Helper Phage

The following is based on the λZAP cDNA synthesis manual from Stratagene. See also the Stockinger Lab protocol "M13K07 Helper Phage Production."

Background:

Stratagene provides two different helper phages with their λZAP-cDNA synthesis kit:

(1) ExAssist interference-resistant helper phage
(2) VCSM13

ExAssist is used to excise, *in vivo*, the pBluescript phagemid from the λ Uni-ZAP XR vector. To do this efficiently and without contaminating the excised phagemid with the ExAssist helper phage, this methodology utilizes the Stratagene *E. coli* strain SOLR. This is because ExAssist helper phage contains an amber mutation (UAG) that requires an amber suppressor tRNA in order to grow. When plated on the SOLR nonsuppressing strain (Su-) strain, only the excised phagemid is permitted to grow. However to propagate (amplify) the ExAssist helper phage requires using the E. coli strain XL1-Blue MRF′ because this strain harbors the supE44 mutation, which provides a glutamine suppressor tRNA. The VCSM13 helper phage is used strictly to produce single stranded DNA from the already excised phagemid. (This is also what M13K07 is used for.)

Storing the Helper Phage:

Stratagene supplies both helper phages in 7% DMSO and recommends storing them at -80°C. As long as there is a –80°C stock in the lab, the amplified lab prep can be stored at +4°C.

Titering the Helper Phage:

Streak out E. coli strain XL1-Blue MRF′ onto an LB_{Tet15} plate.

Pick a single colony of XL1-Blue MRF′, inoculate into LB and grow to OD_{600} = 1.0. (No need to include the antibiotics in the overnight cultures.)

Meanwhile prepare a serial dilution of the phage in TE buffer. Expected number of phage should be about 10^{10} pfu/ml; therefore dilute phage to create dilutions 10^{-4} to 10^{-7}.

	Dilutions				
	10^{-2}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Phage	10µl (stock)	10µl (10^{-2})	100µl (10^{-4})	100µl (10^{-5})	100µl (10^{-6})
TE	990µl	990	900µl	900µl	900µl

Note:

When preparing serial dilutions of bacteriophage, it is usually much more accurate to dilute 10µl into 100 to create a 10^{-1} dilution or 10µl into 1000µl to create a 10^{-2} dilution rather than to dilute 1 into 10µl or 1µl into 100µl because one tends to obtain grossly exaggerated titers resulting from small pipetting errors. This is particularly critical for the first dilution.

Add 200µl of XL1-Blue MRF′ cells at OD_{600} = 1.0 to individual sterile culture tubes (the number of phage dilutions you plan to plate) in a test tube rack.

Add 100µl of each serial dilution of helper phage to the culture tubes containing the XL1-Blue MRF′ cells.

Place the test tube rack into a 37°C water bath for 15 minutes to allow the helper phage to attach to the cells.

Meanwhile melt NZY Top Agarose in the microwave and allow it to cool to ~48/50°C. You need to pay close attention to the bottle containing the top agarose because it can quickly boil over; alternatively use a low power setting on the microwave.

At the conclusion of the 15-minute incubation, add ~3 ml of the NZY top agarose to the test tube containing the helper phage & E.coli. Remove the tube from the rack (which is still sitting in the water bath) and give it a quick flick of your wrist mixing the contents and immediately pour it onto an NZY plate. (I usually do three plates at one by pipetting 10ml of the NZY top agarose from the bottle, dispensing ~3.3 to one tube, ~3.3 to second tube and then another ~3.3 to a third tube. I then pour the plates starting with the first tube I put the NZY into.)

Allow the top agarose to cool (~5 minutes).

Invert the plate and incubate overnight at 37°C.

Count the number of plaques.

Determine the titer; i.e., pfu/ml using the formula:

$$\frac{\text{(Number of plaques (pfu) X Dilution Factor) X 1000ul/ml}}{\text{Volume plated (µl)}}$$

Where the volume plated (in µl) refers to the volume of the helper phage solution added to the cells.

Amplifying the Helper Phage:

Streak out E. coli strain XL1-Blue MRF′ onto an LB_{Tet15} plate.

Pick a single colony of XL1-Blue MRF′ and inoculate into 10ml 2X YT and grow until $OD_{600} = 0.3$ (~2.5 x 10^8 cells/ml). (No need to include the antibiotics in the overnight cultures.)

Add helper phage at a multiplicity of infection (MOI) of 20:1 (phage-to-cells ratio). Do this for both ExAssist and VCSM13.

Note: (If amplifying VCSM13 helper phage, add kanamycin to a final concentration of 25µg/ml to the medium 30 minutes after the helper phage and cells have been allowed to grow together.)

Grow the culture at 37°C with vigorous aeration (~300RPM) for 8 hours.

Heat the culture to 65°C for 15 minutes.

Spin down the cell debris and transfer the supernatant to a fresh tube.

Titer the helper phage produced.

ExAssist should be 7.5 x 10^{10} to 1.0 x 10^{12} pfu/ml and
VCSM13 should be 1.0 x 10^{11} to 1.0 x 10^{12} pfu/ml

Store at +4°C (Add DMSO to 7% for storage at –80°C).

Notes: Several steps greatly aid the ease and success of pouring good phage onto plates:

(1) Position the 37^{o}C and 48^{o}C water baths right next to each other with enough room in front of them for you to work. Wipe this area down with 70% EtOH prior to plating.

(2) Use plates that have been prewarmed to 37^{o}C (for one hour or more).

(3) Use sterile glass pipettes that have been prewarmed to ~50^{o}C.

(4) Have a pipette holder in the work area.

Media:

LB_{Tet15} plates

		100ml
Bacto Tryptone	1.0g	
Yeast Extract		0.5g
NaCl		1.0g
dH_2O		100ml
Adjust pH to		7.5 with NaOH
Agar		1.5g
Autoclave		
Cool to 48/50°C		
Tetracycline (12.5mg/ml)	120µl	

Tetracycline (12.5mg/ml)

Tetracycline	0.125g
ddH_2O	5.0ml
EtOH	5.0ml
Filter sterilize	
Store at –20°C	

(Tetracycline is light sensitive so keep plates in the dark)

NZY

	1000ml
NZ Amine	10.0g
Yeast Extract	5.0g
NaCl	5.0g
$MgSO_4 \cdot 7H_2O$	2.0g
dH_2O	1000ml
Adjust pH to	7.5 with NaOH (~2 pellets)
Autoclave	

NZY Agar Plates

	1000ml
NZ Amine	10.0g
Yeast Extract	5.0g
NaCl	5.0g
$MgSO_4 \cdot 7H_2O$	2.0g
dH_2O	1000ml
Adjust pH to	7.5 with NaOH (~2 pellets)
Agar	15.0 g
Autoclave	
Cool and pour plates	

NZY Top Agarose

NZY liquid medium	250ml
Agarose	1.75g
Autoclave	

2X YT

	1000ml
Bacto Tryptone	16.0g
Yeast Extract	10.0g
NaCl	10.0g
dH_2O	1000ml
Adjust pH to	7.5 with NaOH (~3-4 pellets)
Autoclave	