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Procedure for plant transformation

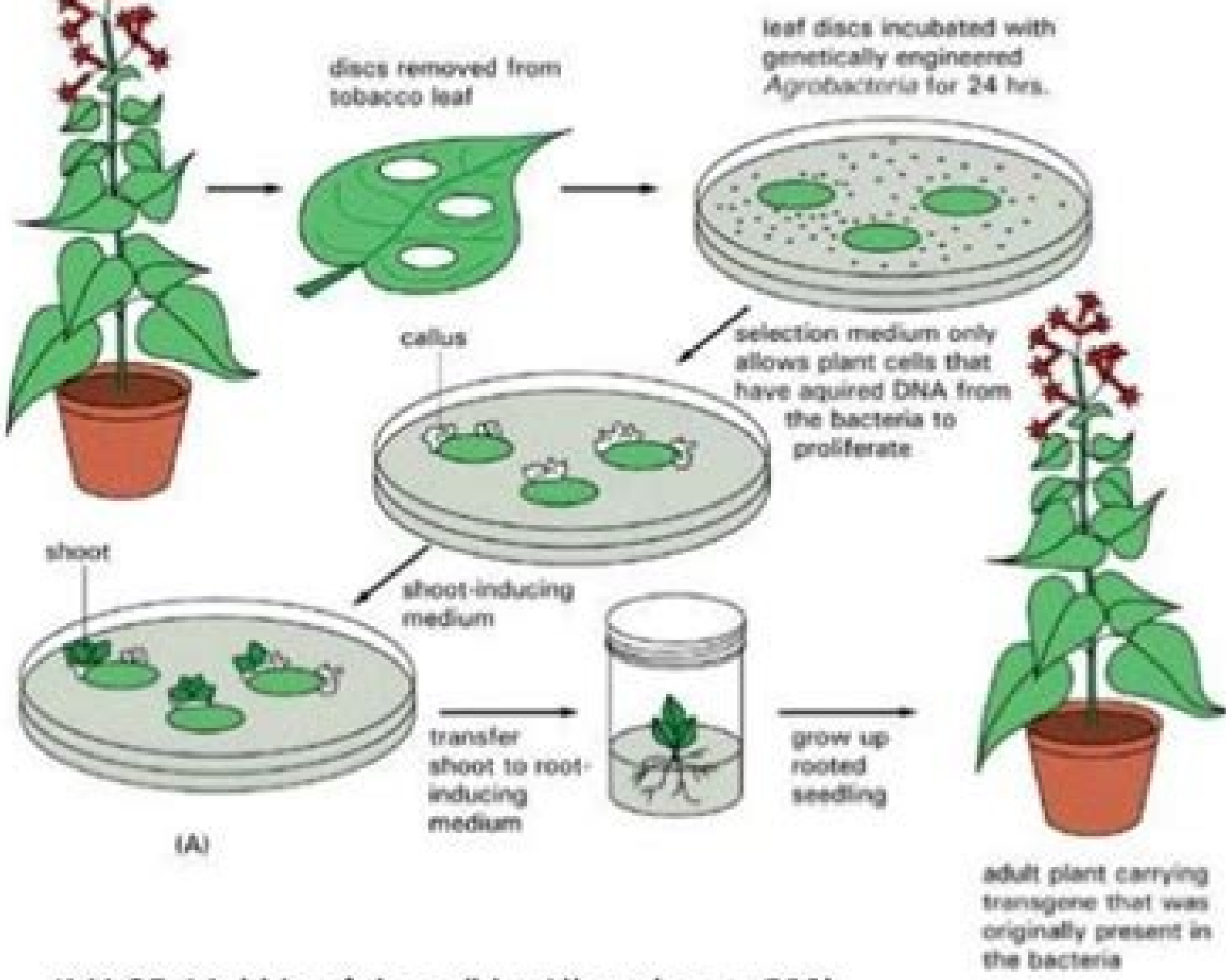
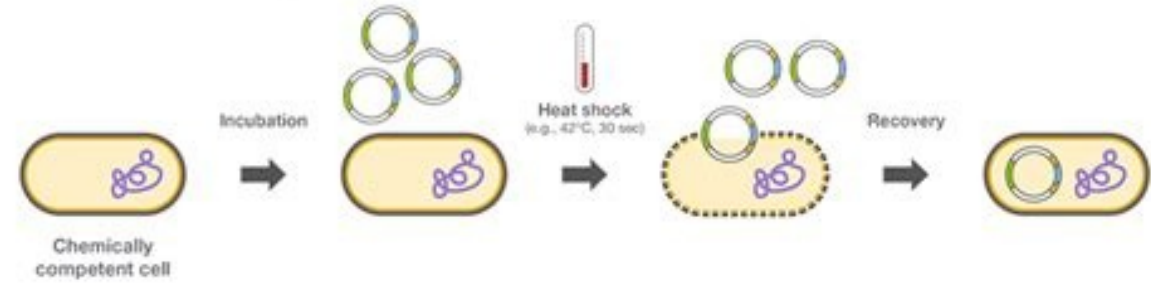
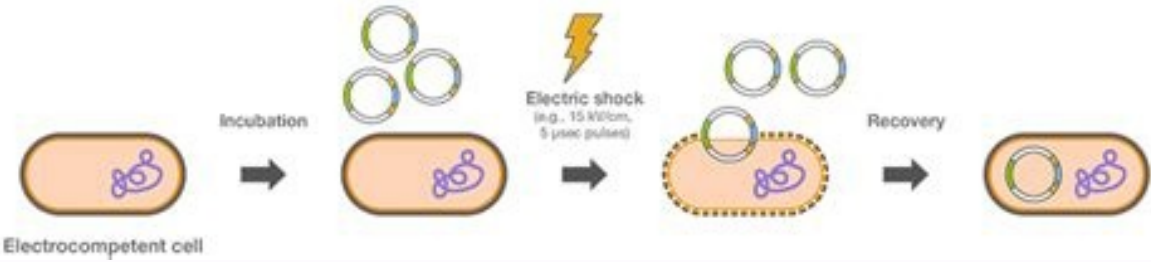


IMAGE: Mol bio of the cell by Albert (pg no:599)

Chemical transformation

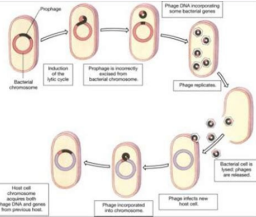
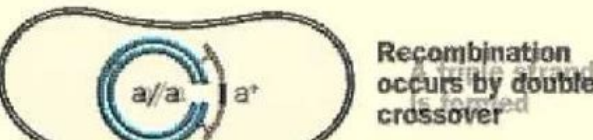


Electroporation



Overall Transformation Process

1. The plasmid vector must be cut with a restriction endonuclease (aka: restriction enzyme)
2. DNA ligase joins the DNA fragment & vector DNA
3. Host cell is made competent so can plasmid can enter
4. Transformed cells are grown on selection media



To obtain a high processing efficiency, it is essential that the growth of the cells is in the medium registry phase at the time of collection - which generally occurs at OD600 between 0.4 and 0.9, with the optimal value depending on the Volume of culture, tension and protocol. First of all, the cells are incubated with ice dna for 5 ... 30 minutes in a polypropylene tube. These preparations minimize the batch-to-batch variability and significantly simplify the efficient propagation of the cloned DNA. Ski-wheeled cells are then returned to ice for 2 minutes before the next step (Figure 3a). Figure 3. For smaller volumes of cells in smaller tubes, thermal shock interval, which The ratio of the cell suspension volume depends on the surface, it should be reduced. 300 colonies form after night incubation. Efficiency in terms of transformation = (300 cfu / 0.00625 l) X (100 Å, l/l / 200 Å, l/l) x 5 = 1.2 x 105 CFU / Å, l/lcolonies must be further shielded for the presence of plasmid and The correct sequence desired if necessary (see colony screening methods). The prolonged incubation should be avoided, since often translates into fusion of large colonies and the appearance of surrounding colonies sensitive to delicate children (called satellite colonies) due to the antibiotic breakage around large colonies. To calculate the efficiency of the transformation, divide the number of transformers from the amount of DNA added and cellular dilution factor (if performed), using the following formula: with DNA Ligated, the quantity of DNA added to the cells can also be determined by the configuration of the reaction of the binding, from dilution of DNA (if performed) and the volume of the DNA for transformation, using the following formula: 50 Ng of DNA Ligata in a reaction of 20 l/l. In this approach, from 10 to 20 pearls are positioned on the plate after applying the cell suspension and the plate has gently turned so that the cell suspension is diffused by the beads (Figure 7b). This culture of start-up and the subsequent larger culture are carefully monitored monitored Growth activates continuously measuring optical density at 600 Nm (OD600). Bacterial transformation using chemically competent cells and thermal shocks, and (b) electrocompetent cells and electroporsation.electroporation involves the use of an electroporator to expose competent cells and DNA to a short pulse of a high voltage electric field (Figure 3B). A single-use format is commercially available to enable transformation and recovery in the same tube and to evade the need for freezing and thawing cells. The choice depends on the efficiency of the requested transformation, of the experimental objectives and resources available (see the selection of competent cells). However, if a very high number of colonies is provided, the cell suspension can be diluted up to 1: 100 in S.O.C. Medium before plating to avoid the formation of a bacterial lawn. Fig. Typically, the electroporation of bacteria uses 0.1 cm cuvettes (20 "80 Å, ~ volume) and requires resistance on the field of> 15 kV / cm. Figure 4. Preparation of competent cells E. Use of S.O.C. Middle, instead of Lennox L Broth (LB broth), can increase the formation of colonies transformed from 2 to 3 times [5]. Bacterial transformation is a key step in molecular cloning, whose goal is to produce more copies of a recombinant DNA molecule. The propagation strains of Bacteriophage M13 carriers do not require this step. 5. To obtain the best results, the cell aliquot after the initial preparation in single-use volumes to minimize freezing and thawing. The ligature DNA mixtures must be Column and resuspended in water or buffer you to remove protein Å

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