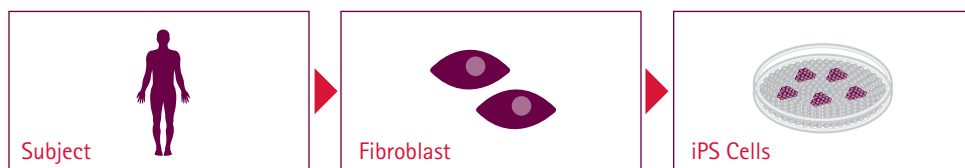


Simplifying the iPSC Workflow



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 iPS 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 iPS cells 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.

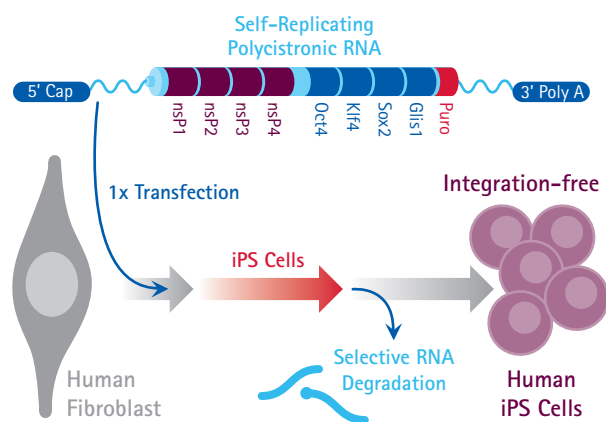


Figure 1.

How Simplicon™ Technology works.

The Evolution of Reprogramming Technologies

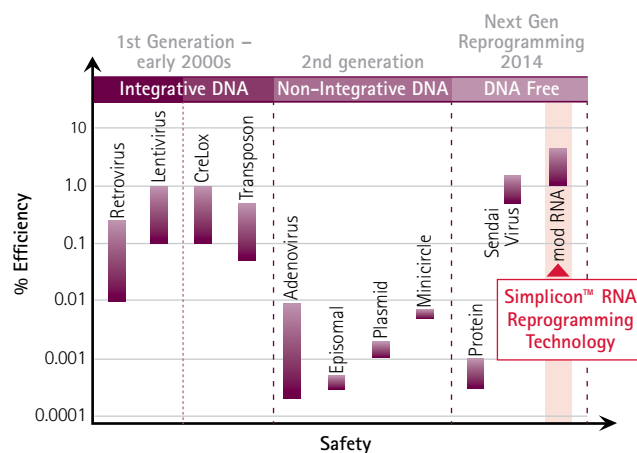


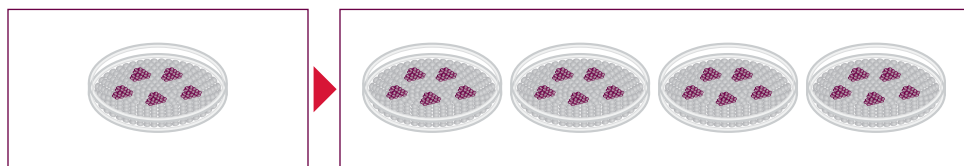
Figure 2.

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.merckmillipore.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, TGFβ1 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.

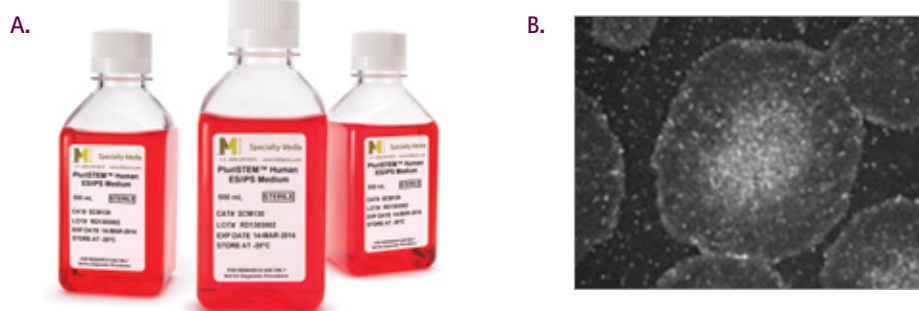


Figure 3.

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

Every other day medium change and skip weekends!

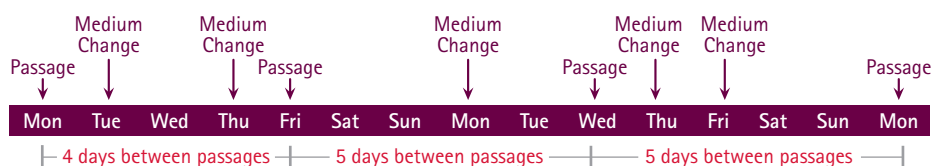


Figure 4.

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

New technology feature:

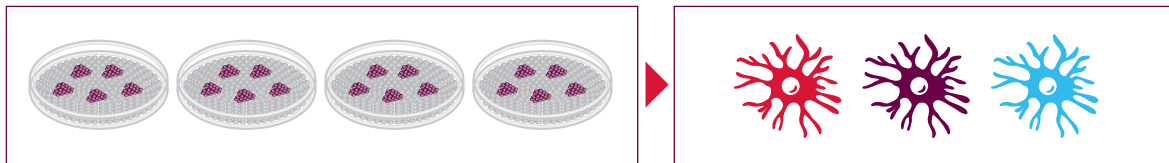
PluriSTEM®-XF Human ES/iPS Medium

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.merckmillipore.com/pluriSTEM



03 Differentiate



Features and benefits:

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

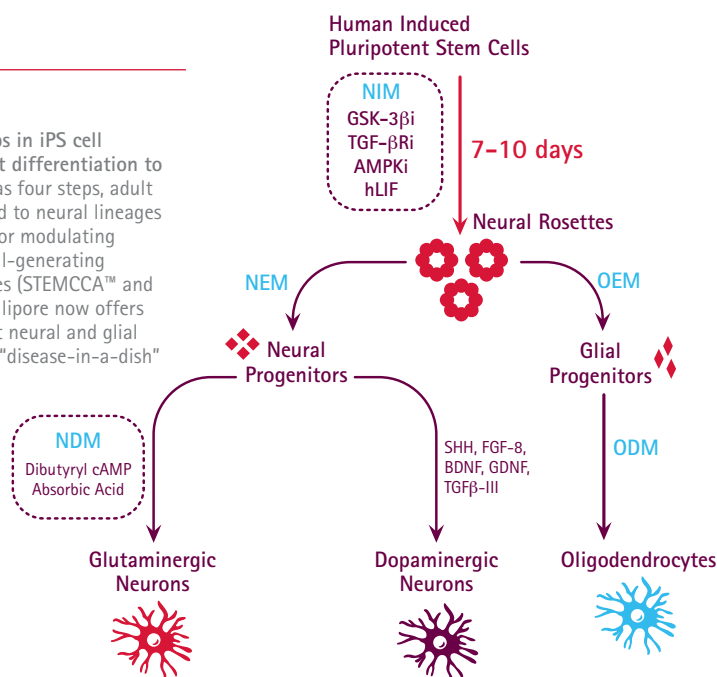
Neural Induction Media

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.

Merck 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.

Figure 5.

Workflow showing all steps in iPS cell generation and subsequent differentiation to neural lineages. In as few as four steps, adult fibroblasts can be converted to neural lineages using media formulations for modulating cell fate. Along with iPS cell-generating reprogramming technologies (STEMCCA™ and Simplicon™ kits), Merck Millipore now offers media to generate different neural and glial subtypes from iPS cells for "disease-in-a-dish" researchers.



Are you a DIY differentiator?

Did you know Merck 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.merckmillipore.com/growthfactors

For a complete listing of Calbiochem® small molecules and inhibitors, visit: www.merckmillipore.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!

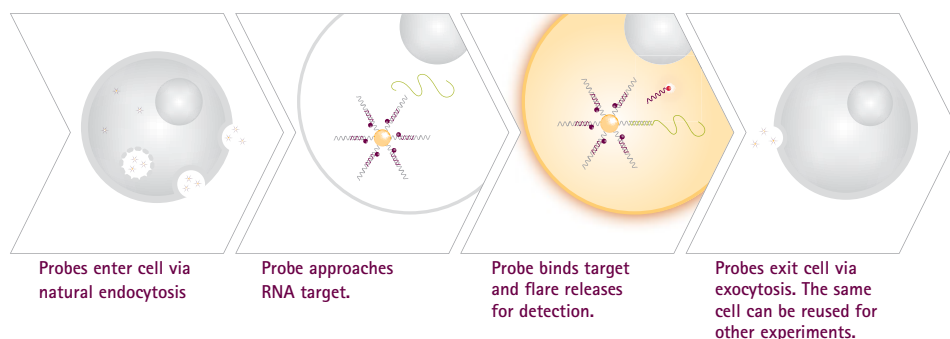


Figure 6.

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.merckmillipore.com/smartflare or scan QR code (right).

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

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CASE STUDY

Transgene-free, Xeno-free iPS Cell Generation

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

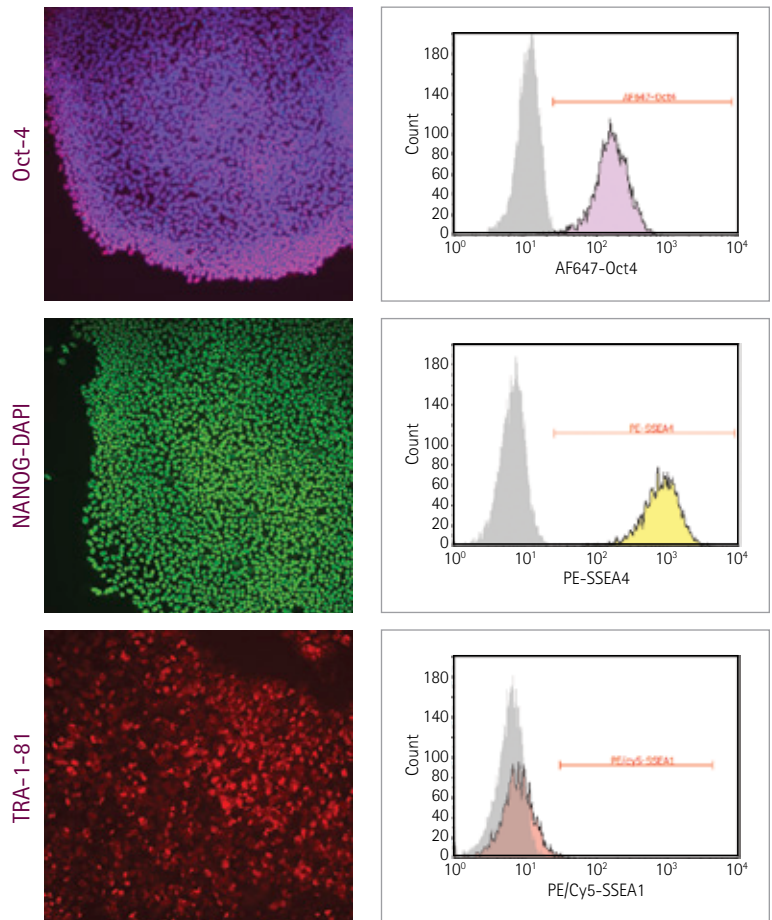
Bridging the gap between basic stem cell research and translational clinical research requires generating patient-specific pluripotent stem cells:

- 1) In a controlled system without host genome integration
- 2) Without the use of viral intermediates
- 3) Expanded rapidly in defined, animal-organ-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 in PluriSTEM®-XF medium maintain proper pluripotent expression of OCT-4, Nanog, TRA-1-81, and SSEA-4.

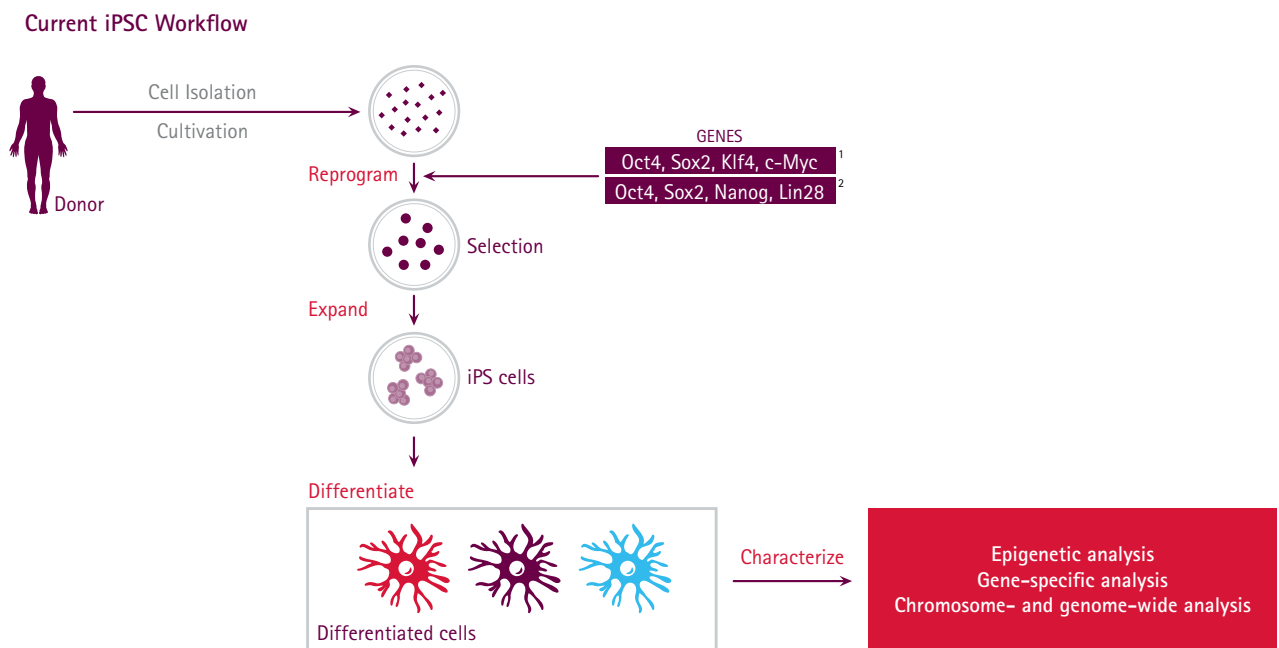


Think Stem Cells. Think Merck Millipore.

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, Merck 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, Merck 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.



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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