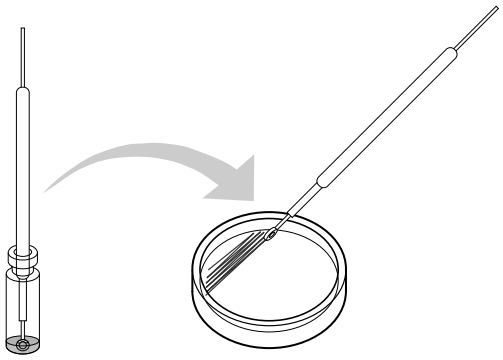
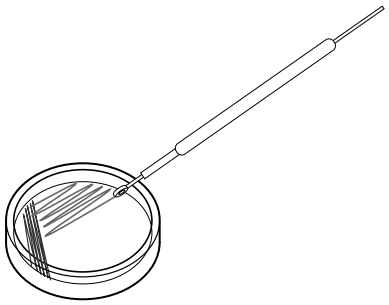
Lesson 2 Cloning

1. Insert a sterile inoculation loop into the rehydrated bacterial library. Insert the loop straight into the vial and do not tilt the vial. Remove the loop and streak for single colonies as illustrated at right.

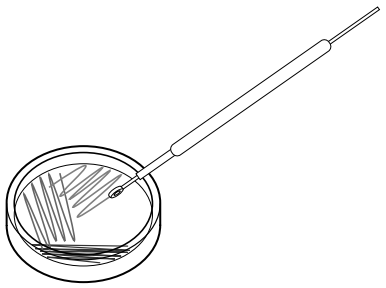


2. Streaking takes place sequentially in four quadrants. The first streak is to just spread the cells out a little. Go back and forth with the loop about a dozen times in the small area shown.

3. For subsequent streaks the overall idea is to use up as much of the surface area of the plate as possible. Rotate the plate approximately 45° (so that the streaking motion is comfortable for your hand) and start the second streak. Go into the previous streak about two times and then back and forth as shown for a total of about 10 times.

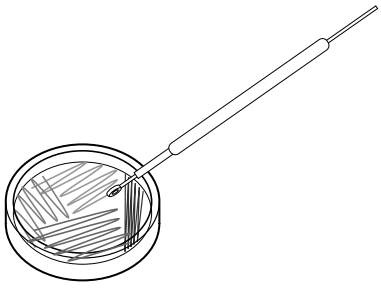


4. Rotate the plate again and repeat streaking.



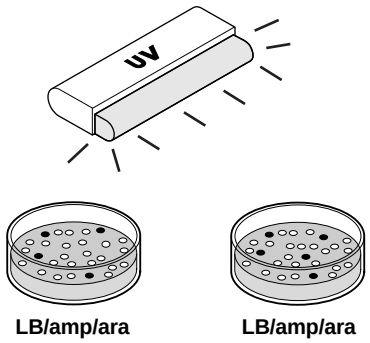
5. Rotate the plate for the final time and make the final streak. Repeat steps 1–5 with the second LB/amp/ara plate and the same inoculation loop.

6. Culture the plates overnight in a 37° incubator. Place the plates upside down in the incubator.

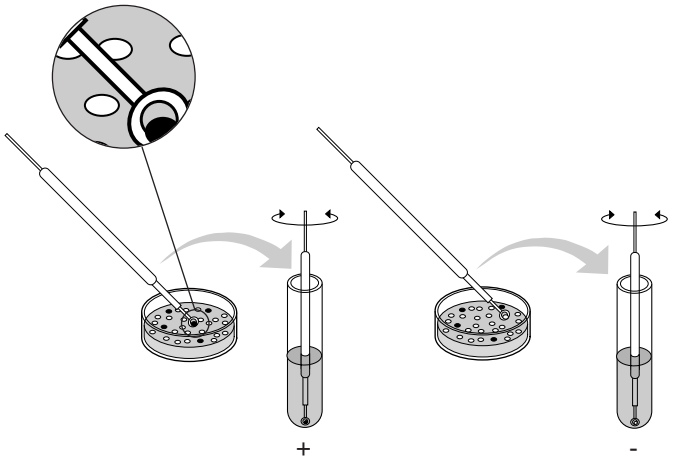


Lesson 3 Screening and Liquid Cultures

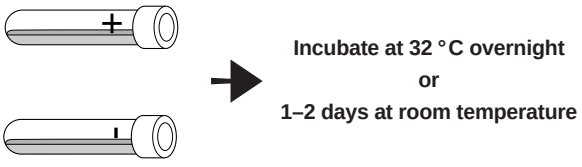
1. Remove the plates from the incubator and examine using the UV light. Identify several green colonies that are not touching other colonies on the plate. On the bottom of the plate, circle several isolated green colonies. Likewise, underline several white colonies that are also separated from other colonies on the plate.



2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a circled green colony and immerse it in the "+" tube. Using a new sterile loop repeat for an underlined white colony and immerse it in the "-" tube (it is very important to pick only a single colony). Spin the loop between your index finger and thumb to disperse the entire colony.

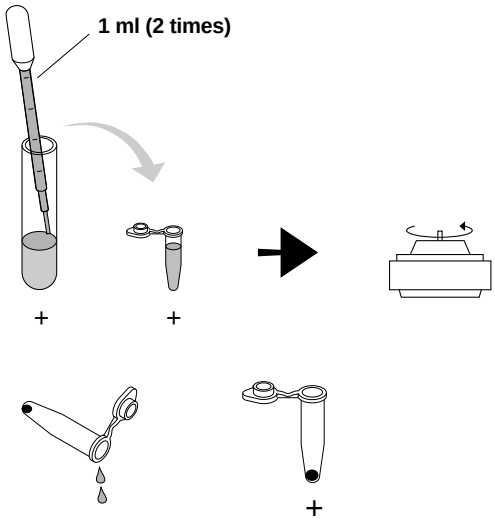


3. Place the tubes in the shaking incubator or on the shaking platform and culture overnight at 32 °C or 1–2 days at room temperature. If a shaker is not available, shake the tubes vigorously, then place horizontally in the incubator and culture for 1–2 days.



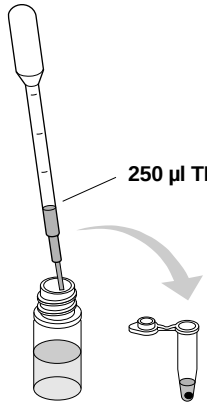
Lesson 4 Purification Phase 1
Bacterial Concentration

1. Label one microtube "+" with your name and class period. Remove your liquid cultures from the shaker and observe with the UV light. Note any color differences between the two cultures. Using a new pipette, transfer 2 ml of "+" liquid culture into the "+" microtube. Spin the microtube for 5 minutes in the centrifuge at maximum speed. The pipette used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

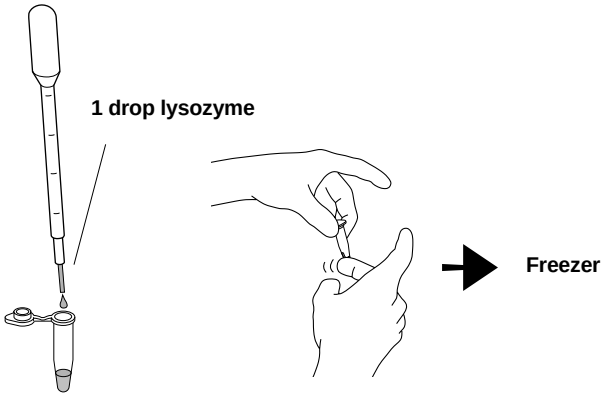


2. Pour out the supernatant and observe the pellet under UV light.

3. Using a rinsed pipette, add 250 µl of TE buffer to the tube. Resuspend the pellet thoroughly by rapidly pipetting up and down several times.



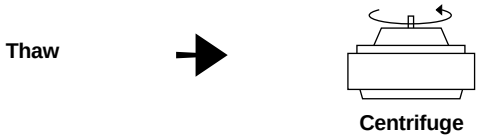
4. Using a rinsed pipette, add 1 drop of lysozyme to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.



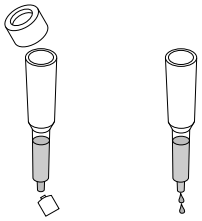
5. Place the microtube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

Lesson 5 Purification Phase 2
Bacterial Lysis

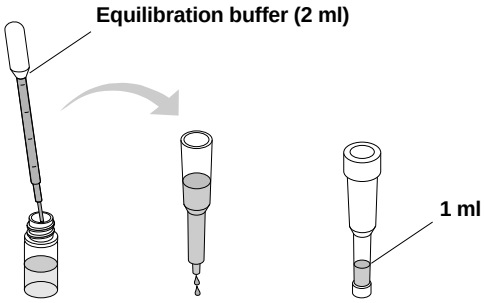
1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.



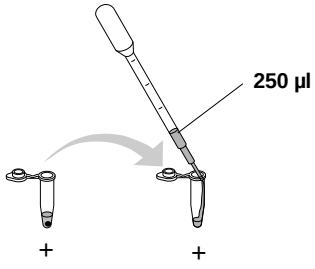
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



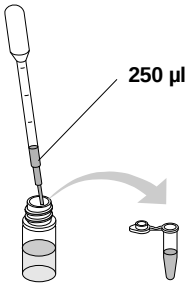
3. Prepare the column by adding 2 ml of Equilibration buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipette, transfer 250 µl of the "+" supernatant into a new microtube labeled "+". Again, rinse the pipette well for the rest of the steps of this lab period.

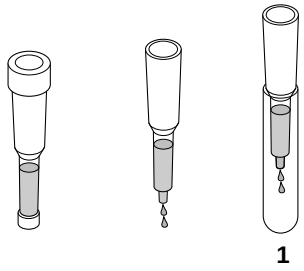


5. Using a well rinsed pipette, transfer 250 µl of Binding buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.

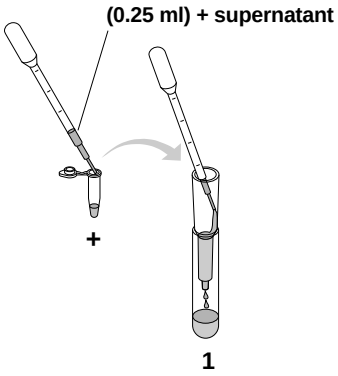


Lesson 6 Purification Phase 3
Protein Chromatography

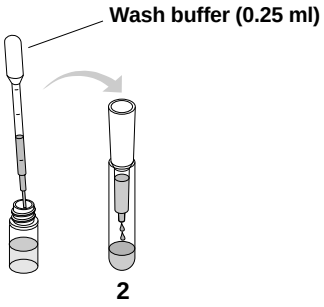
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix, proceed to the next step below.



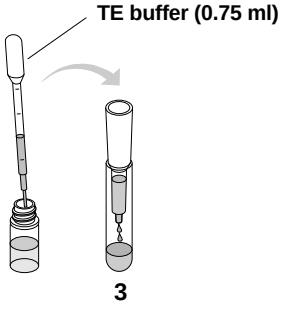
2. Using a new pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping, transfer the column to collection tube 2.



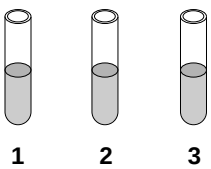
3. Using the rinsed pipette, add 250 µl of Wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



4. Using the rinsed pipette, add 750 µl of TE (Elution) buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.



5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm® or Saran Wrap® the tubes and place in the refrigerator until the next laboratory period.



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