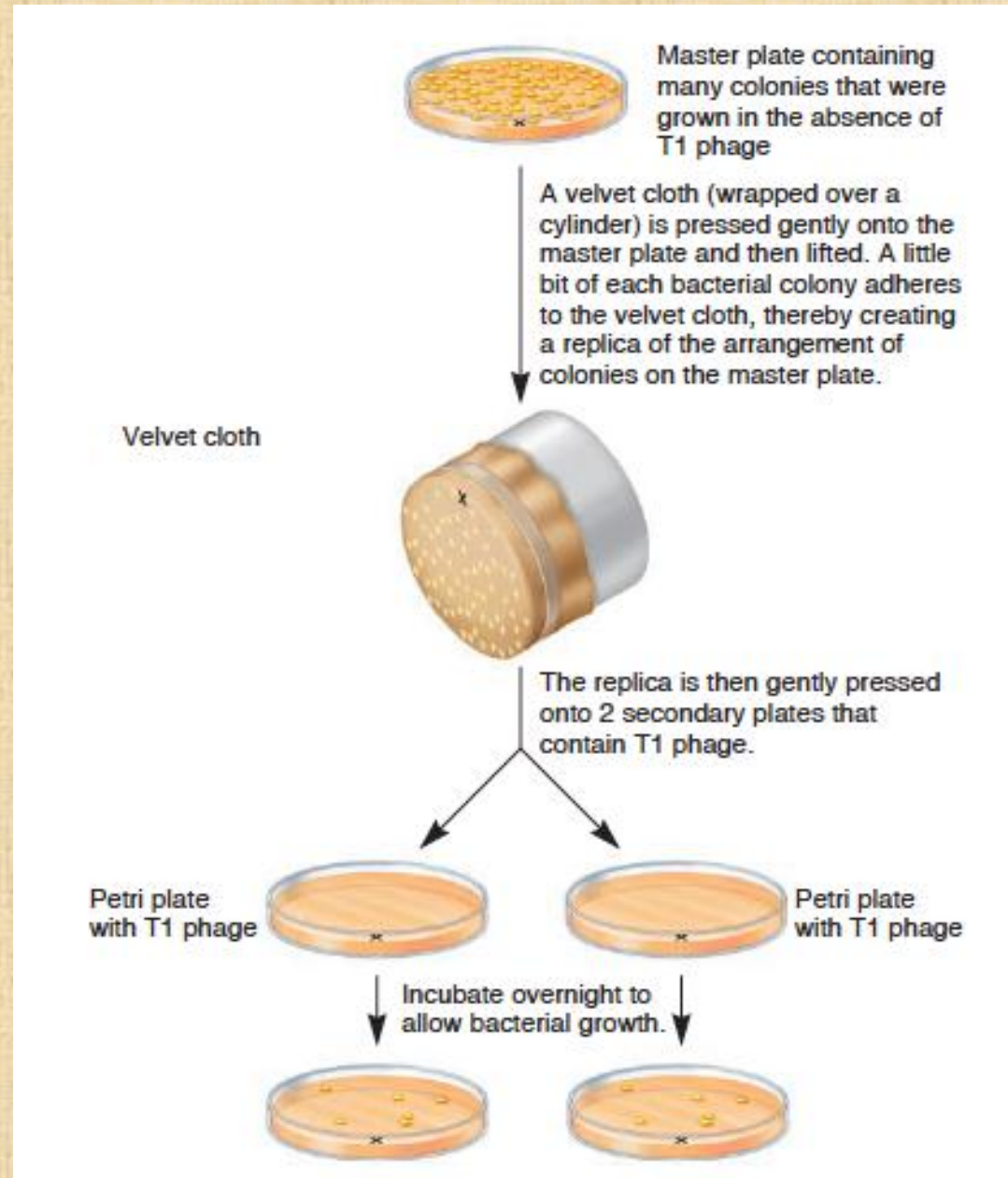


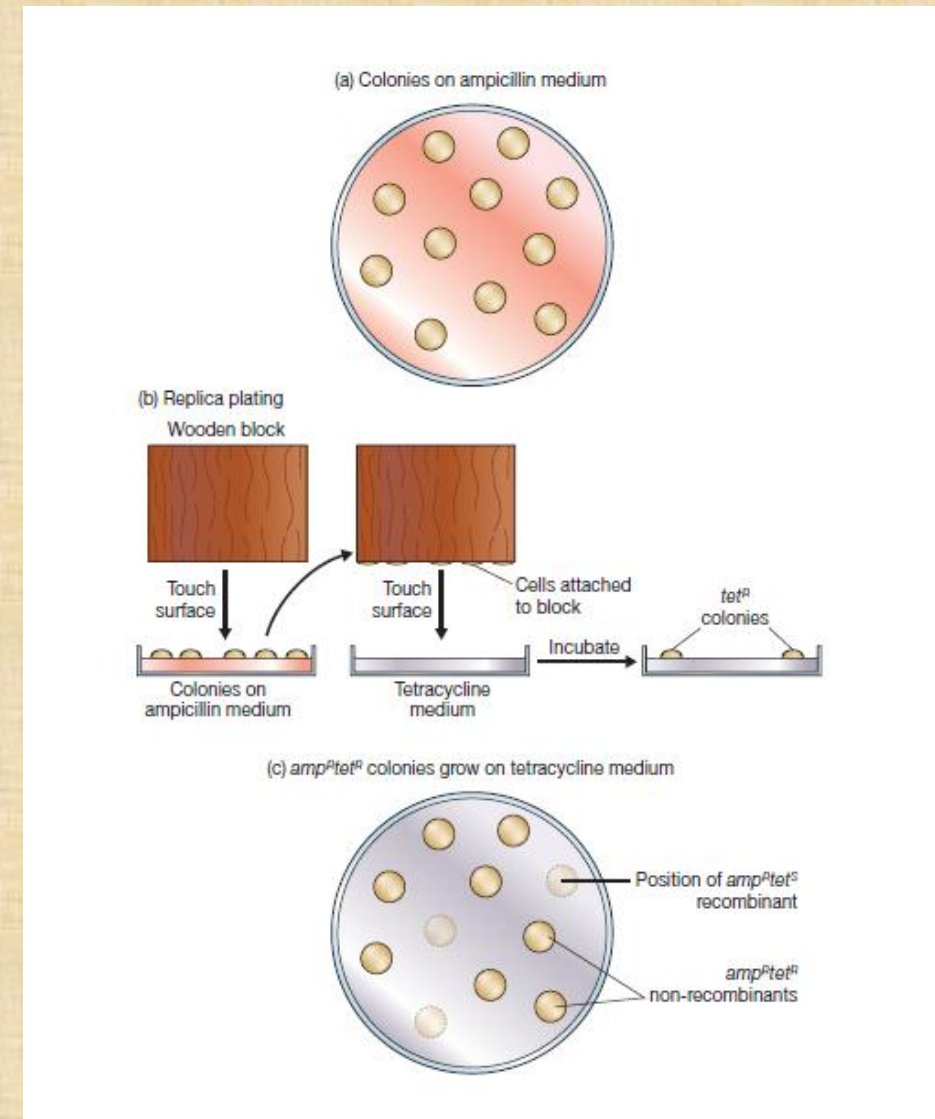
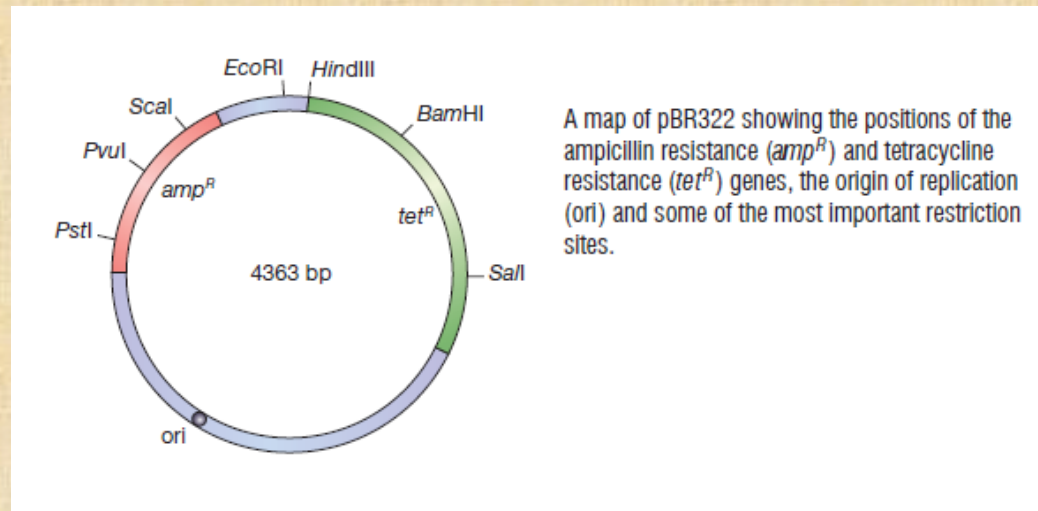
Replica Plating Technique

- A method used to produce identical patterns of bacterial colonies on a series of petri plates.
- The purpose of replica plating is to be able to compare the master plate and any secondary plates, typically to screen for a desired phenotype (Screenable phenotypes include antibiotic resistance and auxotrophic mutants).
- Replica plating is especially useful for "negative selection".

Method



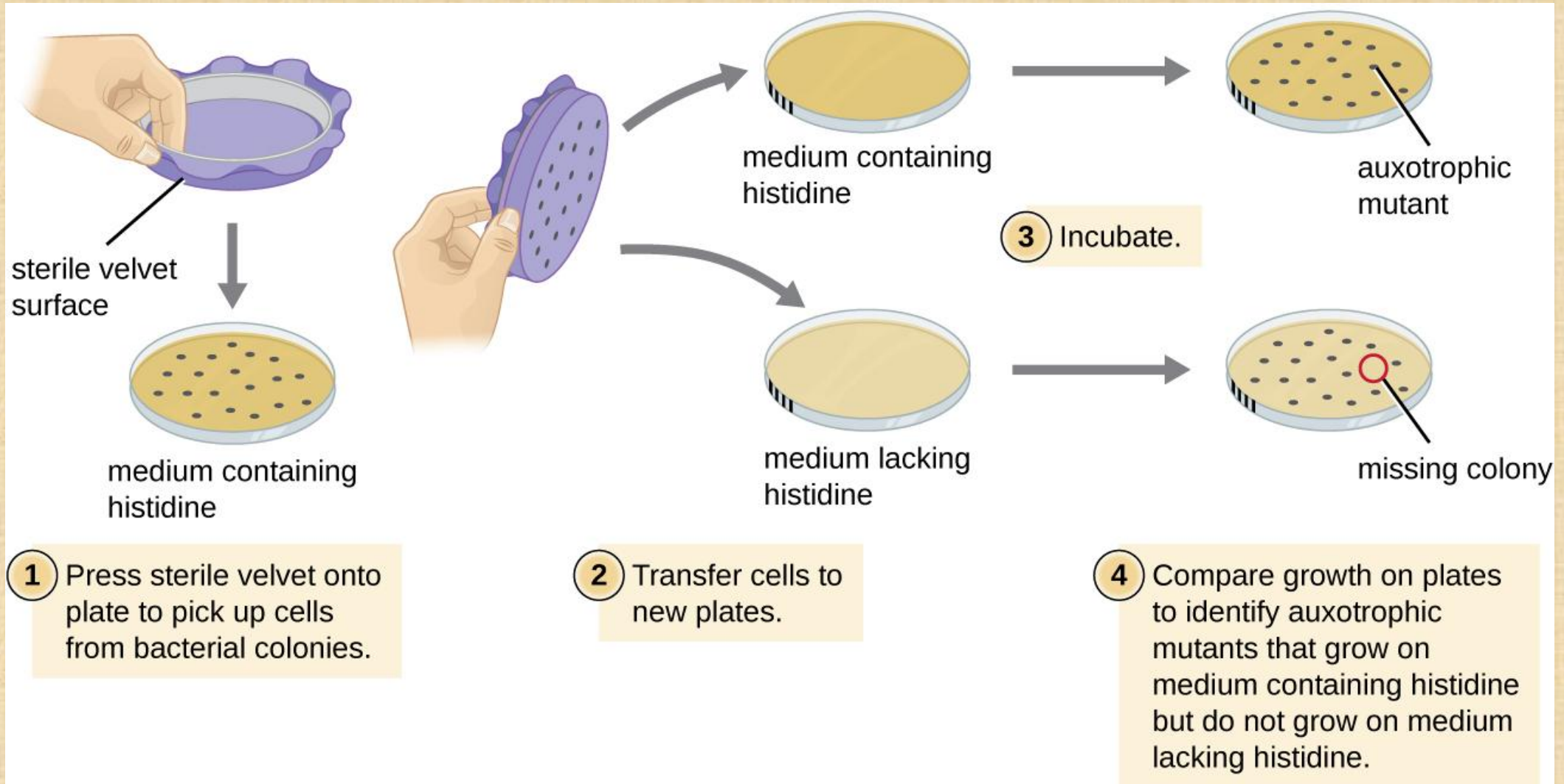
Screening for pBR322 recombinants by Insertional inactivation of tetracycline resistant gene.



Isolation of Yeast auxotrophic mutants.

- Several marker genes used in yeast genetics confer resistance against antibiotics or other toxic compounds.
- Selection for strains that carry such marker genes requires the addition of these toxic compounds to the growth media. In addition to their toxicity, the price of many of these compounds precludes their use in large-scale processes. Moreover, even in resistant strains, the presence of antibiotics may affect cellular function.
- An alternative is the use of marker genes that complement specific nutritional requirements. Some of the most commonly applied marker genes are wild-type alleles of yeast genes that encode key enzymes in the metabolic pathways towards essential monomers used in biosynthesis.
- An example is the *URA3* gene, which encodes orotidine-5'-phosphate decarboxylase, an essential enzyme in pyrimidine biosynthesis in *Saccharomyces cerevisiae*.
- Similarly, the *HIS3*, *LEU2*, *TRP1*, and *MET15* marker genes encode essential enzymes for de novo synthesis of the amino acids l-histidine, l-leucine, l-tryptophan, and l-methionine, respectively.

Isolation of Histidine auxotrophic mutants



Isolation of Leucine auxotrophic mutants

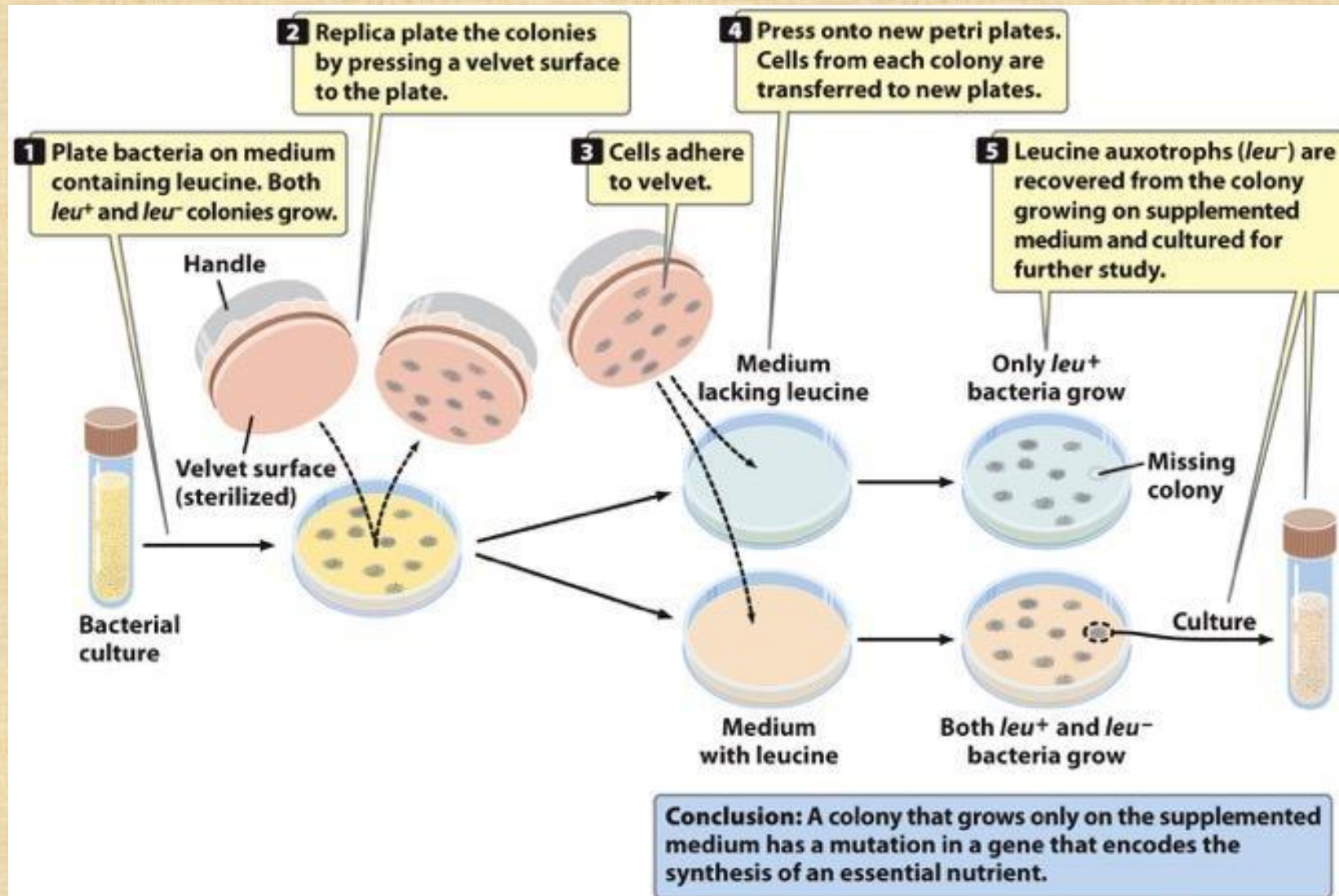


Figure 9.3
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References



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