Protocol: Basic Microbiology Techniques

Materials required:

- Bacterial culture
- Diluent (SM buffer (100 mM NaCl, 25 mM Tris-HCl pH 7.5, 8 mM $MgSO_4$, 0.01% (w/v) gelatin) or sterile broth)
- Petri plates containing solid culture medium
- Bottles containing sterile broth
- Tube racks, pipettors, sterile tips, loops
- Aseptic working area (near a flame or in an aseptic cabinet)

Before you begin

1. Your hands are the most likely source of contamination when working aseptically. Make sure your hands are clean.
2. Disinfect your bench top and light your Bunsen burner.
    a. The aseptic method of working by a flame will provide a clean working area of about a 12" radius around the flame.
    b. Try to keep all aseptic materials: open plates, tubes, pipette tip boxes, etc. within this radius.
    c. Keep the burner flame moderate (about 2-3" high). Make sure the burner is not directly underneath the shelf. Do not keep any flammable materials (paper towels, post-it notes, etc.) on the shelf over the burner.

Bacterial culture

1. Subculturing a culture to a new plate
    a. Place the culture-containing plate face-down near the flame.
    b. Have the new plate that you wish to subculture to next to the plate culture, also face-down.
    c. With your left hand, lift the bottom, agar-containing portion of the plate culture out of the lid and hold it face-up at an angle.
    d. Using the loop in your right hand, touch a single colony on the plate culture.
        i. You do not need to scrape the whole colony up.
    e. Place the plate culture back face-down into its lid.
    f. With your left hand, pick up the bottom of the new plate and streak the loop across the agar surface in a zig-zag motion, and place it back face-down into its lid.

2. Inoculating a liquid culture
    a. Have a blue-top glass culture tube ready nearby in a rack.
        i. Before starting, practice removing and replacing the tube caps with two fingers; you may wish to leave the caps loosened sitting on top of the tubes to make this easier.
    b. Unscrew the top of a broth bottle and place the cap face up on the bench near the flame.
    c. Insert a sterile 10 ml plastic pipette into a bulb and hold in your right hand (if you are right-handed).
        i. Only touch the pipette with your hands in the unmarked area near the top of the pipette; no not touch the numbered area.

ii. Do not allow the pipette to touch any other surface.
iii. Do not hold the pipette in the flame: it will melt.

d. Holding the bottle of broth in your left hand, insert the pipette into the bottle and withdraw 10 ml of broth.
e. Dispense 3 ml of broth into the glass culture tube.
    i. Hold the tube in your left hand and, using 2 fingers, remove the blue cap.
    ii. Briefly flame the top of the tube (1 second max).
    iii. Slowly dispense 3 ml of broth into the tube.
    iv. Replace the cap.
f. Inoculate with a single bacterial colony from a plate culture.
    i. Open the plate culture as you did before and touch a single colony with a sterile loop.
    ii. Open the glass culture tube and insert the loop so that the tip is submerged in the medium.
    iii. Shake the loop gently and briefly, remove and discard. Flame the tube top and re-cap the culture tube.

Making serial 10-fold dilutions

1. Make serial 10-fold dilutions of phage.
    a. Aseptically place microcentrifuge tubes (one for each dilution step you will need) in a row in a tube rack.
    b. Using the P-1000 pipettor, aliquot 900 µl of diluent (buffer or sterile broth) into each tube.
    c. Using the P-200 pipettor, transfer 100 µl of your phage stock into the first microcentrifuge tube. Dispose of the pipette tip. Close the tube and vortex; label this tube as the 10^{-1} dilution.
    d. Open the 10^{-1} dilution tube and transfer 100 µl into the next tube. Close, vortex and label as the 10^{-2} dilution.
    e. Open the 10^{-2} dilution tube and transfer 100 µl into the next tube. Close, vortex and label as the 10^{-3} dilution.
    f. Repeat this procedure until you reach the last tube.