Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytical Biochemistry 339 (2005) 61–68
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Tagged polymerase chain reaction subtractive hybridization for the enrichment of phage display random peptide libraries

Alexander W. Tarr[a], Steven P. Boneham[a], Anna M. Grabowska[b], Jonathan K. Ball[a,*]

[a] *Institute of Infections, Immunity, and Inflammation, School of Molecular Medical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK*
[b] *Cancer Research Unit, School of Surgical Sciences, University of Nottingham, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK*

Received 13 October 2004
Available online 12 January 2005

Abstract

Affinity selection of phage display peptide libraries is routinely used for isolating peptides capable of binding a range of molecules, including antibodies and receptors. This process is most successful when the selecting molecule is relatively pure, for example, a monoclonal antibody. However, isolation of peptides able to bind to target molecules present in a complex mixture is more difficult because the affinity selection process isolates peptides capable of binding to all molecules present in the mixture. Here we describe the development of a tagged polymerase chain reaction (PCR) subtractive hybridization method that is universally applicable for the targeted isolation of peptides able to bind to unique molecules within a complex mixture. We also describe a discriminatory limiting dilution PCR method that can be used to optimize hybridization conditions.

Keywords: Subtractive hybridization; Random peptide libraries; Positive enrichment; DNA selection; Tagged PCR; Phage display; Phage libraries

Random peptide phage display libraries are powerful tools for the identification of peptides able to mimic interactions among a range of biological molecules [1]. This approach has successfully identified peptides that interact specifically with a range of antibodies [2,3], cellular receptors [4,5], and DNA [6]. The technique uses recombinant phage genetically modified to display a randomly determined peptide on its surface; useful libraries consist of millions of phage, each potentially carrying a distinct peptide. Peptides able to bind specifically to the target molecule are then enriched by multiple rounds of biopanning. This process involves incubating the library with the target molecule. Nonbinding phage are removed in a washing step, and then bound phage are recovered [1]. This technique has been most useful where the target molecule used for selection is relatively pure. When the target molecule is present in a background of other biomolecules, such as a specific antibody present in polyclonal serum or a receptor expressed on a cell surface, enriched phage recognize the multitude of molecules present. Complex biopanning procedures are required to differentiate between peptides reacting with the molecule of interest and irrelevant molecules. These include performing depletion steps using an appropriate control that contains the common nontarget molecules but lacks target molecules, as well as performing detailed screening and counterscreening experiments [7]. A major limitation of this process is the small percentage of nonspecific phage that can be depleted in any one reaction. To overcome this problem, multiple rounds of selection and counterselection are used. This can result in a bias toward selection of peptides by factors other than affinity for the target molecule. To minimize these biases, Bartoli and coworkers

* Corresponding author. Fax: +44 115 970 9233.
E-mail address: jonathan.ball@nottingham.ac.uk (J.K. Ball).

0003-2697/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2004.12.020

[8] developed a DNA-based selection technique that used the physical link between the phage DNA and expressed peptide. They generated various phage pools selected against hepatitis C virus antibody positive and negative sera, using the phage DNA to perform a series of enrichment and depletion hybridization steps. This strategy enabled these authors to identify peptides commonly recognized by hepatitis C virus positive sera.

Many molecular techniques have been developed for the comparison of pools of DNA molecules capable of specifically enriching differentially expressed sequences present in a background of common DNA [9]. The process of subtractive hybridization is central to these methods. Subtractive hybridization is a valuable tool that can be used to identify and enrich nucleic acids that are differentially present in two different nucleic acid pools. Subtractive hybridization is defined as the hybridization between two nucleic acid populations that are closely related. Physical or chemical tagging of one of the nucleic acid pools (the driver) enables the depletion of common complementary sequences from the other nucleic acid pool (the tester). This leads to enrichment of unique or overexpressed sequences present in the tester nucleic acid pool. The rate-limiting factor during hybridization is the quantity of nucleic acid available to form interactions. Hybridization efficiency has been vastly improved by the introduction of polymerase chain reaction (PCR)-based[1] techniques able to regenerate large quantities of DNA, and current techniques can enrich low-copy target nucleotide sequences many thousand-fold. Here we describe a new method that combines subtractive hybridization with tagged PCR amplification of depleted phage libraries to enrich individual phage isolates differentially expressed between two pools. We also describe the development of a PCR-based assay to measure prevalence of target sequences before and after subtractive hybridization, enabling the optimization of hybridization conditions suitable for the enrichment of target nucleotide sequences derived from any random library.

Materials and methods

Generation of phage library pools

Phage clones isolated from the M13 bacteriophage random peptide libraries, PhD.7-mer and PhD 12-mer (New England Biolabs), were used to generate stocks of phage clones, each containing 1×10^8 phage/μl. The driver pool contained phage expressing a nontarget 7-mer peptide. The tester pool contained the phage expressing the target peptide diluted 1:1000 in a background of the driver phage, whereas the control driver pool contained phage expressing an irrelevant peptide. The nucleotide sequence corresponding to the peptide insert of each phage clone was known.

Amplification of peptide insert-specific DNA

The general scheme for the tagged PCR subtractive hybridization method is shown in Fig. 1. Initially, a fragment of DNA encompassing the random peptide inserts was PCR amplified. The tester pool was amplified using sense primer TS1 (*TCGGATCGCCTTT*CTATTCTCA CTCT) with 5′-biotinylated antisense primer ASB1 (GCCGAACCTCCACC). To enable selective amplifica-

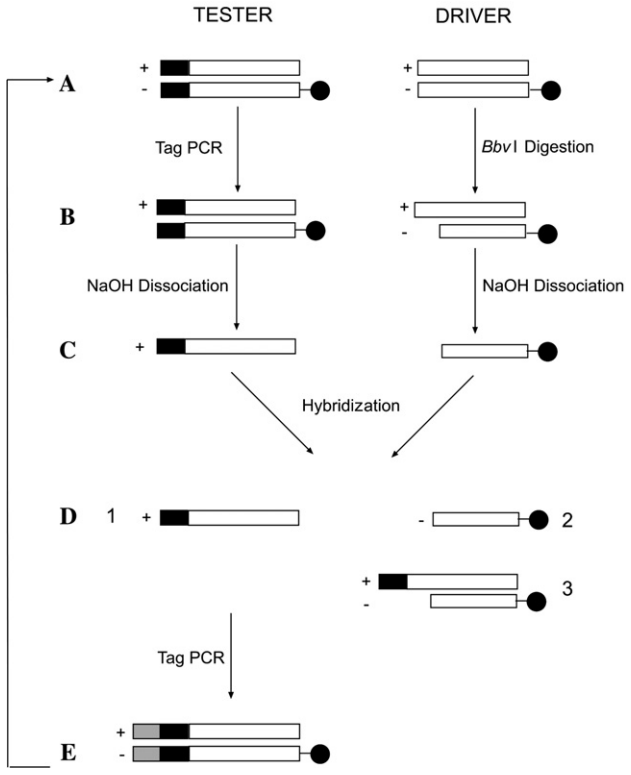

Fig. 1. Schematic of the hybridization protocol. (A) PCR amplicons were generated from tester and driver phage pools. Both pools were amplified using a common biotinylated antisense primer. Tester samples were amplified using a tagged sense primer bearing a 5′ primer-binding site for posthybridization amplification. Driver samples were amplified with a tagged primer bearing a *Bbv*I recognition site. (B) Biotinylated products were immobilized on streptavidin-coated magnetic beads. Driver was digested with the enzyme *Bbv*I to remove the sense primer sequence. (C) Both tester and driver were alkali denatured, and the sense strand of tester and antisense strand of driver were recovered. (D) Hybridization was performed with known quantities of tester and driver, with the driver being attached to streptavidin-coated beads. Three resulting populations of DNA were formed: (1) unhybridized single-stranded tester, (2) unhybridized single-stranded driver, and (3) tester/driver hybrids. (E) The single-stranded tester DNA was recovered and amplified with the tag primer to ensure that driver sequences were not carried through. This amplicon was used to generate more single-stranded tester sample for further rounds of hybridization.

[1] *Abbreviations used:* B&W, binding and washing; EDTA, ethylenediamine tetraacetic acid; TE, Tris EDTA; TMAC, tetramethyl ammonium chloride; SDS, sodium dodecyl sulfate; TBE, Tris–borate–EDTA.