High-throughput hybridoma cloning and screening for antibody discovery

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OVERVIEW

- Monoclonal antibodies used for research, diagnosis, and treatment of diseases rely on the development of stable, high producing hybridomas. Thousands of hybridomas must be cloned and screened to identify the ones that exhibit optimal growth and secrete large amounts of the desired antibody. Manual selection and further downstream functional screening is time consuming and prone to error.
- Mouse hybridoma cells, SJK 287.37 (IgG secreting) and 123-10 (IgM secreting), were cultured in liquid hybridoma media and then seeded in semi-solid hybridoma media with Fluorescein CloneDetect Mouse IgG (Fc) Specific. A ClonePix 2 automated colony picker was used to select and pick IgG secreting colonies based on morphology and fluorescence intensity.
- The selection of picked IgG secreting colonies was confirmed by performing IgG (Fc) Specific ELISA and using a SpectraMax i3X multi-mode microplate reader for absorption measurements.
- Picking colonies based on fluorescence makes it possible to identify colonies secreting the desired antibodies during the initial screen, limiting downstream assays to the best clones saving significant time and resources.

INTRODUCTION

Hybridomas cultured in semi-solid media are immobilized allowing them to grow and form distinct clonal colonies. This improves laboratory efficiency by making it possible to seed and screen many cells in the same well. Adding fluorescently labeled antibodies to the semi-solid media allows the identification of antibodies secreted by the hybridomas.

Here we present a fluorescent method to screen and pick hybridomas secreting IgG antibodies. This method can be easily modified to detect other specific antibodies. Hybridomas were cultured in semi-solid media containing CloneDetect fluorescein-labeled mouse IgG (Fc) specific antibody. A ClonePix2 was used to image, screen, and pick colonies based on morphology and fluorescence intensity. Picked colony growth was monitored by imaging on a CloneSelect Imager. SpectraMax i3x was used to measure antibody secretion of the picked colonies by ELISA. By fluorescently selecting colonies, the best colonies were identified during the initial screen, limiting downstream assays to the best clones saving significant time and resources.

METHODS

CELL CULTURING

SJK 287.38 and 123-10 hybridoma cells were incubated in hybridoma liquid media at 37°C. Next, the hybridomas were seeded into semi-solid hybridoma media containing CloneDetect Mouse IgG (Fc) specific fluorescein labeled antibody. 2mL per well of this semi-solid mixture was pipetted into 6-well plates. The 6-well plates were incubated at 37°C for 9 days.

COLONY SELECTION AND PICKING

White light and fluorescence images of the 6-well plates were captured with the ClonePix 2. Morphology and fluorescence gates were set to select the best clones secreting IgG antibody. The picked colonies were transferred to a 96-well plate containing liquid hybridoma media.

MEASUREMENT OF GROWTH

Each 96-well plate was read on a CloneSelect Imager to determine well confluency and colony growth rates. The 96-well plates were read on Day 0, 3, 5, and 7.

CONFIRMATION OF IgG COLONY SELECTION AND PICKING

After 7 days of incubation at 37°C, the cell culture supernatant from each 96-well plate was transferred to a new 96-well plate. A 5-fold dilution of each culture supernatant was run on mouse IgG (Fc) specific ELISA. Each sample was run in duplicate. An 8-point standard curve was used to calculate the antibody concentration. Absorption measurements were performed on SpectraMax i3x. The antibody production was then normalized by the confluency of the well on Day 7.

INSTRUMENT OVERVIEW

ClonePix 2 Automated Colony Picker

- Accurate, automatic colony picking avoids errors associated with limiting dilution
- Select cells with optimal expression levels with improved ranking consistency. Increase probability of finding optimal producers and select cells

CloneSelect Imager

- Consistent determination of cell confluence and cell number estimation
- Generation of growth curves
- Label-free, white light imaging of living cells

SpectraMax i3x

- User-upgradeable application modules including cellular imaging
- Sensitivity across spectrum with Spectral Fusion™ Illumination
- Expanded dynamic range with cooled PMT

CLONEPIX 2 IgG FLUORESCENCE SCREENING

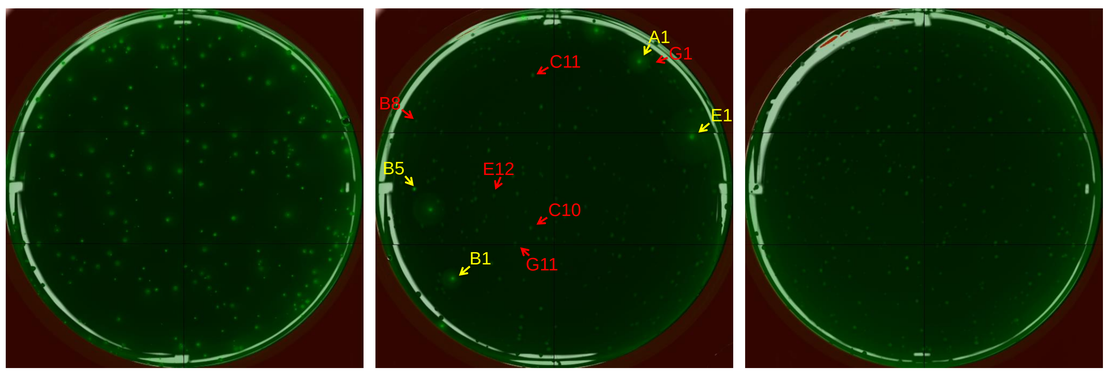

Figure 1. ClonePix 2 fluorescence images captured from a 6-well plate. A well with SJK287.38 colonies only (left), a mixture of SJK287.38 and 123-10 colonies (middle) , and 123-10 colonies only (right) are shown. The colonies that display fluorescence are secreting IgG antibodies as detected by the CloneDetect fluorescein-labeled mouse IgG (Fc) specific antibody. In the middle well, the labels show the 96-well plate location the picked colony was deposited into. Picked colonies that showed fluorescence are in yellow font while picked colonies that had no fluorescence are in red font. The downstream analysis of this subset of colonies is shown below. There were colonies that showed fluorescence but were not picked due too close proximity to another colony or the edge of the well.

IgG ANTIBODY PRODUCTION CONFIRMED BY ELISA

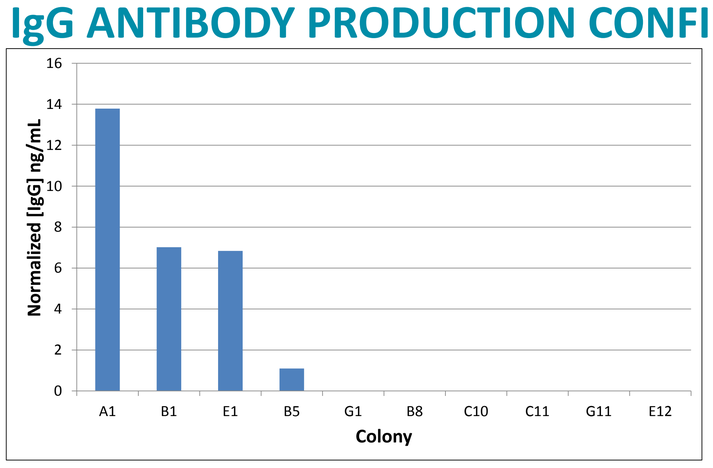

Figure 2. Each colony IgG antibody productivity was determined by performing ELISA on clone supernatants. Values are normalized to 100% confluence. ELISA results confirm that the ClonePix 2 screened and accurately picked colonies secreting IgG antibodies.

GROWTH CURVES OF PICKED COLONIES

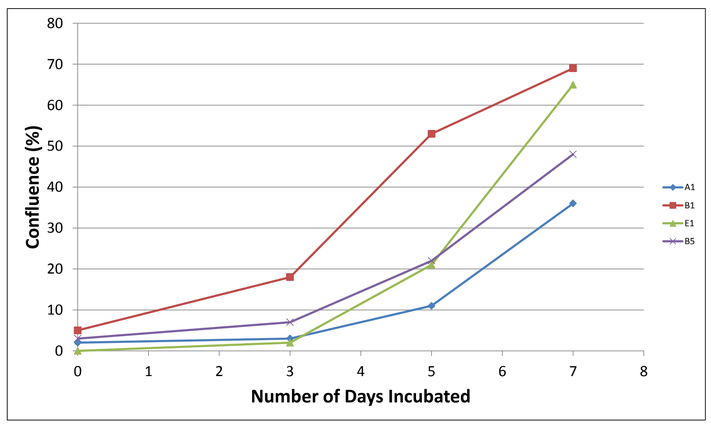

Figure 3. Growth curves of the picked IgG secreting colonies as determined by the CloneSelect Imager. Colonies B1 and E1 show are the optimal colonies based on a balance between IgG antibody production and growth rate.

CONCLUSIONS

- The use of semi-solid media and fluorescence provides a faster and easier solution for hybridoma cloning and screening.
- The use of automation for imaging, screening, and picking colonies shortens the time needed to develop stable, high producing hybridomas

MOLECULAR DEVICES