VIEWPOINT

Ensuring high-quality iPSCs to maximize drug discovery success

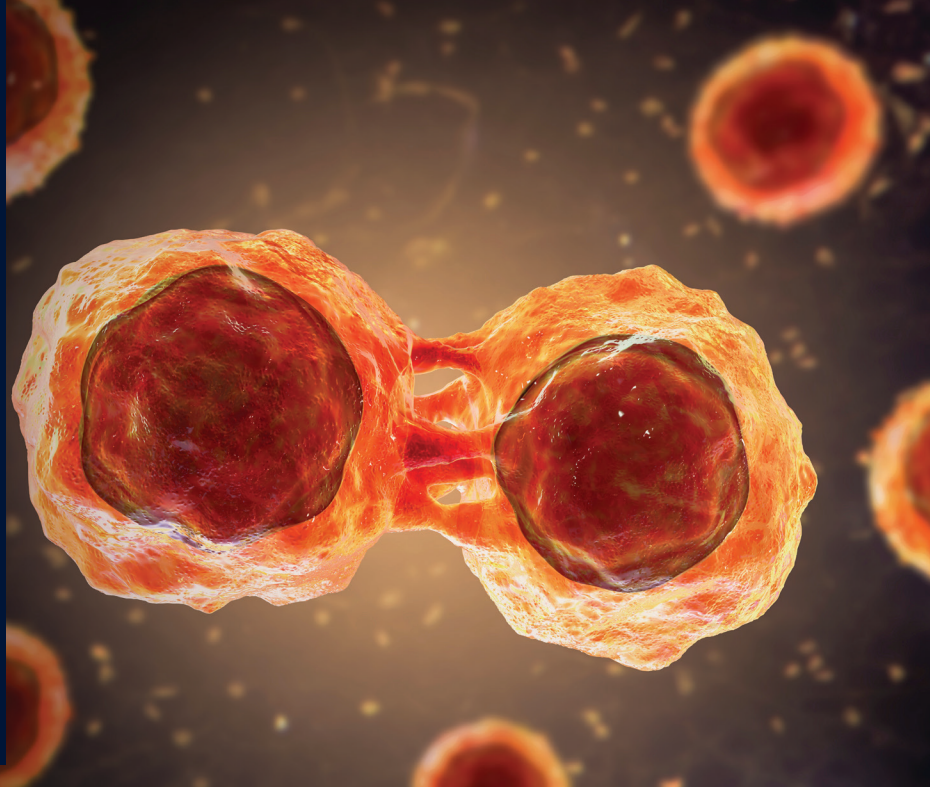
Syngene

Putting Science to Work

Overview

The discovery of induced pluripotent stem cells (iPSCs) has opened a new channel for novel drug discovery, disease modeling, and toxicity studies. As much as iPSCs are an attractive choice in the drug discovery space, it is very important to follow best practices for iPSC research in order to maximize their potential.

In this point of view, we discuss the rigorous measures established at Syngene for tissue procurement, iPSC reprogramming, systematic cell banking, inventory management, and quality control for every iPSC batch. These measures are crucial for ensuring the delivery of high-quality iPSCs across application areas to maximize drug discovery success.



Introduction

iPSC stem cells have shown immense potential in regenerative medicine, disease modeling, high throughput drug discovery, and *in vitro* toxicology studies. Currently, there are multiple clinical trials underway involving iPSC-based cellular products, mainly for visual disorders, neurodegenerative disorders, cardiovascular diseases, and metabolic disorders. Although iPSC-based cell therapy holds a lot of promise and has emerged as a novel modality to revolutionize the field of drug discovery, it requires extensive experience and stringent management to generate and deliver high-quality iPSCs to customers. Starting with high-quality iPSCs is the first step towards drug discovery success.

Key considerations for quality control

iPSC cell morphology

iPSC cells have a distinguished morphology, as shown in Panel A, characterized by compact colonies with a clear boundary and uniform morphology. A routine check of iPSC culture by morphology is the easiest and fastest way to ensure that your iPSC cell cultures are pluripotent. At this stage, it is very important to watch out for spontaneous differentiation of iPSC colonies, as seen in Panel B, where a patch of fibroblast-like cells (shown with red stars) would start to differentiate from one side of the colony, resulting in the iPSC colony no longer having a uniform boundary.

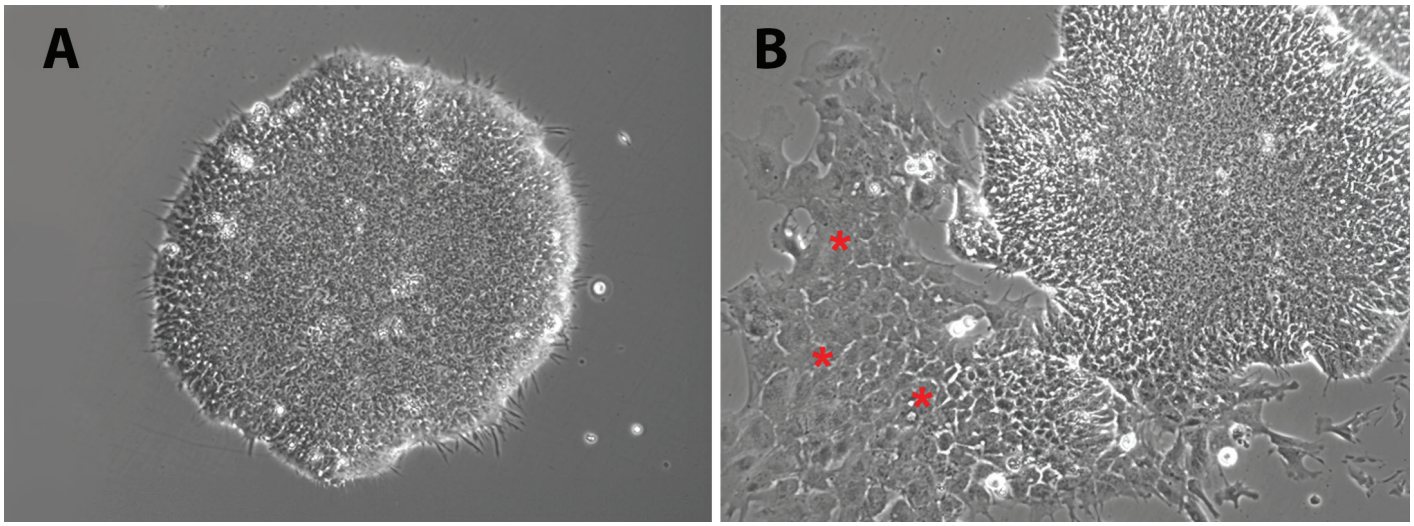


Figure 1: Representative images of iPSC morphology to visually distinguish between high-quality pluripotent iPSC colony (1A) and poor-quality differentiated iPSC colony (1B).

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iPSC pluripotency check

It is imperative to check iPSC cultures regularly for expression of pluripotency markers by Immunocytochemistry (ICC) or flow cytometry. Some common pluripotency markers tested include Oct4 and Sox2 (nuclear markers), SSEA4, and TRA-1 60 (cell surface markers). Below is a representative example of a pluripotency check of iPSCs by flow cytometry (A) and ICC (B). Both the assays presented below show that these iPSC cultures are highly pluripotent.

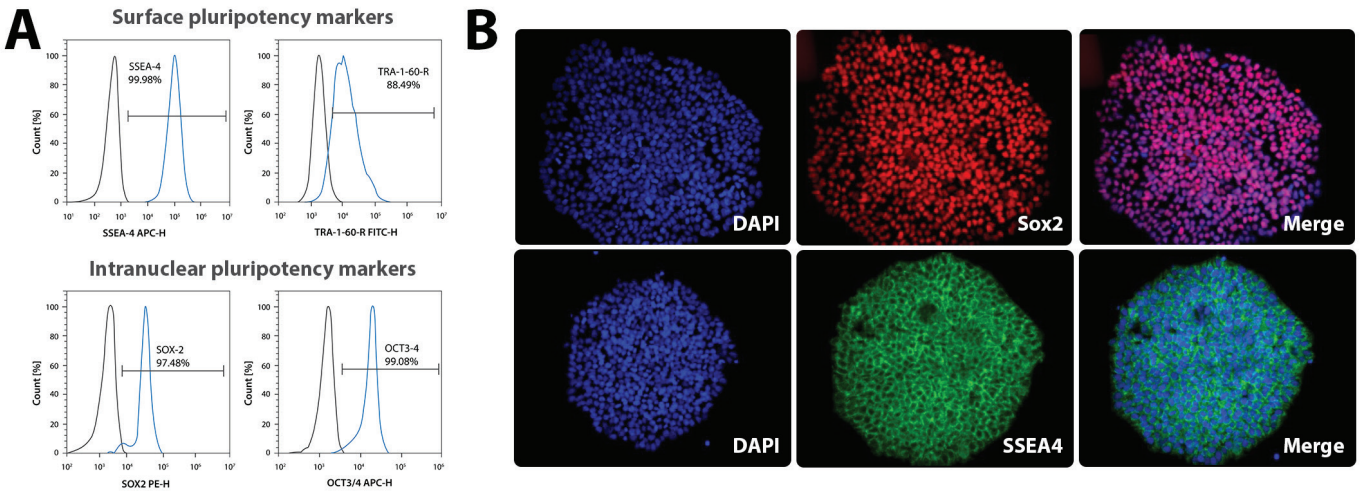

Figure 2: (A) Flow cytometry plots showing high expression of all four pluripotency markers.
(B) Immunocytochemistry (ICC) images showing strong expression of Sox2 and SSEA4 pluripotency markers in iPSC colonies.

Trilineage differentiation potential of iPSCs

As much as it is essential to check the iPSCs/iPS stem cells regularly based on the expression of pluripotent markers, it is crucial to access the differentiation potential of iPSCs into all three lineages (ectoderm, mesoderm, and endoderm). If iPSCs are not fully reprogrammed from the somatic tissue, they may still show high pluripotency but will not differentiate well into all three lineages.

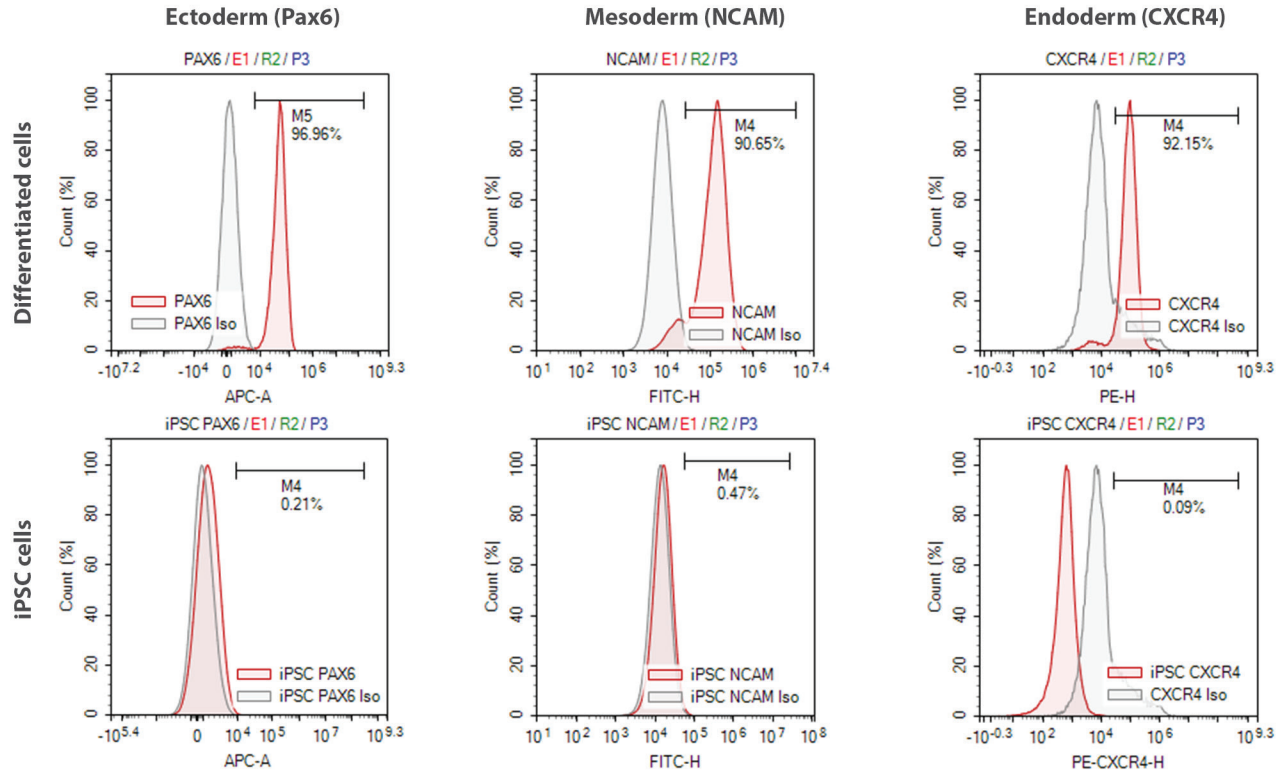

Figure 3: Flow cytometry plots showing the high expression of trilineage markers in differentiated cells.

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iPSC pluripotent stem cells were subjected to trilineage differentiation analysis, and the differentiated cell lineages were assessed by staining with lineage-specific markers either by flow cytometry (top panel) or ICC (bottom panel). Both the assays show that Syngene iPSCs can successfully differentiate into all three lineages with high purity. As part of quality control, iPSC cells are stained with lineage-specific markers. The absence of lineage-specific marker expression in iPSCs indicates the specificity of the studies.

Checking iPSCs for genetic abnormalities

It is vital to check iPSC cultures for any genetic abnormality after reprogramming and at regular intervals while maintaining the iPSC cultures. Karyotyping is one of the standard methods to test for any chromosomal abnormality. Other genetic testing methods commonly used are HLA typing and STR typing to keep the cell line authenticity in check. If these abnormalities are not checked regularly, it could impact the growth kinetics of iPSC culture. Any results produced from poorly characterized iPSCs can be misleading.

Sterility check of iPSCs

iPSC cultures must be routinely checked for bacterial, fungal, and mycoplasma contamination. Unlike bacterial and fungal contamination, which are easy to visualize, mycoplasma contamination is a silent killer. The mycoplasma contamination can go unnoticed for days together and could severely compromise cell health and growth kinetics. Syngene has sensitive PCR detection tools and a dedicated mycoplasma testing team to check all the iPSC cultures for mycoplasma contamination regularly. It is crucial to work with myco-free cultures to ensure the quality of the assays performed with the cells.

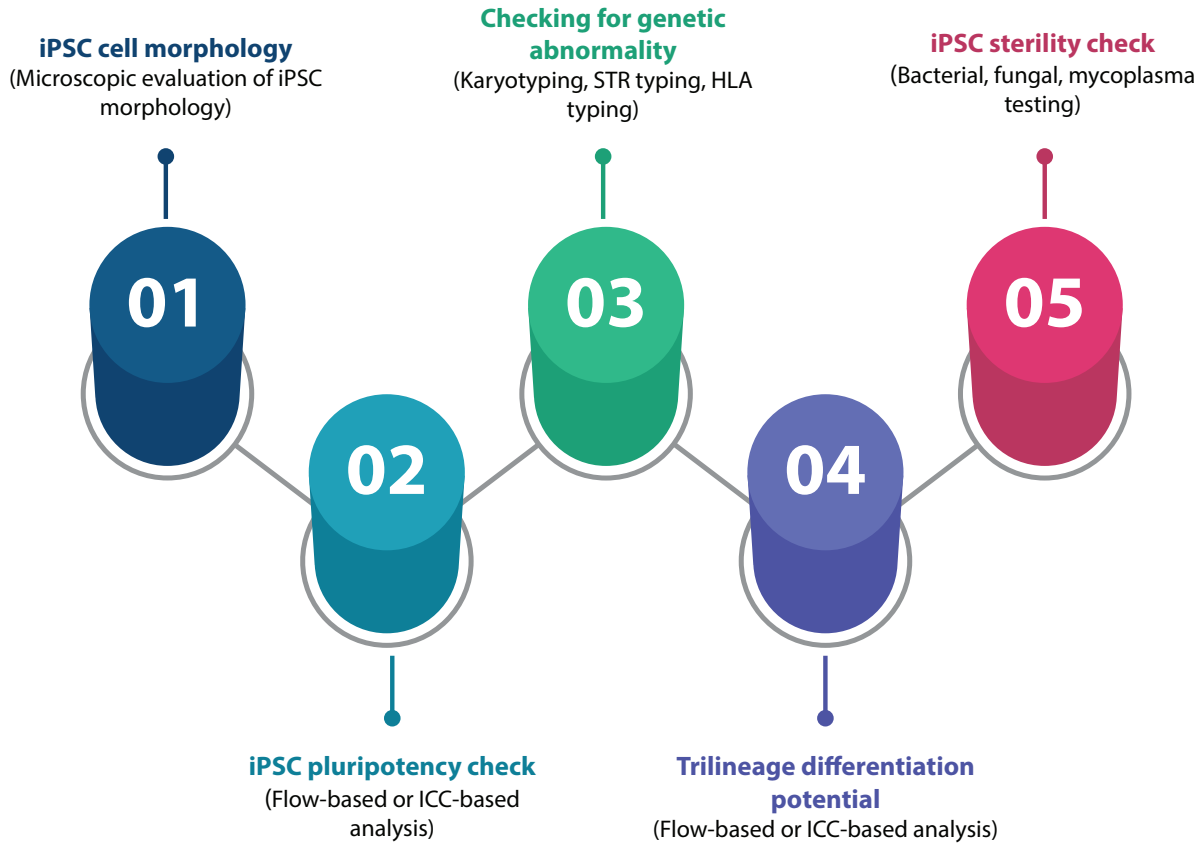

Figure 4: *Five pillars to ensure high quality iPSCs*

Syngene's capability in iPSCs

Syngene has an effective management system to generate and distribute premium quality iPSCs. The system comprises four sections:

1. iPSC generation

The first prerequisite for iPSC stem cell generation is to obtain human somatic tissues like PBMCs and skin fibroblasts. Syngene has MTAs with hospitals for the availability of starting material required for iPSC reprogramming. The donor recruitment methods, consent form, and sample collection procedures comply with ethical, legal, and regulatory authorities. We have a methodical screening process to screen donors to ensure the quality of each sample. Syngene has well-established standard operating procedures and technical expertise that guarantee high reprogramming efficiency and generates feeder-free, integration-free iPSC cells customized to customer needs.

2. iPSC characterization

Syngene has an extensive 11-parameter Quality checklist that involves

- Pluripotency check,
- Sterility testing,
- Chromosomal abnormality and authenticity

S.NO.	Test	Category
1	Morphology	Pluripotency
2	Pluripotent markers	
3	Trilineage differentiation	
4	Mycoplasma testing	Sterility testing
5	Endotoxin testing	
6	Sterility testing (Bacteria, fungi)	
7	Karyotyping	Chromosomal abnormalities and authenticity
8	HLA typing	
9	STR typing	
10	Sendai remnant check	To confirm absence of Sendai virus
11	Viability – Freeze thaw analysis	Viability check

Figure 5: Syngene's iPSC Quality checklist

3. iPSC banking

Each iPSC cell line at Syngene has a unique identifier to distinguish it. Each iPSC line nomenclature is linked to the donor identity for easy traceability. For each line, we have a fully characterized master bank and a working bank using all QC parameters. Our automated inventory helps us bank and dispense the vials seamlessly. All our iPSC stocks are stored in vapor phase LN2 storage tanks, the optimum storage condition for iPSC cells. All our LN2 tanks are carefully monitored 24/7 and have auto-refilling in case of temperature fluctuation to ensure high cell viability upon revival.

4. iPSC distribution

Syngene has a structured procedure to QC the iPSC cell stock before distribution. Clinical outcome assessment (CoA) is provided with every iPSC shipment that contains details of all the QC checks done on every batch of cells. These measures ensure that we deliver high-quality iPSCs without fail.

Conclusion

Using iPSC stem cells for biomedical research is challenging yet rewarding. iPSC-based research has opened several avenues for treating multiple diseases in the drug discovery space. Although there are many approaches to reprograming and maintaining iPSCs in culture, what remains crucial is maintaining the quality of the iPSCs.

Syngene has an experienced and dedicated iPSC team, including a state-of-the-art iPSC facility. Over the years, we have delivered several successful preclinical projects using iPSCs. Our systematic end-to-end procedure comprises required MTA, permissible donor consent, unique identification of each line with traceability to the respective donor, quality control of each iPSC line, batch CoAs, regulated inventory, and data management. This ensures the best quality iPSCs regardless of application area, making us a reliable partner for iPSC-based services to accelerate drug discovery.

About the authors

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Mamta Jain has 11+ years of experience in Drug discovery after completing her Ph.D. in Molecular and Cell Biology. She is currently leading the novel target validation, therapeutic antibody discovery, and iPSC research at Syngene. She has supported several clients in the drug discovery space at Syngene over the last three years. She has a Ph.D. degree from Jawaharlal Nehru Center for Advanced Scientific Research (JNCASR), Bangalore, and a post-doctoral experience from the European Union.

To know about our iPSC services, **contact our experts**

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About Syngene

Syngene International Ltd. (BSE: 539268, NSE: SYNGENE, ISIN: INE398R01022) is an integrated research, development, and manufacturing services company serving the global pharmaceutical, biotechnology, nutrition, animal health, consumer goods, and specialty chemical sectors. Syngene's more than 6000 scientists offer both skills and the capacity to deliver great science, robust data security, and quality manufacturing, at speed, to improve time-to-market and lower the cost of innovation. With a combination of dedicated research facilities for Amgen, Baxter, and Bristol-Myers Squibb as well as 2.2 Mn sq. ft of specialist discovery, development and manufacturing facilities, Syngene works with biotech companies pursuing leading-edge science as well as multinationals, including GSK, Zoetis and Merck KGaA.

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