P1 transduction

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[1]In-house protocol

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1 *Works for me* This protocol is published without a DOI.

Eadewunm

PROTOCOL CITATION

Elizabeth Fozo 2020. P1 transduction. **protocols.io**
https://protocols.io/view/p1-transduction-bpznmp5e

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Prepare P1 phage

1 Inoculate a single colony into 5 ml LB medium (+ appropriate antibiotic if needed)

2 Shake at **37 °C** overnight

3 Dilute to be 0.01 in 10 ml LB medium + **50 µl** of 1 M CaCl2 + 0.2% glucose final

4 Grow at **37 °C** for 60 minutes (don't want the cells to get much above 0.1 OD600)

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5 Add **10 µl** , **50 µl** , or **100 µl** $P1_{KC}$ phage lysate

6 Grow at **37 °C** until lysed (ideally~3 hr)

7 Add **200 µl** drops chloroform, vortex for 30 seconds

8 Spin at ? for 10 minutes

9 Transfer the supernatant into a new 15 ml tube

10 Add 100 ul chloroform

11 Store in fridge

P1 transduction

12 Inoculate a single colony into 5 ml LB medium (+ appropriate antibiotic, if needed)

13 Shake at **37 °C** overnight

14 Spin down cells

15 Resuspend in 5 ml MC buffer (10 mM MgCl2, 5 mM CaCl2)

16 Set the transduction mix in a 1.5 ml Eppendorf tube

Tube#1	Cells	P1 lysate
1	0.1 ml	_
2	0.1 ml	10 ul
3	0.1 ml	50 ul
4	0.1 ml	100 ul
5	-	100 ul

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17 Incubate at RT for 20 min without shaking

18 Add **20 µl** 1 M NaCritrate

19 Shake at **37 °C** for 1 hr

20 Plate **100 µl** on LB plate + appropriate antibiotic

21 Incubate plate at **37 °C** overnight

22 Examine colonies = transductants

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