SBINSIGHTS

Methods for Generating iPS Cells

SBI Partners with StemRD

Disease-Specific iPS Cells Help Reveal Disease Mechanisms

New Webinar Series Launched

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EMPOWER YOUR RESEARCH

Dear SBI Customer,

Welcome to the 2nd volume of SBI's quarterly e-newsletter, **SBINSIGHTS**. The focus of this issue is induced pluripotent stem cells (iPSCs). We have a series of articles highlighting methods of generating iPS cells, products for growing all types of stem cells, and highlights on the types of research questions that can be addressed by using iPS cells.

SBI has also recently launched a new free webinar program. Our webinars cover many different topics and are usually held on Thursday mornings. Take a look at the back page of this newsletter for the upcoming schedule, or go to the SBI website to reserve your spot. http://www.systembio.com/webinars

If you have anything that you would like to contribute to **SBINSIGHTS**, please contact tech@systembio.com. We would enjoy hearing your comments and questions. If you have published a paper or presented your work at a meeting or conference using products or services from SBI, let us know! We would love to hear your feedback and may even feature your work in a future volume.

We hope you enjoy reading **SBINSIGHTS**.

Amy Mendenhall

Amy Mendenhall, Ph.D.
Associate Technical Support Manager
Editor, **SBINSIGHTS**

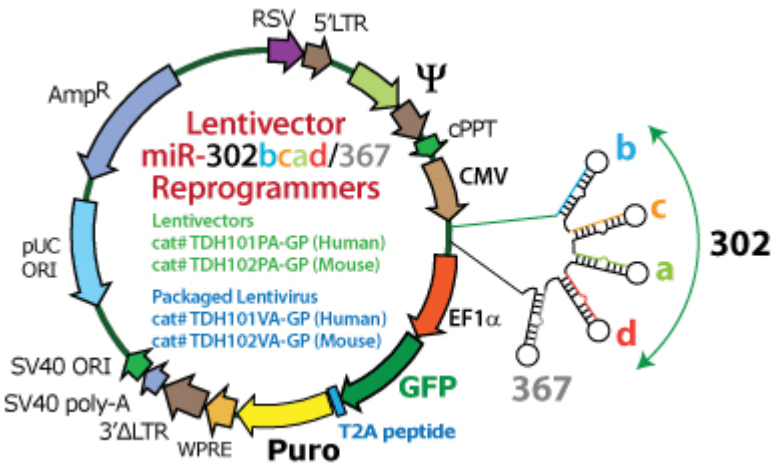
Which method is best for induced pluripotent stem cell generation?

Since Yamanaka's group first showed that fibroblasts could be reprogrammed into induced pluripotent stem (iPS) cells by retroviral delivery of four transcription factors: Oct4, Sox2, Klf4, and c-Myc (OSKM), alternative reprogramming approaches have been developed aiming for better efficiency, reproducibility and safety. Although the concept is simple, reprogramming is an extremely slow and inefficient process that is affected by several variables. Those variables include donor cell type and proliferation ability, genetic background, reprogramming cocktail choice, factor delivery method, and even cell culture conditions. Here, we discuss the impact of reprogramming cocktail and gene delivery method on the efficiency, reproducibility and safety of iPSC generation.

The reprogramming cocktail

Transcription factors. Reprogramming can be achieved by forced expression of combinations of different transcription factors that are important for cell proliferation and pluripotency. Yamanaka's group first generated iPS cells in mouse and human by retroviral expression of four transcription factors, Oct4, Sox2, Klf4 and c-Myc (OSKM). Thomson's group also generated human iPS cells with a set of four partially overlapping four transcription factors, Oct4, Sox2, Nanog and Lin28 (LNSO). It was recently published that another transcription factor, Glis1, can effectively promote the reprogramming of somatic cells during iPSC generation. The selection of transcription factors for reprogramming depends on the starting cell type, factor delivery method and downstream applications.

microRNAs. Anokye-Danso et al. have recently shown in the journal Cell Stem Cell that lentiviral expression of the miR302/367 cluster rapidly and efficiently reprograms mouse and human somatic cells to iPS cells without exogenous transcription factors. In the same journal, Miyoshi et al. have published that direct transfection of mature double strand microRNAs of miR-200c plus miR-302 and miR-369 family can reprogram mouse and human somatic cells to pluripotency. In addition, some microRNAs have been shown to enhance reprogramming efficiency in combination with transcription factors.



Small molecules. Small molecules that alter DNA methylation or chromatin modifications improve reprogramming efficiency in various cell types. DNA methyltransferase inhibitor, 5'-azacytidine, and histone deacetylase inhibitors, such as valproic acid (VPA), hydroxamic acid (SAHA) and trichostatin A (TSA), have been shown to improve reprogramming in mouse embryonic fibroblasts. Vitamin C also significantly improves reprogramming in part by alleviating cell senescence and inducing DNA demethylation.

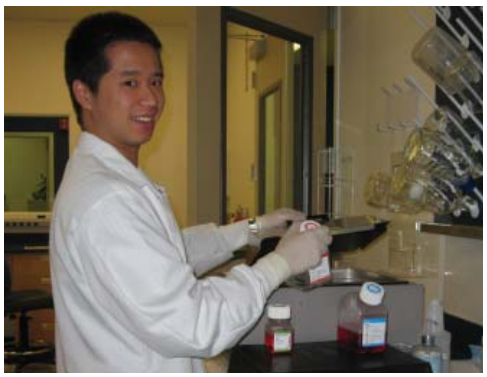
Factor delivery method

Depending on the goal of research, different delivery systems can be applied with variable levels of efficiency and safety. The table below compares some of the most commonly used integrative and non-integrative delivery systems that SBI offers or will offer in the near future for reprogramming. In general, integrative methods are more risky because the permanent genetic changes could produce oncogenic transformation of the source cells. Non-integrative technologies are safer, although are not as efficient in successfully reprogramming source cells to the pluripotent state.

Delivery Method	Cat. #	Factors Delivered	Efficiency	Safety
Retrovirus	SR200VA-1	OSKM	Efficient	Viral, Integrative
Lentivirus	SR10076VA-1	OSKM	Efficient	Viral, Integrative
	TDH101VA-G	miR-302bcad/367	Highly efficient	Viral, Integrative
Minicircle	SRM100PA-1	LGNSO	Inefficient	Transgene-, vector-, and integration-free
	TDH101MN-G	miR-302bcad/367	TBD	Transgene-, vector-, and integration-free
PiggyBac	PB400A-1	OSKM	Average efficiency	Transgene-free after excision
	PB-MIR302 and PBQM-MIR302	miR-302bcad/367	Average efficiency	Transgene-free after excision
mRNA	Coming Soon	OSKML	Highly efficient	Transgene-, vector-, and integration-free
Protein	Coming Soon	OSKM	Inefficient	Transgene-, vector- and integration-free

Experience with Reprogramming

Labs that have not tried reprogramming previously may consider first trying one of the most-efficient and well-characterized reprogramming method: the retroviral delivery using OSKM factors. SBI's retroviral reprogramming factors are a great tool for getting started with reprogramming because they are pre-mixed and easy to use. Once reprogramming using this technique has been perfected, the lab could then transition into using one of the other integration-free, but lower-efficiency methods.



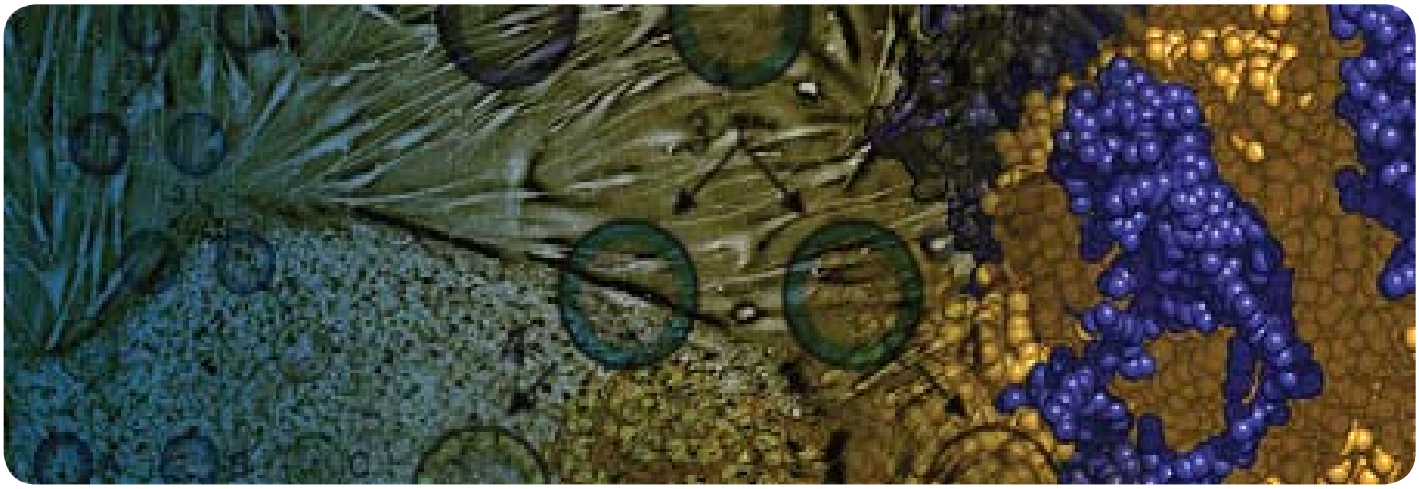
For using retroviral or lentiviral reprogramming factors, SBI follows the NIH guidelines for BSL2, and recommends wearing a lab coat and gloves. The viruses used for the reprogramming factors are self-inactivating and non-replicative, so that after the source cells have been transduced, the cells are not infectious.

References

1. Takahashi, K. and Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006, 126: 663–676.

2. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Zhang Y, Yang W, Gruber PJ, Epstein JA, Morrisey EE. Highly Efficient miRNA-Mediated Reprogramming of Mouse and Human Somatic Cells to Pluripotency. Cell Stem Cell. 2011 Apr 8;8(4):376-88.

3. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, Nishikawa S, Tanemura M, Mimori K, Tanaka F, Saito T, Nishimura J, Takemasa I, Mizushima T, Ikeda M, Yamamoto H, Sekimoto M, Doki Y, Mori M. Cell Stem Cell. 2011 Jun 3;8(6):633-8.



Growing Stem Cells Has Never Been Easier

StemRD and SBI partner to offer media and growth factors

In late June, SBI and StemRD formed a partnership to provide researchers access to new highly purified and bioactive growth and differentaition factors and stem cell growth media. SBI will be offering StemRD's proprietary MesenGro® which is a chemically-defined, serum-free and xeno-free medium for the expansion of human mesenchymal stem cells (hMSCs). MesenGro offers a good alternative to using fetal bovine serum (FBS) for culture of hMSCs since FBS is largely undefined.

In addition to MesenGro, SBI will offer growth factors from the Wnt, Hedgehog, TGF beta, and Notch signaling pathways. These growth factors are prepared using a novel and proprietary process that enables production of highly-pure and biologically active growth factors. All growth factors produced are checked for purity using HPLC and LC/MS. For a full listing of all growth factors available, see: http://www.systembio.com/stem-cell-research/media-growth-factors/ordering

"We at SBI are looking forward to offering a broad range of products for use in growing all types of stem cells and this partnership will benefit our customers so that they can find everything they need for stem cell culture in one place," says Gang Li, Ph.D. of SBI's Stem Cell department. *"The reagents developed by StemRD are of very high quality and our cells respond well to the growth conditions provided by them. The MesenGro saves time because the tissue culture plates do not need to be coated with other extracellular matrix proteins or growth factors."*

StemRD

Have you recently published an article using SBI products?

If so, we would like to know! Please contact tech@systembio.com with a PDF of your publication from 2010 or 2011 and SBI will give you a code for a $500 product credit on your next order.

Disease-specific iPS cells offer a way to study disease mechanisms and discover potential therapies

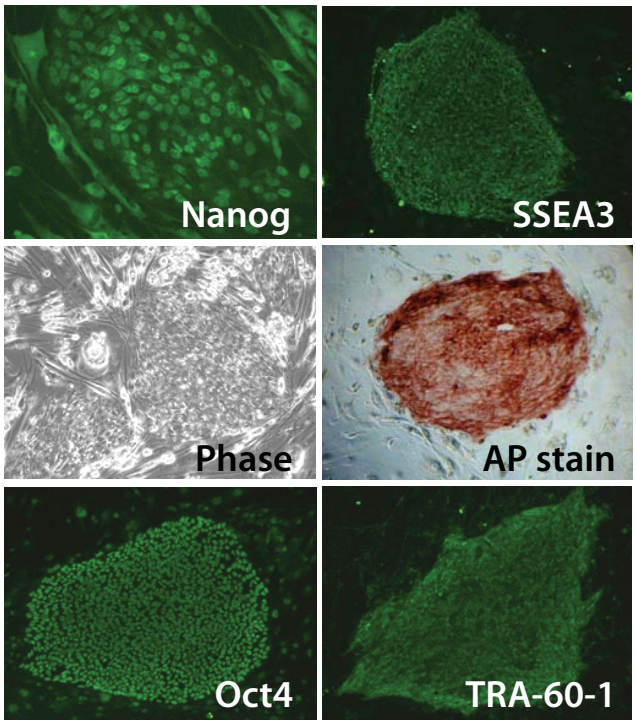
Muscular Dystrophy (MD) is a group of genetic disorders that are characterized by progressive skeletal muscle weakness, defects in muscle proteins, and death of muscle cells and tissue. The main cause of the Duchenne and Becker types of muscular dystrophy is the inability to properly create the protein dystrophin. The Dystrophin gene, that encodes the dystrophin protein, is the longest known gene, covering 2.4 megabases at locus Xp21. The dystrophin protein is found in muscle fiber membranes, where it joins the membrane actin filaments. Dystrophin functions in two ways: it provides mechanical stabilization and regulates calcium levels.

Duchenne Muscular Dystrophy (DMD) is the most common childhood form of muscular dystrophy. The absence of the dystrophin causes damage of the muscle sarcolemma that eventually leads to death of muscle cells. Muscle fibers undergo necrosis and are ultimately replaced with adipose and connective tissue. Becker Muscular Dystrophy (BMD) is a less severe variant of Duchenne muscular dystrophy and is caused by the production of a truncated, but partially functional form of dystrophin.

iPS cell lines derived from patient somatic cells can be used to study disease mechanisms, and also be used for drug screening and potentially for cell therapy. Kazuki et al., (2010), has shown a complete correction of the genetic deficiency occurring in DMD with iPS cells derived from a mouse model (mdx) of Duchenne muscular dystrophy and a human DMD patient using a Human Artificial Chromosome (HAC) with full-length human genomic dystrophin sequence (DYS-HAC). The mutation of dystrophin in iPS cells was corrected by transferring the DYS-HAC via microcell-mediated chromosome transfer (MMCT). DMD patient- and mdx-specific iPS cells were validated pluripotent and human dystrophin expression was detected in muscle-like tissues. In chimeric mice from mdx-iPS (DYS-HAC) cells, DYS-HAC was detected in all tissues examined, with tissue-specific expression of dystrophin.

SBI has recently produced several disease-specific iPS cell lines including one for muscular dystrophy (cat# SC604A/B-MD). The MD iPS cell line was generated from human dermal fibroblasts from a single donor harboring a deletion in the Dystrohpin gene at exons 3-6. Other disease-specific iPS cell lines offered include Type I Diabetes, and Metachromatic leukodystrophy (MLD), a neurodegenerative disorder. Soon, SBI will also have disease-specific iPS cells for Type II Diabetes, amyotrophic lateral sclerosis (ALS), and glioblastoma. These additions to the SBI catalog can help other groups in their disease-mechanism and potential therapy research.

Muscular Dystrophy iPS cell line



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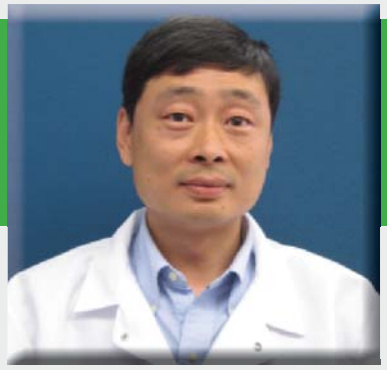

Meet Gang Li, Ph.D.

Dr. Li is the Manager of the Stem Cell Biology Department and has been working here since 2009.

Where did you do your training?

I completed my undergraduate and graduate education in Chemistry at Nanjing University in China. In 2001 I moved to SUNY, Albany to do my postdoc in Neuroscience, where I studied the GluR2Q glutamate receptor and its involvement in amyotrophic lateral sclerosis. In 2005 I moved to the Gladstone Institute of Neurological Disease in San Francisco, where I worked on the role that ApoE plays in differentiation of neuronal stem cells. It was here that I obtained all of my expertise in generating and differentiating induced pluripotent stem cells.

What do you like about working at SBI?

SBI offers a family-like working environment. It creates a community in the workplace that leads us to help each other like brothers and sisters. SBI also provides us a creative and collaborative working environment, which allows us to choose our projects independently.

Meet Danqiong Sun, Ph.D.

Dr. Sun is a Research Scientist in the Stem Cell Biology Department and has been working here since 2010.

Where did you do your training?

I got my bachelor's degree in Biological Sciences in China Agricultural University in Beijing, China. I went to graduate school in the Department of Biochemistry, Kansas State University. My Ph. D research was focused on muscle satellite cells (muscle-specific stem cells) and myogenic differentiation. At the beginning of 2009, I moved to the Gladstone Institutes, University of California, San Francisco for my postdoc training. My research was on neural degenerative diseases, such as Alzheimer's Disease and Frontotemperal Dementia. There I utilized biochemistry assays, transgenic mouse models and induced pluripotent stem cell (iPSC) models to decipher the mechanisms of those diseases.

What do you like about working at SBI?

The innovative spirits. People at SBI pay close attention to the leading-edge scientific techniques and developments. We move very fast to translate them into products and services that will advance scientific research further and faster. At SBI, I feel that we are in a positive feedback loop with the front runners in the scientific research field.

How can SBI's Stem Cell Department help you?

Custom Generated Induced Pluripotent Stem Cells

SBI's Stem Cell Department can generate iPS cells from your source cells using retroviral, lentiviral, or minicircle reprogramming methods. Choose from Yamanaka factors, Thompson factors, or microRNA reprogramming. Source cells can be from healthy or diseased tissue, and SBI can validate all produced iPS cell lines. For more details, contact your local sales representative or international distributor.

Technical Assistance in Growing iPS Cells or Reprogramming Source Cells

Let our Stem Cell biologists help you get started in your iPS cell research. They can help you with experimental design, cell culture techniques and provide you with technical resources on reprogramming methods. Contact tech@systembio.com to schedule a teleconference.

Other Stem Cell Products	Cat. #	Used For
AP Staining Kit	AP100B-1 AP100R-1 AP100D-1	Alkaline Phosphatase is a universal pluripotency marker for all types of pluripotent stem cells including embryonic stem cells, embryonic germ cells, and induced pluripotent stem cells. The pluripotent status of stem cells can be characterized by a high level of AP expression
MycoQuick	MQ100A-1	The MycoQuick mycoplasma detection kit is capable of detecting mycoplasma infection in cell cultures in less than three hours. The system can detect mycoplasma from both cell lysates and cell culture media. MycoQuick can detect as little as 10 copies of mycoplasma DNA.
Pluripotency Reporters	SR2007x Series	Pluripotency response reporters are lentiviral-based constructs that have the Oct4 CR4 or Sox2 SRR2 transcriptional response elements driving expression of GFP and firefly luciferase. Measure transcriptional responses to these transcription factors to help characterize iPS cells.
Differentiation Reporters	SR100xx Series	Monitor differentiation of your iPS cells to specific cell lineages using these lentiviral-based constructs. Cell specific promoters drive expression of GFP fluorescence and zeocin resistance.

We want to hear from you!

Have you used any of SBI's pGreenFire pathway reporter constructs ? If so, we would like to get some feedback from you. Please take our 5 minute survey here: http://www.surveymonkey.com/s/2KY2TGC

LIVE WEBINARS

All webinars are free and open to the public. Space is limited, so register on-line at:
http://www.systembio.com/webinars

CytoTracers and BioLuminescent Imaging with Lentiviral Constructs from SBI

July 28, 2011, 8:00 AM Pacific Time

This webinar will go through some of the challenges of in vivo molecular and cellular trafficking and will present some solutions from SBI. We will talk about construct design, experimental best practices, and show some successful examples of bioluminescent imaging using SBI's products.

Advances in Induced Pluripotent Stem Cell Research Tools

August 4, 2011, 8:00 AM Pacific Time

This webinar on iPS cell technology is for people who are new to iPS cell culture techniques and who would like to learn more about the basics of iPS techniques. Attendees will learn the various ways of reprogramming source cells to iPS cells. They will be able to understand the pros and cons of diferent iPS reprogramming methods and become familiar with human iPS cell culture techniques. We will also learn how to successfully characterize iPS cells.

Nonintegrative Sustained Expression using Minicircle Vector Technology from SBI

August 18, 2011, 8:00 AM Pacific Time

Minicircles are episomal DNA vectors that are produced as circular expression cassettes devoid of any bacterial plasmid DNA backbone. Their smaller molecular size enables more efficient transfections and offers sustained expression over a period of weeks as compared to standard plasmid vectors that only work for a few days. This webinar will give an overview of minicircle technology and also provide examples of uses for minicircle technology. We will go over construct design and choices, production of minicircles, transfection of target cells, and in vivo uses for minicircles.

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