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A Novel Helper Phage Enabling Construction of Genome-Scale ORF-Enriched Phage Display Libraries

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Abstract

Phagemid-based expression of cloned genes fused to the *gIIIP* coding sequence and rescue using helper phages, such as VCSM13, has been used extensively for constructing large antibody phage display libraries. However, for randomly primed cDNA and gene fragment libraries, this system encounters reading frame problems wherein only one of 18 phages display the translated foreign peptide/protein fused to phagemid-encoded gIIIP. The elimination of phages carrying out-of-frame inserts is vital in order to improve the quality of phage display libraries. In this study, we designed a novel helper phage, AGM13, which carries trypsin-sensitive sites within the linker regions of gIIIP. This renders the phage highly sensitive to trypsin digestion, which abolishes its infectivity. For open reading frame (ORF) selection, the phagemid-borne phages are rescued using AGM13, so that clones with in-frame inserts express fusion proteins with phagemid-encoded trypsin-resistant gIIIP, which becomes incorporated into the phages along with a few copies of AGM13-encoded trypsin-sensitive gIIIP. In contrast, clones with out-of-frame inserts produce phages carrying only AGM13-encoded trypsin-sensitive gIIIP. Trypsin treatment of the phage population renders the phages with out-of-frame inserts non-infectious, whereas phages carrying in-frame inserts remain fully infectious and can hence be enriched by infection. This strategy was applied efficiently at a genome scale to generate an ORF-enriched whole genome fragment library from *Mycobacterium tuberculosis,* in which nearly 100% of the clones carried in-frame inserts after selection. The ORF-enriched libraries were successfully used for identification of linear and conformational epitopes for monoclonal antibodies specific to mycobacterial proteins.

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Introduction

Phage display is a powerful technique for studying protein-ligand interactions and identification of immunodominant regions using gene fragment libraries. In addition, it has been exploited for epitope mapping and construction of large antibody libraries to select desired binders with improved affinities [1,2].

Among different phage display systems, gIIIP of the filamentous bacteriophage M13 is most widely employed. The gIIIP is a 406 amino acid protein with a maximum of five copies per phage. It comprises three functionally distinct domains: N1, N2 and CT, which are separated by glycine rich linkers [3]. These domains play a crucial role in infection and phage assembly; however, peptides and proteins can be inserted at the boundaries between the gIIIP domains without affecting the infectivity of the phage [4,5]. For gIIIP-based display, vectors based on phagemid carry the gene encoding *gIIIP* under the control of a regulated promoter, with the foreign DNA cloned between a signal sequence and the *gIIIP* coding sequence [6–8]. Phage production is initiated by infection with a helper phage (such as VCSM13), which provides all of the proteins necessary for the replication and assembly of phage particles. The extruded phage particles encapsulate phagemid single-stranded DNA and display two types of gIIIP protein: one encoded by the helper phage (native gIIIP protein) and the other encoded by the phagemid (gIIIP fusion protein).

The use of phage display technology in constructing cDNA libraries has been challenging due to the stop codons and the polyA tail present in full-length mRNA [9,10]. Using randomly primed cDNA fragments can alleviate this limitation, however, the majority of clones remains out-of-frame (17 out of 18 possible frames). This problem is also encountered in gene fragment libraries made from random fragments of gene/genome sequences. Also, during the construction of complex antibody libraries, PCR is employed at multiple steps. PCR generated errors result in a large number of cloned antibody fragments either having stop codons or out of frame mutations, thus reducing the quality of the libraries. Consequently, large libraries with several million to billion clones are constructed; however, the effective functional population of in-frame clones in these libraries is only 5–6%. Further, when used for affinity selection, these libraries suffer from non-specific interactions leading to poor enrichment of desired clones [11]. The success of selection of specific interactions can be remarkably increased if the quality of input library is improved.