Protocol for DNA purification from reaction mixture

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. Put EB (elution buffer) at 65 degree water bathing.
2. Add a 3:1 volume of Binding Buffer to the reaction mixture (e.g., for every 100 ul of reaction mixture, add 300 ul of Binding Buffer). Mix thoroughly. **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.
3. 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.
4. Add 600 ul washing buffer (WB) before centrifugation at 13000 rpm for 1 min. Pour off the liquid into beaker.
5. **Centrifuge at 13000rpm for 10 min** to spin the ethanol down.
6. 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.
7. Add 30-50 ul elution buffer (EB) to elute the DNA.
8. Get 5 ul of the eluted sample to identify with electrophoresis.

**Note:**
1. 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*