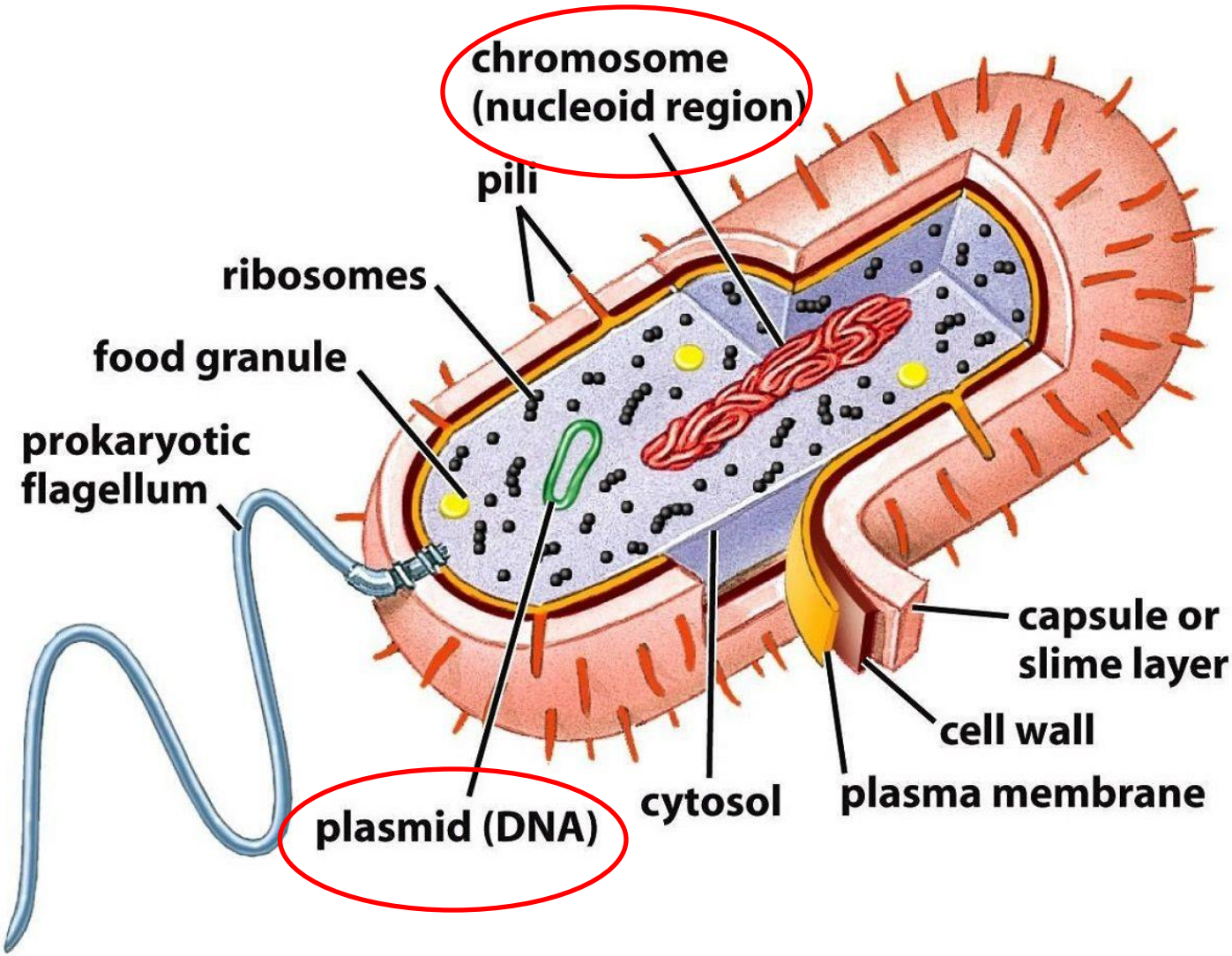
Shoreline
COMMUNITY COLLEGE

Recombinant Protein Production in Bacteria

Module 2, Lesson 2

Bacterial Cell

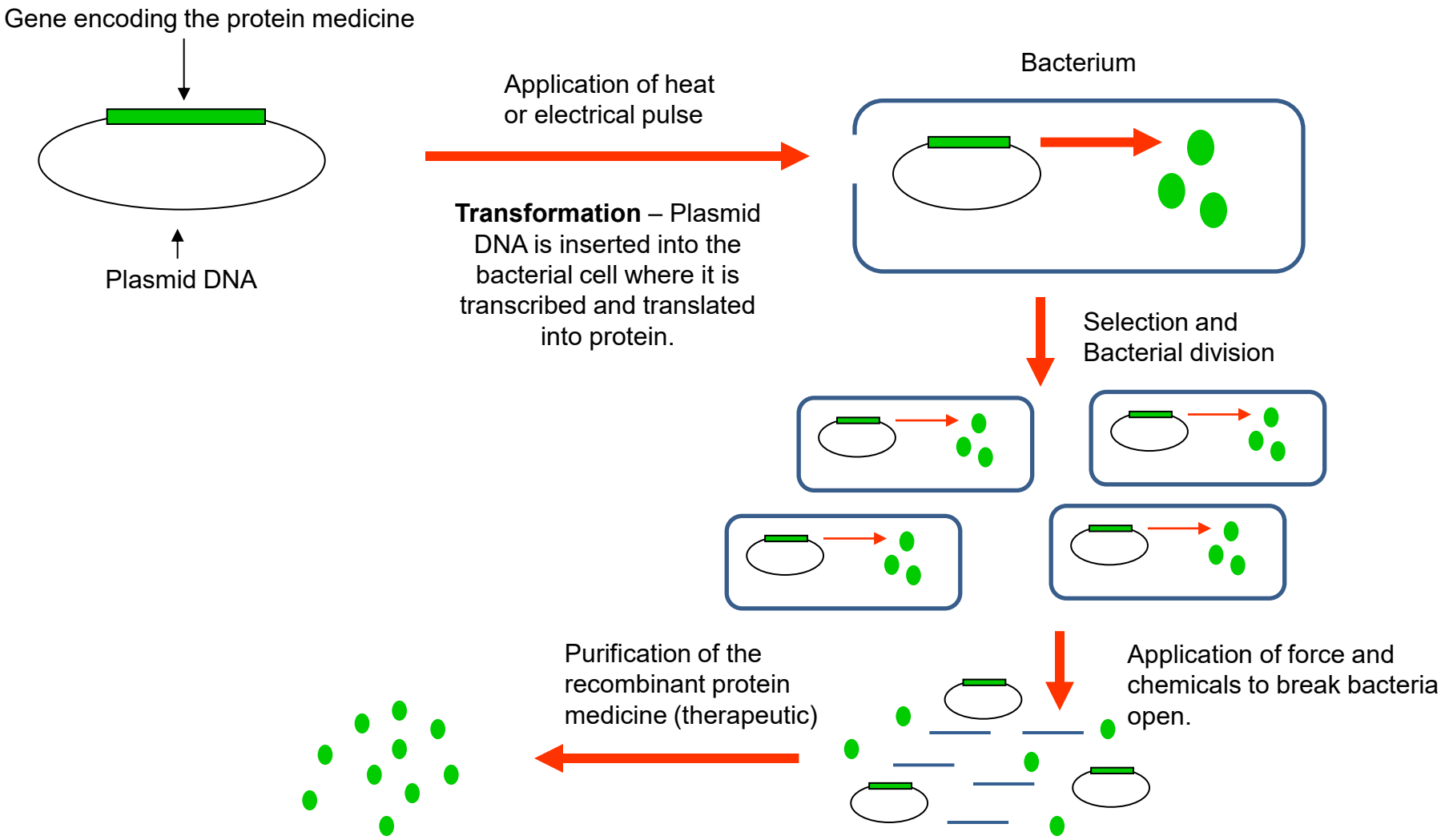


Pinterest.com

The Simplest Cell to Produce Recombinant Proteins in is a Bacterium (Prokaryote)

- They grow/divide quickly.
- They are reasonably inexpensive to grow.
- It is easy to scale up production.

In Order to Produce Large Amounts of Recombinant Therapeutics We Must Grow Many Cells



Bacterial Strain

You will be using the DH5α *E. coli* strain.

This strain of *E. coli* was developed for laboratory cloning use from the K12 *E. coli* strain by Douglas Hanahan.

The strain *E. coli* K-12 is a debilitated strain which does not normally colonize the human intestine. It has also been shown to survive poorly in the environment, has a history of safe commercial use, and is not known to have adverse effects on microorganisms or plants.

E. coli strain DH5α is considered to be non-pathogenic and unlikely to survive in host tissues and cause disease.

https://pubmed.ncbi.nlm.nih.gov/11123478/

Cell Growth

Need to facilitate cell growth

Optimal growth conditions – nutrients, pH, dO_2, temperature etc

Need to control cell growth

Prevent contamination

Need to be able to measure and predict cell growth

Cell Culture Requirements for Bacterial Growth

- A suitable vessel – flask, plate, bioreactor/fermenter etc
- A substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones
- Regulation of the physio-chemical environment (pH buffer, osmotic pressure, temperature)

Bacterial Culture Media Ingredients

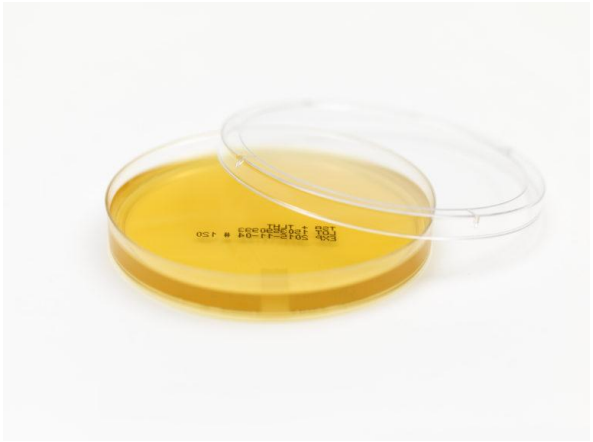
- Carbon source
- Nitrogen source
- Salts
- Buffers
- Growth factors
- Microelements
- Vitamins
- Amino Acids
- Selection factors (dyes, antibiotics)



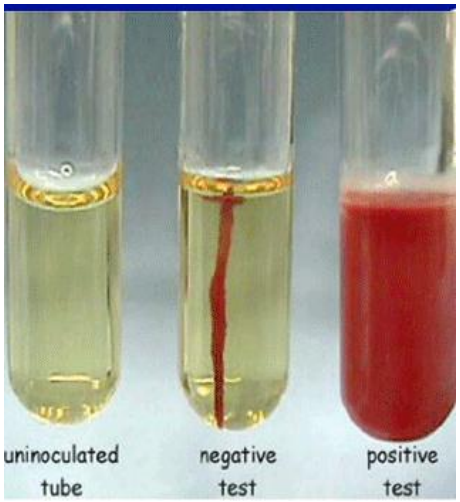
The media you will use is LB (Lysogeny Broth). It contains all the nutrients needed to grow the E. coli strains used in biomanufacturing

Types of Bacterial Media

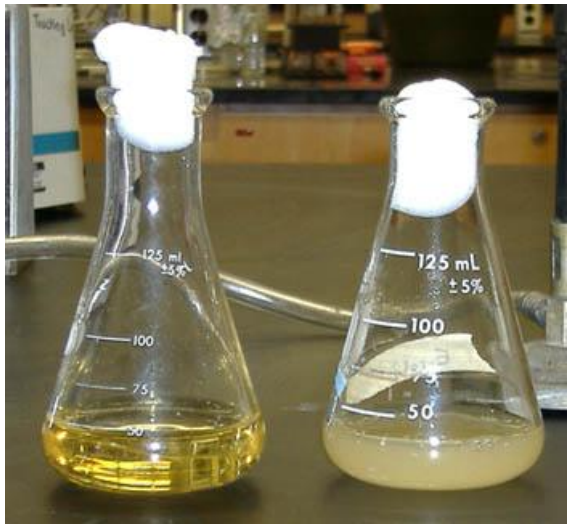
Solid
(*Agar added)



Semi-solid



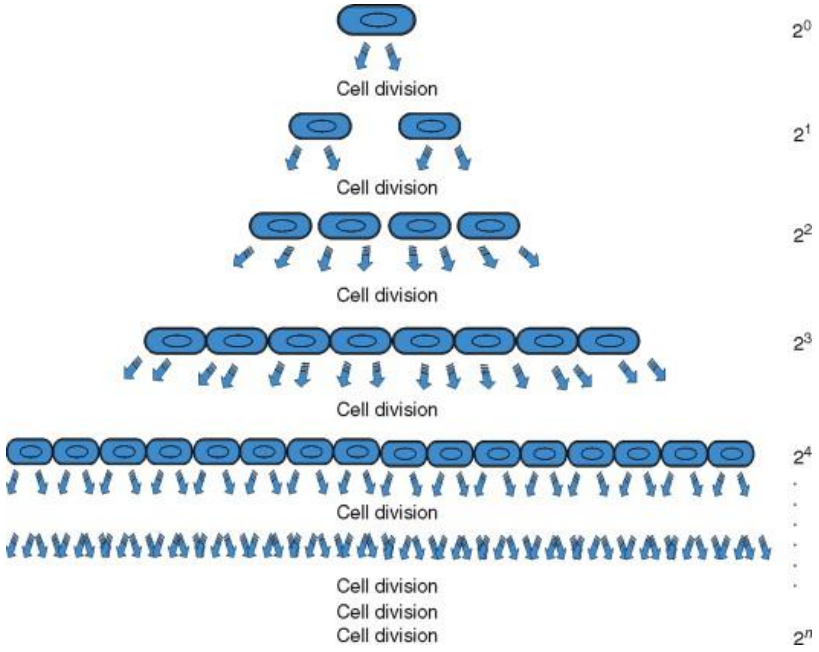
Liquid
(Broth)



* Agar – a solidifying agent isolated from red algae. It is a mixture of agarose and agaropectin, polysaccharides mainly composed of galactose. It is solid at 40^{o}C and melts at 85^{o}C. Most microorganisms are not able to degrade it.

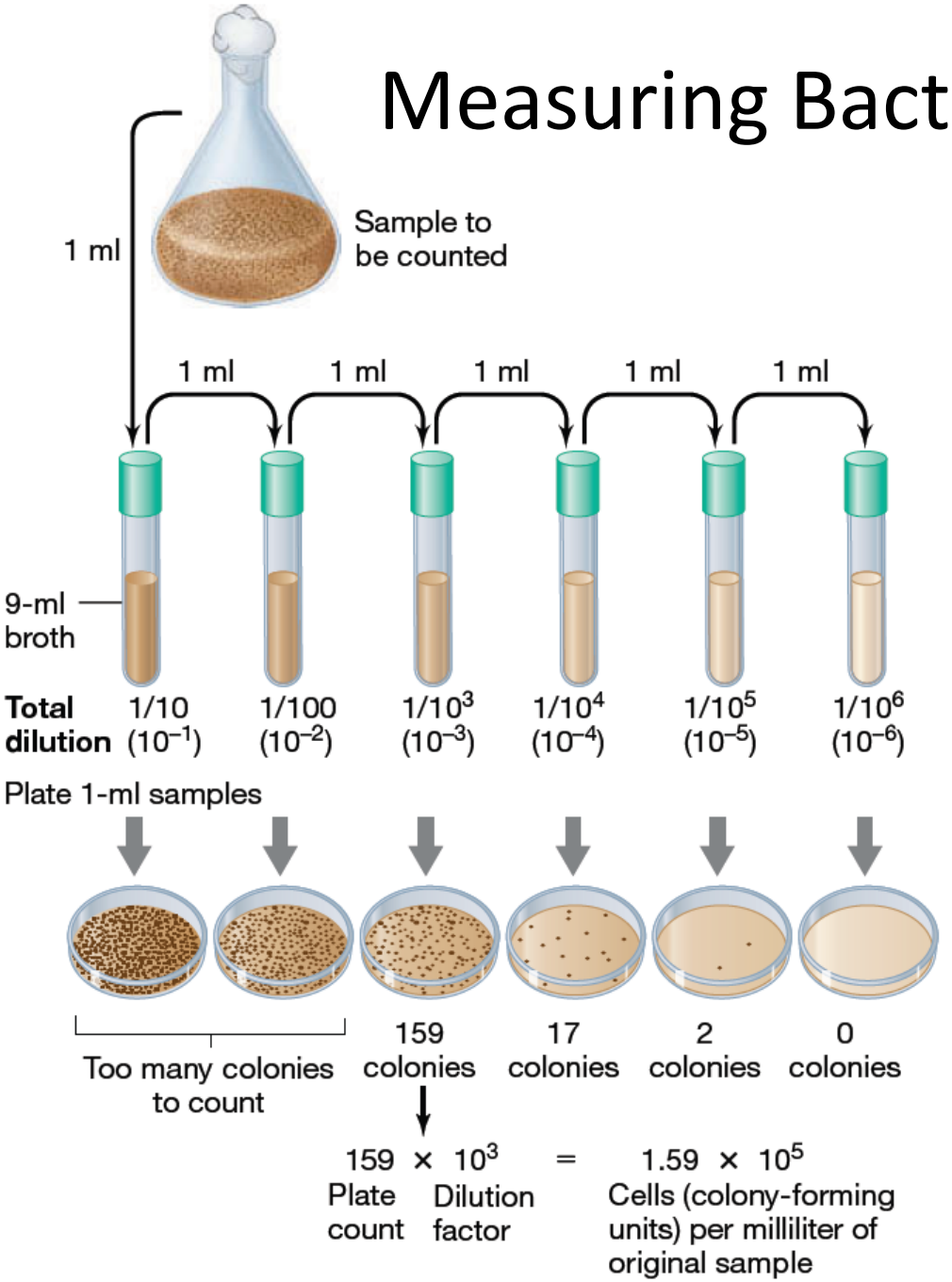
Bacterial Growth Curve Phase
Exponential (or log) Phase

The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation.



Sciencedirect.com

Measuring Bacterial Cell Growth



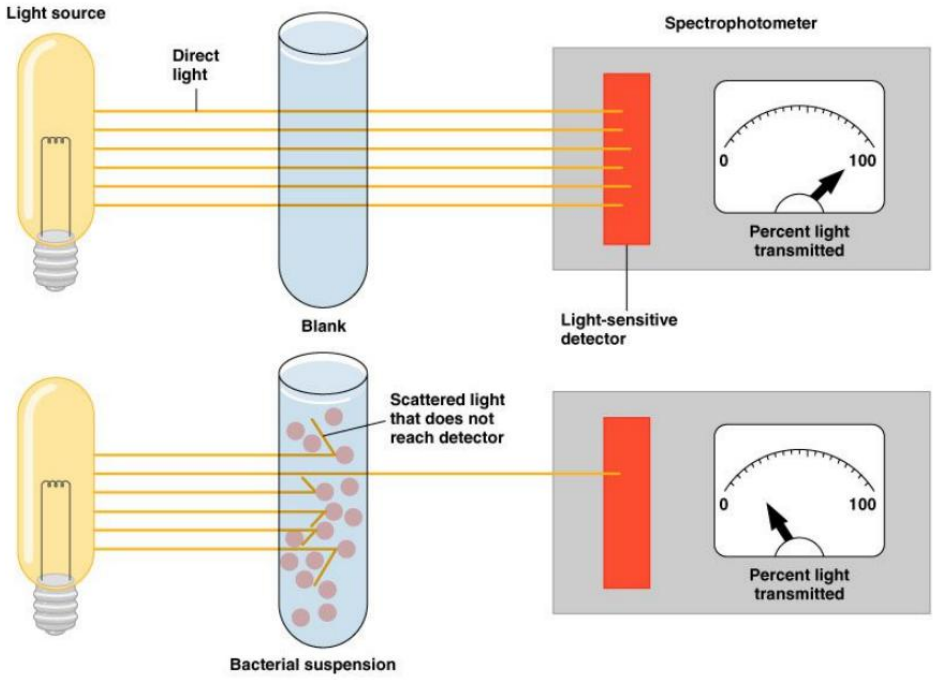
Viable or Colony Count

Counting individual bacterial colonies on agar plates. Each colony is generated by a single bacterium.

Measuring Bacterial Cell Growth

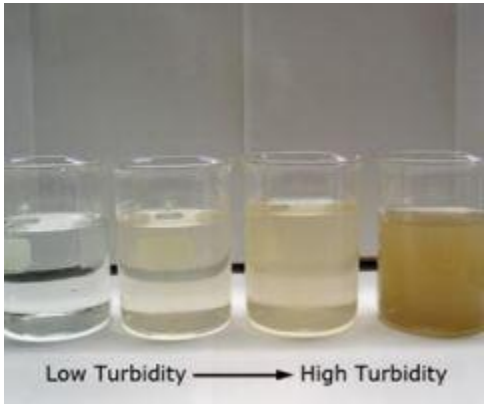
Turbidity measurement -

This can be done with a spectrophotometer. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or **optical density** of a suspension of cells is directly related to cell mass or cell number. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria.



Optical Density

Optical Density



Cram.com

Mathematical Prediction of Cell Growth

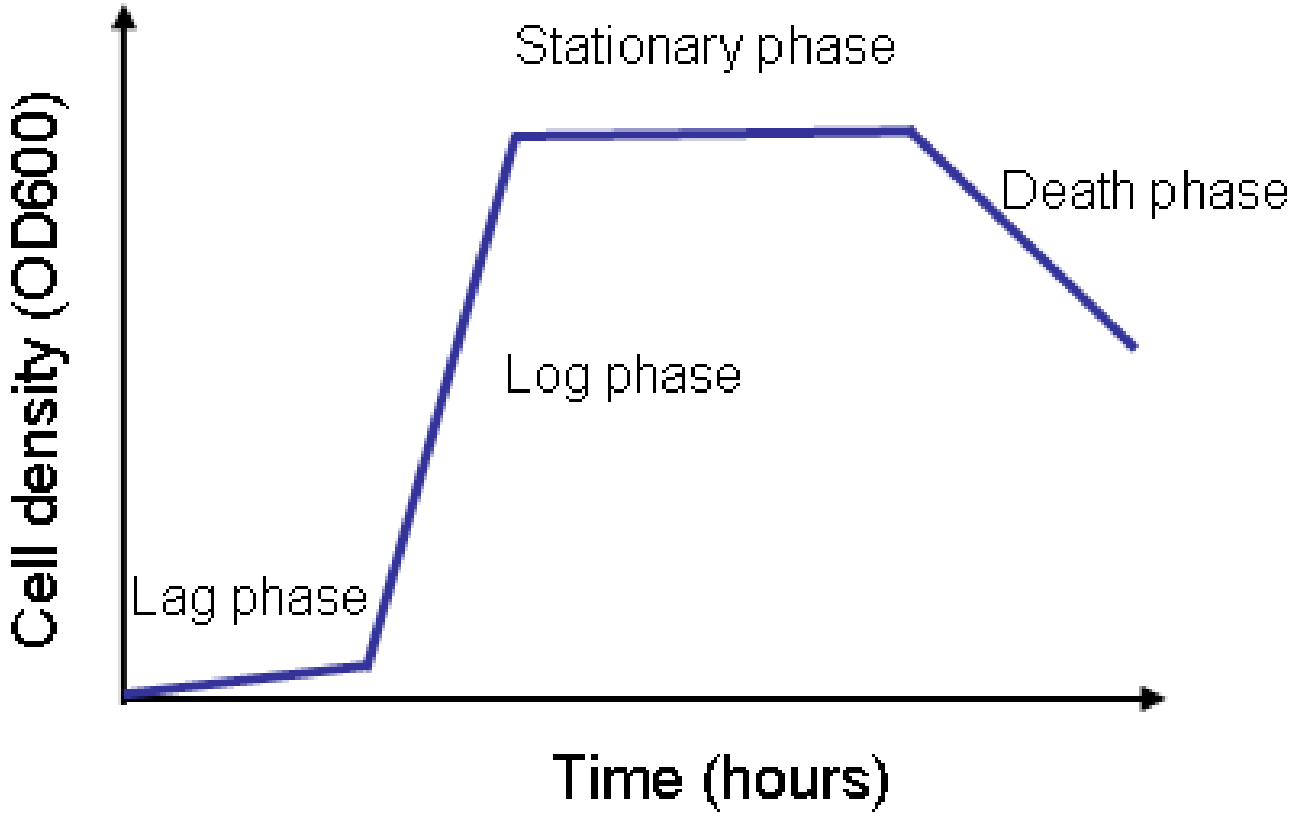
In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals.

Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.....2^n (where n = the number of generations). This is called **exponential growth**.

Bacteria do not remain in exponential growth indefinitely. If nutrients are limiting the growth rate slows.

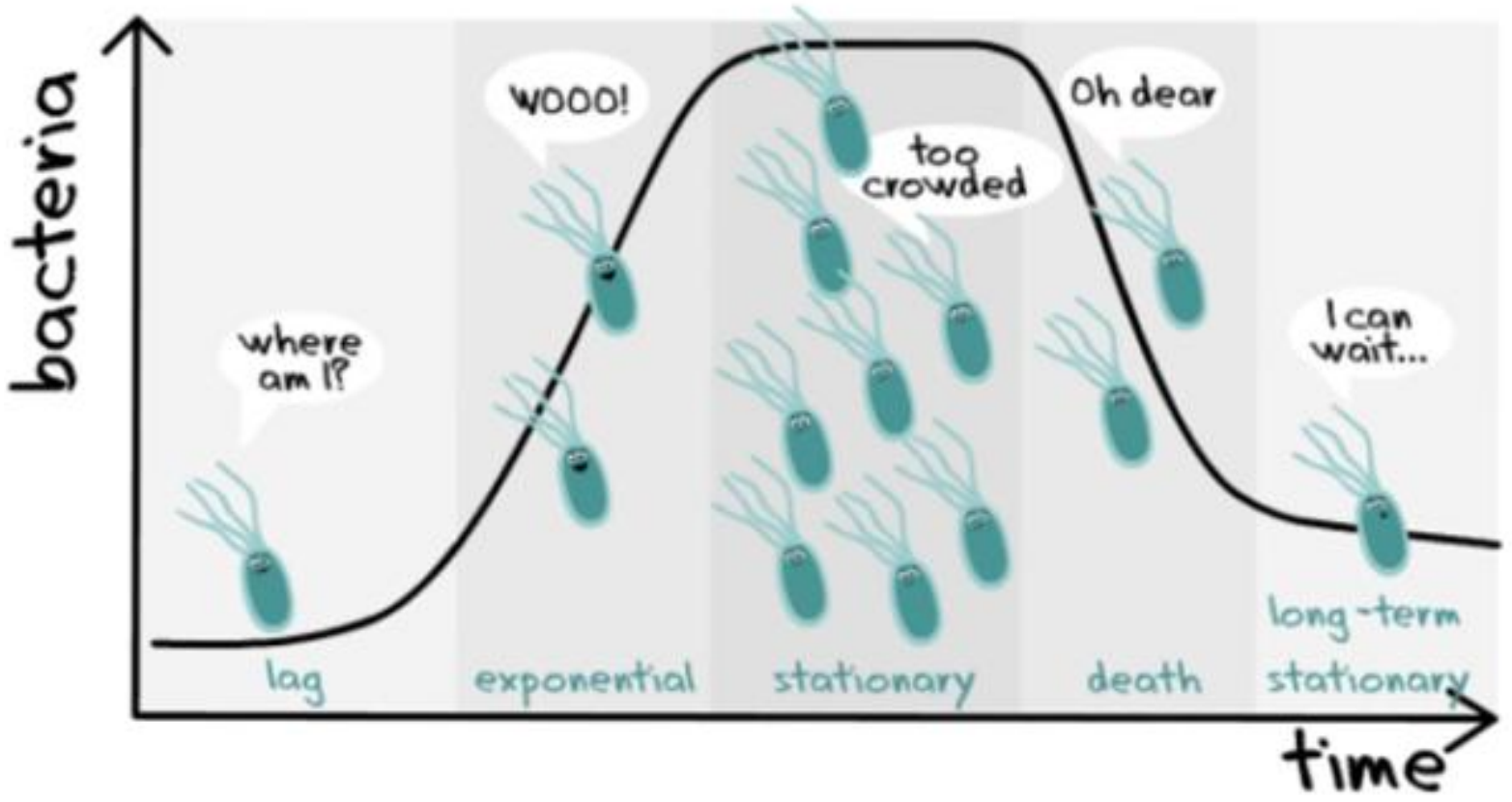
When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve**

Bacterial Growth Curve



https://rtd-test-project.readthedocs.io/

Bacterial Growth Curve



Bacterial Growth Curve
Lag Phase

The phase that occurs before newly inoculated cells begin to divide. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

Bacterial Growth Curve
Log Phase

Log phase, or exponential growth phase, is the time when the cells are dividing by binary fission (ie each cell divides into two new cells) and doubling in numbers after each generation time.

The log phase continues until nutrients are depleted.

Bacterial Growth Curve
Stationary Phase

The phase reached when exponential growth can no longer be maintained. Population growth is limited by one of three factors:

- exhaustion of available nutrients
- accumulation of inhibitory metabolites or end products
- exhaustion of space, in this case called a lack of "biological space“

Exponential growth cannot be continued forever in a batch culture (e.g. a closed system such as a test tube or flask).

Bacterial Growth Curve
Death Phase

If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. During the death phase, the number of viable (live) cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Mathematical Model

The rate of exponential growth of a bacterial culture is expressed as the generation time (G) or the doubling time (T_d) of the bacterial population.

Generation time (G) is defined as the time (t) per generation (n = number of generations).

G = t/n is the equation from which calculations of generation time (below) derive.

Mathematical Model

Calculation of Generation time or Doubling Time (G or T_d)

$$T_d = \frac{t}{n}\ (minutes, hs, days)$$

t = time
n = number of generations

So, if your bacteria double 4 times in 2 hr:

Generation/Doubling time =
2hr = 120 min
120 min/4 generations = 30 minutes

Mathematical Model

The bacterial Division/Generation time = 30 minutes

If you start with a single bacterium, how many bacterial will you have after 6 hours?

$$N(t) = N_0 \; x \; 2^n$$

x = multiplication sign

N = Number of bacteria

N(t) = Number of bacteria at time t

N_0 = Number of bacteria at time 0 – ie at the start

2 – refers to the doubling rate

n = the number of generations

Vocabulary

LB - Lysogeny broth is a nutritionally rich medium primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended LB to stand for lysogeny broth, but LB has also come to colloquially mean Luria broth, Lennox broth, or Luria–Bertani medium.

Cell Culture Vessel – A container cells can be grown in: flasks, plate, bioreactor

Agar - a solidifying agent isolated from red algae

Prokaryote - a microscopic single-celled organism that has neither a distinct nucleus with a membrane nor other specialized organelles

Viable – Living, alive

Vocabulary

Inoculation - The act of introducing a microorganism or a suspension of microorganisms (e.g. bacteria) into a culture medium

Exponential Growth - growth whose rate becomes ever more rapid in proportion to the growing total number or size

Lag Phase - The phase of growth that occurs before newly inoculated cells begin to divide

Exponential or Log Phase - The phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression.

Stationary Phase - The phase of growth reached when exponential growth can no longer be maintained.

Death Phase – The phase in which in which the viable cell population declines

Generation or Doubling time – The time it takes a population of cells to double in number.