RAMA UNIVERSITY

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Course: B. Tech Biotechnology
Sub Code: BBT-515

Semester: 5th
Sub Name: Plant Biotechnology

LECTURE 3

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PETRI DISH PLATING TECHNIQUE:

1. A suspension of purely single cells is pre-pared aseptically from the stock cell suspen-sion culture by filtering and centrifugation requisite cell density in the single cell suspension is adjusted by adding or reduc-ing the liquid medium.

2. The solid medium (1.6% 'Difco' agar added) is melted in water bath.

3. In front of laminar air flow, the tight lid of falcon plastic petri dish is opened With the help of sterilized Pasteur pipette 1 5 ml of single cell suspension is put an equal amount of melted agar medium when it cools down at 35°C, is added in the single cell suspension

4. The lid is quickly replaced and the whole dish is swirled gently to disperse the cell and medium mixture uniformly throughout the lower half of the petri dish.

5. The medium is allowed to solidify and the petri dish is kept at the inverted position.

6. The cultures are incubated under 16hrs light (3,000 lux, cool white) or under con-tinuous dark at 25°C.

7. The petri dishes are observed at regular in-tervals under inverted microscope to see whether the cells have divided or not.

8. After certain days of incubation, when the cells start to divide, a grid is drawn on the undersurface of the petri dish to facilitate counting the number of dividing cells.

9. The dividing cells ultimately form pin-head shaped cell colonies within 21 days of incu-bation.

10. The plating efficiency (PE) can be calculated from the counting of cell colonies by the following formula:

PE = Number of colonies per plate/Number of total cell per plate x 100

11. Pin-head shaped colonies, when they reach a suitable size, are transferred to fresh me-dium for further growth.

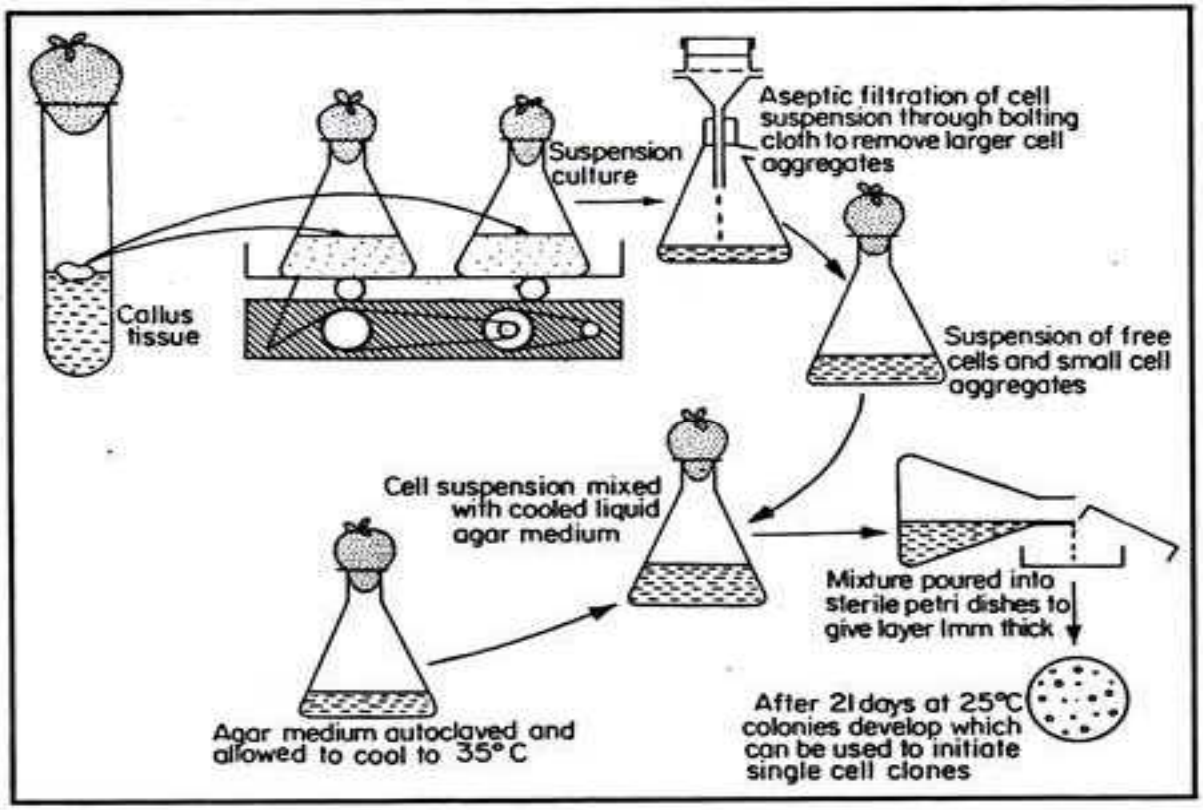

Fig 9.2

Procedure for obtaining single cell clones using a petri dish plating technique

https://www.biologydiscussion.com/plant-tissues/single-cell-culture/single-cell-culture-5-methods-with-diagram-plant-tissue-culture/14699

QUIZ