



Automated clonal selection of high-producing hybridoma colonies from methylcellulose-based, semi-solid medium using the cell separation robot CellCelector™

Monoclonal antibodies are important tools in research, diagnostics and therapy. Thus it is necessary to speed up the selection of high-producing hybridoma cell lines as it is a bottleneck in process development. Using the CellCelector™ to automatically detect and harvest *in situ* screened hybridoma colonies from semi-solid medium provides a rapid, simple and cost-effective alternative for faster cloning and recloning of hybridoma cell lines.

Technology

The CellCelector™ (fig. 1a) is a robot for automated cell harvest. The patented system consists of an inverted microscope (1) equipped with a motorized stage (2) and CCD camera (3), an exchangeable robotic arm (4) as main functional tool and a deck tray for disposable tips (5), capillaries and destination plates (6). The imaging software (fig. 2) enables detection of cells by predefined spectral and morphological parameters. After the culture vessel is scanned, harvest and documentation can be done automatically, based on the generated particle list. The harvest tools provide the collection of adherent or suspension cells as well as colonies in semi-solid media via mechanically detachment and aspiration. Special polished metal capillaries (fig. 1b) are used to scrape off adherent cells via a crosswise movement of the motorized stage. The scrape tool is also suitable for picking colonies from semi-solid medium. The parameters for the harvest can be fine-tuned for the users special application.

Automated detection and harvest of *in situ* screened hybridoma colonies from semi-solid medium

Monoclonal antibodies have a broad applicability. For research or diagnostics antibodies are used as labeling and detection molecules in flow cytometry, immunohistochemistry, western blotting or enzyme-linked immunosorbent assay (ELISA). Used as therapeutic proteins they provide the treatment of various cancers and autoimmune diseases.

Once a hybridoma clone is isolated and cultured, regularly recloning is necessary due to the tendency to develop heterogeneity in the cell line.

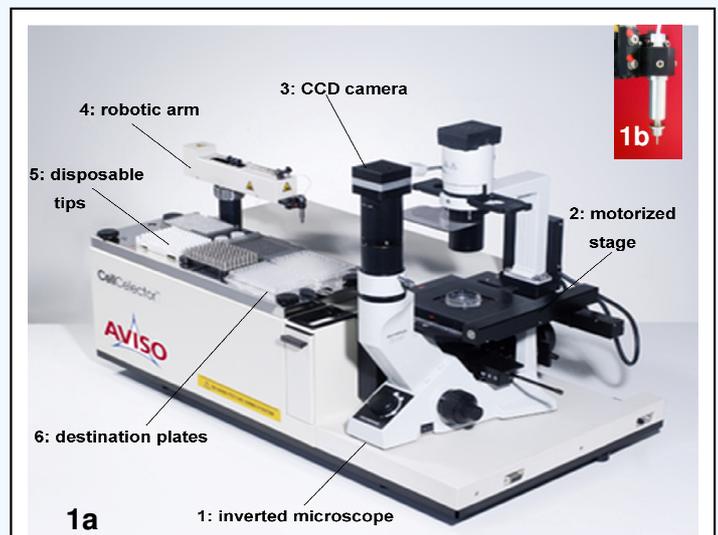


Figure 1: a: Assembly of AVISO's CellCelector™, robot for automated cell harvest, **b:** Scrape tool, harvest tool for adherent cells and colonies from semi-solid medium

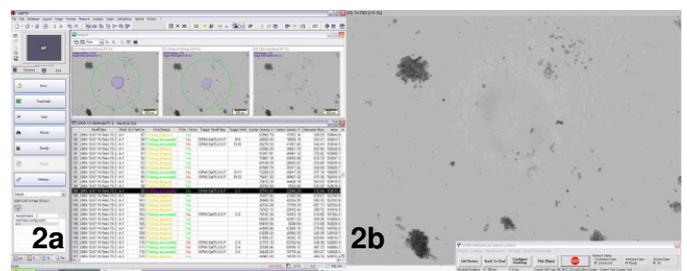


Figure 2: Screenshot of the CellCelector™'s control and imaging software, **a:** Picking list and automatically documented snapshots before and after picking of colonies, **b:** hybridoma colonies in methylcellulose medium on live-image

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In higher passages a decrease in productivity and increase in growth rate is observed. If the cell line is not re-cloned more slowly growing high-producers are probably overgrown by low- or non-producing hybridomas. Therefore, fast and simple methods for discovery and maintenance of high-producing hybridoma cell lines are needed. The establishment of high-producing hybridoma cell lines by traditional cloning and screening procedures is a time-consuming process and includes hazardous aspects [1]. The combination of a cloning procedure in semi-solid medium [2], a modified fluorescent labeling technique [3,4] and high-throughput automation using the CellCelector™ provides a time-saving and reliable alternative. For (re)cloning the cells are seeded in methylcellulose-based, semi-solid (HAT-selection) medium containing commonly available fluorochrome-conjugated anti-IgG antibodies for the detection of IgG secreted by colonies formed (fig. 3a). A ring of fluorescent precipitates around positive colonies is generated (fig. 3b). After 7-10 d of incubation the dish is transferred to the CellCelector™. In bright-field detected colonies are checked for fluorescence, sorted by quality factor (i.e. area of fluorescence over area of colony in bright-field), automatically picked under bright-field microscopic observation and transferred to the destination well plate with transfer rates of 94 % ± 5.5 %. The whole process is automatically documented (fig. 4). A monoclonal transfer of colonies was assured with 93 % ± 2.6 %. The survival rate of successful transferred colonies of 89 % ± 9.7 % was comparable to the manual picking process. Since all relevant clones are harvested no further incubation of the donor dish is necessary.

After the detection antibodies were washed out by passaging the clones, supernatants of the cultures were analyzed for produced antibodies by ELISA. The high productivity of harvested clones and thus the efficiency of the detection method could be confirmed (data not shown). Related to the manual picking procedure from semi-solid medium the alternative method is found to be superior by the following aspects:

- ✓ Incubation time of donor dishes prior to harvesting is reduced.
- ✓ Colonies are harvested under culture conditions (37 °C, 5 % CO₂). Both pH and viscosity of the methylcellulose based medium are maintained.
- ✓ Colonies are harvested automatically and under microscopic observation.
- ✓ All relevant clones are harvested. No further incubation of the donor dish is necessary.
- ✓ Monoclonality of picked colonies is assured. No further rounds of recloning are required.
- ✓ The amount of harvested clones and thus screening efforts are reduced by sorting out low- and non-producers *in situ*.
- ✓ Heterogeneity in monoclonal grown colonies is visualized.
- ✓ Documentation allows the backtracking of subclones to the colony in the donor dish.

One should notice that three clone-specific objectives should be tested before starting: growth behavior in non-supplemented semi-solid medium, specificity of antibody detection and parameters for the harvest.

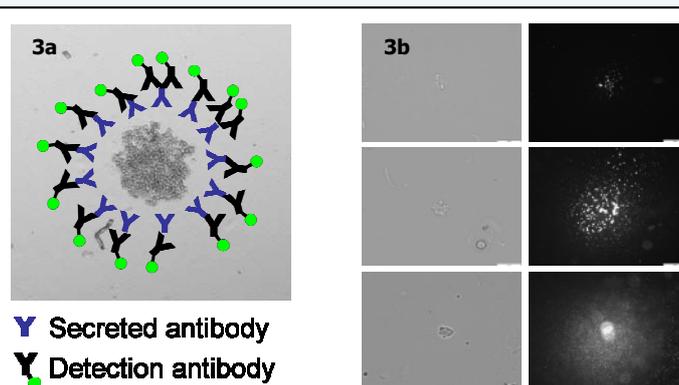


Figure 3: a: Scheme of labeling method in semi-solid medium: secreted antibodies are precipitated and visualized by fluorochrome-conjugated detection antibodies. A ring of fluorescent precipitates is formed around positive colonies. b: Colonies of a rabbit hybridoma clone in semi-solid medium labeled with FITC-conjugated anti-rabbit-IgG antibodies; left in bright-field, right under fluorescence. Distinct detection of heterogeneity in productivity of single clones shows specificity and efficiency of the labeling technique. No unspecific stained artifacts were noticed. Scale bars = 100 µm

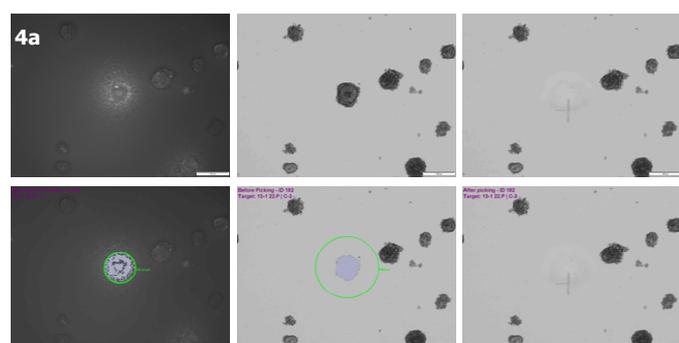
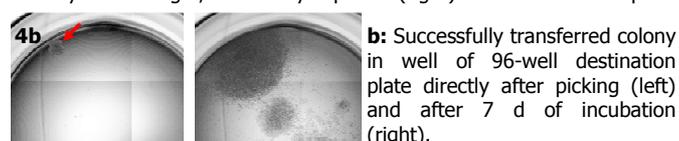


Figure 4: a: Automatically documented pictures during the harvesting process: Upper images show the appearance of labeled colonies under fluorescence (left), before (middle) and after picking (right). Below the same images are shown with detections and overlays, made by the software. Left: The producing colony is identified by fluorescence detection. Middle: A pre defined area around the target is checked for the occurrence of further colonies. If no other colony is detected in the vicinity of the target, the colony is picked (right). Scale bars = 500 µm



Conclusion

The presented method shortens and simplifies the process of (re)cloning for establishment and maintenance of stable hybridomas. The crucial advantage compared to bright-field-microscopy based harvest of hybridomas from methylcellulose medium is the opportunity to differentiate high-, low- and non-IgG-producers while minimizing the number of clones which have to be picked and saving time-consuming follow-up screening procedures.

References
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