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FEATURE ARTICLE

IN THIS ISSUE

T7Select® Phage Display System: A powerful new protein display system based on bacteriophage T7

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Phage display is a powerful technique for identifying peptides or proteins that have desirable binding properties. In this method, a peptide or protein is displayed on the surface of a phage as a fusion to a protein that is normally found in the phage particle. The first phage vectors suitable for surface display were made by Smith and coworkers, using filamentous phage (1). They also developed simple procedures for selecting phage displaying peptides or proteins that bind to particular targets. Such phage can be selected from large libraries of variants.

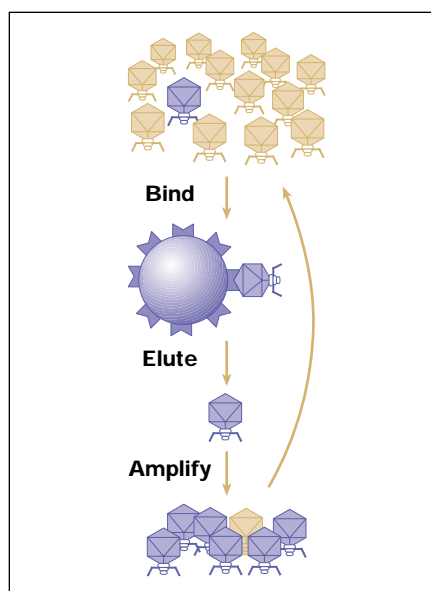
This system is easy to use and has the capacity to display peptides up to about 50 aa in size in high copy number, and peptides or proteins up to about 1200 aa in low copy number.

Both the peptide or protein and its coding sequence are selected at the same time, since the displayed peptide or protein responsible for the binding is encoded in the genome of the bound phage. Phage display has been used to identify peptides that bind to receptors, substrates or inhibitors of enzymes, epitopes, improved antibodies, altered enzymes, and cDNA

clones, and new applications are continually arising (2).

We have developed a new phage display system based on bacteriophage T7. This system is easy to use and has the capacity to display peptides up to about 50 amino acids in size in high copy number (415 per phage), and peptides or proteins up to about 1200 amino acids in low copy number (0.1–1 per phage). T7 is a double-stranded DNA phage that has been extensively studied (3, 4).

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Vector	Use	Display Number	Display Limit (amino acids)	Host
T7Select [®] 415-1	peptides	415	40–50 aa	BL21
T7Select1-1	peptides or proteins	≤ 1	900 aa	BLT5403
T7Select1-2	peptides or proteins	≤ 1	1200 aa	BLT5403

Table 1. Phage display vector features.

Phage assembly takes place inside the *E. coli* cell and mature phage are released by cell lysis. Unlike the filamentous systems, peptides or proteins displayed on the surface of T7 do not need to be capable of secretion through the cell membrane, a necessary step in filamentous phage assembly (5).

T7 has additional properties that make it an attractive display vector. It is very easy to grow and replicates more rapidly than either bacteriophage λ or filamentous phage. Plaques form within 3 hours at 37 °C and cultures lyse 1–2 hours after infection, decreasing the time needed to perform the multiple rounds of growth usually required for selection. The T7 phage particle is extremely robust, and is stable to harsh conditions that inactivate other phage. This expands the variety of agents that can be used in biopanning selection procedures, which require that the phage remain infective. T7 is actually an excellent general cloning vector. Purified DNA is easy to obtain in large amounts, a high-efficiency *in vitro* packaging system is available (6), and the DNA is completely sequenced (39,937 bp), so restriction or DNA sequence analysis of clones is quite straightforward.

T7 structure and assembly

T7 is an icosahedral phage with a capsid shell composed of 415 copies of the T7 capsid protein (gene 10) arranged as 60 hexamers on the faces of the shell and 11 pentamers at the vertices (4). Attached at the remaining vertex is the head-tail connector (gene 8), a short conical tail (genes 11 and 12) and 6 tail fibers (gene 17) (shown schematically in Fig. 1).

The phage assembly process is similar to that of other double-stranded DNA phages (7). DNA is packaged into a procapsid shell made up of scaffolding protein (gene 9), capsid protein, the head-tail connector, and an internal protein structure (genes 13, 14, 15, and 16). The DNA is packaged from linear concatemers, and as the DNA enters the procapsid shell the scaffolding protein

is released and a conformational change occurs in the shell to form the mature particle. Tail and tail fibers attach at the head-tail connector vertex.

The T7Select[®] Phage Display System uses the T7 capsid protein to display peptides or proteins on the surface of the phage. The capsid protein is normally made in two forms, 10A (344 aa) and 10B (397 aa). 10B is produced by a translational frameshift at amino acid (aa) 341 of 10A, and makes

T7Select vectors

There are two basic types of T7Select phage display vectors: the T7Select415 vector for high-copy number display of peptides, and the T7Select1 vectors for low-copy number display of peptides or larger proteins (Table 1). In all of the vectors, coding sequences for the peptides or proteins to be displayed are cloned within a series of multiple cloning sites following aa 348 of the 10B protein (Figs. 2 and 3). The natural translational frameshift site within the capsid gene has been removed, so only a single form of capsid protein is made from these vectors.

Functional peptides up to 39 amino acids have been displayed from T7Select415. Expression of the T7Select415 capsid gene

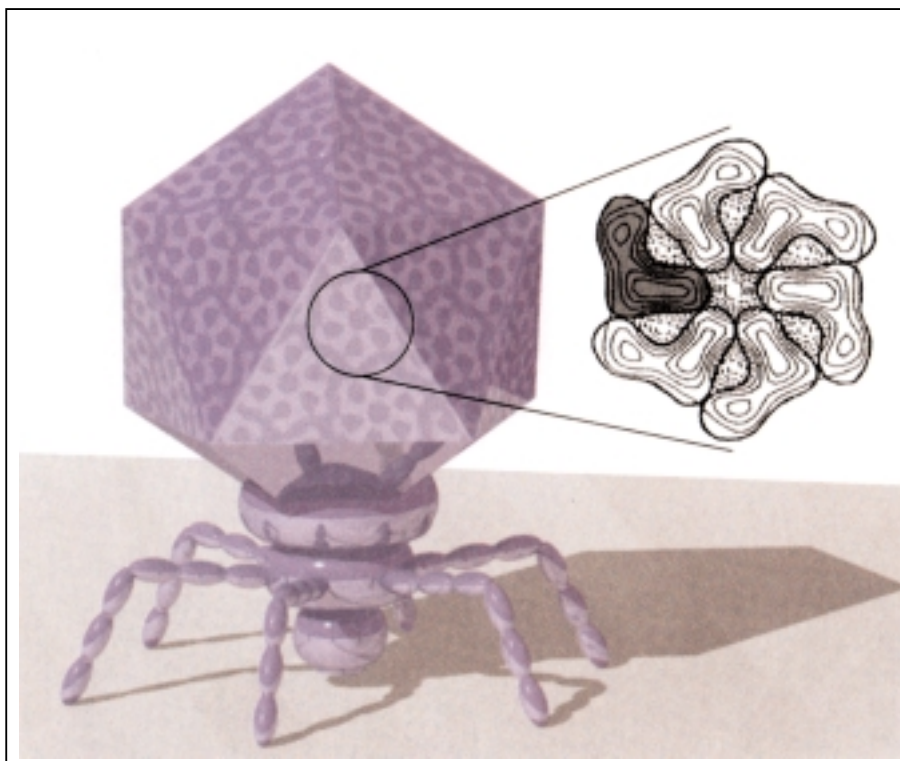


Fig. 1. Structure of the T7 phage particle.

The capsid shell, head-tail connector, tail, and tail fibers are shown schematically. The diffraction pattern from polyheads (4) showing a hexamer capsid unit has been fit onto the surface of the icosahedral particle (diameter approx. 55 nm). The monomer units are in gray.

up about 10% of the capsid protein (8). However, functional capsids can be composed entirely of either 10A or 10B, or of various ratios of the proteins (9). This finding provided the initial suggestion that the T7 capsid shell could accommodate variation, and that the region of the capsid protein unique to 10B might be on the surface of the phage and could be used for phage display.

is controlled by the same strong phage promoter ($\phi 10$) and translation initiation site (*s10*) as in wild-type phage (Fig. 3), and the capsid/peptide fusion protein is produced in large amounts during infection. T7Select415 clones usually grow well on normal T7 laboratory hosts, such as *E. coli* BL21. The capsid shell of the phage is composed entirely of the capsid/peptide fusion

protein, thereby displaying 415 copies of peptide on the surface of the phage. High copy number display is desirable wherever a strong signal is useful, such as in epitope mapping. It may also be important for obtaining peptides that at best bind only very weakly to their targets.

Functional proteins up to slightly more than 1000 amino acids have been displayed from T7Select[®]1-1 vectors. The T7Select1-2a,b,c series provides multiple cloning sites in all three reading frames. Peptides or proteins are displayed in low copy number (about 0.1–1 per phage) from these vectors, which makes them suitable for the selection of proteins that bind strongly to their targets. In order to obtain low-copy display, the promoter of the capsid gene was removed and the translation initiation site was altered (Fig. 3). The capsid mRNA is still produced from phage promoters located further upstream of the gene, but production of capsid protein is greatly reduced. T7Select1 phage are grown on a complementing host (BLT5403) that provides large amounts of the 10A capsid protein from a plasmid clone. The 10A gene in the com-

The phage maintains infectivity following treatment with 1% SDS, 5 M NaCl, up to 4 M urea, 2 M guanidine-HCl, 10mM EDTA, reducing conditions (up to 100 mM DTT), and alkaline conditions (up to pH 10).

plementing plasmid and the capsid gene in the vectors have been engineered to minimize any recombination between the genes.

Cloning in T7Select vectors

Procedures for cloning in T7Select vectors are similar to those for cloning in bacteriophage λ vectors. Vector arms are prepared and ligated with target inserts, the resulting DNA is incubated with an *in vitro* packaging extract, and the phage products are used for infection of a suitable host. The multiple cloning sites in the T7 vectors (Fig. 2) are compatible with many existing vectors, including the pET vectors used in the T7 expression system.

The target DNA inserts usually contain a limited region coding for variant amino acids. The size of the library required to



have a good chance of including all variants increases with the number of varied amino acids. For example, a complete heptapeptide library has $20^7 = 1.28 \times 10^9$ unique heptapeptides. The capacity to construct large libraries in any cloning system depends on the overall efficiency of cloning and packaging (phage) or transformation (plasmids). The vector arms and T7 packaging extracts provided in the T7Select System routinely produce $> 10^8$ recombinant plaques per μg of arms. This efficiency is 10- to 50-fold higher than usually observed with λ cloning systems, and comparable to the optimal efficiency of plasmid systems. The high-efficiency T7 packaging extracts (2×10^9 plaques per μg intact DNA) are made with a specially designed phage that reduces the non-recombinant cloning background to below 0.1%.

For verification of performance, the T7Select Cloning Kits include a positive control target DNA, which encodes the 15 aa S•Tag[™] peptide. S•Tag recombinants are easily detected with a rapid, chemiluminescent plaque lift assay using the T7Select Biopanning Kit.

Examples of peptide and protein display

We have displayed a variety of biologically active peptides and proteins from the T7Select vectors. Peptides displayed in

high copy number (415 per phage) from T7Select415 include:

- S•Tag (15 aa) from pancreatic ribonuclease A
- HSV•Tag[®] epitope (11 aa) from Herpes Simplex Virus glycoprotein D
- Streptavidin-binding peptide (10 aa) (10)
- RGD peptide (8 aa) from adenovirus penton protein (11)
- Thrombin cleavage site (7 aa) from pET vectors
- HSV•Tag + His•Tag[®] sequences (39 aa)

These peptides were cloned on DNAs that added from 10–39 aa to the 10B capsid protein (measured from aa 348, the last naturally occurring aa). In each case, the display of functional peptide was verified by an appropriate binding assay. The use of the thrombin cleavage site enabled us to demonstrate directly that all 415 copies of peptide appear to be on the surface of the phage and can be clipped off by thrombin without reducing the infectivity of the phage (Fig. 4).

Peptides or proteins displayed in low copy number (0.1–1 per phage) from T7Select1 vectors include:

- *E. coli* β -galactosidase (1015 aa)
- T7 RNA polymerase (873 aa)

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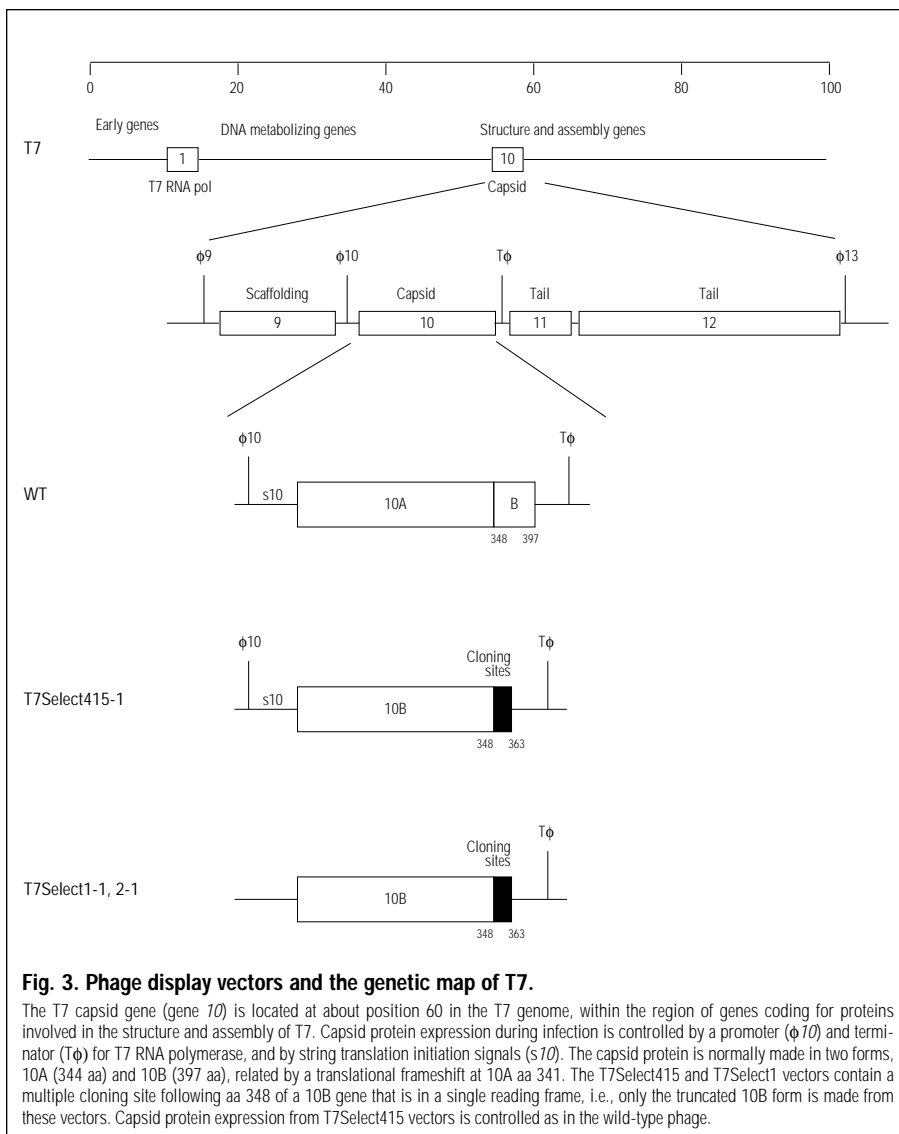


Fig. 3. Phage display vectors and the genetic map of T7.

The T7 capsid gene (gene 10) is located at about position 60 in the T7 genome, within the region of genes coding for proteins involved in the structure and assembly of T7. Capsid protein expression during infection is controlled by a promoter ($\phi 10$) and terminator ($T\phi$) for T7 RNA polymerase, and by string translation initiation signals ($s10$). The capsid protein is normally made in two forms, 10A (344 aa) and 10B (397 aa), related by a translational frameshift at 10A aa 341. The T7Select415 and T7Select1 vectors contain a multiple cloning site following aa 348 of a 10B gene that is in a single reading frame, i.e., only the truncated 10B form is made from these vectors. Capsid protein expression from T7Select415 vectors is controlled as in the wild-type phage.

- scFv single-chain antibody (257 aa)
- T7 endonuclease (149 aa)
- S•Tag (15 aa)
- HSV•Tag (11 aa)

In each case, display was verified by either a binding assay or an enzymatic assay. Phage displaying T7 endonuclease appear to have about the same enzymatic activity as purified T7 endonuclease (12) (Fig. 5). On the other hand, the activity of β -gal phage could be easily detected using a standard enzymatic assay, but was about 250-fold lower than the measured copy number of the β -gal. This difference presumably reflects the fact that β -gal is active only as a tetramer. Clearly, not all displayed enzymes will be active “phagezymes.” This will depend on whether the enzyme can be active with a phage fused to its N-terminus

and, where the phage has been purified, whether the enzymatic activity survives the purification. For example, phage displaying T7 RNA polymerase is recognized by polyclonal antibody bound to the polymerase, but we have not yet been able to establish enzymatic activity for this phage.

Biopanning selection

We have carried out standard biopanning experiments with phage displaying the S•Tag in high copy number or the HSV•Tag in either low or high copy number. S•Tag phage yielded a nearly 10^6 -fold enrichment after two rounds, and $> 10^7$ -fold enrichment after four rounds of biopanning and growth, when the phage was originally mixed with control phage in a ratio of 1 to 2×10^7 (Fig. 6). The HSV•Tag phage gave similar results. When the HSV•Tag was

displayed in low copy number (0.5 per phage, Fig. 7) and the phage mixed with control phage in a ratio of 1 to 10^6 , the HSV•Tag phage constituted about 25% of the population after three rounds of selection. This represents a 50- to 100-fold enrichment for each round of selection.

The stability of the T7 phage particle enables the use of a variety of elution conditions during biopanning. The phage maintains infectivity following treatment with 1% SDS, 5 M NaCl, up to 4 M urea, 2 M guanidine-HCl, 10 mM EDTA, reducing conditions (up to 100 mM DTT), and alkaline conditions (up to pH 10). The phage is not stable to low pH (below 4 or so), a condition often used in biopanning for filamentous phage. Biopanning requires both binding and elution conditions that maintain the infectivity of the phage. The wide range of conditions available for T7Select® biopanning should expand the range of targets that can be used successfully. The T7Select Biopanning Kit provides the materials necessary for testing your own biopanning procedures, using phage that display the S•Tag peptide.

Characteristics of the system

Large proteins cannot be cloned in T7Select415, the high copy number display vector. The largest peptide yet displayed from T7Select415 is 39 aa long. It seems likely that peptides up to at least 50 amino acids will work, since this will create a capsid protein about the same size as the wild-type 10B protein. The capacity of T7Select415 is clearly sufficient for displaying structurally constrained peptides and

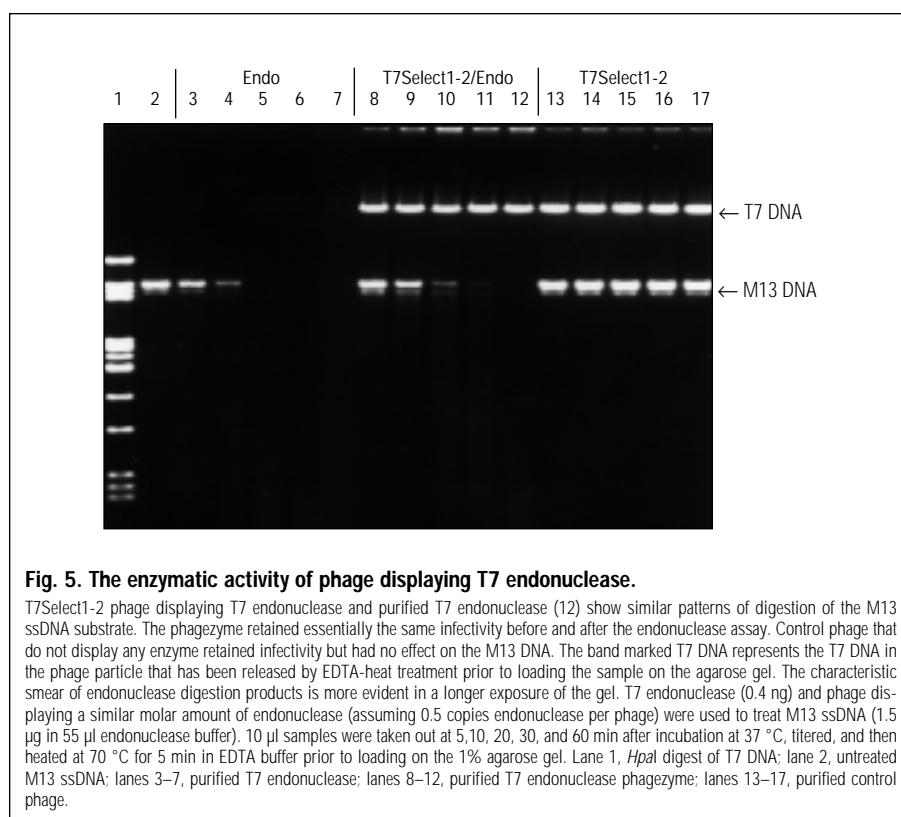
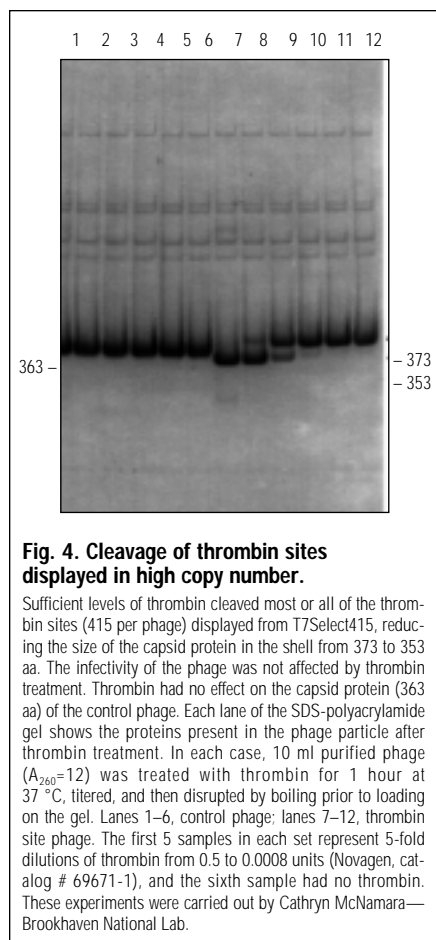
Displayed peptides and proteins do not need to be capable of export through the periplasm and the cell membrane, as in filamentous systems.

peptides whose biological activity requires longer stretches of amino acids.

T7 phage with capsids made entirely of 10B grow poorly, and this may also occur with some T7Select415 phage. We know that even some small peptides cannot be cloned in T7Select415, although the rules for exclusion are not yet understood.

Exclusion because of either the size or the sequence of the peptide presumably occurs because the resulting 10B/peptide fusion protein cannot assemble into a capsid shell. T7Select[®]415 phage are normally grown on the *E. coli* host BL21, where the fusion protein is the only source of capsid protein. In some cases, growth inhibition can be relieved by growing the phage on BLT5403, where large amounts of 10A capsid protein is provided from a plasmid. The capsid shell of these phage will be composed of a mixture of 10A and 10B/peptide fusion protein.

The largest protein yet displayed from the low copy display T7Select1 vectors is 1015 amino acids. The primary limitation on size is the DNA cloning capacity of the vector (3.6kbp, 1200 aa for T7Select1-1; 2.7kbp, 900 aa for T7Select1-2 vectors). However, it may be that phage displaying proteins of more than about 600 amino acids often grow poorly. This rule of thumb is at least consistent with our observations of the behavior of phage displaying a variety of proteins, including a series of N-terminal



fragments of β -galactosidase (71, 271, 431, 691, 833 or 1015 aa). T7Select1 phage that grow poorly can accumulate deletions within the capsid fusion gene, since they are grown on a complementing host (BLT5403) that provides the 10A protein from a plasmid. The 10A gene in the plasmid is controlled by a T7 promoter. In some cases, we have relieved growth inhibition by growing the phage on BLT5615, where plasmid expression of 10A is controlled by the *lacUV5* promoter.

The copy number of display from T7Select415 grown on BL21 is fixed by the number of capsid proteins in the T7 capsid shell (415). The display number from T7Select1 vectors is not similarly fixed. It presumably depends on the ratio of expression of the capsid fusion protein from the vector and the 10A protein from the complementing host (BLT5403 or BLT5615), and also on the efficiency of assembly of the fusion protein into the capsid shell. Copy numbers per phage measured by Western analysis have been 0.5 for HSV•Tag (Fig. 7), 0.3 for T7 ssDNA binding protein, 0.2 for β -galactosidase, and 0.1 for T7 RNA polymerase.

Summary

The T7Select Phage Display System uses bacteriophage T7 as a display vector, and has a number of attractive features:

- T7 is easy to grow
- Large display libraries can be made using the T7Select packaging extracts
- Peptides up to 50 aa can be displayed in high copy number (415 per phage)
- Peptides and larger proteins up to 1200 amino acids can be displayed in low copy number (0.1–1 per phage)
- Displayed peptides and proteins do not need to be capable of export through the periplasm and the cell membrane, as in filamentous systems
- T7 is extremely stable, expanding the variety of agents that can be used in biopanning

The capacity of phage display systems will certainly overlap, and each will have its own limitations that depend on the growth properties of the phage. We have given the same attention to detail and quality control with the T7Select system as with the

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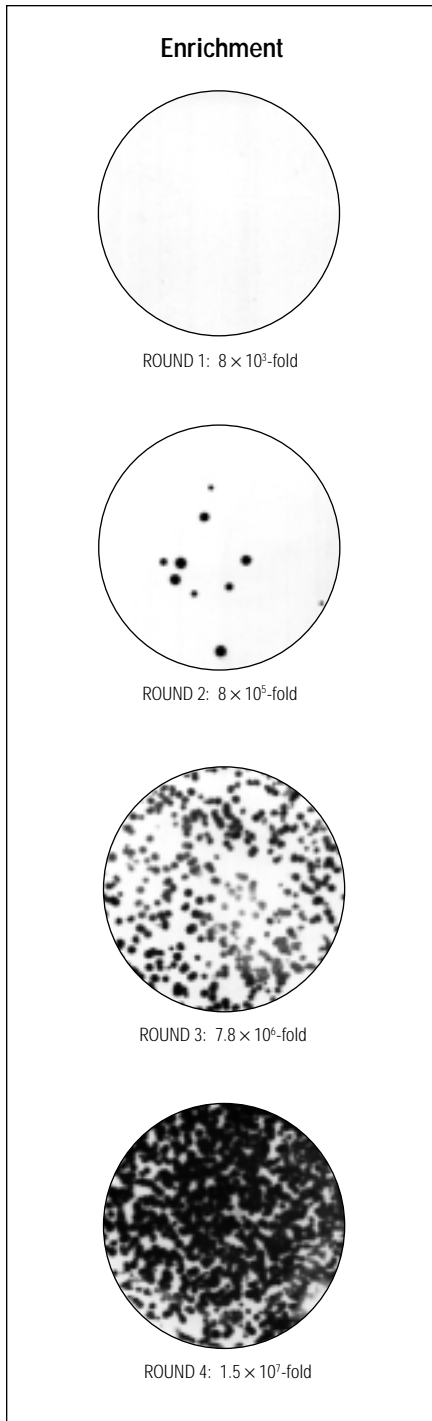


Fig. 6. Biopanning selection of T7Select415 phage displaying the S•Tag sequence.

The S•Tag phage was enriched approximately 10^6 -fold after two rounds of selection, and almost all of the phage contained the S•Tag after four rounds of selection. The experiments were performed starting with a mixture of phage lysate containing S•Tag phage and control phage in a ratio of $1:2 \times 10^7$ (1×10^9 total infective phage). The phage were allowed to bind to S-protein coating a microtiter well (96-well plates) for 30 min at room temperature. Unbound phage were removed by washing with 1X PBST (phosphate-buffered saline, 0.1% Tween-20). Bound phage were eluted with PBST containing 1% SDS and used to grow a new lysate by infecting a mid-log BL21 culture. Four rounds of selection were performed, and the identity of the phage in the lysate after each round was detected by plaque lifts followed by chemiluminescent visualization using the T7Select Biopanning Kit.

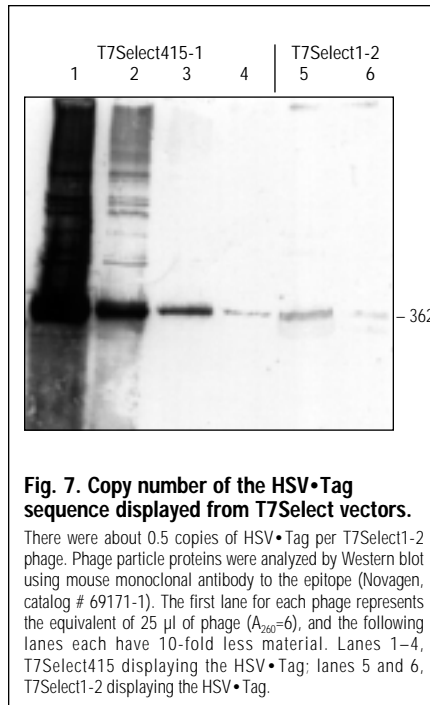



Fig. 7. Copy number of the HSV•Tag sequence displayed from T7Select vectors.

There were about 0.5 copies of HSV•Tag per T7Select1-2 phage. Phage particle proteins were analyzed by Western blot using mouse monoclonal antibody to the epitope (Novagen, catalog # 69171-1). The first lane for each phage represents the equivalent of 25 μ l of phage ($A_{260}=6$), and the following lanes each have 10-fold less material. Lanes 1–4, T7Select415 displaying the HSV•Tag; lanes 5 and 6, T7Select1-2 displaying the HSV•Tag.

pET expression system, and believe that T7Select® is the system of choice for phage display.

Acknowledgments

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* The T7Select® System is covered by US patents 5,223,409, 5,403,484 and other patents pending. The system is sold for research use only. Any commercial use of the T7Select System, including the discovery or development of commercial products, requires licenses from Dyax Corp. and Novagen, Inc.