Scan It. Spot It. Prove It.

The Efficient Way To Monoclonality.

SYNENTEC Innovation Put To Work

HighReSOLUTIONS+

A Tiny Cell Makes All The Difference

Clone Imaging and Proof of Monoclonality

To verify that a cell line that was bred for the production of a pharmaceutical protein is clonal, it is imperative that it originated from a single cell.

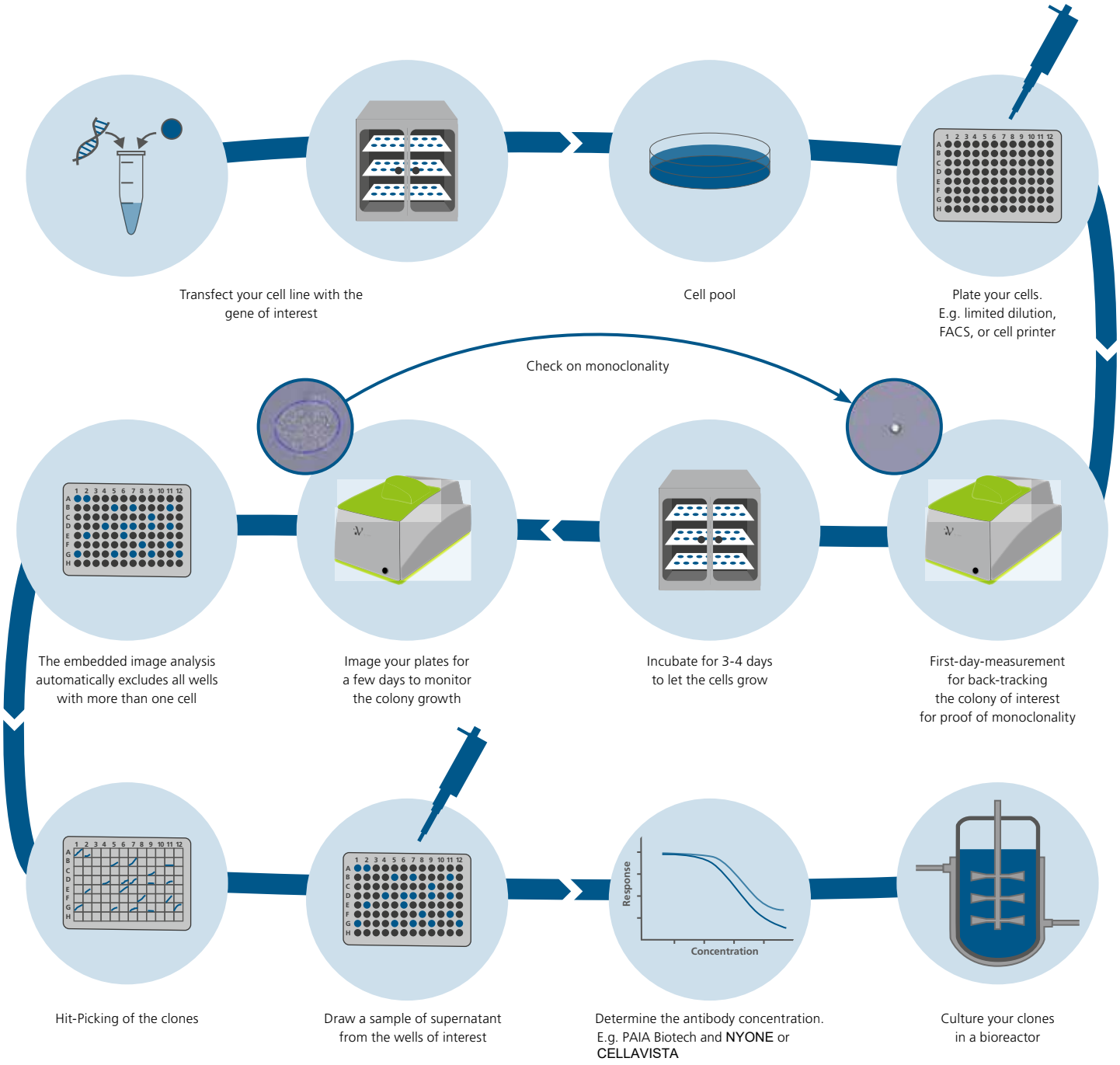
Manual methods of single cell identification are lengthy, cumbersome and often faulty. After all, these methods involve humans looking down microscopes. Therefore, it is becoming increasingly common for cell line developers to demand highly resolved image based evidence of a monoclonal cell line.

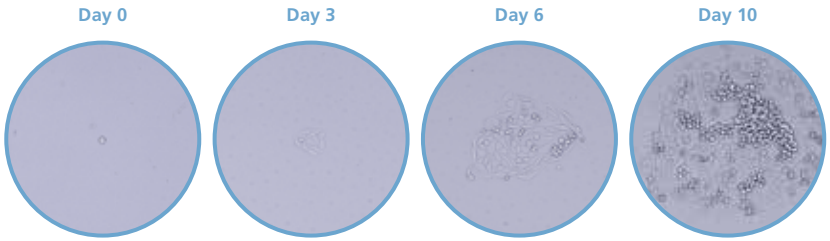
In this Note you will learn more about the innovative methods and technologies that SYNENTEC provides, to help you confidently iden-tify single cells on the day of seeding (FASCC).

Resolution Matters

Many automated imaging systems simply do not have the resolution necessary to provide a picture quality that allows the observer to decide whether it is a single cell, a doublet or even debris on the micro plate he is looking at.

With a resolution below 1 micron/pixel, our automated cell imagers **CELLAVISTA** and **NYONE** provide the highest picture quality amongst cell imaging Systems on the market.
The exceptionally high resolution of both devices allows complete traceability of a cell line and provides unequivocal evidence about its monoclonality. This is especially important because a wrong decision based on a unclear/blurry image can cause great economic losses and could delay the development process for months, since it might involve the need for a lengthy second round of sub-cloning.


Transfect your cell line with the gene of interest
Cell pool
Plate your cells.
E.g. limited dilution, FACS, or cell printer
Check on monoclonality
First-day-measurement for back-tracking the colony of interest for proof of monoclonality
Incubate for 3-4 days to let the cells grow
Image your plates for a few days to monitor the colony growth
The embedded image analysis automatically excludes all wells with more than one cell
Hit-Picking of the clones
Draw a sample of supernatant from the wells of interest
Response
Concentration
Determine the antibody concentration.
E.g. PAIA Biotech and NYONE or CELLAVISTA
Culture your clones in a bioreactor



Single Cell Cloning (SCC)

Can you afford not to be sure?

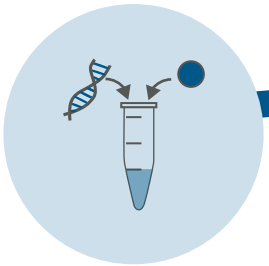
The aim of SCC is to identify and isolate wells where a single cell was seeded and to monitor its growth rate. Due to the fact that a single cell is a tiny object and may look different depending on the cell line, status of the cell cycle etc. it is very important to track your colony back to the seeding day and to display the situation in the best achievable resolution on that particular day. This will then provide you with sufficient proof that your growing colony in fact derived from a single cell or from a doublet.

On the first day, just after the seeding of the cells, the entire plate is scanned in bright field. A seamless stitching of all images inside the well will guarantee that no area for the entire well is missed. The image analysis is turned off not to pick up too many debris or well edge artefacts. After scanning all plates, they will be placed inside an incubator for the next 3-4 days. During this time, the cells will divide or split into a little colony. The plate will be measured again and all colonies are picked up by our software.
An automated filter algorithm will exclude all wells containing more than just a single colony. This will reduce the amount of data to be stored to a minimum and will speed up the scanning time for the next run. After finishing your culture run or during the measurements the operator can track back to the first day or seeding day to confirm monoclonality.

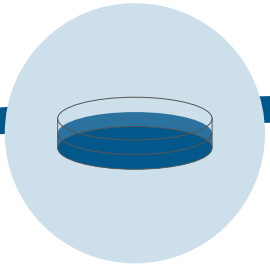
Take an even closer look

HighRes Single Cell Cloning

The difference between HighRes SCC and conventional SCC is that the scan on the seeding day is performed with high resolving power and the colony outgrowth of the following days in a lower resolution. Otherwise, the procedure is the same as outlined above.

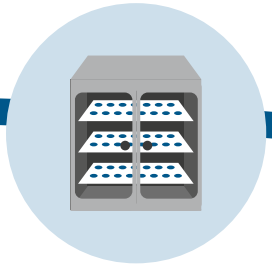


Transfect your cell line



Cell pool

Stain with non-toxic,
animal free fluorescence dye e.g.
Calcein-AM



Incubate for 20 minutes

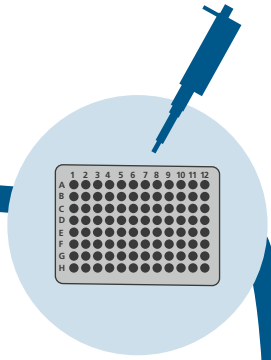


Plate your cells.
E.g. limited dilution,
FACS, or cell printer

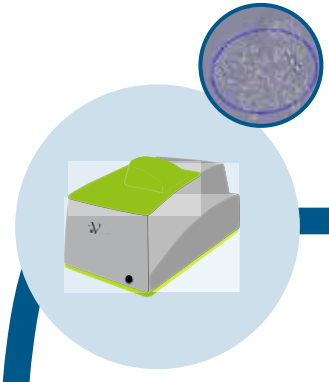
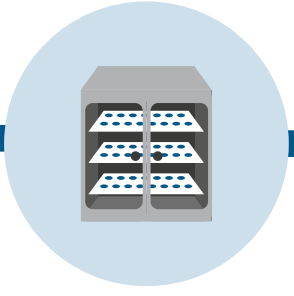
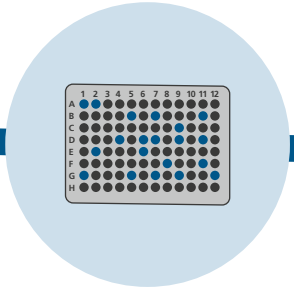


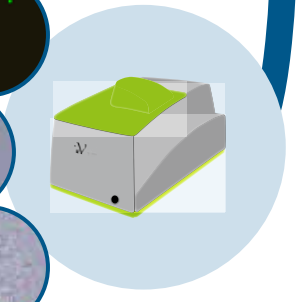
Image your plates for
a few days to monitor
the colony growth



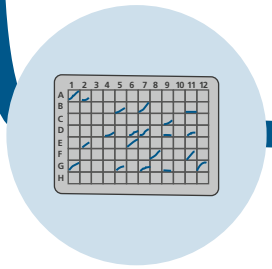
Incubate for 3-4 days
to let the cells grow.



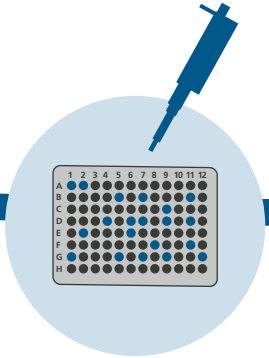
The embedded image analysis
automatically excludes all wells with
more than one cell



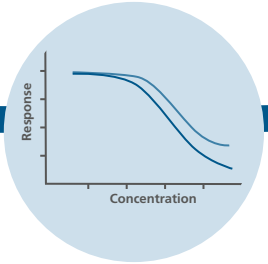
3 phase scanning
Pick up fluo cells (1)
Confirm with high resolution,
exclude doubles (2)
Start confluence measurement (3)



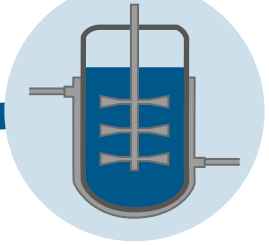
Hit-Picking of the clones



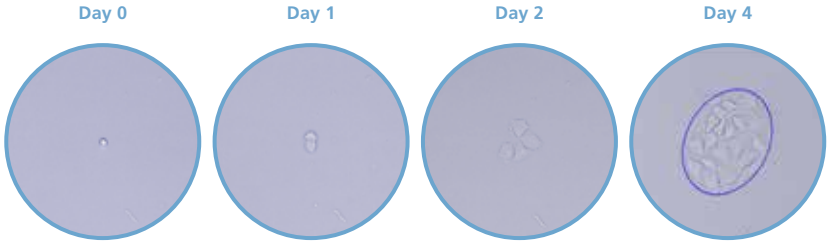
Draw a sample of supernatant
from the wells of interest



Determine the antibody concentration.
E.g. PAIA Biotech and NYONE or
CELLAVISTA



Culture your clones
in a bioreactor



Fluorescence Activated Single Cell Cloning (FASCC)

Next Level Certitude

Since throughput becomes more and more a bottleneck in cell line development, common cell imaging systems handle this issue by using a low magnified lens to enlarge the field of view or bin camera pixels to speed up the download of each image frame. This procedure results in less overhead per well but unfortunately lowers the image quality/resolution (information/content of the image). Due to this fact SYNENTEC developed a method that utilizes throughput as well as higher image resolution, which is needed for an unambiguous proof on monoclonality: FASCC – Fluorescence Activated Single Cell Cloning.

Our advantage over the traditional single cell cloning method arises from our capability to automatically switch between hardware magnifications (lenses) to combine speed with higher resolution. In FASCC we utilize two different resolutions and an additional bright field channel as a further option.

With regard to the throughput, a fast pre-scan will be issued in the first phase of the FASCC procedure. The pre-scan is operating in a low resolved high speed scanning mode and will detect the fluorescence signal of Calcein-AM or Cell Tracker stained cells. Both dyes are non-toxic and will disappear within 3-4 hours. The goal of Cell Tracker and Calcein-AM is an enzymatic reaction, which induces both dyes to emit fluorescence light to indicate a viable organism. This pre-scan will usually take below 2 minutes for an entire 384-well-plate.

After the entire well scan the embedded image analysis tool will exclude all wells seeded with more than just one cell or any number of cell count the user sets up. The subsequent post-scan or post-HighRes scan will pick up all pre-scan results and proceed by centering all regions of interest (e.g. single cells) right in the middle of each image. The images taken at the post-scan are now highly resolved and are offering a detailed view inside of each cell. This unique tool presents an unambiguous proof of monoclonality without guesses or doubts.

CELLAVISTA

Your Next Employee Of The Month

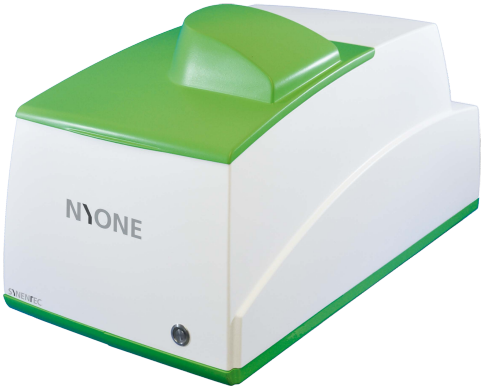
Commonly used in automated and high throughput environments, CELLAVISTA is able to image about 250 plates a day. Equipped with 5 different magnifications, CELLAVISTA is able to image organisms like zebrafish as well as cell fragments inside of a nuclei. The wide range of achievable resolutions makes this an ideal tool for research as well as high throughput screening.
CELLAVISTA is set up with 6 different excitation sources and 6 different emission channels with a chromatic spectrum from UV (Hoechst/DAPI) to RED (Alexa647)

Your benefits

- Run a complete scan of a 96-well micro plate within 2 minutes.
- Achieve brilliant image quality using specialized optics and laser-based auto focus mechanisms.
- Perform automated multi processor image analysis and acquisition at the same time utilizing the full potential of the pc.
- Benefit from comprehensive graphical output, including whole-plate overviews, histograms, scatter plots and time charts.
- Obtain complete documentation of cellular growth.
- Save time and resources compared to conventional microscopy.
- Obtain reliable results using non-invasive techniques.
- Use CELLAVISTA System for all your cellular assays, bead assays, etc.
- Automated data and image export function

Typical Applications

- Single cell cloning
- Cell confluence
- Suspension cell count
- FACS seeding efficiency control
- Cell nuclei count and characterization
- Transfection efficiency
- Microplate quality control
- Viral plaque assays
- Fluorescent cell profiling
- Cell death and apoptosis assays



NYONE

A *My* Perspective For Your Research

NYONE is a fully automated cell imager designed to simplify the screening, detection and classification of cells as an integral step of your research, development and production process. Mechanical robustness, optical quality and functionality are balanced in a way that you will soon recognize NYONE as a reliable member of your team. And its small footprint requires only minimal space on your bench.

NYONE is equipped with 3 different lenses, achieving resolutions down to 360 nm/pixel, 3-4 excitaion sources (UV to RED) and up to 6 different emission filters. All operating procedures are fully automated and can be embedded into a completely automated enviroment.

Your benefits

- Accurate measurements using non-invasive brightfield and fluorescence imaging
- Adherent and suspension cells
- Complete documentation of cellular growth
- Excellent well edge illumination of microplates
- Ultrafast electronic switched excitation sources – less than 5 ms switching time
- Runs a complete scan of a 96-well microplate in about 5 minutes.
- Intuitive workflow to setup your experiment.
- Ultrafast filter wheel – 40ms position changing time. Well suited for FRET assays
- Achieves extraordinary image quality using specifically designed optic and laser autofocus mechanisms.
- Performs automated high-quality image analysis in parallel with image acquisition.
- Uses the flexibility of automated image analysis to improve the handling of your cellular assays.
- Automated data and image export function

Typical Applications

- Single Cell Cloning
- FASCC
- Confluence assays
- Cell counting
- Cell proliferation assay
- Live/Dead assay
- Apoptosis assay
- Viability
- And many more to come

»Take A Quick Cellfie«

Contact us

Regardless if you need application support or technical service for your system, SYNENTEC will reply to your request within 24 hours after receipt of enquiry. The most convenient and quickest way to get an overview of your request is by using **TeamViewer**, a remote control tool to be installed on the systems computer which will give us an impression what is at issue.

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Let us make your research more efficient.