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TXTL phage production

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We use this protocol and it's working

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Abstract

This protocol explains how to use the Abor BiosciencesSigma 70 TXTL mix to produce bacteriophages. This protocol works for bacteriophage T7. It has been tested on phage lambda. However, it seems that the current protocol does not work for phage lambda.

Materials

Arbor BiosciencesmyTXTL® – Sigma 70 Master Mix
Phage genome
60% W/W PEG-8000 solution
150 µM ProteinGamS
10 mM dNTPs mix
GFP positive control plasmid (should be under a sigma 70 promoter)

Safety warnings

As phages can be produced using this protocol, watch out whether working with bacteriophages is allowed in your lab. While *E. coli* bacteriophages are not dangerous to humans, they can be dangerous to experiments of others.

1 Calculate ratios of ingredients. TXTL master mix should always be ¾ of the reaction. Concentrations of other ingredients can be varied, but these are the tested concentrations:

	Ingredient	Concentration
	PEG-8000	3%
	GamS	3.3 µM
	dNTPs	0.5 mM

The remaining part of the reaction should consist of the DNA of choice and MQ water.

As a positive control for the TXTL reaction GFP can be used. Make sure that the GFP plasmid has a sigma 70 promoter and that the sample is RNase free. For the Arbor Biosciences GFP positive control plasmid the recommended concentration is 5 nM. A reaction volume of 12 µL is recommended by Arbor Biosciences. Reactions can be carried out in 96 wells plates or in eppendorfs. When using a 96 wells plate, make sure to seal the plate properly to prevent contamination.

Note

Concentration of DNA used can be varied. Recommended DNA concentration according to literature is 0.25 nM of DNA for phage T7. [1] Lower DNA concentrations do also work. T7 phage production has been observed at concentrations as low as 0.04 nM. For different phages, different genome concentrations might be optimal.

1 Rustad, M., Eastlund, A., Jardine, P., & Noireaux, V. (2018). Cell-free TXTL synthesis of infectious bacteriophage T4 in a single test tube reaction. Synthetic Biology, 3(1), ysy002.

2 After pipetting the ingredients together, the mixture should be incubated for 12:00:00 to 16:00:00 at 30 °C. Other temperatures might be optimal for different phages.

3 To check for phage production, a plaque assay can be done.

Protocol

NAME

Plaque assay

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PREVIEW

3.1 Streak LB plate with E. coli (strain should susceptible to phage lambda. Strains that include a prophage lambda are likely to be resistant. Strains LE392 and DH10B have been used with this protocol) and incubate overnight at 37 °C

3.2 Pick a colony from this plate and use it to inoculate 10 mL of LB. Incubate this culture at 37 °C until OD reaches 2-3. Overnight culture is recommended.

3.3 Make a range of dilutions . Mix 100 µL of phage dilution with 200 µL of E. coli culture. Then incubate for 10 minutes at room temperature. After 00:10:00 add 3 mL of liquid soft LB agar (LB agar with 0.7% agar) (50 °C).

3.4 Pour the resulting mixture on LB plates (preheat plates at 37 °C). Spread the mixture on the plate by moving it. Make sure to work quickly to avoid clumps of solidified agar.

3.5 Incubate for 00:15:00 at room temperature. Afterwards turn the plates over and incubate overnight at 37 °C .

3.6 Use plates with 30-300 plaques to determine phage concentration. Calculate the Plaque Forming Units (PFU)/mL by the following formula: PFU/mL = N × 1/DF × 1/V.

N is the number of plaques of lysis counted on the plate (expressed as PFU); DF is the dilution factor and V is the volume of phage dilution poured on the plate.