

Simplifying the iPSC Workflow



01 Reprogram



With Simplicon™ RNA Reprogramming Technology, you can:

- Create integration-free iPS cells using synthetic RNA
- Reprogram efficiently and easily using a single transfection step
- Achieve controlled elimination of reprogramming factors

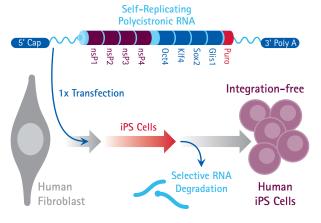
Simplicon™ RNA Reprogramming Technology Single transfection. Virus-free iPSCs.

Drawbacks of current iPSC generation methods, involving virus or multiple transfections, include experimental variability, questions of biosafety, and known and unknown effects of introducing viruses into experimental systems.

We challenge you to rethink your reprogramming strategy. The Simplicon™ RNA Reprogramming Technology utilizes a single, polycistronic, synthetic, self-replicating RNA strand that is sufficient for generating high numbers of human iPSCs with a single transfection step¹. The single RNA contains the four reprogramming factors, OCT-4, KLF-4, SOX-2 and GLIS1, and allows for efficient reprogramming without viral intermediates or host genome integration. The efficiency of this new technology has been shown to range from 0.3% to 1.1%, depending on fibroblast proliferation rate.

References

- 1. Yoshioka N et al. Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell. 2013 Aug 1;13(2):246-54.
- 2. Bernal JA. RNA-based tools for nuclear reprogramming and lineage-conversion: towards clinical applications. J Cardiovasc Transl Res. 2013 Dec;6(6):956-68.



The Evolution of Reprogramming Technologies

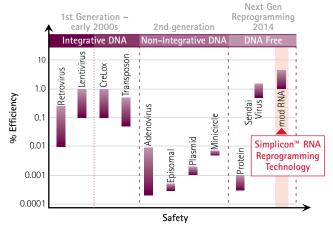


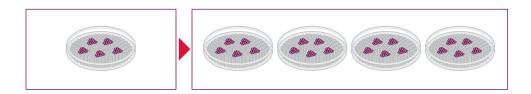
Figure 1. How Simplicon™ Technology works.

Figure 2.

Safest and most efficient. The evolution of reprogramming technologies has culminated in the development of synthetic RNA-mediated reprogramming (extreme right), representing the safest and most efficient method for iPS cell generation². *Illustration adapted from Juan A. Bernal, J. of Cardiovasc. Trans. Res.* (2013) 6:956–968, July 2013



For more information, please visit the link below or scan QR Code (left): www.emdmillipore.com/simplicon



Features and benefits:

- Feeder-free, serum-free, defined cell culture system for human ES/ iPS cells
- Less frequent cell feeding (every other day, weekend-free) reducing cost and overall cell culture time
- Low total protein and growth factor concentrations
- High viability and proliferation in single cell passaging

PluriSTEM® Human ES/iPS Medium

Feed less. Discover more.

This defined medium maintains human pluripotent stem cells in feeder-free and serum-free conditions with less frequent feeding and cell culture time. The proprietary formulation uses Activin-A, $TGF\beta 1$ and b-FGF to promote stem cell self-renewal and potent small molecule combinations to inhibit unwanted spontaneous differentiation along with Human Serum Albumin (HSA) to aid in overall colony morphology.



Figure 3.
PluriSTEM® Human ES/iPS Medium (A) and H9 human ES cells cultured in PluriSTEM® medium (B).

Every other day medium change and skip weekends!

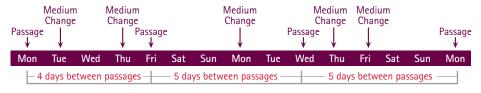


Figure 4.
With PluriSTEM® medium there is no need for weekend feeding.

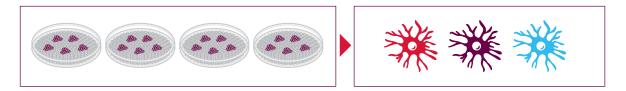
New technology feature:

PluriSTEM®-XF Human ES/iPS Medium

Interested in translational stem cell research? Maintain your pluripotent stem cells in completely humanized cell culture conditions with PluriSTEM®-XF Human ES/iPS Medium.



www.emdmillipore.com/pluriSTEM



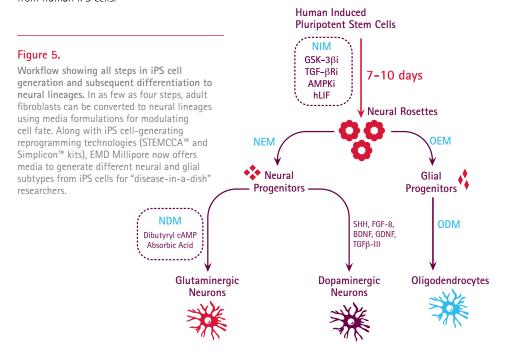
Features and benefits:

- Small molecule media based on Dual-SMAD inhibition
- Convenience
- Cost savings
- Optimized formulations for specialized cell differentiation

Neural Induction Media

The discovery that somatic cells could be converted into induced pluripotent stem (iPS) cells with the expression of four transcription factors has created an exciting new area of stem cell biology research. Many human diseases that previously lacked a comprehensive *in vitro* cellular model can now be modeled using patient-derived iPS cells. Neurological diseases, such as Alzheimer's disease, Parkinson's disease, and autism were some of the first diseases modeled using an iPS cell approach. Highly enriched and scalable neural progenitor cells (NPCs) derived from iPS cells are attractive alternatives to tissue-isolated primary cells and immortalized neural stem cell lines for biomedical research.

EMD Millipore offers ready-to-use differentiation media that incorporate supplements, small molecule inhibitors, and growth factors to easily produce neural progenitors and specific neural and glial subtypes from human iPS cells.



Are you a DIY differentiator?

Did you know EMD Millipore has an extensive portfolio of recombinant proteins and small molecule inhibitors and activators that are highly validated for all your stem cell research needs?

For a complete listing of growth factors and cytokines, including standard, animal-free and xeno-free recombinant proteins, visit: www.emdmillipore.com/growthfactors

For a complete listing of Calbiochem® small molecules and inhibitors, visit: www.emdmillipore.com/inhibitors







Discover the benefits of incorporating SmartFlare™ Probes into your stem cell experiments:

- Monitor reprogramming status of cells based on RNA content
- Characterize differentiated stem cells by looking at RNA expression in real time
- Obtain relative quantification of RNA content at single cell resolution when combined with flow cytometry
- Target RNA sequences for detecting markers that were previously difficult to study
- Sort stem cells based on RNA expression

SmartFlare™ Live Cell RNA Detection Probes

Instead of relying on antibody markers and sacrificing precious samples for characterization, you can now easily use RNA markers, especially in cell types that lack surface markers. And you can even use the same exact cells for downstream manipulation and analysis!

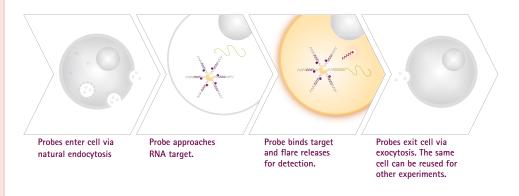


Figure 6.

SmartFlare™ Live Cell RNA Detection Probes work by entering the cell using the cell's own endocytosis process. The probe circulates within the cell and binds to the complementary RNA sequence, simultaneously releasing a fluorophore, illuminating the cells for detection. Over time, the probes exit the cells, leaving the cell unchanged and free for downstream analysis.

Product spotlight:

The best way to try out SmartFlare™ technology for your stem cell characterization: consider the SmartFlare™ NANOG probe (Cat. No. SF-875), validated to detect the mature mRNA in live cells. The SmartFlare™ NANOG Probe has even been used to sort cancer stem cells!



To see how, visit www.emdmillipore.com/smartflare or scan QR code (right).

Using antibodies in Western blot, ICC, IHC or flow cytometry?

Choose from our 10,000+ monoclonal and polyclonal antibodies.

With the expertise of Upstate® and Chemicon®, EMD Millipore provides validated antibodies with breadth and depth in major research areas: Neuroscience, Epigenetics, Immunology, Cell Signaling, Cancer, Cell Structure, and Stem Cell research backed by excellent service and support.



Transgene-free, Xeno-free iPS Cell Generation

Simplicon™ RNA Reprogramming & PluriSTEM®-XF Human ES/iPS Medium

Bridging the gap between basic stem cell research and translational clinical research requires generating patient-specific pluripotent stem cells:

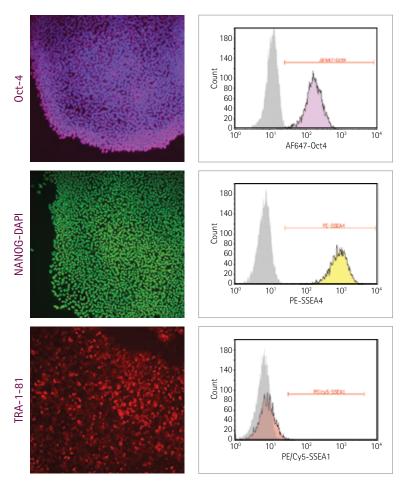
- 1) In a controlled system without host genome integration
- 2) Without the use of viral intermediates
- 3) Expanded rapidly in defined, animal-orgin-free culture conditions.

Combining the Simplicon™ RNA Reprogramming technology with PluriSTEM®-XF Human ES/iPS medium, it is now possible to effortlessly create "transgene-free" iPS cells in completely xeno-free cell culture conditions.

Figure 7.

Transgene-free human iPS cells generated with Simplicon™
RNA Technology and expanded

RNA Technology and expanded in PluriSTEM®-XF medium maintain proper pluripotent expression of OCT-4, Nanog, TRA-1-81, and SSEA-4.





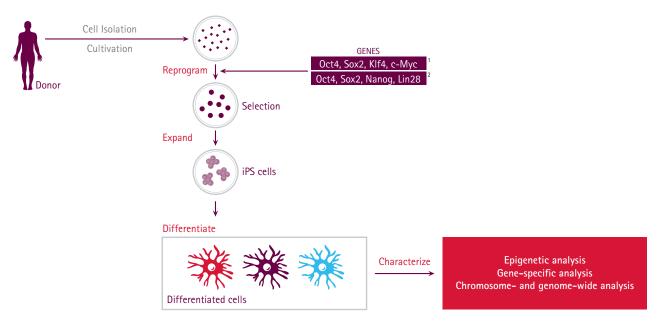
Think Stem Cells. Think EMD Millipore.

A ready source of induced pluripotent stem cells (iPS cells) is critical to the effective study of differentiation pathways or the investigation of the therapeutic potential of iPS cells. More convenient and reliable than embryonic stem cells, which are often more heterogeneous, hard to culture and subject to regulation, iPS cells offer a significant opportunity to not only propel, but dramatically enhance the efficiency and effectiveness of stem cell research overall.

Because the iPS cell generation process is sensitive to multiple variables, EMD Millipore supports the entire workflow with resources including fibroblasts validated for efficient reprogramming, optimized, defined media for expansion, novel reagents for characterizing protein and RNA cell fate markers and the most efficient, safest technology for iPS cell generation.

As a tools provider and partner in research, EMD Millipore is committed to the advancement of life science research and therapeutic development. Our offering includes a number of new products for the iPS cell workflow including reprogramming kits, culture media, and characterization tools. These products provide proven solutions for a range of applications and are backed by extensive technical support.

Current iPSC Workflow



- 1. Takahashi et al. (2007)
- 2. Yu et al. (2007)

Illustration adapted from Alexandra Rolletschek and Anna M. Wobus Biol. Chem., Vol. 390, pp. 845-849, September 2009



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