

Plasmid Preparation Procedure

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- › Bacteria culture
- › Microfuge Tubes
- › TENS
- › Sodium Acetate
- › 95% Ethanol
- › 70% Ethanol
- › TE
- ›

Procedure

Main Procedure

- ✓ 1. Transfer 1.5 mL bacterial culture to a labeled microfuge tube
- ✓ 2. Centrifuge for 30 seconds to pellet bacteria
- ✓ 3. Pour out most of the growth medium. Leave approximately 100 λ in the tube.
- ✓ 4. Resuspend the pellet by shaking, vortexing, or tapping the tube vigorously.
Make sure the bacteria are completely resuspended and no clumps remain before continuing.
- ✓ 5. Add 300 λ TENS to the tube
- ✓ 6. Mix well by inversion for 2 minutes. Do not shake the tube.
The solution should get viscous
- ✓ 7. Add 150 λ Sodium Acetate and mix well.
- ✓ 8. Centrifuge for 3 minutes
- ✓ 9. Transfer the supernatant (liquid) to a clean, labeled microfuge tube.
- ✓ 10. Add 1 mL 95% Ethanol to the tube and mix well by inversion. You may see DNA as faint as white strands in the liquid.

The tube should be nearly full of liquid.

Sharpie brand pens have an ink that is soluble in Ethanol. Make sure your labels do not get removed from the tubes accidentally.

- ✓ 11. Centrifuge for 5 minutes.

Place the tubes in the centrifuge with the hinges out to make finding the pellet easier.

- ✓ 12. Pour out the 95% Ethanol.

- ✓ 13. Add 0.5 mL 70% Ethanol and mix well by tapping or vortexing.

- ✓ 14. Centrifuge for 5 minutes.

Place the tubes in the centrifuge with the hinges out to make finding the pellet easier.

- ✓ 15. Pour out the 70% Ethanol.

- ✓ 16. Place the tubes upside down and allow them to dry completely.

Residual Ethanol can inhibit restriction digestion or PCR..

- ✓ 17. Resuspend the pellet in the 25 λ TE.

Rinse the side of the tube with the TE to resuspend any DNA not at the bottom of the tube.

- ✓ 18. Use 10 λ of the DNA in a restriction digest of 1 λ for PCR.