Journal of Immunological Methods 438 (2016) 67–70

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Technical note

Refined protocol for generating monoclonal antibodies from single human and murine B cells

Irvin Y. Ho [a,1], Jeffrey J. Bunker [b,c,1], Steven A. Erickson [b,c], Karlynn E. Neu [a,c], Min Huang [a], Mario Cortese [d], Bali Pulendran [d,e], Patrick C. Wilson [a,c,*]

[a] Department of Medicine, Section of Rheumatology, Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, 924 E 57th Street R422, Chicago, IL 60637, USA
[b] Department of Pathology, University of Chicago, 929 E 57th Street W503S, Chicago, IL 60637, USA
[c] Committee on Immunology, University of Chicago, USA
[d] Emory Vaccine Center, Yerkes National Primate Research Center, Atlanta, GA 30329, USA
[e] Department of Pathology, Emory University School of Medicine, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 1 September 2016
Accepted 2 September 2016
Available online 4 September 2016

Keywords:
Monoclonal antibodies
Single-cell PCR
Human antibodies
Gibson assembly
Antibody expression

ABSTRACT

Generating monoclonal antibodies from single B cells is a valuable tool for characterizing the specificity and functional properties of humoral responses. We and others developed protocols that have facilitated major advances in our understanding of B cell development, tolerance, and effector responses to HIV and influenza. Here, we demonstrate various refinements and dramatically reduce the time required to produce recombinant antibodies. Further, we present new methods for cloning and isolating antibodies from cells with lower immunoglobulin mRNA levels that may be resistant to traditional techniques. Together, these refinements significantly increase single-cell antibody expression efficiency and are easily integrated into established and novel pipelines.

1. Technical note

Antibodies are the primary mediator of humoral immunity, so they are a recurrent focus of scientific inquiry and medical discovery. In recent years, characterization of humoral responses by production of monoclonal antibodies from single cells has enabled rapid advances in B cell biology due to the high throughput and efficiency of these methods [1–3]. These include the isolation of large numbers of broadly neutralizing antibodies against diverse pathogens like HIV and influenza, which are revolutionizing vaccine and therapeutic design. Single cells from a population of interest are isolated using flow cytometry then immunoglobulin genes from each cell are cloned into a vector for protein expression. The resulting antibodies are used for downstream assays to study their specificity, function, and repertoire characteristics.

A popular use for this involves screening panels of monoclonal antibodies, allowing a clonal assessment of the specificities present in a population of interest. Antibodies derived from plasmablasts, the temporary effector cells that peak 7 days after an immune response, present an opportunity to study acute infection and vaccination [4,5]. Memory B cells give access to affinity-matured antibodies, especially those formed during chronic or repeated infections [6,7]. An advantage of this method over traditional serum or hybridoma methods is that rare single cells with desirable antibodies can be isolated. Characterization of individual monoclonal antibodies yields data on specificity, binding conformation, reactivity breadth and potency, and protective capacity. Studies using these methods have shed light on potential targets for universal vaccine development and provide benchmarks for evaluating future therapeutics.

Here, we show several refinements for single-cell cloning and antibody expression that eliminate bottlenecks by replacing various single-sample manipulations with high-throughput alternatives. For large-scale projects, these changes are easily implemented and noticeably increase the pace at which antibodies can be generated. Further, our methods enable high-throughput isolation and characterization of antibodies from cells with relatively low immunoglobulin expression levels such as naïve or memory B cells. Limitations that are inherent to generating antibodies from single cells remain, including cell fragility (plasmablasts cannot be frozen and thawed) and the time-consuming nature of sorting cells and transfecting individual antibodies. Additionally, the lack of PCR error-correcting mechanisms necessitate redundancy measures and occasional redoes. Instructions and primers for

* Corresponding author at: Committee on Immunology, University of Chicago, USA.
E-mail addresses: irvin@uchicago.edu (I.Y. Ho), jeffbunker@uchicago.edu (J.J. Bunker), sae@uchicago.edu (S.A. Erickson), karlynn@uchicago.edu (K.E. Neu), huang2@uchicago.edu (M. Huang), mario.cortese@emory.edu (M. Cortese), bpulend@emory.edu (B. Pulendran), wilsonp@uchicago.edu (P.C. Wilson).
[1] Irvin Y. Ho and Jeffrey J. Bunker made equal contributions to this work.

http://dx.doi.org/10.1016/j.jim.2016.09.001
0022-1759/

creating both fully human and chimeric (murine variable region and human constant region) antibodies are included.

2. Methods

This section describes the steps that are substantially different from our previously published protocol [3]. A complete protocol, which includes other modifications and quality-of-life changes, is included as supplementary material. Table 1 compares the bench time required for each step for our old protocol and this new protocol.

We applied this procedure to human plasmablasts isolated seven days after seasonal influenza vaccination or pneumococcal vaccination. We validated the cloning efficiency of the new protocol (Fig. 1a) and confirmed the production of vaccine-specific antibodies by ELISA (Fig. 1b, c).

Peripheral blood samples were collected in accordance with the University of Chicago Institutional Review Board (#09-043-A) and informed consent was obtained from all subjects.

2.1. cDNA preparation

To prepare template cDNA from lysed cells, a non-specific cDNA synthesis kit is used rather than immunoglobulin-specific primers. With specific primers, for each sample plate only one of the light chain genes (lambda or kappa) can be reverse transcribed, and antibodies that use the other gene are lost. With non-specific primers, both lambda and kappa chain DNA can be recovered from the same plate.

Important: use RNase-free precautions.

2.1.1. Direct cDNA synthesis from sorted and lysed cells

This is efficient for most cell types, especially plasmablasts. Non-plasmablast human cells may experience slightly diminished PCR efficiency.

Note: Sort cells into 10 μl/well of catch buffer (for ten half-plates: 5 ml RNase-free water, 50 μl 1 M Tris pH 8.0, 125 μl RNasin; make fresh each time)

1. Create the master mix.

cDNA reaction (1 well)	
5× buffer mix	3 μl
Maxima enzyme mix	1.5 μl
5% IGEPAL	1.5 μl

2. Aliquot 6 μl master mix into each well with catch buffer, for a total reaction volume of 16 μl. Run the following program. Store plates at −20 °C.

25 °C for 10 min
50 °C for 30 min
85 °C for 5 min
4 °C forever

2.1.2. cDNA synthesis after RNA purification

Purification with SPRI beads removes debris and other contaminants, increasing the efficiency of cDNA synthesis and subsequent PCRs [8]. A variety of murine non-plasmablast cells amplify very inefficiently; SPRI purification increases the yield of naïve cells, small intestinal IgA plasma cells, and colonic IgA plasma cells by 41%, 17%, and 127% respectively (Fig. 2). However, this technique may be useful for all plates that have low PCR efficiency.

Note: Do not sort cells into catch buffer. Instead, use 5 μl/well of TCL buffer and 1% beta-mercaptoethanol (vol/vol). Plates can be flash-frozen on dry ice and stored at −80 °C.

1. Warm RNase-free SPRI beads to room temperature.
2. Add 10 μl nuclease-free water to each sample, then add 33 μl SPRI beads to each sample. Cover plate to prevent contamination and incubate at room temperature for 10 min.
3. Place the plate on top of the magnetic plate and incubate, covered, at room temperature for 5 min.
4. Wash the plate 3× with 75 μl 80% EtOH, incubating 30 s each cycle. Upon final aspiration, air dry for 8 min and remove from the magnetic plate.
5. Begin cDNA synthesis by resuspending beads in 10 μl/well reverse transcription mix #1, and thermocycle.

cDNA reaction #1 (1 well)	
10 mM dNTPs	1.25 μl
Oligo $d(T)_{18}V$	1 μl
Template RNA pellet	–
Nuclease-free water	To 10 μl

65 °C for 5 min
4 °C forever

6. Incubate on ice for 1 min, then add reverse transcription mix #2; aliquot 10 μl/well and cycle. Store plates at −20 °C.

cDNA reaction #2 (1 well)	
5× SuperScript IV RT buffer	4 μl
100 mM DTT	1 μl
RNaseOUT	0.5 μl
SuperScript IV reverse transcriptase	0.25 μl
Nuclease-free water	to 10 μl

50 °C for 1 h
80 °C for 10 min
4 °C forever

2.2. Cloning

After cDNA preparation, two rounds of PCR are performed to amplify immunoglobulin genes for sequencing. The products of the first round are used as templates in a cloning PCR reaction to generate DNA for

Table 1
Comparison of the bench time required to perform the steps in the protocol.

Smith, nature methods, 2009	Step	New protocol
Unchanged	Blood draw and flow cytometry	Unchanged
Unchanged	cDNA prep	Unchanged (RNA bead purification: add 45 min/plate)
Unchanged	PCR	Unchanged
1 day, 100 sequences	Prepare expression vector	**15 min, 96 sequences**
1 h, 24 sequences	Transformation	**1 h, 96 sequences**
Unchanged	Plasmid DNA preparation	Unchanged
Unchanged	Cell culture and transfection	Unchanged
1 day, 24 antibodies	Antibody purification	**45 min, 24 antibodies**