

Protocol for making bacteria to feed dictyostelids

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I. Important Issues

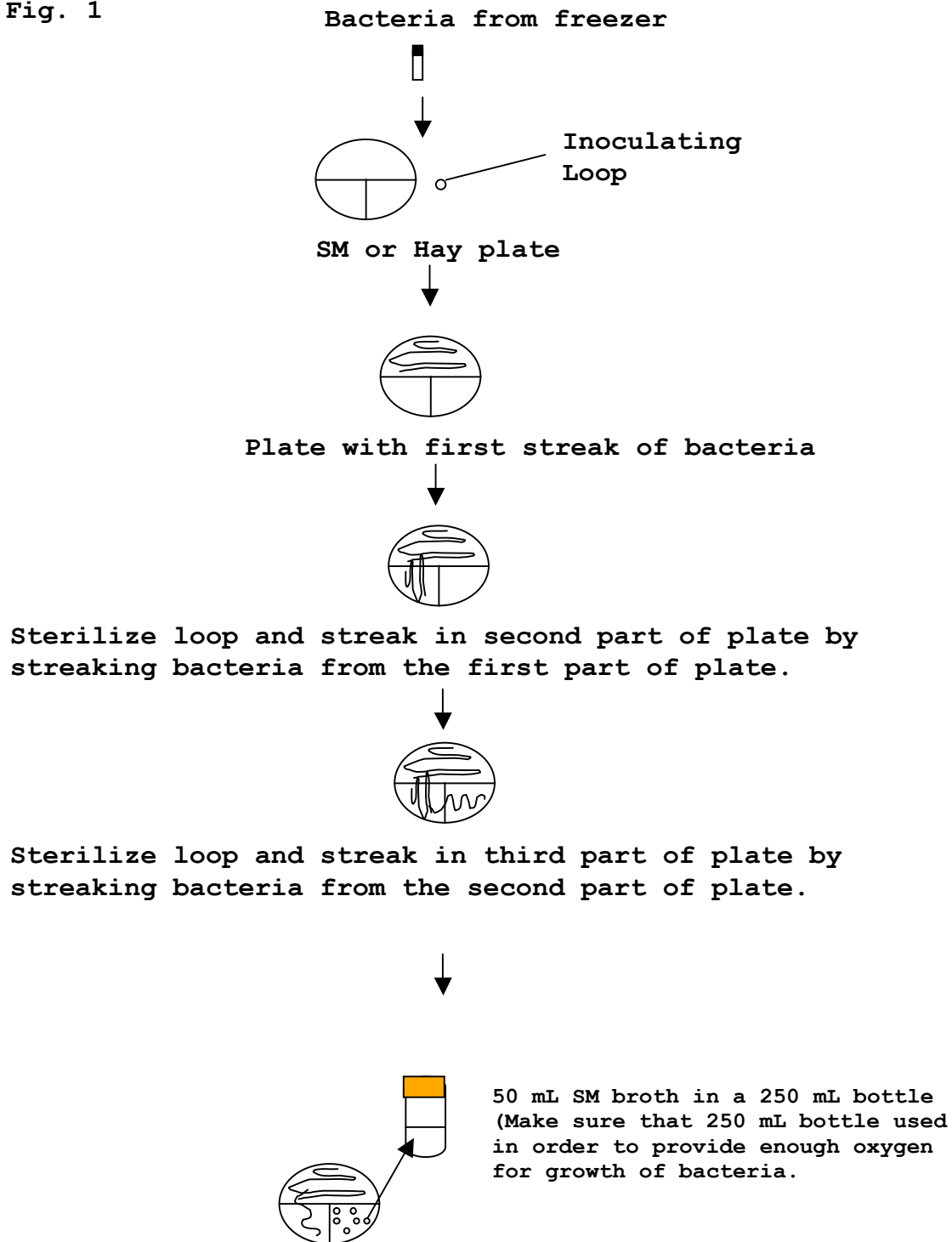
1. It is important to ensure that bacteria from a single colony is used for several reasons. By using clonal bacteria we are able to make sure that the clone we use is healthy and able to grow on its own.
2. When the bacteria is grown up on a plate, its appearance is very important. The bacteria colonies should appear as matte circles. If the bacteria is globular and shiny it should not be used because that is an indication that the bacteria is mucoid, which the *Dictyostelium* will have trouble eating.
3. The plate used to grow up the bacteria should not have any type of contamination on it as this could spread to the bacteria and later contaminate the dicty plates. The liquid bacteria should also be free of contamination before used.
4. *Klebsiella aerogenes* (KA) is the bacteria most commonly used in our lab but *E. coli* is also used.

II. Methods- The general method is to keep a plate with a clonal isolate in the refrigerator, return to that plate to pick a clonal isolate for liquid culture, and start a new plate from frozen stocks every 2-3 months. This keeps deleterious evolution of the bacterial culture to a minimum.

1. Growing on an SM or Hay plate- SM plates are the typical plates used to grow up bacteria; however, a hay plate can be used when you want to slow the growth of the bacteria.
 - a. Invert an SM or Hay plate and divide into 3 parts using a Sharpie (see Fig 1).
 - b. Label the plate with the date, bacteria strain, and your initials
 - c. Take a single colony from another plate using a loop and plate out a three way streaking using the method shown in Fig. 1. A loopful can also be taken from frozen stock and plated in the same manner.
 - d. Leave the plate on the bench for two days until single colonies are visible.
 - e. Parafilm the plate and place it in the refrigerator upside down.
 - f. The plate will be good for 10-14 days.
2. Growing in liquid- The bacteria is transferred and grown in liquid before being used for experiments.
 - a. Pour up to 50mL of SM broth into a 250mL flask or bottle.
 - b. Using a loop, transfer a single colony from a plate to the flask.
 - c. The bacteria will be ready the next day and can be kept on the bench for 10-14 days.
3. Storing in the Freezer
 - a. Place 1mL of fresh liquid bacteria into a freezer vial containing a 60% glycerol solution.
 - b. Label the vial with the strain, date frozen, and your initials and place in a -80°C freezer.

- c. A new plate should be started with bacteria from the freezer every two months.

Fig. 1



Next day, take a single colony and put in 50 mL SM broth. Store bottle on lab bench, the bacteria will be ready for use in 2 days. It can then be used for 10-14 days. (Plate can be kept in the refrigerator and lasts up to two weeks.)