# A FOOLS GUIDE TO MINIPREPS WITH DAVID DANKORT AS YOUR GUIDE

**Reagents needed:**

14ml tubes with 3ml LB + antibiotic
Qiagen solutions: P1 (with RNAse), P2 and ice cold N3 sol$^{n}$ (these can be made or can be used from kits)
Chloroform, isopropanol, 70% ethanol, 100% ethanol,
TE (with 1ug/ml RNAse)

**Day 1**

1. Inoculate bacteria by picking up a colony using a p20/p200 tip and dropping it into a Falcon tube 2059 containing3ml LB+antibiotics and grow overnight (or 6hours for Mach1/Turbo bacteria)

**Day 2**

1. Remove tip, and save this aliquot of bacteria for later use.

   If you don't have a bacterial stock, either save 10ul in a labeled eppendorf or spot to an LB plate (again containing drug of choice).
2. Pellet bacteria in the Falcon tube by centrifugation at 3000-4000 rpm for 7 min.
3. Resuspend pellet in **200ul of P1 sol$^{n}$**.

   With large numbers of samples just put P1 solution in and place back into incubator and shake for a few minutes while you label centrifuge tubes used in step 6&8. If this doesn't work well drop a tip in and use it to disperse bacteria. The tip need not be removed. Just leave it behind at step 6.
4. Add **200ul of sol$^{n}$ P2**, vortex briefly and incubate 5min at RT
5. Add **300ul of sol$^{n}$ N3**, vortex briefly and incubate 5min on ice.

   (you can use chilled or room temp N3 at this point.)
6. Add **400ul of chloroform**, vortex and pour contents to labeled microcentrifuge tube.

   Do not worry about getting every microliter - it doesn't matter, your success will be binary.
7. Centrifuge at 15000 rpm for 5 min.
8. With a p1000, remove 500ul of top phase to a 1.7 ml microcentrifuge tube.

   Do not worry about getting every ul it doesn't matter. The key thing here is to minimize the amount of "crud" transferred. Coloured tubes make seeing the DNA pellet a little easier although with time you won't even look for it.
9. Fill tube with **isopropanol**, mix and incubate at RT for 5-10min.
10. Centrifuge at 15000 rpm for 5-10 min.
11. Rinse pellet in 70% ethanol (squirt bottle) and centrifuge at 15000 rpm for 2 min.
12. (optional) Rinse pellet in 100% ethanol and centrifuge at 15000 rpm for 2 min.
13. Remove traces of ethanol and dry in 50-65°C heat block.
14 Resuspend in **150ul of TE** containing **1ug/ml RNAse A**.
15. Use 5-10ul per restriction digest.

## A SHORT GUIDE TO MINIPREPS WITH DAVID DANKORT AS YOUR GUIDE

**Reagents needed:**

14ml tubes with 3ml LB + antibiotic
Qiagen solutions: P1 (with RNAse), P2 and ice cold N3 sol$^{n}$
Chloroform, isopropanol, 70% ethanol, 100% ethanol,
TE (with 1ug/ml RNAse)

**Day 1**

1. Inoculate 3ml of bacteria Falcon tube 2059 and grow overnight (or 6hours for Mach1/Turbo)

**Day 2**

1. Save aliquot and pellet by centrifugation at 3000-4000 rpm for 7 min.
2. Add **200ul of P1 sol$^{n}$** and resuspend pellet
3. Add **200ul of sol$^{n}$ P2**, vortex briefly and incubate 5min at RT
4. Add **300ul of sol$^{n}$ N3**, vortex briefly and incubate 5min on ice.
5. Add **400ul of chloroform**, vortex and pour contents to labeled microcentrifuge tube.
6. Centrifuge at 15000 rpm for 5 min.
7. Remove 500ul of top phase to a 1.7 ml microcentrifuge tube.
8. Fill tube with **isopropanol**, mix and incubate at RT for 5 min.
9. Centrifuge at 15000 rpm for 5-10 min.
10. Rinse pellet in 70% ethanol and centrifuge at 15000 rpm for 2 min.
11. (optional) Rinse pellet in 100% ethanol and centrifuge at 15000 rpm for 2 min.
12. Remove traces of ethanol and dry in 50-65°C heat block.
13 Resuspend in **150ul of TE** containing **1ug/ml RNAse A**.
14. Use 5-10ul per restriction digest.

**>>>>>>>>>>>>>>>>>>>>>>>>>>**

## SEQUENCING MINIPREPS

(this will clean up the DNA further, it is not always necessary to do so before sequencing)

1. Take 100ul of miniprep DNA and add **700ul of soln QG**.
2. Add **100ul isopropanol** and mix
3. Add 700ul to top of QIAquick column (from Qiagen's Qiaquick gel extraction kit).
4. Centrifuge at 15000 rpm for 20sec.
5. Discard flow through.
6. Repeat steps 3-5 to purify remaining DNA
7. Add **750ul PE buffer**, centrifuge at 15000 rpm for 20sec and discard flow through.
8 Repeat step 8 once.
9. Transfer QIAquick column to a new tube and spin at 15000rpm for 2-4 min.
10. Transfer QIAquick column to a clean, labeled 1.5ml eppendorf tube.
11. Add **100ul EB buffer** and centrifuge at 15000 rpm for 2 min.
12. Quantify using nanodrop and adjust to the desired concentration.