

# Protoplast Culture : Purification

## Lecture II

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Protoplast purification : **WHY???**

Incubation mixture: Contains Protoplast, undigested tissues, cell debris & enzymes

Remove debris, enzymes and undigested tissues

Debris: By filtration

Enzyme: By washing (involve centrifugation)

Intact protoplast: by Centrifugation

Protoplast

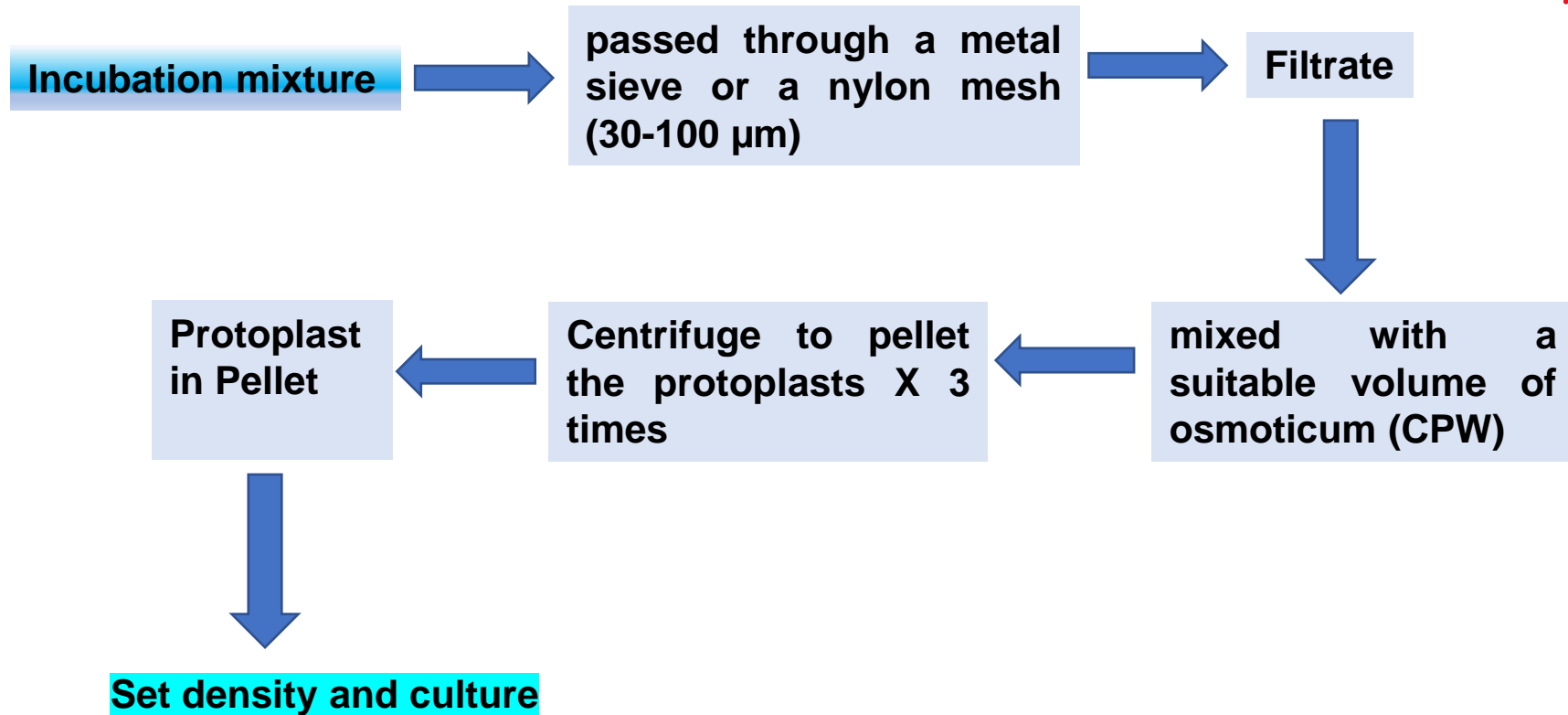
Test viability

Culture to regenerate/fusion

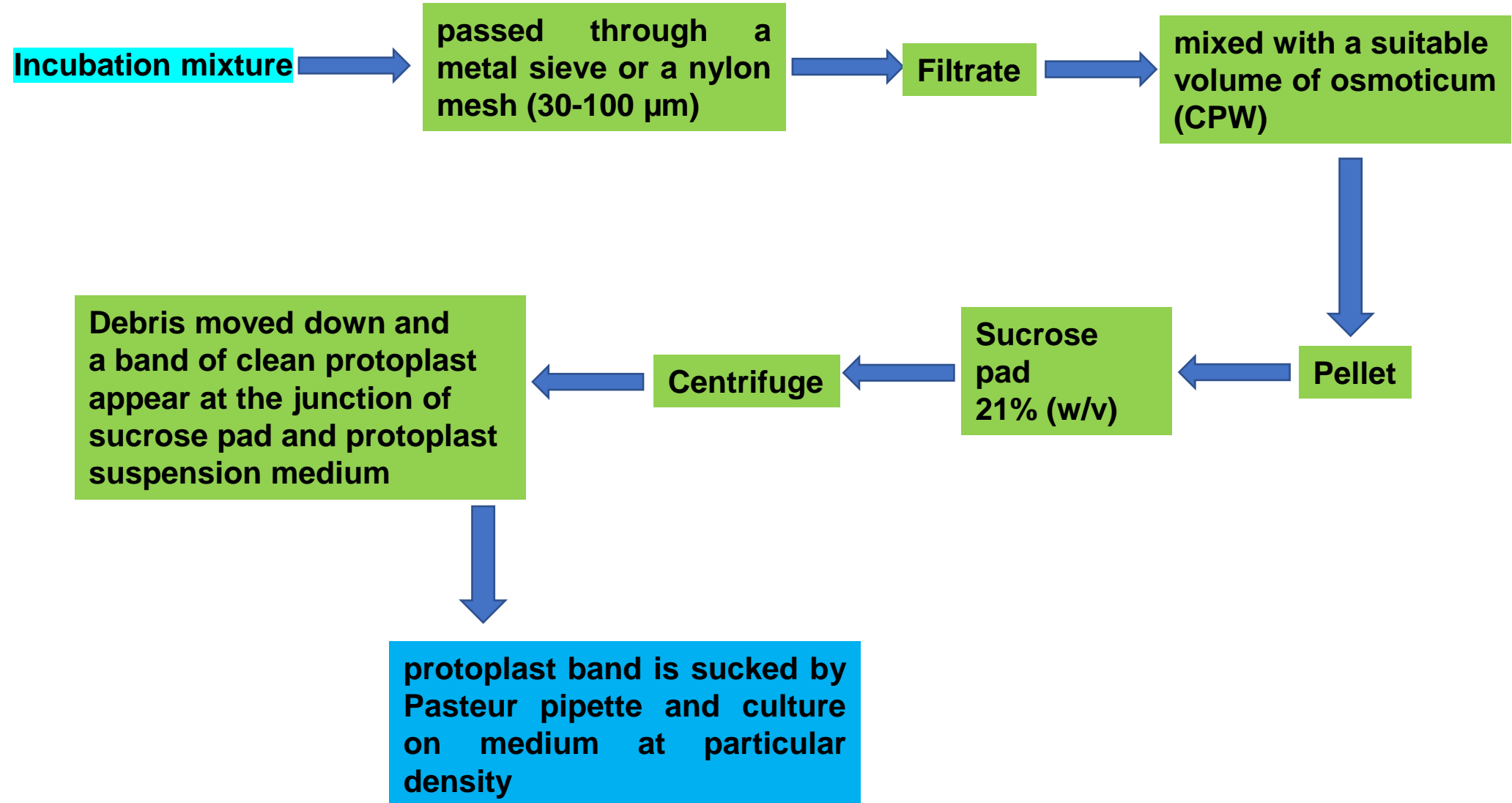
## Purification Method

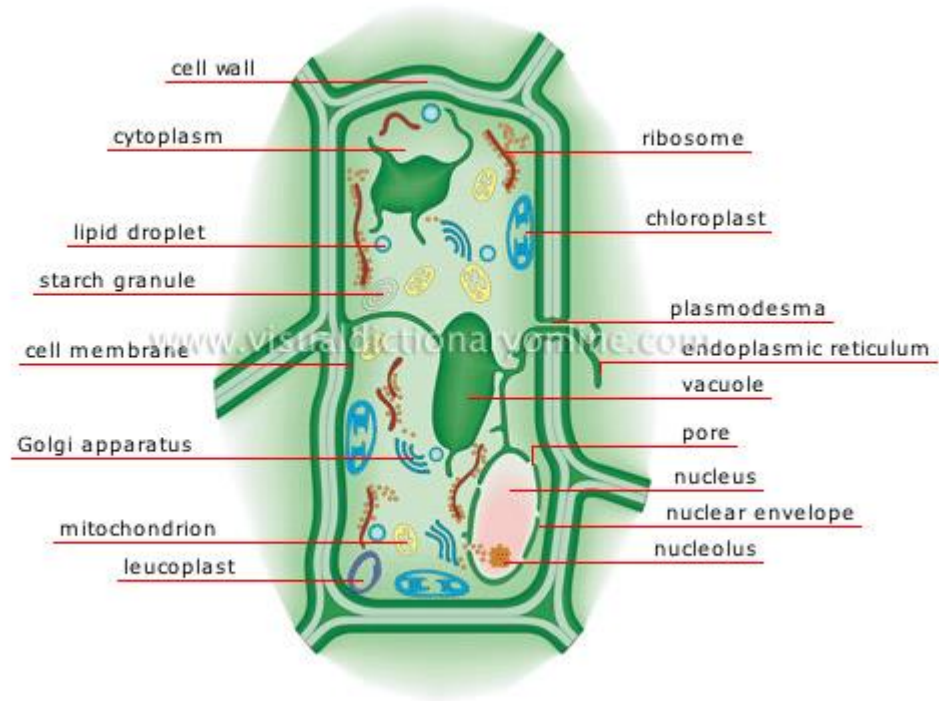
Can be purified by

**Method 1**



## Method 2





**Plant cell**



**A photomicrograph of a plant protoplast**

# Protoplast viability

## Viability can be tested by

### ➤ **Fluorescein diacetate staining 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.

### ➤ **Evans Blue test**

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

### ➤ **TTC reduction**

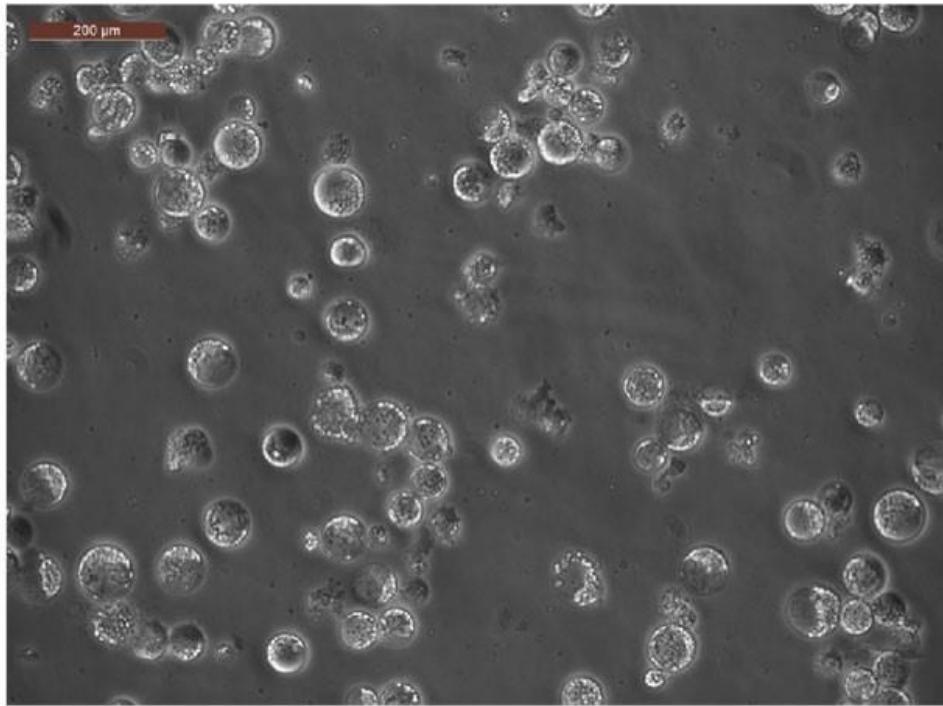
Reduction of 2,3,5 triphenyl tetrazolium chloride (TTC) to the red dye formazan

### ➤ **Phase contrast microscopy**

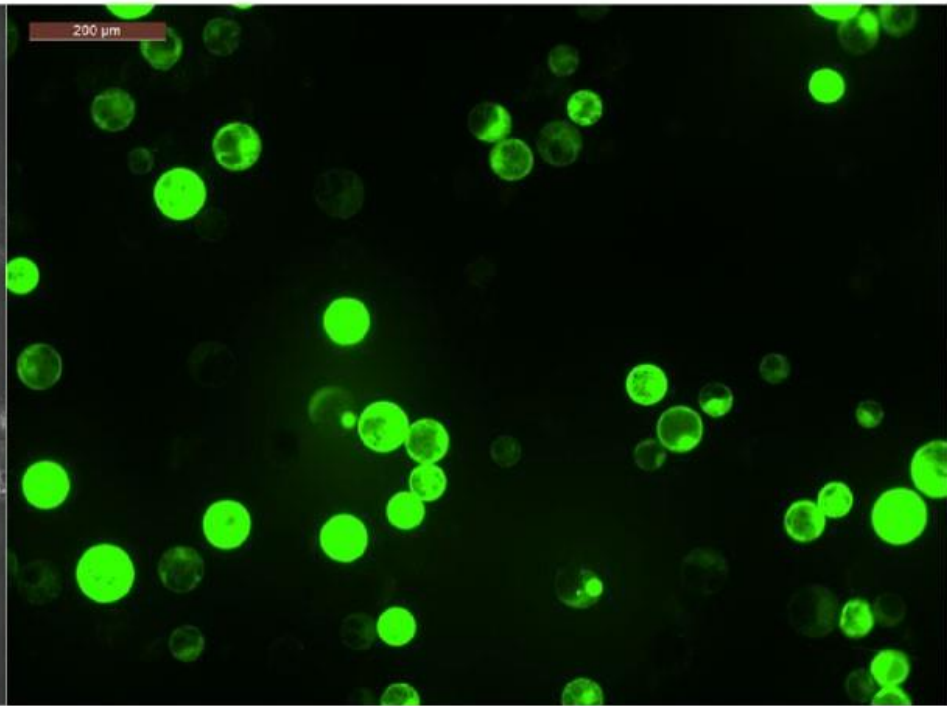
Cytoplasmic streaming and the presence of healthy nucleus

➤ *Protoplast viability* can also be detected by monitoring oxygen uptake of cells by oxygen electrode, which shows respiration.

Bright



Fluorescence



Protoplast after FDA test FDA Test

# Protoplast Culture

## Protoplasts culture techniques

The culture requirements and the culture methods are same for both protoplasts and single cells.

The main difference is the requirement of suitable osmoticum for protoplasts until they regenerate a cell wall.

Isolated protoplasts are either cultured in liquid or semisolid agar or agarose media plates, sometimes the protoplast is first grown in liquid media and then transferred into the agar media plates.



**Thank You**