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Ethanol precipitation protocol from a book p.286 at the bench

(updated protocol: EtOH is about 70% in the final conc. ,-80 C substituded by on ice incubation)

Given the small volumes, done in the pcr tubes

0 Samples were incubated at 80 C for 20 min to inactivate restriction enzymes

1 To a maximum volume of 450 uL of DNA in water add 1/10 volume of 3 M Na-Ac, pH 4.8. Invert to mix.

2 Add two volumes of 95% or 100% EtOH. Invert to mix.

Vol. of DNA	**10**	**uL**
Vol. of NaAc	**1**	**uL**
Vol. of EtOH	**24.75**	**uL**

3 Precipitate DNA by placing the sample in the cold. Overnight: -20 C; 30 min: -70 C; 5 min: dry ice. (I did on Ice 0C about half an hour or hour)

4 Centrifuge at least at 12,000 rpm for 15-30 min at 4 C.

5 Decant or aspirate and discard supernatant. Drain the tubes by inverting and leaving upside down on the paper towel.

6 Wash the pellet with cold 70% ethanol, Centrifuge at least at 12,000 rpm for 15 min at 4 C.

7 Dry as in step 5.

8 Resuspend the DNA in ddH2O. (In Angela's protocol 10-15 uL)

8' Book reccommends resuspension in TE buffer, pH 8.0 (10 mM Tris-HCl, 0.1 mM EDTA).

Comment: Difficult to dissolve, kept for ` 40 min at 60C in the thermal block with shaking

% stock	ml	H2O, ml	final %
1	559	250	0.691
0.95	1	0.3571	0.7

goal 0.7

pcm184 gell purified		
Vol. of DNA	**10**	**uL**
Vol. of NaAc	**1**	**uL**
Vol. of EtOH	**24.75**	**uL**

pcr4 gell purified		
Vol. of DNA	**10**	**uL**
Vol. of NaAc	**1**	**uL**
Vol. of EtOH	**24.75**	**uL**