

# **Cellular Totipotency**

## **Lecture I: Isolation of cell**

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# What is Totipotency

**Cellular Totipotency:** Potential of a cell with intact and functional to regenerate into a complete organism by dedifferentiation and redifferentiation

**Dedifferentiation:** Differentiated to undifferentiated

**Redifferentiation :** Meristematic to differentiated

# The Process

1

- **Isolation of Cell**

2

- **Culture of Cell**

3

- **Regeneration into plantlet**  
**Either Organogenesis or Somatic embryogenesis**

# **Lecture I**

## **Cellular totipotency: Isolation of cell**

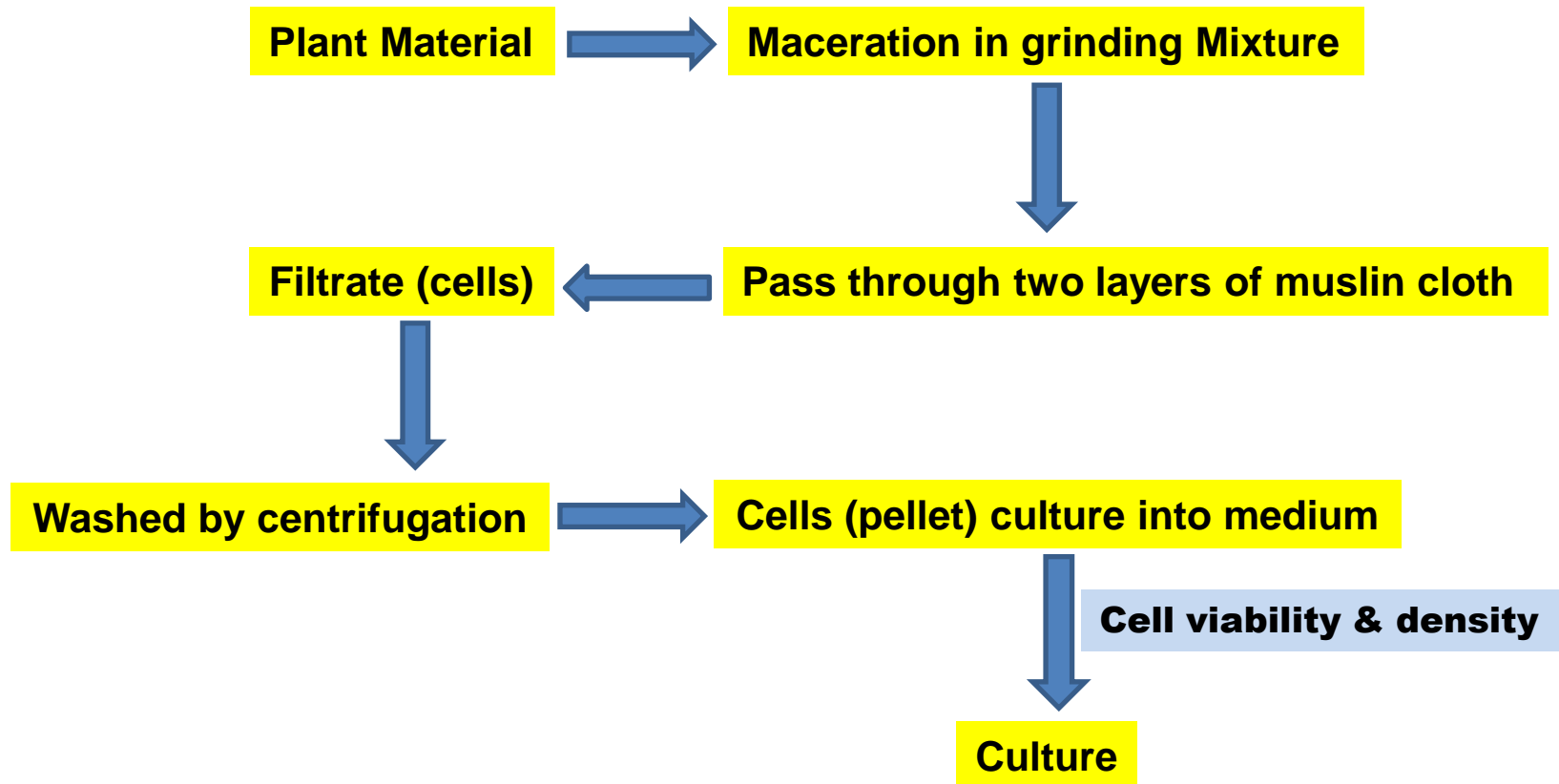
# Isolation of Single Cell

## Two methods

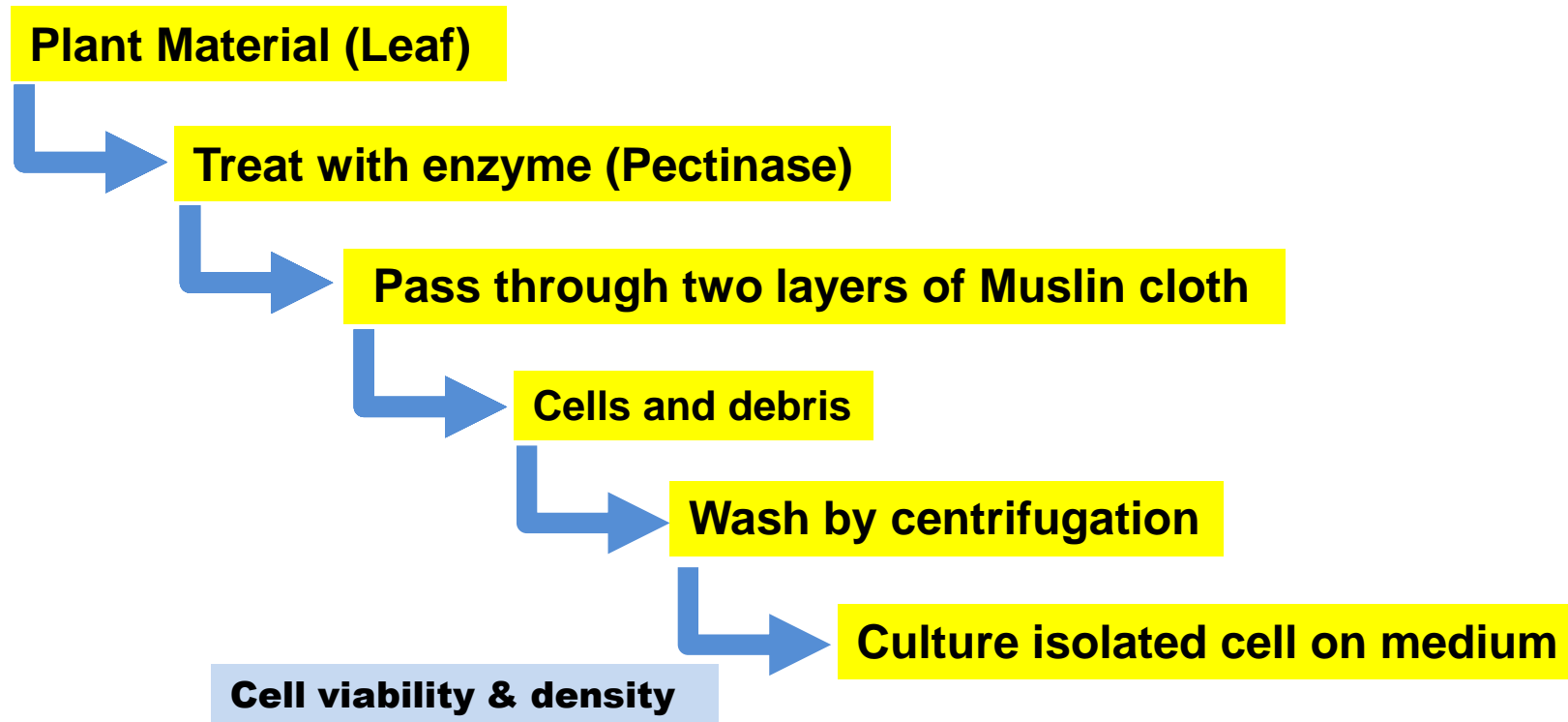
- **Mechanical method**

- **Enzymatic method**

## Mechanical Method



## Enzymatic Method



## Assessment of Viability

### ➤ **Phase contrast microscopy**

Cytoplasmic streaming and the presence of healthy nucleus

### ➤ **Reduction of tetrazolium salt**

Reduction of 2,3,5 triphenyl tetrazolium chloride (TTC) to the red dye formazan

### ➤ **Fluorescein diacetate (FDA) method**

FDA accumulates inside the plasmalemma of viable cell/protoplasts. Live cell/protoplasts contain esterases which cleave FDA to release fluorescein which fluoresces yellowish-green using fluorescence microscopy within 5 min. FDA dissociates from membrane after about 15 min. It is used at a concentration of 0.01% dissolved in acetone.

### ➤ **Evan's blue staining**

When the cells are treated with a dilute (0.025%) solution of Evan's blue the damaged cells take up the stain but the intact and viable cells exclude it and, thus, remain unstained



# **Plant material**

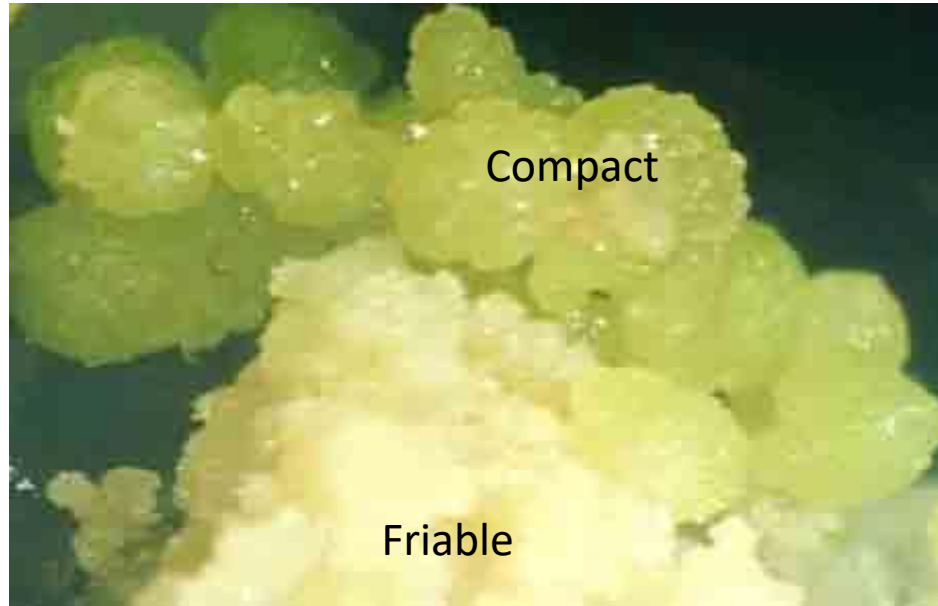
## **Effecting isolation of cells**

**Enzyme concentration: Amount of plant material**

**Incubation condition: Temperature, pH**

**Plant material: In vivo (intact) & In vitro**

## In Vitro Plant Material



## **Reference**

**Bhojwani S.S. and Razdan M.K. (2006) Plant Tissue Culture: Theory and Practice, A revised edition: Elsevier Science B. V. The netherlands**