

An Optimized Media Solution for Antibody Production in Hybridomas

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INTRODUCTION

Monoclonal antibodies (mAb) have the remarkable ability to recognize individual protein species, which make them extremely useful as potential therapeutic candidates. They have revolutionized the treatment and diagnosis of cancer and other diseases, making them the fastest growing market in the biomedical industry.

To obtain monoclonal antibodies, B cells and myeloma cells are fused together to create hybridoma cells, which are then isolated as single cells to ensure monoclonality. The cloning of hybridomas is a crucial step in the generation of monoclonal antibodies because it facilitates the selection of stable and high-producing clones. In the set of experiments presented here, individual hybridoma cells were immobilized in semi-solid media and allowed to propagate into distinct colonies in the presence of CloneDetect (a fluorescently-labeled mouse IgG-specific antibody). This process prevents the overgrowth of the fast-growing colonies, ensuring colonies of monoclonal origin, while simultaneously screening for antibody productivity. This is a more efficient process than limiting dilution, which requires two or more separate steps to select and screen for mAbs.

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 and compare this solution to a similar suite of media from one of our competitors.

MATERIALS & METHODS

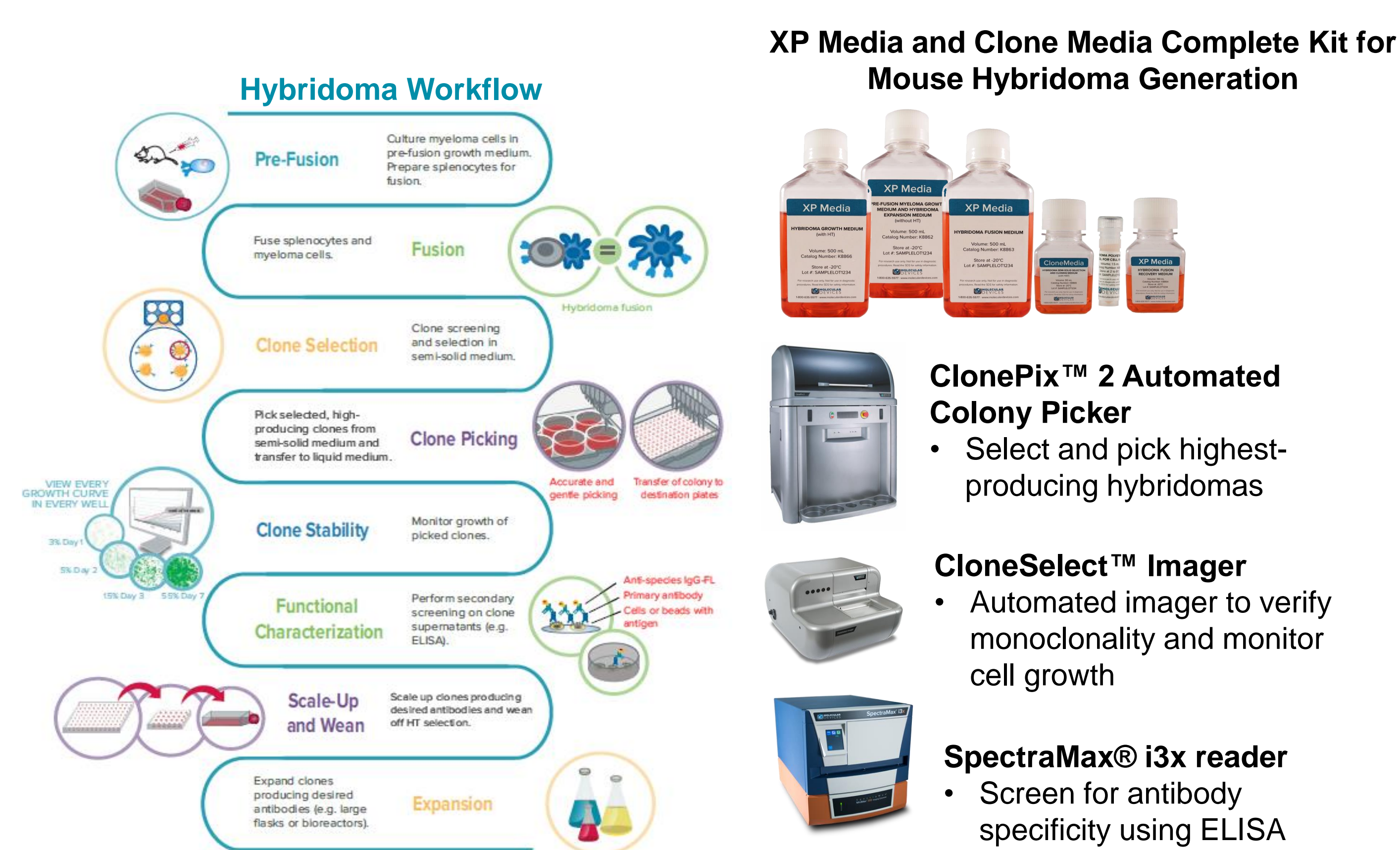


Figure 1. Cell line development workflow for screening antibody-producing hybridomas

COLONY SELECTION AND PICKING

Hybridomas were seeded into CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT) 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 backcalculate the antibody concentration. Absorbance measurements were performed on the SpectraMax i3x reader.

HYBRIDOMA GROWTH IN SEMI-SOLID MEDIUM

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. CloneMedia's performance is comparative to Product X in terms of number of colonies, size and hybridoma viability.

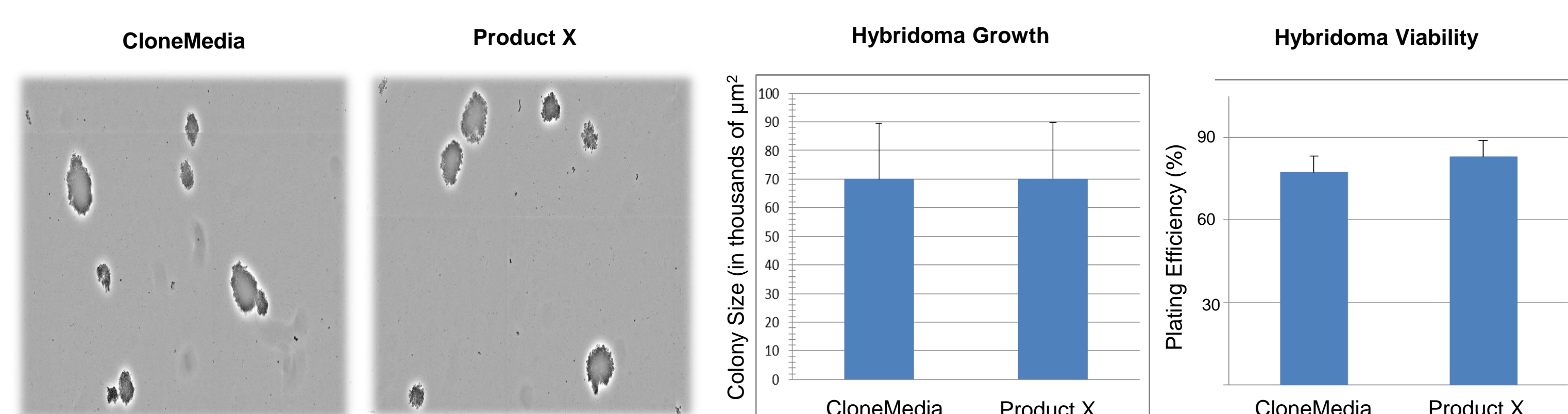


Figure 3. Hybridomas cultured in semi-solid media. Hybridomas were plated in CloneMedia semi-solid media from Molecular Devices and in semi-solid media from Competitor X. Using CloneSelect Imager (CSI), images and data of the colonies were taken on day 7 (shown on left). The average size of colonies was measured along with the viability from both conditions and reported on the right.

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 captures fluorescence images of antibody secretion from hybridomas (Figure 4) and subsequently ranks and picks colonies based on the FITC intensity (highest expressors), which greatly reduces the time to discovery by decreasing the number of sub-cloning steps required.

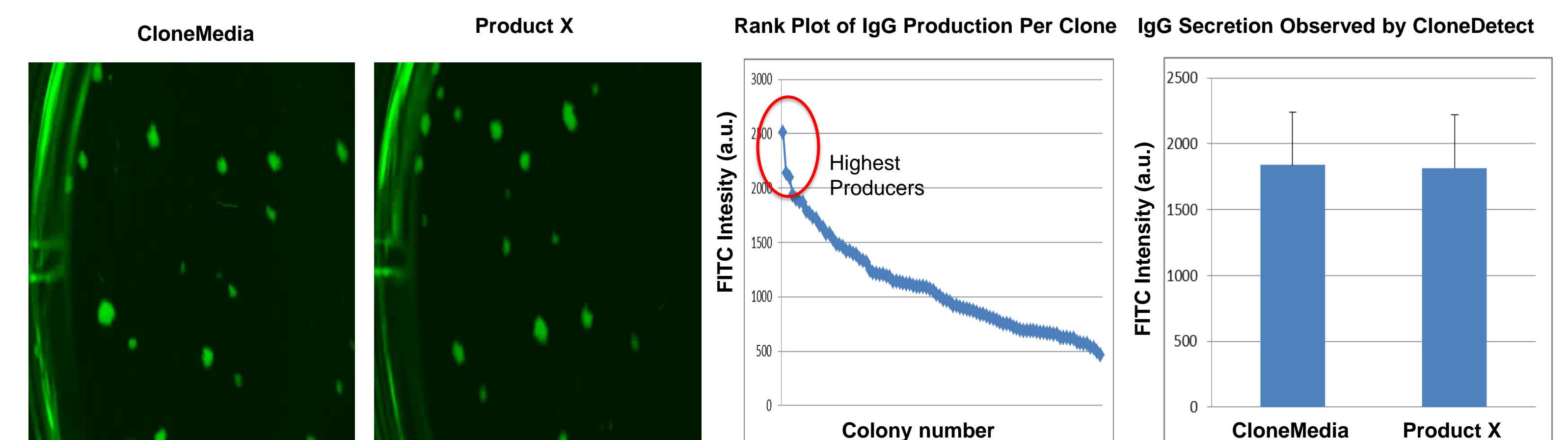


Figure 4. Selection and picking of highest-producing colonies. Fluorescence images of hybridomas plated in semi-solid medium with CloneDetect-FITC were captured with the ClonePix 2 System to assess total IgG production. Clones were ranked based on their FITC intensity and subsequently picked into a 96-well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

GROWTH AND AB PRODUCTION IN LIQUID MEDIA

Hybridomas were then cultured in XP Media Hybridoma Growth Medium and monitored for stable growth. 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

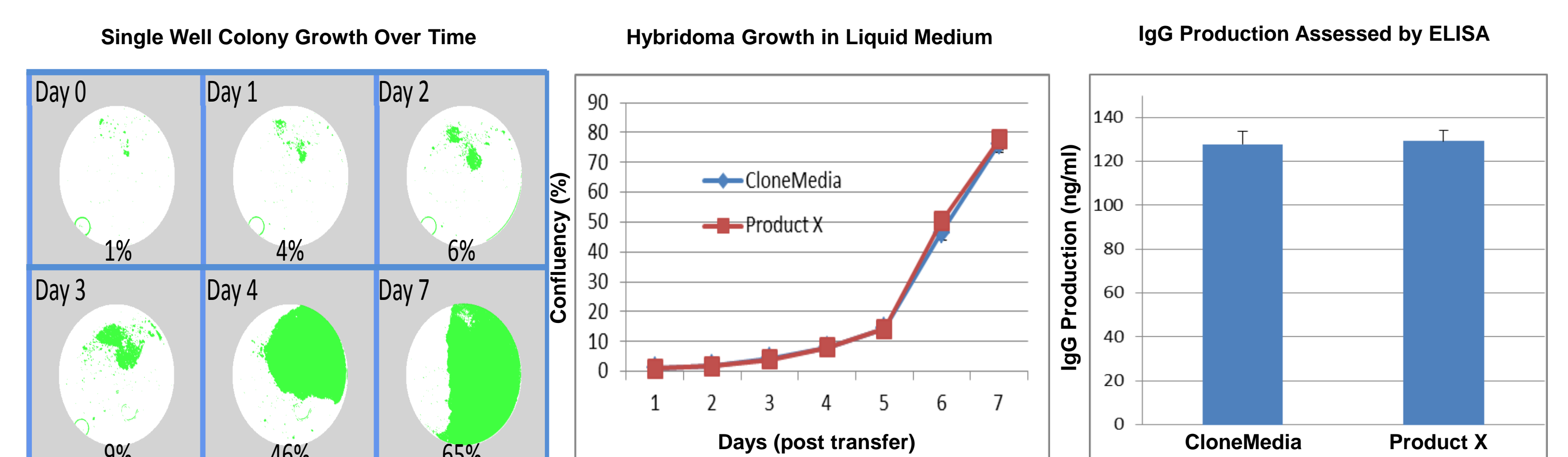


Figure 5. Growth and antibody production of hybridomas. 96 top-producing clones were picked from semi-solid media into a 96-well plate, and their growth was monitored over 7 days on the CloneSelect Imager.

ADVANTAGES OF CLONING IN SEMI-SOLID MEDIUM

Aside from shortening the antibody discovery process, it has been shown that the viability and survival of hybridoma cells is vastly improved when suspended in a semi-solid medium compared to limiting dilution or FACS sorting. Furthermore, when semi-solid cloning is combined with the ClonePix 2 System, researchers are able to increase throughput while decreasing time to discovery and consumable costs.

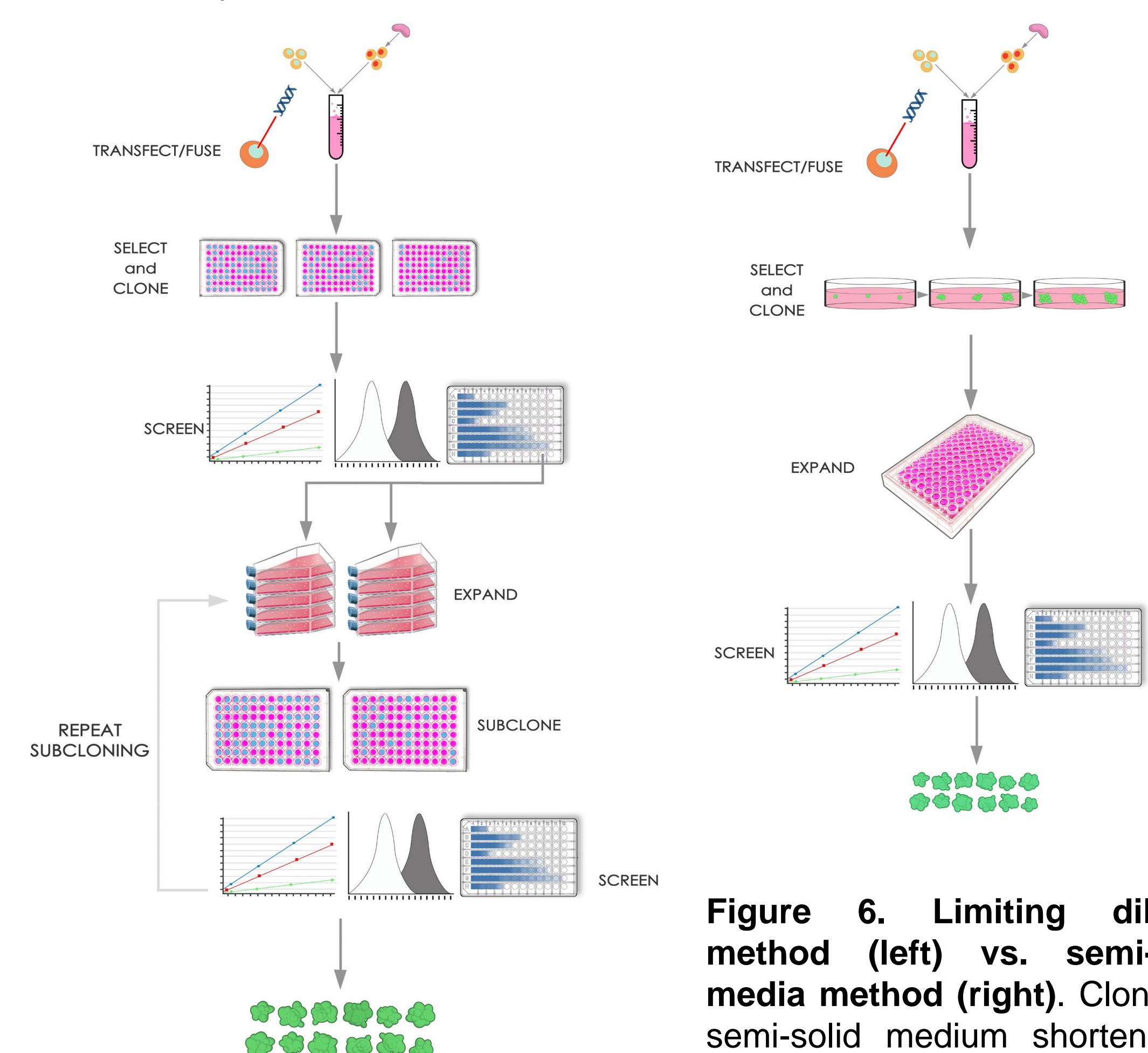


Figure 6. Limiting dilution method (left) vs. semi-solid media method (right). Cloning in semi-solid medium shortens the time to discovery by reducing subcloning steps.

CONCLUSIONS

- XP Media and CloneMedia provide a simple (no extra supplements required), yet comprehensive solution from hybridoma generation to production.
- XP Media and CloneMedia's performance of monoclonal antibodies are on par with the leading vendor.
- Cloning in semi-solid medium and 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.