

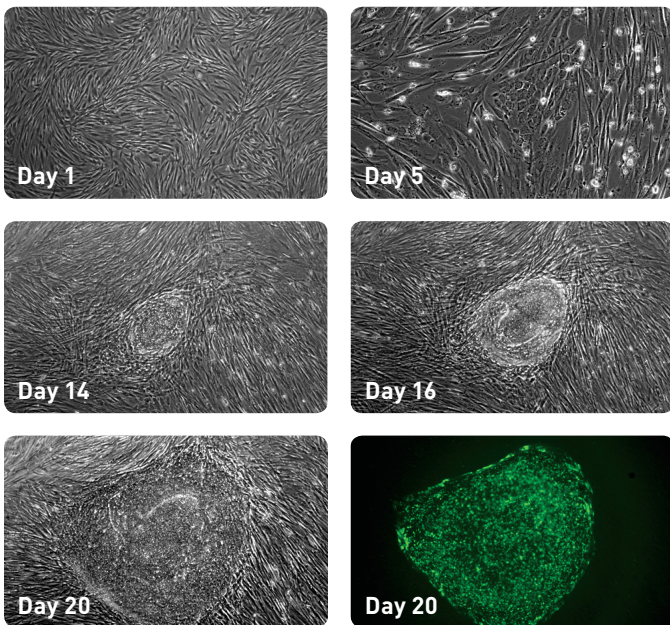
# mRNA Reprogramming System

The fastest and safest non-integrating reprogramming technology

## CUTTING-EDGE TECHNOLOGY

The ability to reprogram mature human tissue into induced pluripotent stem (iPS) cells has potentially profound implications for the future of science and medicine, as these cells hold the promise of treating cell-based diseases and defects, including diabetes, congenital heart disease, Alzheimer's disease, and many others.

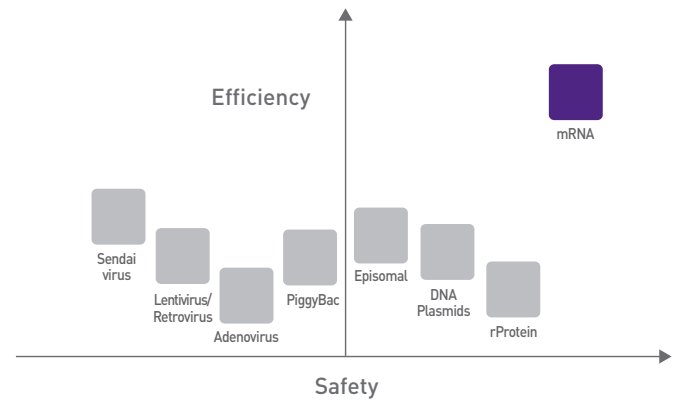
Patient-specific iPS cells offer a unique chance to model human disease and expand the field of personalized medicine. The Stemgent mRNA Reprogramming System is the fastest, safest, and most efficient method for generating integration-free, virus-free, clinically relevant iPS cell lines from mature human cells.



**Figure 1.** In collaboration with the laboratory of Dr. Rudolf Jaenisch, human iPS cell lines were generated from dermal fibroblasts from an adult patient with Parkinson's disease. Time-course phase-contrast images show morphology changes up to Day 20, and uniform expression of TRA-1-81 pluripotency marker at Day 20. All images taken at 10X magnification.

## SAFE, VIRUS-FREE REPROGRAMMING

The Stemgent mRNA Reprogramming System is the most advanced technology to date for reproducibly generating human iPS cell lines, with reprogramming efficiencies and kinetics far superior to DNA- and viral-based methods (Figure 2). The mRNA Reprogramming System eliminates virus bio-containment and safety issues, and carries no risk for insertional mutagenesis, an inherent concern with DNA-based reprogramming methods.



**Figure 2.** Comparison of standard reprogramming methodologies.

mRNA proves to be the most efficient and safest reprogramming method as compared to all viral- and DNA-based methods.

## ADVANTAGES

- No risk of insertional mutagenesis
- Non-viral application eliminates bio-containment concerns
- Fast reprogramming kinetics
- High reprogramming efficiency
- No screening required to eliminate viral remnants

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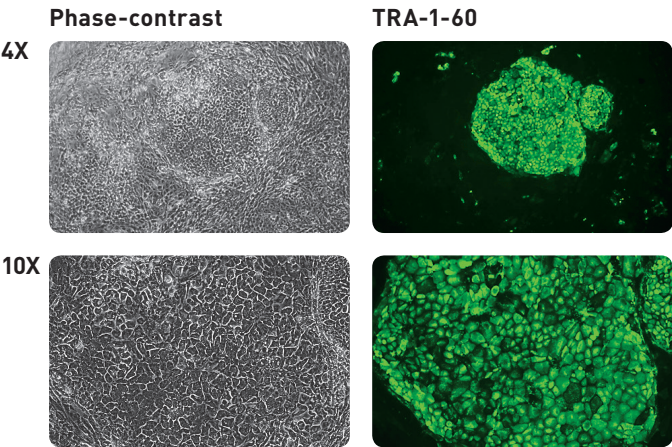
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## HIGHLY EFFICIENT REPROGRAMMING

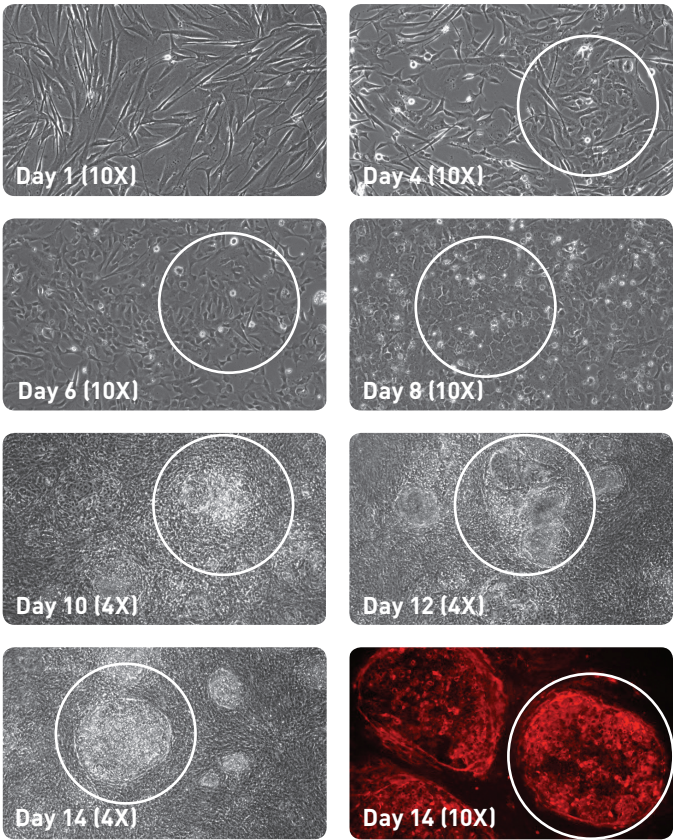
The Stemgent mRNA Reprogramming System provides efficiencies greater than 1% as compared to other methods, which yield reprogramming efficiencies varying from 0.00001 to 0.01%. In addition to increased yield of colonies and fast reprogramming kinetics, mRNA reprogramming does not require laborious multi-step passaging or screening for viral or genomic integration once the new colonies are derived (Table 1).

	Efficiency	Integrating	Screening
mRNA	>1%	No	No
Sendai virus	0.01-1%	No	Yes
Lentivirus/Retrovirus	0.001-0.01%	Yes	Yes
Adenovirus	0.0001-0.001%	Possible	Yes
Episomal/Minicircle	0.0001%	Possible	Yes
Protein	0.00001%	No	No

**Table 1. Benefits of using the Stemgent mRNA Reprogramming System compared to other reprogramming methods.** Optimized to provide efficiencies greater than 1% over existing reprogramming methods, the Stemgent mRNA Reprogramming System generates high-quality iPS cell lines that do not require additional screening to check for viral contaminants or insertional mutagenesis of the target cells' genome.



**Figure 3. A human iPS cell colony at Day 18 generated using the mRNA Reprogramming System.** Human iPS cell colony morphologies can be seen in the phase-contrast images above. Occasionally, colony boundaries may be difficult to visualize due to the high density nature of the culture at this stage in reprogramming. However, the use of live staining antibodies (such as Stemgent StainAlive™ DyLight™ 488 Mouse anti-Human TRA-1-60) enables rapid identification of reprogrammed cells prior to colony picking and expansion.



**Figure 4. Time-course images of the generation of human iPS cells using the Stemgent mRNA Reprogramming System show expected morphology of emerging iPS cells.** Phase-contrast images show the progression of morphology changes throughout reprogramming. The iPS cell colonies stain positively for TRA-1-60 pluripotency marker expression on Day 14.

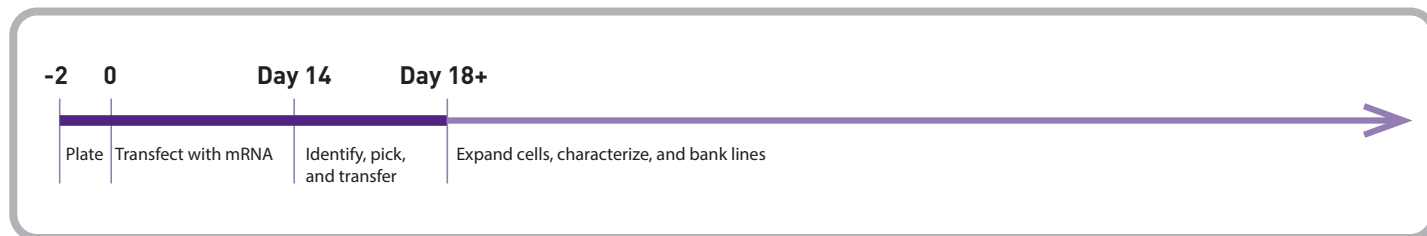
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## FAST GENERATION OF FOOTPRINT-FREE IPS CELL LINES

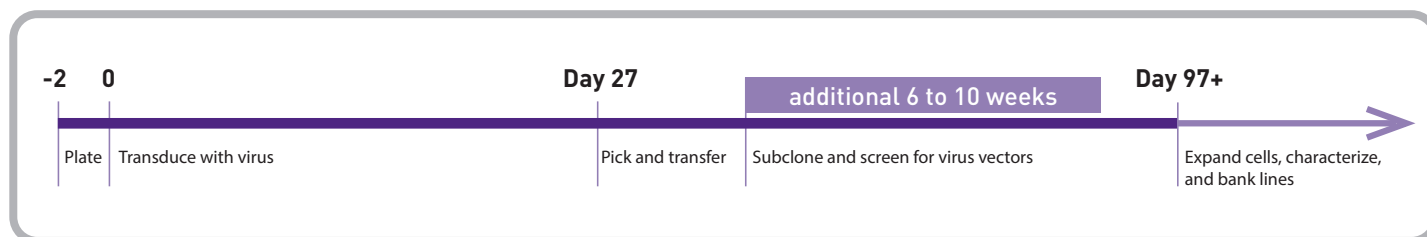
With fast reprogramming kinetics and non-viral application, reprogramming with mRNA produces clinically-relevant iPS cell lines months earlier than other systems that require additional work to screen new cells for the presents of contaminating viral remnants. The total time needed to generate a characterized iPS cell line using virus-based systems can take up to 25 weeks, whereas the Stemgent mRNA Reprogramming System generates virus-free, integration-free iPS cell lines in less than 2 weeks. This system enables the generation of fully characterized and banked iPS cell lines ready to use in as little as 9 weeks (Figure 5).

### mRNA



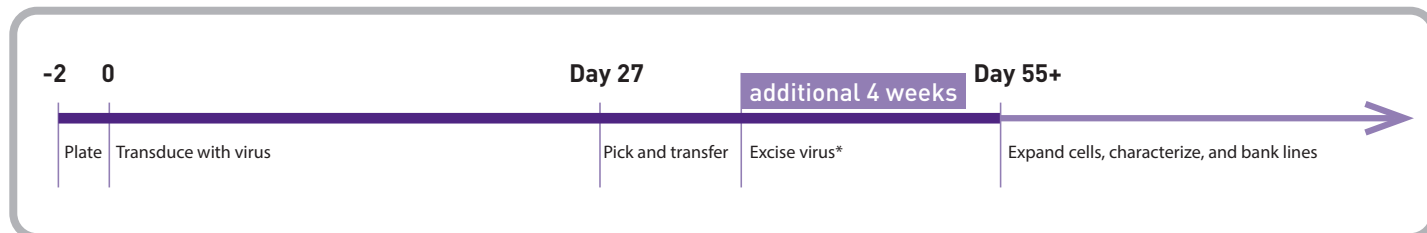
mRNA reprogramming timeline generated using Stemgent mRNA Reprogramming System protocol.

### Sendai virus



Sendai virus reprogramming timeline generated using CytoTune™ user guide protocol.

### Lentivirus



\*Optional step to excise virus transgenes with Cre/loxP.

**Figure 5. mRNA reprogramming experimental timeline compared to other reprogramming systems.** The Stemgent mRNA reprogramming protocol has been optimized to ensure that human iPS cell colonies are ready to expand in less than 20 days - months faster than when reprogramming with lentivirus, Sendai virus, and other viral or potentially integrating methods, which require additional laboratory time for cloning, selecting, and screening of cells to confirm that viral remnants no longer exist.

**Note:** Timelines are provided as a guideline for experimental planning and actual timelines can vary based on the cell type and experimental conditions.

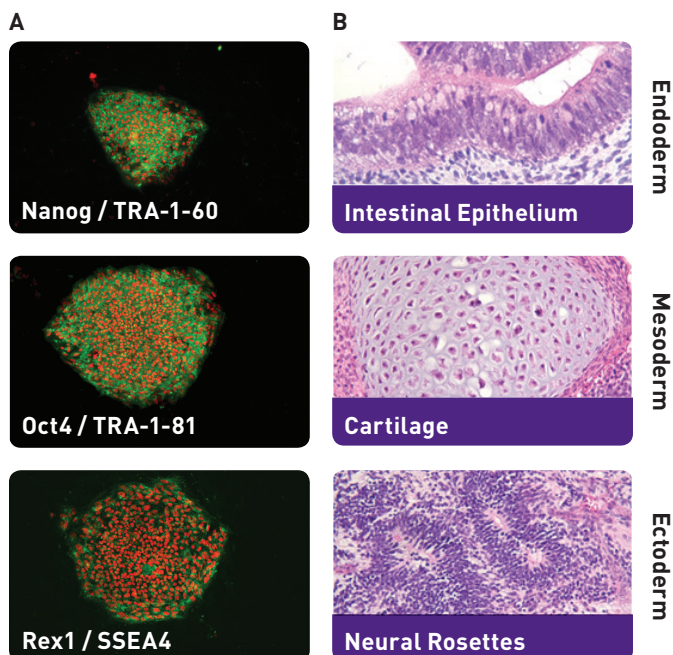


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## HIGH-QUALITY iPS CELL LINES

The mRNA Reprogramming System yields footprint-free, stable iPS cell lines. The initial reprogrammed cultures contain homogeneous iPS cell colonies that exhibit uniform pluripotency marker expression. The new iPS cell lines are readily established, requiring no cloning or small molecule treatment to eliminate partially reprogrammed colonies.



**Figure 6. iPS cells express pluripotency markers and form teratomas.** Human iPS cell lines generated by reprogramming BJ fibroblasts with the Stemgent mRNA Reprogramming System are shown to maintain a normal karyotype (data not shown), express appropriate pluripotency markers (A), and demonstrate the ability to form teratomas containing tissues of all three developmental germ layers (B).

## REPRODUCIBLE RESULTS

Fully validated and optimized on human BJ fibroblasts as a control cell line, the Stemgent mRNA Reprogramming Kit includes five mRNA reprogramming factors, a nuclear GFP marker for monitoring transfection efficiency, the Stemgent Pluriton™ Reprogramming Medium, B18R recombinant protein to reduce the innate cellular immune response, and a reproducible, easy-to-use protocol and user manual.



For a thorough review of the major steps of mRNA reprogramming and to download the full protocol, visit [www.stemgent.com/mrna](http://www.stemgent.com/mrna).

## PRODUCT ORDERING INFORMATION

Description	Cat. No.	Qty.
<b>Stemgent mRNA Reprogramming Kit</b> Includes: Stemgent mRNA Reprogramming Factors Set, Stemgent Pluriton™ Reprogramming Medium, B18R Recombinant Protein	00-0071	1 kit
<b>Stemgent mRNA Reprogramming Factors Set: hOKSML</b> Includes: Oct4, Klf4, Sox2, c-Myc, Lin28, and nGFP mRNA	00-0067	1 set
<b>Pluriton™ Reprogramming Medium</b> Includes: Pluriton™ Medium, Pluriton™ Supplement	00-0070	500 ml

**PLACE AN ORDER at**  
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or call 1.877.228.9783.

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