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Immunological Screening of λ-Phage cDNA Expression Libraries

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1. Introduction

When trying to obtain a cDNA clone to a novel protein, the only handle one may have is an antibody that recognizes the protein of interest. Consequently, the obvious approach is to screen a λ-phage expression library using the antibody. In general, polyclonal sera give better results, but a mixture of monoclonal antisera could be used instead. Fortunately, a number of convenient commercial vectors are available that can be used to generate an expression library where the cloned inserts are induced to produce protein within bacteria, usually as a fusion protein with β-galactosidase. Alternatively, it may be possible to buy a library of a cell line or tissue known to express the protein of interest in reasonable abundance. The library should have a complexity of at least 10^6, since in theory only one in six inserts will clone in the orientation and frame required to produce protein, although modern vectors often allow directional cloning. Thus, one in three of the clones to the protein of interest may be recognized by the antibody. Attention should also be given to the average size of the inserts, particularly for a large protein, since even polyclonal sera to the full protein may only recognize one epitope, which could lie at the N-terminus. Random-primed libraries tend to contain more N-terminal sequences and fewer extraneous sequences such as long 3'-untranslated regions than oligo-dT-primed libraries, but the former are rarely directional. A suitable compromise may be to screen a mixed library that has been generated using both priming methods, and many of these are available commercially.

Having obtained the library, the essence of the technique is to infect a bacterial lawn much as for DNA probe screening. Initially, expression of the cloned inserts is suppressed in case the protein products are toxic. Once phage lysis is

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detected, the plates are overlaid with nitrocellulose filters and switched to conditions that will allow fusion protein production onto the filter. Subsequently, the filter can be probed with the antibody as for a Western blot to detect phage plaques producing the cognate protein. False positives can be excluded by making replica filters. Repeated rounds of screening will allow the purification of a cDNA clone to the protein of interest.

2. Materials

2.1. Library Plating and Replica Lifts

1. Phage expression library of known titer: The protocol described here is suitable for λgt11 *(1)* or λZAP (Stratagene, La Jolla, CA) libraries.
2. Appropriate bacterial host, e.g., Y1090 (amp^R) for λgt11; XL1-blue (tet^R) for λZAP
3. Bacterial culture media and well-dried plates: L-broth with 10 m*M* $MgSO_4$, 0.2% maltose for growing plating cells; L-broth agar or NZY agar plates; top agarose. 0.7% agarose in L-broth with 10 m*M* $MgSO_4$, 0.2% maltose
4. SM phage dilution buffer: 100 m*M* NaCl, 10 m*M* $MgSO_4$, 50 m*M* Tris-HCl, pH 7.5, 0.01% gelatin. Autoclave.
5. Isopropyl β-D-thiogalactopyranoside (IPTG). 1*M* stock in water. Store at –20°C
6. Nitrocellulose filters; 132- and 82-mm circles
7. TBST: 20 m*M* Tris-HCl, pH 7.5, 150 m*M* NaCl, 0.05% Tween-20
8. Block: TBST plus 1% bovine serum albumin (BSA)

2.2. Antibody Screening

1. High-titer polyclonal primary antibody or two or more monoclonal antisera
2. Bacterial lysate.
3. Antibody detection system: usually requiring incubation with an enzyme-conjugated secondary antibody followed by either a color reaction (e.g., Stratagene's Picoblue immunodetection kit) or a photochemical system (e.g., Amersham's [Amersham, UK] ECL kit). Commercially available screening kits often contain positive controls (phage clones and appropriate primary antibodies). If the antibody to the protein of interest is in short supply, it is a good idea to run through the technique using these controls before experimenting with a precious antiserum!

3. Methods

Before using the antibody to screen a library, it is important to check its specificity on Western blots and to determine the optimum dilution factor and incubation conditions (*see* Note 1). It is also important to check that there is minimal crossreaction with proteins in an *Escherichia coli* lysate to avoid too high a background. If the antibody does show some recognition of bacterial proteins this can usually be pre-absorbed out (*see* Note 2), and mild crossreactivity will be progressively reduced if the antibody dilution is kept

and reused in subsequent screens. This is particularly desirable if the serum is precious—a mouse polyclonal serum, for example.

3.1. Library Plating and Replica Lifts

1. Grow a fresh overnight culture of host bacteria in L-broth with maltose. Spin cells down and resuspend in a half volume of SM. Keep at 4°C until required
2. Plate out 10^6 plaques at 50,000/15-cm Petri dish using the plating cells (600 µL/plate) from above and top agarose (Note 3). Incubate plates upside down at 42°C for 3–4 h until the plaques are just visible as pinpricks on the bacterial lawn.
3. Soak one set of 132-mm nitrocellulose circles in 10 m*M* IPTG, and pat dry. Taking one plate at a time from the 42°C incubator, quickly lay a numbered damp (but not wet) filter on each plate trying to avoid air bubbles, and place in a 37°C incubator for 2–4 h (Note 4). The temperature of the agar should not drop below 37°C, or protein production will be resuppressed.
4. Prepare a further set of IPTG-soaked filters and 500 mL each of TBST and of Block. Again working with one plate at a time, stab through the first filter, and agar with a syringe needle four or five times to orient the two. Peel off this filter and place in a large lunch box or plastic container containing the TBST Place a second numbered filter on the agar as before, and return to the 37°C incubator for a further 1–3 h (*see* Note 4). Before removing these filters, place each plate on a light box, and use a permanent marker pen to mark dots over the needle holes visible in the agar. After removing the filters, it is wise to use a needle to pierce through the inked dots!
5. On removal, each set of filters should be rinsed briefly in TBST—this will reduce background—and then transferred individually to the Block Swirl the container as each filter is added to prevent them from sticking together. Filters can be left in Block at 4°C overnight (and probably up to 2–3 d as long as they do not dry out). The plates should be wrapped in cling film and stored at 4°C until the screening is complete.

3.2. Antibody Screening

1. Make an appropriate dilution of the primary antibody in 50 mL of fresh Block Incubate five to six filters at a time for 1 h at room temperature (*see* Note 1) on a slow shaker. An ideal container is a 150-mm high-sided Petri dish. Again, remember to swirl the dish as a filter is added to ensure there is always a layer of fluid between each. If large supplies of antisera are available, set up sufficient parallel incubations to probe all the filters; otherwise, probe in sequential batches. Afterward, the antibody dilution can be stored at –20°C and reused for subsequent screens, although it may need "pepping up" with some fresh antibody occasionally.
2. Remove the filters to a large container of TBST. These can be left until all the filters have been incubated with antibody. Wash all the filters three times for 5 min each wash in a large excess of TBST, ensuring that they do not stick together during shaking.

3. Dilute the detection system's antibody–enzyme conjugate into Block, and incubate as recommended (usually 1 h at room temperature)
4. Wash the filters as in step 2.
5. Give the filters a final rinse in Tris-buffered saline to remove Tween-20.
6. Develop the filters using either color detection or chemiluminesence. In either case, a small number of spots should appear (*see* Note 3). Check that spots are duplicates by orienting the stab holes—for detection systems using color development, this can most easily be done by tracing the orientation holes and the positive spots onto pieces of clear plastic. For photochemical detection, it is important to include fluorescent markers on the mounting used to autoradiograph the filters to enable you to orient the film to the filters.
7. Pick all duplicate positive signals (*see* Note 1), and replate out a dilution on 9-cm agar plates as described in Chapter 14. By repeating the processes, you will ultimately be able to pick a pure plaque for further authentication.

4. Notes

1. The library screen is essentially a Western blot, so it is important to do a series of these to determine the optimum incubation conditions (usually 1 h at room temperature) and the appropriate dilution factor for the primary antibody. Excess antibody will only give an unworkable background. It is also important to assess whether the washing conditions described will be sufficient to detect only the protein of interest in a blot using total cell extract. If a number of other proteins are also detected, then the antibody is probably unsuitable to use for screening since too many unrelated clones will be purified. However, more stringent washing may improve the situation, and if so, these conditions should also be used in the screen. Sometimes a purified protein used to raise an antibody contains a low-level contaminant that proves to be very antigenic, giving a bright signal on the Western in addition to the protein of interest. In this situation, it is possible to proceed, but when picking plaques after the first-round screen, it will be important to select both bright and faint spots to ensure that the interesting clones are not overlooked. Another issue is that if the protein of interest is thought to be covalently modified (phosphate, carbohydrate), then it is wise to check that the antisera will still recognize the native protein, since obviously these modifications will not be reproduced in the bacterially made protein during the screen.
2. Polyclonal antisera are often reactive with bacterial and phage proteins. This can be tested by dotting some phage-infected bacterial lysate (often provided in screening kits) on the corner of a Western blot prior to blocking or running some on a spare lane in the initial sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. If a strong signal is observed, it will be necessary to preabsorb out the reacting antibodies. This can be done by incubating three to four strips of nitrocellulose (5 × 10 cm) in bacterial lysate and then blocking them for 1 h at room temperature before rinsing three times in TBST. Dilute a reasonable amount of the primary antibody 1:5 in TBST, and incubate this with one of the filters for 15 min at room temperature. Remove the filter, and discard and replace with

another. Repeat until all of the prepared filters have been used. Store the final antibody solution, and recheck on a Western for the appropriate dilution and also that the crossreactivity has been reduced sufficiently.

3. Top agarose is much easier to use than top agar, which frequently sticks to the nitrocellulose and tears when the filters are removed.
4. Optimal incubation times are difficult to determine, since so many variables will influence the amount of protein and the ease of detection of the signal (density of plaques on the plate, specific activity of antibody for protein, protein size, etc.). I readily detected clones to a 70-kDa protein using a 3-h incubation for the first lifts and a 2-h incubation for the second lifts. As the plaques grow and the bacteria in the middle lyse, less and less protein is made in the center of the plaque Consequently, after development, the final signals often do not look like uniform spots, but more like "donuts," that is, a bright ring around a clear center

Reference

1 Huynh, T. V., Young, R. A., and Davis, R. W. (1988) Constructing and screening cDNA libraries in λgt10 and λgt11, in *DNA Cloning A Practical Approach*, vol 1 (Glover, D. M , ed), IRL, Oxford, UK, pp 49–78