

# Accepted Manuscript

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>

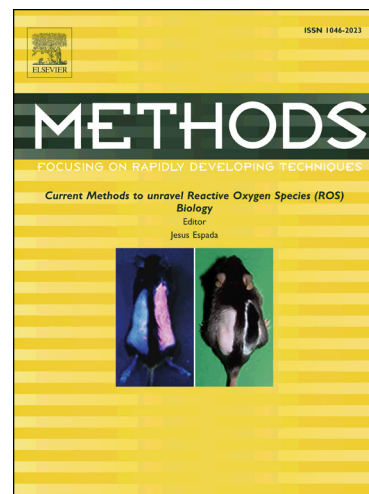
Reference: YMETH 4107

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>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

# 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; C $\kappa$ , constant kappa light chain; C $\lambda$ , 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  $\beta$ -D-1-thiogalactopyranoside; J segment, joining segment; J $\lambda$ , 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;

Download English Version:

<https://daneshyari.com/en/article/5513653>

Download Persian Version:

<https://daneshyari.com/article/5513653>

[Daneshyari.com](https://daneshyari.com)