DNA Gel extraction protocol

Here is a suggested protocol; this protocol can be used to purify a wide range of DNA fragments with recoveries of >80%. The bolded should be noticed for a nice DNA extraction.

1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade.

Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 ml tube and weigh. Record the weight of the gel slice.

2. Add a 3:1 or 6:1 volume of Solution Buffer B2 to the gel slice (volume:weight)

Incubate the gel mixture at 50 degree for 5-10 min until the gel slice is completely dissolved.

3. Pour the solution to a fresh adsorption column. Centrifuge at 8000rpm for 30 sec.

Pour off the liq uid in the collection tube. For critical samples, repeat the operation above.

4. Add 500 ul washing buffer (WB) before centrifugation at 9000 rpm for 30 sec. Pour off the liquid in the collection tube. Repeat once.

5. Centrifuge the empty column and tube at 9000 rpm for 1min.

6. Put collection tube in a new tube. Add 15-40 ul elution buffer (EB) to elute the DNA, incubate at room temperature for 1-2 min. Store the purified DNA at -20° C.

Tips:

1. Extract the gel as soon as you excise the gel slice.

2. If the purified DNA will be used for cloning, avoid UV dama ge of the DNA by minimizing the UV exposure to a few seconds or keeping the gel slice on a glass or plastic plate during UV illumination.

3. If a large amount of DNA is purified or if the volume of the binding reaction is greater tha n 1.5 ml increase the incubation time of the binding step to 15 min.

References:

*Current protocols in molecular biology