Large-scale DNA preps using DMAE columns

(Michael Lin, March 1998)

This protocol is almost identical to the Qiagen maxiprep protocol. The DNA you get from it is as pure as and sometimes better than Qiagen by several criteria: A260/A280 ratios, sequenceability, and transfection efficiency. Yields from 200 ml 2xTY culture range from 200 to 1500 ug, depending mostly on the efficiency of plasmid replication.

According to the data sheet for Fractogel DMAE, "the pH-values of the functional groups of this type of ion exchanger are 1.2-1.5 pH-units below that of the DEAE groups [such as those in Qiagen columns]. This is why they are particularly suited for the separation of strongly acidic bioplymers — especially nucleic acids — from less acidic components... Fractogel EMD DMAE gel is resistant to acid, base, and alcohols. The material can be cleaned for reuse without any sample cross-contamination."

I have had excellent results with plasmids of up to 21 kb, but preps of 36 kb plasmids have been small and impure.

Quick protocol for advanced users

All column steps are performed with one full column volume of solution.

1. Equilibrate column with equilibration buffer (50 mM MOPS 7.0, 500 mM NaCl.).
2. Resuspend 1-2g bugs in 8 ml P1 (50 mM Tris 7.5, 10 mM EDTA, 100 ug/ml RNase A).
3. In 50 ml conical, lyse with 8ml P2 (200 mM NaOH, 1% SDS) <10min.
4. Precipitate protein by mixing in 8 ml cold P3 (3M KOAc pH 4.8-5.5). Spin 15,000g 10min.
5. Apply supernatant to column, filtering out floating debris with cheesecloth.
6. Wash column twice with wash buffer (50 mM MOPS 7.0, 800mM NaCl, 10% ethanol).
7. Elute with elution buffer (50 mM Tris 7.5, 1.2M NaCl.).
8. Precipitate DNA with 0.6-1 vol isopropanol.
9. Wash pellet with 70-80% ethanol, resuspend DNA in buffer of choice.
10. Regenerate the column by successively applying 1 M NaOH, 2-3 M NaCl, and either water, if you will be re-using the column right away, or storage solution (100 mM NaCl, 20% ethanol), if you will be storing the column. Store in a 50 ml conical at 4˚.

Locations and catalog numbers of materials

Polyethylene frits: Alltech #211416 for 100/$36, in Michael's cabinet. Plastic 25ml columns: Alltech#210425 for 100/$39, in Michael's cabinet. Fractogel EMD DMAE-650(M): EM Separations #16884-7 for 50ml/$90 or #16884-1 for 500ml/$595, on Michael's shelf. RNAse A: Sigma R5503 for $144.50/1g, in dessicated stocks in bottom door shelf of -20˚ freezer by centrifuge room.

Detailed protocol for beginners

Columns: To pack a new DMAE column, get two polyethylene frits and a plastic 25ml column. Using a pair of tweezers, push one frit all the way to the bottom of the column. Then pipette 6ml Fractogel DMAE on top. Slowly draw a vacuum from the bottom until the liquid level reaches near the top of the settled resin. (If the vacuum is too strong, the resin will get packed too tightly and gravity drip will therefore be too slow. For comfort, you can place the column on a compatible vacuum manifold, e.g. the blue Promega Wizard manifold.) Push the second frit down carefully so it rests flat at the top of the resin and

does not squeeze the resin too much. Add some equilibration buffer and draw the vacuum through slowly until there are no more air pockets visible in the resin. The column should now be wetted all the way through and thus allow a modest rate of gravity drip. From this point on, do not let columns dry out. Columns left overnight at any step will usually stay moist.

Solutions: All the solutions are made of common components. It is important to use MOPS rather than Tris in the wash buffer because the pH of Tris will drift upwards, which favors elution. Stock solutions of autoclaved 2.5M NaCl and 1M MOPS 7.0 (made by titrating a solution free MOPS acid with 10M NaOH followed by filter sterilization) are useful to have around.

1. Spin down bugs from 100-200ml 2xTY or 200-400ml LB (about 1-2g) for 10min 4° 5000g. While bugs are spinning down, begin equilibrating the DMAE column with equilibration buffer (50mM MOPS 7.0, 500mM NaCl).
2. Resuspend bugs thoroughly in 8ml P1. Shaking the solution in the centrifuge bottle works fine. (P1 is the same as Wizard Resuspension Buffer. To make it, dissolve 6.1g Tris base and 3.7g EDTA•2H2O in 1L water, adjusting the pH to 8.0 with HCl. Add 100mg of RNase A that had been dissolved in 10ml TE and boiled 15min to inactivate contaminating DNase.)
3. Move the suspension into a 50ml conical and lyse bugs at RT with 8ml P2. Invert the tube to mix in the P2. Stop lysis at 10min or when solution clears, whichever is sooner. (P2 is the same as Wizard Lysis Buffer. To make it, dissolve 8g NaOH and 10g SDS in 1L water.)
4. Precipitate protein with 8ml cold P3 (3M KOAc pH 4.8-5.5). Invert until the lysate becomes clear and well separated from the precipitated protein. Spin 15,000g 10min. (To make P3, dissolve 295g KOAc in about 700ml of water. Add glacial acetic acid until the pH is 5.5, i.e., about 110ml. Add water to 1L.)
5. Apply supernatant to column, filtering out floating debris with cheesecloth.
6. Wash column twice with wash buffer. (Wash buffer is 50mM MOPS 7.0, 800mM NaCl, 10% ethanol. Be careful with the NaCl solutions; 800mM NaCl is close to the concentration where DNA begins eluting from the column.)
7. Elute with elution buffer (50mM Tris 7.5, 1.2M NaCl.).
8. Precipitate DNA with 0.6-1vol isopropanol. Carefully pour off the supernatant. The pellet will be clearer and more fragile than a Qiagen pellet, and often breaks before you can get all the supernatant off.
9. You can transfer the remainder of the supernatant containing pellet fragments with a pipette to an eppendorf, which you can then spin down to collect the pellet fragments, and then perform ethanol washs on both the 50ml conical and the eppendorf. Alternatively, you can do a high-volume ethanol wash of the 50ml conical containing some isopropanol.
10. Regenerate the column by successively applying 1M NaOH, 2-3M NaCl, and either water, if you will be re-using the column right away, or storage solution (100mM NaCl, 20% ethanol), if you will be storing the column. Store the column in a 50ml conical at 4°. Because Fractogel is an synthetic polymer, it is less supportive for bacterial growth than regular matrix materials such as dextran or dextrose. However, the ethanol-containing storage buffer further prevents contamination. Only flat-capped 50ml conicals, such as the purple-capped VWR brand conicals, can fit the column inside.