Establishment of a quality-driven manufacturing process to reprogram human donor material into human induced pluripotent stem cells (iPSCs) to support drug discovery

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Abstract

Since its discovery, induced pluripotent stem cell (iPSC) technology has been utilized by researchers seeking to build human-relevant *in vitro* disease models[1]. Human iPSC technology has enormous potential for drug discovery, enabling researchers to understand disease mechanisms and screen potential therapeutics on physiologically relevant cells at scale.

Key to unlocking the full potential of iPSC technology at scale is the establishment of an effective Quality Management System (QMS) to regulate the consistency, purity and functionality of iPSCs and their supporting media and supplements. At Axol Bioscience, we have spent the last decade investing in our quality manufacturing capabilities to create iPSCs under ISO 9001-accredited standards. Our QMS is key to this process, from establishing full donor licensing and consent to characterizing the endpoint cells for use in advanced *in vitro* models.

Framed by our experience generating over 50 lines at scale (both from healthy control and patient donor material) here we describe our experience establishing standardized reprogramming techniques and quality control checks of pluripotency, genomic stability and karyotyping. With these robust quality systems in place, iPSC reprogramming can be utilized at scale to support a new generation of robust *in vitro disease* models, to develop better therapies for patients.

Introduction

In 2006, Yamanaka et al. first described the development of pluripotent stem cells from the skin cells of adult mice[2]. Researchers have since built on this to produce iPSCs from adult skin or blood cells, which can then be differentiated into endpoint cells and used in advanced *in vitro* disease models, for research and drug discovery. The key to building better *in vitro* human disease models is ensuring stringent quality control procedures for the cells that power them; high-quality, consistent cells improve confidence in these models, generating useful data outputs for drug discovery.

Methods

Ethical Consent

We utilize full consent agreements and UK ethics-approved protocols when collecting donor samples, enabling genetic sequencing, gene editing and commercial use.

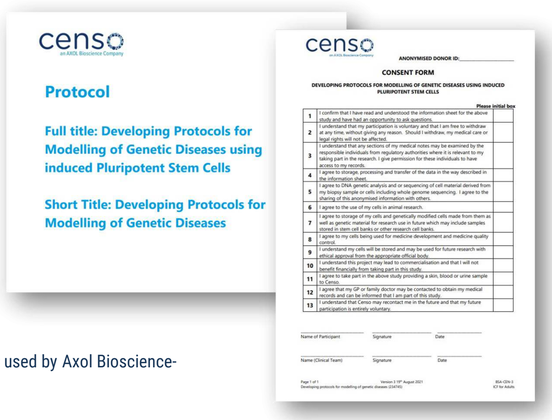

Figure 1. Primary tissue collection agreement protocol and consent form template as used by Axol Bioscience-affiliated collection sites.

Primary tissue reprogramming and validated clone generation

The flow chart below outlines the steps in the creation of high-quality iPSC lines, with the corresponding QC step (matched to those in table 1).

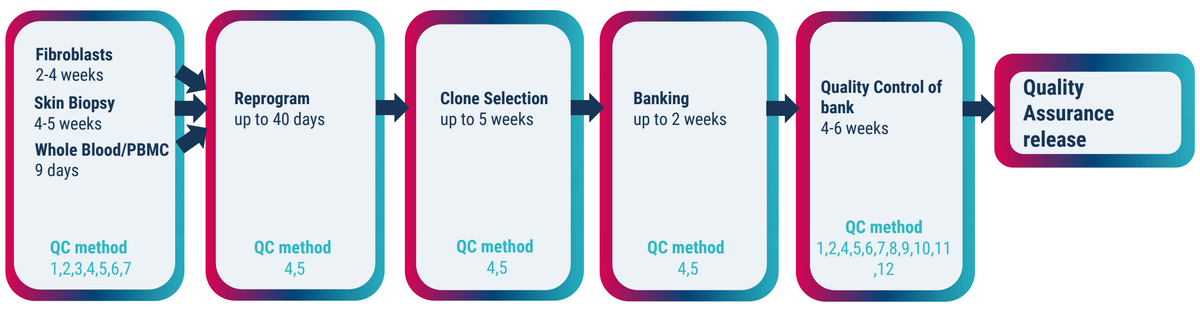

Figure 2. Flow diagram of iPSC creation showing the key steps and time frames. For each step, the corresponding quality control test(s) is highlighted.

Quality Control Test	Method	QC
Microbiology	Sterility by broth	1
	Mycoplasma PCR/qPCR	2
	Viral screen of primary cells	3
Viability/Growth profile	Visual record/Images	4
	Record of growth	5
Cell line identity	Genetic lesion	6
	Short Tandem Repeat analysis	7
Clearance of reprogramming factors	qPCR analysis	8
Karyology	G-Banding	9
	aGH analysis	10
Pluripotency assessment	Flow cytometry analysis	11
Differentiation potential	Embryoid body Scorecard	12

Table 1. The key Quality Control tests we typically use for iPSC generation.

We use Sendai virus methodologies to generate stable clones, enabling comparative data and standardization. Our scientists carry out STR analysis and genetic lesion confirmation on both the primary tissue and the resultant iPSC to ensure the sample is as expected and comparable. We also clearly define pass/fail criteria that align to those outlined by the ISSCR Standards Document[3].

Quality Assurance

A key aspect of our ISO 9001-accredited QMS is the full traceability and standardization of our work. We have developed controlled documentation, including the procedures and protocols we work to on our QMS, to ensure that all products are manufactured to the same high standards for each completed axoLines iPSC.

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QMS process

Key to our QMS is Q-Pulse, a system that we use for documentation storage and version control. We have programmed the system to hold all documentation types required for our quality processes. For iPSCs, the quality control checks are recorded in the associated standard operating procedure (SOP) and traced through the input of information requested on the corresponding form(s). Once signed off by a quality representative, a certificate of analysis (CoA) is created to confirm if the product has passed our specifications.

In the case of reprogramming human donor material into iPSCs, the scientist will fill out a report with their findings on the characterization and performance of the specific cell line. This report, together with the SOP, form(s) and CoA, is held and controlled on the QMS. This also enables us to pre-assign dates for data checks and/or ad hoc updates, ensuring that we are always keeping our processes and procedures up to date.

QC data generation and results

For every iPSC line we generate, we produce a certificate of analysis (CoA) summarizing results from our quality control (QC) procedures. Example data is presented below. Here we have used primary human fibroblasts from a patient with Nasu-Hakola disease to generate axoLines iPSC line Censoi078-A. This batch has passed all QC criteria and is therefore released for use.

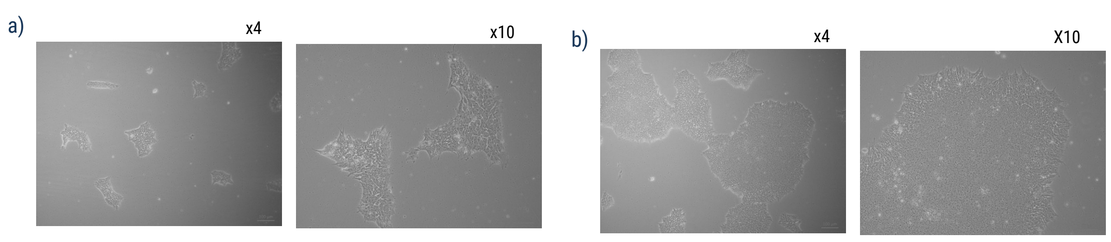

Figure 3. Microscopy results to characterize iPSC phenotype. We capture images at 24- and 48-hours post-thaw as well as pre-passaging, enabling comparison to future passages and to future working banks. a) Microscopy image at 4x and 10x magnification, 48 hours post-thaw, passage 22. b) Microscopy image at 4x and 10x magnification at confluency.

Quality Control Testing			
Test	**Test Method**	**Specification**	**Result**
Sterility	qPCR for mycoplasma	Not detected	Pass
	PCR for HIV-1, HIV-2, HBV and HCV	Negative	Pass
	Inoculation for microbiological growth	Not detected	Pass
Viability	Visual assessment	Growth to confluency in <7 days	Pass
Vector clearance	qPCR for Sendai backbone	Not detected	Not detected at passage 17
Confirmation of genetic mutation	Targeted sequencing of the relevant TREM2 region	Matches expected	Heterozygous TREM2, c.150G>T p.(Trp50Cys)

Table 2. QC results table. The sterility, viability and specification details are used to define the pass/fail status for the test methods shown.

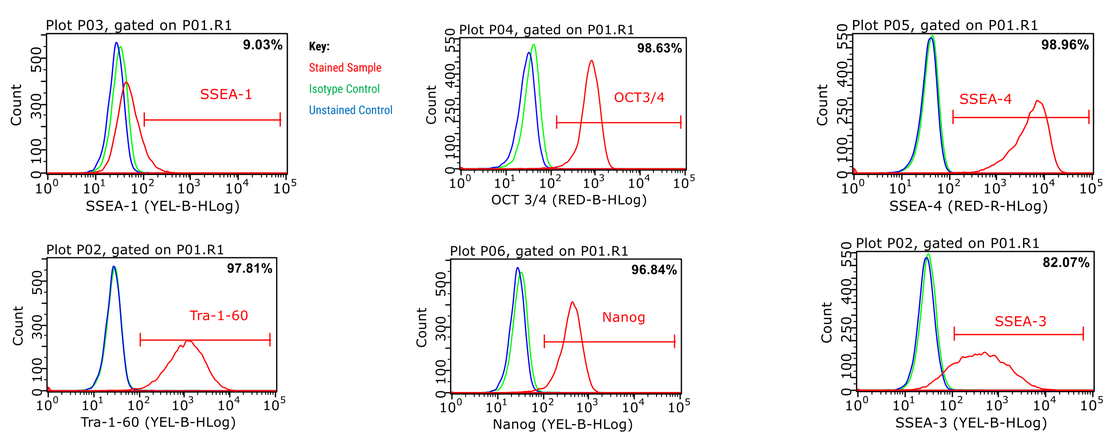

Figure 4. Flow cytometry data on stem markers to quantitatively define homogeneity of the clone. For each marker, specific antibodies are compared to unlabelled cells and isogenic control antibodies. The pass/fail criteria are shown below.

Test		Marker	Specification	% Positive	Result
Phenotype	Flow cytometry	SSEA-1 (differentiation marker)	<10%	9.03%	Pass
		OCT3/4 (pluripotency marker)	>90%	98.63%	Pass
		SSEA-4 (pluripotency marker)	>90%	98.96%	Pass
		TRA-1-60 (pluripotency marker)	>80%	97.81%	Pass
		NANOG (pluripotency marker)	>90%	96.84%	Pass
		SSEA-3 (pluripotency marker)	>70%	82.07%	Pass

Table 3. Flow cytometry data for each marker and the specification used to either pass or fail the clone.

a)

Quality Control Testing			
Test		**Specification**	**Result**
Cytogenetics	aCGH	No pathogenic Copy Number Variations (CNVs) detected	Pass
		No more than 5 aberrant CNVs have been detected which are >100kb and classified as non-pathogenic	Pass - 4 aberrant CNVs detected
		Mosaicism not detected	Pass

b)

Chromosome	Start	Stop	Size [kb]	Gain/Loss	# Probes	Classification
7	70'257'735	71'075'600	817.87Kb	Gain	53	Uncertain significance
8	1'531'691	4'972'779	3.44Mb	Loss	251	Uncertain significance
9	124'423'026	124'533'406	110.38Kb	Loss	9	Uncertain significance
20	8'639'165	8'784'975	145.81Kb	Loss	15	Uncertain significance

Table 4. Array CGH data analysis to define the karyology of the clone. a) Table of the specifications used to assess the data, using *in silico* comparison to known published genetic variants. b) Individual probe data for the 4 aberrant Copy Number Variations (CNVs) detected on analysis.

Conclusion

With over 10 years of experience in iPSC reprogramming, we have developed a quality-focused approach with an ISO 9001-accredited QMS. This has enabled us to manufacture over 50 lines from patient and healthy control donors, with full documentation, traceability and standardization.

By using the same reprogramming methods with rigorous quality control, comparative data can be drawn from line to line. This is the first step to building more human-relevant *in vitro* disease models at scale, in an array of functional assay formats including microfluidic devices and microphysiological systems.

Human iPSC technology has the potential to transform drug discovery through the generation of useful *in vitro* models at scale. But to realize this potential, we must prioritize the quality and consistency of the cells that power them, to ultimately produce better human disease models.

References

1 Rowe, R.G., Daley, G.Q. Induced pluripotent stem cells in disease modelling and drug discovery. *Nat Rev Genet* **20**, 377–388 (2019). https://doi.org/10.1038/s41576-019-0100-z
2 Scudellari, M. How iPS cells changed the world. *Nature* **534**, 310–312 (2016). https://doi.org/10.1038/534310a
3 www.isscr.org/standards-document

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