# Plasmid Preps/DNA Cleanup Protocols

Protocol handbooks for Qiagen kits can be downloaded from the following web page:

Qiagen: http://www1.qiagen.com/literature/

For the Invitrogen maxi prep kit, please be directed to this website:

https://catalog.invitrogen.com/index.cfm?fuseaction=iProtocol.viewUnit&treeNodeID=61666E8D00953D157C184E4B97DAED29&objectid=F9060ED5A16DEF0BF21379999FFA2CC0

If there are no mini prep columns available for your use, you can follow Colin's protocol for mini/maxi preps:

## Modified Maxi Prep protocol:

Buffers 1 and 3 should be kept on ice

1. Spin 250ml of culture (in sterile 500ml bottles) for 10 minutes at 4˚C at 6000g (~6000 rpm)
2. Suck off sup. & resuspend in **10ml of buffer P1**. Get completely into solution- (vortex lightly if need be)
3. Add **10ml of buffer P2**
   Invert at least 3 times
   **Room Temp for 5 minutes**
4. Add **10ml of buffer P3**
   Swirl for 10 seconds
   **Ice for 20 minutes (optional)**
5. Spin at **9000 rpm for 10 minutes at 4˚C**
6. While tubes are spinning, set up a column for each maxiprep ("500") Add **10 ml of QBT equilibration buffer** to column and allow it to flow through
7. Empty sup from spin into a gauze mesh, then allowing to flow into column
8. Once the sup runs all the way through the column, wash column **twice with QC buffer** (filling entire column)
9. Elute into fresh **50 ml conical** with 15ml of QF buffer
10. Add **10.5ml of isopropanol (2-propanol)**
11. Spin in blood centrifuge for **15 minutes at level "4 to 6"**
12. Take off sup, but DON'T TOUCH THE PELLET!
13. Add **300µl of TE buffer,** mix and transfer into **1.5ml eppendorf**.
14. Wash original Conical with **200µl of TE,** mix and transfer into the eppendorf.
15. Add **25µl 3M NaCl2** and then **1ml of ice cold ETOH**
16. Spin **4000 rpm for 2 minutes**

17. Remove sup carefully,
18. Wash with **ice cold 70% ETOH**
19. Spin **4000 rpm for 2 minutes**
20. Take off sup and air dry in hood
21. Dilute DNA in 150μl of H20 and 150μl of TE buffer
22. Spec and dilute to 1μg/μl

## Columnless Mini Prep-

Spins @ 14000 RPM
Buffers 1 and 3 should be kept on ice

23. Spin 1.5ml of culture for 2 minutes
24. Suck off sup. & resuspend in **100μl of buffer P1**
25. Add **200μl of buffer P2**
    Invert 3 times
    **Incubate room temp for 5 minutes**
26. Add **150μl of buffer P3,**
    Shake hard for 10 seconds
    **Incubate on ice for 5 minutes**
27. Add **400μl of chloroform**
    **Vortex**
    **Spin 5 minutes**
    Remove white interface w/ pipette tip
28. Add **400μl of phenol**
    **Vortex**
    **Spin 5 minutes**
    Take off top layer & place into fresh tube
29. Add **280μl of isopropanol (2-propanol)**
30. Spin for **15 minutes**
31. Take off sup
32. Add **500μl of ice cold 70% ETOH**
33. Spin for **15 minutes**
34. Take off sup and air dry in hood
35. Dilute DNA in 25μl of H20 and 25μl of TE buffer