**Date:**
**Names:**

# Plasmid Prep

____ 1. Transfer 1.5ml bacterial culture to a **labeled** microfuge tube. (pour directly from test tube into microfuge tube)

____ 2. Centrifuge for 30 seconds to pellet bacteria

**-Always place hinges out in centrifuge**

____ 3. Pour off (into bleach) most of the growth medium into bleach. Leave approximately 100µl in the tube.

____ 4. Resuspend the pellet by shaking, **vortexing**, or tapping the tube vigorously. (make sure the pellet is completely resuspended)

____ 5. Make TENS (makes 15)

____ a. To 4.5 ml TE add:

- 250µl 10% SDS
- 250µl 2N NaOH

____ 6. Add 300µl TENS (make fresh every time) to the tube.

____ 7. Mix well by inversion for 2 minutes. Do not shake the tube.

- The solution should get viscous (snotty)

____ 8. Add 150µl Sodium Acetate and mix well.

____ 9. Centrifuge for 3 minutes.

____ 10. Transfer the supernatant (liquid) to a clean, **labeled** microfuge tube.

- Label while in the centrifuge

____ 11. Add 1m (1000µl) 95% Ethanol to the tube and mix well by inversion. You may see DNA as faint white strands in the liquid. The test tube should be nearly full. Make sure label does not come off because **Ethanol takes off sharpies**.

____ 12. Centrifuge 5 minutes to pellet DNA.

____ 13. Pour off the 95% Ethanol into bleach.

____ 14. Add 0.5 ml (500µl) 70% Ethanol and mix well by tapping or vortexing.

____ 15. Centrifuge 5 minutes to pellet DNA.

____ 16. Pour off the 70% Ethanol into bleach.

____ 17. Place the tubes upside down to allow to dry **completely**. (best if left overnight)

- Leftover ethanol can inhibit restriction digestion of PCR.

____ 18. Resuspend the pellet in 25µl TE.

- Rinse off the side of the tube with TE to resuspend any DNA not on the bottom.

____ 19. Use 10µl of the DNA in a restriction digest or 1µl for PCR.

NOTE: **Always** label! Label on top of microfuge tube and on tape for test tubes. Use black sharpie.

# Parts

| Tube Number | Part | Antibiotic Resistance |
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