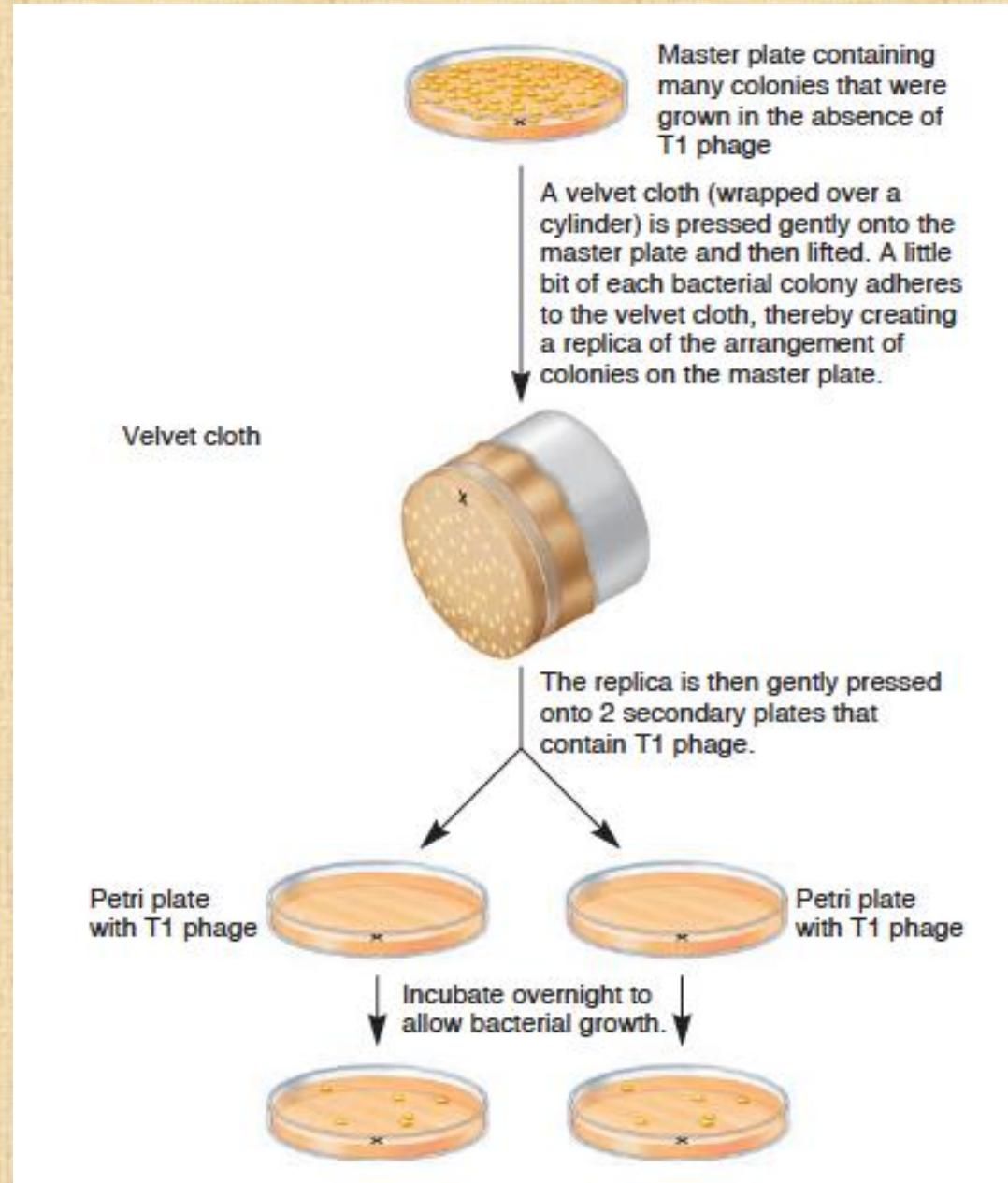


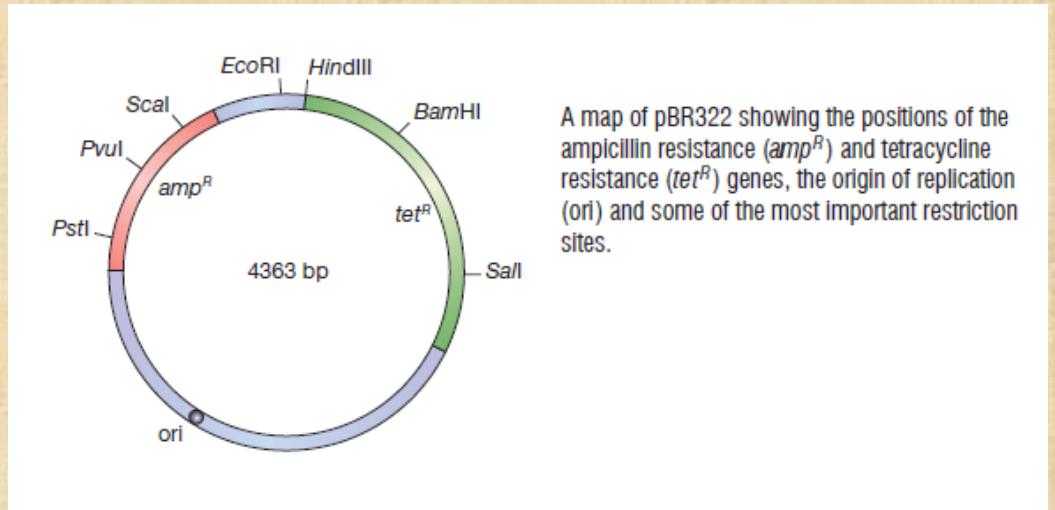
# Replica Plating Technique

- 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".

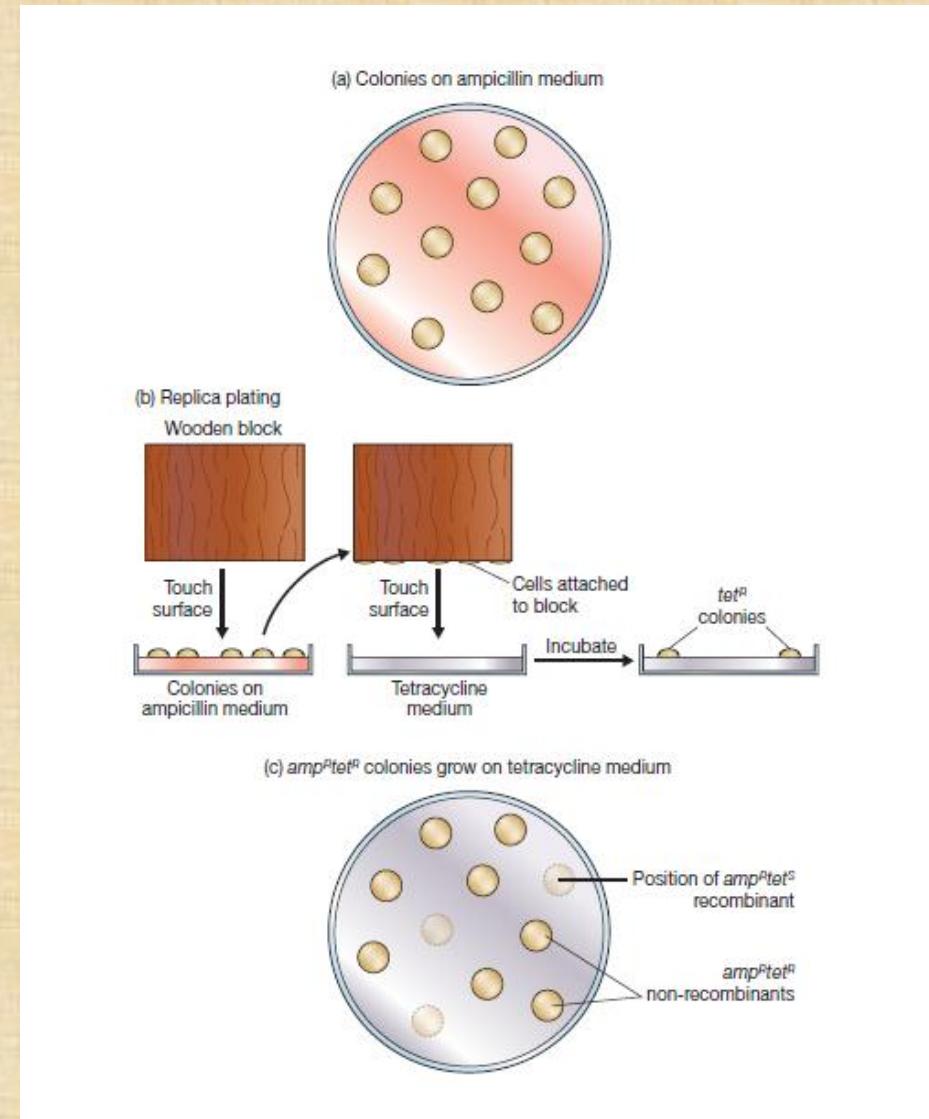
# Method



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



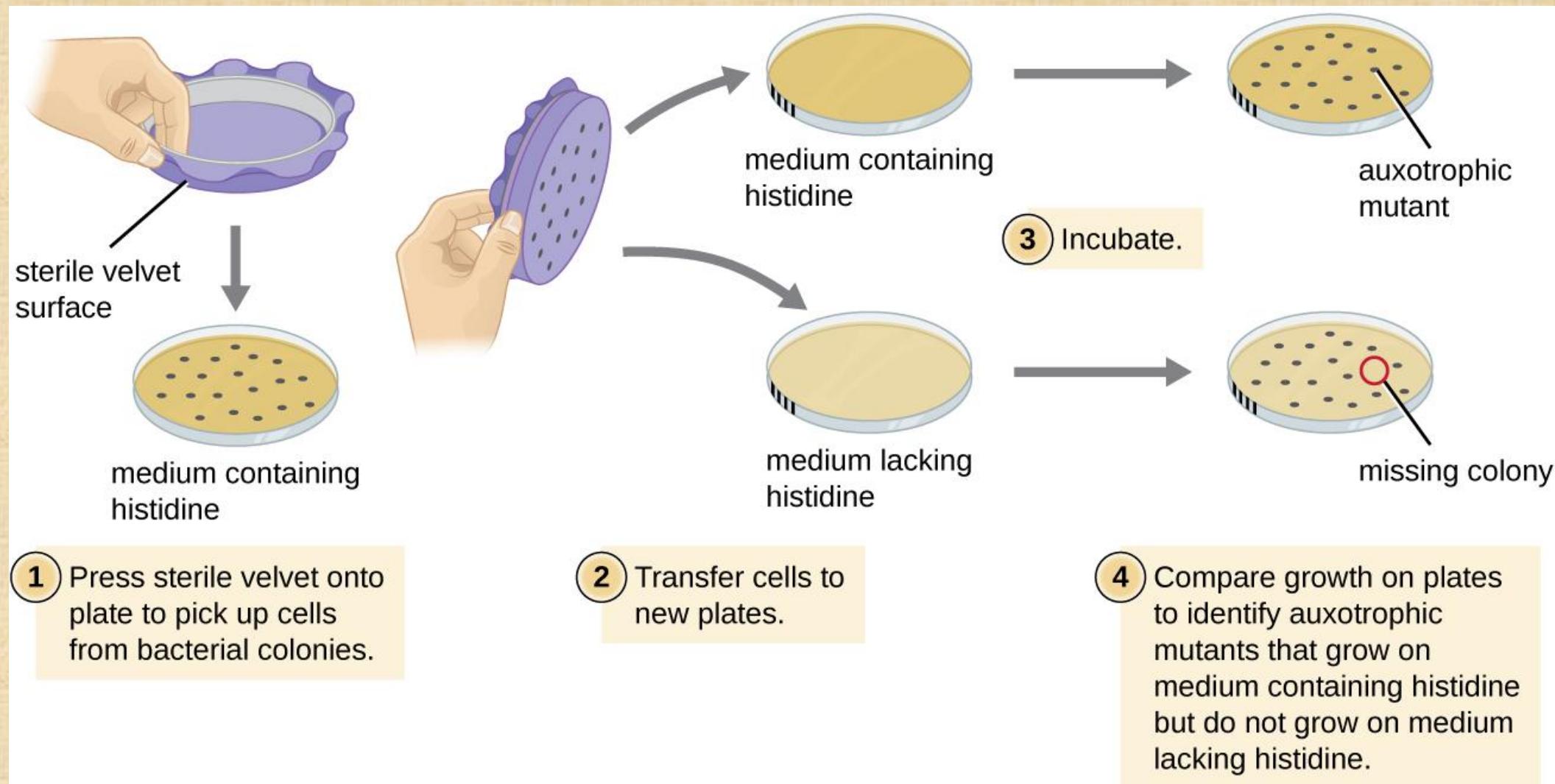
A map of pBR322 showing the positions of the ampicillin resistance ( $amp^R$ ) and tetracycline resistance ( $tet^R$ ) genes, the origin of replication (ori) and some of the most important restriction sites.



## **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

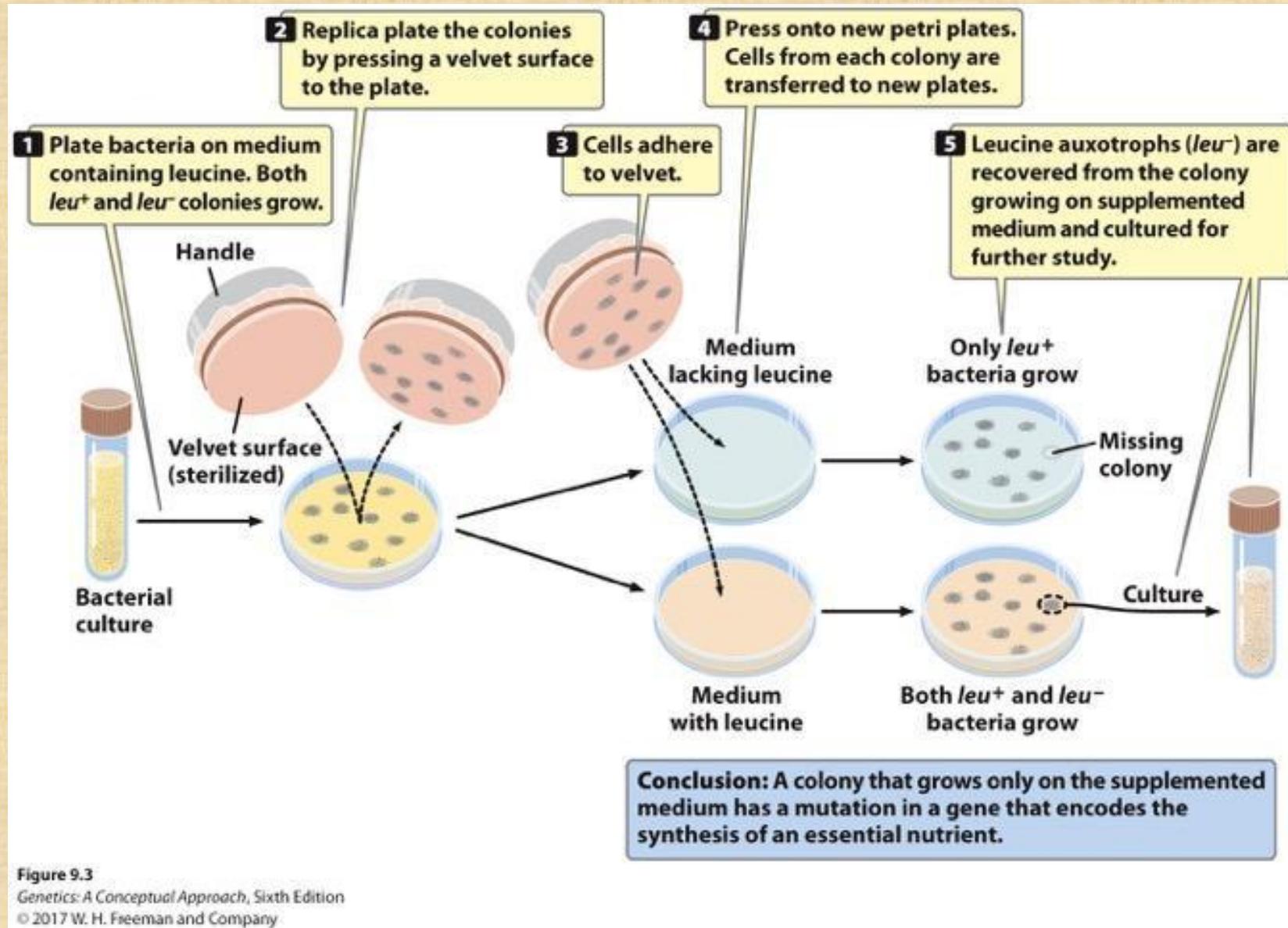


Figure 9.3

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# References



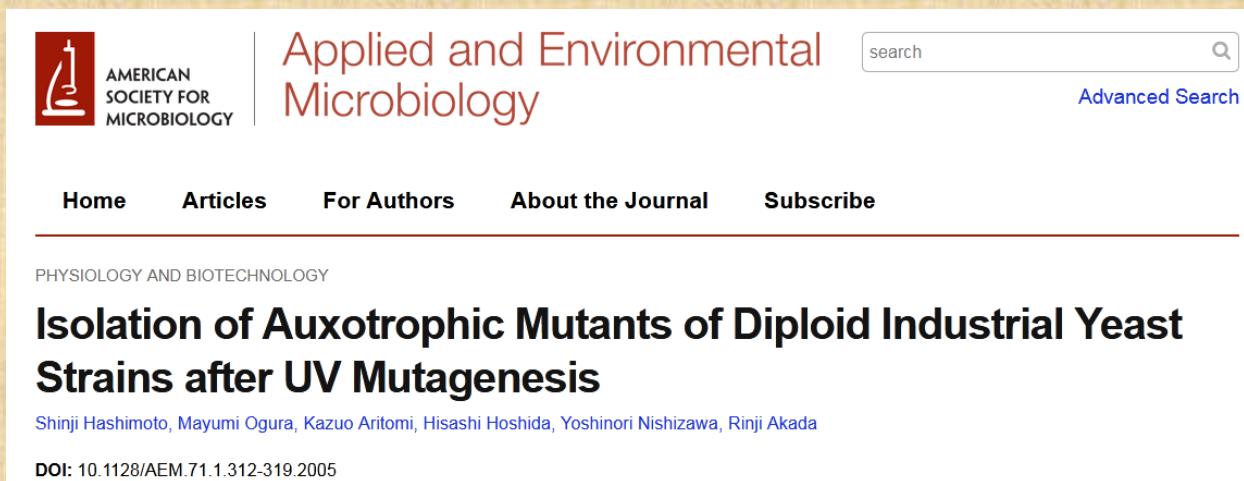
The image shows the homepage of the journal Applied and Environmental Microbiology. At the top left is the American Society for Microbiology logo (a stylized 'A' inside a red square) and the text 'AMERICAN SOCIETY FOR MICROBIOLOGY'. To the right is the journal title 'Applied and Environmental Microbiology' in red. A search bar with a magnifying glass icon is positioned above a link to 'Advanced Search'. Below the header is a navigation menu with links to 'Home', 'Articles', 'For Authors', 'About the Journal', and 'Subscribe'. A horizontal red line separates this from the main content area. In the content area, the word 'Minireview' appears in small grey text. The title of the article is 'Auxotrophic Yeast Strains in Fundamental and Applied Research' by Jack T. Pronk. The DOI is listed as 10.1128/AEM.68.5.2095-2100.2002.

Minireview

**Auxotrophic Yeast Strains in Fundamental and Applied Research**

Jack T. Pronk

DOI: 10.1128/AEM.68.5.2095-2100.2002



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PHYSIOLOGY AND BIOTECHNOLOGY

**Isolation of Auxotrophic Mutants of Diploid Industrial Yeast Strains after UV Mutagenesis**

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