97.5.2

プラスミドのミニプレップ

(written by Dr. 白尾)

Stock Solution (one liter) in 4℃

Buffer A:	0.05 M glucose	20% (1.1 M) glucose	22.7 ml
	0.025 M Tris-Cl pH8	1 M Tris (pH8.0)	12.5 ml
	0.01 M EDTA	0.5 M EDTA	10 ml

Buffer B: 5 M KAc, pH4.8
(To make the KAc solution, mix 2 vol. of 5M HAc with 1 Vol. of 5M KAc. Acetic acid is 17.4 M.)

(This protocol is for 1.5 ml 5 hour culture from 2 ml culture)

1. Spin down cells, 2 min 12K rpm, 4℃ by ;using eppendorf tube.
2. Resuspend in 100µl in buffer A completely. Let stand 5 min at room temperature
3. Add 200 µl of 0.2 N NaOH, 1% SDS: mix gently. **Don't use Vortex**. (This solution should be made fresh each time. using 10 N NaOH stock.) Let stand 5 min on ice.
4. Add 150µl cold buffer B; mix gently and let stand 5 min on ice. **Don't use Vortex.**
5. Spin 1 min in Eppendorf tube. (This removes SDS, protein, and tangled, denatured chromosomal DNA.)
6. Save supernatant, and add 2 Vol.. of 100% EtOH (900µl). Let stand 2 min at room temperature, then spin 1 min.
7. Rinse pellet with 70 % EtOH, then dry.
8. Resuspend pellet in 100 µl of 1x TE.
9. Add 2 µl of 1µg/µl RNase and incubate 37℃for 30 min. **(Do it in the other room)**
10. Add equal amount of phenol and mix well..Then spin down 1 to 5 min and save supernatant.
11. Add equal amount of chloroform and mix well. Then spin down 1 min and save supernatant.
12. Add 1/10 vol. of 3 M sodium acetate and 2 vol of 100 % EtOH. Incubate in cold and spin down 15 min at 13K.

97.5.2

13. Rise pellet with 70 % EtOH, then dry.
14. Resuspend pellet in 50 µl of 1x TE.