

# Get the advantage

Proven antibody discovery solutions from target validation to scale up

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[moleculardevices.com/antibodydiscovery](http://moleculardevices.com/antibodydiscovery)

# Introduction

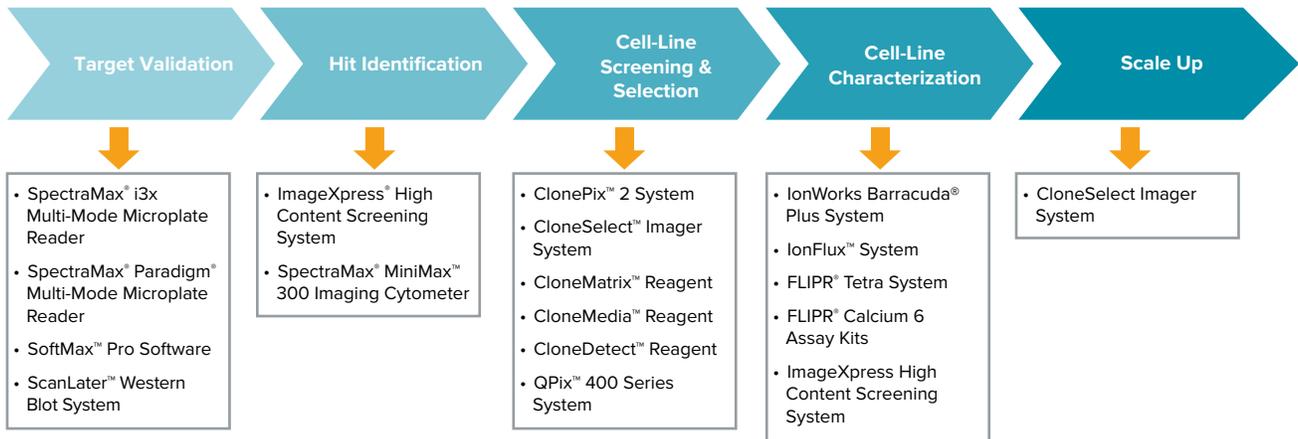
Monoclonal antibodies (mAbs) continue to enjoy intense interest as potential therapeutics. With antibody-drug conjugate and bispecific antibody products recently reaching the market, the use of mAbs will remain a key part of therapeutic development. Critical steps in the discovery and scale-up process can still be a challenge, but high-quality monoclonal antibody producing cell lines are within your reach. Accelerate your antibody discovery with our product portfolio specifically designed to shorten cell line development time and deliver stable clones with higher affinities and expression levels.

## Accelerate Your Time to Market with Comprehensive, Industry-Proven Solutions You Can Count On

We offer a complete solution that addresses all stages of Antibody Discovery research—from target validation, screening and clone selection to characterization and process scale up. Our comprehensive

portfolio suite is designed to optimize your productivity to get safe and efficacious therapeutics to market faster. With Molecular Devices as your partner, you'll get the expertise developed through working with leading antibody discovery groups and the solutions you need to optimize your productivity and accelerate your time to market.

- Automated screening & selection of high-expression cell lines
- Novel antibody internalization and binding assays
- Easy expression assays
- Objective verification of monoclonality
- Leading provider with decades of experience



**Figure 1: Molecular Devices' range of instrument, software and reagent solutions related to the validation, screening, characterization and production of monoclonal antibodies.**

# Target Validation

Taking protein expression measurement to a new level and expanding western blot capabilities, Molecular Devices detection platforms have emerged as important tools in validating targets in antibody discovery.

## Measuring Protein Marker Expression with Imaging Cytometry

Monitoring cell proliferation and protein expression are fundamental to many target validation assays but traditional methods require tedious labeling. The SpectraMax i3x with MiniMax 300 Imaging Cytometer allows you to count cells without the use of harmful dyes. Once transfected with GFP, SoftMax Pro Software can count the number of transfected vs un-transfected cells with our automated analysis tool. In addition, the SpectraMax i3x with MiniMax 300 Imaging Cytometer option provides visualization and information beyond simple fluorescence intensity in the well. Measured areas of fluorescence are localized to the cells expressing the markers of interest, and SoftMax Pro Software makes plotting dose response curves and IC<sub>50</sub> determination easy.

## Improved Western Blot Detection

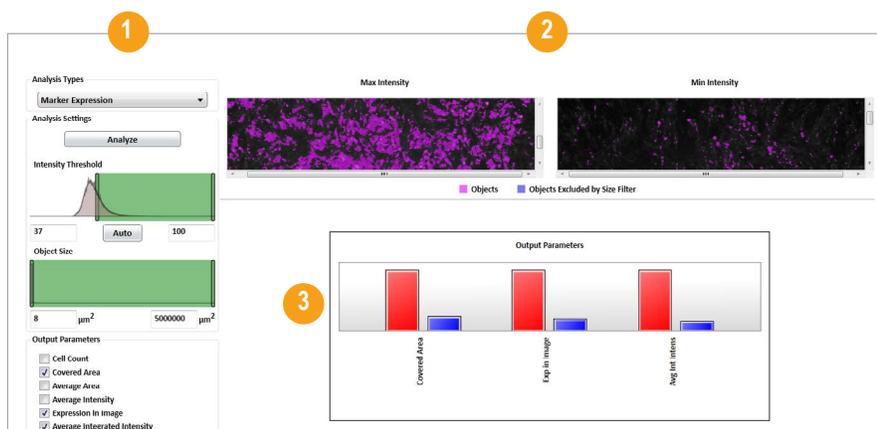
Improving protein quantitation and expanding workflow flexibility compared to traditional western blots drove development of the ScanLater Western Blot System. Used with the SpectraMax i3x and the SpectraMax Paradigm Multi-Mode Microplate Readers, the system uses a Europium secondary antibody to allow blots to be read for over 30 days. The blots are very stable and can be stripped and re-probed with additional antibodies. With Softmax Pro Software integration, quantification can occur directly after read.

### Resources:

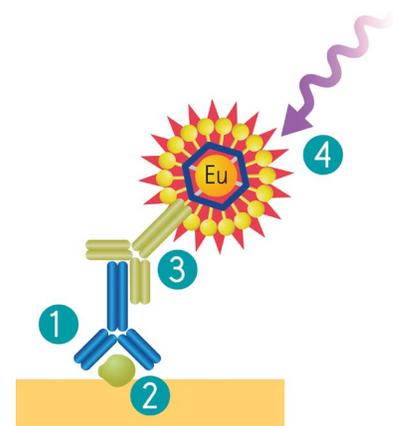
- Download Application Note: **Alternatives to DAPI staining: imaging and counting live cells.**
- Download Application Note: **Optimizing GFP transfection with SpectraMax i3 Multi-Mode Detection Platform.**

- Easily measure marker expression levels per cell or per well
- Improve protein quantitation with western blots in a plate reader
- Suits a large variety of applications: fluorescence detection or StainFree™ Technology

- Download Application Note: **StainFree technology for cellular image analysis without fluorescent dyes.**
- Download Application Note: **Measuring marker expression with imaging cytometry on a plate reader.**
- Download Data Sheet: **Detection and quantitation of protein with ScanLater Western Blot Detection System.**



**Figure 2: Test analysis parameters on positive and negative samples before plate read.** The SoftMax Pro Software interface has several visual components to help set up optimal parameters for image segmentation: 1: Adjust background intensity and object size thresholds to identify fluorescent cells. 2: Visualize resulting segmentation masks of positive and negative wells to see if further adjustment is needed. 3: Choose output parameters that yield large differences between positive and negative wells as seen in bar graphs. The Marker Expression Protocol is used to identify cell areas labeled with the fluorescein-conjugated anti-VCAM antibody. The presence of VCAM-1 indicates a positive inflammatory response.



**Figure 3: The ScanLater Western Blot System workflow.** Use an existing primary antibody (1) for binding to the protein of interest (2). Eu-labeled ScanLater Secondary Antibody (3) binds to the primary antibody. Detection with the ScanLater TRF Western Blot Detection System (4).

# Hit Identification

Discover the advantages of antibody binding assays with high content imaging. Take your antibody discovery beyond traditional ELISA and Fluorometric Microvolume Assay Technology (FMAT) assays. Our ImageXpress Micro High-Content Imaging System enables your antibody discovery with the ability to multiplex different cell types in the same well for faster development of higher-expressing cell lines.

ELISAs may use denatured epitopes, potentially leading to the generation of antibodies with poor affinities to conformational epitopes. Since the ImageXpress Micro High-Content Imaging System uses intact cells, antigens are presented in their natural conformation, improving your antibody discovery. Additionally, because you are free to select a range of excitation and emission wavelengths for any assay, you can incorporate multiplexing to measure multiple targets and cell types in the same assay simultaneously. This enables specificity and cross-reactivity measurements at the same time.

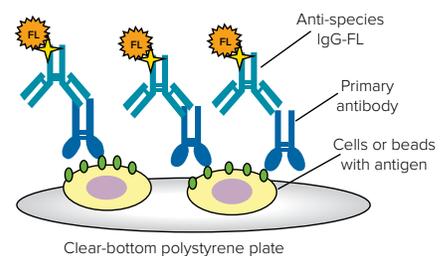
## Cell Counting and Morphology Visualization Utilizing Microplate Imaging Cytometry

Cell numbers are often indirectly estimated with benchtop microplate readers using luminescence or

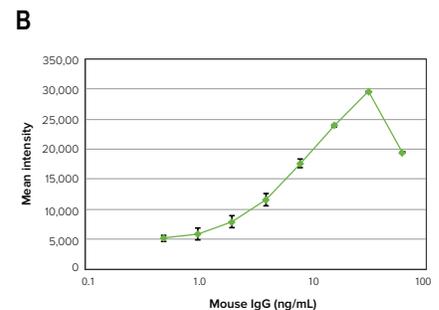
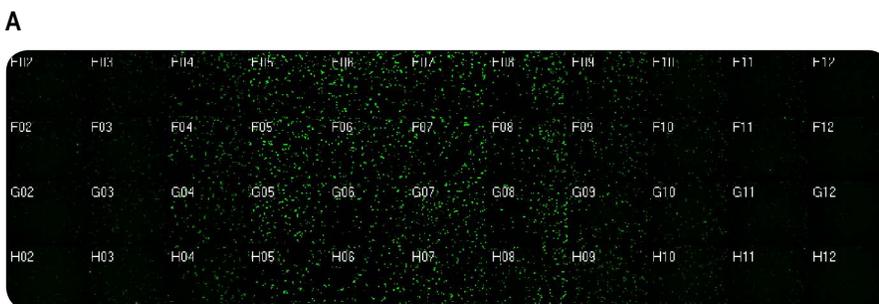
fluorescence readouts and assays for detecting ATP or cell viability dyes. While these assays can yield acceptable approximations, they do not provide visual information such as uniformity of growth, pipetting artifacts, or changes in cell size or morphology. The SpectraMax MiniMax 300 Imaging Cytometer provides sensitivity that is > 100X more than a plate reading assay. Images from multiple sites may be acquired to detect down to 1 cell per well. MetaXpress® software makes the data acquisition and analysis easy.

The patent-pending StainFree™ Cell Detection algorithm enables cell confluency and cell counting measurements on the SpectraMax MiniMax 300 Imaging Cytometer, eliminating destructive stains and saving you valuable time and money.

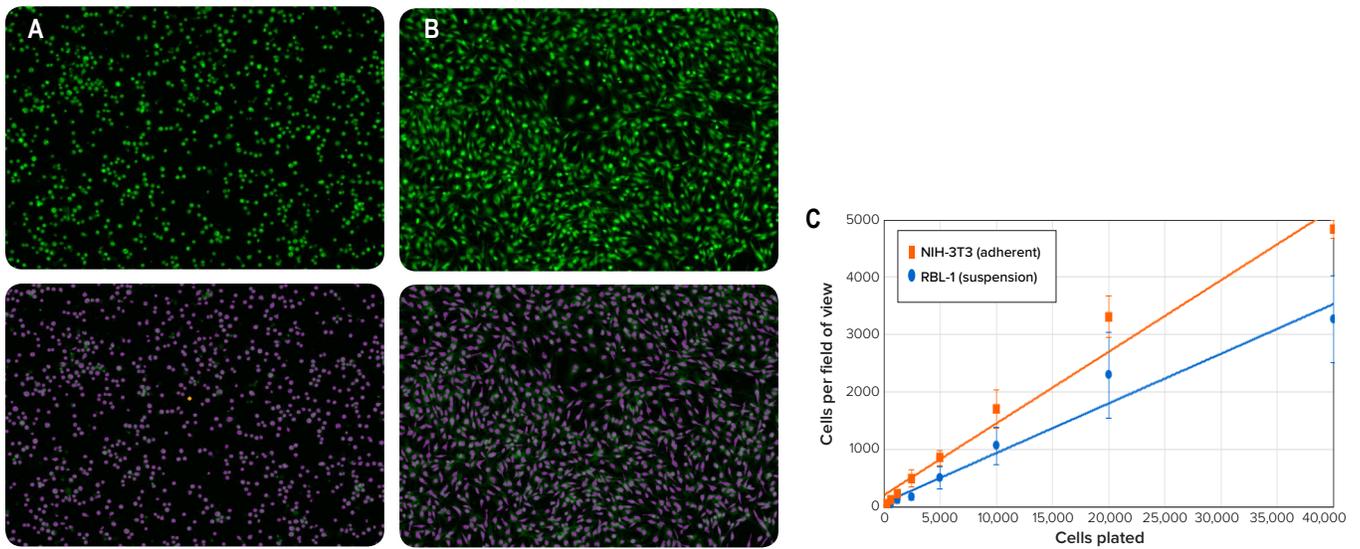
- Multiplexed screening of hybridoma supernatants
- Wavelength flexibility and multiplexing
- Time savings and enhanced specificity with no-wash formats
- Compatibility with adherent cells, suspension cells and beads
- Exquisite sensitivity for detecting low-abundance antigens



**Figure 4: Homogeneous antibody binding assay.** In homogeneous antibody binding assays cells or antibody-coated beads are added to microplate wells. A primary antibody of interest is added, followed by a secondary detection antibody.



**Figure 5: Primary antibody titration.** (A) Wells were run in quadruplicate, with an isotype antibody control in column 12. Seven-micron beads coated with goat anti-mouse antibody were combined with varying concentrations of mouse IgG analyte and added to the wells of a microplate followed by AlexaFluor™ 488-labeled secondary antibody and incubated at room temperature for one to two hours. Plates were imaged with a 10X PlanFluor objective. Thumbnail images of the wells clearly shows a dose-response of binding. (B) Image analyses using MetaXpress software determined an analyte lower limit of detection (LLD) of 0.5 ng/mL (30 pg/well) and a linear response of approximately 1–31 ng/mL. Note the well-known prozone or “hook effect” associated with homogeneous binding assays.



**Figure 6: Suspension and adherent cells are counted over four orders of magnitude.** (A) Suspension cells (RBL-1 leukemia cells) imaged with fluorescently labeled nuclei. The purple mask overlaid on the cell image illustrates the segmentation of the objects that were identified and measured. (B) Adherent cells (NIH-3T3 fibroblasts), imaged and segmented similarly as above, show that cells with very different morphology can be detected correctly with the SpectraMax MiniMax 300 Imaging Cytometer. (C) Both suspension and adherent cells can be quantitated for up to 4 orders of magnitude. The  $R^2$  of a linear curve fit is  $>0.96$  for both dilutions even after a period of cell growth overnight.

#### Resources:

- Download Application Highlight: [Homogeneous antibody binding assays with high content imaging.](#)
- Download Data Sheet: [Cell counting and morphology visualization utilizing microplate imaging cytometry \(MiniMax\).](#)

# High-Throughput Cell Screening and Selection

Don't miss your best clones. Use the ClonePix System to automatically screen and select high-producing cell lines. Then use the CloneSelect Imager System to select only fast-growing monoclonal cell lines.

- Accelerate selection of high valued clones
- Find rare high-secreting clonal cell lines
- Rapidly ensure monoclonality
- Objectively assess cell confluence
- Freely use adherent or suspension cells

The ClonePix and CloneSelect Imager Systems are an essential part of a seamless workflow for finding rare high-secreting clonal cell lines (see Figure 7). Use CloneMedia Reagent, our semi-solid media, with the ClonePix and CloneSelect Imager Systems to ensure formation of discrete clonal colonies for hybridoma and such recombinant cell lines as CHO, HEK and others. Once colonies are formed, the ClonePix System automatically ranks thousands of colonies to select only the high producers. Further, the CloneSelect

Imager System is used to normalize productivity, assess confluency and verify monoclonality to select only the optimal colonies that originated from a single cell.

The ClonePix and CloneSelect Imager Systems improve efficiency and productivity by eliminating the need for manual, time consuming limiting dilution or disruptive and expensive cell sorting techniques. Monoclonal populations of high-secreting cells are established in a much shorter timeframe, removing

many of the bottlenecks associated with producer cell-line optimization and downstream scale up for protein production.

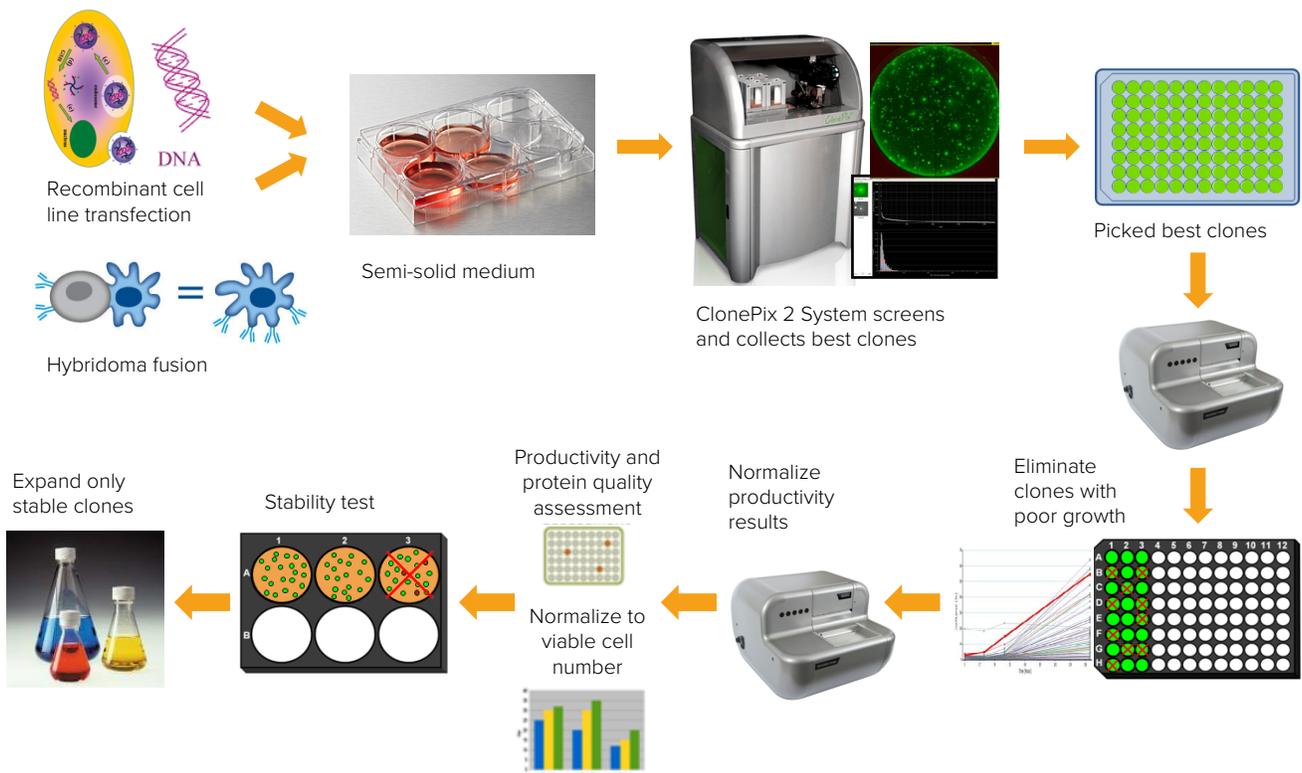


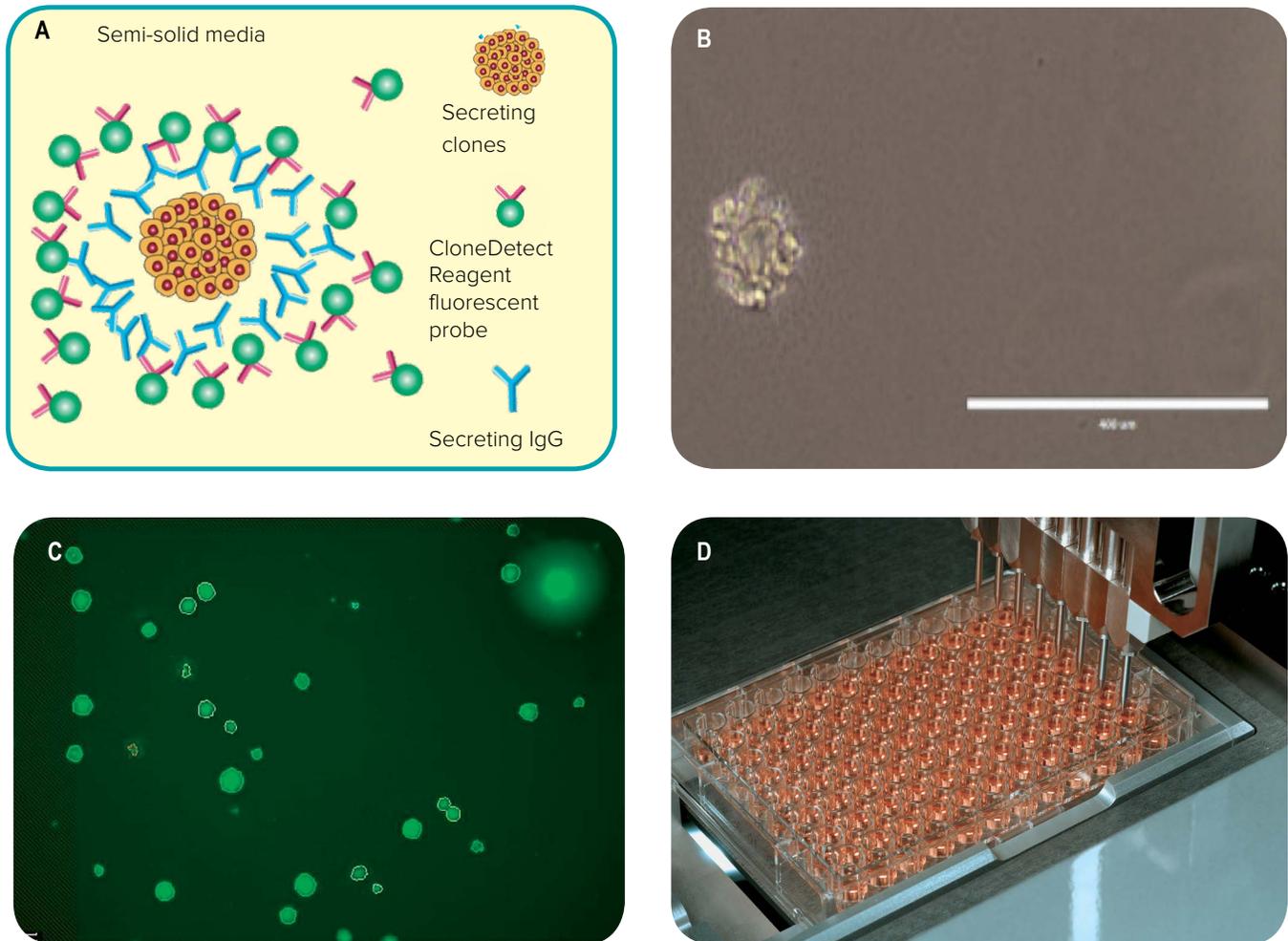
Figure 7: The ClonePix 2 and CloneSelect Imager Systems in the antibody discovery workflow.

### ClonePix Technology at the Heart of Faster Antibody Discovery

Using the ClonePix System is simple in principle, but powerful in practice. Simply plate mammalian cells in semi-solid medium, leave colonies to form and let the ClonePix System screen and pick only the highest value candidates using proprietary measurements of productivity or antigen specificity.

Using CloneMedia Reagent ensures a high probability of monoclonality, maintaining high clonal diversity and making it easy to isolate rare, high expressing clones. Additionally, expanding pre-grown colonies decreases the number of empty wells, ensuring that 96 clones are screened per plate.

The ClonePix System automatically images (in white light and fluorescence), selects, and picks mammalian cell colonies based upon a number of parameters such as size, roundness and proximity to neighbors, or far more specific information such as quantitative protein secretion or specific protein production.



**Figure 8: ClonePix technology principle.** (A) Conceptual overview of secreted protein detection from individual clones grown suspended in a methylcellulose matrix. (B) White light image of precipitated protein around a clone (in this case, antibody surrounding a growing hybridoma clone). Note the optical clarity of the image and spherical formation of the clonal colony (important for colony integrity) produced using CloneMedia Reagent. (C) ClonePix System detection and selection of target clones. (D) After automated picking of clones, the ClonePix System transfers the isolated target clones to destination plates.

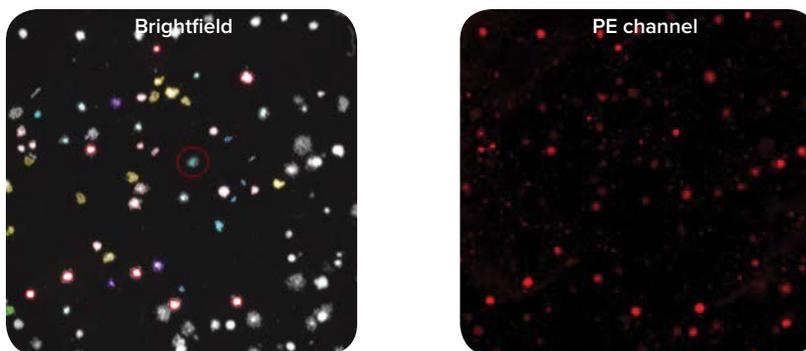
## Rapid Selection of GPCR Expressing Cells

The ClonePix System provides a new method to rapidly assess GPCR target protein expression levels in mammalian cell lines. The ClonePix System can reliably detect variable expression of GPCR clones. Moreover, fluorescence intensity has been shown to correlate positively with the magnitude of GPCR-mediated changes in cytoplasmic calcium levels as measured by the FLIPR Tetra System, caused by differences in the expression of membrane bound G-protein coupled muscarinic receptor M1.

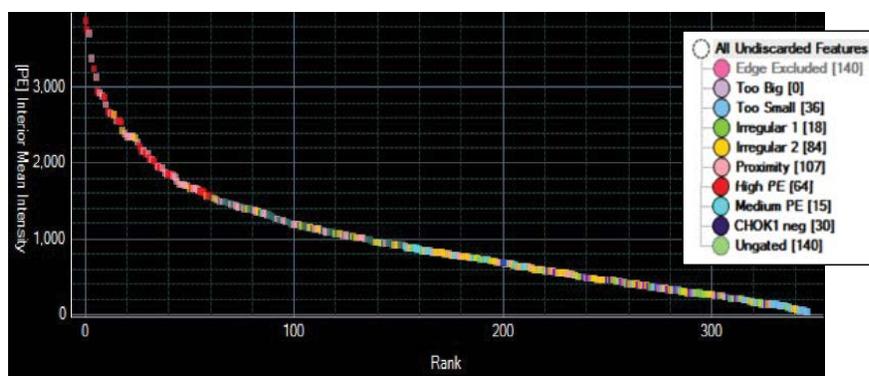
The ClonePix System can be efficiently used to detect and pick GPCR expressing clones while the CloneSelect Imager System enables selection of only the high-growing colonies. Positive high-growing colonies can be used as a source for GPCR proteins for subsequent generation of antibodies.

## Resources

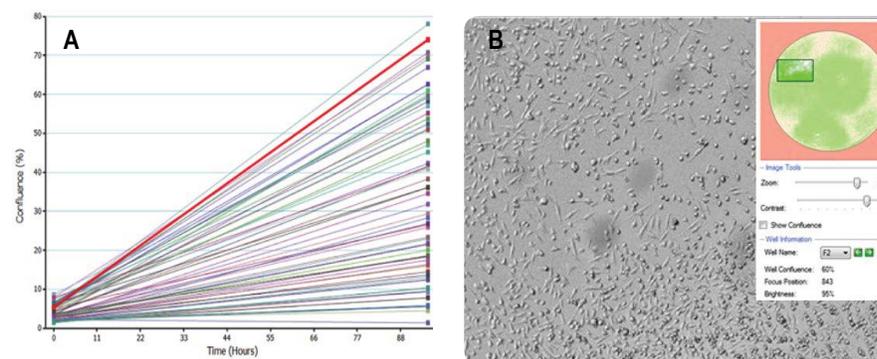
- Watch Webinar:  
[Identification and selection of GPCR cell lines with the ClonePix 2 System](#). Barbara Robertson, BMS and Alison Glaser, Molecular Devices.
- Download Application Highlight:  
[Rapid selection and development of GPCR expressing mammalian cell lines using novel ClonePix technology](#).
- Download Application Highlight:  
[Rapid automated selection of mammalian cell colonies by cell surface protein expression](#).
- Download Poster:  
[Rapid selection and development of GPCR expressing mammalian cell lines using novel ClonePix technology](#).
- Download Datasheet:  
[FLIPR Calcium 6 Assay Kits](#)
- Watch Video:  
[Micro robots drive Bayer's high-tech vision](#).



**Figure 9: Detection of CHO-M1 cells on the ClonePix 2 System.** The ClonePix 2 System reveals diverse levels of fluorescent intensity with CHO-M1 cell line, demonstrating it can distinguish between various levels of expression of GPCR M1 protein. Colonies recognized by the software are outlined in color under the brightfield channel. Fluorescence intensity is calculated based on the physical location of colonies.



**Figure 10: Fluorescent ranking of CHO-M1 expression.** Cells were grouped to identify and separate colonies based on morphology and fluorescence intensity. Acceptable morphology for picked colonies was based on size, shape, and proximity to neighboring colonies. Morphologically ideal colonies were ranked by interior fluorescence intensity and gated into one of four fluorescence groups: high, medium, CHO-K1 negative, and un-gated. The CHO-K1 negative group was defined by the background signal intensity. All colonies identified in the group were confirmed to originate from the corresponding negative control well. Un-gated colonies are colonies that had low levels of signal but above the background.



**Figure 11: Rapid confluency measurements to select high-growing clones.** Colonies picked by the ClonePix 2 System were deposited into 96-well Greiner plates containing 200  $\mu$ L Ham's F12 media + 10% FBS + G418 for CHO-M1 picked clones and the same media conditions without G418 for CHO-K1 clones. These 96-well plates were imaged on the CloneSelect Imager System to verify transfer and growth (A). Cells were cultured for one week then analyzed on the CloneSelect Imager System to ensure the cells had proliferated (B). Cells were subsequently transferred to 384-well plates for functional verification of expression by the FLIPR Tetra System using the FLIPR Calcium 6 Assay.

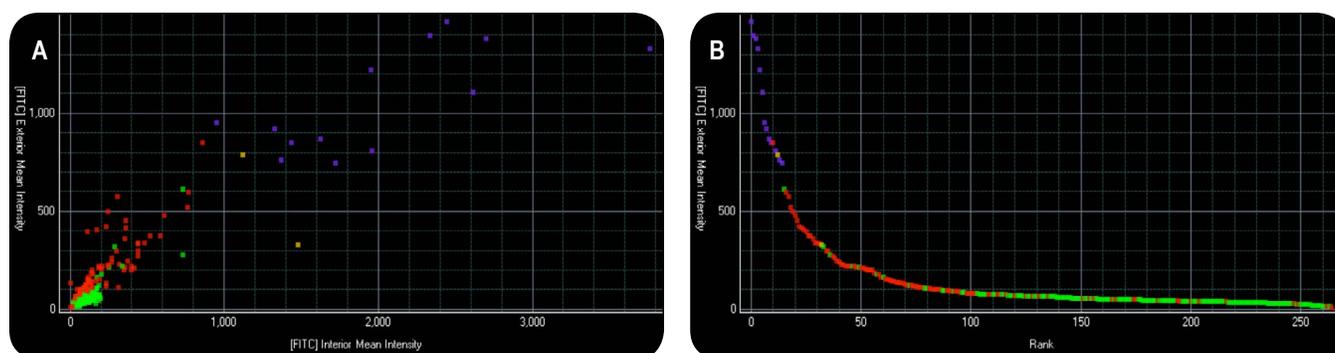
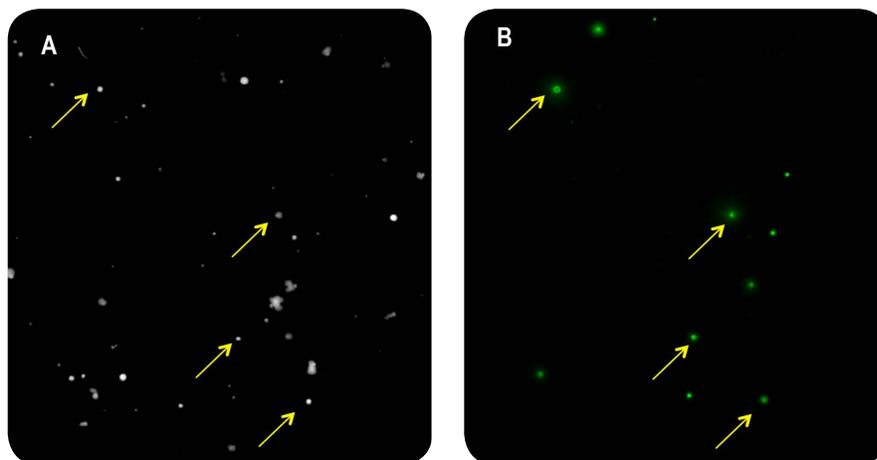
### Enhanced Development of Virus-Specific Hybridomas

The ClonePix System can accelerate and improve your hybridoma screening by decreasing manual manipulation and providing high sensitivity detection of secreting clones.

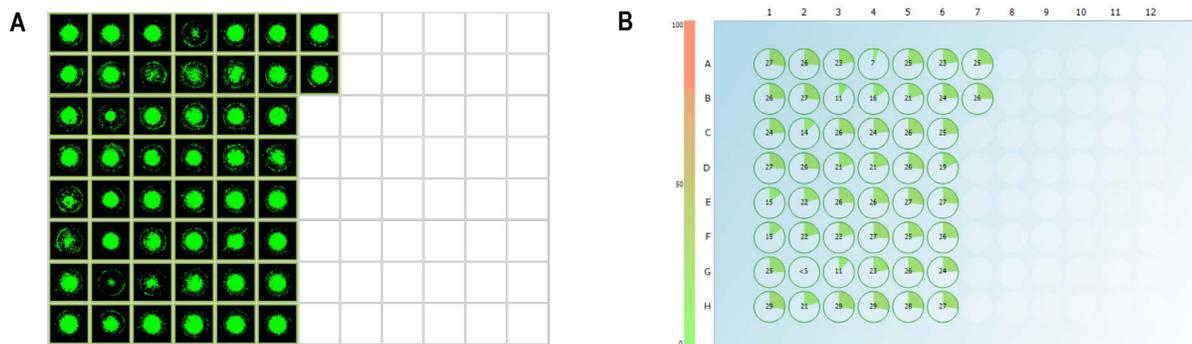
The ClonePix and CloneSelect Imager Systems are being employed in high-throughput screening, selection and growth assessment of hundreds of sub-cloned colonies from parental hybridoma material (historically, parental line yields were less than 1 mg/L of mAb).

This highly efficient automated clone selection, rescue and stabilization of a high-titer hybridoma cell line produces a highly specific antibody to an immunogenic viral antigen.

**Figure 12: Hybridoma cells were plated at 200 cells/mL in 6-well plates in CloneMedia Semi-Solid Media Reagent (for hybridomas/myelomas).** Fluorescently conjugated CloneDetect Reagent was added to enable *in situ* detection of secreted IgG. Colonies were imaged on a ClonePix System in white light at 150 ms (A) and fluorescence at 500 ms (B). Variability of FITC signal as quantitated by Interior Mean Intensity and Exterior Mean Intensity of the parent line indicate an unstable, non-monoclonal hybridoma cell line. Based on the ranking plot data, only clonal, high-secreting colonies were selected.



**Figure 13: Visualization of IgG secretion in parental hybridoma cell line.** (A) Scatter plot shows a linear correlation between Exterior and Interior Mean Intensity suggesting that the IgG is being secreted properly (otherwise immobilized on the cell surface with high Interior and low Exterior), and the low IgG yielding clones are due to a heterogeneous population of variable secretors (i.e., only a few hybridomas, 5–6% of the total population, are producing IgGs, while the majority of the clones are growing without IgG expression). (B) Ranking plot shows clone selections in purple.



**Figure 14: CloneSelect Imager Software analysis enables identification of optimally growing clones.** Image analysis of the subset of selected clones from Figure 13 demonstrates assessment of colonies' cellular morphology and growth characteristics (A) as well as estimation of colony number and colony area for each well (B), thereby efficiently tracking colony growth.

### Verification of Monoclonality and Uniform IgG Secretion

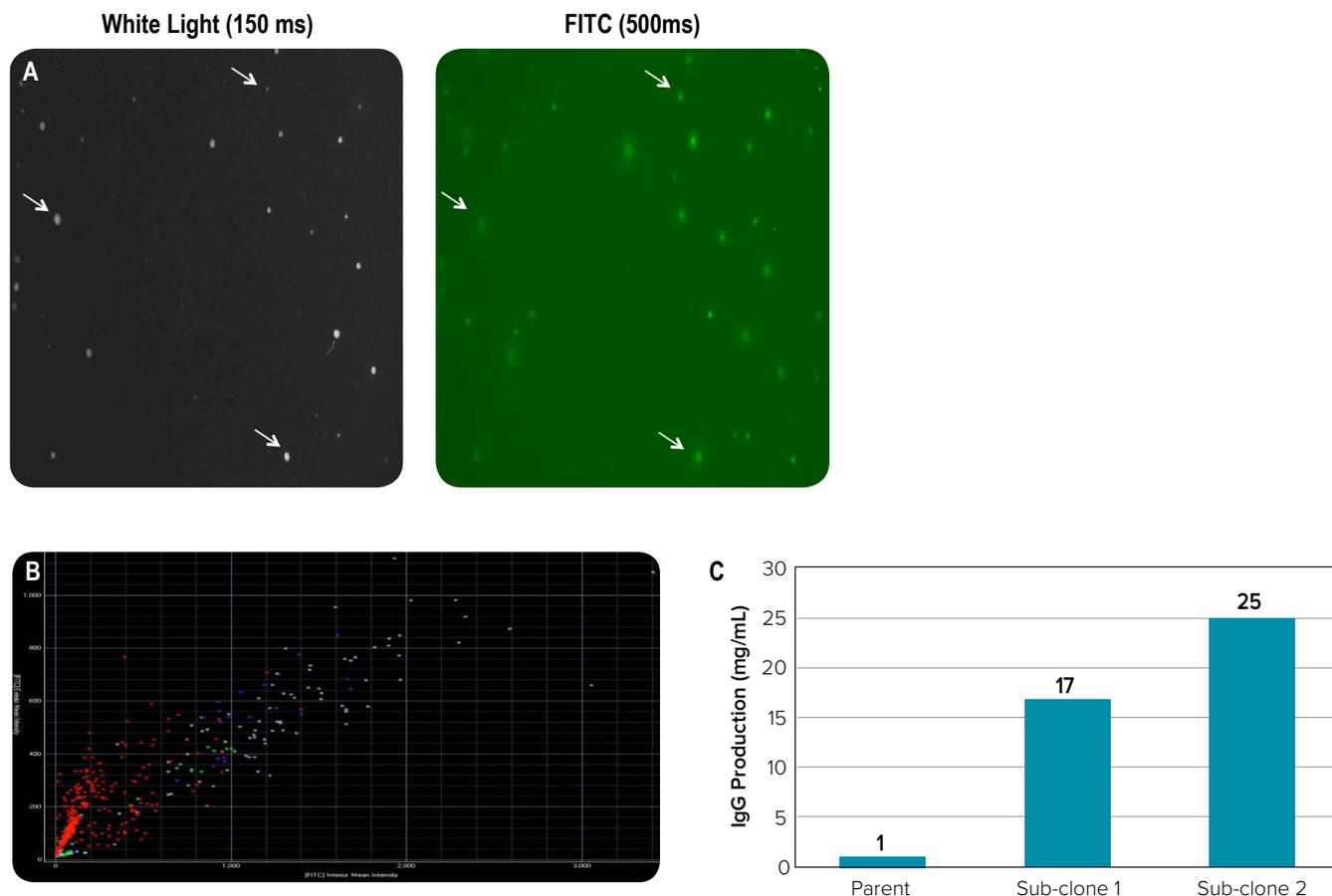
The ClonePix System makes it easy to verify production of high-yield clones. In this example, two sub-clones were expanded and re-plated at 200 cells/mL in Semi-Solid CloneMedia Reagent with CloneDetect Reagent. Visualization and analysis by the ClonePix Software suggested that IgG was being secreted properly and higher IgG yields were observed due to a homogeneous population of uniform secretors.

### Increasing Production Capacity on Novel ds-DNA Viral Hybridoma Sub-Clones

By utilizing the ClonePix System, poorly-producing parental clones can be re-screened and higher-producing, stable sub-clones rescued. In this instance, total yields were quantified after Protein G column purification. Both novel sub-clones had shown a dramatic improvement in IgG production (17– 25 mg/L) over historic yields of the parent clone (~1 mg/L).

### Resources:

- Download Application Highlight: [Enhanced development of virus-specific hybridomas using ClonePix and CloneSelect technologies.](#)
- Watch Webinar: [The application of the ClonePix and CloneSelect technologies for hybridoma discovery.](#) Jason Goldstein PhD, AbiPointe Biotechnology Webinar.



**Figure 15: Sub-cloning of parental hybridoma by the ClonePix System results in uniform IgG secretion at higher yields.** Subset data for one sub-clone is shown. (A) A significant improvement in % FITC-positive colonies as a result of sub-cloning is observed as compared to the parental clone (refer to Figure 12A, B). IgG secretion was detected at seven days growth by 100 U/mL of CloneDetect Reagent. (B) Scatter plot shows a linear correlation between Exterior and Interior Mean Intensity; the slope is shifted towards the Y-axis indicating greater uniformity, while more clones in the upper quadrants signify the presence of more highly FITC-positive clones. (C) Sub-cloning by the ClonePix System resulted in a dramatic increase in cell line titer.

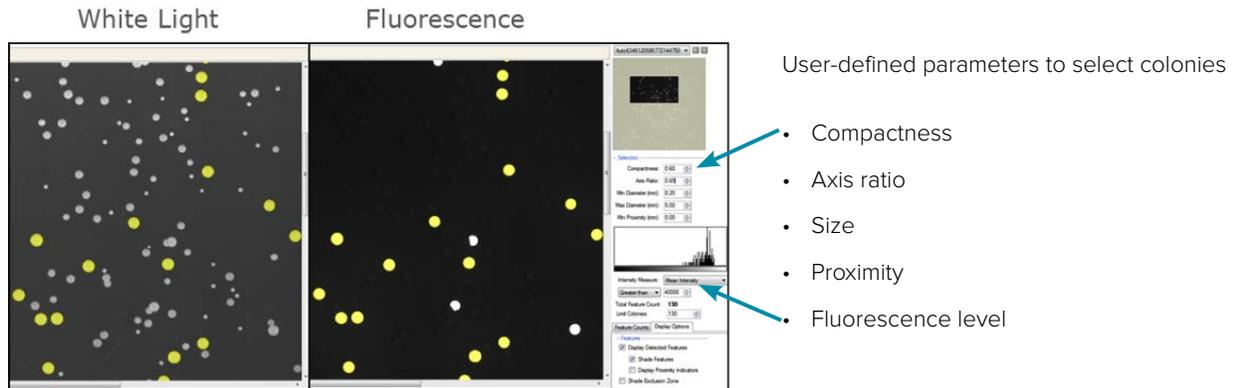
### High-Throughput Phage Library Screening

The development of phage display revolutionized antibody drug discovery. The method is often used to isolate highly specific therapeutic antibody

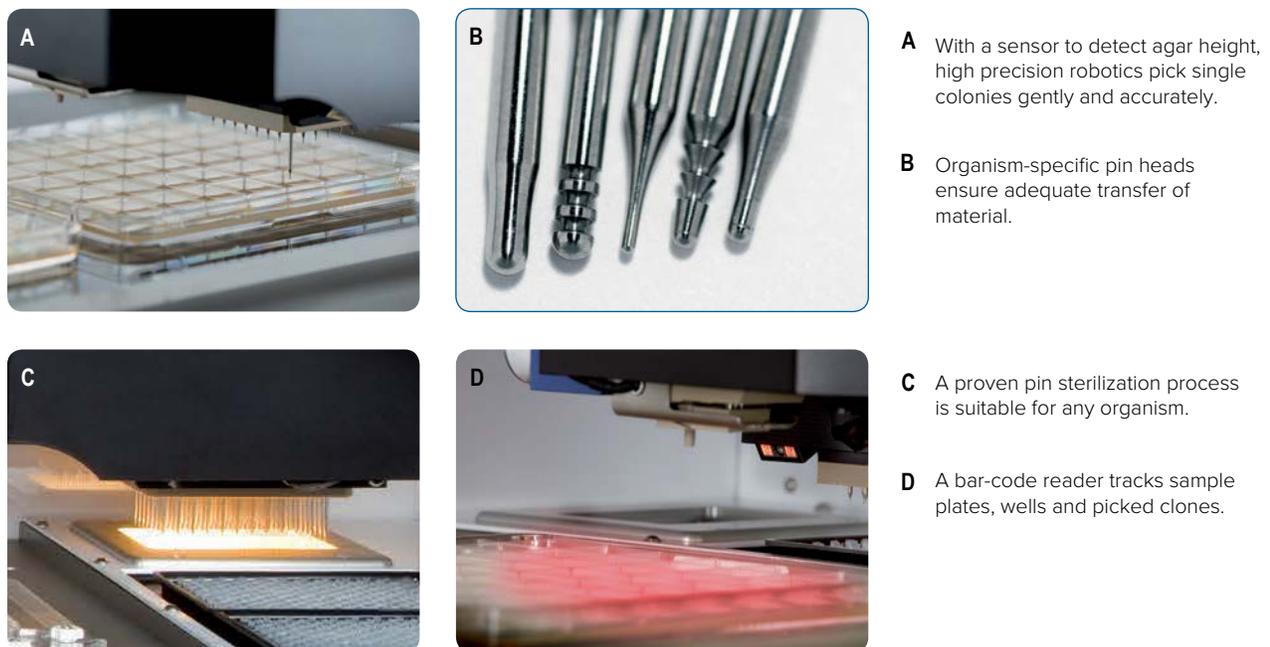
leads to develop anti-cancer or anti-inflammatory therapeutics.

A typical phage library contains about  $10^9$ – $10^{11}$  variants, making it very difficult and time consuming to find the right candidate with traditional screening

technologies. The automated QPix 400 Series Microbial Colony Picking Systems can screen 3000 clones per hour in white light or fluorescence and select clones based on user-defined parameters such as compactness, axis ratio, size, proximity and fluorescence level.



**Figure 16: QPix 400 Series Microbial Colony Picking Systems offer you the unique option to simultaneously detect colonies and quantify fluorescent markers in a pre-screening step before picking.** Colonies are selected according to user-defined parameters: compactness, axis ratio, size, proximity and fluorescence level.



**Figure 17: A typical QPix 460 Microbial Colony Picking System workflow.**

### Intracellular Localization Studies

Understanding how a therapeutic antibody may be internalized after binding to a target is critical for antibody-based drug discovery in oncology. The MetaXpress Transflour Module allows accurate quantitation of vesicular structures. The Transflour-based co-localization Journal allows quantitation of time-dependent accumulation of fluorescently-labeled antibodies in intracellular compartments.

### IonFlux Systems in Nanobody Characterization

IonFlux Systems were used in the identification and characterization of highly potent and selective anti-Kv 1.3 nanobodies (single domain antibody) developed by Ablynx Pharmaceuticals.

### Functional Validation of CHO-K1 and CHO-M1 Clones Picked Using the ClonePix System and the FLIPR Calcium 6 Assay Kit

Membrane-bound G-protein coupled muscarinic receptor expression level was evaluated using anti-M1 antibody conjugated with PE from a newer and an older passage of CHO-M1 cells. The expectation is that the expression level of M1 GPCR will be significantly lower in the older passage than in the newer passage. Parental CHO-K1 cells were also included in the study as negative control.

Upon activation of GPCRs by ligands, receptor conformation is changed triggering G-protein activation inside the cell. An active G-protein has the potential to induce various cascades of intracellular messengers including calcium.

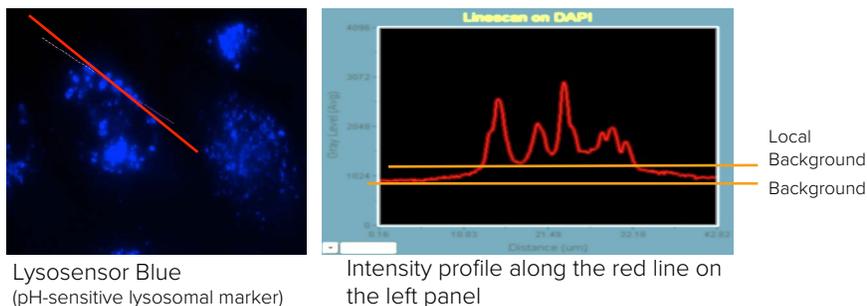
Cells from each group picked by the ClonePix System were evaluated for functional activity using the FLIPR Tetra System. Calcium-sensitive fluorescent dye (FLIPR Calcium 6 Assay Kit) was used to

assess changes in cytoplasmic calcium via activation of a G-protein coupled IP3-sensitive pathway by carbachol at 40 nM throughout this feasibility study. Carbachol at 40 nM is the  $EC_{50}$  concentration based on historic agonist concentration response curves, determined empirically.

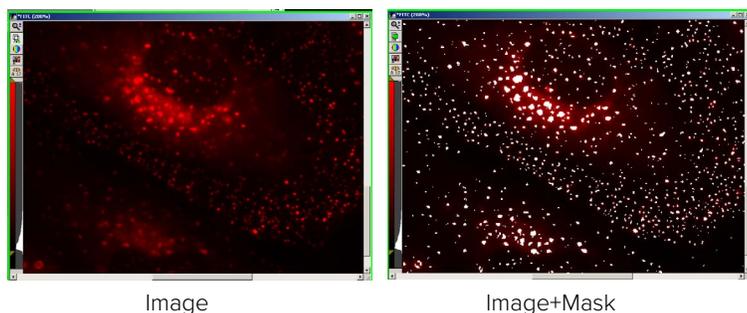
Since each group (except the mixed medium and low fluorescence picked clones) was seeded on a separate 384-well plate, changes in baseline fluorescence intensity were normalized to background fluorescence reads of each 384-well plate before adding 40 nM carbachol.

These results support a positive correlation between membrane-bound G-protein coupled muscarinic receptor expression level and functional activity. The lack of calcium fluorescent signal in the CHO-K1 negative control group further confirms that the ClonePix System can accurately distinguish between clones with and without expression of the M1 GPCR on the cell surface.

### Vesicles Area Intensity profile: higher local background



### HUVEC cells infected with BacMam expressing EEA1-RFP



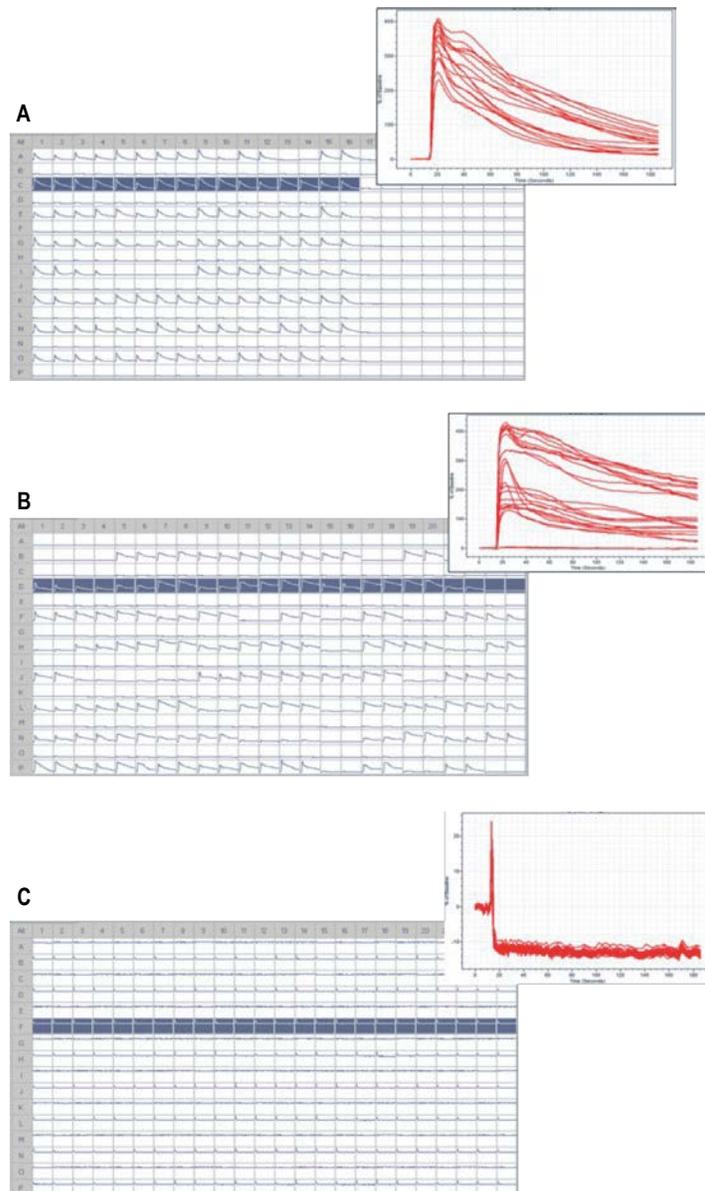
Measurements available in MX Transflour Module with Adaptive Acquisition Background(TM) algorithm

1. Vesicles Integrated Intensity
2. Vesicles Average Intensity
3. Vesicles Area

**Figure 18: Measurement of internalization of anti-M1 antibody into CHO-M1 cells.** The Transflour Module is well suited for segmentation and quantitation of internalized antibodies within vesicles. Data courtesy J. Andreev, Regeneron.

## Resources:

- Watch Webinar:  
**Application of Molecular Devices HCA tools for antibody drug discovery at Regeneron.**  
Julian Andreev, PhD, Regeneron Pharmaceuticals, Inc.
- Watch Webinar:  
**Automated patch clamp in drug discovery.** Daniel Jannsen, PhD, Ablynx Pharmaceuticals.
- Watch Webinar:  
**Identification and selection of GPCR cell lines with ClonePix 2 System.**  
Barbara Robertson, BMS and Alison Glaser, Molecular Devices.
- Download Application Highlight:  
**Rapid selection and development of GPCR expressing mammalian cell lines using novel ClonePix technology.**
- Download Poster:  
**Rapid selection and development of GPCR expressing mammalian cell lines using novel ClonePix technology.**



**Figure 19: Validation of ClonePix System clone selection using the FLIPR Tetra System.** The FLIPR Tetra Instrument performs high-throughput, functional cell-based assays and is the system of choice in drug discovery for evaluating changes in intracellular calcium detected through the use of fluorescent calcium-sensitive reporter dyes (FLIPR Calcium 6 Assay Kit). (A) In the CHO-M1 high-fluorescence picked clones, carbachol produced a four-fold increase in fluorescent read from background. (B) In the mixed CHO-M1 medium- and low-fluorescence picked clones, carbachol produced a four-fold and a two-fold increase in fluorescent read from background respectively. (C) In the CHO-K1 negative group, carbachol failed to elicit any significant change in fluorescent read from background.

# Quality Control for Scale Up

Be ready to scale your production process through careful monitoring of cell growth and verification of monoclonality.

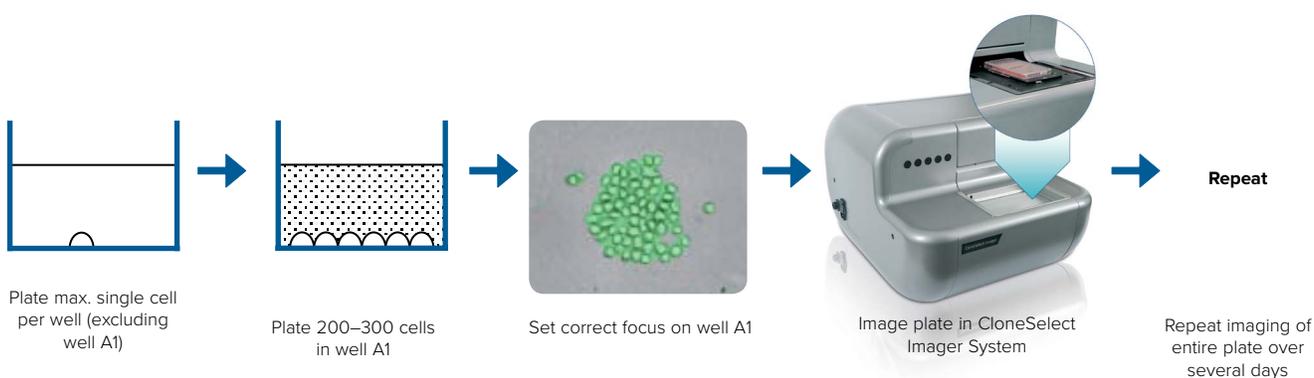
## Produce Consistent Results in Less Time

When developing cell lines for therapeutic antibody production, it is crucial from a quality and regulatory perspective to ensure that the cell line originates from a single progenitor and is therefore monoclonal.

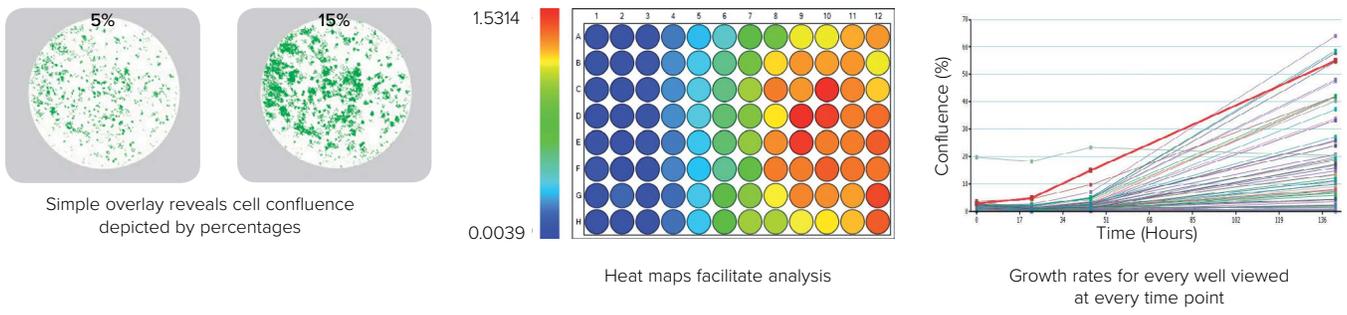
Traditional cloning methods, such as limiting dilution and FACS, use statistical analysis to determine a confidence level for monoclonality. The CloneSelect Imager System, utilizing noninvasive, white light imaging, verifies monoclonality based on objective image analysis after single-cell sorting.

Furthermore, rapid, quantitative measurement of cell confluence and generation of well-by-well growth curves is easy, helping to make your production process faster and more efficient.

- Label-free white light imaging of living cells
- Physiologically-relevant label-free cell detection suitable for adherent and suspension cells
- Consistent and objective growth rate determination
- Fastest cellular imaging at 90 seconds per 96-well plate
- Flexible integration for high-throughput operation



**Figure 20: A typical CloneSelect Imager System workflow for monoclonality verification and cell growth assessment.** Simply plate your cells, set correct focus on well A1 and image your plates over a period of 14 days. The growth (image) history of each well can be tracked back to its starting point — providing evidence of monoclonality.



**Figure 21: Rapid image generation and clear data analysis.** The CloneSelect Imager System replaces time-consuming, subjective manual inspections by providing objective measurement of cell confluence in every well. The heat map visually displays cell confluence, while cell growth rate can be viewed and tracked in every well in every plate.

**Monoclonality Verification**

After initial seeding, the CloneSelect Imager System can image every well at any time point, and a Loci of Growth function enables viewing only those wells that contain a single colony. Seed one cell per well and image at any point.

- Focus on wells with a single locus of growth and view image history to verify monoclonality
- Verify colony origin by tracking the image history of each well

**Resources:**

- Download Application Note: [Monoclonality verification on CloneSelect Imager System.](#)
- Watch Video: [Micro robots drive Bayer's high-tech vision.](#)

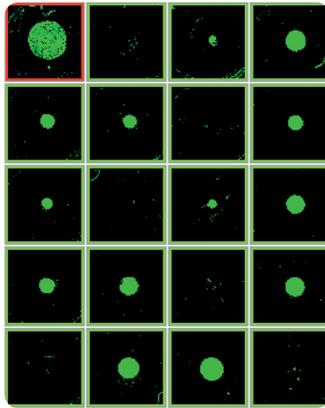
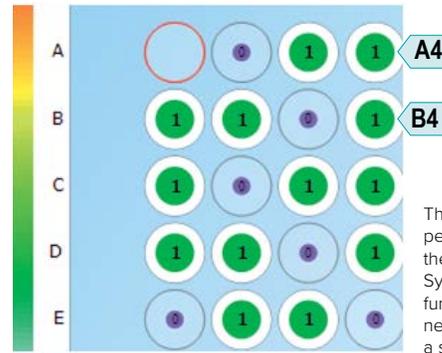
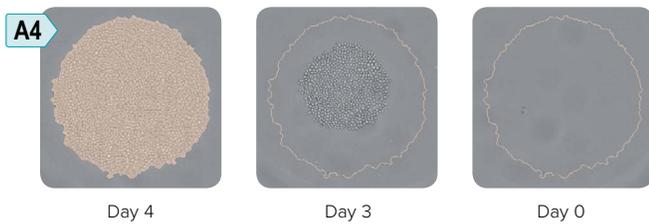


Plate thumbnails — the green overlay highlights where colonies have been identified.

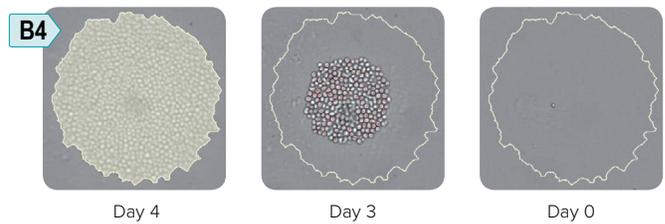


The number of colonies per well is identified by the CloneSelect Imager System's Loci of Growth functionality. You only need to review wells with a single colony.

**Two cells on Day 0 – not monoclonal**



**One cell on Day 0 – monoclonal**



**Figure 22: Verification of monoclonality — viewing the origin of a colony.** The growth (image) history of each well can be tracked back to its starting point — providing evidence of monoclonality.

# Antibody Discovery Solutions



**ImageXpress Micro XLS  
Widefield High Content  
Screening System**



**SpectraMax i3x Multi-Mode  
Microplate Reader**

**SpectraMax MiniMax 300  
Imaging Cytometer**



**IonFlux Automated Patch  
Clamp System**



**QPix 400 Series Microbial  
Colony Picking Systems**



**SpectraMax Paradigm  
Multi-Mode Microplate  
Reader**



**FLIPR Tetra High  
Throughput Cellular  
Screening System**



**ClonePix 2  
System**



**IonWorks Barracuda  
Plus System**



**CloneSelect Imager  
System**



**FLIPR Calcium Assay Kits**



**ScanLater Western Blot  
Cartridge**



**CloneMatrix Reagent and  
CloneMedia Reagent**



**CloneDetect Reagent**

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