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λgt11 DNAの調整（１０ｍｌ培養）

(written by Dr. 白尾)

A: Stock solution
1. 1 mg/ml RNase A in water.
2. 0.5 mg/ml DNase I

1. Make complete lysis plate (10 cm in diameter) and add 5 ml of SM.

2. After incubation at 4°C for over night, collect the liquid and add $CHCl_3$.

3. Transfer 200 μl o/n LE392 (Y1090) to a sterile tube. Add 5 μl of the bacteriophage stock (1/10 or 1/100 dilution). Mix by tapping the tube. incubate 20 min at 37°C.

4. Add 10 ml of LB medium and incubate for 2-5 hours at 37°C with constant, vigorous shaking. Bacteria should have been lysed and you could have seen the debris of bacteria.

5. When the bacteria was lysed enough, add 1.0 ml of 5M NaCl and add drops of $CHCl_3$, and incubate further 5 min.

6. Collect the supernatant after centrifuge at 3000 rpm for 15 min.

7. Add 1.1g of PEG6000 and store at 4°Cfor over night with gentle rotation. (Use rotator.)

8. Remove the supernatant as completely as possible (You can even wipe the inside of tube with kimwipes or with cotton buds.) and collect the precipitate after centrifuge at 3000 rpm for 15 min.

9. Suspend the precipitate in 0.35 ml of SM solution and transfer to 1.5 ml tube.

10. Add 1 μl of RNase A (1 mg/ml) and 1 μl of DNaseI (0.5 mg/ml), and incubate at 37°C for 30 min.

11. Add 0.1 ml of 5 x STEP and incubate at 50°C for 15 min.

12. Extract with phenol and phenol/$CHCl_3$ each twice or more if the interphase is not clear.

13. Add two volumes of 100% ethanol. Wash with 70% ethanol and dry.