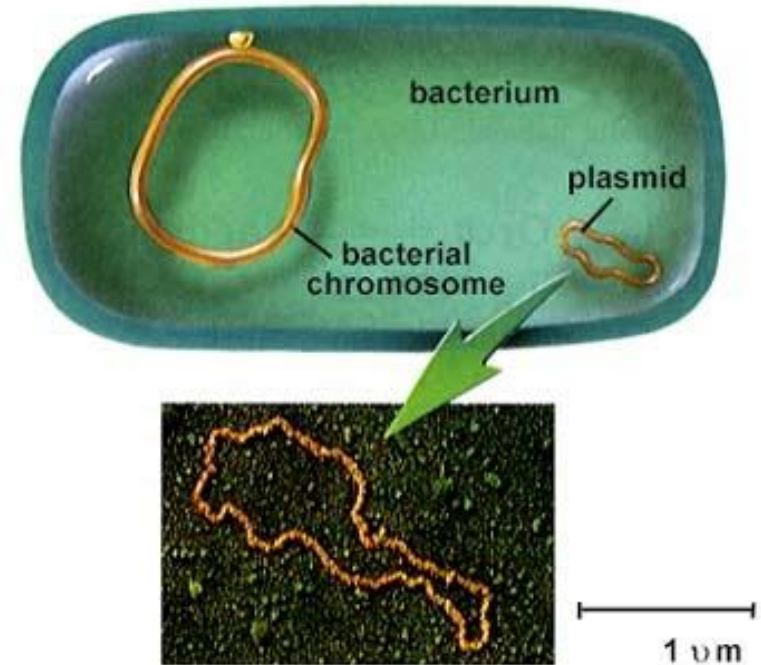
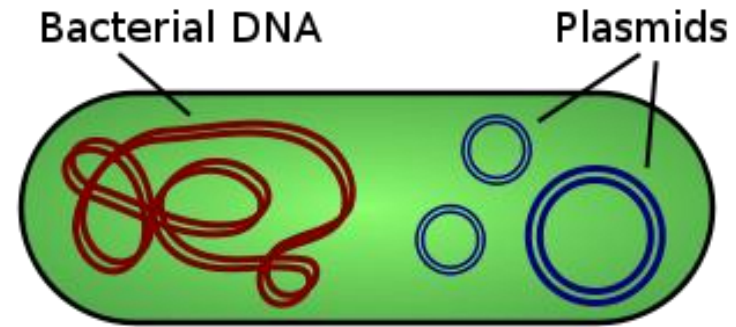


Plasmid Isolation and Types of Bacterial media

By: Amal Alamri

What is the Plasmid?

- Small double stranded, close circular DNA.
- Can be isolated from bacterial cells
- It replicate independently of the bacterial chromosom.
- Plasmid are not life formes.
- Like viruses viroids, they depend on host proteins for maintainance and replicate functions
- Rang size from 1- 200 kb



Plasmids are:

1- Found in **wide variety** of bacterial species.

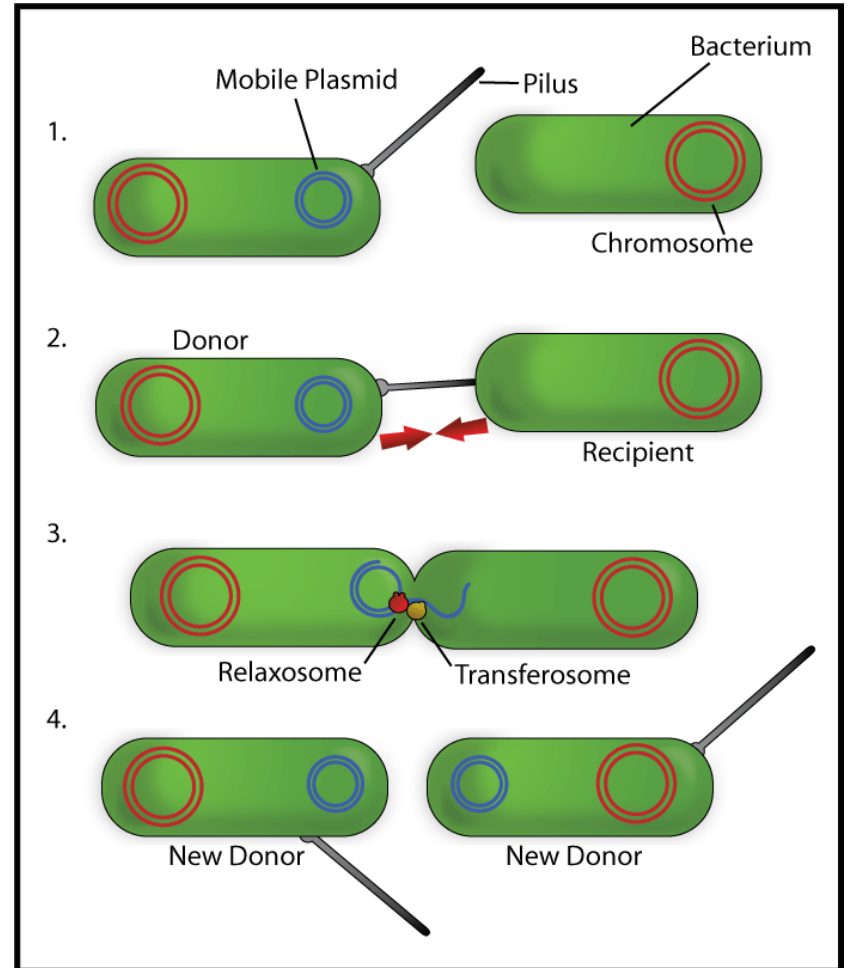
2- It is **extra chromosomal** element that behave as accessory genetic units which replicate and inherited independently of the bacterial chromosome.

3- Dependent, to a greater or lesser extent, on the enzymes and proteins encoded by their host for their replication and transcription.

4- Frequently **contain genes coding for enzyme that are advantageous to the bacterial host**. These genes specify a remarkably diverse set of traits, many of which have a great medical significance.

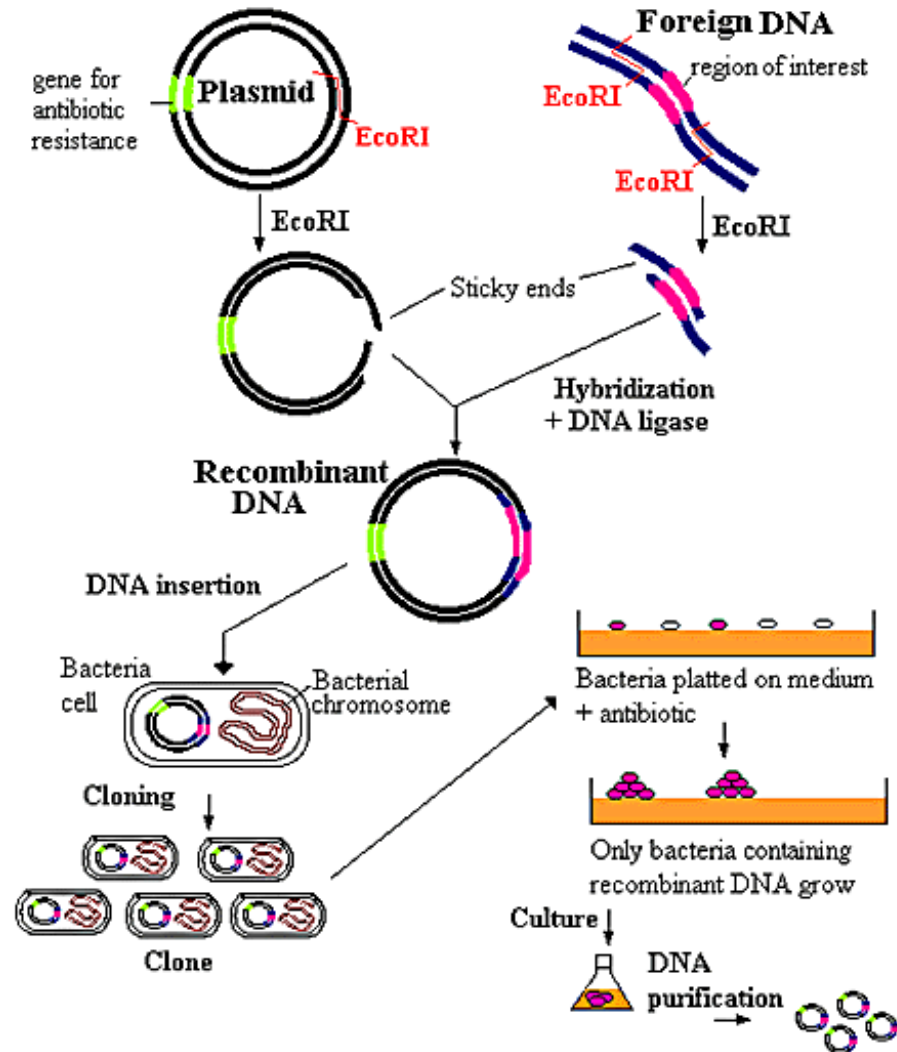
Classes of plasmids which can be advantageous for the host

- 1-Virulence plasmid encoding **toxin gene**.
- 2-**Drug resistance** plasmids that confer resistance to antibiotics.
- 3-Plasmids encode gene required for **bacterial conjugation**.



Application on Plasmids

- Gene therapy
- Polymerase Chain reaction
- Molecular cloning



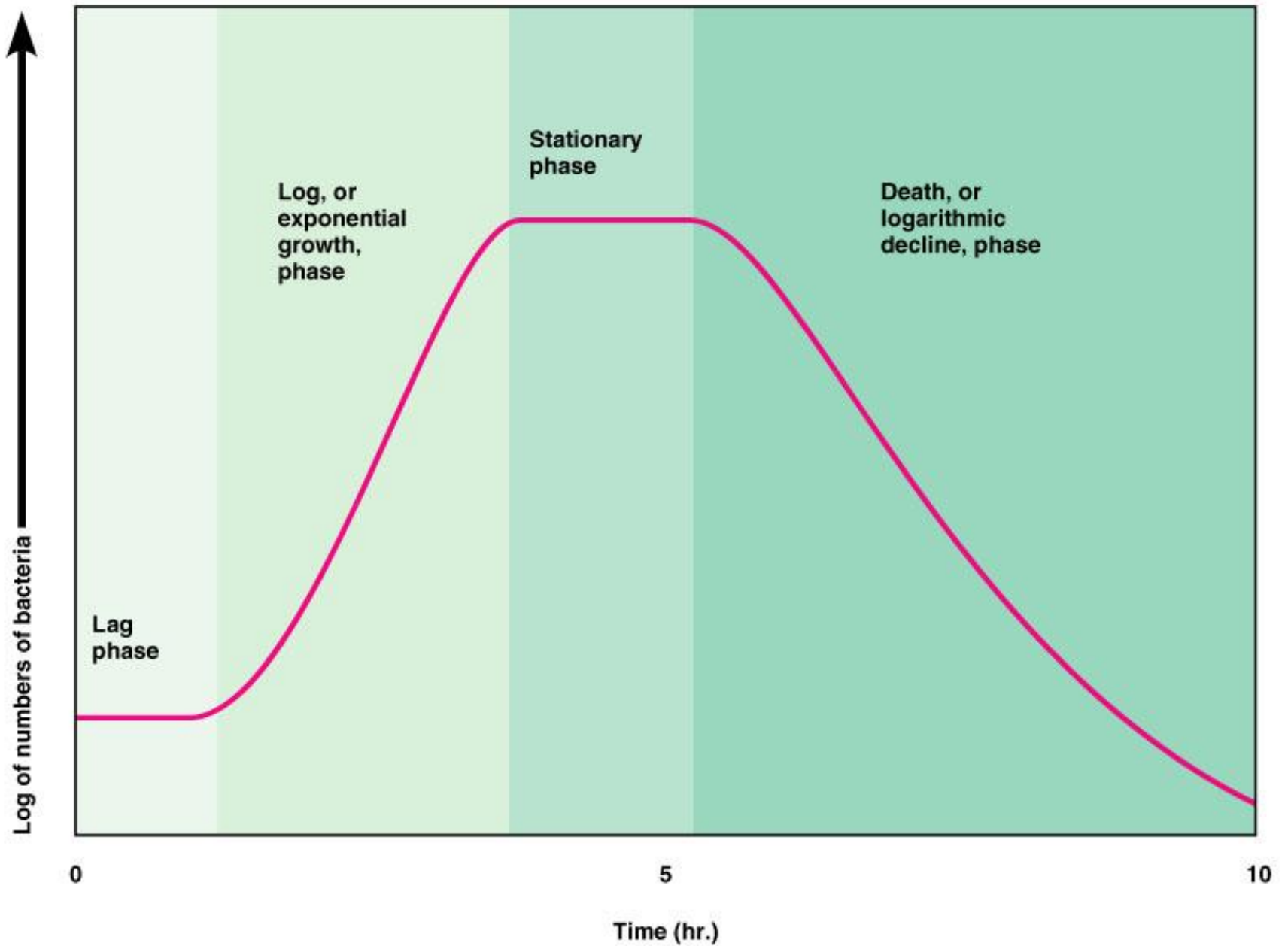
Cloning into a plasmid

Steps to purifying plasmids:

- 1- Growth of bacterial culture
- 2- Harvesting and lysis of the bacteria.
- 3- Purification of plasmid DNA

1- Growth of bacterial culture

- Plasmid should be purified from bacterial culture that have been inoculated with a single transformed colony picked from an agar plate.
- Usually they colony is transformed to a small starter culture, which is grown to late log phase. Aliquots of this culture can be used to prepare small amount of plasmid DNA for analysis.
- The transformed bacteria should be grown in selective conditions, i.e. in the presence of the appropriate antibiotic

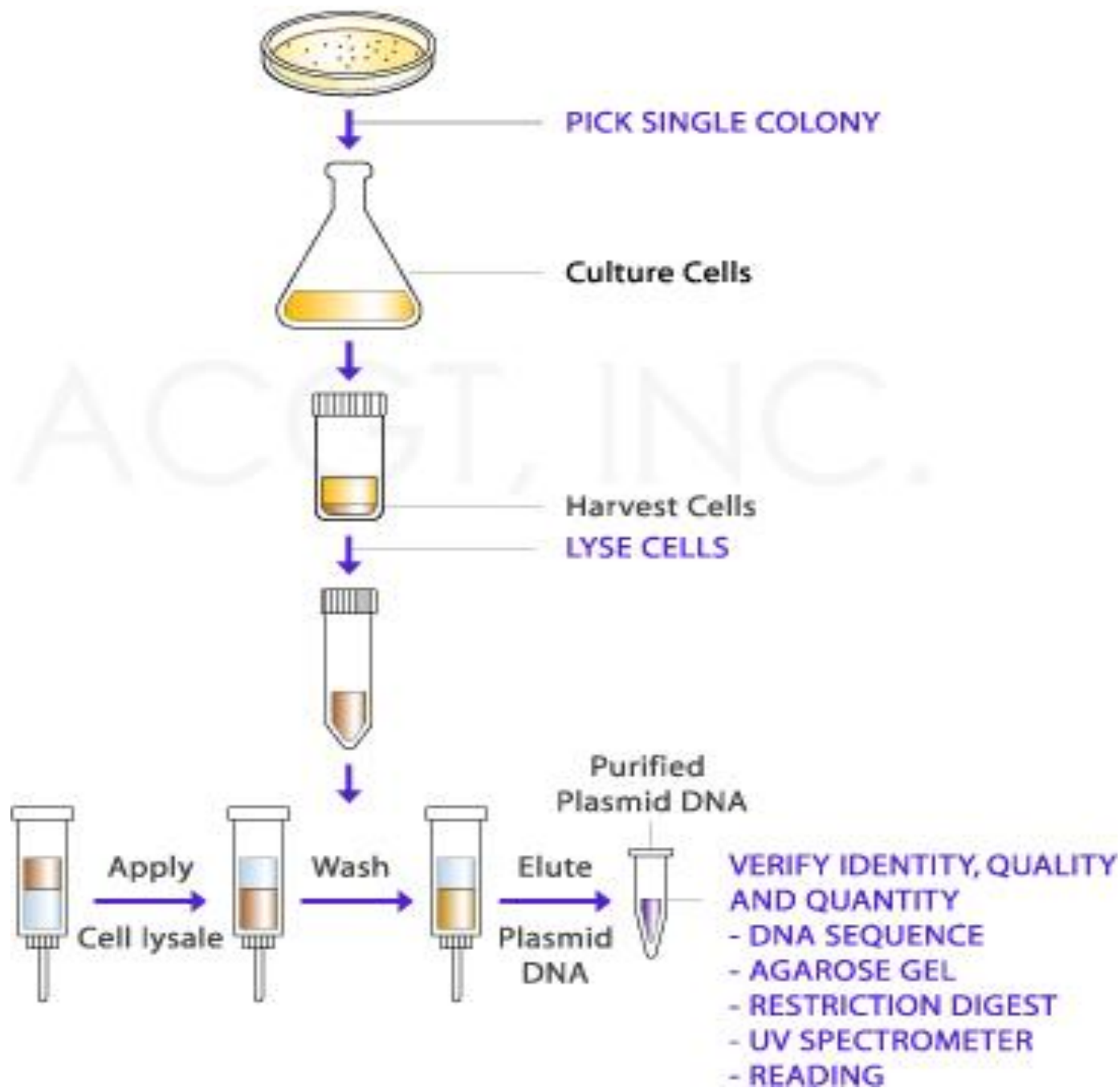


2- Harvesting and lysis of the bacteria.

Bacteria are recovered by centrifugation and lysed by any one of large number of methods, including treatment with detergent, alkali, organic solvent, and heat. The choice among these methods depends on three factors:

- The **size** of plasmid
- The **strain** of E.coli
- The **technique** used to subsequently purify the plasmid DNA

3- Purification of plasmid DNA



What is the growth medium?

A **growth medium** or **culture medium** is a **liquid** or **gel** designed to support the growth of microorganisms or cells. There are different types of media for growing different types of cells.

There are **two major** types of growth media:

1- cell culture media:

which use specific cell types derived from plants or animals

2-microbiological culture:

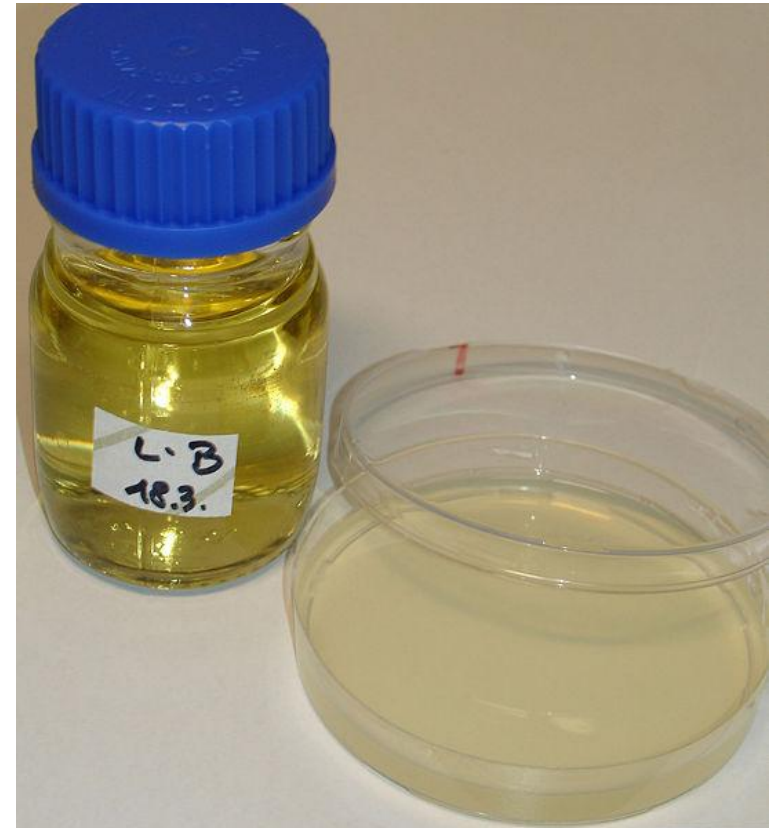
which are used for growing microorganisms, such as **bacteria** or yeast. The most common growth media for microorganisms are either **liquid as LB** or **solid as agar plates**

Types of Bacterial Media

The most common growth media for microorganisms are :

1-nutrient broths (liquid nutrient medium) or LB medium (Luria-Broth).

-often mixed with **agar** and poured into Petri dishes to solidify. These agar plates provide a **solid medium** on which microbes may be cultured. They remain solid, as very few bacteria are able to decompose agar. **Bacteria grown in liquid cultures often form colloidal suspensions.**



LB medium bottle and LB agar plate

Luria broth (LB),

- a nutritionally rich medium ,is primarily used for the growth of bacteria.
- These media have been widely used in molecular microbiology applications **for the preparation of plasmid DNA** and recombinant proteins.
- There are several common formulations of LB. Although they are different, they generally share a somewhat similar composition of ingredients used to promote growth, including the following:
 - 1- **tryptone** which provide Peptides and peptones.
 - 2- **yeast extract** which provide Vitamins and certain trace elements
 - 3- **NaCl** which are provided by Na^+ for transport and osmotic balance