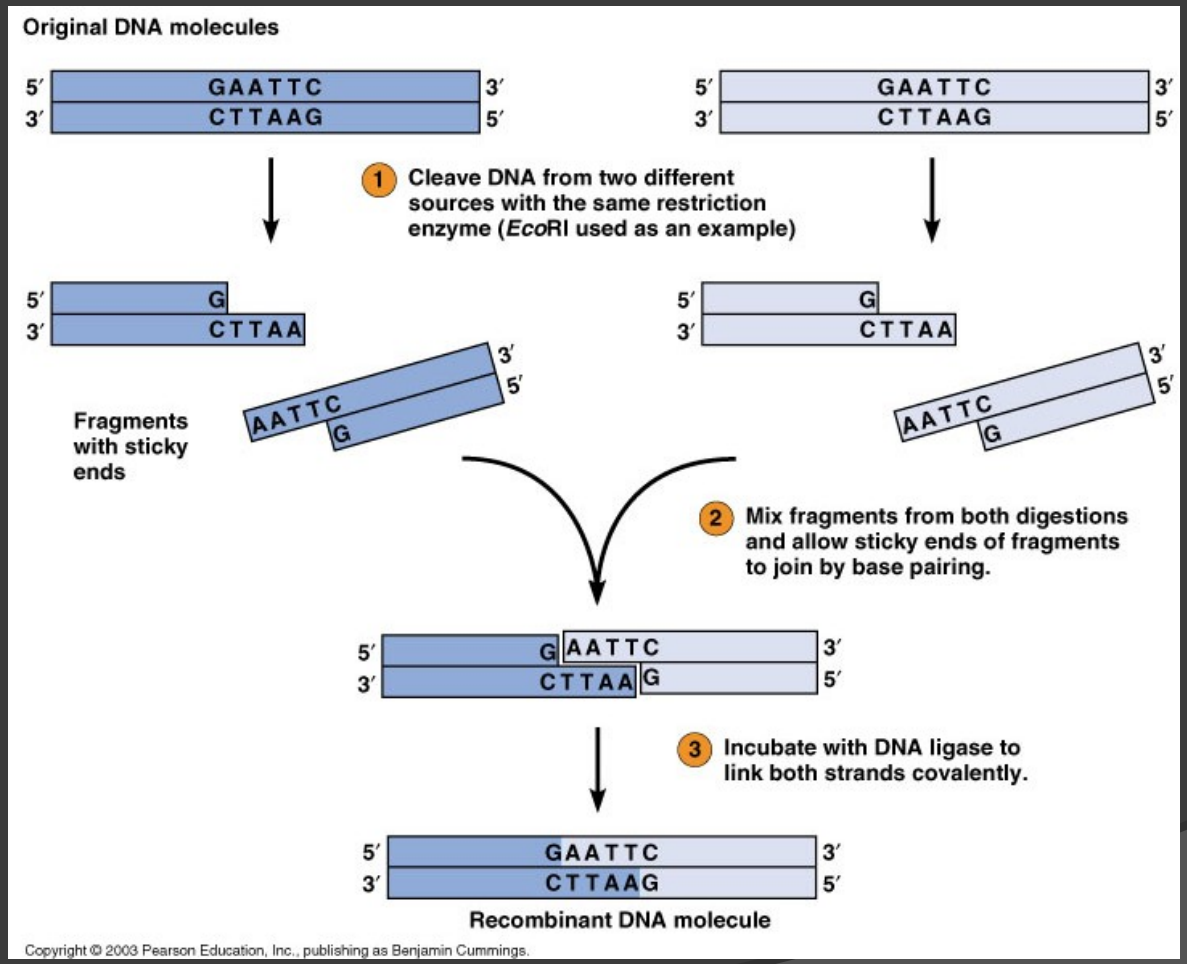
# Types of Cloning Vectors

- **Plasmid** - an extrachromosomal circular DNA molecule that autonomously replicates inside the bacterial cell; cloning limit: 100 to 10,000 base pairs or 0.1-10 kilobases (kb)
- **Phage** - derivatives of bacteriophage lambda; linear DNA molecules, whose region can be replaced with foreign DNA without disrupting its life cycle; cloning limit: 8-20 kb
- **Cosmids** - an extrachromosomal circular DNA molecule that combines features of plasmids and phage; cloning limit - 35-50 kb
- **Bacterial Artificial Chromosomes (BAC)** - based on bacterial mini-F plasmids. cloning limit: 75-300 kb
- **Yeast Artificial Chromosomes (YAC)** - an artificial chromosome that contains telomeres, origin of replication, a yeast centromere, and a selectable marker for identification in yeast cells; cloning limit: 100-1000 kb

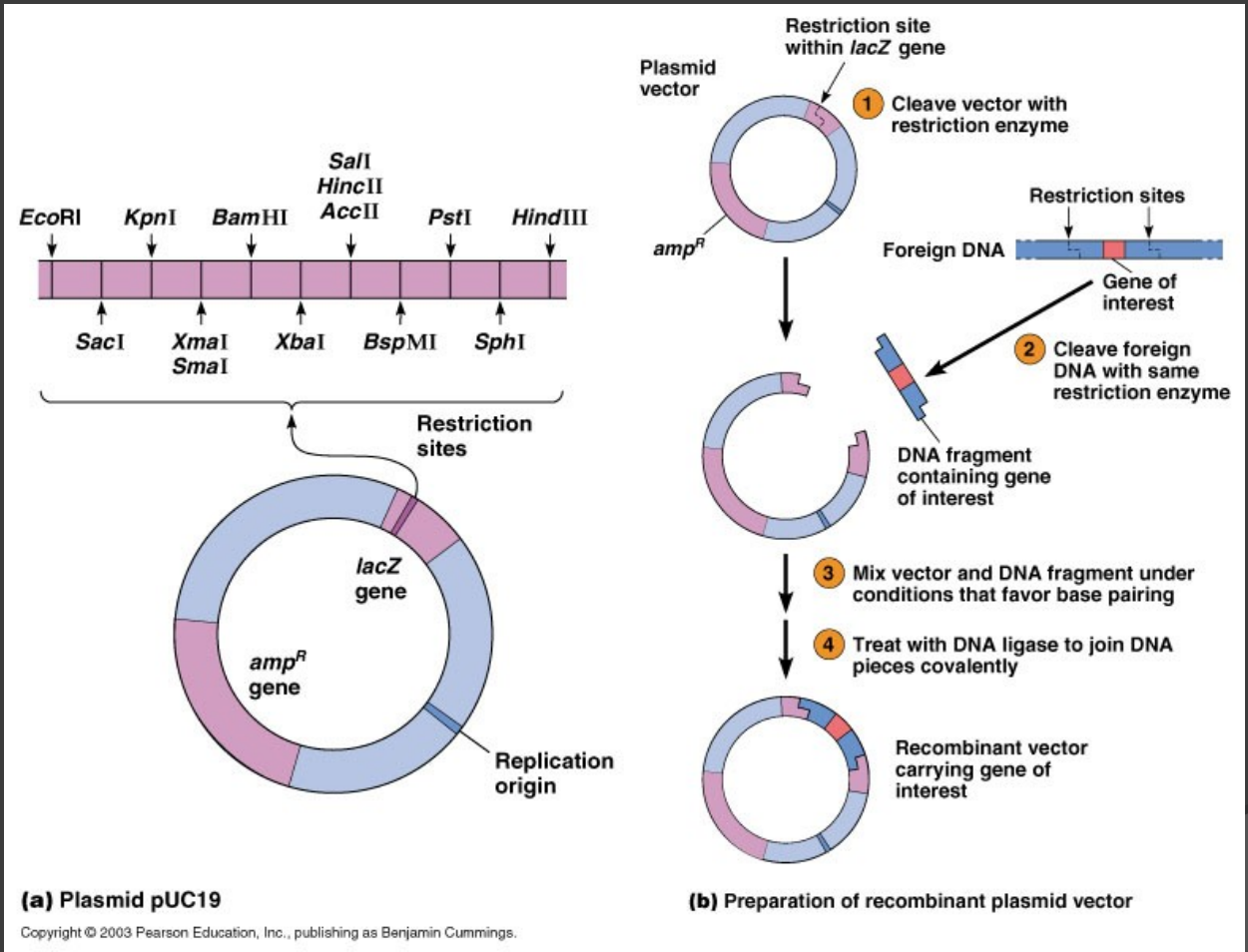
# General Steps of Cloning with Any Vector

- prepare the vector and DNA to be cloned by digestion with restriction enzymes to generate complementary ends (exception Topo cloning see later slides)
- ligate the foreign DNA into the vector with the enzyme DNA ligase
- introduce the DNA into bacterial cells (or yeast cells for YACs) by transformation
- select cells containing foreign DNA by screening for selectable markers (usually drug resistance)

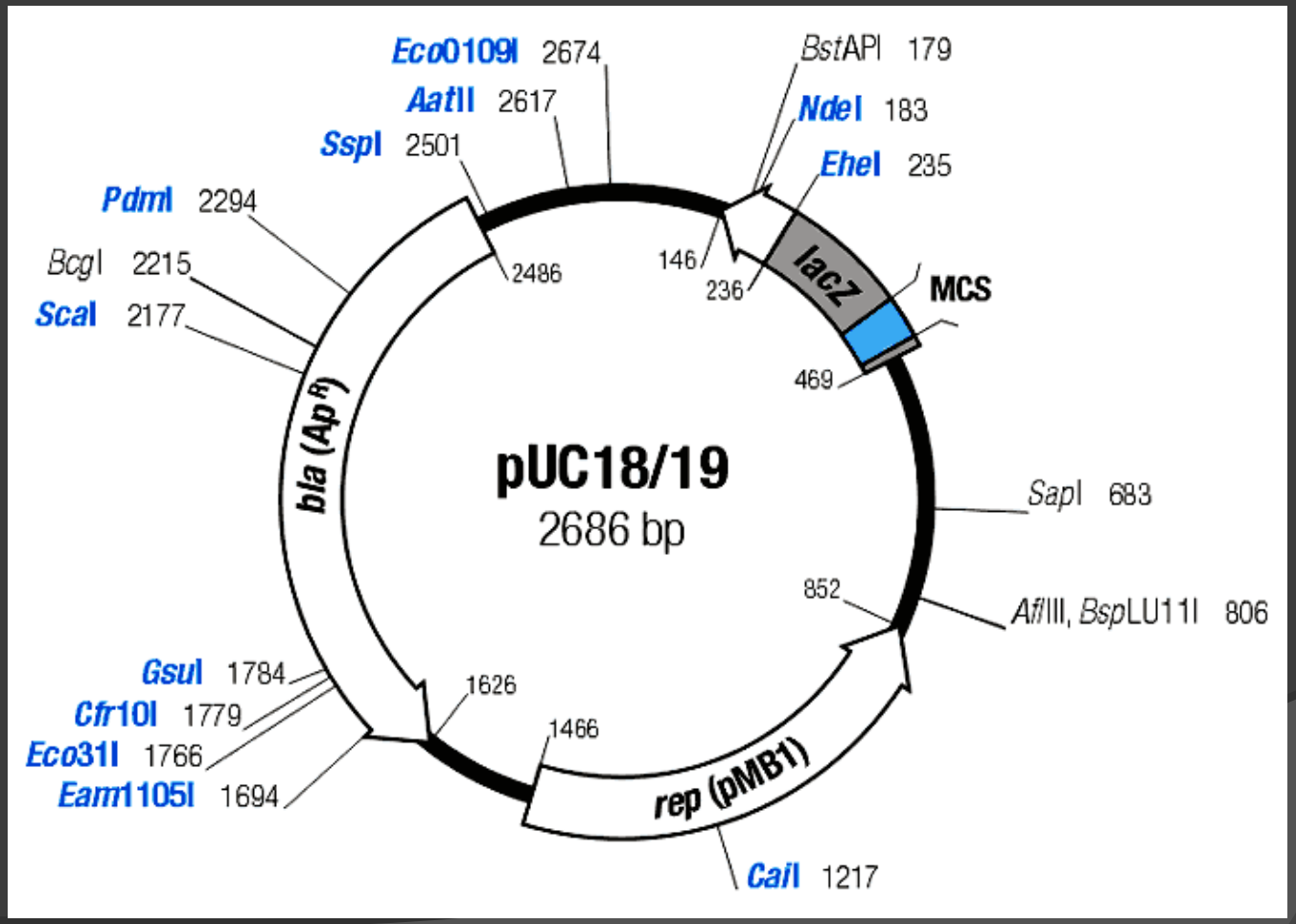
# Restriction & Ligation



# Insertion of Restriction Fragment into Vector



# The pUC 18 or 19 Cloning Vector



# Transformation and Selec