

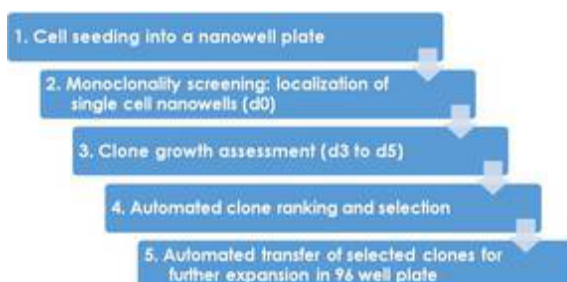
Novel CellCelector one-round single cell cloning method with an integrated monoclonality proof

The ALS CellCelector™ single cell and colony picking platform can now be used for high-throughput single cell cloning allowing fast generation of clonal production cell lines with one cloning round while providing robust in-process image-verified monoclonality proof. With **integrated clonality** and **clone viability assessments** as well as **high outgrowth rate** after clone transfer to 96 or 384 well plates the CellCelector single cell cloning technology represents an advantageous alternative to limiting dilution, FACS sorting or single cell printing techniques. The method has been developed in collaboration with [ProBioGen AG](#).



CellCelector nanowell-based single cell cloning workflow

The method uses standard-format 24 well CellCelector Nanowell plates (sold by ALS) with thousands of tiny nanowells on the bottom of each well.



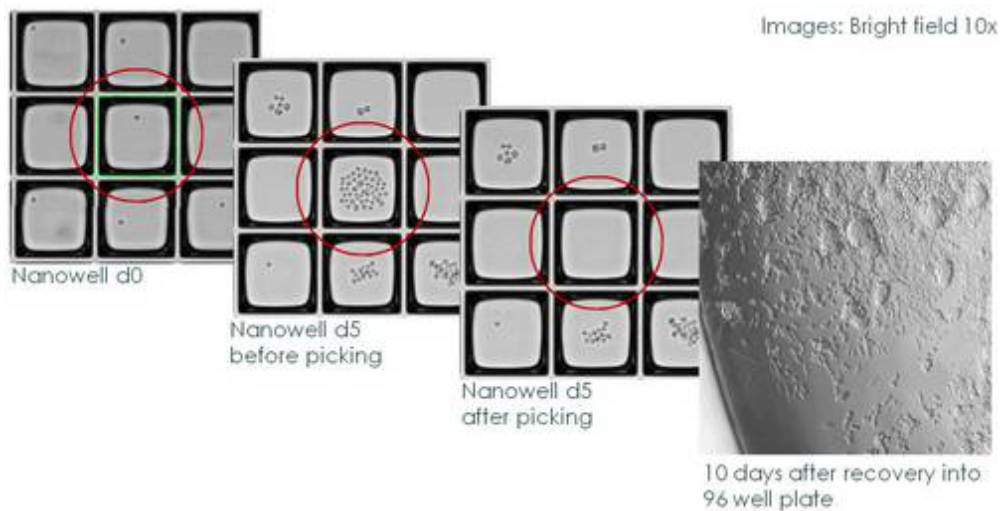
Cell seeding is performed in a way similar to that used for conventional cell culture plates. Cells are captured randomly by nanowells following Poisson distribution.

Bright field scanning of seeded wells followed by the automated identification of all single cell nanowells provides a robust and documented image-based monoclonality proof.

Depending on number of seeded cells 400 to 600 nanowells are usually occupied by single cells. If necessary several plate wells can be seeded in parallel allowing up to ~14,000 single cell nanowells per cloning experiment.

After monoclonality scan cells are let to grow 3 to 5 days in an incubator to reach 20 to 50 cells per clone. Contrary to a traditional methylcellulose-based approach the CellCelector nanowell-based method allows the colony growth in liquid media with the clones situated just 200 μm from each other (center-to-center) while efficiently prevented from mixing by nanowell walls.

After growth the nanowell plate is scanned again and viable clones resulting from single cells are automatically selected and transferred to 96 or 384 well plates for further growth and productivity assessment.



Automated monoclonality assessment: robust single cell detection in nanowells

In a conventional single cell cloning paradigm single cells are seeded in 96 well plates making difficult reliable automated single cell detection in bright field as cells are often settled at the very edge of the well. Thus the monoclonality status of a given clone at day 0 is usually checked manually and retroactively once the clone has grown. Instead of looking for a single cell within a large well bottom area which is >105 times larger than the surface occupied by a cell, in CellCelector approach the cells are clearly visible in 200 μm large nanowells and can be reliably software-detected just after seeding even when they touch the nanowell border.



CellCelector HT-NIC method advantages

- Single cloning round resulting in faster cell line development (by 5 to 9 weeks)
- Integrated image-verified monoclonality proof and viability assessment
- High image quality for robust automated label-free single cell detection
- Selection of clones by outgrowth and/or by fluorescence
- 100% selective clone recovery without cross contamination
- High outgrowth efficiency both in nanowells (cell grow together) and after clone transfer
- Compatibility with various cell lines (CHO, HEK293, NS0, etc.)
- Significant cost savings on consumables, media and incubator storage space: just one nanowell plate per cloning experiment
- Easy cell density or media optimization in parallel up to 24 samples within one plate
- Easy-to-change disposable single-use capillaries
- Simple to use and intuitive software with integrated traceability
- No routine maintenance necessary

Controlled sterile environment

CellCelector can be placed within a standard biological safety cabinet for sterility or installed in [ALS Incubator FlowBox™](#) with laminar flow, temperature, humidity and CO₂ control.

Recommended configuration for single cell cloning

- CellCelector with single cell picking module, 10x objective and standard deck tray
- Set of glass capillaries and CellCelector Nanowell plates allowing 30 or more cloning experiments
- Optional: 2 channel (FITC, TRITC or compatible) fluorescence module for methodology green-red tests or fluorescence-based assays.
- Optional: Biosafety cabinet class II customized for CellCelector™ or ALS FlowBox™ incubator.

CellCelector platform having open architecture other modules, accessories and consumables can be added to fit other applications (e.g. semi-solid media cloning, CRISPR single cell sorting/cloning, molecular analysis etc.)

[Please contact us for more information about CellCelector cloning method or request a quote and/or demo.](#)