

REVISED MINIPREP PROCEDURE TO MIMIC MAXIPREPS:

- Differences from kit insert highlighted in bold.
 1. **2x-25mL (+antibiotic) overnight cultures.**
 2. Spin down cells at 2700RCF for 15m. Discard supernatant.
 3. Add 250uL P1 to pellet, resuspend. **Because of residual LB, this brings volume to ~600uL.**
 4. **Divide into 2 Aliquots in 2mL (blunt bottom) eppendorf tubes.**
 5. Add **300uL** (two volumes) of P2 to each, invert slowly 4-6 times.
 6. Add **400uL** N3, immediately invert slowly 4-6 times.
 7. Microcentrifuge at 13,000 rpm for 10 minutes. Meanwhile, label miniprep columns.
 8. Transfer supernatant of each aliquot to separate miniprep columns. Careful not to take white pellet.
 9. Pass through miniprep column by microcentrifuging 45 sec at 10,000 rpm, or in benchtop microfuge for 1 minute.
 10. Discard buffer from bottom of column.
 11. Add 500uL PB to column, microcentrifuge 45 sec at 10,000 rpm, or in benchtop microfuge for 1 minute.
 12. Discard buffer from bottom of column.
 13. Add 700uL PE to column, microcentrifuge for ~1 minute.
 14. Discard PE from bottom of column, microcentrifuge at *13,000 rpm for 1 minute to remove all traces of ethanol in PE. This is a key step.* Meanwhile, label eppendorf tubes.
 15. Insert top of first column into 1.5mL eppendorf tube. Add 50uL EB dropwise to center of first column.
 16. Let sit for 1 minute, then microcentrifuge at 13,000 rpm for 1 minute.
 17. **Insert top of second column into same eppendorf.**
 18. **Add EB from bottom of eppendorpf dropwise to center of second column (double elution with same EB to concentrate the DNA).**
 19. Let sit for 1 minute, then microcentrifuge at 13,000 rpm for 1 minute.

For all minipreps, make a gel sample of 4uL DNA, 2uL 6x Loading Dye.
Run a 1% Agarose gel, take a picture, save image.