Scfv Library Construction Guide

ScFv Library Platform
ALPHA LIFETECH INC.

1. Introduction

To date, generation of single-chain fragment variable (scFv) has become an established technique used to produce a completely functional antigen-binding fragment in bacterial systems. The advances in antibody engineering have now facilitated a more efficient and generally applicable method to produce Fv fragments. Basically, scFv antibodies produced from phage display can be genetically fused to the marker proteins, such as fluorescent proteins or alkaline phosphatase. These bifunctional proteins having both antigen-binding capacity and marker activity can be obtained from transformed bacteria and used for one-step immunodetection of biological agents. Alternatively, antibody fragments could also be applied in the construction of immunotoxins, therapeutic gene delivery, and anticancer intrabodies for therapeutic purposes.

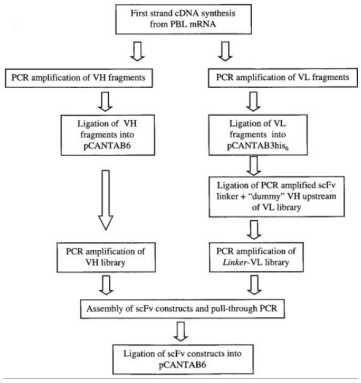

Fig. 1. Scfv library construction protocol flow chart.

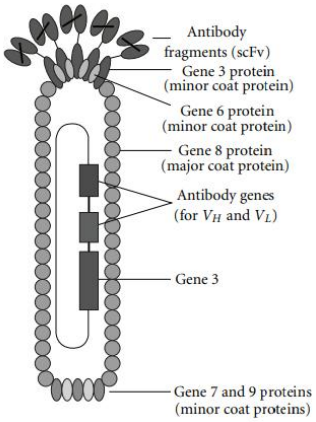

Fig 2 Structure of a filamentous phage displaying scFv fragments on its surface

2. Scfv 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 and VL, 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 and VL gene fragments are ligated in a suitable phagemid for single-chain variable fragments (scFv) 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 scFv, 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.

3. Advantages of Phage-Displayed Single- Chain Variable Fragment (scFv)

1. Phages are more stable and can be stored up to several years at 4◦C.
2. Secondly, they can be produced rapidly and inexpensively just by infecting the E. coli.
3. Their genes can be easily manipulated and lastly they can be produced by circumventing hybridomas and immunization
4. Higher affinity mutants of scFv (single chain fragment variable) can be generated through site directed mutagenesis which is much easier and simpler to be performed.
5. In contrast to soluble scFv (single chain fragment variable), phage-displayed scFv (single chain fragment variable) can be used directly during mice immunization to produce anti-idiotypic antibodies without the use of adjuvants. This is because phage particles are also good immunogens.

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