A42: Protocol of Gel Fragment Extraction with MIDSCI Kit
10/7/08 by TCH

Kit Contents (MIDSCI Order # IB47101)
- DF Buffer
- Wash Buffer
- DF Column
- 2ml Collection Tube
- Elution Buffer

Excise the agarose gel slice containing relevant DNA fragment and remove extra agarose to minimize the size of the gel slice (It is better to use TAE buffer to make the gel rather then TBE buffer)

Step 1 Gel Dissociation
- Transfer up to 300mg of the gel slice into a 1.7 microcentrifuge tube;
- Add 500ul of DF Buffer to the sample and mix by vortex;
- Incubate at 55-60°C until the gel slice has completely dissolved (about 5-10min). During the incubation, invert the tube 1-2 times.
- Cool the dissolved sample mixture to room temperature.

Step 2 DNA Binding
- Place a DF Column in a 2ml Collection Tube.
- Apply 800ul of the sample mixture from the previous step into the DF Column.
- Centrifuge at full speed (13,000rpm) for 30 seconds.
- Discard the flow-through and place DF Column back in the Collection Tube.
- If the sample mixture is more the 800ul, repeat this DNA Binding Step.

Step 3 Wash
- Add 600ul of Wash Buffer (ethanol added) into the DF Column.
- Centrifuge in full speed for 30 seconds.
- Discard the flow-through and place the DF Column back in the Collection Tube
- Centrifuge again for 3 minutes at full speed to dry the column matrix.

Step 4 DNA Elution
- Transfer the dried column on a new microcentrifuge tube (1.7ml tube can be used here)
- Add 15-50ul of Elution Buffer or water in the center of the column matrix.
- Let stand for 2 minutes until the Elution Buffer or water is absorbed by the matrix.
- Centrifuge for 2 minutes at full speed to elute the purified DNA.

Caution
- DF Buffer contains guanidine thiocyanate which is a harmful irritant. During operation, always wear a lab coat and disposable gloves.
- Add ethanol to Wash Buffer prior to initial use.