Fab Library Construction Guide

Fab Library Platform
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1. Introduction

Fab libraries, in which light-chain (LC) and heavy-chain (HC) variable region genes are cloned into a phagemid vector and subsequently displayed on the surface of the filamentous phage particle, have been widely used for the isolation of antibodies (Abs) with specificity for haptens, foreign antigens (Ags), and self Ags. Immune Fab libraries, in which lymphoid tissue from individuals who, perhaps because of disease, have mounted an immune response to particular Ags, have been used in the recovery of Fabs with binding specificity for a number of clinically relevant Ags including c-erbB-2 and p53. Fab libraries are thus valuable as a means whereby the genes for Abs of interest can be immortalized and propagated. This enables information to be gathered regarding the Ab, including structural features, V-gene usage, and the nature of the immune response in the individual. Additionally, the isolated Abs can be used to evaluate immunogenic epitope(s) of the Ag. Furthermore, the Abs themselves provide potentially useful diagnostic or therapeutic agents. The isolation of Fabs from combinatorial libraries is thus valuable in contributing to the understanding of Ab–Ag interactions, as well as the nature of the in vivo immune response.

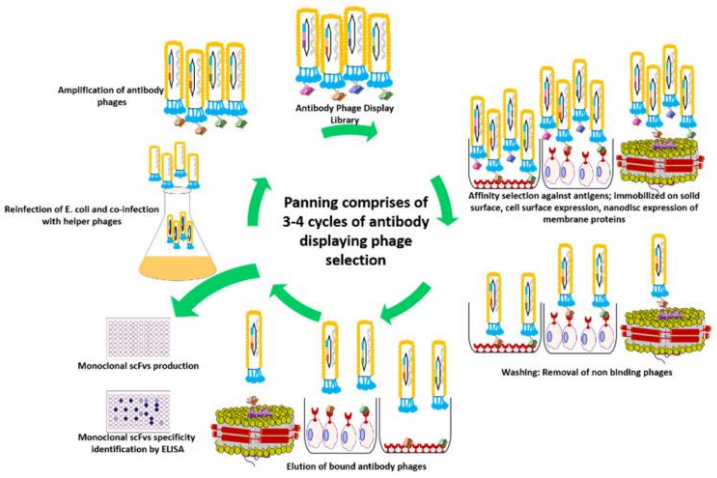

Fig 1 Panning of an antibody phage display library for the generation of high-affinity antibodies

2. Fab Library Construction Basic Process

1. Starting with the extraction of mRNA from B-cells isolated from blood, spleen, tonsils, and tumor tissue samples.
2. Using oligo (dt) primers or random primers, cDNA is synthesized from mRNA through reverse transcription-polymerase chain reaction.
3. Defined PCR primer sets, designed on the basis of species consensus sequence and anneal to the conserved region of V-gene families or constant domain, are used to amplify the heavy chain and light chain corresponding region (VH, CH, VL and CL, respectively) genes within a given immunoglobulin repertoire from cDNA pools, thus revealing the all antibody specificities in a particular individual.
4. The PCR-amplified VH-CH and VL-CL gene fragments are ligated in a suitable phagemid for Fab library generation, respectively.
5. In the case of synthetic antibody phage libraries, the initial few steps are not needed, and library diversity is increased through the precise introduction of degenerate DNA into CDR encoding regions, thus rivaling or exceeding than that of natural immune repertoire.
6. The engineered phagemids are transformed into suitable bacteria (e.g. TG1), providing a suitable environment for recombination of antibody fragments.
7. For rescuing the recombinant phagemid harboring the gene of inserts like Fab, the transformed bacteria are infected with helper phages like M13 that are well-adapted for the exposition of antibody-variable scaffolds.
8. This results in a library of phages, where each phage is expressing a unique antibody fragment on its surface as a phenotype while possessing the vector with specific nucleotide sequences within as respective genotypes.
9. The antigen is presented in its native form either immobilized on a plastic surface or expressed on the cell surface.
10. Antibody library displaying phages are incubated with antigen, and non-bound or weakly bound phages are removed by washing.
11. The tightly bound phages are recovered by elution via trypsin or pH shift for the re-infection of E. coli cells.
12. Following the coinfection with helper phages, new phage particles are produced for usage in subsequent rounds of panning. This cycle continues for 3–4 rounds, leading to enrichment of binders.

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