MINIPREP

ITO Kei, 1993 October

1. incubate bacteria in 2 ml LB.
   37°C, O/N.
2. mix the media and bacteria well.
3. take 1.5 ml into microtubes / keep 0.5 ml at 4°C.
4. cfg. **4°C, 12000g, 30 sec**.
5. decant out the medium *completely*.
   remove the remaining medium with a pipette.
6. add **100 ul Solution I**.
   vortex
7. add **200 ul Solution II** (freshly prepared).
   invert the tubes 10 times to mix the solution gently, *don't vortex!*
   store on ice, 3-5 min. *(not longer)*
8. add **150 ul ice-cold Solution III**.
   invert the tubes 10 times, keep the tube in an inverted position for 10 sec to disperse Solution III through the lysate.
   store on ice, 3-5 min.
9. cfg. **4°C, 12000g, 5 min**.
10. prepare microtubes with **500ul phenol:CIAA** (25:24:1)
11. transfer the cfg. supernatant to phenol:CIAA.
    vortex
    cfg. **4°C, 12000g, 2 min**.
12. prepare microtubes with **900ul EtOH**
13. transfer the cfg. upper phase to EtOH.
    vortex
    store at R.T. 2 min.
14. cfg. **4°C, 12000g, 5 min**.
15. decant out the supernatant *completely*.
    remove the remaining solution with a pipette.
16. add **180 ul 70%, EtOH**.
17. cfg. **4°C, 12000g, 5 min**.
18. remove the supernatant *completely* with a pipette.
19. dry the pelet for 10 min.
20. dissolve the pelet in **30 ul TE**
21. store the DNA at 4°C

Solution I

50 mM	glucose	0.9 g	glucose (=dextrose)
25 mM	Tris Cl (pH 8.0)	2.5 ml	1 M Tris Cl
10 mM	EDTA	2.0 ml	500 mM EDTA
			H_2O to 100 ml

autoclave, store in 4°C

Solution II

0.2 N	NaOH	5 ml	0.4N NaOH
1 %	SDS	5 ml	2% SDS

mix just before use. (200 * tube number * 1.1) ul

Solution III

5 M KOAc	60 ml	29.44 g KOAc	14.72 g KOAc
acetic acid	11.5 ml	11.5 ml	5.75 ml
H_2O	28.5 ml	to 100 ml	to 50 ml

do not autoclave! (Filteration is not needed, either.)