



The Chinese University of Hong Kong
Non-confidential Abstract of Technology Disclosure

Title:

Frame-shifting PCR for Germline Immunoglobulin Genes Retrieval and Antibody Engineering

CUHK Ref. No.:

03/SCI/158

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Patent Status:

US Patent Allowed

Non-confidential abstract:

Library of phage-displayed single chain variable fragment (ScFv) of antibody has been use for deriving tailor-made antigen-specific monoclonal antibody (mAb) in the last decade. Furthermore, affinity enhancement of ScFv can be achieved by in vitro mutation. However, in addition to the shortage of retrieving a smaller repertoire pool, the use of mRNA as antibody template is also hampered by the inability of retrieving immunoglobulin genes (Ig) mRNA of poor immunogenic targets, e.g. haptens, and Ig genes that masked by class selection, clonal deletion, negative selection, self-tolerance and non-productive exon joining.

Here a novel platform technology for antibody engineering was developed. Briefly, a set of degenerated primers, which covers most of Ig genes, was used to recover the variable regions of immunoglobulin heavy and κ/λ -light chains (VH & VL- κ/λ) from sorted CD+19 lymphocytic genomic DNA with a semi-nested PCR method. Moreover, a supplementary PCR step was used to recover defective Ig genes resulting from non-productive exon joining and to introduce diversity into the CDR3 region of immunoglobulin by mimicking somatic recombination.

Feasibility of applying current method for preparation of antigen-specific antibody was confirmed by having constructed a small scFv phage-displayed library (5.16×10^5 recombinants) from CD19+ cells that derived from a Balb/C mouse which was immunized with phenyl-oxazolone (phOx) - chicken serum albumin (CSA) conjugate. After 5 rounds of panning against phOx conjugated to bovine serum albumin (BSA), potential candidate clones (9.7×10^5 recombinants) were identified. Clones (288) were randomly picked and their reactivity against phOx were determined by phageELISA. Forty-four highly reactive clones, of which reactivity towards phOx were 1.5-fold higher than the mean value of the sample set, were isolated and further analyzed. After sequence determination, phylogenic analysis suggested that the derived Ig genes were grouped into different classes and significant sequence variations were found within the CDR3 region of Ig genes in each class. Furthermore, with the use of phOx-BSA conjugate as free ligand, competitive phageELISA indicated significant differences in affinity among different clones.

In conclusion, our approach offers a fast and simple way to retrieve the variable region of Ig genes and simultaneously introducing sequence diversity in the CDR3 region of antigen recognition domain.

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