**How to do maxi-preps with ClC-3 cDNA.**

1. Elute plasmid from filter paper using TE buffer (note: I usually sent plasmids on filter paper rather than liquid).

2. Transform DH5alpha using normal heat shock transformation protocol and plate onto LB agar with 100 ug/mL ampicillin.

3. Incubate at 37C for 24-36 hours, until many small colonies (and a few large ones) are visible.

4. Pick several of the small colonies, NOT the large ones. The large colonies are almost always negative. I usually pick 12 small colonies to guarantee I get at least a few good ones. Inoculate 5mL LB broth + 100 ug/mL ampicillin for each colony.

5. Incubate with shaking at 37C. What you are looking for are cultures which are growing slowly. Check tubes after 12 hours and discard any which appear to be growing at a "normal" rate.

6. Continue incubating the slowly-growing cultures until they reach an OD600 of 0.5-1.0, which should take 24-36 hours. Do NOT grow them to full turbidity. Discard any cultures which have no growth after 36 hours.

7. For the slightly turbid cultures, spin down 3 mL of bacteria and miniprep pellet, performing the additional washes recommended in Qiagen protocol (otherwise do everything else the same). Elute with 50 uL TE buffer.

8. Measure DNA concentration of minipreps and take note of which colony/colonies had the best yield. You want at least 250 ng/uL. If your highest yield is less than 200 ng/uL, I recommend starting over with new colonies, because your maxiprep yield will be low (with high contamination).

9. Take the best colony, and use 1mL of culture to inoculate 500mL or 1000 mL LB broth + 100 ug/mL ampicillin.

10. Incubate with shaking at 37C for 24-36 hours (again, to an OD600 of 0.5-1.0). Spin down entire culture into 1 or 2 pellets. If you're not doing the maxiprep right away, freeze the pellet at -80C.

11. Maxiprep pellets using a high-purity DNA prep, such as the Qiagen kit, and using a protocol for low copy number plasmids. Due to the large volume of bacteria and relatively low quantity of plasmid DNA, there will be much more gDNA/RNA contamination than a normal prep, so the extra work is worth it.

12. Reconstitute DNA pellet in 500 uL TE buffer and check quality on a gel. That is, linearize 50ng plasmid DNA, load into a 5mm lane, run it out, stain with EtBr, and check with UV. Band should be very thick and bright. If it's not, start over (after you stop crying).

So... it's a lot more steps than a normal maxiprep, but this is still far better than spending a month doing a bunch of failed maxipreps, or worse, doing experiments with a maxiprep that is mostly contamination.