stemgent

A REPROCELL BRAND

Review Article:

The Past, Present and Future of iPSC Establishment

www.reprocell.com

REPROCELL

bioserve • stemgent • alvetex • biopta

1. The History of iPSC Establishment

In 2006, a Japanese research group led by Shinya Yamanaka created the very first induced pluripotent stem cells from murine fibroblasts [1, 2]. In this revolutionary study, mature cells were transfected with a retroviral vector containing four transcription factors: Oct4, Sox2, Klf4 and c-Myc. These factors reversed the differentiation process underwent by these cells, transforming them back to a pluripotent state. The following year, Yamanaka's group used the same methodology to create pluripotent cells from human fibroblasts – and the human Induced Pluripotent Stem cell (hiPSC) was born[3, 4].

This method of establishing iPSCs is known as *reprogramming* and has since been optimized to improve its efficacy and safety. Thanks to over a decade of research, we now know that safe and effective reprogramming is dependent upon low transgene persistence, high chromosome stability[6], and reduced clone variation; the latter being a critical consideration for iPSCs used in regenerative medicine[7].

The original methodology detailed by Yamanaka, which utilized episomal vectors, is now avoided as it carries a high risk of the DNA transgene being incorporated into the chromosome[8,5]. Introduction of the Sendai Virus vector in 2009 meant that this could be negated – however, long term transgene persistence represented a further obstacle[9].

Today, the Gold Standard of iPSC establishment is RNA Reprogramming – a methodology first described in 2010[10,11]. During RNA reprogramming, transgenes are introduced to target cells as mRNA rather than DNA. The reprogramming factors RNA is then translated in the cytoplasm, and subsequently degraded meaning RNA-iPSCs possess no transgene persistence (Figure 1). Further benefiHigh chromosomal stability, and there is minimal variation amongst clones. This novel methodology is therefore suited perfectly for regenerative medicine and industrial applications.

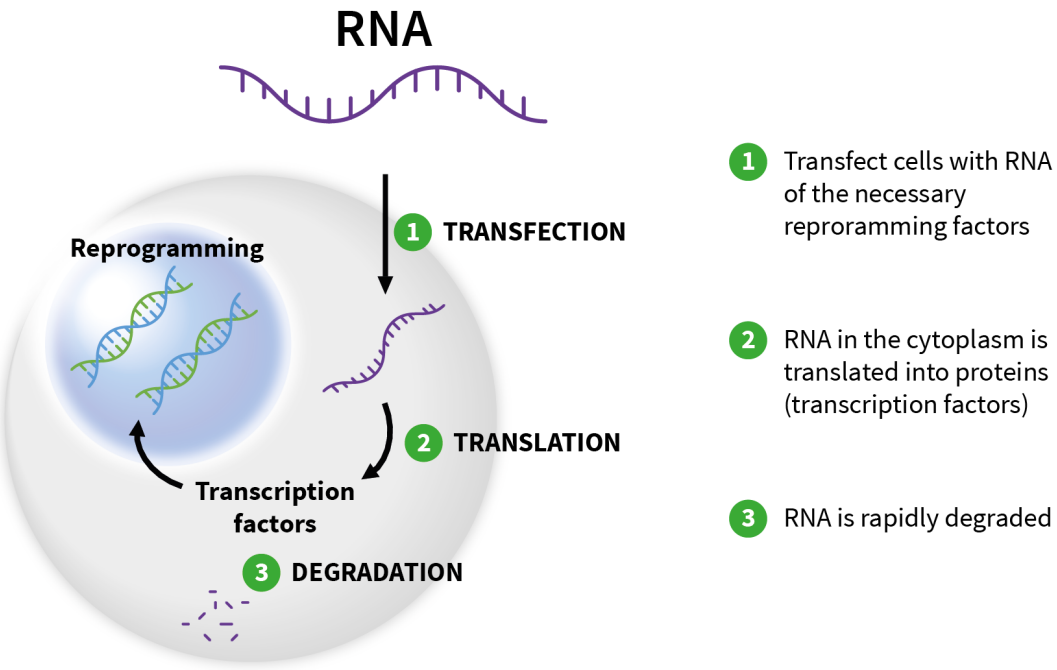

Figure 1: RNA-reprogramming methodology.

2. The Evolution of REPROCELL's Reprogramming Technology

REPROCELL's RNA reprogramming method has constantly evolved, ranging from our first-generation product, launched in 2012, to our third-generation technology today (Figure 2).

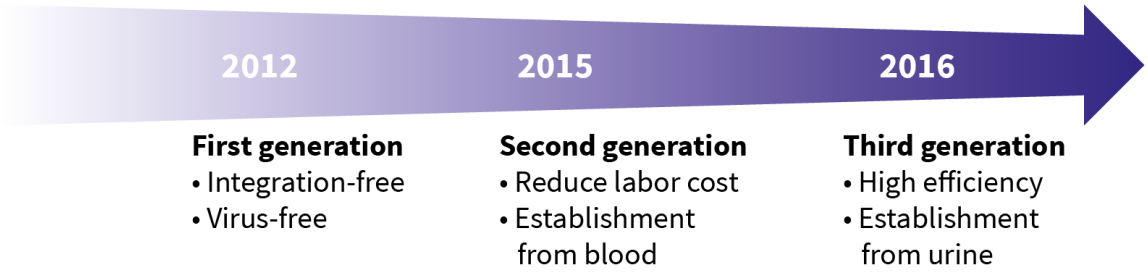

Figure 2: Evolution of RNA reprogramming methodology.

Our first-generation RNA reprogramming method made it possible to create iPSCs with high genomic stability and low transgene persistence by using RNA at the time of reprogramming factor transfection. However, the challenges were that gene transfection was laborious, the efficiency of establishment was low, and iPSCs could only be established from fibroblasts.

These obstacles were overcome by the development of our second-generation RNA reprogramming methodology. As this used self-replicating RNA reprogramming efficiency was improved, and hiPSCs could now be established from patient blood samples.

Our latest RNA reprogramming method has increased the efficiency of iPSC establishment further by suppressing the interferon response induced when RNA is transfected into donor cells. Using this third-generation methodology, iPSCs can be established from urine samples, a process which is dramatically less invasive than the use of fibroblasts or blood (Figure 3). This has made it easier to prepare iPSCs from children and adults who cannot donate skin and blood.

A further advantage of RNA-iPSCs is their ability to differentiate into the three germ layers via induction of differentiation *in vitro* or teratoma formation (Figure 4). This allows differentiation into cardiomyocytes, hepatocytes, and neurons, which was previously deemed impossible. It is also easier to create retinal cells from iPSCs created from RNA than conventional methods[12].

3. The Advantages of RNA-iPSCs

Low Transgene Persistence

RNA-iPSCs use an RNA transgene which is rapidly degraded following translation. This means there is low transgene persistence compare with other reprogramming methods, with the quantity of transgene RNA undetectable after 2-3 cell cycles (Figure 3).

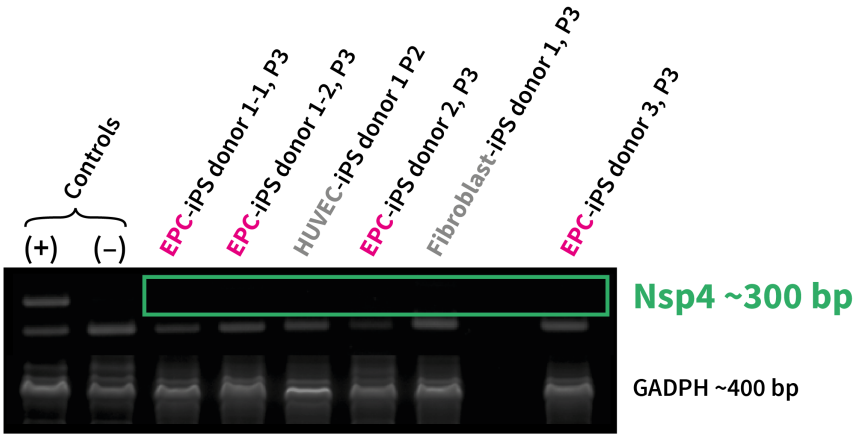

Figure 3: Transgene is undetectable after 3 cycles.

High Chromosomal Stability

The viral vector methodology developed by Takahashi et al (2006) resulted in incorporation of the transgene into the genome. This led to major safety problems including unexpected mutations in the host DNA and oncogenesis. Reprogramming methods that use DNA transgenes cannot eliminate the danger genome integration, making it necessary to test iPSCs before use. By contrast, iPSCs created via RNA reprogramming can be used without concern over genomic integration; a study by Schlaeger et al found that the frequency of chromosomal aneuploidy in RNA-iPSCs is significantly reduced compared with iPSCs created using retroviral, Sendai Virus, or episomal vectors[13].

The presence of chromosome Copy Number Variations (CNV) in cells, such as karyotype abnormality and gene polymorphism, indicate an increased risk of oncogenesis. CNVs therefore represent a major barrier in the application of iPSCs in regenerative medicine. Table 1 shows that RNA-iPSCs possess significant less CNV mutations than those created using conventional reprogramming methods. However, CNV occurrence is not limited to cell line establishment; it can also happen during iPSC cultivation. Our results demonstrate that the use of RNA reprogramming will make it possible to easily create hiPSCs free of genomic damage.

Low Clone Heterogeneity

Due to variation among human iPS cell-lines, a selection method must be used to screen for high quality iPSCs — even when cells are derived from the same donor[7]. RNA-iPSCs show less variation than those created via Sendai Virus vector[14] and show a similar gene expression pattern to embryonic stem cells, which are the gold standard for pluripotency[8]. Human iPSCs created though conventional methods also display different differentiation potential, while RNA-iPSCs do not. This means that iPSCs created using RNA reprogramming have equal tendency to become hepatocytes, cardiomyocytes, or neurons (Figure 6). These results show that using RNA-iPSCs it is possible to create differentiated cells with low clone heterogeneity, which is particularly significant when considering production of iPSCs from precious specimens — this is important for their effective use and commercial adoption.

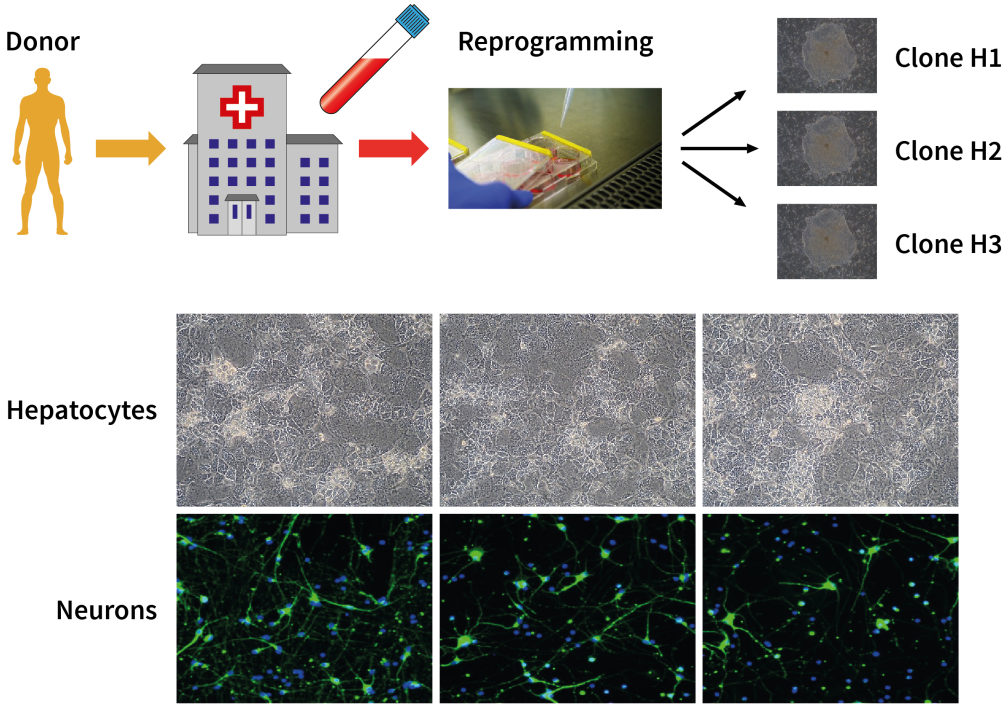

Figure 4: RNA-iPSCs show reduced inter-clonal variation compared with conventional techniques.

4. The Future of Reprogramming Technology

More than 10 years have passed since Takahashi *et al* created the first human iPS cell-line. Today, the introduction of RNA reprogramming as significantly increased the efficacy of hiPSC establishment, with REPROCELL's third-generation technology remaining the most effective reprogramming methodology on the market.

Whilst iPSCs have the potential for application in full-scale commercial use and regenerative medicine, the reprogramming and differentiation methodology requires further optimization. Safety is a major consideration when considering the use of iPSCs clinically and can be improved by increased chromosomal stability and low transgene persistence; of course, RNA- iPSCs are greatly advantageous in this respect. The use of RNA-technology also benefits iPSC production: an important consideration in commercialization. With the ability to now create RNA-iPSCs from urine, it is expected that their range of applications will increase dramatically.

5. References

1. Takahashi *et al*. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:4 (2006).
2. Wernig *et al*. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448:7151 (2007).
3. Takhashi *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:5 (2007).
4. Yu *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:5858 (2007).
5. Okita *et al.* Generation of germline-competent induced pluripotent stem cells. *Nature* 448: 7151 (2007).
6. Ministry of Health, Labor and Welfare Health Policy Bureau Research and Development Division. Points for certified special committees for regenerative medicine to consider when evaluating tumorigenicity assessment in provision plans of regenerative medicine using human pluripotent stem cells - at the tenth meeting of the special select committee for regenerative medicine. Document 3-1 (2016).
7. Mandai *et al*. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *New English Journal of Medicine* 376:11 (2017).
8. Okita *et al*. A more efficient method to generate integration-free human iPS cells. *Nature Methods* 8:5 (2011).
9. Agu *et al*. Successful Generation of Human Induced Pluripotent Stem Cell Lines from Blood Samples Held at Room Temperature for up to 48 hr. *Stem Cell Reports* 5(4 (2011).
10. Warren *et al*. Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA. *Cell Stem Cell* 7:618 (2010).
11. Yakubov *et al*. Reprogramming of Human Fibroblasts to Pluripotent Stem Cells using mRNA of Four Transcription Factors. Biochemical Biophysical Research Communication. 394:189 (2010).
12. Sridhar *et al*. Robust Differentiation of mRNA-Reprogrammed Human Induced Pluripotent Stem Cells Toward a Retinal Lineage. *Stem Cells Translational Medicine* 5:4 (2016).
13. Schlaeger *et al*. A comparison of non-integrating reprogramming methods. *Nature Biotechnology* 33:1 (2015).
14. Paull *et al*. Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nature Methods* 12:9 (2015).

REPROCELL USA Inc
9000 Virginia Manor Road
Suite 207
Beltsville, MD 20705
USA

Tel: +1 301 470 3362
Email: info-us@reprocell.com

REPROCELL Europe Ltd
Thomson Pavilion, Todd Campus
West of Scotland Science Park
Acre Road
Glasgow G20 0XA
UK

Tel: +44 (0)141 465 3460
Email: info-emea@reprocell.com

REPROCELL India Ltd
3-1-135/1A, CNR Complex
Mallapur
Hyderabad 500 076
Telangana
India

Tel: +44 (0)141 465 3460
Email: Bhargavi.Gurram@reprocell.com

REPROCELL Inc (Japan)
MetLife Shin-yokohama 381,
Bldg. 9F
3-8-11, Shin-yokohama
Kohoku-ku, Yokohama
Kanagawa 222-0033
Japan

Tel: +81 45 475 3887
Email: info-asia@reprocell.com