METHODS **24,** 240–246 (2001)
doi:10.1006/meth.2001.1185, available online at http://www.idealibrary.com on IDEAL®

# Screening Phage-Displayed Combinatorial Peptide Libraries

Brian K. Kay,*[,1] Jeremy Kasanov,* and Montarop Yamabhai†

**Department of Pharmacology, University of Wisconsin—Madison, 1300 University Avenue, Madison, Wisconsin 53706-1532; and †School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakon Ratchasima 30000, Thailand*

Among the many techniques available to investigators interested in mapping protein–protein interactions is phage display. With a modest amount of effort, time, and cost, one can select peptide ligands to a wide array of targets from phage-display combinatorial peptide libraries. In this article, protocols and examples are provided to guide scientists who wish to identify peptide ligands to their favorite proteins. 

One fruitful method of mapping protein–protein interactions is to screen phage-displayed combinatorial peptide libraries for peptide ligands. Only a modest amount of time, effort, and resources are needed to screen a library displaying a billion different peptides by affinity selection. Peptide ligands have been isolated to a wide array of proteins, such as 14-3-3 proteins (1, 2), calmodulin (3–5), cell surface receptors (6–10), EH domains (11–13), heat-shock proteins (14, 15), integrins (16), PDZ domains (17, 18), SH3 domains (19–21), vascular endothelial growth factor (VEGF) (22, 23), viral proteins (24, 25), and WW domains (26, 27), to name a few. Phage-displayed combinatorial peptides are also very useful in mapping the epitopes of both monoclonal and polyclonal antibodies (28, 29).

Once peptides have been isolated they can be used for a variety of purposes. First, the primary structures of the selected peptides often resemble the primary structure of the natural interacting partner of the protein target. We have termed this phenomenon "convergent evolution" (30). Therefore, a useful approach for mapping protein–protein interactions can be to isolate peptide ligands to a target protein and identify candidate interacting proteins in a sequenced genome by computer analysis. Second, synthetic forms of the selected peptides can serve to antagonize the interaction between particular proteins, both *in vitro* and *in vivo* (31). Third, in a number of cases (8, 9, 32) peptide ligands have been isolated for cell surface receptors that have agonist activity, even though the peptides are much smaller than the native hormones and share no sequence similarity. Fourth, the peptides can serve as displaceable probes that one can use to format high-throughput screens of small molecule libraries for drug discovery (33, 34).

## GENERAL CONSIDERATIONS

In this article, we present simple, step-by-step protocols for screening phage-displayed combinatorial peptide libraries. Many types of protein targets, including enzymes (34, 35), have been used successfully to affinity select binding phage. In our hands, we routinely isolate peptide ligands to approximately one-half of the targets we test, with only a modest investment of time (1–3 weeks) and material (100–500 $\mu$g). When successful, the selected peptides can be used to probe blotted SDS–polyacrylamide gels (36, 37), screen cDNA expression libraries (38), and affinity select proteins from complex mixtures (39).

While peptides have been displayed on several of the capsid proteins of bacteriophage M13, proteins III and VIII are the most popular fusion partners. Protein III (pIII) is a minor capsid protein, present in five copies at one end of the filamentous particle, where it functions to infect bacteria. Fortunately for molecular biologists,

[1] To whom correspondence should be addressed. Fax: (608) 262-1257. E-mail: bkkay@facstaff.wisc.edu.



1046-2023/01 $35.00

display of peptides or proteins at the N terminus of pIII does not interfere with the folding of the three globular domains of pIII (40, 41), which are essential for bacterial infection. Protein VIII (pVIII) is the major capsid protein, with approximately 2500 copies per particle. Due to the crystalline nature of this protein on the virus surface (42, 43), mature pVIII tolerates only short (i.e., 9-mer) peptides at its N terminus. While the protocols provided below can be used to screen combinatorial peptide libraries displayed at the N terminus of either pIII or pVIII, one difference to point out is that peptides selected from pIII or pVIII display libraries can differ in their dissociation constants for the same target. Typically, peptides isolated from pIII libraries have low micromolar (1–10 $\mu$M) dissociation constants, whereas peptides isolated from pVIII libraries have midmicromolar (i.e., 10–100 $\mu$M) dissociation constants. This quantitative difference in the binding strength is a consequence of the avidity effects caused by the high valency of peptides displayed on pVIII. When one is interested in isolating the strongest binding peptides from a phage-display library, one option is to use phagemids (42, 44–46), which yield viral particles with reduced numbers of displayed peptides.

While bacteriophage M13 is well suited to the display of exogenous peptides and proteins as fusions to its capsid proteins, it is also possible to use bacteriophage lambda ($\lambda$) (47, 48) or T7 (49) as display vectors. Peptide ligands to various protein targets have been identified from ribosome-displayed libraries (50–52), peptides immobilized on plastic beads (53) or crowns (54), or libraries of soluble peptides (55). However because of its simplicity, minimal cost, and ease of manipulation, (56), bacteriophage M13 has proven to be an extremely popular and useful vector system for selecting peptide ligands from combinatorial libraries (Fig. 1).

## MATERIALS

1. Phage libraries: New England BioLabs (Beverly, MA; www.neb.com) sells M13 bacteriophage libraries displaying $X_7$, $X_{12}$, and $CX_7C$ peptides, where X is any amino acid encoded by NNK codons and C is cysteine. Each library has a complexity of $\sim 10^9$ members.

2. 2xYT media: 10 g tryptone, 10 g yeast extract, and 5 g NaCl dissolved in 1 L $H_2O$; autoclave; for bottom and top agar add Bacto-agar to final concentrations of 1.5 and 0.8%, respectively.

3. Microtiter plate tape: Continental Laboratory Products, Catalog No. 2415. Alternatively plastic wrap can be used.

4. Phosphate-buffered saline (PBS): 137 mM NaCl, 3 mM KCl, 8 mM $Na_2HPO_4$ 1.5 mM $KH_2PO_4$; a 10x stock solution consists of 80.0 g NaCl, 2.0 g KCl, 11.5 g $Na_2HPO_4{\cdot}7H_2O$, and 2.0 g $KH_2PO_4$ dissolved in 1 L $H_2O$ and then autoclaved. The pH should be ~7.5.

5. Wash buffer (PBS–Tween 20): To 1 liter of 1x PBS add 1 mL Tween 20

6. ELISA-ready microtiter plates (Costar).

7. Blocking solution: Add BSA to 1% in wash buffer. One can purchase blocking reagents such as Superblock from Pierce Chemical Company (Rockford, IL; www.piercenet.com).

8. 100 mM $NaHCO_3$: 8.3 g $NaHCO_3$ dissolved in 1 L $H_2O$; autoclave.

9. 50 mM glycine–HCl: To prepare a 1 M stock solution dissolve 111.6 g glycine in 1 L $H_2O$, and adjust the pH to 2.0 with HCl. After autoclaving the solution, dilute to 50 mM with sterile $H_2O$. The final pH should be ~2.0.

10. Neutralization solution: 200 mM $NaHPO_4$ (pH 7.5); mix 16 mL of solution A (0.2 M $NaH_2PO_4{\cdot}H_2O$) and 84 mL of solution B (0.2 M $Na_2HPO_4{\cdot}7H_2O$).

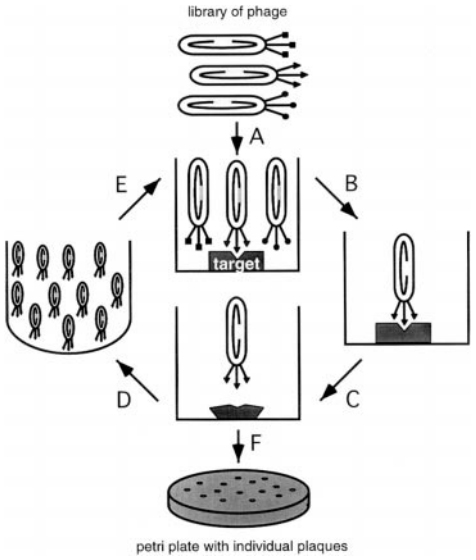


FIG. 1. General scheme of selecting phage from a phage-displayed combinatorial peptide library. Six steps are shown. In step A, an aliquot of the library is introduced into microtiter plate wells that have been previously coated with target protein. After a 2-h incubation (step B), the nonbinding phage are washed away. The bound phage are then recovered (step C), by denaturing or destroying the target with exposure to pH 2, pH 12, 15 mM DTT, 1% DMF, 6 M urea, or trypsin; remarkably, M13 bacteriophage particles remain intact under these conditions. After the phage particles are transferred to another tube and the pH (or trypsin) neutralized, bacteria are infected and more phage particles produced (step D). The amplified phage are then rescreened (step E), to complete one cycle of affinity selection. After three or more rounds of screening, the phage are plated out (step F) such that there are individual plaques (clones) for additional analysis.

11. Horseradish peroxidase-conjugated antiphage antibody: Pharmacia Biotech (Piscataway, NJ).

12. ABTS (2′,2′-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) Solution: Add 220 mg ABTS to 1 L of 50 mM citric acid (10.5 g citrate monohydrate in 1 L $H_2O$, pH adjusted to 4 with approximately 6 mL of 10M NaOH). Filter-sterilize and store at 4°C; stable for at least 6 months. Immediately before using an aliquot, add 30% $H_2O_2$ to 0.05% final concentration.

13. 2% IPTG: 0.2 g isopropyl-$\beta$-D-thiogalactopyranoside dissolved in 10 mL $H_2O$; filter-sterilize and store aliquoted at −20°C.

14. 2% X-Gal: 0.2 g 5-bromo-4-chloro-3-indoyl-$\beta$-D-galactoside dissolved in 10 mL dimethyl sulfoxide (DMSO) or dimethylformamide (DMF). Limit exposure to light and store at −20°C.

15. Gene III sequencing primer, upstream of cloning site: 5′- ATT CAC CTC GAA AGC AAG CTG-3′

16. Gene III sequencing primer, downstream of cloning site: 5′- CTC ATA GTT AGC GTA ACG-3′

17. Aerosol resistant tips: Promega (Madison, WI; www.promega.com)

18. DH5$\alpha$F′: Life Technologies, Inc. (www.lifetech.com)

## METHODS

### Immobilization of Targets onto ELISA-Ready Microtiter Plates

1. Add 1–10 $\mu$g of target protein(s) in 100 $\mu$L 100 mM $NaHCO_3$ (pH 8.5) onto microtiter wells of three ELISA-ready plates. (Plate 1 will be used in the first-round panning, while plates 2 and 3 will be used for the second and third rounds, respectively.) Use a control target protein, such as streptavidin or a GST fusion protein to the Src SH3 domain. Leave a space between different targets to prevent cross-contamination.

2. Seal the wells with tape to avoid evaporation, and incubate the plates at room temperature for 1 h. Use either strips of Scotch Tape to cover individual wells, or microtiter plate-sized tape or plastic wrap to cover the entire plate.

3. Add 150 $\mu$L blocking solution to each well to block nonspecific binding. Seal the wells with tape and incubate the first plate at room temperature for 1 h. Incubate the second and third plates overnight at 4°C.

### Affinity Purification of Binding Phage: First-Round Panning

4. Wash the wells three times with PBS–0.1% Tween 20, which can be introduced into the wells by pipetting or with a plastic squeeze bottle. The wash solution can be flicked into the sink after each wash. Remove residue liquid by slapping the dish against a clean Kimwipe. Do not let the wells dry out completely.

5. Add 25 $\mu$L of a combinatorial peptide library (concentrated to $\sim 10^{14}$ plaque-forming units (PFU)/mL, with a complexity of $10^9$) in 125 $\mu$L PBS–0.1% Tween 20 to each well. (To ensure successful isolation of a particular peptide, we use 2500 copies of each phage in the first round of panning. Seal the wells and incubate the plate at room temperature for 2 h.

6. Remove nonbinding phage by washing the wells five times as described in step 4.

7. Elute bound phage by adding 50 $\mu$L of 50 mM glycine–HCl (pH 2.0) to each well and incubating the plate at room temperature for 15 min. Neutralize the solution by transferring eluted phage to a new well containing 50 $\mu$L neutralization solution.

### Amplification of Recovered Binding Phage

8. Dilute 30 $\mu$L of an overnight culture of F′ *Escherichia coli*, such as DH5$\alpha$F′, in 3 mL sterile 2xYT. Add 290 $\mu$L of the eluted phage and incubate the culture at 37°C, with vigorous agitation, for 8 h. To minimize proteolytic degradation of displayed peptides, do not incubate longer than 16 h.

### Affinity Purification of Binding Phage: Second and Third-Round Panning

*Second Round*

9. Collect the amplified phage by spinning out cells at 4°C, 4000*g* for 10 min and transfer the phage supernatant to a new tube (4-mL Falcon).

10. Wash the wells of the second plate three times with PBS–0.1% Tween 20 by flicking the wash solution into the sink. Remove residue liquid by slapping the dish against a clean Kimwipe. Add 150–200 $\mu$L amplified phage ($\sim 10^{10}$ PFU) from the first-round panning to each well.

11. Seal the wells and incubate the plate at room temperature for 2 h.

12. Wash the wells five times as described in step 10.

13. Elute bound phage by adding 50 $\mu$L of 50 mM glycine–HCl (pH 2.0) to each well and incubating the plate at room temperature for 15 min. Neutralize the solution by transferring eluted phage to a new well containing 50 $\mu$L neutralization solution.

14. Dilute 30 $\mu$L of an overnight culture of F′ *E. coli*, such as DH5$\alpha$F′, in 3 mL sterile 2xYT. Add 290 $\mu$L of the eluted phage and incubate the culture at 37°C, with

vigorous agitation, for 8 h. To minimize proteolytic degradation of displayed peptides, do not incubate longer than 16 h.

*Third Round*

15. Collect the amplified phage by spinning out cells at 4°C, 4000*g* for 10 min and transfer the phage supernatant to a new tube (4-mL Falcon).

16. Wash the wells of the second plate three times with PBS–0.1% Tween 20 by flicking the wash solution into the sink. Remove residue liquid by slapping the dish against a clean Kimwipe. Add 150–200 $\mu$L amplified phage ($\sim 10^{10}$ PFU) from the second round of panning to each well.

17. Seal the wells and incubate the plate at room temperature for 2 h.

18. Wash the wells five times as described in step 10.

19. Elute bound phage by adding 50 $\mu$L of 50 mM glycine–HCl (pH 2.0) to each well and incubating the plate at room temperature for 15 min. Neutralize the solution by transferring eluted phage to a microcentrifuge tube containing 50 $\mu$L neutralization solution. This represents the output population of binding phage from three rounds of affinity purification.

## Isolation of Affinity-Purified Phage Clones

20. Perform a 10-fold serial dilution of each recovered phage on a microtiter plate (U-bottom). Add 180 $\mu$L PBS into the wells (rows A–E) with an eight-channel pipettor. The number of the columns should equal the number of targets.

21. Add 20 $\mu$L of each recovered phage to be titered in row A. Mix by pipetting up and down.

22. Transfer 20 $\mu$L of the phage from row A to row B. Mix and continue the dilution series through row G. (Before starting step 22, melt the top agar in a microwave and keep it at 55°C).

23. Add 10 $\mu$L of diluted phage from rows A–E with 200 $\mu$L DH5$\alpha$F′ overnight culture into a 4-mL sterile Falcon tube. Premix 3 mL of molten 0.8% top agar with 30 $\mu$L 2% IPTG and 30 $\mu$L 2% X-Gal; keep at 55°C. Add the 3 mL of agar mixture to each tube containing diluted phage, invert several times, and pour onto a 2xYT Petri plate that has been prewarmed at 37°C. Allow the plates to then sit undisturbed for a few minutes (~5 min), until the top agar hardens.

24. Incubate the plates inverted at 37°C overnight, then keep in 4°C until ready to pick up the isolated phage clones. Phage are viable as plaques for at least 1 month if the plates are kept at 4°C.

## Propagation of Individual Phage Clones

25. Dilute an overnight culture of DH5$\alpha$F′ 1:100 into sterile 2xYT. For each isolated plaque to be propagated, add 3 mL of the mixture into 15-mL tubes.

26. Pick (touch and twist) and inoculate the blue isolated plaques into each 15-mL tube with sterile long wooden toothpicks. Incubate the tubes at 37°C, with vigorous agitation, for 8–10 h.

## Confirmation of Binding Activity of Affinity-Purified Phage Clones by ELISA

27. For each phage clone to be tested, add 1 $\mu$g of target protein and 1 $\mu$g of a negative control protein, such as unrelated bacterial fusion partner proteins, and BSA in 100 $\mu$L of 100 mM $NaHCO_3$ (pH 8.5) into adjacent microtiter wells.

28. Seal the wells with tape to avoid evaporation, and incubate the plate at room temperature for 1 h.

29. Add 150 $\mu$L blocking solution to each well to block nonspecific binding. Seal the wells with tape and incubate overnight at 4°C.

30. Pellet the bacterial cells in the culture tubes by centrifugation at 4°C, 4000*g* for 10 min. Transfer the phage supernatant into a new tube. Keep the bacterial pellet for preparation of replicative form DNA (step 36).

31. Wash the wells three times with PBS–0.1% Tween 20 by flicking the wash solution into the sink.

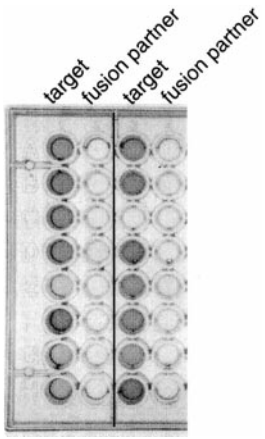


FIG. 2. Phage ELISA. Individual plaques are propagated in 2xYT culture medium and the phage particles are separated from the bacteria by centrifugation. The supernatant is then distributed into pairs of microtiter plate wells, which have been previously coated with target (GST-Src SH3 domain) and fusion partner (GST). Binding of particles to the immobilized proteins is detected immunologically with an antiphage antibody conjugated to horseradish peroxidase. In a successful experiment, many of the target (but not the negative control) wells develop a blue color when incubated with the ABTS substrate and, hence, are considered to contain binding phage, ready for sequencing.

Remove residue liquid by slapping the dish against a clean Kimwipe. Do not let the wells dry out completely.

32. Add 100 $\mu$L of each recovered phage into a separate pair (target/negative control) of wells and incubate for 2 h at room temperature. Keep the rest of the supernatant as a phage stock in 4°C (for a long-term storage, add glycerol to a final concentration of 20% and keep at −70°C).

33. Wash the wells five times as described in step 31.

34. Dilute horseradish peroxidase-conjugated anti-phage antibody 1:5000 in PBS–Tween 20. Add 100 $\mu$L of the diluted conjugate to each well. Seal the wells and incubate the plate at room temperature for 1 h. Wash the wells five times as described above.

35. Add 100 $\mu$L ABTS reagent containing 0.05% $H_2O_2$ to each well. Incubate the plate at room temperature until the color reaction develops (10–30 min) (Fig. 2). Quantify the reaction by measuring the absorbance at 405 nm with a microtiter plate reader. Positive signals give optical density (OD) values in the range 0.5–3.0, while negative signals are in the range 0.05–0.3.

### Preparation of Plasmid from Positive Phage Clone for DNA Sequencing

36. Use the cell pellet of *bona fide* binding phage to prepare double stranded DNA for automated fluorescence DNA sequencing. Follow the procedure in the

**Peptide Ligands of the SH3 domain of Src**

| | | |
|---|---|---|
| STAVSFRFMPGGGGAFYST | RPVPPIT | RPSR |
| STRWSHSWPGYVGGANPSPAT | RPLPTRP | SR |
| STAHSLWDWGTFSGVSHK | SRLPPLP | TRPSR |
| STAPWGLRVAHEGGVLK | RPLPIPP | VTRPSR |
| STNVWVTGSVIARGAQS | RPLPIPP | ETRPSR |
| STNDVDWMHMWNSGGPH | RRLPPTP | ATRPSR |

RPLPxxP

**Peptide Ligands of the SH3 domain of Crk**

| | | |
|---|---|---|
| GQPAGDPDP | PPLPAK | F |
| FEQTGV | PLLPPK | SFKY |
| IFGDPP | PPIPMK | GRSL |
| SNQGSI | PVLPIK | RVQY |
| NYVNALPPG | PPLPAK | N |
| SSDPER | PVLPPK | LWSV |
| HFGPSK | PPLPIK | TRIT |
| DWKVPEPPV | PKLPLK | Q |
| ATSEGL | PILPSK | VGSY |
| NANVSAPRA | PAFPVK | T |
| EMVLG | PPVPPK | RGTVV |
| AGSRHP | PTLPPK | ESGG |
| SVAADP | PRLPAK | SRPQ |

PxLPxK

**Peptide Ligands of Troponin C**

VDYLKDKLISLA
VADVKRKILGLA
VDELKLKLSTLV
VEELRGALLSLK
LDYLKSSLLHLG
LELLKESMRSLA
EAWRGRLWDLAK
VGELKEMMRTLA
LTELKEKLSQLN

$\frac{VD}{LE}$xLKxxLxxLA

FIG. 3. Examples of peptides isolated from phage-displayed combinatorial peptide libraries that bind to targets. The Src SH3 domain binding phage were isolated from a 22-mer library (21), the Crk SH3 domain binding phage were isolated from a $x_6PxxPx_6$ library (59), and the troponin C phage were isolated from a 12-mer combinatorial library (60). The flanking sequences in the 22-mer isolates and the fixed prolines in the $x_6PxxPx_6$ library are underlined. The sequences have been aligned with gaps to highlight the consensus (shown below) for each set of peptide ligands, with x denoting any amino acid.

QIAprep Spin Plasmid handbook. Elute DNA with 50 $\mu$L sterile $H_2O$.

37. Measure DNA concentration by reading the OD value at 260 nm (1 OD = 50 $\mu$g/mL).

38. Add 0.7 $\mu$g of DNA, 10 pmol primer (1 $\mu$L of 10 pmol/$\mu$L gene III downstream primer), and sterile $H_2O$ to bring the total volume to 20 $\mu$L into a 0.65-mL microcentrifuge tube.

39. Label each tube with the sender's name, submission date, and sample number. Submit to a sequencing facility for fluorescent dideoxynucleotide sequencing.

## NOTES

1. Many phage-display combinatorial peptide libraries use the NNK coding scheme, where N is an equimolar mixture of A, C, G, or T and K is an equimolar mixture of G or T nucleotides. In this scheme, 32 codons encode one stop codon (TAG), which is suppressed in DH5$\alpha$F′ bacteria with a glutamine, and all 20 amino acids, which are represented once (C, D, E, F, H, I, K, M, N, Q, W, Y), twice (A, G, P, V, T), or thrice (L, R, S).

2. For positive controls, we recommend the following targets [and their phage-displayed motifs]: streptavidin [HP(Q/M)], Src SH3 domain [RxLPxLP or PxxPxR], FLAG MAb M2 [DYKxxD], and troponin C [(V/L)(D/E)xLKxxLxxLA]. Figure 3 lists the peptides and motifs of peptides which were affinity selected for three different targets.

3. In some instances, targets become denatured when affixed on the surface of microtiter plate wells and fail to isolate any phage. To overcome this limitation, one should chemically biotinylate the target and then capture target:phage complexes formed in solution with immobilized avidin or streptavidin. Biotinylation of a target with EZ-link-sulfo-NHS-LC-LC biotin (Pierce) should be according to the manufacturer's directions. Protocols for affinity selecting phage with biotinylated proteins can be found elsewhere (57).

4. Before sequencing the inserts of any recovered phage, one should verify by ELISA that the phage bind specifically to the target of interest. It is possible to isolate false, such as phage that bind to glutathione *S*-transferase (GST) or the plastic surface (58).

5. To maintain the sequence diversity among the binding phage, one should not do more than four rounds of selection. Additional rounds of selection most likely select for phage that grow, infect, or elute better, rather than phage that bind better to their targets.

## REFERENCES


1. Wang, B., Yang, H., Liu, Y. C., Jelinek, T., Zhang, L., Ruoslahti, E., and Fu, H. (1999) *Biochemistry* 38, 12499–12504.
2. Henriksson, M. L., Troller, U., and Hallberg, B. (2000) *Biochem. J.* 349, 697–701.
3. Adey, N. B., and Kay, B. K. (1996) *Gene* 169, 133–134.
4. Pierce, H. H., Adey, N., and Kay, B. K. (1996) *Mol. Div.* 1, 259–265.
5. Nevalainen, L. T., Aoyama, T., Ikura, M., Crivici, A., Yan, H., Chua, N. H., and Nairn, A. C. (1997) *Biochem. J.* 321, 107–115.
6. Goodson, R. J., Doyle, M. V., Kaufman, S. E., and Rosenberg, S. (1994) *Proc. Natl. Acad. Sci. USA* 91, 7129–7133.
7. Yanofsky, S. D., Baldwin, D. N., Butler, J. H., Holden, F. R., Jacobs, J. W., Balasubramanian, P., Chinn, J. P., Cwirla, S. E., Peters-Bhatt, E., Whitehorn, E. A., Tate, E. H., Akeson, A., Bowlin, T. L., Dower, W. J., and Barrett, R. W. (1996) *Proc. Natl. Acad. Sci. USA* 93, 7381–7386.
8. Wrighton, N. C., Farrell, F. X., Chang, R., Kashyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K., and Dower, W. J. (1996) *Science* 273, 458–464.
9. Cwirla, S. E., Balasubramanian, P., Duffin, D. J., Wagstrom, C. R., Gates, C. M., Singer, S. C., Davis, A. M., Tansik, R. L., Mattheakis, L. C., Boytos, C. M., Schatz, P. J., Baccanari, D. P., Wrighton, N. C., Barrett, R. W., and Dower, W. J. (1997) *Science* 276, 1696–1699.
10. Sarrias, M. R., Whitbeck, J. C., Rooney, I., Spruce, L., Kay, B. K., Montgomery, R. I., Spear, P. G., Ware, C. F., Eisenberg, R. J., Cohen, G. H., and Lambris, J. D. (1999) *J. Virol.* 73, 5681–5687.
11. Salcini, A. E., Confalonieri, S., Doria, M., Santolini, E., Tassi, E., Minenkova, O., Cesareni, G., Pelicci, P. G., and Di Fiore, P. P. (1997) *Genes Dev.* 11, 2239–2249.
12. Paoluzi, S., Castagnoli, L., Lauro, I., Salcini, A. E., Coda, L., Fre, S., Confalonieri, S., Pelicci, P. G., Di Fiore, P. P., and Cesareni, G. (1998) *EMBO J.* 17, 6541–6550.
13. Yamabhai, M., Hoffman, N. G., Hardison, N. L., McPherson, P. S., Castagnoli, L., Cesareni, G., and Kay, B. K. (1998) *J. Biol. Chem.* 273, 31401–31406.
14. Takenaka, I. M., Leung, S. M., McAndrew, S. J., Brown, J. P., and Hightower, L. E. (1995) *J. Biol. Chem.* 270, 19839–19844.
15. Sauk, J. J., Coletta, R. D., Norris, K., and Hebert, C. (2000) *J. Cell Biochem.* 78, 251–263.
16. Kraft, S., Diefenbach, B., Mehta, R., Jonczyk, A., Luckenbach, G. A., and Goodman, S. L. (1999) *J. Biol. Chem.* 274, 1979–1985.
17. Gee, S. H., Sekely, S. A., Lombardo, C., Kurakin, A., Froehner, S. C., and Kay, B. K. (1998) *J. Biol. Chem.* 273, 21980–21987.
18. Fuh, G., Pisabarro, M. T., Li, Y., Quan, C., Lasky, L. A., and Sidhu, S. S. (2000) *J. Biol. Chem.* 275, 21486–21491.
19. Cheadle, C., Ivashchenko, Y., South, V., Searfoss, G. H., French, S., Howk, R., Ricca, G. A., and Jaye, M. (1994) *J. Biol. Chem.* 269, 24034–24039.
20. Rickles, R. J., Botfield, M. C., Weng, Z., Taylor, J. A., Green, O. M., Brugge, J. S., and Zoller, M. J. (1994) *EMBO J.* 13, 5598–5604.
21. Sparks, A. B., Quilliam, L. A., Thorn, J. M., Der, C. J., and Kay, B. K. (1994) *J. Biol. Chem.* 269, 23853–23856.
22. Fairbrother, W. J., Christinger, H. W., Cochran, A. G., Fuh, G., Keenan, C. J., Quan, C., Shriver, S. K., Tom, J. Y., Wells, J. A., and Cunningham, B. C. (1998) *Biochemistry* 37, 17754–17764.
23. Binetruy-Tournaire, R., Demangel, C., Malavaud, B., Vassy, R., Rouyre, S., Kraemer, M., Plouet, J., Derbin, C., Perret, G., and Mazie, J. C. (2000) *EMBO J.* 19, 1525–1533.
24. Dyson, M., and Murray, K. (1995) *Proc. Natl. Acad. Sci. USA* 92, 2194–2198.

25. BouHamdan, M., Xue, Y., Baudat, Y., Hu, B., Sire, J., Pomerantz, R. J., and Duan, L. X. (1998) *J. Biol. Chem.* 273, 8009–8016.
26. Linn, H., Ermekova, K. S., Rentschler, S., Sparks, A. B., Kay, B. K., and Sudol, M. (1997) *Biol. Chem.* 378, 531–537.
27. Tommasi di Vignano, A., Di Zenzo, G., Sudol, M., Cesareni, G., and Dente, L. (2000) *FEBS Lett.* 471, 229–234.
28. Scott, J. K., and Smith, G. P. (1990) *Science* 249, 386–390.
29. Yip, Y. L., and Ward, R. L. (1999) *Comb. Chem. High Throughput Screen.* 2, 125–138.
30. Kay, B. K., Kasanov, J., Knight, S., and Kurakin, A. (2000) *FEBS Lett.* 480, 55–62.
31. Cochran, A. G. (2000) *Chem Biol.* 7, R85–94.
32. Wrighton, N. C., Balasubramanian, P., Barbone, F. P., Kashyap, A. K., Farrell, F. X., Jolliffe, L. K., Barrett, R. W., and Dower, W. J. (1997) *Nat. Biotechnol.* 15, 1261–1265.
33. Kay, B. K., Kurakin, A., and Hyde-DeRuyscher, R. (1998) *Drug Discov. Today* 3, 370–378.
34. Hyde-DeRuyscher, R., Paige, L. A., Christensen, D. J., Hyde-DeRuyscher, N., Lim, A., Fredericks, Z. L., Kranz, J., Gallant, P., Zhang, J., Rocklage, S. M., Fowlkes, D. M., Wendler, P. A., and Hamilton, P. T. (2000) *Chem Biol.* 7, 17–25.
35. Kay, B. K., and Hamilton, P. T. (in press) *Comb. Chem. High Throughput Screen.*
36. Hoffman, N. G., Sparks, A. B., Carter, J. M., and Kay, B. K. (1996) *Mol. Div.* 2, 5–12.
37. Adey, N. B., and Kay, B. K. (1997) *Biochem. J.* 324, 523–528.
38. Sparks, A. B., Hoffman, N. G., McConnell, S. J., Fowlkes, D. M., and Kay, B. K. (1996) *Nat. Biotechnol.* 14, 741–744.
39. Smith, G. P., Petrenko, V. A., and Matthews, L. J. (1998) *J. Immunol. Methods.* 215, 151–161.
40. Holliger, P., and Riechmann, L. (1997) *Structure* 5, 265–275.
41. Lubkowski, J., Hennecke, F., Pluckthun, A., and Wlodawer, A. (1998) *Nat. Struct. Biol.* 5, 140–147.
42. Kishchenko, G., Batliwala, H., and Makowski, L. (1994) *J. Mol. Biol.* 241, 208–213.
43. Petrenko, V. A., Smith, G. P., Gong, X., and Quinn, T. (1996) *Protein Eng.* 9, 797–801.
44. Bass, S., Greene, R., and Wells., J. (1990) *Proteins: Struct. Funct. Genet.* 8, 309–314.
45. Malik, P., and Perham, R. N. (1997) *Nucleic. Acids Res.* 25, 915–916.
46. Petrenko, V., and Smith, G. (1997) *Chem. Rev.* 97, 391–410.
47. Sternberg, N., and Hoess, R. (1995) *Proc. Natl. Acad. Sci. USA* 92, 1609–1613.
48. Mikawa, Y. G., Maruyama, I. N., and Brenner, S. (1996) *J. Mol. Biol.* 262, 21–30.
49. Sche, P. P., McKenzie, K. M., White, J. D., and Austin, D. J. (1999) *Chem Biol.* 6, 707–716.
50. Mattheakis, L. C., Bhatt, R. R., and Dower, W. J. (1994) *Proc. Natl. Acad. Sci. USA* 91, 9022–9026.
51. Roberts, R. W., and Szostak, J. W. (1997) *Proc. Natl. Acad. Sci. USA* 94, 12297–12302.
52. Liu, R., Barrick, J. E., Szostak, J. W., and Roberts, R. W. (2000) *Methods Enzymol.* 318, 268–293.
53. Lam, K. S., and Lebl, M. (1998) *Methods Mol. Biol.* 87, 1–6.
54. Geysen, H. M. (1990) *Southeast Asian J. Trop. Med. Public Health* 21, 523–533.
55. Houghten, R. A., Pinilla, C., Appel, J. R., Blondelle, S. E., Dooley, C. T., Eichler, J., Nefzi, A., and Ostresh, J. M. (1999) *J. Med. Chem.* 42, 3743–3778.
56. Cesareni, G., Castagnoli, L., and Cestra, G., (1999) *Comb. Chem. High Throughput Screen.* 2, 1–17.
57. Smith, G. P., and Scott, J. K. (1993) *Methods Enzymol.* 217, 228–257.
58. Adey, N. B., Mataragnon, A. H., Rider, J. E., Carter, J. M., and Kay, B. K. (1995) *Gene* 156, 27–31.
59. Sparks, A., Rider, J., Hoffman, N., Fowlkes, D., Quilliam, L., and Kay, B. (1996) *Proc. Natl. Acad. Sci. USA* 93, 1540–1544.
60. Pierce, H. H., Schachat, F. H., Lombard, C. R., and Kay, B. K. (1998) *J. Biol. Chem.* 273, 23448–23453.