Ph.D.™ Peptide Display Cloning System

NEB #E8101S

20 µg

Version 2.0_2/23

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Ph.D. Peptide Display Cloning System Components

Kit components should be stored at -20°C:

M13KE gIII Cloning Vector, 20 µg, 1 mg/ml

M13 Extension Primer
2,150 pmol, 40 pmol/µl

Introduction

M13KE is a simple M13mp19 derivative into which cloning sites have been introduced at the 5´ end of gene III for display of short peptidesequences as N-terminal pIII fusions. The sequence of M13KE is available at neb.com; see DNA Maps and Sequences. Because this is a phage, rather than a phagemid vector, all 5 copies of pIII on the surface of each virion will be fused to the cloned peptide. Displayed proteins longer than 30–50 amino acids may have a deleterious effect on the infectivity function of pIII, therefore this vector is suitable only for the display of peptides and small proteins (unpublished observations). The small insert size does not appreciably attenuate phage replication, allowing the vector to be propagated as phage, rather than as a plasmid (i.e., titer for plaques, not colonies). **Thus**, **the vector carries neither a plasmid replicon nor antibiotic resistance**. This simplifies the intermediate amplification steps during panning considerably, as it is not necessary to express antibiotic genes before plating, and helper phage is not required. The steps necessary to clone a peptide library into M13KE are outlined below. To clone a single peptide sequence, reactions can be scaled down appropriately.

Preparation of Electrocompetent Cells

Ph.D. phage display peptide libraries are constructed and amplified in *E. coli* K12 ER2738 (NEB #E4104). Alternative F´ *E. coli* strains may be used but have not been tested. Refer to Appendix B, General M13 Methods. This procedure will generate enough vials of electrocompetent cells for test ligations and large-scale library production. Electroporation efficiencies should be at least 1 x 10^9 transformants/µg M13KE. Note that cells will have lower efficiency results with ~7 kb M13KE than with a typical 2–3 kb plasmid. On the day the cells will be used (Steps 6–7), inoculate a culture of *E. coli* K12 ER2738 and grow until turbid. Also, pre-warm three LB/IPTG/Xgal plates.

1. **Day 1:** From glycerol stock of a robust growing F´ *E. coli* strain, streak a fresh LB/Tet agarose plate. Incubate overnight at 37°C for 10–16 hrs.
2. **Day 2:** Inoculate two liquid cultures (2 x 20 ml) with individual colonies from day one plate. Shake overnight at 250 rpm, 37°C.

3. **Day 3:** Inoculate 6 liters of LB medium (1L per flask, recommend using 4-liter Erlenmeyer or 2.8-liter Fernbach flasks to maximize aeration) with 1/100 – 1/200 volume (5–10 ml per liter) of overnight culture of *E. coli* K12 ER2738. Shake vigorously (> 250 rpm) until cultures reach an OD_{600} of 0.5–1.0, approximately 3 hrs.

4. **Harvest Cells:** Transfer each culture to a centrifuge bottle (6 in total) and chill on ice for 30 minutes. Then, harvest cells by centrifugation 5000 x *g* for 15 minutes at 4°C. Discard the supernatant.

5. **Wash Cells**:
    a) Suspend each pellet in 1 liter of ice-cold autoclaved H_2O (Milli-Q or equivalent). Centrifuge 5000 x *g* for 15 minutes at 4°C. Discard the supernatant.

    b) Suspend each pellet in 0.5 liter of ice-cold autoclaved 10% (v/v) glycerol in water. Combine in 3 x 1 L bottles and centrifuge 5000 x *g* for 15 minutes at 4°C. Carefully, discard as much of the supernatant as possible without disturbing the pellet.

    c) Suspend each pellet in 40 ml of ice-cold autoclaved 10% (v/v) glycerol in water. Combine in a single bottle (120 ml total) and centrifuge 8000 x *g* for 10 minutes at 4°C. Discard supernatant.

    d) **Final re-suspension**: Suspend the pellet in 12 ml of ice-cold 10% glycerol. Dispense into 120 x 100-µl aliquots and immediately freeze in dry ice or dry ice-ethanol bath. Store at –80°C.

6. **Electrocompetence Test:** Electroporate 1 ng of M13 RF DNA (e.g., M13KE or M13mp19), diluted (~1 ng/µl) in a low ionic strength buffer (such as TE or water) into an aliquot of electrocompetent cells that is thawing on ice. Follow manufacturer's instructions. Suggested parameters (Bio-Rad Gene-Pulser) are: 25 µF, 200 Ω, 2.5 kV. Immediately add 1 ml of SOC medium and incubate with shaking 250 rpm, 37°C for 45 minutes. This gives the cells sufficient time to recover without allowing phage production and infection of untransformed cells, which could result in inflated electroporation efficiencies.

7. Prepare 10^2-, 10^3-, and 10^4-fold dilutions of the outgrowth in LB. Transfer 10 µl of each dilution to a test tube containing 3 ml of Top Agar (equilibrated at 45–50°C) and 200 µl of turbid *E. coli* K12 ER2738 culture. Vortex briefly and immediately spread on LB/IPTG/Xgal plates. Incubate overnight at 37°C and count blue plaques the next day. Note, with exception of the infection step, this plating resembles Phage Titering as described in Appendix B.

Example efficiency calculation:

If 12 pfu are observed on 10^3 dilution plate:

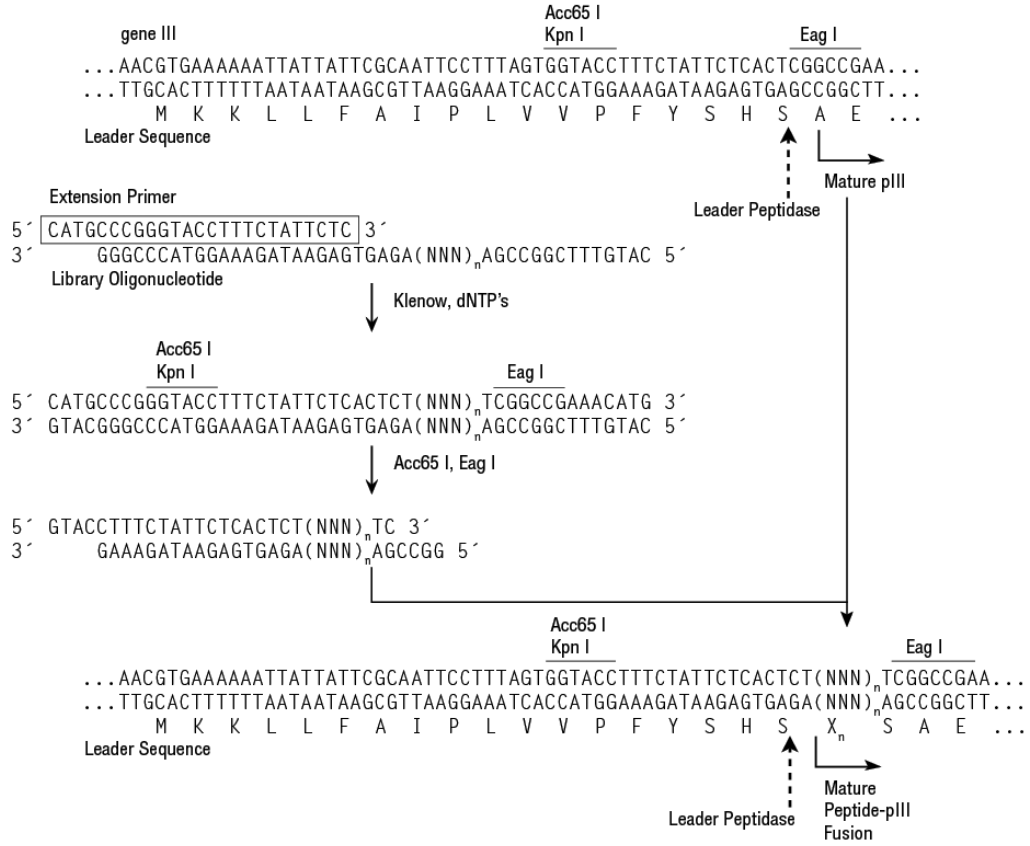
(12 pfu/10 µl) x (10^3 dilution/1 µl outgrowth) x (1100 µl total outgrowth/0.001 µg M13KE) = 1.32 x 10^9 pfu/µg M13KE efficiency

Design and Cloning of Synthetic Oligonucleotide Inserts

The following procedure (adapted from references 1 & 2) is specific for the M13 cloning vector, M13KE, but could easily be adapted for other phage (but NOT phagemid) vectors. More details can be found in reference 3. Detailed protocols for gel purification and phenol:chloroform extractions of nucleic acid reactions may be found in reference 5.

1. Design. Refer to figure to design a library oligonucleotide. The sequence VPFYSHS preceding the leader peptidase cleavage site is part of the pIII signal sequence and should not be altered. The first residue of the displayed peptide will immediately follow this sequence. For randomized positions, relative representations of each amino acid can be improved by limiting the third position of each codon to G or T (= A or C on the synthetic library oligonucleotide). Include a short spacer sequence between the randomized segment and the first native pIII residue to improve target accessibility to the displayed peptide, e.g., the spacer Gly-Gly-Gly between the random peptide and the Ser-Ala-Glu (SAE) shown in figure below. The oligonucleotide should be synthesized on a minimum of 0.2 µmol scale, gel-purified, and accurately quantitated by measuring the OD_{260} in a spectrophotometer (1 absorbance unit at 260 nm = 20 µg/ml of single stranded DNA).

2. Prepare duplex. Anneal 5 µg of the library oligonucleotide with 3 molar equivalents of the universal extension primer 5´-d(CATGCCCGGGTACCTTTCTATTCTC)-3´ (approximately 4 µg for a 90-nucleotide library oligonucleotide) in a total volume of 50 µl TE containing 100 mM NaCl. Heat to 95°C and cool slowly (15–30 minutes) to less than 37°C in a thermal cycler or water bath.



Construction of a peptide library in M13KE. Schematic shows the sequence of the peptide cloning site as well as the strategy for designing and cloning a peptide library into M13KE. The sequence of the extension primer is outlined. N = A, G, C or T; X = any user defined or randomized amino acid.

3. **Extend** the annealed duplex as follows (mix in the given order)

H_2O	119 µl
NEBuffer 2 (10X) (NEB #B7002)	20 µl
Annealed duplex	50 µl
10 mM dNTPs (NEB #N0447)	8 µl
DNA Polymerase I, Large (Klenow) Fragment (5 units/µl) (NEB #M0210)	3 µl
	200 µl

Incubate at 37°C for 10 minutes, then 65°C for 15 minutes. Save 4 µl for later analysis (Step 5).

4. **Digest duplex**

Extension Reaction	196 µl
H_2O	154 µl
NEBuffer r3.1 (10X) (NEB #B6003)	40 µl
Acc65I (10 units/µl) (NEB #R0599)	5 µl
EagI-HF (20 units/µl) (NEB #R3505)	5 µl
	400 µl

Incubate at 37°C for 3–5 hours. Purify the DNA by phenol/chloroform extraction, chloroform extraction and ethanol precipitation.

5. **Gel-purify** the digested duplex on an 8% nondenaturing polyacrylamide gel, including 4 µl of the undigested duplex as well as DNA marker, such as NEB #N3032. Visualize by ethidium bromide staining, and excise the digested duplex from the gel, minimizing UV exposure time. Mince the excised band and elute the DNA by shaking overnight in several volumes of 250 mM Tris-acetate, pH 6, 1 mM EDTA, 0.1% SDS at 37°C.

6. Briefly microfuge to separate the gel fragments from the elution buffer and transfer the supernatant to a clean tube. Repeat wash to improve yield, if desired. Purify the DNA duplex from the supernatant by phenol/chloroform extraction, chloroform extraction and ethanol precipitation. Resuspend the pellet in 50 µl of TE and quantitate a small amount by PAGE or spectrophotometrically. One microgram of purified insert is more than sufficient for a library of complexity 10^9.

Preparation of Acc65I and EagI-HF Digested M13KE Vector

Digest 10–20 µg of M13KE Vector with the same enzymes used to prepare the insert above:

H_2O	330 µl
NEBuffer r3.1 (10X) (NEB #B6003)	40 µl
M13KE (1 µg/µl) (NEB #N3541)	10 µl
Acc65I (10 units/µl) (NEB #R0599)	10 µl
EagI-HF (20 units/µl) (NEB #R3505)	10 µl
	400 µl

Gel purify using standard methods, such as the Monarch DNA Gel Extraction Kit, NEB #T1020 or Monarch PCR and DNA Clean-up Kit, NEB #T1030 or β-Agarase I, NEB #M0392. Quantitate a small amount of purified cut vector on an agarose gel or spectrophotometrically.

Ligation of Digested Synthetic Oligo Duplex and M13KE Vector, Followed by Transfection of *E. coli* K12 ER2738 Electrocompentent Cells

1. Small scale optimization of ligation conditions (3 ligations). For a 20 µl ligation, combine:
   - 50 ng of cut vector
   - 3:1, 5:1 and 10:1 molar excess of cut duplex;
   - 2 µl of T4 DNA Ligase Buffer (10X) (NEB #B0202)
   - 200 units (3 Weiss units) of T4 DNA Ligase (400 U/µl) (NEB #M0202)

   Incubate at room temperature for 2 hour. Heat-kill at 65°C for 15 minutes. Store at -20°C or immediately test in cells.

2. Electroporate 1 µl of each ligation into 100 µl of electrocompetent *E. coli* K12 ER2738 or other F^+ strain (see Competent Cell Preparation above, Step 6). Outgrowths are carried out in 1 ml of SOC medium for 45 minutes at 37°C with shaking.

3. Dilute outgrowths. Prepare 10, 100, and 1000-fold dilutions of the outgrowth in LB. Transfer 10 µl of each dilution to a test tube containing 3 ml of top agar (equilibrated at 45–50°C) + turbid *E. coli* K12 ER2738 culture. Vortex briefly and spread on LB/IPTG/Xgal plates. Incubate overnight at 37°C and count blue plaques the next day.

4. Scale-up. Determine conditions for large scale ligation using the highest plaque/µg ratio to desired library complexity. For example, a library with a complexity of 10^9 clones may require 5 µg M13KE ligation reaction, if the small-scale ligations yield a ratio of 2 x 10^8 plaques/µg of vector. Use no more than 500 µl per individual ligation reaction; use multiple tubes if necessary.

5. Purify the large-scale ligation by phenol/chloroform extraction, chloroform extraction and ethanol precipitation. Wash with cold 70% ethanol to desalt. Resuspend the DNA in low salt buffer and electroporate as described above. To reduce the likelihood of cells picking up more than one DNA sequence, the ligation should be divided and electroporated using as many cuvettes as convenient. For example use 10 µg digested M13KE in ligations and follow with 100 electroporations, using 3 µl of resuspended ligated DNA per 100 µl of electrocompetent cells.

6. Outgrowth. After each electroporation, add 1 ml of SOC to the cuvette immediately. For high-complexity libraries it may be convenient to pool SOC outgrowths in groups of five. Each outgrowth (or pool of five) should be incubated for 30–45 minutes

(no longer) before amplification. Titer several outgrowths or pools (as in Step 3 above) prior to amplification to obtain library complexity.

7. Amplification. Amplify the electroporated cells by adding 20 ml of pooled SOC outgrowths to 1 liter of early-log cells (OD_{600} 0.01–0.05) in LB medium. Incubate with vigorous aeration (250 rpm) at 37°C for 4.5 to 5 hours. Centrifuge at 5000 x g for 20 minutes at 4°C. Transfer the supernatant to a clean bottle and discard the cells.

8. PEG precipitate phage library. Recover the phage from the supernatant by adding 1/5 volume of 20% PEG/2.5 M NaCl and incubating overnight at 4°C. Pellet the phage by centrifugation at 5000 x g for 45 minutes at 4°C. Discard the supernatant. Thoroughly resuspend the phage pellet in 100 ml of TBS by gently rocking ~1–3 hour or overnight at 4°C. Remove residual cells by centrifugation at 5000 x g for 10 minutes at 4°C. Transfer the supernatant to a new tube and discard the pellet. Re-precipitate the phage by adding 1/5 volume of 20% PEG/2.5 M NaCl and incubating for 1 hour at 4°C. Centrifuge at 5000 x g for 20 minutes and discard the supernatant.

9. Resuspend library in TBS-glycerol. Resuspend the final library in 10–40 ml of TBS by gentle rocking for 24–48 hours at 4°C. For long-term storage, add an equal volume of sterile glycerol, mix thoroughly and store at –20°C. The titer of the library should remain constant for several years at this temperature. Further amplification of the library is not recommended, as sequence biases will increase upon reamplification.

Phage Display Library Usage

The Ph.D. Phage Display Peptide Library Manual has general protocols for phage panning: Solution-phase Panning with Affinity Bead Capture and Surface Phase Panning. For complex applications such as whole-cell, *in vivo* or inorganic material panning, seek relevant methodology in the current literature.

Appendix A

Media and Solutions

LB Medium

Per liter: 10 g Bacto-Tryptone, 5 g yeast extract, 5 g NaCl. Autoclave, store at room temperature.

IPTG/Xgal Stock:

Mix 1.25 g IPTG (isopropyl-b-D-thiogalactoside) and 1 g Xgal (5-Bromo-4-chloro-3-indolyl-b-D-galactoside) in 25 ml DMF (dimethyl formamide). Store at -20°C.

LB/IPTG/Xgal Plates:

1 liter LB medium + 15 g/l agar. Autoclave, cool to < 70°C, add 1 ml IPTG/Xgal stock per liter and pour. Store plates at 4°C in the dark.

Top Agar:

Per liter: 10 g Bacto-Tryptone, 5 g yeast extract, 5 g NaCl, 7 g Bacto-Agar (or eletrophoresis grade agarose). Autoclave, dispense into 50 ml aliquots. Store solid at room temperature, melt in microwave as needed.

Tetracycline Stock (suspension):

20 mg/ml in 1:1 Ethanol:Water. Store at -20°C. Vortex before using.

LB+Tet Plates:

LB medium + 15 g/l Agar. Autoclave, cool to < 70°C, add 1 ml Tetracycline stock and pour. Store plates at 4°C in the dark. Do not use plates if brown or black.

SOC Electroporation Outgrowth Media:

Per liter: 20 g Bacto-Tryptone, 5 g yeast extract, 0.5 g NaCl. Dispense in 100 ml aliquots and autoclave. Prior to use add 0.5 ml 2 M $MgCl_2$ and 2 ml 1 M glucose (both sterile) per 100 ml. Store at 4°C for several months.

Appendix B

General M13 Methods

It is important to note that unlike phage lambda, M13 is not a lytic phage. Plaques are caused by diminished cell growth rather than cell lysis and are turbid rather than clear. Plating on IPTG/ Xgal media is strongly recommended to facilitate visualization of plaques.

Strain Maintenance

1. The recommended *E. coli* host strain *E. coli* K12 ER2738 (F´ *proA*$^{+}$*B*$^{+}$ *lacI*^{q} Δ*(lacZ) M15 zzf::Tn10(Tet*^{R}*)/fhuA2 glnV* Δ*(lac-proAB) thi-1* Δ*(hsdS-mcrB)5* is particularly well-suited for M13 propagation. *E. coli* K12 ER2738 is a recA^{+} strain, but we have never observed spontaneous *in vivo* recombination events with M13 or phagemid vectors. Commercially available F^{+} strains such as DH5αF´and XL1-Blue may be substituted for *E. coli* K12 ER2738 but have not been tested with NEB's vector system. Any strain used should be supE (*GlnV*) to suppress amber (UAG) stop codons within the library with glutamine.

2. Because M13 is a male-specific coliphage, it is recommended that all cultures for M13 propagation be inoculated from colonies grown on media selective for presence of the F-factor, rather than directly from the glycerol culture. The F-factor of *E. coli* K12 ER2738 contains a mini-transposon which confers tetracycline resistance, so cells harboring the F-factor can be selected by plating and propagating in tetracycline-containing medium. Tetracycline does not need to be added to media during phage amplification but should be used on all solid media growth and overnight liquid cultures. Streak out *E. coli* K12 ER2738 from the supplied glycerol culture onto an LB+Tet plate. Invert and incubate at 37°C overnight and store wrapped with parafilm at 4°C in the dark for a maximum of 1 month. We recommend that fresh glycerol stocks of *E. coli* K12 ER2738 be prepared from liquid cultures and archived at -80°C for extended Ph.D. Library projects.

3. Serial dilution of *E. coli* K12 ER2738 cultures for infection will give erratic results. If titer or amplification results are inconsistent, first streak a fresh *E. coli* K12 ER2738/LB+Tet plate.

Avoiding Phage Contamination

The library cloning vector M13KE differs from wild-type filamentous phage vector in that the lacZα-peptide cloning sequence (which permits blue/white screening) has been inserted in the vicinity of the (+) strand origin of replication, resulting in a longer replication cycle. In addition, display of foreign peptides as N-terminal fusions to pIII (which mediates infectivity by binding to the F-pilus of the recipient bacterium) may attenuate infectivity of the library phage relative to wild-type M13. As a result, there is the possibility of in vivo selection for any contaminating wild-type phage during the amplification steps between rounds of panning. In the absence of a correspondingly strong in vitro binding selection, even vanishingly small levels of contamination can result in a majority of the phage pool being wild-type phage after three rounds of panning.

1. The potential for contamination with environmental bacteriophage can be minimized by always using **aerosol-resistant pipette tips** and wearing gloves for all protocols.

2. Because the library cloning vector M13KE is derived from the common cloning vector M13mp19, which carries the lacZα gene, phage plaques appear blue when plated on media containing Xgal and IPTG. Environmental filamentous phage will typically yield colorless plaques when plated on the same media. These plaques are also larger and "fuzzier" than the library phage plaques. **It is recommended to plate on LB/IPTG/Xgal plates for all titering steps and, if white plaques are evident, picking ONLY blue plaques for sequencing.** Note, many times the presence of some white or clear plaques is unavoidable.

3. Severe contamination (white plaques present in large numbers) can lead to contamination of subsequent panning experiments. To prevent this, all solutions should be re-autoclaved; solutions containing heat-labile components should be remade. The work area including incubators should be wiped down with ethanol. Pipettors should be disassembled, and the parts soaked in detergent, rinsed carefully with sterile water, and reassembled.

Phage Titering

The number of plaques will increase linearly with added phage only when the multiplicity of infection (MOI) is much less than 1 (i.e., *E. coli* cells are in considerable excess). For this reason, it is recommended that phage stocks be titered by diluting prior to infection, rather than by diluting cells infected at a high MOI. Plating at low MOI will also ensure that each plaque contains only one DNA sequence.

1. **Day culture.** Inoculate 5–10 ml of LB with *E. coli* K12 ER2738 from a plate and incubate with shaking 4–8 hrs until mid-log phase, OD_{600} ~ 0.5.

2. **Prepare top agar and LB/IPTG/Xgal plates**. While cells are growing, melt Top Agar in microwave and dispense 3 ml into sterile culture tubes, one per expected phage dilution. Maintain tubes at 45–50°C. Pre-warm, for at least 30 minutes, one LB/IPTG/Xgal plate per expected dilution at 37°C until ready for use.

3. **Phage Dilutions.** Prepare 10 to 10^{3}-fold serial dilutions of phage in LB; 1 ml final volumes are convenient. Suggested dilution ranges: for amplified-PEG/NaCl concentrated phage culture supernatants, 10^{8}–10^{11}; for unamplified panning eluates, 10^{1}–10^{4}. Use aerosol- resistant pipette tips to prevent cross-contamination and use a fresh pipette tip for each dilution.

4. **Pre-plating Infections**. When the culture in Step 1 is turbid, dispense 200 µl into microfuge tubes, one for each phage dilution. To carry out infection, add 10 µl of each phage dilution to each tube of cells, vortex quickly, and incubate at room temperature for 1–5 minutes.

5. **Plating**. Transfer the infected cells one infection at a time to culture tubes containing warm Top Agar. Vortex briefly and IMMEDIATELY pour culture onto a pre-warmed LB/IPTG/Xgal plate while maintaining sterile conditions. Gently tilt and rotate plate to spread top agar evenly. Allow the plates to cool for 5 minutes, invert, and incubate overnight at 37°C.

6. **Count plaques the next day**. Count blue plaques on plates. Multiply each number by the dilution factor for that plate to get phage titer in plaque forming units (pfu) per 10 µl. *For example, if there are 91 pfu on a 10^9 dilution plate, the titer result would be 9.1 x 10^9 pfu/µl for the stock.*

Storing of M13 Phage Solutions

Panning experiments may be interrupted at several points in the protocol. Phage in suspension with NaCl/PEG may be stored for several weeks at 4°C. Eluted phage in neutralized buffer may be stored at 4°C for up to 1 week. Amplified phage may be stored in neutral buffer for up to 3 weeks with, either the addition of 0.02% NaN_3 or incubation at 65°C for 15 min to kill residual *E. coli*. Amplified phage may be stored long term (5+ years), by adding an equal volume of sterile glycerol, vortexing and storing at –20°C. It is not necessary to store phage at temperatures below –20°C, however, if required, single use aliquots of phage stocks may be flash frozen and thawed once without significant loss of titer. This follows for either glycerol-free -20°C storage or -70/-80°C storage with or without glycerol.

Appendix C

Ph.D. Cloning System FAQs

Q: Can I display antibody fragments or proteins at the N-terminus of pIII coat protein with M13KE?

A: In the Ph.D. Phage Display System each copy of pIII displays the encoded peptide sequence. For this reason any insert that interferes with phage particle assembly or pIII function will not make viable phage. Typically, phagemid systems, where a second copy of native coat protein is provided by helper phage, are used to display protein-sized inserts on M13. We consider 25–50 amino acids to be the maximum range for this system. To some extent peptide display is also sequence and secondary structure dependent.

Q: What are the differences between pIII and pVIII display?

A: Filamentous phage display systems are generally based on N-terminal fusions to the coat proteins pIII or pVIII. pIII is present at 5 copies per virion, of which all 5 can be fused to short peptides without interfering with phage infectivity. The major coat protein pVIII is present at ~2700 copies per virion, of which ~10% can be reliably fused to peptides or proteins. As a result, peptides expressed as pIII fusions are present at low valency (1–5 copies per virion), while pVIII fusions are present at high valency (~200 copies per virion). The increased avidity effect of high valency pVIII display permits selection of very low affinity ligands, while low valency pIII display limits selection to higher affinity ligands. The Ph.D. Phage Display Cloning System is designed to make pIII fusions (5 copies of the peptide per virion). NEB's pre-made libraries are the pentavalent pIII display format.

Q: What type of strain do I use with the Ph.D. Phage Display Cloning System?

A: Any robust F´ or male *E. coli* strain may be used with M13. We prefer *E. coli* K12 ER2738 (NEB #E4104) or NEB Turbo Competent *E. coli* (High Efficiency) (NEB #C2984). Cells may be made competent or purchased that way. For the most complex libraries, electrocompetent cells must be used (eff. ≥ 10^9 transformants per µg M13). See our protocol for making electrocompetent cells in the Ph.D. Phage Display manual or purchase from NEB's line of electrocompetent cells.

Q: I have done all the standard troubleshooting for my cloning and transfection steps and I still cannot get the desired clone in M13KE?

A: Make sure the pIII leader sequence is intact and in-frame with pIII coat protein in your design). Assuming that the reading frame is correct, the insert may not allow the production of viable phage either due to issues with export into the cell periplasm or function of pIII coat protein after phage assembly. Inserts larger than 25–50 amino acids and those with N-terminal cysteines or positively charged residues are known to be problematic. These issues may be probed by subcloning the insert into another vector with KpnI/EagI sites.

References

Cloning Phage Display Libraries in M13

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The Ph.D. Phage Display Peptide Library Manual, available at www.neb.com, has general protocols for phage panning: Solution-phase Panning with Affinity Bead Capture and Surface Phase Panning. For complex applications such as whole-cell, *in vivo* or inorganic material panning, seek relevant methodology in the current literature.

Ordering Information

NEB #	PRODUCT	SIZE
E8101S	Ph.D. Peptide Phage Display Cloning System	20 µg

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	4/22
2.0	Updated Step 5c (page 2) to 20 ml	2/23

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