# Automated harvest of induced pluripotent stem cell colonies and colony fractions using the cell separation robot CellCelector™

**The discovery of the unique features of stem cells and induction of pluripotency in somatic cells revealed promising new strategies for therapies of degenerative diseases, disease modeling or drug discovery. Stem cell cultivation still depends on the use of feeder cells. The application of the CellCelector™ to pick stem cell colonies or colony fractions provides a highly precise alternative to manual picking procedures.**

## 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 objects from semi-solid or solid media. The single cell tool (fig. 1b) works with a glass capillary attached to a pipetting system filled with mineral oil. The glass capillary is available in different diameters up to 220 µm. The tool enables the aspiration of small volumes in microliter range. It is suitable to aspirate semi-adherent cells grown in multilayers. The parameters for the harvest can be fine-tuned for the users special application.

## Automated harvest of induced pluripotent stem cell colonies and colony fractions

Self-renewal and pluripotency are unique features of stem cells. Various studies focus on the application of stem cells for alternative therapies of degenerative diseases, disease modeling or drug discovery [1-4]. New opportunities emerge from patient-specific induced pluripotent stem cells (iPSC) [5] generated from reprogrammed somatic cells [6].

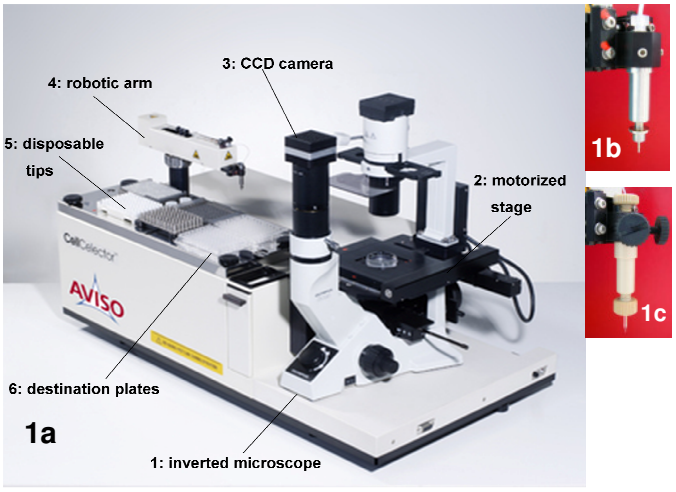


**Figure 1: a:** Assembly of AVISO's CellCelector™, robot for automated cell harvest. **b:** Scrape tool for harvest of stem cell colonies, **c:** single cell tool for harvest of stem cell colony fractions

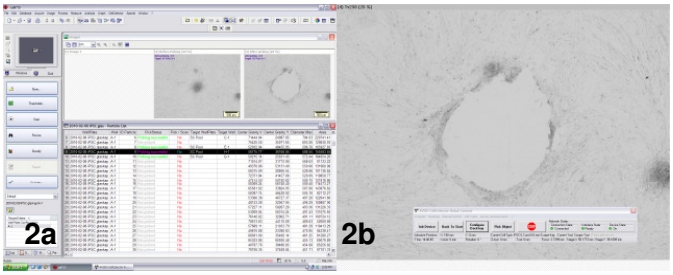


**Figure 2:** Screenshot of the CellCelector™'s control and imaging software. **a:** Picking list and automatically documented snapshots before and after picking of iPSC colonies **b:** stem cell culture on live image with hole where the colony was picked.

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Currently applied stem cell culture methods depend on the use of feeder cells to create the specific environment for maintenance of pluripotency. Colonies are picked manually to passage the cells, which poses a time-consuming and tedious task. To provide feeder cell-independent growth conditions Matrigel (BD Biosciences) can be applied. The pluripotency of human embryonic and induced pluripotent stem cells cultured on the complex protein matrix of Matrigel could be maintained [6,7]. For long term maintenance of feeder cell free stem cells Matrigel might be a very cost-intensive solution.

As shown in previous studies of murine and human embryonic stem cells the CellCelector™ could be successfully applied for both, the passaging of stem cell colonies and the selective isolation of stem cell colony fractions [8,9]. For experiments shown here human iPSC cultured on a human fibroblast feeder cell layer have been used. The colonies could be detected selectively based on a combination of user-defined parameters.

Harvest of stem cell colony fractions was enabled by the single cell tool. The stem cells were aspirated with an 80 µm glass capillary at multiple positions of the colony (fig. 3). This is also a user-defined functionality of the harvesting tools. Transferred feeder-freed stem cells appeared suspended or formed small aggregates in the destination wells directly after picking.

For picking of the entire colony the scrape tool was applied (fig. 4). The feeder layer around the colony could be cut through by the scrape movement. The colony was than aspirated and transferred to a 96-well destination plate. After picking the colonies were either replated on a human fibroblast feeder cell layer (fig. 5d) or cultured in absence of a feeder layer in the destination plate (fig. 6d).

Automatically harvested stem cell colonies showed no significant differences to manually picked colonies of the same passage. Vitality and growth behavior were also comparable.

After harvest with the CellCelector™ colonies were stained for the pluripotency associated markers nanog, Oct4, SSAE3, SSAE4, TRA1-60 and Sox2 in various combinations as shown in figures 5 and 6. Even in absence of the feeder cell layer colonies appeared pluripotent after 4 weeks of incubation.

## Conclusion

Using the CellCelector™ for automated selection and harvest of iPSC colonies and colony fractions provides a simple and reliable alternative to manual picking procedures. The different tools are applicable for passaging colonies or obtaining feeder cell free stem cells.

Picking of defined positions of colonies also allows the isolation of pluripotent cells from colonies with differentiating areas.

Sophisticated imaging software enables the selective detection of the colonies grown on feeder cells.

The laminar flow cabinet of the CellCelector™ maintains sterility and culture conditions (37 °C, 5 % $CO_2$) during the harvesting process. Vitality, proliferation and pluripotency of iPSC used for these experiments were not affected by automated picking.

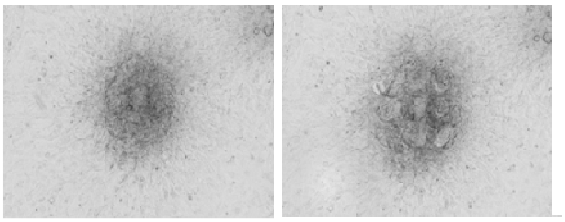

**Figure 3:** Automatically documented pictures during the harvesting process using a glass capillary. iPSC are selectively aspirated at multiple positions of the colony. image width = 1000 µm

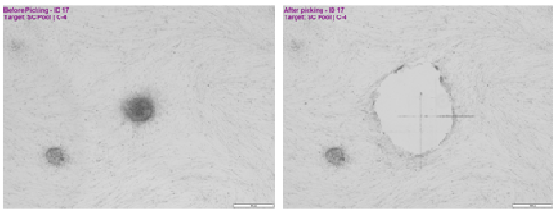

**Figure 4:** Automatically documented pictures during the harvesting process using scrape capillaries. Entire iPSC colonies can be picked. The feeder cell layer is cut thought by the scrape movement. scale bars = 500 µm

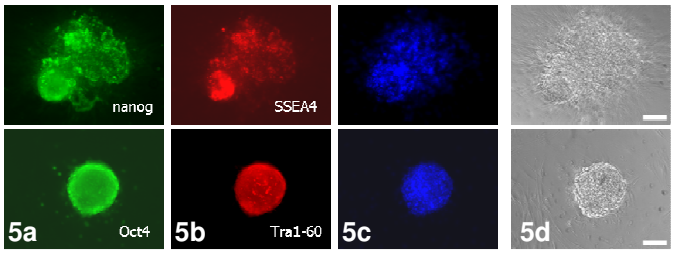


**Figure 5:** On feeder cells replated iPSC colonies. **a, b:** After 4 weeks incubation the cells were immunocytochemical stained for pluripotency associated markers (nanog, SSAE4, Oct4, TRA1-60). **c:** DAPI counter stain. **d:** phase contrast images. scale bars = 200 µm

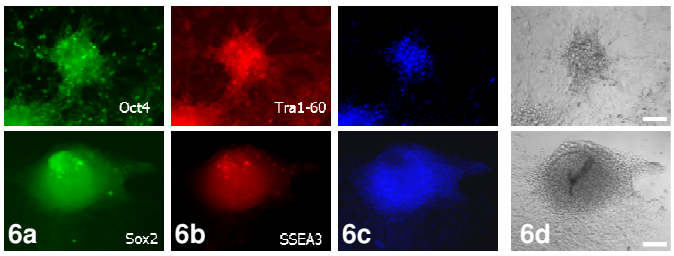


**Figure 6:** iPSC colonies cultured in absence of a feeder cell layer. **a, b:** After 4 weeks incubation the cells were immunocytochemical stained for pluripotency associated markers (Oct4, TRA1-60, Sox2, SSAE3). **c:** DAPI counter stain. **d:** phase contrast images. scale bars = 200 µm

**References**

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