Contents

Alkaline Lysis

Once DNA is introduced and carried in bacteria, we would like to isolate the DNA again for further manipulation. In order to do so, bacteria containing the plasmid of interest is grown in a liquid culture of nutrient rich broth made of yeast extract called Luria-Bertani Broth (**LB**). These cultured bacteria are grown until they are of a high concentration over night. They are harvested through centrifugation and the broth is removed. The resulting pellet of bacteria is resuspended in a physiological buffer containing the chelator EDTA. A **chelator** is a chemical that removes divalent cations like Ca^{2+} or Mg^{2+} from solution. This is significant because divalent cations are necessary for DNA digesting enzymes to be active. By chelating the ions, the DNA we ultimately wish to purify will be safe from degradation.

After resuspension of the bacteria, an alkaline solution of 0.1N NaOH is mixed into the bacterial mix. This solution also contains an ionic detergent called sodium dodecyl sulfate (**SDS**) that aids in denaturing proteins and disrupting their interactions with the DNA. The mixture becomes viscous as the bacteria burst open and their contents leak into the solution. This basic solution is then neutralize with a potassium acetate buffer at pH5.5. As the solutions mix together, the pH approaches 7 and the potassium interacts with the SDS to cause a precipitation of the genomic chromosomal DNA and proteins. In order to separate the precipitate from the solution, the mixture is centrifuged at high speed to pellet the genomic DNA and protein. The **supernantant**, or solution, is transferred to a column containing a **silica membrane**. Under high salt conditions, DNA adheres to glass or silica. By passing the solution through this column, the plasmid DNA in the supernatant is trapped onto the silica membrane and removed from solution. Additional washes are used to removed stray contaminants and remove the excessive salt. Plasmid DNA is finally removed from the column through **elution** by a low salt buffer. This low salt buffer is Tris pH 8 with EDTA (**TE**). Plasmid DNA can be stored stably in TE buffer in the freezer for extended periods.

Exercise 1: Plasmid DNA Mini-Prep by Alkaline Lysis

1. **Inoculate** 2 ml of rich medium (LB, YT, or Terrific Broth) containing the appropriate

antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking. (This is what you were provided)
    1. Each group should take 2 cultures
2. **Centrifuge** culture tubes directly at maximum for 5 minutes.
    1. If incapable of spinning in these tubes, transfer 1.5 ml of the culture into a microfuge tube (Eppendorf Tube).
    2. Centrifuge at maximum speed for 30 sec.

3. When centrifugation is complete, pour the broth solution into a container of bleach
4. **Resuspend** the bacterial pellet in 250 µl of ice-cold P1 solution by vigorous shaking and transfer back into a microcentrifuge tube.

    - P1 is a physiological solution of 50mM Tris at pH 8
    - P1 contains a Chelator called EDTA
        - chelators bind up excess divalent cations that are required for DNAse activity

5. **Lyse**: Add 250µl of P2 solution to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube gently five times. Do not vortex! Store the tube on ice.

    - This is the lysis buffer containing the detergent Sodium Dodecyl Sulfate and NaOH

6. **Neutralize:** Add 350 µl of ice-cold P3 solution. Close the tube and disperse lysis solution by inverting the tube several times. Store the tube on ice for 3-5 minutes.

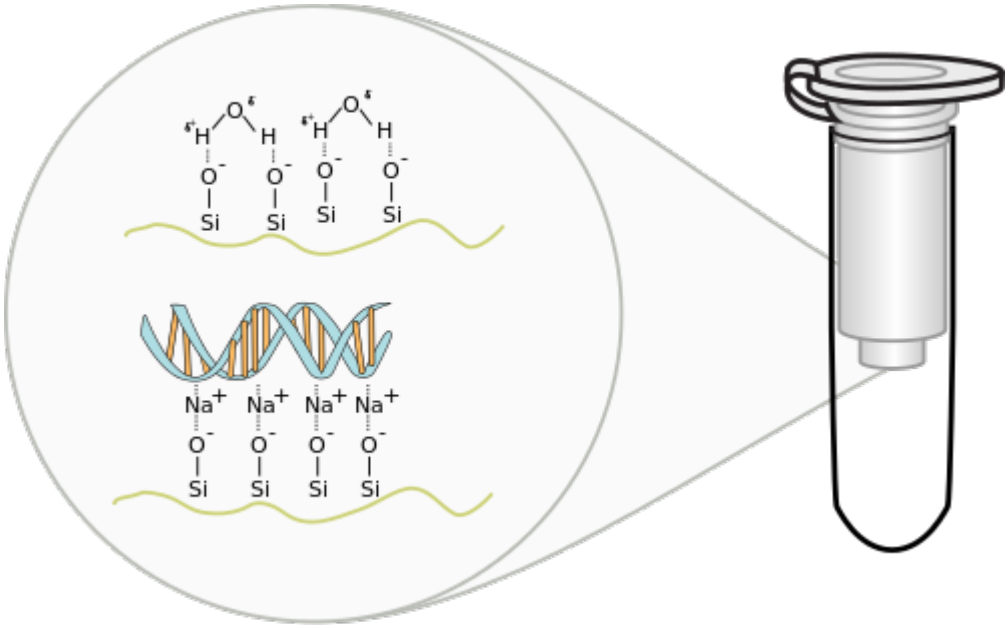
    - This is the neutralization buffer containing Potassium Acetate
    - Neutralization restores pH to near 7 and also causes the precipitation of genomic DNA and proteins into a gloopy mess (snot-like)

7. Centrifuge the bacterial lysate at maximum speed for 5 minutes in a microfuge.

    - Snot-like substances should be tightly packed into a pellet at the bottom of the tube after this step
    - the solution or supernatant contains the plasmid DNA

8. **Column Purification of DNA**: Transfer the supernatant to a fresh tube with silica-membrane column

    - DNA likes to bind to glass under high salt conditions
    - the white membrane is made of a glass fiber



9. Centrifuge the supernatant through column for 1 minute at maximum speed in a microfuge.
    - The DNA will be bound to the membrane on the column (silica)
10. **Wash**: Discard flow-through and place column back into waste tube. Wash column with 500 µl PE. Centrifuge the supernatant through column for 1 minutes at maximum in a microfuge.
    - PE is a solution that helps to wash away the non-specifically bound substances
11. Discard flow-through and place column back into waste tube. Wash Column with 700 µl PE. Centrifuge the supernatant through column for 1 minutes at maximum in a microfuge. Discard flow-through and repeat spin to dry column.
12. Place column in a fresh centrifuge tube and **Elute** the nucleic acids in 50 µl of TE (pH 8.0) by binding for 1 minute and spinning at maximum speed for 1 minute.