Large-scale phagemid conversion on solid medium

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An important element of genomic analysis programs is the investigation and systematic sequencing of cDNA populations. The method we propose is designed to isolate a large number of different representative cDNAs from phagemid libraries with no perturbation of their relative amounts.

Modified λ phages, called phagemids, have been designed for rapid and efficient subcloning of recombinant fragments (1-4), phage display technology (5), and also DNA delivery (6,7). We have used a phagemid (λ TriplEx2™; BD Biosciences Clontech, Palo Alto, CA, USA) based on the *Cre/LoxP* system (8,9) and propagated it in *Escherichia coli* XL1-blue (10). *Cre* is a recombinase that specifically recognizes a DNA sequence named *LoxP*. The cloning vector is a λ phage bearing a plasmid sequence surrounded by two *LoxP* sites. When λ TriplEx2 phage is introduced into *E. coli* BM25.8, a strain that expresses *Cre*, the phagemid is converted into its plasmid, which can be amplified in the bacteria. This plasmid can then be extracted for sequencing or other procedures. Normally, these types of phagemids are mainly used for subcloning particular sequences after library screening, and the conversion process is done in liquid media. Large-scale conversions are also done in liquid media. Under these conditions, the relative abundance of different sequences in the population may be artifactually altered, because phage replication will depend on the length and/or cytotoxicity of the inserted sequence.

In our research program, we wanted to rapidly isolate plasmids carrying cDNAs that were representative of the *in vivo* mRNA population for sequencing. For this purpose, we have developed a technique that allows the conversion of phagemid to plasmid on solid media. This technique presents

numerous advantages: (i) the yield of conversion is nearly 100%; (ii) in 1 day, a single person can convert a large number of clones (12,000) and manually subculture 10% of them; (iii) plasmids are easily extracted and sequenced; and (iv) as no amplification of the primary library is necessary, no bias is introduced into the cDNA population profile, and moreover, toxic cDNA sequences can be detected, isolated, and amplified in the λ phage vector for subsequent characterization.

The protocol for use of λ TriplEx2 is as follows. A primary cDNA library is plated on predried solid LB medium at a density of 2–2.2 plaque-forming units (pfu)/cm² and grown overnight. At the same time, a preculture of *E. coli* BM25.8 is grown overnight at 31°C in LB medium plus 10 mM MgSO₄. The BM25.8 overnight culture is diluted 1:40 in LB medium plus 10 mM MgSO₄ and grown until OD_{600 nm} = 1. Then 1 M MgCl₂ is added to a final concentration of 10 mM.

A drop of the BM25.8 bacterial culture is placed on a hydrophobic surface (such as a plastic sheet or a Petri dish cover) using 15 μ L culture/cm² of surface. A nitrocellulose filter with 0.45 μ m porosity [either Optitran®, from Schleicher & Schuell (Keene, NH, USA) or Hybond®-C Pure from Amersham Biosciences (Piscataway, NJ, USA)] is then deposited on the drop of bacteria so that the entire filter is impregnated (Figure 1). The filter

Figure 1. Summary of the filter conversion method. Orientation of the word "NITRO" shows the orientation of the nitrocellulose filter during each stage of the manipulations.

is then inverted (bacteria side up) and dried on 3MM filter paper (Whatman, Clifton, NJ, USA) until no trace of liquid remains (several seconds). The filter is placed bacteria side down on a Petri dish containing the λ TriplEx2 lysis plaques and incubated for 15 min at 31°C. A longer incubation time (up to 1 h) may be preferable if the lysis plaques are very small. The filter is then removed and placed plaque side up on an LB plate containing at least 100 μ g/mL ampicillin and cultured overnight at 31°C.

Each plaque will give rise to a colony carrying the corresponding plasmid. Single colonies can be replicated in selective medium (Terrific Broth plus antibiotic) or stored as desired. We have used yellow Gilson cones (Gilson, Middletown, WI, USA) to transfer individual colonies into 100 μ L of medium in 96-well microtiter plates for culture and storage as glycerol stocks. Two-milliliter cultures were used for plasmid extraction and direct sequencing. More than 12,000 clones have been isolated from three different cDNAs libraries with this method.

To further explore conversion conditions, we have tried other types of support like filter paper or Hybond-N (Amersham Biosciences), but it appears that conversion does not work properly on these supports. On the other hand, it is possible to prepare ready-to-use nitrocellulose conversion filters that can be stored. After deposition of bacteria on the filters, we have subjected them to lyophilization and stored them at 4°C. After 2 days, we have successfully used these filters for conversion without any loss of activity. Thus, it appears that preparation of the conversion filters can be uncoupled from the library conversion itself.

This efficient method of conversion can be applied to other binary vectors, such as λ SCREEN™ and λ BlueSTAR™, both from Novagen (Madison, WI, USA) (3). For the latter vector, which is designed for genomic libraries, the conversion yield cited by Novagen in liquid media is 10%, which is very low. The very high-throughput observed with our technique should greatly increase the utility of the λ BlueSTAR vector for production of genomic libraries.

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Viability assay for *Dictyostelium* for use in drug studies

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The eukaryotic cells of *Dictyostelium discoideum* are widely used for studies in cellular and molecular biology (1). The organism has been especially useful for elucidating the molecular mechanisms that underlie cell motility and chemotaxis (2). Recently, *D. discoideum* has taken on an important new role in investigations of resistance to anticancer drugs and the discovery of new drug targets (3-6). For example, it has been used to identify novel genetic pathways involved in the response to cisplatin (4), and its endogenous morphogen differentiation-inducing factor-1 (DIF-1) has been

identified as an antileukemic agent (7). Several features make this organism ideal for these studies, including (i) its well-studied biology and haploid genome, which is almost completely sequenced; (ii) the ability to do homologous gene replacement and random insertional mutagenesis; (iii) the ease and low cost of culture (compared to animal cells) and rapid doubling time; and (iv) its high level of genetic conservation with humans.

Assessing the effects of drugs on living cells requires frequent and accurate measurements of cell viability over several orders of magnitude. *D. discoideum*