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. Put EB (elution buffer) at **65 degree** water bathing.
3. Add a 3:1 volume of Solution Buffer to the gel slice (volume:weight) (e.g., add 300 ul of Binding Buffer for every 100 mg of agarose gel). Incubate the gel mixture at 60 degree for 5 min at least **until the gel slice is completely dissolved**. Mix the tube by inversion every few minutes to facilitate the melting process. **Check the color of the solution.** A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 ul of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
4. Pour the solution to a fresh adsorption column. Centrifuge at 13000rpm for 1 min. Pour off the liquid in the collection tube. **For critical samples**, repeat the operation above.
5. Add 600 ul washing buffer (WB) before centrifugation at 13000 rpm for 1 min. Pour off the liquid into beaker.
6. **Centrifuge at 13000rpm for 10 min** to spin the ethanol down.
7. Put the column into a fresh EP tube. If necessary air-dry the pellet for 10-15 min to avoid the presence residual ethanol in the purified DNA solution. **Residual of ethanol in the DNA sample may inhibit downstream enzymatic reactions.**
8. Add 30-50 ul elution buffer (EB) to elute the DNA.
9. Get 5 ul of the eluted sample to identify with electrophoresis.

Notes:

1. Extract the gel as soon as you excise the gel slice.
2. If the purified DNA will be used for cloning, avoid UV damage 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 than 1.5 ml increase the incubation time of the binding step to 15 min.

References:

*Current protocols in molecular biology