

# LAMDA DNA MINIPREP

## BEFORE STARTING EXPT:

DNase I on ice

Make phage prep buffer I (see below)

Water bath @ 65 C

Total time for one phage split into two tubes: 5 1/2 hr

## PROTOCOL:

1. Grow 33 mL of phage plate lysate for this prep. Place lysate in sterile large tube. (You will end up with ~33 mL if have three large plates of confluent lysis in which 10 mL of SM has been added to each.)
2. Add 33 $\mu$ L of DNase I (stock 10 mg/mL). Any leftover culture can be stored over chloroform as a lysate.
3. Let sit at RT for 15 min, or at 4 C overnight.
4. Split the lysate into two tubes (16.5 mL each). Add 4.3 mL of phage prep buffer I ( 0.3 M Tris-HCl pH = 9.5, 0.15 M EDTA, 1.5% SDS) to each tube.
5. Incubate at 65-70 C for 15 min then cool down at RT or on ice.
6. Add 2.8 mL (a total of 5.6 mL in the two tubes) 8 M KOAc and keep on ice for 15 min.
7. Spin down precipitate for 20 min @ 3000 rpm.
8. Decant the supernatant into two new tubes (23-24 mL will end up in each tube). Add 9.5 mL isopropanol to each tube. Let sit at RT for 2-5 min.
9. Spin down the phage DNA for 20 min @ 3000 rpm.

10. Discard the supernatant and let the pellets air dry by inverting the tubes for a few minutes. Resuspend both pellets in TE so that the resulting volume of the DNA solution is ~100
11. Transfer to an Eppendorf tube; add 1  $\mu$ L of RNase (10 mg/mL) and incubate @ 37 C for 30 min.
12. Phenol extract, phenol-chloroform extract, chloroform extract. Use equal volumes and spin for 3 seconds in microfuge to separate phases.
13. Add NaCl to 0.1 M, then add 1 volume of isopropanol and let sit at RT for 2-5 min.
14. Spin for 5 min in microfuge; discard the supernatant.
15. Wash with 70% ethanol and spin again for one minute.
16. Dry pellet under vacuum; resuspend in 100  $\mu$ TE.

Phage Prep Buffer I

	[ i	[ j]	Vi	Vf
Tris pH 9.5	1 M	0.3 M	3 mL	
SDS	10%	1.5%	1.5 mL	
EDTA	0.5 M	0.15 M	3 mL	
ddH <sub>2</sub> O			<u>2.5 mL</u>	
<b>Total: 10 mL</b>				