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Expression Library Screening

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1. Introduction

When trying to identify a clone within a cDNA library, which may contain a coat protein (CP) gene, one useful technique may by immunological screening, using antibodies raised against either purified virus or isolated CP. Antibody screening can be carried out on a cDNA cloned into a wide range of vectors, including plasmids and phage-based vectors. Indeed, a whole plethora of commercial vectors are now available that have been optimized for generating expression libraries, including λ-gt11, λZAP (Stratagene, La Jolla, CA). However, antibody screening can be carried out on the simplest of plasmid vectors, based on the principle that, if the plasmid uses blue/white color selection, then a percentage of the cDNA inserts will be expressed as a fusion protein with β-galactosidase when the cells are induced with IPTG. The method described within this chapter will deal with such a plasmid screen, with readers directed to λ-screening chapters by Somssich and WeiBhaar in *Plant Gene Isolation **(1)*** and Hurst in *cDNA Library Protocols **(2)***, and (one of the original and best descriptions) by Huynh et al. in *DNA Cloning: A Practical Approach **(3)***, all being good references for suitable lambda protocols. A typical immunological screen is shown in **Fig. 1**, for a pUC13 vector ***(4)***. Double-stranded cDNA to the carlavirus, Helenium virus S (HelVS) was ligated into *Sma*I digested pUC13 vector and transformed into competent *Escherichia coli.* Colonies were screened with both nucleic acid probes using HelVS specific (^{32}P) first-strand cDNA (**Fig. 1A**) and also using HelVS polyclonal antisera (**Fig. 1B**). Two clones at positions 19 and 23, designated pHel19 and pHel23, were detected using polyclonal antisera. Both clones were further analyzed as to the possible size of the CP-coding region that was being expressed by polyacrylamide gel electrophoresis (PAGE) and Western analysis of total

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