Human pluripotent stem cell (hPSC) lines are now commonly used across the globe. In your lab, you might rely on hPSCs to uncover answers about human development, to study the effects of drugs, or maybe as a tool to delve deeper into human disease. As the body of knowledge around these cells grows, it is increasingly challenging to keep track of best practices, including standards for handling and information reporting. These discrepancies have downstream impacts on the reliability and reproducibility of research outcomes. Understanding the current state of hPSC research is the first step toward a collective effort to improve as a field.

To that end, STEMCELL Technologies conducted a survey of nearly 600 scientists, asking them to help highlight the needs and challenges in the hPSC field and to gather ideas on how to achieve greater reproducibility. From their responses, we learned a lot about how hPSCs are currently being used, and what can be done to increase the impact that they can have in fundamental research and clinical settings. Some key applications of hPSCs reported by respondents include disease modeling (65%), organoids (56%), genome editing (44%), reprogramming (37%), drug discovery and toxicity screening (37%), cell banking (33%), cell therapy research (28%), and suspension culture (14%). As shown by the sum of these percentages exceeding 100%, most researchers reported pursuing two or more applications, highlighting the importance of considering the entire workflow when embarking on hPSC-related research.
Directed differentiation of hPSCs to terminal cell types is a key component of many of the aforementioned applications. The most commonly derived cells were neural stem and progenitor cells (40%), mature neurons (36%), glial cells (22%), cardiomyocytes (21%), endothelial and angiogenic cells (15%), smooth and skeletal muscle cells (13%), hematopoietic cells (13%), neural crest cells (12%), and immune cells (10%). There were many other cell types reported, though none by more than 10% of respondents. The sum of percentages again exceeds 100%, clearly demonstrating that many researchers are differentiating hPSCs into more than one terminal cell type.

Despite the widespread use of hPSCs in research, when respondents were asked if reproducibility is a major concern in the field, 85% agreed or strongly agreed. The most frequently reported factors contributing to irreproducibility were cell line variability and inconsistent or incomplete reporting. While other factors, such as experimental design and operator variability, were rated as less critical, the vast majority of respondents still agreed that each factor contributed to some extent.

**What are the key factors that contribute to irreproducibility?**

- Complexity of protocols and/or experimental design
- Inconsistent quality control standards
- Inefficient protocols and/or experimental design
- Cell line variability

*Image illustrating cell types differentiated from hPSCs.*
Managing cell line variability during maintenance can be tricky, especially as most labs reported maintaining between 5 - 30 cell lines at any given time, with some respondents reporting over 500. While variation between cell lines is to be expected, proper reporting and standardized quality control measures can help limit variability and ensure that experimental results are valid.

To ensure the quality and consistency of downstream applications, it is critical to properly characterize your cell lines. A clear majority of respondents consider various cell quality attributes such as pluripotency, gene and marker expression, genomic integrity, and cell line identity as “critically important”. However, many are only performing a subset of screening tests. At a minimum, it is routinely recommended that all hPSC cell lines are tested for cell identity, sterility (including from mycoplasma), karyotype, expression of undifferentiated markers, and functional pluripotency. Performing only one or two tests may not be sufficient to determine the quality of hPSCs.

To learn more about hPSC quality or to read the in-depth results from our survey, please visit www.stemcell.com/psc-cell-quality.

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