12mer194 array DNA preparation

1. Grow 4 L of Dh5a ecoli in Terrific Broth with phosphate salts (see CSH protocol, pg 87 ntbk3)
   1. Transform fresh and streak plate: **Zeocin selection**, grow over night
   2. Partially scrape plate and start 100 mL starter in the morning, grow ~8 hr
   3. Split starter 4 ways and grow overnight
   4. Spin down pellets and freeze, or go straight to the gigaprep
2. Gigaprep DNA using the quigen kits
   1. I usually do all the addition of all the lysis buffers in the large centrifuge bottles so I can spin down the cell junk with ease
3. Digest with EcoRV, XhoI, StuI, ApaL1 for two days (pg 93, notebook 3)

4 mL plasmid, 8mg total

1 mL 10x cutsmart buffer

40 uL each: XhoI, StuI, EcoRV, ApaLI

H2O to 10 mL

37deg, overnight

check digestion on a 1% agarose gel, add more enzymes and digest for another day if necessary (it usually is).

1. Gel purify DNA out of 5% native PAGE (pg 97, ntbk 3)
   1. I borrowed two sets of the large gel plates from the Narlikar lab
      1. Each set holds 100 mL and I use the combs with two large bucket wells
      2. In each well load no more than 2 mg of DNA, 1 mg might even be too much. Anymore and each band will contains DNA fragments from the other bands regardless of how long the gel is run
   2. Run small amount of DNA in side wells with indicator dye
      1. Cut these off and stain with EtBr
      2. Reassemble gel on UV light box and cut bands
   3. Crush and soak gel
      1. Crush gel through 3 or 10 mL syringe
      2. Resuspend in enough 1xTE to cover the gel and freeze
      3. Double the volume with fresh 1x TE and rock at least overnight
      4. Filter through a 50mL conical vacuum filter (also borrowed form Narlikar lab)
      5. EtOH precipitate DNA

5% native PAGE

12.5 mL 40% acrylamide (37.5:1)

10 mL 10x TBE

H2O to 100 mL

1 mL 10% APS

100 uL TEMED

Run at 200 V, 4 hours, 1xTBE, room temp