Macherey-Nagel Maxiprep Protocol:

In advance: Thaw one tube of Tris elution buffer (kept in the freezer near Maria's bench)
for each maxiprep you are planning. Also make sure you have at least 5 ml of 70%
ethanol (made from pure ethanol and ddH20, filtered into a sterile container) for each
maxiprep you are doing. Make sure that the LYS buffer does not contain white particles,
if it does, warm it to dissolve them, then cool down to room temperature.

1. Grow a 500 ml culture overnight.

2. Spin down at 6000 x g for 15 minutes.

3. Discard supernatant and place pellets on ice.

4. Add 20 ml cold RES buffer (to which Rnase has been added) to each pellet. Resuspended pellet by stirring and carefully pipetting up and down with a 25 ml pipette until the pellet is fully dissolved and no pellet clumps remain on either the inside of the centrifuge tube or on the pipette's tip.

5. Add 20 ml LYS buffer to pellet. Screw on cap and gently mix by inverting 5 times. Do not overmix or vortex, as this could shear the DNA. Incubate at room temperature for 4-5 minutes.

Important: Do not let the lysis time exceed 5 minutes. The Macherey-Nagel guide warns on page 23: "Prolonged exposure to alkaline conditions can irreversibly denature and degrade plasmid DNA and liberate contaminating chromosomal DNA into the lysate."

6. Set up columns so that they can drain into a container. Equilibrate columns by applying 25 ml of EQU buffer around the top rim of the paper filter cup. Dribble a thin stream of EQU around the rim until the full volume is applied.

7. Add 20-22 ml NEU and gently invert tube 15 times.

8. Once EQU has drained through columns, carefully pour the neutralized cell lysate
mixture into the center of the paper filter. If you mus wait until the EQU has drained through, mix the lysate by inverting 3-4 times, and then apply. Allow the lysate to drain all the way through. This can sometimes take some time. Be

patient.

9. Rinse lysate completely through column by applying another 25 ml of EQU around the rim of each filter, again in a circular manner. Allow to drain through completely. Discard paper filter cups containing the cellular debris.

10. Wash column with 40 ml of WASH buffer.

11. Transfer columns to a secure place where they can drain into a clean 50 ml falcon
tube. Elute DNA from column using 20 ml of ELU buffer.

12. Add 14 ml isopropanol. Vortex well and let sit for 2 minutes.

13. Pull the plunger out of a fresh 30 ml syringe. Screw a blue finalizer filter onto
the end of the syringe. Pour the isopropanol/DNA mixture into the syringe and insert the plunger. Gently force the liquid through, catching the waste liquid in a beaker. If there is a lot of DNA, sometimes this can require a certain amount of strength and patience.

14. Unscrew the finalizer and place it on a clean kimwipe. Remove the plunger from
the syringe. Rescrew the finalizer onto the syringe. Pour 5ml of filtered 70% ethanol into syringe, reinsert plunger, and force the ethanol gently through.

15. Remove the finalizer to a clean kimwipe. Dry the inside of the syringe with a clean kimwipe or use a new syringe. Screw the finalizer back onto the syringe. Hold yet another clean
kimwipe over the end of the finalizer. Force 30 ml of air gently through the finalizer to remove the ethanol. You will see some on the kimwipe, and if you turn the finalizer over you will see that it is still damp. Keep removing the finalizer, filling the syringe with air, and replacing the finalizer onto the syringe and blowing air through it gently until the paper on the bottom of the finalizer looks very dry and white. This might take 7 or 8 changes of air. Be patient, as it is important not to get ethanol into the final DNA prep.

NOTE: Trace contamination of your DNA with ethanol can lower the amount of protein you get from a transient transfection in mammalian cells so THIS STEP IS CRUCIAL. Be sure to dry the finalizer as much as possible!

16. Elute the DNA out of the finalizer into a clean 1.5 ml eppendorf tube as follows:

Fill a clean 1 ml syringe with 1 ml of Tris buffer. Wet the finalizer to redissolve the purified DNA by pushing half of the Tris through. Balance the 1 ml syringe carefully on the eppendorf collection tube. Wait 4-5 minutes. Carefully and gently push the rest of the Tris through and collect the eluate in the eppendorf tube. Remove the syringe, load it with air, and put it back on the finalizer. Keep flowing air through until all of the Tris has been collected in the tube. Note: sometimes if you look at the bottom of the finalizer, it will still look damp, and you will see that you have not collected a full ml of eluate. In this case, take a fresh 30 ml syringe, fill it with air, and gently blow the air through the finalizer. Be sure not to use too much force or you can splash DNA out of the collection tube.

17. Invert the tube several times to mix DNA solution before taking the OD.

NOTE: When specking the DNA on the nanodrop please note that for pure DNA the ideal ratio of 260 and 280 nm is 1.8. If you notice that the ratio for your maxiprep is higher than 1.8, this could mean there is residual ethanol in your sample or RNA contamination. The Average maxiprep 260/280 ratio is ~1.83-1.86.