

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