High-Throughput Hybridoma Generation and Selection for Antibody Discovery

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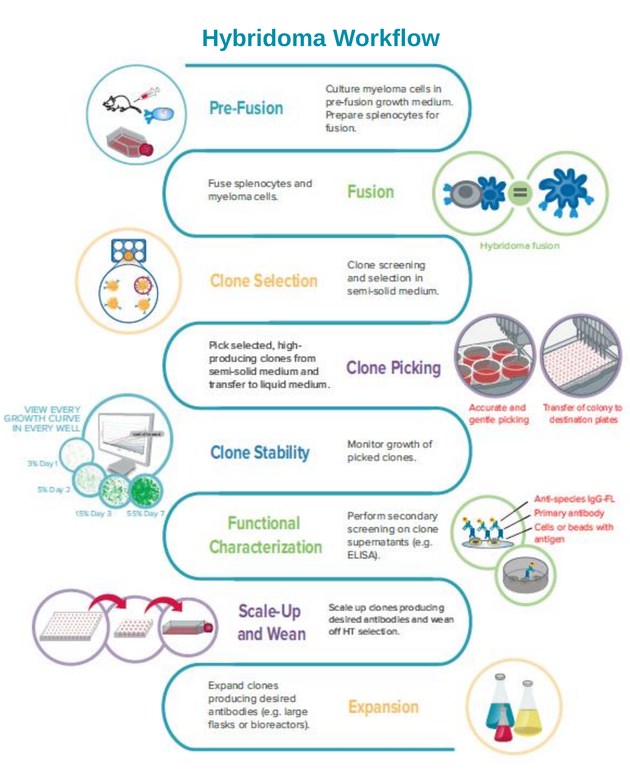
INTRODUCTION

During the last decade, the rising demand for monoclonal antibodies (mAbs) has led to a boom in the introduction of biotherapeutic proteins to the market. A total of 96 recombinant protein therapeutics have been approved between 1986 and 2011. The global market is expected to grow 12.5% from 2014 to 2019 to a $4 billion market. As growth continues, the need to bring down cell line development costs and shorten the time to market is more critical than ever. In this poster, we present an overview of the complete solution for high-throughput hybridoma cloning and screening for antibody discovery provided by Molecular Devices.

MATERIALS & METHODS



XP Media and CloneMedia Complete Kit for Mouse Hybridoma Generation

ClonePix™ 2 Automated Colony Picker
- Select and pick highest-producing hybridomas

CloneSelect™ Imager
- Automated imager to verify monoclonality and monitor cell growth

SpectraMax® i3x reader
- Screen for antibody specificity using ELISA

Figure 1. Cell line development workflow for screening antibody-producing hybridomas

HYBRIDOMA GENERATION BY PEG FUSION

Myeloma cells grown in XP Media Pre-Fusion Myeloma Growth Medium and Hybridoma Expansion Medium (without HT) were fused with splenocytes using the Hybridoma Polyethylene Glycol (PEG) for Cell Fusion. Prior to fusion, it is important to wash the splenocytes in serum-free XP Media Hybridoma Fusion Medium, otherwise fusion efficiency is greatly diminished. Hybridomas were then cultured in XP Media Hybridoma Fusion Recovery Medium for 24 hours before plating in semi-solid media. Figure 2 illustrates the fusion efficiencies observed following 5 independent hybridoma generation experiments.

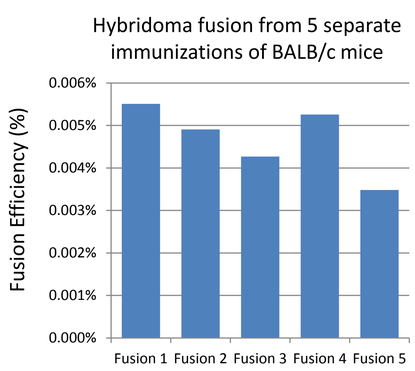


Figure 2. Fusion efficiency of hybridomas. The efficiency of PEG fusion of hybridomas was calculated by dividing the number of colonies recorded in semi-solid media by the splenocyte count prior to fusion. Consistent fusion efficiencies were observed across 5 independent experiments and are comparable with hybridoma fusion efficiencies from other techniques.

COLONY SELECTION AND PICKING

Hybridomas were seeded into CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT) media containing 1% CloneDetect Mouse IgG (Fc) specific fluorescein-labeled antibody. 2 mL 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 before picking. White light and fluorescence images of the 6-well plates were captured with the ClonePix 2 System. Morphology and fluorescence gates were set to select the best 96 clones secreting IgG antibodies. The picked colonies were transferred to a 96-well plate containing XP Media Hybridoma Growth Medium (with HT).

MEASUREMENT OF GROWTH

Each 96-well plate was imaged in white light on a CloneSelect Imager to determine well confluency and colony growth rates. The 96-well plates were read on Day 0, 1, 2, 3, 4, 6, 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 10-fold dilution of each culture supernatant was tested using an ELISA to measure mouse IgG (Fc). Each sample was run in duplicate. A 4-parameter standard curve was used to back calculate the antibody concentration. Absorbance measurements were performed on the SpectraMax i3x reader.

SUPERIOR HYBRIDOMA GROWTH IN SEMI-SOLID MEDIA

CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT) is a semi-solid methylcellulose-based medium containing supplements to promote the growth of single-cell hybridomas into colonies in the presence of the selection agents hypoxanthine, aminopterin, and thymidine (HAT) as shown in Figure 3. Colonies grown in CloneMedia are larger in size and more densely packed, indicating better growth and viability, respectively, than Product X.

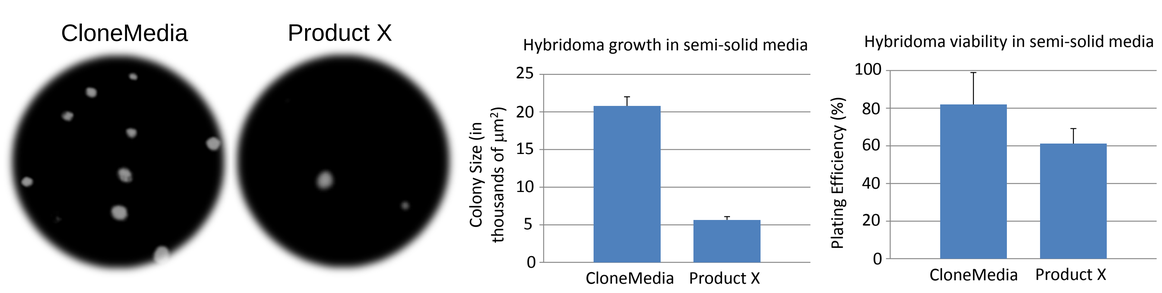


Figure 3. Hybridomas cultured in different semi-solid media. Hybridomas were plated in CloneMedia semi-solid media from Molecular Devices and in semi-solid media from Competitor X. A greater number of clones were able to grow in CloneMedia when compared to Product X as shown in the white light images taken of colonies on the ClonePix 2 System. Hybridoma viability and colony size were also better in CloneMedia.

SIGNIFICANT TIME SAVINGS BY HIGH-THROUGHPUT FLUORESCENCE SCREENING OF ANTIBODY PRODUCTION

The addition of a fluorescently-labeled antibody such as FITC-labeled CloneDetect to semi-solid media allows for fluorescent screening of antigen specificity or total IgG production. The ClonePix 2 System fluorescently images antibody secretion of hybridomas (Figure 4) and subsequently ranks and picks colonies based on the FITC intensity. This allows the ClonePix 2 System to select only the highest expressors amongst thousands of colonies, which greatly reduces the mAb workload by decreasing the number of sub-cloning steps required.

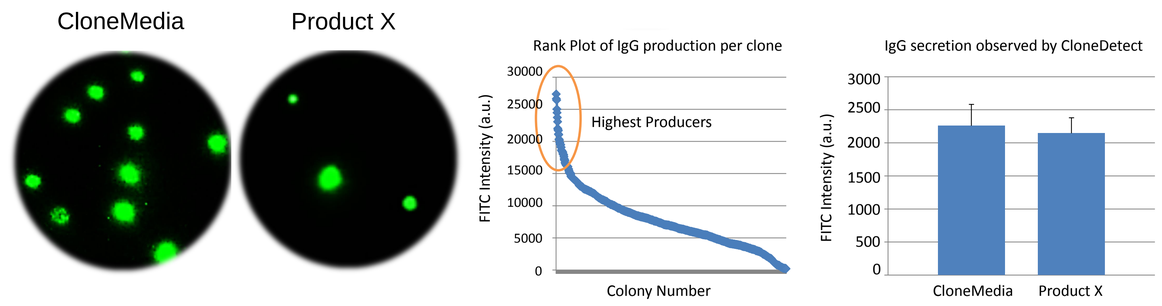


Figure 4. Selection and picking of highest-producing colonies. Fluorescence images of hybridomas plated in semi-solid medium with CloneDetect-FITC were captured with the ClonePix 2 System to assess total IgG production. Clones were ranked based on their FITC intensity and subsequently picked into a 96-well plate for further characterization.

BETTER GROWTH AND AB PRODUCTION IN LIQUID MEDIA

Hybridomas were then cultured in XP Media Hybridoma Growth Medium and showed superior growth and antibody productivity compared to products from another vendor (Figure 5). The proliferation of high-secreting clones enables more efficient workflows by increasing the number and quality of candidates moved on to the scale-up stage.

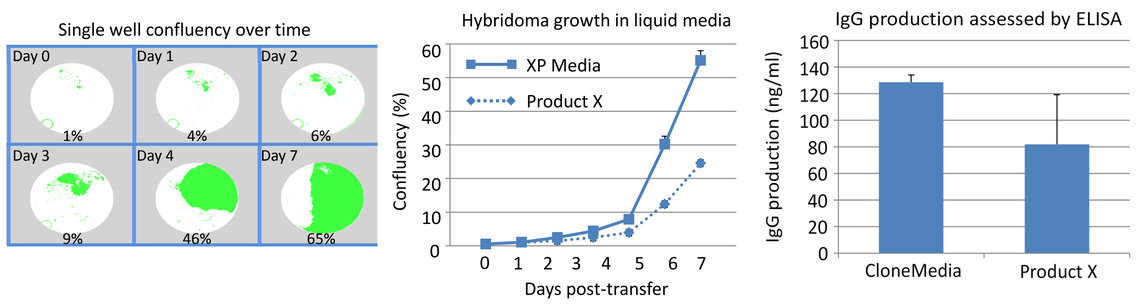


Figure 5. Growth and antibody production of hybridomas. 96 top producing clones were picked from semi-solid media into a 96-well plate and their growth was monitored over 7 days on the CloneSelect Imager. Hybridomas grown in XP Media grew 2X as fast and showed improved antibody production over Product X.

CONCLUSIONS

- XP Media and CloneMedia provide a simple (no extra supplements required), yet comprehensive solution from hybridoma generation to production.
- XP Media and CloneMedia perform significantly better than competitor X on all stages of the hybridoma workflow
- The use of automation for imaging, screening, and picking of clones drastically shortens the time needed to identify desirable clones
- CloneMedia, CloneDetect reagent, and the ClonePix 2 System allow for the automated selection of high-producing clones in one convenient step

MOLECULAR DEVICES