Miniprep - GET Buffer

Solutions/reagents:

- overnight culture
- ice-cold GET buffer
  (50 mM glucose (MW 180), 10mM EDTA, 25 mM Tris-HCl pH 8)
- 0.2M NaOH stored at room temperature
- ice-cold potassium acetate solution
  (3 M potassium acetate, 1.8 M acetic acid, no pH adjustment)
- 95% / 100% ethanol
- 70% EtOH
- TE buffer
- distilled water
- PCA solution
  ((optional)50 parts phenol, 49 parts chloroform, and 1 part amyl-alcohol)
- SDS
- lysozyme
  (optional)

Equipment:

- Centrifuge
- Sterile 2-ml microcentrifuge tubes

Steps:

1. Measure out **2 ml** of overnight culture into a sterile 2-ml microcentrifuge tube.

2. Centrifuge at maximum speed for **1 min** at **room temperature**, gently aspirate out the supernatant and discard it.

3. Add **100 µl** of ice-cold GET buffer.
   Resuspend the pellet by vortexing/by shaking vigorously.

   (Optional)
   Add **10 mg** of lysozyme.
   Store at **room temperature** for **30 mins**.
   This step is essential for lysing gram-positive cells.

4. Measure out **200 µl** of 0.2M NaOH into a sterile 2-ml microcentrifuge tube.
   Add **2 mg** of SDS.
   Vortex the mixture for a few secs.

5. Add alkaline SDS solution to cell suspension.
   Close the tube tightly and gently mix the contents by inverting the tube.
   DO NOT VORTEX! The solution should become clear.

6. Add **150 µl** of ice-cold potassium acetate solution.
   Close the tube tightly and gently mix the contents by inverting the tube.
   DO NOT VORTEX! A precipitate should form.

7. Store the tube **on ice** for **3 - 5 mins**.

8. Centrifuge at maximum speed for **10 mins** at **room temperature** and aspirate out **400 µl** of top layer.
   Transfer top aqueous layer into a sterile 2-ml microcentrifuge tube.
   Discard bottom layer.
   DO NOT PICK UP ANY PRECIPITATE!!!

   (Optional)
   Add **400 µl** of PCA solution.
   Close the tube tightly and gently mix the contents by inverting the tube.
   Centrifuge at maximum speed for **3 mins** at **room temperature** and aspirate out the top layer.
   Transfer top aqueous layer into a sterile 2-ml microcentrifuge tube.
   Discard bottom layer.
   This helps remove any residual proteins.

9. Add **900 µl** of 95% / 100% ethanol.
   This is to precipitate the plasmid DNA.

10. Store at **-80°C** for **30 mins**.
    Use the -80°C freezer.

11. Centrifuge at maximum speed for **10 mins** at **room temperature**, gently aspirate out the supernatant and discard it.

12. Add **1 ml** of 70% EtOH.
    Store at **room temperature** for **3 mins**.

13. Centrifuge at maximum speed for **3 mins** at **room temperature**, gently aspirate out the supernatant and discard it.
    Make sure the pellet is toward the outside.

14. Dry the pellet in air for **10 - 15 mins**.

15. *Make sure the pellet is completely dry before this step.*

    Option 1: Add **20 µl** of TE buffer.
    (or)
    Option 2: Add **20 µl** of distilled water.

    Resuspend the pellet by vortexing/by shaking vigorously.
    The DNA will contain RNA contamination, which can be removed by resuspending in TE with RNAse.