Single-step colony assay with autoinduction of scFv expression for the screening of antibody libraries

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ABSTRACT

We present a simple colony assay method for screening antibody libraries based on autoinduction of antibody fragment expression. This protocol eliminates the need for colony size monitoring and a separate induction step for single-chain Fv (scFv) antibody fragment expression. Here, scFvs are expressed in an automatic and timely fashion during the assay, resulting in high yields of positive clones and substantial time savings. The method was used successfully to establish monoclonal scFvs with high affinity and specificity against human IgG.

METHOD SUMMARY

A simple colony assay protocol with autoinduction of antibody fragment expression is described. The method relies on the timely consumption of glucose to ensure sufficient colony size, and subsequent conversion of lactose to allolactose to induce scFv expression.

KEYWORDS:

colony assay • monoclonal antibody • scFv • screening

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Recombinant antibody technology enables the screening of large libraries to generate monoclonal antibodies in a shorter time than conventional methods [1]. Libraries with a vast repertoire and efficient screening are two prerequisites for obtaining monoclonal antibodies with high affinity to the antigen [2,3]. Two approaches for screening currently exist: display strategy and repertoire cloning strategy [4,5].

Phage display is the most common form of display strategy [6,7]; however, other display systems also exist. These include yeast display, whereby single-chain variable fragments (scFvs) are displayed on the surface of yeast cells [8]; mRNA display [9]; ribosome display, which integrates a ribosome, mRNA and an scFv [10]; bacterial inner-membrane display [11]; and mammalian cell surface display [12] for human antibody discovery. In phage display, antibody fragments are joined to the filamentous phage coat protein gp3. The scFv is suitable as an antibody fragment due to its small size and ease of handling. Panning is performed against the fixed antigen to remove weakly bound clones and is then repeated to select the phage whose scFv exhibits highest affinity to the antigen. This method is characterized by several rounds of selection, proliferation and enrichment of positive clones to enable the processing of large libraries. However, it gives rise to substantial background during panning selection, leading to false-positive clones [13,14].

In the repertoire cloning strategy, antibody libraries, including scFv libraries, are expressed in *Escherichia coli*, which are grown on a hydrophilic filter placed on an agar plate [15]. Once colonies form, the filter is transferred to an antigen-coated membrane placed on an agar plate containing isopropyl β -D-1-thiogalactopyranoside (IPTG) to induce scFv production. The scFv produced by the colonies diffuses out, binds to the antigen on the membrane, and its presence is detected by superimposing the spot on

the colony (filter-sandwich assay) [16,17]. A gene encoding the scFv with affinity for the antigen is obtained. The colony assay identifies clones with high reliability by directly observing antibody-antigen binding, thus resulting in a low false-positive rate. In addition, the method can be easily used to screen libraries an order of magnitude larger than using hybridoma technology [4].

In the filter-sandwich assay, the timing for inducing expression is critical. If induction occurs too early, the stress of expression prevents *E. coli* growth and the resulting colony is too small for the assay. At the same time, *E. coli* does not express scFv when the colony grows too large (>1 mm). Therefore, to ensure scFv expression, one needs to carefully monitor colony size and transfer the filter harboring the colonies to the IPTG-containing plate at the right time. This transfer requires delicate manipulation of the filter and frequently causes unwanted stress to *E. coli*. Recently, a single-step colony assay has been developed, in which the filter is first superimposed on the antigen-coated membrane, the colony is formed on the filter, and the assay can then be performed [18]. This method does not require the filter to be transferred and screening can be completed in a single step. However, it still requires colony size monitoring and a separate induction step. Here, we went a step further by developing an autoinduction protocol that eliminates the need for colony size monitoring and a separate induction step for scFv expression.

The procedure used in the single-step colony assay with autoinduction is depicted schematically in Figure 1. A hydrophilic polyvinylidene difluoride filter and the antigen-coated nitrocellulose membrane are placed on the agar plate. *E. coli* transformed with the scFv library are grown to exponential phase and suspended in a solution containing 0.05% glucose and 0.1% lactose to inhibit and induce scFv expression, respectively. Then, cells are spread on the filter and ►

Figure 1. Schematic diagram of the procedure describing the single-step colony assay with autoinduction.

HRP: Horseradish peroxidase; scFv: Single-chain variable fragment.

► incubated. As they consume glucose, *E. coli* form colonies on the filter surface. Colony size increases as glucose becomes depleted and scFv expression is achieved automatically by subsequent conversion of lactose into allolactose [19]. This simple procedure enables the timely induction of expression using allolactose as the inducer. The scFvs diffuse out into the antigen-coated membrane, where those with affinity bind the antigen beneath the colonies. Importantly, scFvs without affinity do not bind the antigen. The filter with the colonies is removed, placed in a fresh plate, and stored for later recovery of the bacteria. Antigen-bound scFvs on the membrane are detected by chemiluminescence using a horseradish peroxidase

(HRP)-conjugated anti-His antibody. The filter with the colonies and the image with the chemiluminescence data are superimposed. Positive clones corresponding to the chemiluminescence signals are identified, transferred to medium and incubated. Plasmids encoding scFvs with affinity for target antigens are purified.

The single-step colony assay with autoinduction was tested by selecting positive clones from an immune scFv library using human IgG as the antigen (Figure 2). *E. coli* were transformed with the scFv library and 4×10^3 cells in a solution containing glucose and lactose were spread on a 10-cm plate. Following incubation for 16 h at 30°C, 3508 colonies were recorded. Colony diameter varied

from 0.5 to 0.8 mm. Colony number and size were almost identical to those on control plates. Antigen binding of scFvs to the lower membrane was detected by chemiluminescence. Spots whose intensity was above 30 (arbitrary units) were considered as positive. In the plate shown in Figure 2, there were 24 such spots. Tight regulation of expression, such as inhibition of leaky expression prior to colony formation (or when the colony size was too small) and induction of expression after colony formation (once the colony was sufficiently large), contributed to the strong signal from positive clones and dispensed with monitoring colony size. This allowed the successful identification of positive clones expressing anti-human IgG scFvs.

20 of the clones with the strongest signal and two clones with no signal were selected for further analysis. Each clone was identified by superimposing the filter and the membrane chemiluminescence image. Cells were picked from the upper filter and cultured to express scFvs. The selected positive and negative clones were cultured at 37°C in LB medium containing 50 µg/ml carbenicillin until they reached an OD₆₀₀ of 0.6. Then, the cells were incubated at 30°C for 6 h in the presence of 1 mM IPTG. Thereafter, they were centrifuged at 5000 × g at 4°C for 5 min, and the supernatants were collected and examined for reactivity against human IgG by ELISA (Figure 3A). Every identified positive clone showed antigen-specific binding activity, whereas no binding to the uncoated ELISA plate blocked with bovine serum albumin was detected. None of the negative clones bound to the antigen or bovine serum albumin. Thus, the colony assay with autoinduction successfully established scFv clones with binding affinity and specificity toward the antigen. The DNA of each scFv from the six clones with the strongest reactivity against the antigen (No. 1, 6, 9, 10, 17, and 18 in Figure 3A) was sequenced. The inferred amino acid sequences of selected clones are shown in Figure 3B. Every clone contained the complete scFv structure consisting of V_H, linker and V_L. All sequences were unique, indicating that diversity of the scFv library was maintained through the assay. These results show that genes encoding scFvs

In summary, we report a simple method that does not require colony size monitoring and a separate induction step for scFv expression. Instead, scFvs are expressed in an automatic and timely fashion during the assay.

► SUPPLEMENTARY DATA

A supplementary protocol accompanies this paper. To view the supplementary protocol please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2018-0195

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