Journal of Immunological Methods 395 (2013) 45–53

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

A mammalian expression system for high throughput antibody screening

Linda Xu, Xiaofang Jin, G. Jonah Rainey, Herren Wu, Changshou Gao *

Department of Antibody Discovery and Protein Engineering, MedImmune, LLC, One MedImmune Way, Gaithersburg, MD 20878, United States

ARTICLE INFO

Article history:
Received 6 January 2013
Received in revised form 20 June 2013
Accepted 21 June 2013
Available online 4 July 2013

Keywords:
Adenovirus expression system
Mammalian expression
High throughput screening
Phage display

ABSTRACT

We describe herein a method to enable high throughput (HTP) screening of libraries of soluble proteins such as phage-derived clones of IgG, scFv-Fc, or other Fc-fusion proteins expressed in mammalian cells via adenovirus transduction. DNA fragments of antibody single chains (scFvs) and fragment antigen-binding (Fabs) from the positive clones of the third round of bacteriophage panning against a target antigen were batch reformatted into scFv-Fc or IgG in an oriP bearing entry vector and then recombined to an adenovirus vector through Gateway technology. The resulting antibody gene-containing adenovirus libraries were added to 96-well plates seeded with mammalian cells at a ratio of 0.7 infectious viral particles per well to establish clonality. Protocol optimization improved the expression of scFv-Fc and IgGs up to 100 μg/mL in 96-well plates, which is sufficient for most antibody characterizations. In addition, 78% of the wells that were positive for protein expression contain only one sequence, indicating successful establishment of clonality in a majority of wells. We have established and optimized a mammalian expression system that produces soluble protein variants in a HTP manner. The system will facilitate developing multiple downstream screening methodologies.

1. Introduction

Phage display is an important approach for antibody discovery and optimization. The most commonly used expression system to produce phage display-derived antibodies for HTP screening is *Escherichia coli* due to its quick turnaround time and high expression levels in certain formats. The potential drawbacks of using *E. coli* as an expression host include the lack of post-translational modifications and poor expression yields of full length IgG. Excessively high levels of translation driven by very strong promoters often lead to expression of misfolded, insoluble, or inactive proteins (Davies et al., 2005; Vendel et al., 2012). Leader sequences have often been employed for secreting antibody fragments into the periplasmic space of *E. coli* (Thie et al., 2008) for functional screening but only with moderate expression level. Moreover, the contamination of endotoxin in the samples produced with *E. coli* restricts the usage of the preparations in certain assays especially in mammalian cell-based functional assays. Mammalian expression systems allow production of active recombinant proteins that overcome these limitations. High level transient expression of recombinant proteins in mammalian systems has been accomplished through the use of plasmids with the capability for episomal replication (Durocher et al., 2002; Davies et al., 2005).

A plasmid mediated HTP transient expression of phage-derived antibodies in IgG format was developed for antibody HTP screening (Jostock et al., 2004). However, transient gene expression requires a significant amount of plasmid DNA. Typical protocols use about 1–1.25 μg plasmid DNA/mL of culture (Baldi et al., 2007). The *E. coli* host that generates the plasmid must be plated for single colonies and hundreds or thousands of plasmid extractions must be conducted prior to high throughput transfection in microtiter plates. This

Abbreviations: HTP, high throughput; scFv, single chain Fv; Fab, fragment antigen-binding; IRES, internal ribosome entry site; VH, heavy chain variable regions; *EBNA-1*, Epstein–Barr virus nuclear antigen 1; CMV, cytomegalovirus.

* Corresponding author. Tel.: +1 301 398 4704; fax: +1 301 398 9704.
E-mail address: gaoc@medimmune.com (C. Gao).

0022-1759/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2013.06.009

process is laborious and expensive. In addition, the final antibody yields are usually less than 10 μg/mL regardless of the formats (e.g., scFv, Fab, scFv-Fc, and IgG). To overcome this problem, we reasoned an expression system in which the mixture of DNAs is introduced into cells at one copy of DNA per cell to maintain the genotype-phenotype linkage while the single copy of DNA is able to replicate in the cell to produce high level of proteins would be ideal. The adenovirus expression system is equipped with these required features. Recombinant adenoviruses have become a popular method to deliver and transiently express genes in a wide range of cell types. Upon uptake by an adenovirus E1 gene-expressing cell line, such as HEK293 cells, the adenovirus can be propagated to high viral copy numbers. Accordingly, adenoviral vectors have been successfully used to express a wide variety of viral and cellular genes in mammalian cells (Berkner, 1988; He et al., 1998; Massie et al., 1998a, 1998b; McVey et al., 2003).

In the current study we report developing an adenovirus mediated high-level expression system and its applications in HTP antibody screening to overcome the laborious and expensive plasmid preparation and transfection required for a transient expression approach. The application of the method described is not limited to libraries of immunological origin, but could be broadened to investigate variants of any Fc-fusion (Nuttall et al., 1999; Hufton et al., 2000), or other soluble protein. The oriP-containing entry vectors were designed to facilitate the batch reformatting of phage-derived antibody fragments (scFv and Fab) into bivalent scFv-Fc and IgG. Gateway technology was then utilized to recombine the scFv-Fc and IgG into the adenoviral destination vector to generate the antibody gene-bearing adenovirus libraries. To maintain genotype and phenotype linkage, we infected cells in 96-well plates with the antibody adenovirus libraries at a ratio of less than 1 infectious viral particle per well. Further protocol optimization improved the expression of scFv-Fc and IgGs up to 100 μg/mL in 96-well plates, which is sufficient for antibody HTP screening and functional characterization. Here we report a HTP mammalian expression system ideally suited to produce thousands of protein variants with minimal effort in a short time frame.

2. Materials and methods

2.1. Vectors, cell lines, media, restriction enzymes, and PCR reagents

The pENTR™ 2B Dual Selection vector and pAd/CMV/V5-DEST vectors were purchased from Life Technologies (cat. no. A10463 and V49320). Cell lines of HEK293E and A549 were obtained from ATCC (cat. no. CRL-10852 and CCL-185) and HEK293A was from Life Technologies (cat. no. R705-07) and were maintained as suggested by the provider. Media used for cell culture were from Life technologies. Restriction enzymes and high fidelity Phusion DNA polymerase were from New England Biolabs.

2.2. Construction of pEN-oriP vectors

The pENTR vector was modified from pENTR™ 2B by introducing an oriP fragment (Yates et al., 1984) and an IgG or scFv-Fc expression cassette to reformat phage derived Fab and scFv into IgG and scFv-Fc, respectively. The oriP fragment was amplified from an in house mammalian expression vector (Dimasi et al., 2009) with primers TGCTGGGGAcagc GCAGGAAAAGGACAAGCAGCGA and CGGTCATTTCGAAACAA CATTGCCTTTATGTGTAACTCTTGGCTG. The oriP PCR product was digested with Sph I and BstB I and ligated into pENTR, which was digested with the same enzymes, to generate pEN-oriP. pEN-oriP-scFv was constructed by insertion of the sequence of human IgG1-Fc into pEN-oriP downstream of the cytomegalovirus (CMV) promoter (Fig. 1A). Similarly, the sequence of human IgG1 constant region was inserted into pEN-oriP to generate pEN-orip-IgG driven by a CMV promoter (Fig. 1B). Restriction enzyme digestion sites of Sfi I/Not I or ApaL I/Sal I have been created at the 5′ or 3′ end of the scFv fragment in vector pEN-oriP-scFv or at the 5′ or 3′ end of the Fab in vector pEN-oriP-IgG, respectively, to facilitate the scFv or Fab library reformatting processes.

2.3. Adenovirus library generation

To generate the pEN-oriP-scFv library, scFv fragments were dissected out by digesting the plasmid of the pools of positive clones from the third round of panning against a membrane anchored multidomain protease on a scFv-phage library (Vaughan et al., 1996) with Sfi I and Not I. The purified ~700 bp scFvs were ligated into similarly digested pEN-oriP-scFv vector (Fig. 1A). The pEN-oriP-IgG library was generated from a Fab phage display library (Dyax) that had been selected similarly to the scFv library above. Fab expression cassettes, including light chains, internal ribosomal entry site (IRES) (Jang et al., 1990), and heavy chain variable regions (VH) were inserted into the vector at ApaL I and Sal I sites to generate a human IgG expressing library (Fig. 1B). The quality of the libraries was assessed by bacterial colony PCR to visualize the size of the inserts. Libraries with <1% incorrect insert size were considered high quality. Complex mixtures of plasmids (the libraries) were prepared from bacteria with a maxi prep kit (Qiagen, cat. no. 12663) and then were recombined with pAd/PL-DEST (destination vector) following the protocol provided by the manufacturer. Briefly, the DNA fragment, containing the expression cassette and oriP, was excised from the pEN libraries with Nhe I and Pvu I to eliminate the contamination of entry vector after recombination and added into the tube with destination vector pAd/PL-DEST and LR recombinase. After overnight incubation, the LR reaction samples (viral library) were transformed into DH5α and plasmids carrying the antibody library were prepared from the transformants. A representative number of colonies was counted for pAd-oriP-scFv and pAd-oriP-IgG library size determination.

2.4. Adenovirus production and transient IgG expression

In accordance with the function of oriP, HEK293E cells, a cell line that stably expresses Epstein–Barr virus nuclear antigen 1 (*EBNA-1*) (Durocher et al., 2002), were used for transfection to produce the adenovirus antibody expression libraries. oriP allows stable episomal replication and long term transient expression of the plasmid in cell lines that express *EBNA-1* (Yates et al., 1984). HEK293E cells were