CONCEPT: MUTANT DETECTION

●Scientists can identify mutants using two different methods of detection:

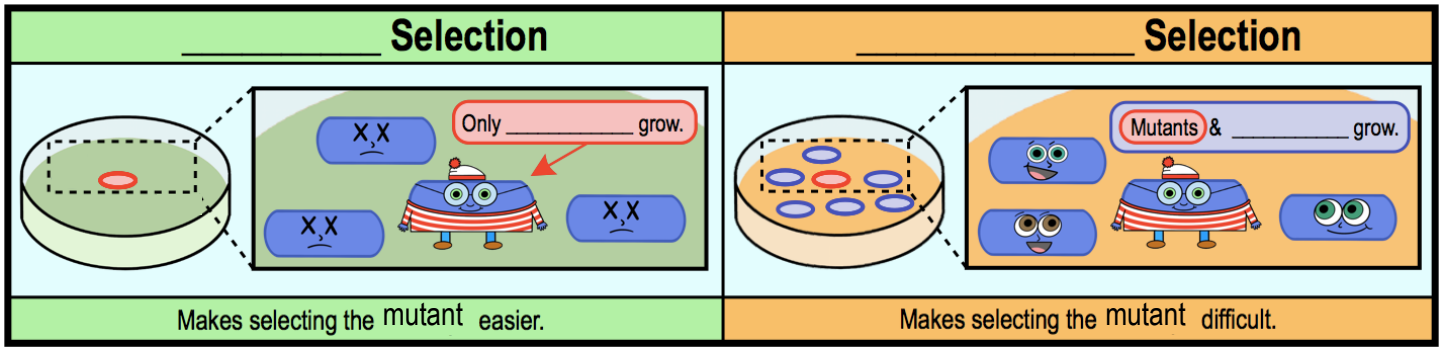
1) **Direct Selection:** only promotes growth of *selectable* mutants (_____ parents) to *select* mutant *directly & easily*.

□ Does NOT work for selecting _____ (since prototrophs always grow where auxotrophs grow).

2) **Indirect Selection:** _____ mutants & parents grow, so mutants must be *indirectly* selected amongst crowd.

□ Typically, a more tedious method only used when required for identifying _____-*selectable* mutants.

□ Indirect identification can be accomplished via the method *replica plating*.



PRACTICE: Which of the following is a major difference between direct and indirect selection of mutants?

a) Direct selection finds selectable mutants while indirect selection finds non-selectable mutants.
b) Direct selection is able to find auxotroph mutants while indirect selection cannot.
c) Direct selection only allows the selected mutants to grow while indirect selection allows parent cells and mutants to grow.
d) Direct selection is a more tedious form of mutant selection than indirect mutant selection.
e) A and C.
f) B and D.

PRACTICE: A scientist has a population of His+ prototroph and His- auxotroph cells and wants to select and separate the auxotrophs from the prototrophs. Which form of selection should the scientist use?

a) Indirect selection.
b) Auxotroph selection.
c) Direct selection.
d) Any of the above forms of selection can be used.

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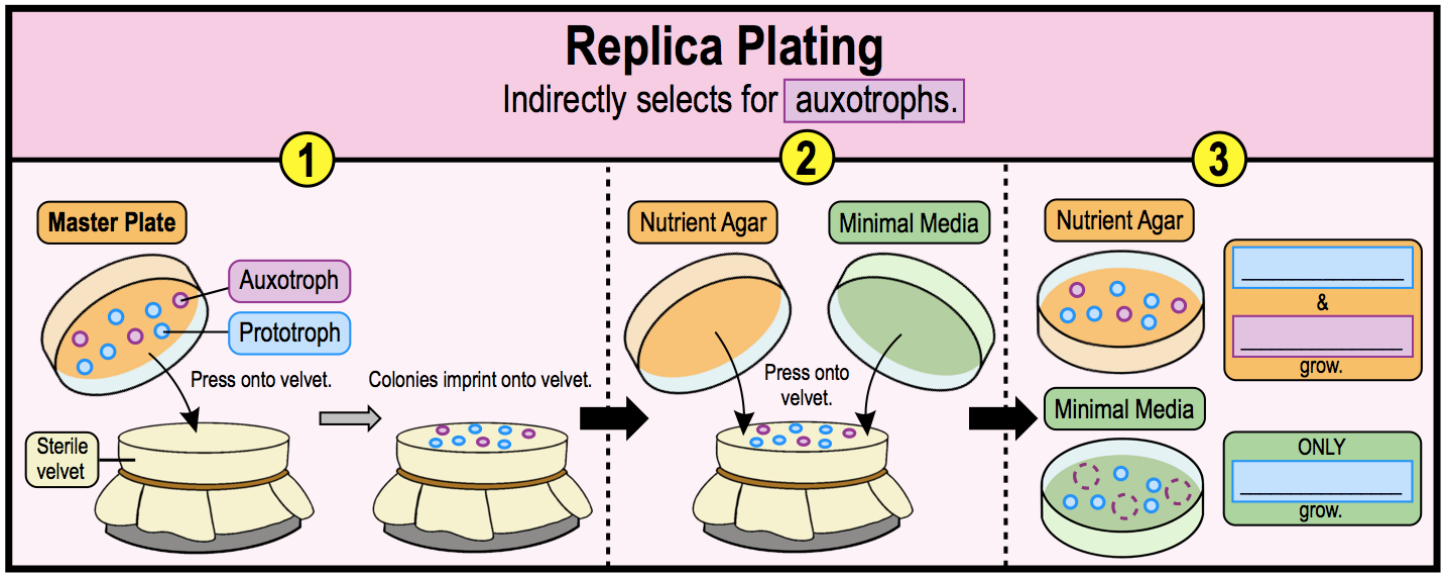
Replica Plating

- **Replica Plating:** technique used to *indirectly select* for _____-*selectable* mutants (cannot be directly selected).
- Involves a series of ____ steps:
  1) Plate *auxotrophs* & *prototrophs* onto _____ *plate* & press master plate onto sterile velvet fabric.
  2) Use velvet fabric to inoculate ____ ***replicate*** agar plates (*nutrient* agar & *minimal media*).
  3) Plates are incubated then observed:
     - □ *Prototrophs* grow on _____ nutrient agar & minimal media plates.
     - □ *Auxotrophs* _____ grow on the nutrient agar (does NOT grow on minimal media).



PRACTICE: Replica plating is an example of which type of mutant selection?

a) Auxotroph selection.
c) Direct selection.
b) Indirect selection.
d) Secondary selection.

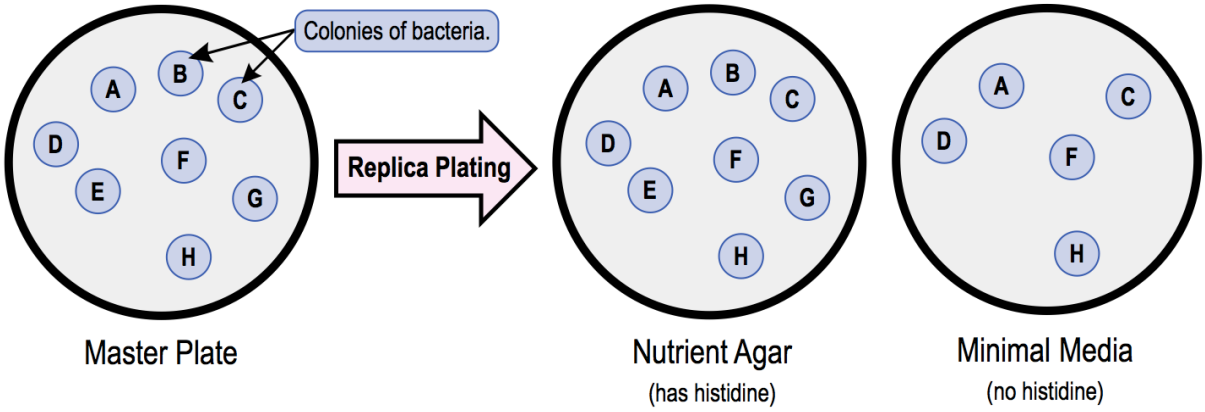
PRACTICE: What types of bacteria will be growing on the nutrient agar and minimal media at the end of a replica plating experiment?

a) The nutrient agar will possess prototroph bacteria, while the minimal media will possess auxotroph bacteria.
b) The nutrient agar will possess both prototroph & auxotroph bacteria, while the minimal media will possess prototroph bacteria.
c) The nutrient agar will possess auxotroph bacteria, while the minimal media will possess the prototroph bacteria.
d) The nutrient agar will possess auxotroph bacteria, while the minimal media will possess both prototroph & auxotroph bacteria.

CONCEPT: MUTANT DETECTION

PRACTICE: Why do prototroph bacteria grow on minimal media but auxotroph bacteria do not?

a) The minimal media does not possess the growth factor the auxotrophs need to grow.
b) The minimal media does not possess growth factors, but prototrophs do not require growth factors to grow.
c) The prototrophs outcompete the auxotrophs for the minimal nutrients found in minimal media.
d) Prototrophs and auxotrophs are not able to grow together on the same plate.
e) A and B.
f) C and D.



Based on the image above answer the following two questions:

PRACTICE: Which of the following colonies of bacteria are composed of His- auxotroph bacteria?

a) A, C, D, F, & H.
b) B, E, & G.
c) A, B, E, & F.
d) All of the colonies are composed of His- auxotroph bacteria.

PRACTICE: What characteristics are true about the colonies living on the minimal media plate?

a) These colonies do not require histidine to grow.
b) These colonies are composed of His+ prototroph bacteria.
c) These colonies do not require growth factors to grow.
d) There are not His- auxotroph bacteria present in these colonies.
e) All of the above statements about the colonies living on the minimal media are true.