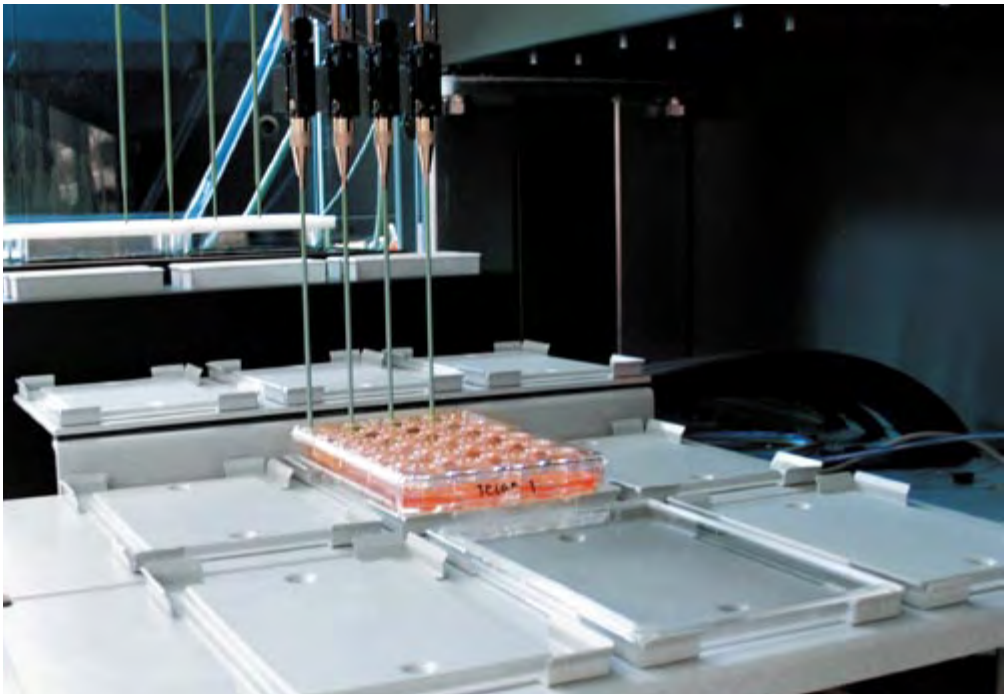
Automated differentiation of stem cells with the Freedom EVO® and Millicell®-24 filter plates

Scientists at Millipore have developed an automated method for the neural differentiation of embryonic stem cells (ESCs) in monolayers, instead of the non-automatable, three-dimensional cell clusters that are typically used. The approach relies on a Freedom EVO® 150 liquid handling platform from Tecan, with Millicell®-24 co-culturing filter plates from Millipore.

Millipore Corporation is a global life science company, headquartered in Massachusetts, USA, that provides technologies, tools and services for bioscience research and biopharmaceutical manufacturing. Millipore has worked with Tecan for a number of years in a variety of laboratory automation projects and many of Millipore's consumable devices are automation-friendly. In this latest collaboration, Libby Kellard, automation specialist at Millipore, and her colleagues have used a Freedom EVO 150 workstation to develop an automated method for the expansion and neural differentiation of mouse embryonic stem cells (mESCs).

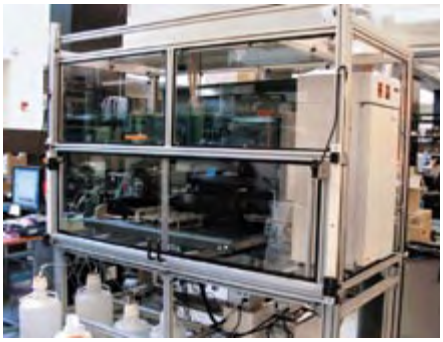


Fixed tips on the liquid handling arm add media to cultured cells in the Millicell®-24 filter plate

ESCs are widely used in a growing number of laboratory research areas, and successful differentiation of cultured ESCs into specific tissues is key to applications in tissue engineering, drug discovery and regenerative medicine. ESCs are usually co-cultured with mouse embryonic fibroblasts during expansion to maintain the ESCs in an undifferentiated state; removal of the fibroblasts is necessary to initiate differentiation. Although culturing of ESCs has been successfully automated in the past, manipulating the differentiation of these cells is not conducive to automation and is typically done manually. Differentiation protocols are labor-intensive and commonly include the use of three-dimensional cell clusters (embryoid bodies) that allow the initial formation of the three germ layers, which develop later into different tissue types. An alternative method has been proposed where ESCs grown as a monolayer can differentiate into neurites under the right conditions.[1] The scientists at Millipore adopted this approach to develop an automated technique for stem cell differentiation using the Freedom EVO workstation with Millipore's Millicell®-24 filter plate. Monolayers of ESCs are indirectly co-cultured with a feeder layer of mouse embryonic fibroblasts, separated by a 1 µM PET membrane that allows easy removal of the fibroblasts to initiate differentiation.

"We developed the original Millicell® product in the early 1980s as a novel cell culturing device with a microporous membrane support," explained Ken Ludwig, group product manager for cell biology devices at Millipore. "Cells can be grown on the membrane, allowing researchers to access both the top and bottom (apical and basolateral) surfaces of the cells. This cannot be done with standard solid plastic cell culturing plates. The microporous membranes are available as single inserts or in 24- and 96-well single units, suitable for high throughput screening applications, including automated workflows."

"The first step was to establish the cell culturing method and expansion using the Freedom EVO," Libby Kellard said. The Freedom EVO includes a specially integrated Liconic incubator with an



The Freedom EVO workstation is housed within a HEPA filter hood to minimize contamination

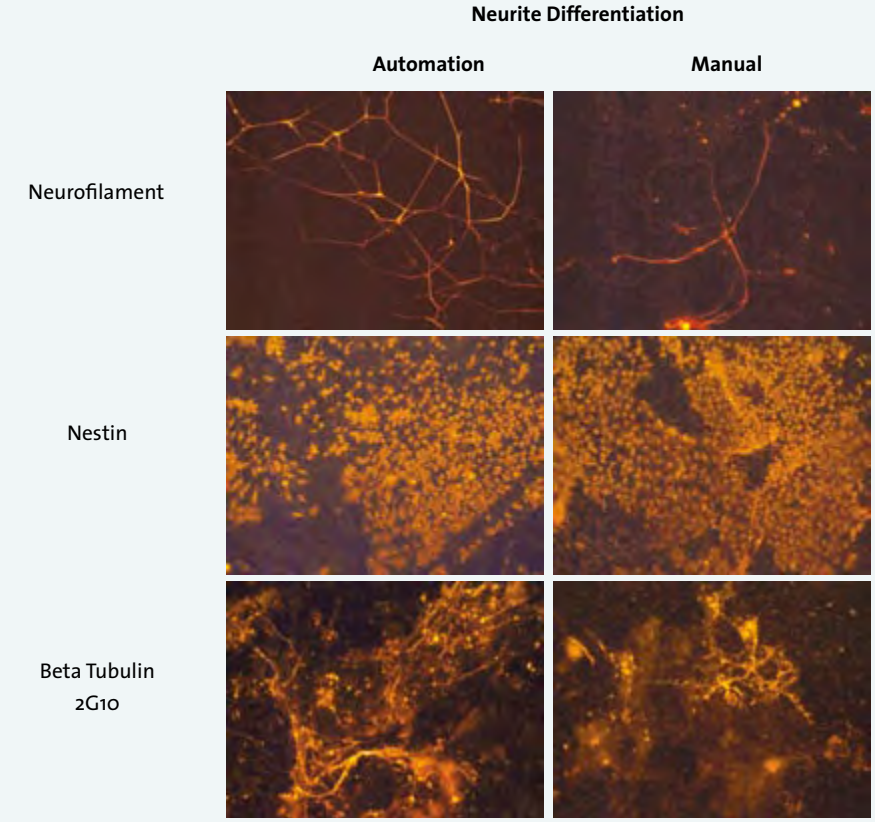
automatic shuttle that brings plates to the deck for the robotic manipulator (RoMa) arm to pick up. Other modules include a tilting rack on the deck that aids removal of media from the feeder wells by the 8-channel liquid handling arm, and cooling and warming racks for the media. The entire platform is housed within a HEPA (high efficiency particulate air) filter hood to minimize contamination.

"The biggest challenge was getting all the equipment fitted, because the engineers had to build the system within the HEPA hood," Libby continued. "It was important to ensure that the co-culturing environment meets cleanroom requirements before we could grow stem cells. We validated the system using standard cell lines such as Caco and MDCK. The people from Tecan were really helpful; they set up the entire system for me and helped with all the initial programming so that if any modifications needed to be made later, I was able to do them myself."

"We found the people at Tecan really easy to work with," Ken added. "They seem very much focused around the customer and the application, and that really helped."

"We have now shown that mESCs can be successfully maintained by the Freedom EVO in an undifferentiated state using the Millicell®-24 filter plates for indirect co-culturing, where mouse embryonic fibroblasts are seeded in a layer that is separated from the mESCs by the 1 µm PET membrane. The filter membrane makes it easy for the Freedom EVO to automatically remove the fibroblast layer, causing cell differentiation, and we achieved successful differentiation of the cells along neural lineages," said Libby.

"There were no discernible differences in cells that were automatically cultured and differentiated when compared with cells that were manually prepared at the same time. We have not yet optimized the program for high throughput but, at the moment, it certainly avoids the need for anyone to come in and feed the cells at the weekend! The system takes care of all the routine culturing steps without any intervention."



mESC cells were expanded for 4 days and then allowed to differentiate for 15 days using the Tecan Freedom EVO workstation. Plates that were handled by automation behaved similarly to plates processed manually. All plates stained positive for Neurofilament, Nestin and Beta Tubulin 2G10. In both cases Neurite differentiation occurred starting with a cell monolayer as opposed to embryoid bodies which is the typical way of producing neurites.

The method is likely to be suitable for all mouse ESCs and could potentially be adapted for other ESC types. "I think it would benefit any stem cell research application where scientists are trying to maintain ESCs over a lengthy period of time," Libby concluded.

Reference

1. *Pachernik J, Esner M, Bryja V, Dvorak P, Hampl A (2002). Neural differentiation of mouse embryonic stem cells grown in monolayer. Reprod Nutr Dev 42: 317-326*

For more information on Tecan's Freedom EVO workstations, visit **www.tecan.com/freedomevo**