**Stem Cell Tools: Aiding a Revolution**

Posted: June 25, 2015 [*Josh P. Roberts*](http://www.biocompare.com/1944-AuthorProfile/3047-Josh-P-Roberts/)

The advancements in culturing [stem cells](http://www.biocompare.com/Cloning-and-Expression/7598-Stem-Cell-Culture-Reagents/) have provided researchers with a greater vision and stronger tools to understand the mechanisms of how cells work. Today, participants in this revolution-in-progress are even able to bypass embryos and obtain pluripotent stem cells (PSCs) by reprogramming cells taken from skin or blood, and from these “induced” PSCs ([iPSCs](http://www.biocompare.com/11023-Cells-Strains/?search=iPSC)) create cells with the theoretical potential to become any cell type found in the human body. They are deriving stem cells from patients with specific diseases and from diverse backgrounds. They are “repairing” stem cells by editing the genomes and adding reporter constructs. And they are using cells differentiated from embryonic stem cells (ESCs) and iPSCs for basic disease research, toxicology screening and regenerative medicine.

Maturation of the stem cell field has meant a drive for standardization and reproducibility not seen in the earlier days of serum, feeder cells and co-cultures. It has meant a host of defined reagents with which to reprogram, passage, freeze, expand, edit and drive stem cells to differentiate, yet with plenty of room to improve. It has meant specialized service providers and training courses. Here are a few things scientists—even those steeped in cell biology and savvy about cell culture—should know about working with stem cells.

## Not just for its own sake

Scientists specializing in both developmental biology and embryogenesis have an interest in studying stem cells in their own right. But “much of the recent interest, and much of the growth in this space, is really driven by the ability to take and convert somatic cells to a pluripotent cell type, and then convert that ultimately to a desired cell type of interest. … Something that can be useful from the perspective of a cellular model or therapy,” says Mark Powers, senior director of R&D in cell biology at [Thermo Fisher Scientific](https://www.lifetechnologies.com/us/en/home/life-science/stem-cell-research.html).

There is a direct relationship between a cell’s pluripotency and its proliferative ability. Thus a principal advantage of ESCs and iPSCs (especially compared with more differentiated cells) is that they are infinitely self-renewing and “just grow and grow and grow,” points out Brad Hamilton, director of R&D at [Stemgent](https://www.stemgent.com/products). “You can make bucket loads of them.”

Another enticement is the ability to derive differentiated cells from a diversity of age, ethnic, lifestyle and disease backgrounds. (Contrast this with the difficulties faced when procuring and culturing primary cells, and the paucity of backgrounds found in continuous cell lines.) From a developmental potential perspective, you’re effectively resetting the iPSCs back to an embryonic state, “but you still have that genetic background or profile,” Hamilton explains. “So whatever caused that disease, whether it’s monogenic or polygenic or multifactorial, the genetic component is still ingrained in the DNA.”

The stem cell is typically the level at which genomic-editing methodologies such as [CRISPR](http://www.biocompare.com/Editorial-Articles/167238-Edit-Your-Way-to-Better-DNA-with-CRISPR-Cas/) and TALENs are applied to “correct” a disease, for example, or to mutate a target for drug screening. The cells can then be used to create genetically identical populations of a desired lineage or even multiple lineages—say, cardiomyocytes and hepatocytes for toxicity testing.

“iPSCs can be stored cryopreserved indefinitely,” notes Emile Nuwaysir, president and chief operating officer of [Cellular Dynamics International](http://www.cellulardynamics.com/), a FUJIFILM company. They can be “thawed, expanded in culture as iPSCs and then differentiated into the cell type of interest.”

## Medium is not rare

By most accounts, a stem cell is a stem cell no matter how it got that way—for practical purposes, it doesn’t matter whether it’s an ESC or an iPSC. But to keep the cells undifferentiated, pluripotent and growing, you need the right cell signaling, in terms of cytokines or small molecules, as well as an appropriate growth substrate, notes Simon Hilcove, senior product marketing manager at STEMCELL Technologies.

There is no shortage of media options, from a host of vendors, all claiming to provide the right balance of factors to support stem cells. Some of the media are formulated with a bare minimum of ingredients (such as insulin, fibroblast growth factor (FGF) and transforming growth factor beta (TGF-beta)), while others contain factors such as albumin, antioxidants, vitamins and nutrients. There are even media, such as L7™ hPSC BulletKit™ Medium from [Lonza](http://www.lonza.com/products-services/bio-research/stem-cells/pluripotent-stem-cells/pluripotent-stem-cells-and-media/l7-hipsc-reprogramming-and-hpsc-culture-system/l7-culture-system.aspx), that are formulated to allow feeding of the cells “every other day, so you don’t have to come every day to maintain these cells, which is the norm,” says Theresa D’Souza, cell biology R&D section manager at Lonza. Thermo Fisher has released its [Essential 8™ Flex](https://www.lifetechnologies.com/us/en/home/life-science/stem-cell-research/induced-pluripotent-stem-cells/essential-6-8-medium.html) Medium Kit, which “enhances the stability of heat-sensitive compounds, including FGF-2, that are present [in] pluripotent stem cell media, to the extent that there is virtually no loss of activity over several days,” says Powers. And[StemCulture](http://stemcultures.com/), a New York-based research company, offers Stem Beads® FGF2, which “delivers a steady release of growth factor into your media of choice,” according to the company’s website.

Depending on the ultimate use for the cells, it may be important to ensure that media are manufactured under Current Good Manufacturing Practice (CGMP) conditions, or that they are xeno- or even animal product-free. Besides regulatory concerns, “the field as a whole generally is moving to more defined surfaces and defined media,” says Hamilton. “The goal is reproducibility. If I’m going to bring in 30 patient samples, I want to know that your protocol will consistently get me the same sort of composition, such that the results I get from whatever screen or assay I’m going to run are strictly just dependent upon the variability of the patient I’m looking at and not due to the process.”

## Rites of passage

Passaging stem cells is not just a matter of trypsinizing them. “There are very specific phenotypes and morphologies that you’re looking for to ensure that you’re maintaining an undifferentiated state,” Powers says.

Typically cells are passaged once every five to six days, and “you need to spend a fair bit of time going through the well and manually scraping away any areas of differentiation and removing them from the culture. If you don’t, your culture will soon be overrun by differentiated cells,” explains Hilcove. Trypsin indiscriminately lifts up all the cells, so STEMCELL Technologies developed ReLeSR™, which selectively releases the stem cells while leaving differentiated cells stuck to the plate. “We routinely keep the differentiation below 5%,” he says.

When differentiation is the goal, look for differentiation media. Several vendors offer media and kits designed to drive stem cells down specific lineage pathways. “Creating standardized and useful differentiation media is in many ways the next frontier around this space,” says Powers. Some of the newest offerings include STEMCELL Technologies’ [STEMdiff™](http://www.stemcell.com/en/Products/Popular-Product-Lines/STEMdiff.aspx) Astrocyte Differentiation and Maturation Kits and STEMdiff™ Dopaminergic Neuron Differentiation and Maturation Kits, and Thermo Fisher’s Gibco® PSC Definitive Endoderm Induction Kit. Noting that competing media may already exist, Powers says that “we’ve found that in some cases existing solutions do not offer the simplest of workflows or leverage recent advances in our knowledge of the field as effectively as researchers might like.”

So it behooves researchers to ask a colleague, post a question, check the literature for what’s worked for others, keep an eye out for products old and new that may meet their needs and/or take a training course (maybe even online). But above all, don’t be afraid to try something new. Be part of the revolution.