

Phage T7 Assembly TX/TL

Aim of the experiment

This protocol is used to assembly phage in vitro method by our homemade cell extract.

Materials

- Buffer B (self-made)
- Cell Extract E (self-made)
- GamS (Arbor)
- dNTPs (NEB)
- Phage DNA (self-isolated)
- Nnuclease-free H₂O

Preparation

1. Prepare Buffer B from the below table.

Concentration (mM)	Reagents
6	Mg-glutamate
100	K-glutamate
3	DTT
1.5	each amino acid except leucine
50	HEPES
1.5	ATP and GTP
0.9	CTP and UTP
0.2 mg/ml	tRNA
0.26	CoA
0.33	NAD
0.75	cAMP
0.068	folinic acid
1	spermidine
30	3-PGA
2%	2% PEG-8000

Procedure

Assembly

1. Keep the reagents on ice. Extract and Buffer must thaw on ice.
2. Mix the reagents in the following order:

Concentration	Reagents
8.9 μ l	Buffer B
3.3 μ M	GamS
0.5 mM	dNTPs
Fill to 20 μ l	Nf H ₂ O
7.1 μ l	Extract E

3. Incubate for 10 minutes on ice.
4. Add 0.5 nM of Phage DNA.
5. Incubate at 29°C for at least 5 hours (overnight possible).
6. Following: Measurement of number of Phages: e.g. Plaque Assay.
7. Prepare also the negative control: mix Buffer B, dNTPs, Extract E and fill it with water (do not add GamS and DNA genome)

Plaque Assay

1. Prepare 1% agar in NZCYM-media plates.
2. Add 4 ml 0.5% Agar in MZCYM-media in falcon tubes and put them on water bath at 48°C.
3. Set a dilution row 10 – 2 to 10 – 10 (1 ml) → use 10^{-4} , 10^{-6} , 10^{-8} , 10^{-10} .
4. add 100 μ l of bacteria culture (from incubator) and 100 μ l of Phage dilution to the 4 ml falcon tube
5. Pour out the solution from the falcon tube on a MZCYM plate and let it incubate overnight at 37°C.

6. Prepare control plate: add 100 μ l of bacteria to 4ml MZCYM in falcon tube and make spot test:

Control	Volume (μ l)	Reagents
positive	5	T7 of current assembled one
positive – positive	5	T7
negative	5	Phage buffer (1xPBS, 1mM $MgCl_2$, 1mM $MgSO_4$)