TECHNICAL BULLETIN

Outsource Your Characterization and Banking of Human Pluripotent Stem Cells

At this time, the hPSC characterization and banking services are only available in the United States. For customers outside of the United States, please contact Customer Service at info@stemcell.com.

Introduction

The human pluripotent stem cell (hPSC) field has grown rapidly since the first isolation of human embryonic stem cells (hESCs) in 1998.[1] The subsequent discovery that human induced pluripotent stem cells (hiPSCs) can be derived by reprogramming adult somatic cells, along with development of more accessible and efficient genome editing techniques, have led to a vast increase in the number of hPSC lines being created and used globally. In 2018, it was estimated that over 10,000 different hPSC lines had been reported,[2] which has led to concerns about the lack of consensus on hPSC quality attributes and reproducibility between research groups.

At the beginning of 2020, STEMCELL Technologies conducted a survey asking scientists to help highlight the needs and challenges in the hPSC field and to gather ideas on how to achieve greater reproducibility. The survey had more than 550 respondents and 85% agreed that reproducibility is a major concern in the field. In the survey report, we found that 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 researchers report that they do not perform testing on all attributes they consider critical. Creating a fully characterized hPSC bank is vitally important to improve reproducibility and ensure the quality and consistency of downstream applications.

Characterize your hPSCs and generate cell banks quickly and reliably using pluripotent stem cell characterization and banking services from STEMCELL Technologies, offered in collaboration with WiCell®. Explore this technical bulletin to learn more about these services, as well as tips on how to prepare your samples to ensure the success of these services.

Types of Services Offered

Banking and Characterization Service

Save time and resources by outsourcing your human induced pluripotent stem cell banking and characterization needs to Contract Assay Services. Submitted samples will be thawed and undergo testing for basic quality attributes (karyotype, identity, and mycoplasma) while the banking process is completed to ensure any issues are identified early. You have the option to choose from 25-, 50-, or 100-vial bank sizes for your research needs. To ensure results are as expected, final characterization of your samples will be performed only after reviewing and communicating the initial results from the basic quality testing. Once confirmation is received, the bank will be fully characterized using the assays listed in the table on the right.

Characterization Service

Get timely and relevant characterization data for your existing hPSC bank or newly derived hPSC lines using Contract Assay Services' Characterization Service. By choosing this option, you can benefit from all the assays under the Banking and Characterization Service above, with the exception of sterility testing (mycoplasma testing included). Sterility testing results are highly dependent on the culture conditions prior to banking and the freezing medium used. Any contact with antimicrobial agents can mask any contamination present in the bank and produce a false negative result.

When choosing this service to characterize an existing bank that you have created, it is important to keep in mind that the service is being carried out on just one vial from the bank. Therefore, the submitted vial should be representative of the whole bank, and there should be no concerns over consistency of material from vial to vial or the quality of the bank (e.g. due to improper banking practices such as failing to vial from a pooled population). The characterization service can only provide information on the samples that are sent; thus, all responsibility lies with you as the sample provider to ensure this service is relevant. If there are concerns about the quality or consistency with the overall bank, please consider using our banking and characterization service to ensure optimal bank quality.

Assays Performed on Samples Submitted for Banking and Characterization

Assay	What It Detects/Analyzes
Thaw Testing	A vial from the bank will be thawed to confirm that the cells are recoverable
Short Tandem Repeat (STR) Analysis	STR polymorphisms for 15 loci plus amelogenin to confirm the identity matches the sample submitted**

G-T-L Karyotype*	• Microscopic genomic abnormalities (> 5 - 10 Mb)
	  • Inversions
	  • Duplications/deletions
	  • Balanced and unbalanced translocations
	  • Aneuploidies
	• 20-metaphase cell counts

SNP Microarray	Submicroscopic genomic abnormalities (< 5 Mb); results reviewed by an American Board of Medical Genetics and Genomics (ABMGG) board certified or board-eligible director
Sterility	• Bacterial or fungal contamination using direct transfer according to USP/EP guidelines • Mycoplasma contamination using PCR that can identify 96 species, including the 6 species that make up 95% of cell culture contaminations[3]
Undifferentiated Cell Marker Expression	Undifferentiated marker (OCT4, SSEA3, SSEA4, TRA-1-60, and TRA-1-81) analysis using flow cytometry
Pluripotency	Trilineage potential using directed differentiation of cells and assessed by flow cytometry for lineage-specific markers

**Assay performed by clinically certified cytogenetic technologists and reviewed by an American Board of Medical Genetics and Genomics (ABMGG) board certified or board-eligible director*

***For the banking and characterization service, STR analysis will be available for both the submitted sample(s) and the cell bank(s) for comparison purposes*

Preparing Samples for Services

The success and timeliness of the services provided rely on the quality of sample(s) being sent. For example, the service cannot be completed if the sample does not recover after thawing, has poor expansion, or the quality of culture is sub-optimal (e.g. high spontaneous differentiation). Below are recommendations on how you can prepare your samples to ensure the service can be performed in a timely and effective manner. The cell vials must be an appropriate size to be stored within a typical 10 x 10 cryobox (e.g. 1.8 - 2 mL cryovial).

Culture Conditions

The service will be performed on the cell culture system of your choice; currently we offer mTeSR™1 (Catalog #85850) or mTeSR™ Plus (Catalog #100-0276) on Corning® Matrigel®. It is common for some cell lines to perform better in certain culture systems than in others, and also for some cell lines to be more sensitive to changes in culture systems. As a result, it is highly recommended that the cell line be provided to us already adapted to the culture system in which you would like the service performed. We also recommend culturing the cell line for at least three passages to avoid any adaptation issues that may arise and subsequently delay the service. Explore how to choose a culture system that is most appropriate for your applications, as well as detailed protocols on how to culture hPSCs.

Cryopreservation

We recommend that hPSCs are cryopreserved as aggregates in an appropriate freezing medium (e.g. Cryostor® CS10 (Catalog #07930) or mFreSR™ (Catalog #05855)) to ensure an efficient thaw and subsequent expansion of material. There should be sufficient material to thaw the submitted vial of cells into one well of a 6-well plate (>1 x 10^5 cells).

Genetic Testing

It has been shown that hPSCs are prone to acquiring cytogenetic changes throughout their time in culture.[4] Studies have shown that these changes are recurrent and can occur sporadically throughout culture, providing genetically variant cells with a selective advantage. The recurrent karyotypic abnormalities detected in hPSC cultures are also observed in many human cancers, raising safety concerns for their therapeutic applications. It is therefore critical that researchers working with hPSCs monitor their cultures frequently to detect unwanted karyotypic changes early. To avoid unexpected cytogenetic results, we recommend screening your sample prior to submission for any recurrent abnormalities using the hPSC Genetic Analysis Kit (Catalog #07550). This is a rapid qPCR-based assay to detect eight recurrent abnormalities prevalent in hPSC cultures. To learn more about important cell quality attributes of hPSCs, visit our Cell Quality Resource Center.

Antibiotics

It is crucially important that any samples submitted for banking and/or characterization are free of any antimicrobial agents. Prior to submission, samples should be cultured for at least three passages in a culture system free of antibiotics; the freezing medium should also be free of antibiotics. The use of antibiotics in cell culture can mask underlying contamination and could affect the success of the characterization service and/or nullify sterility and mycoplasma testing results. All services provided are performed in a culture system free of antibiotics.

Additional Information

Biosafety*

- **Screened for human pathogens:** Samples submitted for testing should be generated from screened donors negative for infectious diseases, including HIV-1, HIV-2, HBV, and HCV.
- **Residual viral vectors:** Samples provided should be clear of any viral vectors used to derive iPSC lines.
- **Handling frozen cell products:** Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling frozen cell products, do not use sharps such as needles and syringes.

Shipping Information

- **Shipping samples for service:** Please refer to the *Submitting Cryopreserved Cells for Characterization and Cell Banking instructions* document. Customers are responsible for costs associated with shipping the starting cell material for the requested services.
- **Receiving banked cells following service completion (Characterization and Banking Service only):** Upon issuance of the service report, customers will be contacted to confirm the scheduled cell bank shipment dates. Our standard shipping date is Tuesday; cell bank shipments should arrive at your facility on Wednesday or Thursday. The cell bank will be returned in two separate shipments to reduce the risk of losing the entire cell bank in case shipping issues arise. If there is any remaining client-submitted starting cell material, those frozen vials will also be included in the cell bank shipment.

**THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.*

References

1. Thomson J et al. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391): 1145–7.
2. Guhr A et al. (2018) Recent trends in research with human pluripotent stem cells: Impact of research and use of cell lines in experimental research and clinical trials. Stem Cell Rep 11(2): 485–96.
3. Kazemiha V et al. (2014) Sensitivity of biochemical test in comparison with other methods for the detection of mycoplasma contamination in human and animal cell lines stored in the National Cell Bank of Iran. Cytotechnology 66(5): 861–73.
4. Amps K et al. (2011) Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol 29(12): 1132–44.