M13 Based Phage Scaffold Prep

This protocol is for the purification of the scaffold DNA strand used to build DNA origami structures.

REAGENTS

- Ampicillin sodium salt (Sigma, cat. no. A0166)
- Isopropyl-b-D-1-thiogalactopyranoside (IPTG; Sigma, cat. no. I6758). ! CAUTION Do not breathe the dust. Avoid contact with skin and eyes.
- Triton X-100 (Sigma, cat. no. T8787) ! CAUTION Harmful. Avoid contact with eyes.
- EDTA (Fisher Scientific, cat. no. E478-1)
- Magnesium chloride hexahydrate 99.995% (Sigma-Aldrich, cat. no. 255777-25G)
- Mops (VWR International, cat. no. BDH4522-500)
- 2xYT Broth Capsules Microbial Medium (Research Products International, cat. no. X15640-500.0)
- Sodium phosphate dibasic anhydrous (Fisher Scientific, cat. no. S375-500)
- Sodium phosphate monobasic anhydrous (Fisher Scientific, cat. no. S397-500)
- Polyethylene glycol 8000 (Sigma Aldrich, cat. no. P4463-1)
- Sodium chloride (Fisher Scientific, cat. no. S271-10)
- Tris base (Fisher Scientific, cat. no. BP152-10)
- UltraPure Agarose
- Glacial acetic acid (Fisher Scientific, cat. no. A491-212) ! CAUTION It is evaporative and corrosive. Wear goggles, lab coat and face mask during experiments. Handle acetic acid inside a hood.
- Isopropanol (Fisher Scientific, cat. no. A415-4) ! CAUTION Flammable. Perform all manipulations under a fume hood.
- Ethanol 200 proof
- Luria Broth (Research Products International, cat. no. L24041-500.0)
- Bacto agar (General Stores, cat. no. 4236)
- Hoechst 33258, 2'-(4-hydroxyphenyl)-5-[5-(4-methylpiperazine-1-yl)benzimidazo-2-yl]bezimidazole, is a synthetic bis-(benzimidazole) derivative developed by the Hoechst Pharmaceutical Co.1 {Sapse:1997uz}

EQUIPMENT

- Petri dishes, 100x15 mm CRITICAL All the equipment used for growing cells should be sterilized.
- Shaker incubator, 37˚C
- BioProducts 96-Well PCR Plate (Fisher Scientific, cat. no. 21-402-441)

- Aluminum sealing tape for 96-well plates (Fisher Scientific, cat. no. 11806)
- Disposable Multichannel Pipetter Basins (Fisher Scientific, cat. no. 13-681-500)
- Gilder fine bar grids (Ted Pella, cat. no. G400)
- Qiagen-tip 10000 (Qiagen, cat. no. 10091)
- Teflon tube FEP (Thomas Scientific, cat. no. 9567K10)
- NMR Shigemi tube
- 12x14 cm gel box, OWL Easycast B2 apparatus (Thermo Scientific)

REAGENT SETUP
- JM101 bacteria (New England Biolabs, cat. no. E4106S) Used as competent receiver cell for making new recombinant phage
- JM109 bacteria (New England Biolabs, cat. no. E4107S); or K91endA if available. Used as primary host cell for growth of M13 phage
- M13mp18 Single-stranded DNA New (England Biolabs, cat. no. N4040S) This is the native M13 DNA, ~7308bp long; it can be used as a positive control on gels
- Loading buffer "QBT" : 50mM MOPS pH 7.0, 750mM NaCl, 15%(v/v) Isopropanol, 0.15%(v/v) Triton X-100. CRITICAL It is highly recommended that all buffers used for chromatography applications be filtered.
- Wash buffer "QC" : 50mM MOPS pH 7.0, 1M NaCl, 15%(v/v) Isopropanol.
- Elution buffer "QF" : 50mM Tris pH 8.5, 1.25M NaCl, 15%(v/v) Isopropanol.
- Folding Buffer 20x : 100mM Tris pH~8.0, 20mM EDTA, 200mM $MgCl_2$.

EQUIPMENT SETUP
- Microscope with polarizer and rotating analyzer.
- Thermal cycler (MJ Research)
- NMR spectrometer equipped with a triple resonance probehead.
- RDCs were recorded on protein labelled with 15N, 13C.
- NMR spectra were processed and analyzed by using NMRPipe and nmrDraw {Delaglio:1995wk}.
- Fitting of the dipolar couplings to the known ubiquitin structure was done by singular-value decomposition (SVD), using the program PALES {Zweckstetter:2000fh}{Zweckstetter:2008bp}. The goodness of fit was assessed by both Pearson correlation coefficient (r) and the quality factor (Q){Anonymous:VE4pcpFz}.

PROCEDURE

NANOMOLE-SCALE PRODUCTION OF M13 BACTERIOPHAGE SINGLE-STRANDED DNA "SCAFFOLD STRAND".

This protocol is written and optimized for a modified bacteriophage M13 genome, 7,308 bases in length previously discribed for DNA origami purposes with JM-109 strains {Douglas:2007ek}. It is also the best protocol known to date for generalized M13 phage cultivation and scaffold harvesting

Creating New Recombinant Phage

1. Transform the recombinant M13 bacteriophage RF dsDNA into JM101 cells.
2. Pre-warm LB agar plate at 37°C for ~30 minutes.
3. Perform "quadrant streak" on LB agar plate.
4. Grown overnight at 37°C on an LB-agar plate.
5. Pick single colony from quadrant streak.
6. Inoculate 50mL LB and incubate during 8h at 37°C and ~250rpm.
7. Pellet bacterial cell by centrifugation 6000g 20minutes.
8. From the supernatant recover the phage by polyethylene glycol fractionation (incubation on ice for 30 min with a final concentration of 4% PEG8000, 0.5 M NaCl), followed by centrifugation.
9. Re-suspend phage in 100 mL of 10mM Tris (pH 8.5) and labeled "pre-inoculation phage".

Growing a Phage Stock

1. Pre-warm LB agar plate at 37°C for ~30 minutes.
2. Perform "quadrant streak" on LB agar plate. With JM109 or K91endA cells
3. Grown overnight at 37°C on an LB-agar plate.
4. Pick single colony from quadrant streak.
5. Inoculate 3mL of 2xYT media at 37°C, grow cells on rotator overnight
6. Using 3mL of the overnight culture, inoculate a 2L flasks containing 300mL of 2xYT medium supplemented with $MgCl_2$ to 5 mM final concentration (1.5mL per 300mL of 2xYT)
7. Shake at 280rpm and 37˚C until OD_{650nm} = 0.4. (takes ~2hr)
8. Add 50 mL of the "pre-inoculation phage" stock. Continue shaking at 37°C for 4 hours at 280rpm.
9. Recover phage as described above and re-suspended in 3 ml of 10 mM Tris pH 8.5 and labeled "inoculation phage".

Large-scale Scaffold Harvesting

1. For nanomole-scale production of phage, follow the same procedure as steps 1-5 above except make a 50mL overnight culture in 2xYT in a 250mL flask.

2. Inoculate 12 x 2L flasks each with 300mL 2xYT with a 1:100 dilution of the overnight culture (3mL per flask)
3. Shake at 280rpm and 37˚C until OD_{650nm} = 0.4.
4. To inoculate, use a 50μL aliquot of p7308 "inoculation phage". Add 600μL of buffer: 10mM Tris pH 8.5, 1mM EDTA.
5. Continue shaking at 37°C for 4 hours at 280rpm.
6. After the 4 hour incubation, harvest phage by spinning cultures in 4x1L bottles (~900mL per bottle) to pellet bacterial cells. Spin at 6000xg, 15 minutes, 4°C.
7. Recover supernatant to fresh centrifuge bottles. Again, there should be ~900mL supernatant in each bottle. Directly add dry NaCl to 30g/L (27g/900mL bottle) and dry PEG 8000 to 40g/L (36g/900mL bottle) to these bottles. Alternatively, one may add these dry to empty centrifuge bottles first, and quickly pour the supernatant into them.
8. Mix with Magnetic stir bar until all PEG has dissolved. CAUTION At this point, the supernatant should be a cloudy suspension. If it is still clear, it is likely that there is little or no phage present.
9. After mixing, incubate supernatant on ice for 30 minutes.
10. Pellet phage: 6000g, 15 minutes, 4°C.
11. Remove the supernatant, but do not discard yet. CAUTION The pelleted phage is the fraction of interest, but it is best to save the supernatant in case some is lost (this happens very frequently, one may re-spin the supernatant in new bottles if recovery is a problem)
12. Allow bottles to sit at an angle for a few minutes, and then remove any additional supernatant with pipet.
13. Actively re-suspend pelleted phage into 1/100 the original culture volume with 10mM Tris pH = 8.5, 1mM EDTA. To achieve this, the pellet in each bottle is first resuspended in 5mL buffer, and then rinsed with an additional 4mL.
14. Transfer resuspended phage to 2x40mL centrifuge tubes roughly 18mL per tube. Spin down to remove residual bacterial cells. 6000xg, 15 minutes, 4°C.
15. Recover supernatant to a 50mL conical tube. PAUSE POINT The sample can be stored at -20°C over night (it probably has been 10-14 hours since your day started)
16. Thaw the harvested phage. When thawed split into 2 x 250mL centrifuge bottles, 18mL per bottle.
17. Add 2 volumes PPB2 per bottle to strip phage protein. PPB2 : 0.2M NaOH, 1% SDS.
18. Mix gently by inverting tube. Do this step quickly. Do not allow reaction to extend beyond 2-3 minutes.
19. Add 1.5 volumes PPB3 to neutralize the NaOH. PPB3 : 3M KOAc pH 5.5.
20. Again, mix gently by inverting bottles.
21. Incubate on ice for 15 minutes.
22. Spin down bottles at 16000xg for 10 minutes at 4°C to remove precipitated SDS.
23. Transfer supernatant to 2 fresh 250mL centrifuge bottles.

24. To each bottle, add 1 volume EtOH, mix.
25. Incubate on ice for 30 minutes.
26. Spin down bottles 16000xg, 30 minutes, 4°C to pellet DNA.
27. Remove supernatant with pipette to minimize loss.
28. Wash each pellet with 20mL 75% ethanol.
29. Pellet DNA once more 16000xg, 10 minutes, 4°C
30. To each pellet, apply 10mL of buffer : 10mM Tris pH = 8.5, 1mM EDTA.
31. Allow to sit for 20-30 minutes, and then actively re-suspend any remaining pellet.
32. Wash each bottle with an additional 5mL of buffer : 10mM Tris pH = 8.5, 1mM EDTA to remove residual DNA.
33. Estimate the concentrations of the re-suspended scaffold DNA on a UV/visible spectrophotometer using an extinction coefficient = 37 mg/ml for A= 1.
34. Transfer to conical and freeze at -20°C