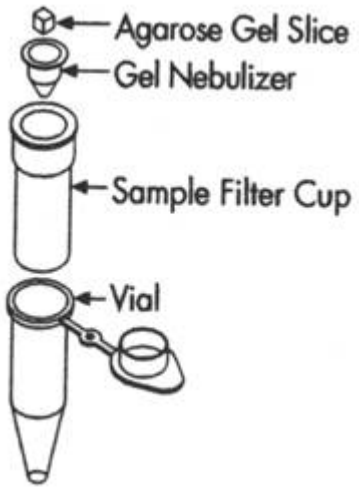
Extract DNA from Agarose Gel Using Montage DNA Gel Extraction Device (Millipore)

Quan Kang 11/5/2003; Updated by JK Park 4/20/04, Commented by TCH

1. Prepare a **six-well** minigel of **0.5% to 0.8%** agarose [**Note: The gel is thicker than regular ones, and you will need 70-100ml to pour such a gel**].
2. Load the DNA digestion mix (usually **100ul** reaction plus **20ul** of 6x GSB dye) [**Note: If you have spare wells, leave at least one blank well between samples in order to minimize potential cross-contaminations**].
3. Run the gel at 60 to 75 voltages for 30 min. to 60 min [**Note: The running time depends on the size of your interested DNA fragment**].
4. Locate the DNA band of interest in the gel using a UV lamp and cut the gel containing the DNA. Trim down the gel piece as much as possible. **The gel slice should be under 100µl in volume or 100mg in mass.**
5. Make sure the montage DAN gel extraction device (a.k.a. ULTRAFREE-DA spin column, Millipore) is assembled as follows:



6. Place the gel slice in the Gel Nebulizer and seal the device with the cap attached to the vial.
7. Spin the assembled device for 10-15 minutes at 5,000×g (~7,500 rpm).

8. Discard the Sample Filter Cup and Gel Nebulizer units.
9. Recover the eluted DNA, and bring the volume to 200ul with ddH2O. Add 100ul 7.5M ammonium acetate.
10. Extract the DNA mix with phenol-chloroform by adding 250ul PC-8, vortexing, and briefly spinning down [**Note: Repeat the PC-8 extractions if dirties still appear at the interphase**]. Transfer the clear DNA solution to a new 1.7ml microfuge tube.
11. Precipitate DNA by adding 4ul seeDNA and 600ul to 700ul of ice-cold 100% ethanol. Centrifuge the sample at top speed for 5 min. Wash the pellet with 70% ethanol twice.
12. Air-dry the pellet. Dissolve the DNA in **12ul** ddH2O, which is ready for use (e.g., use **3ul** per ligation reaction).