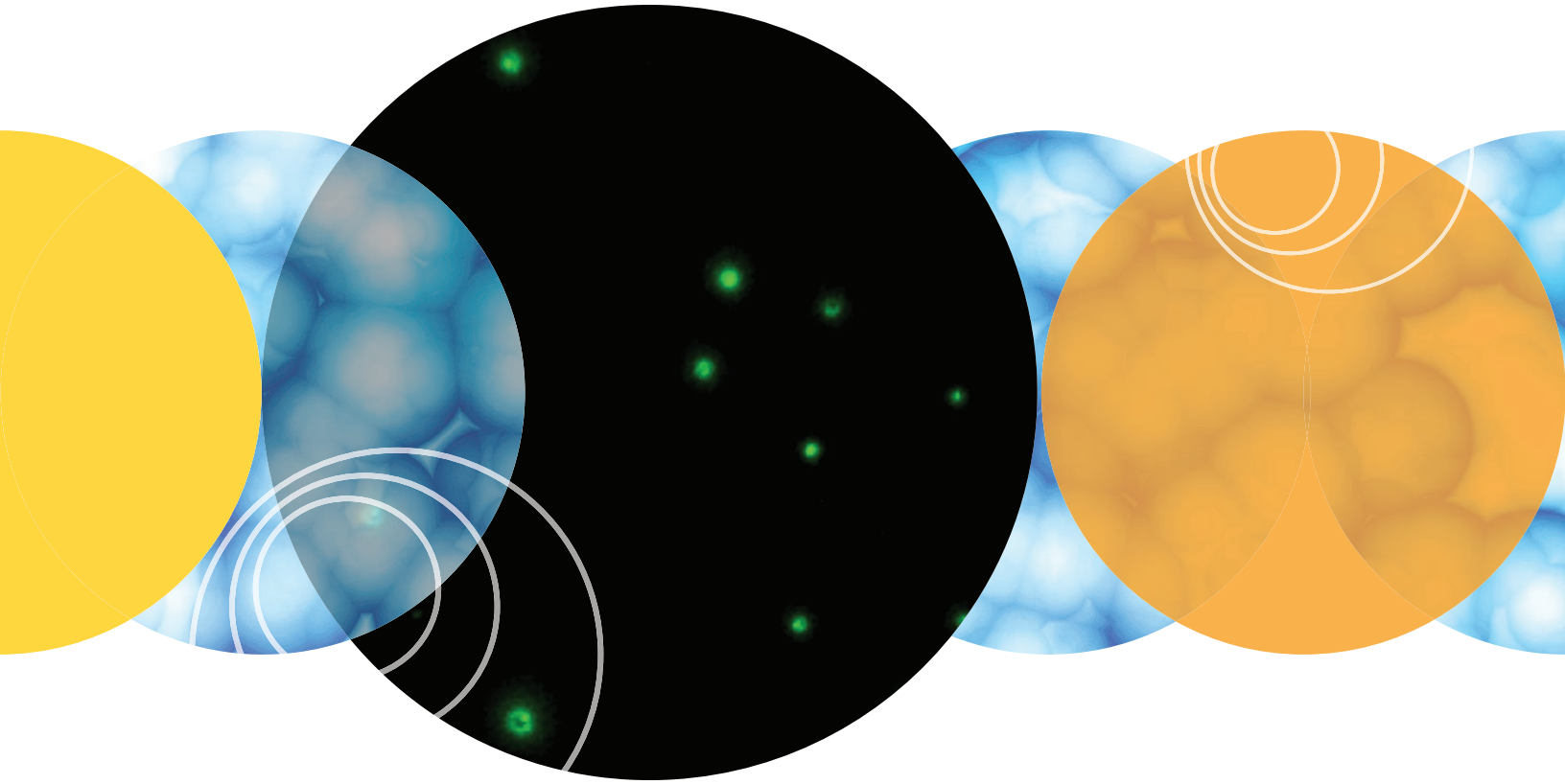
ClonePix™ 2



Redefine clone screening and selection:
transform your cell line development workflow

MOLECULAR DEVICES

KEY BENEFITS

- Screen more clones in less time
- Accurate, automatic colony picking avoids errors associated with limiting dilution
- Excellent image quality allows for screening of stable, high-producing clones
- Increased productivity of a cell line development workflow

Transform development workflows: screen more clones in less time

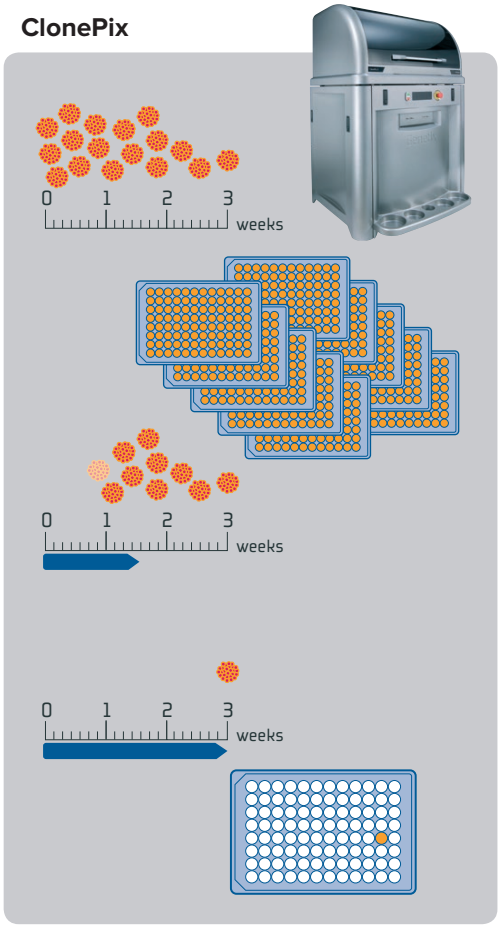
Cut cell line and antibody development times

Screen more clones in less time with the ClonePix system's fully automated workflow. Our users see major improvements both in productivity and overall costs compared to conventional techniques. Now you can reduce your timelines for monoclonal antibody generation by screening 10x more clones in weeks, not months!

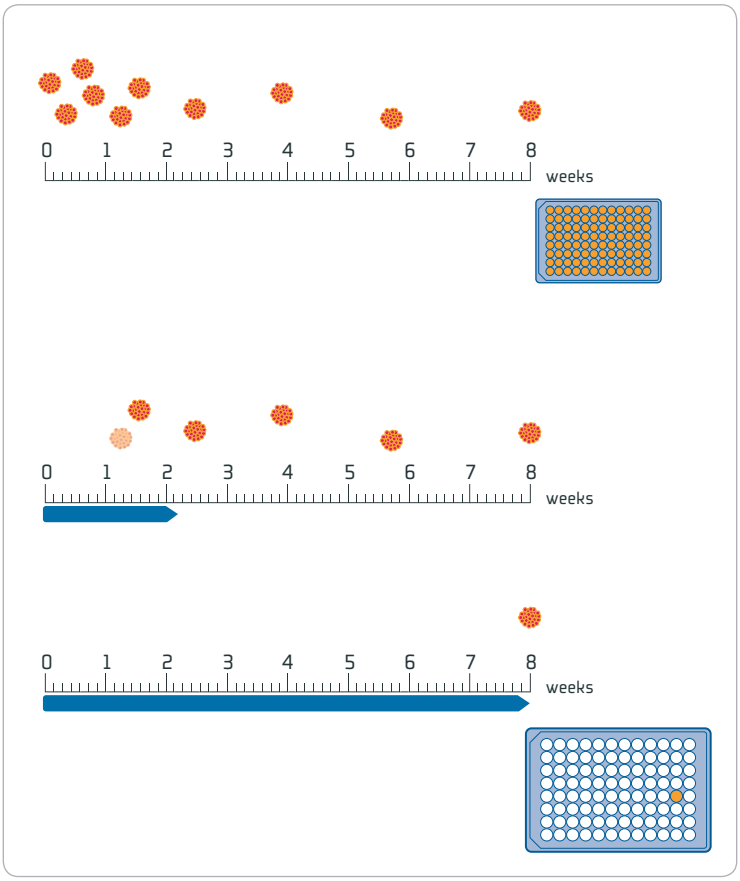
"THE CLONEPIX™ 2 HAS ALLOWED US TO COMPLETELY REDEFINE OUR PROJECT WORKFLOW AND CAPACITY."

Ben Hoffstrom, PhD, Director, Antibody Development Fred Hutchinson Cancer Research Center

ClonePix vs. Limiting Dilution



...screens 10,000 clones in 3 weeks



...screens 1,000 clones in 8 weeks

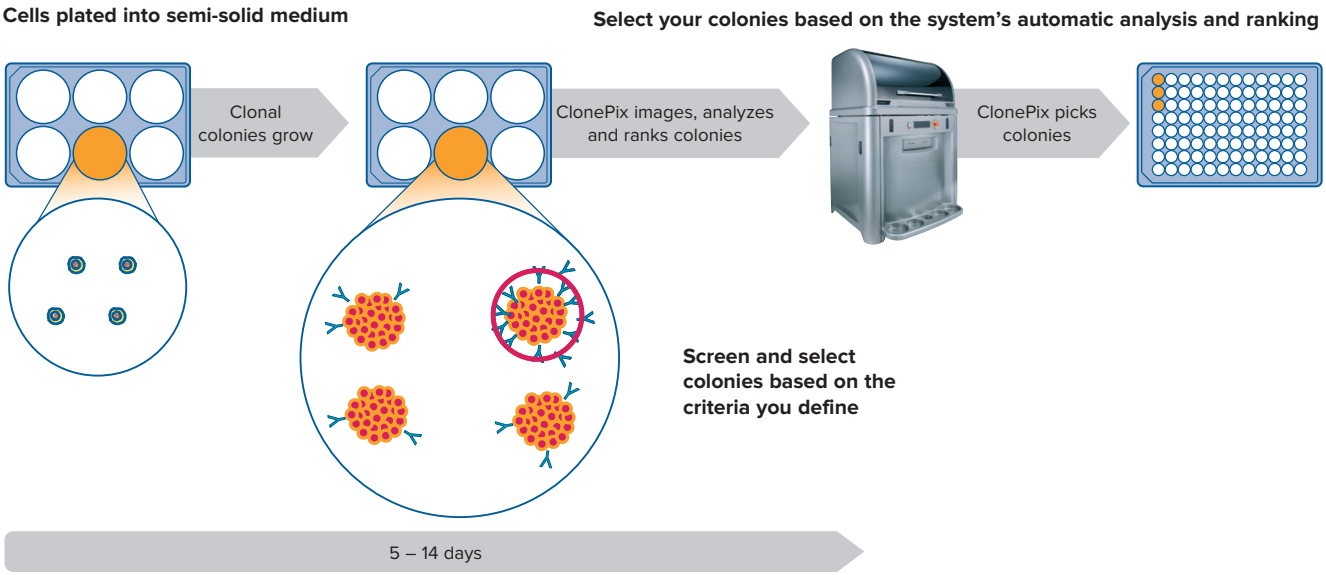
Accelerate selection of cells with optimal expression levels

- Grow your cells in semi-solid media, forming discrete clonal colonies
- Rapid screening of thousands of clones increases your probability of finding optimal producers exponentially
  - Visualize colonies for imaging and picking in white light
  - Observe expression levels using fluorescence imaging
- Choice of detection methods enables:
  - Label-free detection of clones independent of secretion
  - Detection of tagged recombinant proteins and expression markers
  - Fluorescence detection of secreted IgG, IgM, or antigen-specific mAbs from hybridomas
- Objective image analysis lets you select colonies with optimal expression levels and reject poor performers early

SUPPORTS REGULATORY REQUIREMENTS

These products do not contain components of animal origin when you need to work with human IgG: CloneMedia, CloneMatrix, Recombinant CloneDetect, and XP media.

Select and pick with more accuracy and confidence

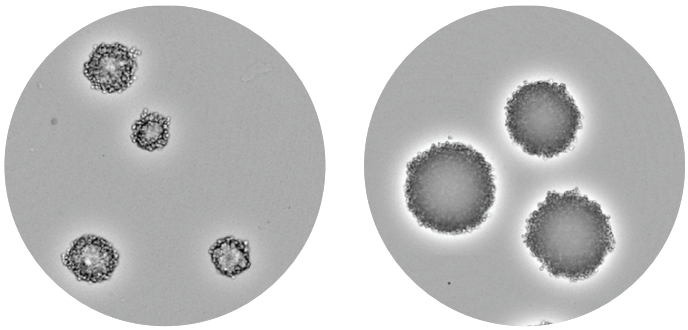


Ensure formation of discrete colonies

Utilizing semi-solid media as a cloning system is a well-established method. Growing cells in CloneMedia makes it easier to plate out large numbers of cells and ensures formation of discrete colonies—facilitating recovery of many independent clones.

Fast-track recovery of independent clones

- Choose from a range of media optimized for use with ClonePix systems
- Compatible with CHO, HEK, CHOK1SV cells and hybridomas
- Flexibility to prepare your specific media using a CloneMatrix concentrate



Colonies growing in CloneMedia. Images captured using CloneSelect Imager. **(Left)** CHO colonies, serum-free suspension-adapted, in CloneMedia CHO Growth A imaged on day 8 post-plating. **(Right)** Hybridoma colonies in CloneMedia Hybridoma/Myeloma imaged on day 7 post-plating.

"EASIER TO PLATE OUT LARGE NUMBERS OF CELLS AND TO RECOVER MANY INDEPENDENT HYBRIDOMA CLONES"

A simple, single-step technique for selecting and cloning hybridomas for the production of monoclonal antibodies (J. Immun. Methods (1982) 50, 161-171)

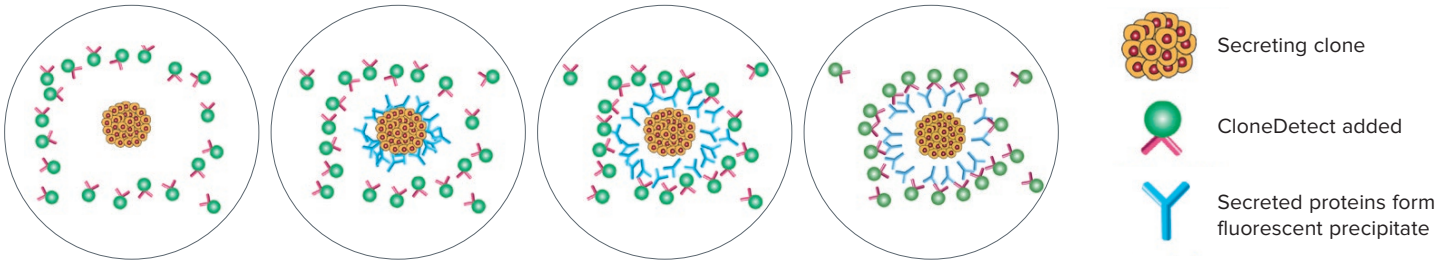
Simplify target protein detection

Detect secretion using fluorescence-based methods

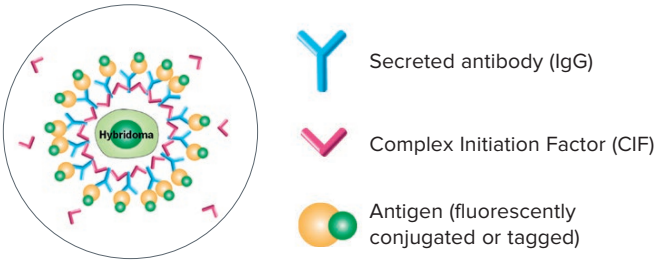
Detecting secretion using fluorescently-conjugated CloneDetect agents enables *in situ* detection of secreted antibodies.

- Select the detection agent that's right for you: CloneDetect anti-human, anti-mouse, anti-rat, and anti-sheep detection agent FITC labels
- Add detection agents directly to semi-solid media
- ClonePix systems image, analyze and rank fluorescence levels across thousands of clones in parallel

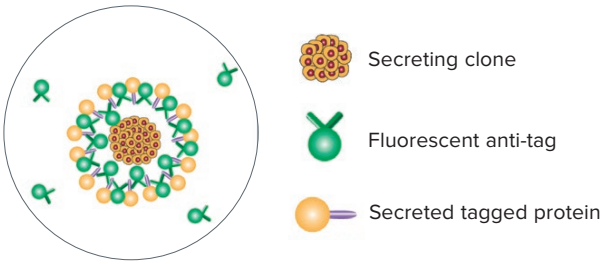
Choice of detection methods



MAb secreting IgG. For *in situ* detection of human antibodies use CloneDetect fluorescently-conjugated agents.



Antigen-specific MAbs from hybridomas. Generate precipitation using fluorescently-conjugated or tagged antigen plus complex initiation factor (CIF).

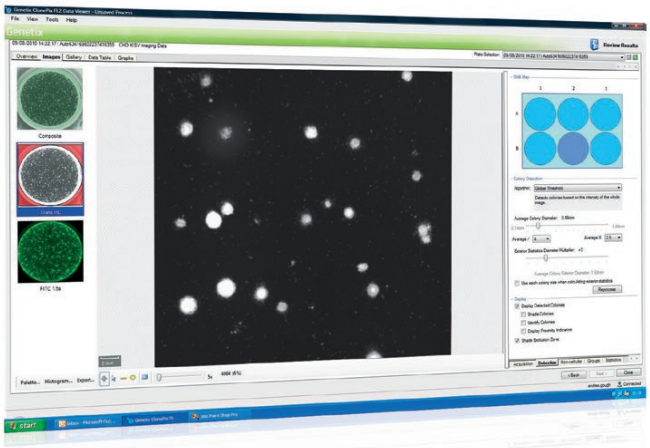


Tagged recombinant proteins. Protein construct contains epitope tag(s), e.g. His, FLAG™ or Fc using tag-specific agents.

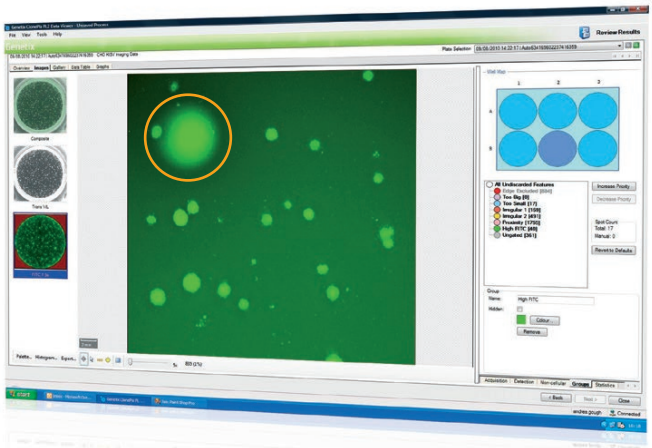
Intelligent imaging and analysis

Imaging

Automatically capture images of all your colonies in white light and secreting colonies with fluorescence.



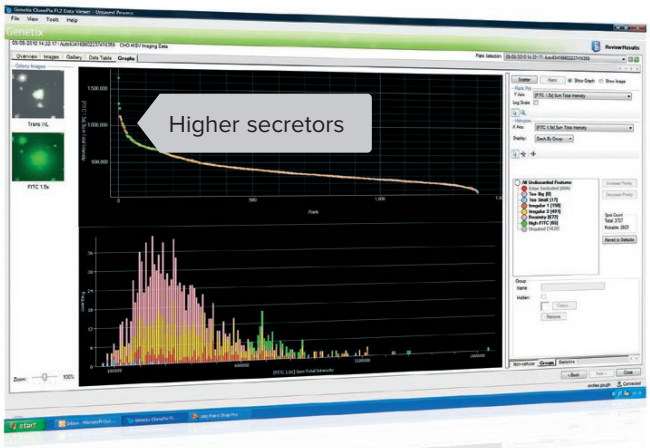
White light suggest selection of the largest clone



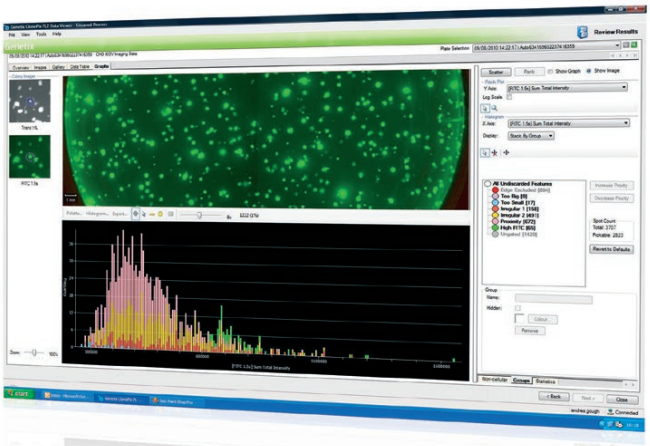
Fluorescence reveals the optimal secretor

Data analysis

- Automatically generate a 2D map of clones and their secretion levels from a series of images generated *in situ*
- Screen and select colonies based on:
  - Size, roundness, and proximity to neighbors
  - Ranking according to fluorescence levels
  - Closely placed colonies ignored via user-controlled “proximity” software setting



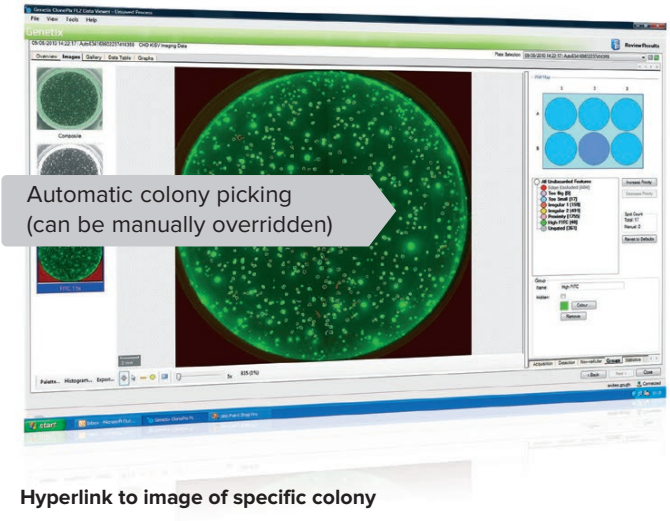
2D ranking plot



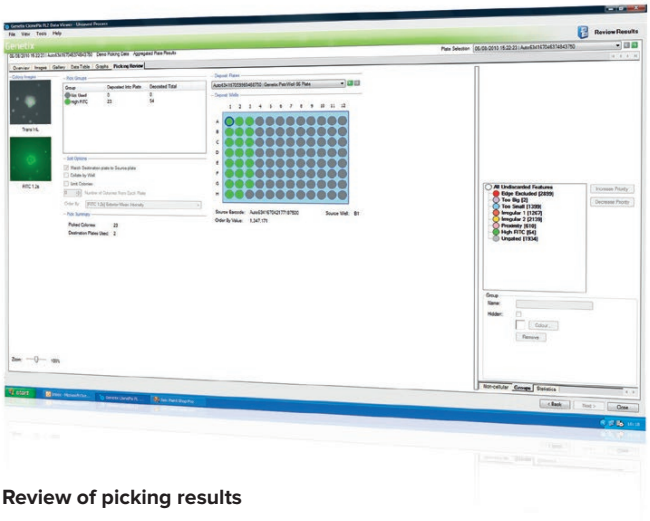
You define selection parameters, the system selects clones

Data tracking

All relevant data associated with each colony (including images taken before and after picking along with their picking coordinates) are automatically saved for review and downstream analysis.



Hyperlink to image of specific colony



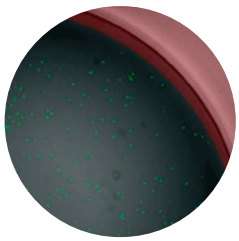
Review of picking results

Picking and transfer

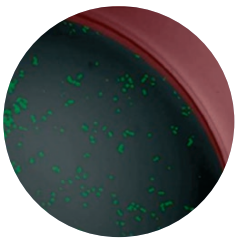
- Define the final picking list from system images and statistics
- The system selects your colonies and transfers each to a well in a 96-well destination plate for growth assay and/or expansion of clonal cells
- Optimal cell growth for cell line expansion after picking is achieved using XP Media and CloneMedia

Accurate and gentle picking

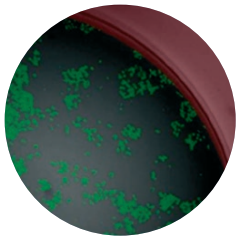
Transfer of colony to destination plates



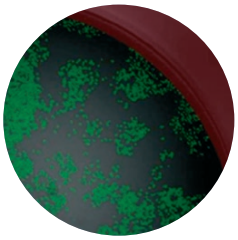
Day 0



Day 2



Day 4



Day 6

Track cell growth using CloneSelect™ Imager. Green represents software overlay applied for automatic confluence determination.

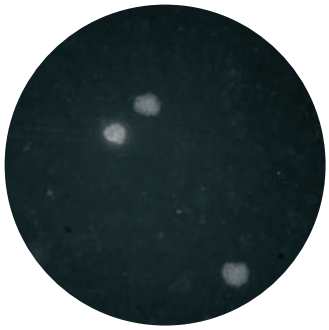
Boost generation of research antibodies

Automate screening and collection of hybridomas from fusions

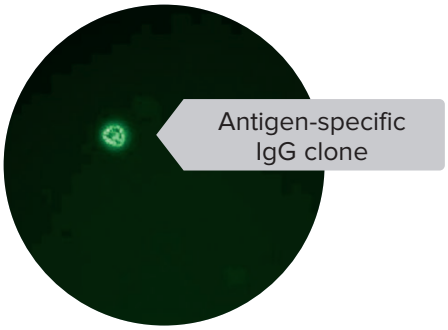
- Efficient processing lets you screen 10,000 clones and pick 1,000 HAT-selected clones per day
- Minimal loss of positive clones downstream and early elimination of negative clones upstream saves time and resources
- Screen *in situ* for antigen specificity and /or IgG secretion across a broad range of antigens: 160 kDa multimeric protein to 2.6 kDa phosphopeptide

Rapidly detect an antigen-specific IgG clone in a population of hybridoma cells

FITC conjugated 60 kDa antigen.



White light

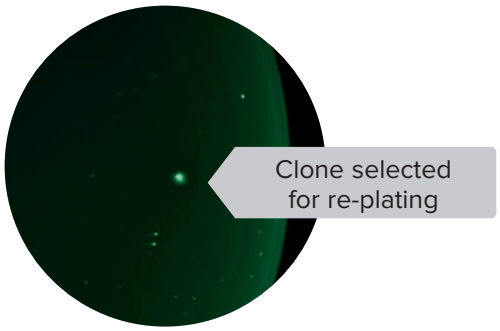


Fluorescence

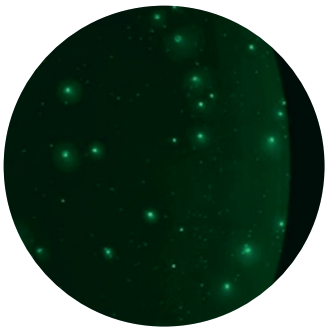
FITC filter set: Excitation 470 + / - 25nm
Emission 535 + / - 25nm

Generate product from unstable clones

Certain hybridoma cell lines show a decline in MAb productivity or are poor secretors. ClonePix system users can successfully and rapidly recover these clones, allowing them to generate product that was impossible by other means.



Cell line showing heterogeneity between sub-clones



Clones visualized after re-plating show homogeneity

"WE HAVE INCREASED OUR FUSION PRODUCTIVITY BY APPROXIMATELY 50%, WHILE DECREASING OUR TIME FROM FUSION TO STABLE CLONE BY 50%"

Dr. Robin Barbour, Prothena Corporation

Elevate productivity for biotherapeutics

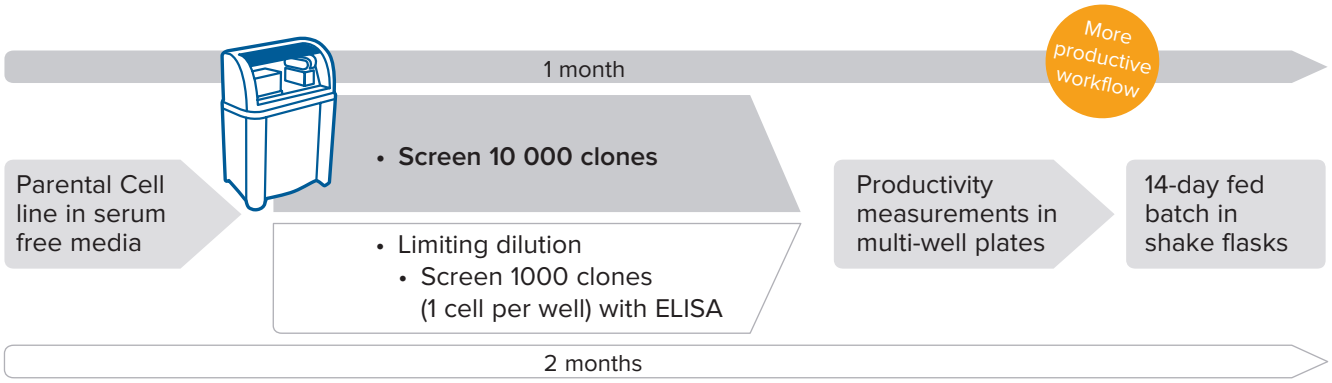
Amplify the probability of finding rare high secretors

More clones can be screened in less time on the ClonePix system than with traditional methods, letting you screen larger cell populations. MedImmune LLC compared the effectiveness of the ClonePix system to an already established limiting dilution process for their cell line development workflow. Real-world results validate the ClonePix system increased their overall productivity.

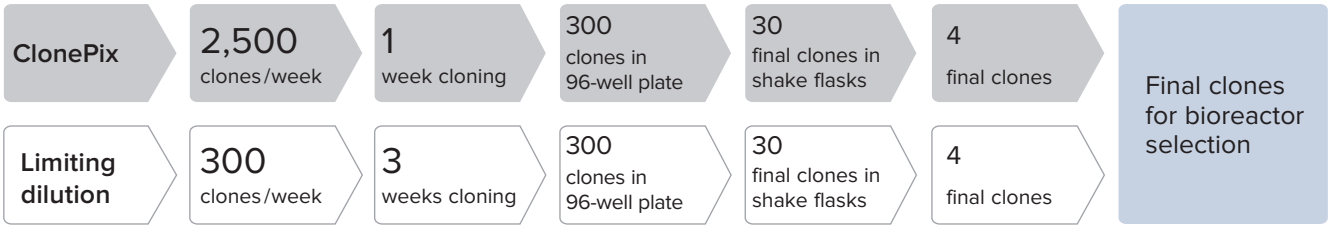
"... A POWERFUL TOOL IN CELL LINE DEVELOPMENT. THIS METHOD MAKES SELECTING THE OPTIMUM PRODUCERS FASTER AND LESS LABOR-INTENSIVE AND SHORTENS CELL LINE DEVELOPMENT TIME."

Dr. Jianguo Yang, Group Leader in Cell Line Development, MedImmune LLC

10,000 clones screened in 1 month, compared to 1,000 in 2 months with limiting dilution



In situ indication of high titer cell lines eliminated unwanted clones from further processes



High titer clones obtained even prior to process optimization (NS/0: 4-5 g/L, CHO: 5-6 g/L)

ClonePix		
Clone	**Titer (g/L)**	**qP (pcd)**
Clone 1	4.5	54.6
Clone 2	4.4	44.6
Clone 3	4.3	49.4
Clone 4	4.0	43.8

Limiting dilution		
Clone	**Titer (g/L)**	**qP (pcd)**
Clone A	2.9	32.7
Clone B	2.8	21.0
Clone C	2.7	20.9
Clone D	2.6	29.0

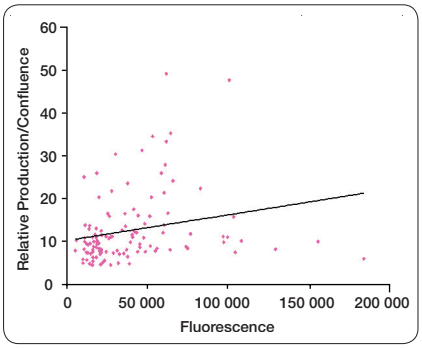
Comparison of top NS/O clones from ClonePix and limited dilution revealed almost a 2x increase qP and titer with ClonePix. Subclones from same parent.

Reveal stable clones faster

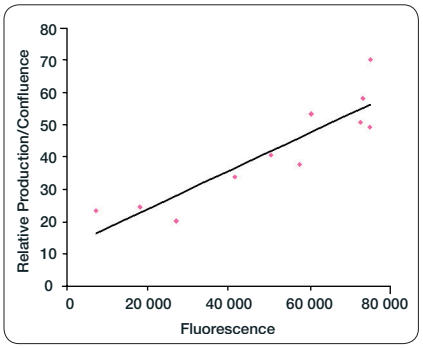
Lack of cell line stability is problematic with early stage transfectants. ClonePix systems can help you isolate stable cell line.

- Select clones and re-plate aliquots into semi-solid media
- Re-image within 4-7 days to verify and compare production rates of the daughter clones. Or, re-screen for sub-clones within 7-14 days.

Selection of stable clones by second round screening

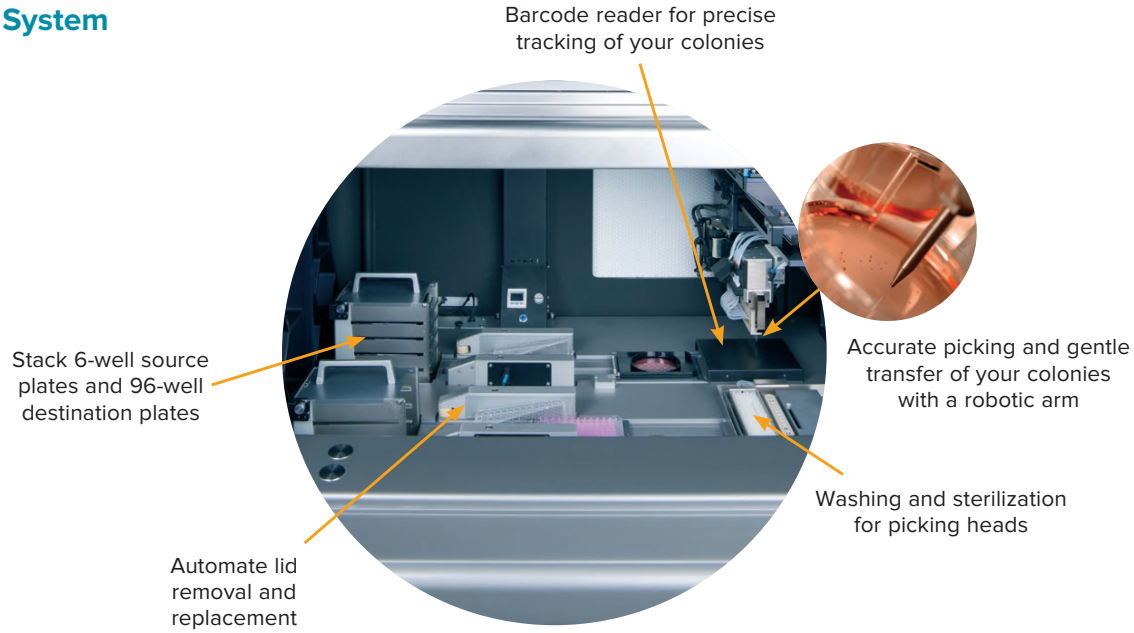


Top 2% of transfected population of suspension-adapted CHO cells collected and assayed for productivity. Confluence values determined by CloneSelect Imager.



Productivity versus fluorescence after second round screening. Confluence values determined by CloneSelect Imager.

ClonePix 2 System



System Specifications	
Imaging	
Software	Dedicated imaging software pre-installed on a high-specification PC, Microsoft Windows 7
White light imaging	• Trans-illumination for imaging low contrast colonies such as adherent monolayers or small colonies in suspension • Epi-illumination for imaging colonies as they are collected
Fluorescence imaging	Software-controlled switching between up to 5 excitation /emission filter pairs (we recommend no more than 3 filters be multiplexed for optimal performance)
Data tracking	Internal barcode reader for source and destination plates enables data tracking for each run
Camera	Integrated 16-bit cooled CCD camera
Imaging speed	6-well microplate: 5 min for 2 wavelengths (standard conditions)
Resolution	Standard: 28 micron; Maximum: 1.5 micron
Instrumentation	
Containment	Fully enclosed working environment with Class 100-type, HEPA filtration
Source plate type	PetriWell-6 plate, PetriWell-1 plate, Greiner 6 well plate, Nunc 6 well plate, Nunc OmniTray
Destination plate type	PetriWell-96 plate, Costar 96 well plate, Greiner 96 well plate, Nunc 96 well plate, Falcon 96 well plate
Source plate capacity	10 plates
Destination plate capacity	10 plates
Picking head	8 x picking pins – each pin independently controlled
Picking pin size	Diameter of picking pins is application specific – F1: suspension cells, F2: adherent cells
Picking speed	> 200 clones per hour
Wash bath	Ethanol wash bath, automatically refilled
Picking system fluids	5 L sterile water supply, 5L waste bottle
Pin drying	Proprietary halogen pin drying station
Instrument dimensions	1010 mm (width) x 900 mm (depth) x 1490 mm (height)
Instrument weight	350 kg
Compressed air specifications	
Air	Clean, oil-free with sub-micron filtration
Minimum operating pressure	6 bar (~90psi)
Minimum operating volume	80 L/min
Optional compressor specifications	
Compressor unit	Clean, oil-free compressor with sub-micron filtration
Dimensions	250 mm (width) x 600 mm (depth) x 750 mm (height)
Weight	60 kg
Minimum operating pressure	6 bar
Minimum operating volume	80 L/min
Noise level	61 dB(A)
Regulatory approval	
Compliance	CE
Quality	ISO9001:2008 certified

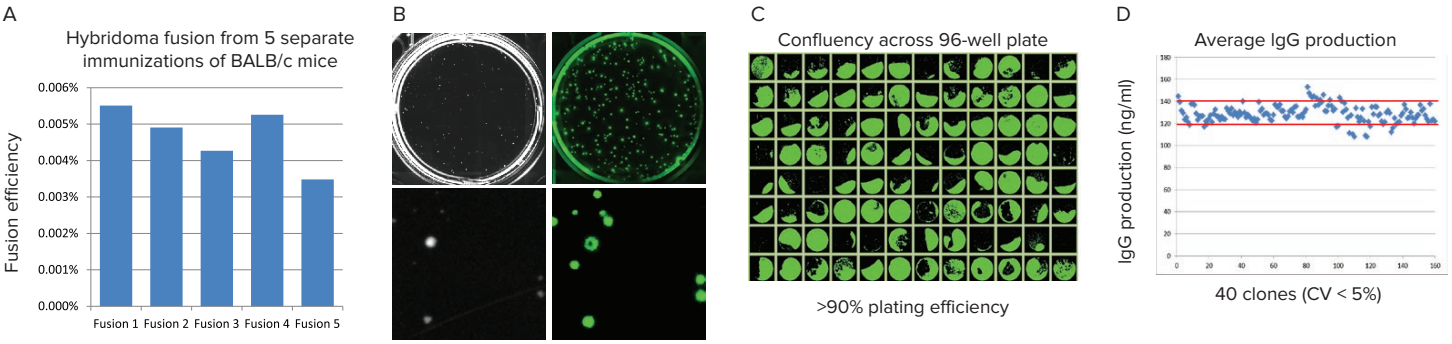
Maximize your hybridoma yield with our complete set of culture media

Culture media for every stage of hybridoma cell line generation

Stable hybridoma cell lines are critical for monoclonal antibody production. Our XP Media and CloneMedia portfolio of products is a complete solution that supports all stages of hybridoma cell line development from fusion to scale up. Optimized to support the selection and growth of hybridoma clones using our ClonePix 2 System, the kit is also compatible with other appropriate methods.

- HAT (hypoxanthine-aminopterin-thymidine) selection and cloning of hybridomas are accomplished in one step, which minimizes both time and materials required
- Not only does the semi-solid CloneMedia method eliminate the possible masking of potentially valuable slow-growing clones by fast-growing clones, but it also reduces or eliminates sub-cloning steps
- Reduce hands-on time when the workflow is combined with the ClonePix 2 System

Optimized growth conditions result in stable antibody production



Hybridomas were generated, selected, and screened using our XP Media and CloneMedia suite of hybridoma media. (A) 5 individual hybridoma fusion experiments were conducted on BALB/c mice, immunized to the same antigen, to assess reproducibility of fusions in XP Media suite of products. Fusion efficiency was calculated by dividing the number of hybridoma colonies detected on the ClonePix 2 System by the number of splenocytes grown in XP Media Pre-Fusion Myeloma Growth Medium and Hybridoma Expansion Medium (without HT). **(B)** Images of hybridomas were captured with the ClonePix 2 System in white light (left panel) and FITC (right panel), after 7 days growth, to determine growth and expression of IgGs, respectively. Colonies grown in the presence of CloneDetect were ranked according to their FITC intensity (indicating total IgG production), with the highest producers picked for further characterization. **(C)** Software detection of cell confluency, indicated by the green overlay, across a 96-well plate allowing for a quick visualization of plating efficiency. Images were collected on the CloneSelect Imager. 87 out of 96 wells grew to a confluency >5% after 7 days (the initial confluency of all wells was <1%) for a >90% plating efficiency. The real plating efficiency may be even higher because slow growing clones may be classified as non-growing using the >5% confluency criteria. **(D)** IgG production plotted per well (show in blue) with red lines indicating 2 s.d. away from the mean. Because these are stable hybridomas, we don't expect a large variation in the total amount of IgG produced per cell, which is confirmed by <5% CV across all clones tested.

The full kit contains*:

XP Media Pre-Fusion Myeloma Growth Medium and Hybridoma Expansion Medium (without HT), P/N K8862

Used to support the growth of myeloma cells before fusion. Also supports expansion of hybridoma clones. Does not contain hypoxanthine or thymidine (HT).

XP Media Hybridoma Fusion Medium, P/N K8863

Used to wash cells before fusion and during fusion process. Does not contain supplements to support growth.

XP Media Hybridoma Fusion Recovery Medium, P/N K8864

Used to promote hybridoma viability after the fusion process but before clone selection. Does not contain hypoxanthine, aminopterin, and thymidine (HAT).

CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT), P/N K8865

Used after fusion of splenocytes and myeloma cells to select and clone hybridomas in one step. Optimized for colony formation. Equally suitable for fresh fusions and for stable hybridoma cell lines.

XP Media Hybridoma Growth Medium (with HT), P/N K8866

Optimized for hybridoma expansion following clone selection and colony picking. Contains hypoxanthine and thymidine (HT) and is used to wean hybridomas off aminopterin from the selection process.

Hybridoma Polyethylene Glycol (PEG) for Cell Fusion, P/N K8868

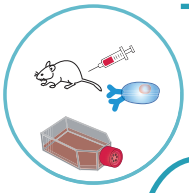
Used for the fusion of mouse splenocytes and parental myeloma cells to generate hybridomas. PEG is present as a 50% solution in DMEM.

CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (without HAT), P/N K8867

Does not contain any selection reagents. If appropriate selective reagent has been added, then the medium can be used after fusion to select and clone hybridomas in one step. Optimized for colony formation.

*Components can be ordered separately. If you are using alternate hybridoma selection methods, then CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (without HAT), P/N K8867, is available. You must add agents for hybridoma selection to this medium before use.

Accelerate your hybridoma cell line development with a complete set of platforms and culture media



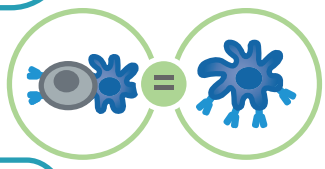
Pre-Fusion

Culture myeloma cells in pre-fusion growth medium. Prepare splenocytes for fusion.

Expansion Medium without HT (K8862), Fusion Medium (K8863)

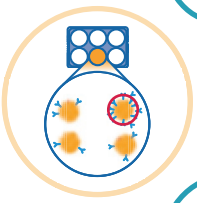
Fusion

Fuse splenocytes and myeloma cells.



Hybridoma fusion

Fusion Medium (K8863), Polyethylene Glycol (K8868), Fusion Recovery Medium (K8864)



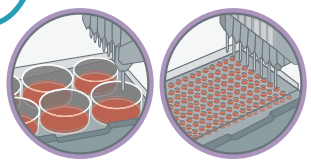
Clone Selection

Clone screening and selection in semi-solid medium.

ClonePix 2 System, CloneDetect, CloneMedia Selection and Cloning Medium with and without HAT (K8865, K8867)

Clone Picking

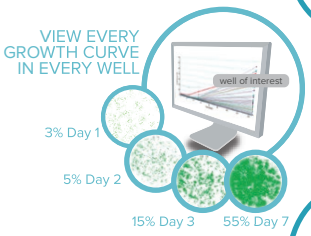
Pick selected, high-producing clones from semi-solid medium and transfer to liquid medium.



Accurate and gentle picking

Transfer of colony to destination plates

ClonePix 2 System, Growth Medium with HT (K8866)



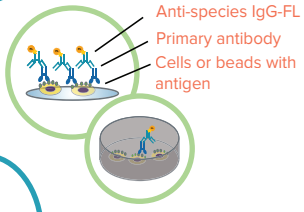
Clone Stability

Monitor growth of picked clones.

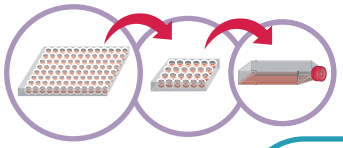
CloneSelect Imager

Functional Characterization

Perform secondary screening on clone supernatants (e.g. ELISA).



SpectraMax i3x or other Molecular Devices microplate readers



Scale-Up and Wean

Scale up clones producing desired antibodies and wean off HT selection.

Growth Medium with HT (K8866) and Expansion Medium without HT (K8862) at 1:1 ratio

Expansion

Expand clones producing desired antibodies.

Expansion Medium without HT (K8862)

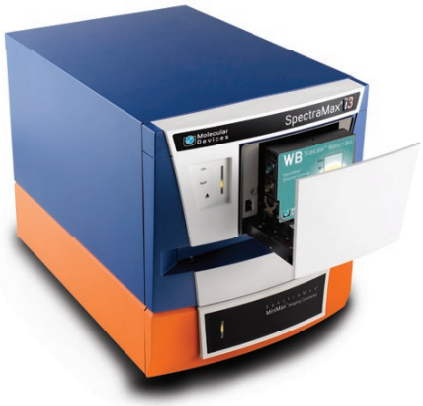
Accelerate cell line development with a range of Molecular Devices platforms

QPix 400 Series Microbial Colony Picker

The QPix™ 400 series of microbial colony pickers offer you the unique option to simultaneously detect colonies and quantify fluorescent markers in a pre-screening step before picking. Our QPix systems are used worldwide in over 600 installations in research institutes, biotech, and pharmaceutical companies. QPix robotics developed a famous reputation for reliability and accuracy in sequencing centers during the Human Genome project.

CloneSelect Imager

With high quality imaging and intelligent image analysis, CloneSelect™ Imager allows you to assess cell confluence objectively and quantitatively. Cell growth is viewed and tracked in every well in every plate.



SpectraMax i3x Multi-Mode Microplate Reader

The SpectraMax® i3x multi-Mode microplate reader measures spectral-based absorbance, fluorescence, and luminescence with the added functionality of modular upgrades for western blot, imaging, and fast kinetics with injectors.

Unrivaled solutions

Our products empower you with unrivaled solutions that utilize imaging and intelligent image analysis to support basic research, pharmaceutical and biotherapeutic development. We are continually establishing industry standards in areas such as picking microbial colonies for genomic studies or screening and selection of mammalian cell lines. Our systems use imaging platforms to monitor cell growth, evaluate cellular responses and quantify protein production. We bring you expertise in robotics, cell and molecular biology, image analysis and interpretation supported by a strong IP portfolio, and are truly committed to the continual development of innovative solutions for life science applications.

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