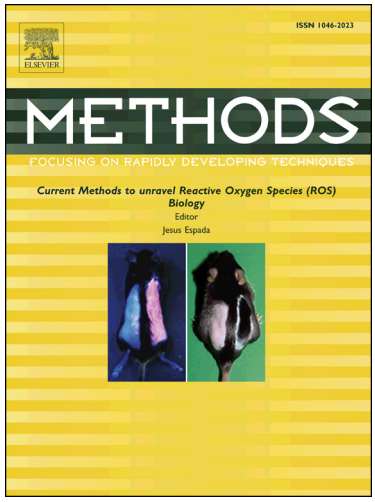

Recombinant Antibody Fragment Production

H. Ma, R. O'Kennedy

PII: S1046-2023(16)30449-2
DOI: http://dx.doi.org/10.1016/j.ymeth.2016.11.008
Reference: YMETH 4107

To appear in: *Methods*

Received Date: 30 September 2016
Revised Date: 14 November 2016
Accepted Date: 15 November 2016

Please cite this article as: H. Ma, R. O'Kennedy, Recombinant Antibody Fragment Production, *Methods* (2016), doi: http://dx.doi.org/10.1016/j.ymeth.2016.11.008

Recombinant Antibody Fragment Production

H. Ma and R. O'Kennedy

Biomedical Diagnostics Institute, Dublin City University, Dublin 9, Ireland.

Abstract

Recombinant antibodies are now very important in both therapeutics and diagnostics and offer significant advantages over conventional antibodies. The generation of a single-chain variable antibody fragment (scFv) (a common and important recombinant antibody format) is used to demonstrate the construction of a recombinant antibody library. An immunotube-based two-day panning approach, using *Escherichia coli* as an expression system, is utilised for antibody screening. The methods used for antibody selection and purification using immobilized metal affinity chromatography (IMAC) are described.

Abbreviations

BCA, bicinchoninic acid; CDR1, 2, complementarity determining regions 1, 2; CFA, complete Freund's adjuvant; Cκ, constant kappa light chain; Cλ, constant lambda light chain; D_H, heavy diversity; DNase, deoxyribonuclease; ELISA, direct enzyme-linked immunosorbent assay; Fab, fragment antigen-binding region; $F(ab')_2$, bivalent fragment antigen-binding region; Fab-scFv, fragment antigen-binding region and single-chain variable fragment fusion; HA, hemagglutinin; HRP, horseradish peroxidase; IFA, incomplete Freund's adjuvant; IMAC, immobilized metal affinity chromatography; IMS, industrial methylated spirits; IPTG, isopropyl β-D-1-thiogalactopyranoside; J segment, joining segment; Jλ, lambda joining; LB, Lysogeny broth; MOPS, 3-(N-morpholino)-propanesulfonic acid; PBS, phosphate buffered saline; PBST, phosphate buffered saline with 0.05% (v/v) Tween-20; PBSM, phosphate buffered saline with milk; PBSTM, phosphate buffered saline with 0.05% (v/v) Tween-20 and milk; PCR, polymerase chain reaction; RT, reverse transcriptase; SB, super broth; scAb, single chain antibody fragment; scFv, single-chain variable fragment; $sc(Fv)_2$, bivalent single-chain variable fragment; SOC, super optimal catabolite; SOE, splicing by overlap extension; TMB, 3,3',5,5'-tetramethylbenzidine;