



Aug 27,  
2019

## Preparation of SLiCE Extract

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Works for me

[dx.doi.org/10.17504/protocols.io.6vbhe2n](https://doi.org/10.17504/protocols.io.6vbhe2n)



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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Y. Zhang, U. Werling and W. Edelmann, "SLiCE: a novel bacterial cell extract- based DNA cloning method," Nucleic Acids Research, vol. 40, no. 8, p. e55, 2012.

- 1 Acquire CellLytic TM B Cell Lysis Reagent (Sigma, B7435).
- 2 Prepare sufficient chloramphenicol stock and streptomycin stock to produce LB agar plates (10 µg/mL streptomycin and 12.5 ug/mL chloramphenicol), 1 L of 2X YT media (10 ug/mL streptomycin), and LB (10 ug/mL streptomycin and 12.5 ug/mL chloramphenicol).
- 3 Prepare sufficient L-(+)-arabinose (Sigma, A3256) to induce at 0.2% (weight/volume) in 50 mL.

Prepare 1 L of 2X YT media as follows:

16 g Bacto-tryptone  
+ 10 g Bacto-yeast extract  
+ 5 g NaCl  
+ base to pH 7.2 (1 M NaOH or 1 M KOH)  
+ \_\_\_\_\_dH<sub>2</sub>O to  
1 L

### Section 2

- 4 Autoclave to sterilize. Once cooled, under sterile conditions add streptomycin to 10 ug/mL. Store at 4 C.
- 5 Streak PPY glycerol stock or fresh culture on an LB agar plate (10 ug/mL streptomycin and 12.5 ug/mL chloramphenicol). Incubate at 37 C overnight.
- 6 Inoculate 1 colony into a 50 mL tube containing 25 mL 2X YT with 10 µg/mL streptomycin. Incubate at 37 C with heavy shaking (~338 rpm) overnight.
- 7 Measure OD<sub>600</sub> of the overnight culture.

- 8 Note on OD600: Use extra media as blank. Ensure culture is evenly mixed when taking OD600 samples. Take out OD600 samples from cultures under aseptic conditions (flame, positive airflow cabinet) Measure OD600 within the photometric linear range for your spectrophotometer (~0.1- 1.0, variable by machine). Dilute sample ~1/10 (with media), measure OD600, and calculate OD600 of culture.
- 9 Using a sterile 250 mL or greater baffled flask, dilute the overnight culture to 0.03 OD600 using 2X YT media with 10 ug/mL streptomycin. Shake at 37 C at ~338 rpm until culture reaches OD600 of 5-5.5.
- 10 Note: Vigorous shaking and aeration are necessary to reach OD600 5-5.5. Measure OD600 over time and calculate doubling time to project endpoint. If doubling takes 40 minutes this will be ~5 h 20m, but growth will slow toward the end. If culture begins to plateau in stationary phase, begin arabinose induction. Cell lysate will function, but at potentially lower ligation efficiency. Obtaining OD600 5-5.5 before induction is ideal.
- 11 Induce by adding 0.2% L-(+)-arabinose to the culture. Continue shaking at 37 C at ~338 rpm for 2 hours to express  $\lambda$  prophage protein Red. Measure final OD600.
- 12 Transfer the remaining ~48 mL of culture into two 50 mL centrifuge tubes. Pellet by centrifugation at 5,000 x g for 20 minutes at 4 C. Decant/aspirate the supernatant.
- 13 Wash each pellet with 50 mL ddH<sub>2</sub>O. Pellet by centrifugation at 5,000 x g for 20 minutes at 4 C. Decant/aspirate the supernatant. Measure the wet weight of each pellet.
- 14 Resuspend each cell pellet (~0.25 g each) in 300  $\mu$ L CellLytic TM B Cell Lysis Reagent (Sigma, B7435). Briefly vortex and spin down. Transfer the resuspended cells into a low-binding 1.5 mL tube, and incubate at room temperature for 10 minutes to allow lysis to occur.
- 15 Centrifuge cell lysates at 20,000 x g for 2 minutes at room temperature to pellet insoluble cell debris. Remove the supernatants from the cell debris into a single 1.5 mL tube, being careful not to disturb cell debris.
- 16 Mix the cell extract with equal volume of 100% glycerol. Dispense into 40-60  $\mu$ L aliquots in low-binding 0.5 mL tubes. Label appropriately as PPY SLiCE extract.
- 17 Store the PPY SLiCE extract at -20 C for <2 months, or at -80 C for long term storage. Aliquots can be thawed on wet ice and refrozen up to 10 times without significant loss of activity.

#### Maintaining the PPY Strain

- 18 Inoculate 1 single colony of the PPY strain from an LB agar plate (10 ug/mL streptomycin and 12.5 ug/mL chloramphenicol) into 5 mL LB (10 ug/mL streptomycin and 12.5 ug/mL chloramphenicol) and incubate at 37 C at 200-338 rpm overnight.
- 19 In a sterile tube add equal volumes of PPY culture and 20% autoclaved glycerol. Mix and store at -80 C.



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