DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF ENGINEERING

& TECHNOLOGY

LT.11. Cell cloning & Micromanipulation

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Cell cloning & Molecular cloning

Cellular cloning

•**Cloning**, the process of generating a genetically identical copy of a **cell** or an organisms.

•Unicellular organisms, such as bacteria and yeast, naturally produce clones of themselves when they replicate asexually by binary fission; this is known as cellular cloning.

•The nuclear DNA duplicates by the process of mitosis, which creates an exact replica of the genetic material.

•Clones are organisms that are exact genetic copies. Every single bit of their DNA is identical.

Molecular cloning

Molecular cloning is a set of techniques used to insert recombinant DNA from a prokaryotic or eukaryotic source into a replicating vehicle such as plasmids or viral vectors.

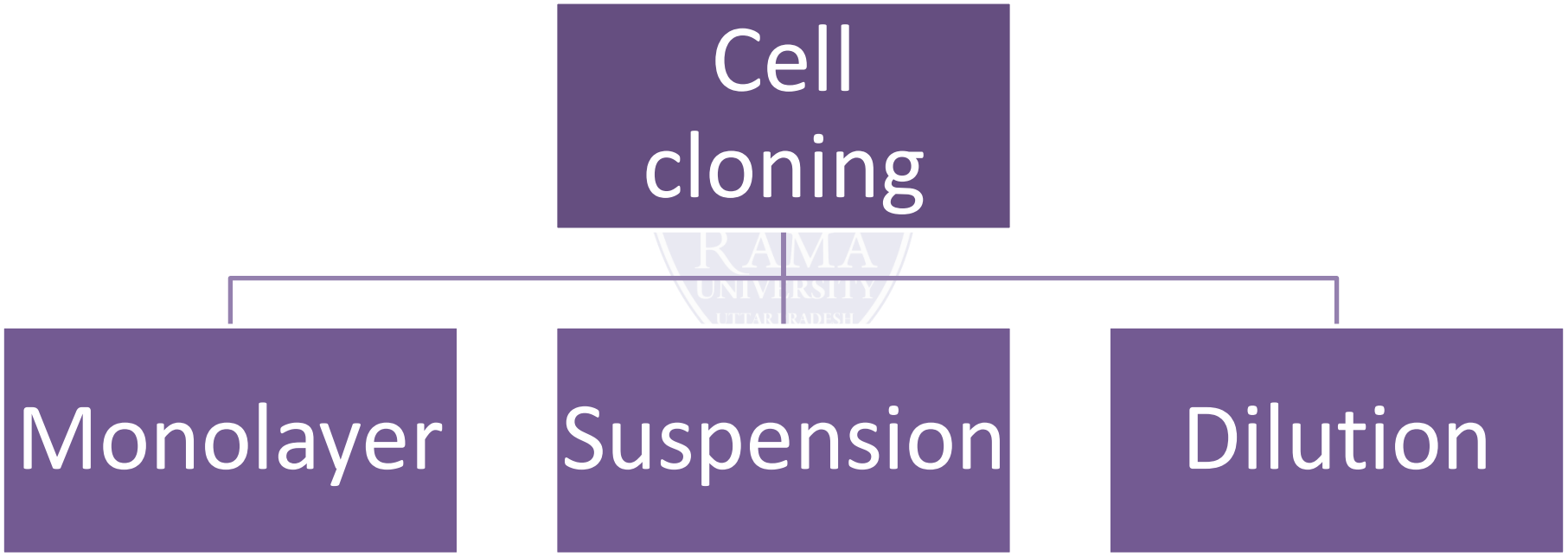
Cloning refers to making numerous copies of a DNA fragment of interest, such as a **gene**.

Cell cloning vs Molecular cloning

Cellular cloning refers to production of clones from single cells which are genetically identical whereas molecular cloning reproduces the desired regions or fragments of a genome, enabling the manipulation and study of genes. These clones are not genetically identical.

Approaches used for cellular cloning

Cellular cloning can be performed by following methods

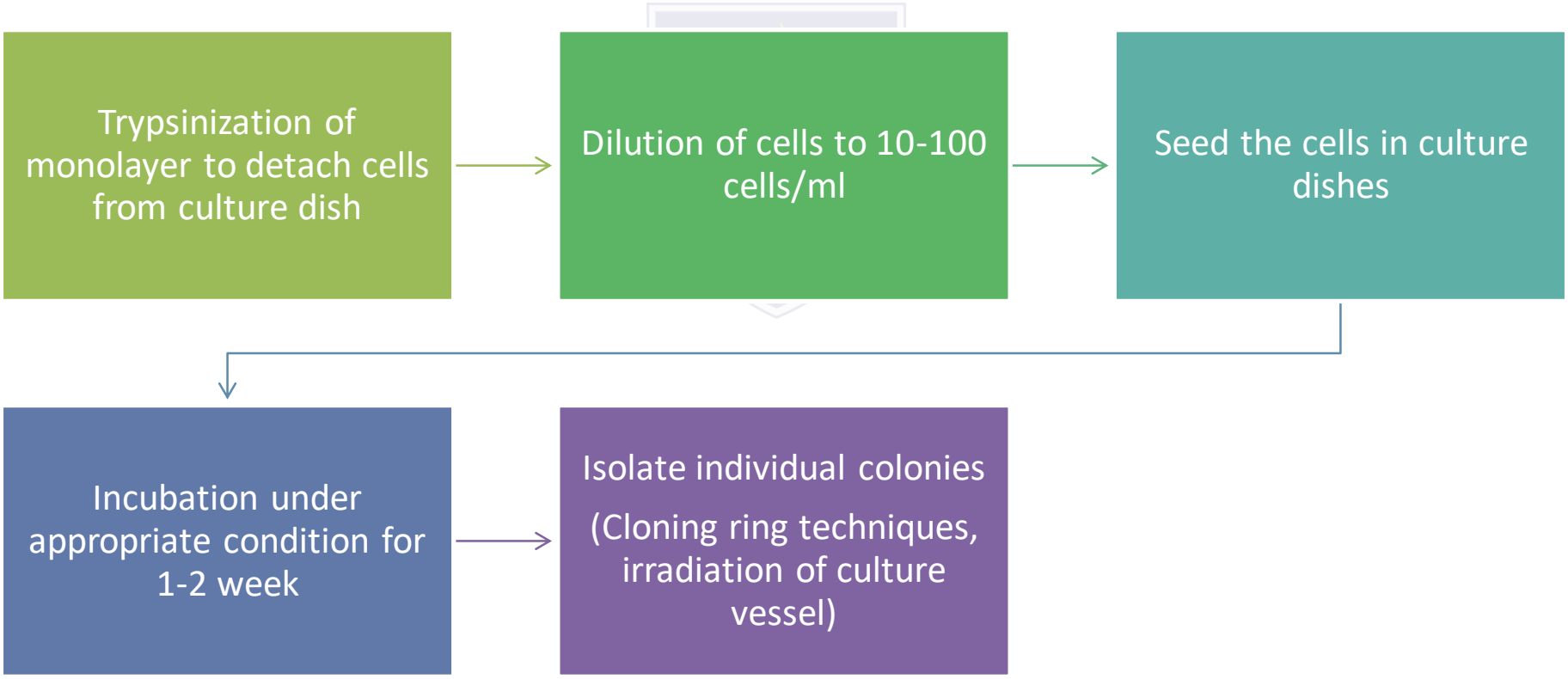


Cell cloning: Monolayer method

Monolayer culture:

Petri dishes, multi-well plates or flasks can be used for cloning by monolayer culture. It is relatively easy to remove the individual colonies of cells from the surfaces where they are attached.

Steps in cloning



Cell cloning: Suspension culture method

•Cloning can also be carried out in suspension by seeding cells into a gel, such as agar or agarose, or a viscous solution, such as Methocel, with an agar or agarose underlay. The stability of the gel, or viscosity of the Methocel, ensures that daughter cells do not break away from the colony as it forms. E.g. transformed Hematopoietic stem cells and fibroblast cells are usually cloned in suspensions.

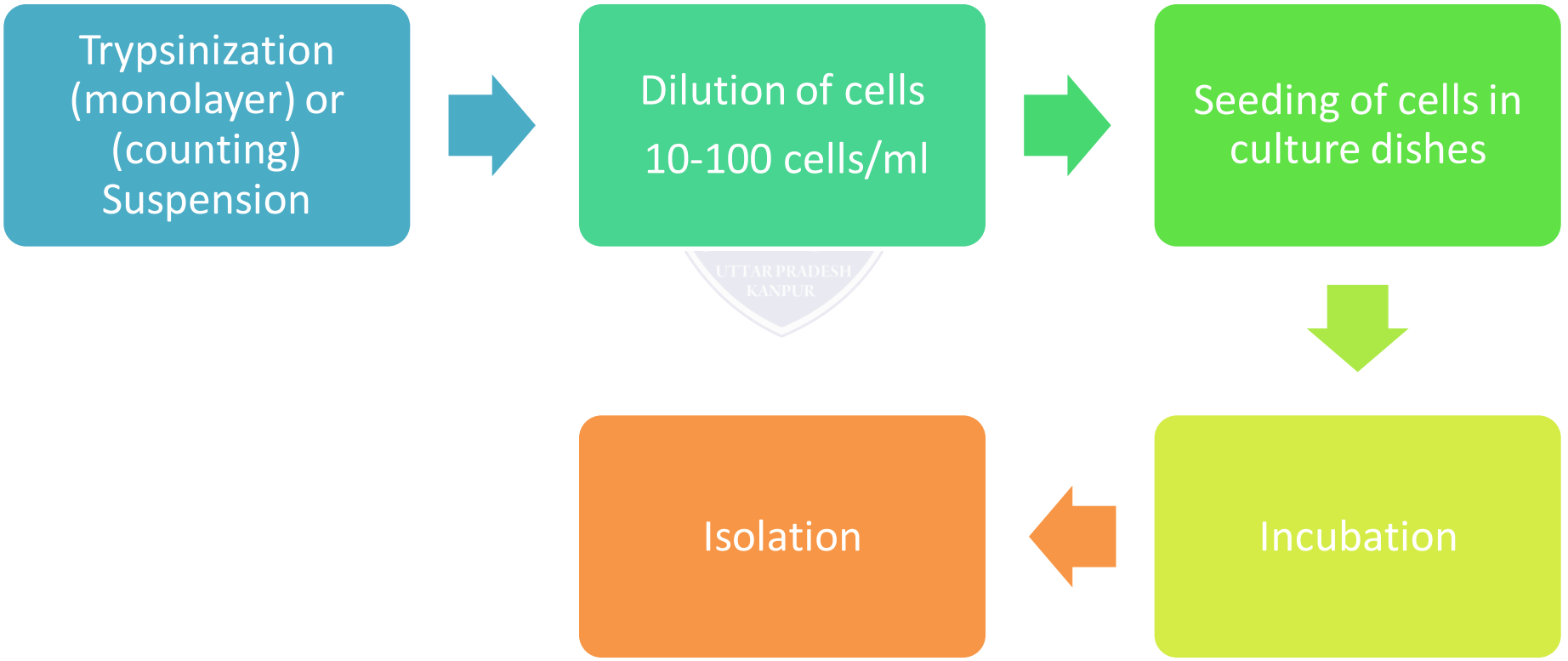
➢Steps are similar to monolayer culture; Except gelling medium is used in suspension

•Cells from suspension culture or monolayers can be used. The monolayer cells have to trypsinized while the suspension cells can be directly used.

•Cells are seeded on to agar medium and incubated for 1-3 weeks.

Cell cloning: Dilution cloning

•Dilution cloning is the technique which is based on the observation that cells diluted below a certain density form discrete colonies. Dilution cloning is the most commonly used technique for cloning of monolayer cells



Isolation of clones & Plating Efficiency

•If the monolayer cells are cloned directly in the multi-well plates, the colonies can be isolated by trypsinization of the individual wells.

•when the cloning is carried out in petri dishes, the colonies can be separated from the medium by placing stainless steel or ceramic rings around the colonies.

Plating efficiency: Plating efficiency represents the percentage of cells seeded at subculture that gives rise to colonies. The plating efficiency and cloning efficiency are said to be identical, if each colony is derived from a single cell. The plating efficiency is around 10% for continuous cell lines, while for primary cultures and finite cell lines, it is quite low — 0.5 to 5% or sometimes even zero. A high plating efficiency is desirable for seeded cells in cell culture.

Micromanipulation

•Micromanipulation refers to the microscopic-level manipulation of cellular organelles using specific tools that can move an object with high precision. Can insert material into living cells

•Micromanipulation techniques evolved to manipulate a specimen delicately or precisely.

•Different micromanipulators include holding pipettes, injectors, and cutting tools.

•Micromanipulation is applied in diverse fields such as electrophysiology, *in vitro* fertilization (IVF), transgenics, and adherent cell research.

•Specifically, optical tweezers are used to monitor the movement and properties of cells, metal particles, and colloids.

Micromanipulation pertinent for *in vitro* fertilization (IVF)

•One of the widely applied fields of micromanipulation is during IVF. Various micromanipulation methods - such as zona dissection, sub-zonal insemination, and direct injection of a spermatozoon into the cytoplasm (ICSI) - are extensively used in assisted reproduction.

•ICSI involves micromanipulating male and female gametes. Another method is opening the zona pellucida using lasers during different stages of pre-implantation to help in hatching, or to remove cells for genetic diagnosis. Blastocysts are also sometimes micromanipulated to shrink the blastocoel, which in turn facilitates its survival.

Test your understanding

1. The clones generated by molecular cloning has identical genetic make up. (True/False)

2. Plating efficiency represents the percentage of cells seeded at subculture that gives rise to colonies. (True/ False)

3. Cloning ring method is used to

   a. Clone cells
   b. Divide cells
   c. Multiply cells
   d. Isolate cells

4. Adherent cells are best cloned as

   a. Monolayer culture
   b. Suspension culture
   c. Either (a) or (b)
   d. None of the above

5. Dilution cloning is the most commonly used technique for cloning of monolayer cells. (True/ False)

6. Transformed cells is cloned as .....

References & Further reading

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Further reading

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