**Alternative DNA Isolation Protocol using ZnCl2**

Solutions required: Equipment required:

5ml lysate 2 Microcentrifuges (25°C and 4°C)

Nuclease mix (DNAaseI + RNAaseA) 37°C heat block

0.5M EDTA 60°C heat block

2M ZnCl2 (filter sterilized) Ice

TES buffer: Nanodrop

 0.1M Tris-HCl, pH 8

 0.1M EDTA

 0.5% SDS

10mg/mL Proteinase K

3M potassium acetate, pH 5.2

Isopropanol

70% ethanol

Nuclease-free water

Day One

1. Gently mix your HVL, then aliquot 5mL of your lysate into a 15 mL conical tube. Give that tube to a GSI or TA who will add 20uL of nuclease mix for you.
2. Once nuclease has been added, gently invert the tube and incubate at 37°C for 10min.
3. Aliquot lysate into 5 microfuge tubes, 1mL each.
4. To each tube, add 20uL of ZnCl2, mix gently by inversion, and incubate at 37°C for 5min. This “precipitates” the phage.
5. Centrifuge at 10,000rpm for 1min to pellet the phage.

STEPS 6 and 7 MUST BE DONE QUICKLY AND EFFICIENTLY

1. KEEP THE PELLET. Remove supernatants by aspiration, but try not to disturb the pellet. Discard the liquid filled pipette tips in the sharps trash.
2. Resuspend pellets in 500uL TES buffer per tube, and incubate at 60°C for 15min. This will denature the capsids, exposing the DNA, while protecting it from the nuclease activity (EDTA in the TES sequesters divalent cations required for nuclease function).
3. Add 1uL of Proteinase K and mix gently. Incubate at 37°C for 10min to completely eliminate any residual nuclease activity.
4. Add 60uL of potassium acetate to each tube. Mix well and leave on ice for 15min. A white, dense precipitate will form. This represents your capsids.
5. Centrifuge at 4°C for 1min at 12,000rpm to pellet the capsids. KEEP THE SUPERNATANTS containing your DNA, and place into new microfuge tubes. You can throw away the tubes with the pellets.
6. Add 500uL of isopropanol to each of the tubes with the supernatant, mix, and leave on ice overnight (or until next lab).

Day Two

1. Centrifuge at top speed for 10min to pellet DNA, and discard the supernatant into a WASTE tube. It is OK if you do not see a pellet.
2. Add 250uL of 70% ethanol in each tube, and spin again for 1min, at top speed. This washes your DNA pellet. Discard supernatants into a WASTE tube.
3. Dry the DNA pellets at room temperature by turning upside-down onto paper towels, tapping out excess liquid, and leaving upside-down until pellets begin to turn clear. The tubes can also be placed in a fume hood or 30⁰C incubator to help with drying. DO NOT RUSH THIS STEP! If not dry enough, you will NOT recover enough DNA!
4. Resuspend the first pellet in 50uL nuclease-free water. Then use that solution to resuspend the next pellet. Continue until all 5 pellets have been resuspended in the same 50uL of water.
5. Check DNA concentration and quality (A260:280 and A260:230) with the Nanodrop.

**Record the DNA concentration and A260:280 and A260:230 ratios in your notebook!!**