



Axol Bioscience Custom Cell Services

Stem Cell Reprogramming
Stem Cell Differentiation
Genome Editing
Sequencing
Cell and Tissue Sourcing

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Custom Cell Services at Axol

WE CAN HELP

We specialise in the supply of human cell culture systems and custom services for disease modelling and drug discovery.

Our expertise includes human induced pluripotent stem cell (hiPSC) generation, as well as providing a variety of custom services to support your individual research needs.

Our aim is to provide you with cutting-edge, physiologically relevant cellular models and systems that are reliable and easy to use, helping you to generate high quality, consistent results with your *in vitro* studies.

MULTIPLE OPTIONS

Find out more about the range of custom services open to you:

- Cell reprogramming - to generate footprint-free iPSCs from your donor cells
- iPSC differentiation - directed differentiation of iPSCs to neural cells, cardiomyocytes and other cell types
- iPSC genome editing - create isogenic and reporter cell lines for disease modelling and drug discovery
- Sequencing - to sequence human samples with any read type and read length
- Cell and tissue sourcing - from both healthy donors and patients, across a wide variety of disease types.

Testimonials

“Axol has provided us with more than a fast and reliable supply of functional cortical human iPSC derived neurons. It has also given us access to the collective expertise of Axol, whom we now view as much a collaborator as a company.

DR ERIC HILL, CLINICAL AND SYSTEMS
NEUROSCIENCE, ASTON UNIVERSITY

“Axol Bioscience has been a valued and important supplier to our lab's research since the company was founded. The cells supplied to us by Axol represent exceptional value for money, but, most importantly, save us a great deal of valuable research time. Throughout our dealings with the company, service and support have always been excellent.

DR PAUL CHARLESWORTH, PHYSIOLOGY,
UNIVERSITY OF CAMBRIDGE

“I've been able to grow all the cells that Axol generated for me from my patient lines. All the cells provided grew well and I'm very happy with the service as well as the quality of the cells.

DR DAVID MILLAR, HUMAN MOLECULAR
GENETICS, CARDIFF UNIVERSITY

Stem Cell Reprogramming

A COMPLETE SUITE OF SERVICES

Our custom reprogramming and characterization services empower you to focus on your research interests and not use up your valuable time and resources generating cells. Simply send us your donor cells (fibroblasts or peripheral blood mononuclear cells (PBMCs)) and we'll use cutting edge reprogramming technologies (non-integrating episomal or Sendai virus reprogramming) to produce

induced pluripotent stem cells (iPSCs) within a short period of time (usually 2 to 3 months). If you are struggling to find the right donor cells for reprogramming, we can also help you source fibroblasts and PBMCs with a wide range of donor backgrounds from our established network.

WHAT WE CAN DO FOR YOU

Our reprogramming service includes:

- ✓ Recovery, passaging, initial testing and banking of donor fibroblasts or PBMCs
- ✓ Cell reprogramming with non-integrating, zero-footprint systems:
 - i. Episomal vectors or
 - ii. CytoTune™-iPS 2.0 Sendai virus
- ✓ Analysis of pluripotency using immunocytochemistry and TaqMan hiPSC Scorecard Assay

Additional 'add-on' services include:

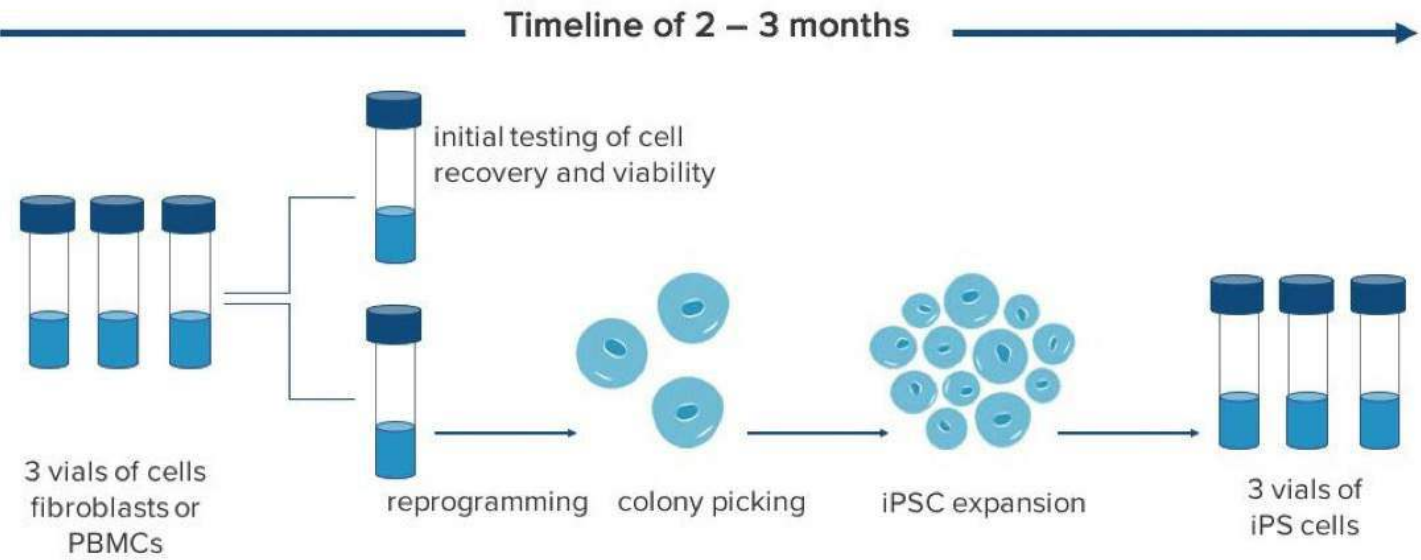
- ✓ Human pathogen testing
- ✓ Short-Tandem Repeat (STR) profiling
- ✓ qPCR analysis for pluripotency markers
- ✓ Karyotyping (G-Banding)

- ✓ Provision of more than one iPSC colony from a single donor
- ✓ Assessment of reprogramming vector clearance
- ✓ Checking expression of additional marker genes by immunocytochemistry
- ✓ Sequencing genomic regions of interest

Related services:

- ✓ Sourcing donor fibroblasts and PBMCs
- ✓ Adapting your iPSCs from a feeder layer to a feeder-free system
- ✓ iPS cell differentiation to multiple important cell lineages and supply of ready-to-use/cryopreserved cells
- ✓ Genome editing and creation of isogenic cell lines with CRISPR-Cas9

OUR WORKFLOW





Reprogramming Somatic Cells

REPROGRAMMING SOMATIC CELLS TO INDUCED PLURIPOTENT STEM CELLS (iPSC): A CELLULAR CAREER CHANGE

Somatic cells can be compared to human beings in that they grow up to perform a specific function in life. While a human being may develop into a world class athlete or a research scientist, a somatic cell can develop into any of the cell types that make up an organism except the germline cells.

Human beings and somatic cells are extremely fortunate to have so much choice available when it comes to deciding on a path, but what happens if we get bored of our chosen career? Although human beings have the option to re-train in a different profession, somatic cells require a little help from the experts to guide them smoothly into their next role.

HOW TO REPROGRAM CELLS INTO iPSCS

A career change for a somatic cell is more accurately referred to as reprogramming and involves conversion of the cell to an induced pluripotent stem cell (iPSC) before differentiation into one of many diverse cell types.

Reprogramming can be achieved by using vectors to integrate DNA into the cell's genome. This method has been shown to have success, but has its drawbacks. Potential issues include insertional mutagenesis and residual expression of reprogramming factors in cellular progeny. Integrating vectors hold little clinical appeal as their use does not preserve the original genomic integrity of the somatic cell.

The need to increase the therapeutic potential of iPSC has been one of the main drivers behind the development of non-integrating vectors. Many different strategies are under investigation, and currently the three most widely used non-integrating reprogramming systems are episomal vectors, Sendai virus vectors or mRNA transfection technology.

Episomal vector reprogramming uses components of the Epstein-Barr virus to facilitate delivery of reprogramming factors into somatic cells. This method is highly successful in generating iPSCs from peripheral blood mononuclear cells (PBMCs) and fibroblasts. It also enables a rapid loss of reprogramming vectors from the cells and is

non-viral so does not require a category II tissue culture laboratory. We use a licensed four-factor episomal vector reprogramming method from iPS Academia, allowing us to provide reprogrammed cell lines to our customers that they can use to advance their research endeavors.

Sendai virus is non-pathogenic to humans and is incapable of integration into the host cell genome. The success rate of Sendai virus vector reprogramming in PBMCs and fibroblasts is high. The technology is associated with low aneuploidy and, although slower, has been found to be a more efficient method than episomal vector reprogramming in generating iPSCs. We license the CytoTune®-iPS 2.0 Sendai virus technology from ID Pharma and offer customers who do not have their own license the opportunity to access a project-specific sub-license as part of our cell reprogramming contract service.

mRNA transfection technology relies on the use of mRNA rather than DNA to deliver reprogramming factors to somatic cells. Although more efficient than the two non-integrating methods discussed previously, the procedure is less popular due to its complexity. Careful consideration must be given to the design of the mRNA to limit activation of an innate immune response to foreign nucleic acids. Repeat administration is also necessary since mRNA only has a short half-life.

ADVANCING RESEARCH AND DEVELOPMENT WITH REPROGRAMMED iPSCS

Human beings have many reasons for wanting a career change, but why would a somatic cell choose to try something new? For somatic cells, it doesn't come down to choice, and for this reason, researchers should be thankful that the human body doesn't contain an HR department organ for somatic cells to complain to.

Reprogramming of somatic cells is instead performed by highly specialised and skilled scientists who are motivated by the possibilities which patient-derived iPSCs hold for disease modeling, drug discovery, regenerative medicine and cell therapy. For example, although continuous cell lines have been used as a research tool by pharmaceutical companies for decades, iPSC-derived cells provide a more biologically relevant model of the *in vivo* system. They also hold huge potential in the generation of patient-specific iPSC- derived tissue grafts, which will dramatically reduce the risk of immune rejection and improve the patient's quality of life.

CONCLUSION

The reprogramming of somatic cells into iPSCs holds great potential for the future, enabling scientists to push forward ground-breaking research and discover the next generation of therapeutics. Why not discover how you can take advantage of this amazing opportunity?

Choosing the Best Reprogramming Approach

Advances in cell reprogramming technology has catapulted the field of stem cell biology and its applications in disease research and therapeutic development, to where it is today. The initial discovery that adult somatic cells could be reprogrammed into human induced pluripotent stem cells (hiPSCs) (Takahashi et al. 2007; Scudellari 2016) has made pluripotent stem cell biology a flourishing research area. Cell reprogramming is not only enabling a better understanding of human disease pathways, but is improving the reliability of *in vitro* drug screening to boost the translatability of disease research into therapies that can directly help patients.

For example, cell reprogramming has led to the creation of hiPSCs carrying the genes of patients with specific disease types, offering researchers an innovative model to discover personalized treatments and further advancing the field of personalized medicine. Moreover, in disease therapy, autologous hiPSC transplants are being used to replace diseased or ineffective cells with reprogrammed healthy new cells, offering lifesaving opportunities to patients with serious blood disorders.

However, the clinical applications of this technology have only been possible since the crucial discovery of non-integrating reprogramming technologies. Unlike traditional integrating methods, these non-integrating systems are able to generate 'clean' hiPSCs that contain no trace of the vectors used to reprogram the cells, and so do not result in mutations within the host cell genome (Kang et al. 2015). As such, non-integrating reprogramming of human somatic cells has now become the gold standard approach for clinical applications.

Here we review the different non-integrating reprogramming systems available to researchers along with the approaches we use at Axol to generate hiPSCs from your cells. This custom service gives you the freedom to choose from different reprogramming technologies, cell origins, and culture systems, so you can acquire a rapid, reliable and costeffective supply of relevant hiPSCs that are best suited to your research objectives.

COMPETENCE

Which reprogramming technology should you use?

For the reasons stated above, at Axol, we use the Epi and SeV technologies to ensure we supply you with a fast, reliable and translatable source of hiPSCs. However, before you decide which one to use, it is essential to consider which of these technologies is best suited to your specific research needs:

Episomal vector reprogramming

Epi technology uses Epstein-Barr virus-derived sequences to facilitate episomal plasmid DNA replication in dividing cells (Yu et al. 2007). The Epi method has a high success rate in generating hiPSCs from healthy or diseased patient donor peripheral blood mononuclear cells (PBMCs) and fibroblast cells, with its reprogramming efficiency having undergone extensive improvements since its conception (Okita et al. 2011; Chou et al. 2011).

The resulting Epi-hiPSCs are ‘footprint-free’ because they exhibit a quick loss of reprogramming vectors, particularly when reprogramming fibroblasts (Schlaeger et al. 2015). This therefore offers an extremely reliable and efficient reprogramming strategy.

This non-viral reprogramming method utilizes Professor Yamanaka’s 4-factor reprogramming capabilities (Takahashi et al. 2007) and is licensed to Axol from iPS Academia, Japan. It does not require a Category II Tissue Culture Laboratory, nor a license for derivatives of the iPSCs.

Sendai-virus reprogramming

Additionally, we can produce hiPSCs using the CytoTune®-iPS 2.0 Sendai-virus (SeV) reprogramming technology (ID Pharma, Japan). Uniquely (as an Axol customer), if you do not have an ID Pharma SeV technology license, you can obtain a project-specific sub-license as part of our cell reprogramming contract service.

Additionally, given that the SeV method is licensed under clear regulatory and intellectual property (IP) guidelines, large pharmaceutical companies find this method particularly appealing.

Although it is a viral method, the Sendai-virus technology is non-integrating, because the RNA virus does not enter the nucleus of the donor cell, so is typically diluted out of cells after 10 passages post-infection (Malik & Rao 2013) (however, this does mean that the SeV method is a lot slower than the Epi method at generating hiPSCs).

The SeV method has been found to be reliable and more efficient than Epi (Schlaeger et al. 2015), requiring fewer starting cells and reprogramming cells within approximately 25 days at an efficiency of 0.1% for PBMCs and 1% for fibroblasts (Malik & Rao 2013). It also requires less time to generate ready-to-use colonies, and SeV-hiPSCs exhibit significantly lower aneuploidy rates than Epi-hiPSCs (4.6% versus 11.5%) (Schlaeger et al. 2015).

Table 1: The benefits of using feeder-layer and feeder-free systems of culturing reprogrammed cells using the Sendai-virus and Episomal vector reprogramming methods.

FEEDER-LAYER SYSTEM	FEEDER-FREE SYSTEM (AXOL'S RECOMMENDATION)
Increases the number of colonies produced	Eliminates the need to use animal materials
Once generated, colonies can be sustained as a stable pluripotent culture for a prolonged period of time <i>in vitro</i>	Better reliability in the culture medium and reprogramming processing, due to the removal of variable components in animal materials
Once generated, the hiPSCs can be adapted to a feeder-free system for easy maintenance, or to a feeder-layer system	Cost effective - no requirement to purchase feeder cells
	Easier maintenance
	Reduces culture time, because there is no need for cells to adapt to a feeder layer



Feeder-free (Axol’s recommendation) or feeder-layer culture system

You have the choice of using either a feeder-free or feeder-layer culture system to generate hiPSCs. The feeder-layer system uses a layer of inactive murine embryonic fibroblast (MEF) feeder cells during reprogramming, whereas the feeder-free system uses defined reagents from non-animal sources.

This is coupled with extracellular matrices to support cell survival, adhesion, and pluripotency, along with factors that promote proliferation. Both systems offer different benefits, so it’s important to consider which culture system best suits your research requirements (Table 1).

STARTING MATERIAL

Which cell should you use?

Many sources of somatic cells are used for generating hiPSCs, such as peripheral blood mononuclear cells (PBMCs), neuronal progenitor cells, keratinocytes, hepatocytes, but these cell sources vary in ease, efficacy, and cost (El Hokayem et al. 2016). We routinely offer reprogramming of two different cell types that are the most easily accessible and reliable in terms of success rates: fibroblasts and PBMCs.

Episomal vector reprogramming

Dermal fibroblasts were the first cell type to be successfully reprogrammed into hiPSCs (Takahashi et al. 2007) and fibroblasts are still widely used and accepted as the gold standard for reprogramming efficiency and differentiation (Li et al. 2014).

Alternatively, PBMCs are emerging as an effective and easily accessible, non-invasive source for patient-specific

hiPSC derivation (El Hokayem et al. 2016). A variety of studies have demonstrated the feasibility of using PBMCs as an accessible resource for cell reprogramming and modeling some diseases, such as Alzheimer’s disease (Yu et al. 2007; Táncos et al. 2016).

There are comparative advantages and disadvantages of using fibroblasts versus PBMCs as the cell source for reprogramming (Table 2) (El Hokayem et al. 2016). We recommend weighing these up before deciding which one to use for your specific research project.

Sendai-virus reprogramming

Whichever cell type you choose to reprogram, you can use our custom cell sourcing service or ready supply of cryopreserved fibroblasts or blood cells. Additionally, both types of cells can be used with either the Epi or SeV reprogramming technologies.

Table 2: The advantages (+) and disadvantages(-) of using fibroblasts and peripheral blood mononuclear cells (PBMCs) for reprogramming.

FIBROBLASTS	PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs)
Gold standard for reprogramming efficiency and differentiation (+)	Reprogramming efficiency is much lower than for fibroblasts. Can yield hiPSCs with undesirable germ lines and raises concerns about blood infections (e.g. hepatitis C, HIV) (-)
Involves an invasive biopsy of donor tissue by specialists (-)	Easily isolated by routine venipuncture by a phlebotomist, with minimal risk to the donor (+)
Have to be expanded <i>in vitro</i> to generate sufficient quantities of cells before reprogramming can occur (-)	Sufficient quantities can be extracted for immediate reprogramming, without the need for expansion <i>in vitro</i> (+)
	Convenient cell source from blood banks, and can be extracted from patients of any age (+)

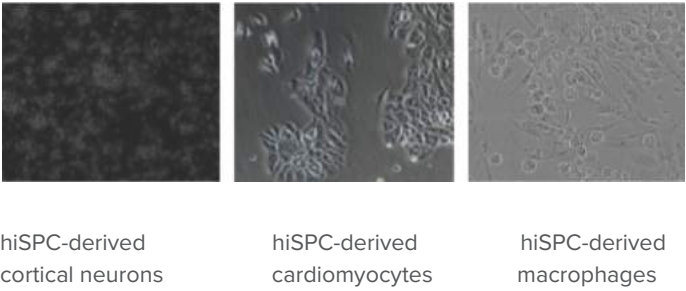
CONCLUSIONS

There are many options to consider when sourcing hiPSCs for your disease modelling research and cell therapy applications. It is clear that non-integrating reprogramming systems offer an advantage over integrating systems, as they provide high quality hiPSCs that typically do not contain any remnants of reprogramming vectors, which is a key requirement for clinical application.


Non-integrating episomal vector and Sendai-virus reprogramming technologies are reliable and efficient options for the generation of hiPSCs, and both fibroblasts and PBMCs can be re-programmed using these technologies. However, each system and cell type has its own advantages and limitations in the generation of hiPSCs, and these need to be assessed to determine which is best suited to your research needs when using our custom reprogramming service.

As experts in cell reprogramming and hiPSC differentiation, we offer collaborative custom services to source a fast, reliable and convenient supply of high quality hiPSCs. All our cells originate from one stable donor, providing you with access to consistently standardized cells. We can also differentiate and edit the hiPSCs (using CRISPR-Cas9 gene editing) to your desired final cell state and function (e.g., neural, immune and cardiovascular cell types) (Figure 1).

Figure 1: Axol can directly differentiate your hiPSCs into different cell types that you need for your research.



Therefore, without depleting your time and resources on generating the cells you need, you can ensure the success and reproducibility of your research, and potentially change the lives of many patients around the world.



Assessing the pluripotency and the differentiation potential of iPSCs

Once a somatic cell has been converted to an induced pluripotent stem cell (iPSC), the next stage in the process can be thought of as an appraisal or performance review. This is essential to confirming that the reprogrammed cell is behaving as expected.

Before a new iPSC line can be registered and banked, extensive characterisation is required. This involves rigorous testing to demonstrate that the iPSCs are pluripotent - able to give rise to cells from all three germ layers. Following this, the differentiation capacity of the cells is validated. The aim of characterisation is to ensure consistent and reproducible results across laboratories, and to address possible safety issues of stem cell-based therapies early in development.

DETERMINING THE PLURIPOTENCY OF IPS CELLS

There are several methods for assessing pluripotency potential. These include detection of an alkaline phosphatase which is specifically related to pluripotency, evaluation of various cellular markers via immunostaining techniques, and use of the widely-cited teratoma assay. In addition, several bioinformatic methods have evolved, providing a more cost-effective methodology to the latter.

Alkaline phosphatase (AP) expression

AP expression is easy to determine since the enzyme can convert a soluble colorimetric reagent to a precipitated state, yielding a rapid visual readout. Placental alkaline phosphatase (hPLAP) is a form of AP that is related to pluripotency and is easily distinguished from other APs through the incorporation of a high temperature step into immunostaining protocols. hPLAP can withstand high temperatures (68°C) which inactivate endogenous APs. This allows its expression to be easily characterized and used as an assay to assess pluripotency.

iPSC maker expression

Marker expression is another approach which is used to confirm pluripotency and is typically performed once hPLAP expression has been identified. Immunostaining techniques are used to detect a panel of markers specific to iPSC physiology and to maintaining these cells in an undifferentiated state. In humans, these markers include octamer-binding protein 4 (Oct4), the transcription factors Nanog and Sox2, tumor-rejection antigens Tra-1-60 and Tra-1-81 and stage-specific embryonic antigen-3 and -4 (SSEA3 and SSEA4), while SSEA1 is commonly used as a negative control. In mice, the main markers of pluripotency are Oct4, Nanog, Sox2 and SSEA1. It is important to point out that such immunostaining data requires careful interpretation. For example, Oct4 exists as two splice variants, Oct4A and Oct4B, yet

only Oct4A is related to pluripotency. It is essential that any Oct4 antibodies that are employed recognise only Oct4A to avoid false positive results.

Teratoma assay

One of the most frequently used methods for evaluating pluripotency is the teratoma assay. This is based on the ability of iPSC to form teratomas (tumours composed of cells derived from all three of the germ layers) in immunodeficient mice. Although currently considered to be a gold standard approach, the teratoma assay is time-consuming, technically challenging and difficult to standardise. A further drawback is that it is not scalable to the increasing number of iPSC lines that are being created. For these reasons, there is a significant need for the development of a cost-effective and animal-free alternative. PluriTest and ScoreCard, developed in 2011, are two potential alternatives.

PluriTest and ScoreCard are bioinformatic assays which provide a molecular signature for pluripotency based on gene expression profiles. The PluriTest database contains transcriptional profiles derived from over four hundred diverse stem cell preparations by microarrays. The ScoreCard database was produced by applying three genomic assays (gene expression profiling, DNA methylation mapping and transcript counting of lineage marker genes in embryoid bodies) to approximately thirty previously derived human embryonic stem cell lines and iPSC cell lines. Both assays provide a reference against which unknown cell lines can be compared and have seen considerable success. ScoreCard has recently evolved to incorporate qPCR, enabling faster, more quantitative assessment of functional pluripotency.

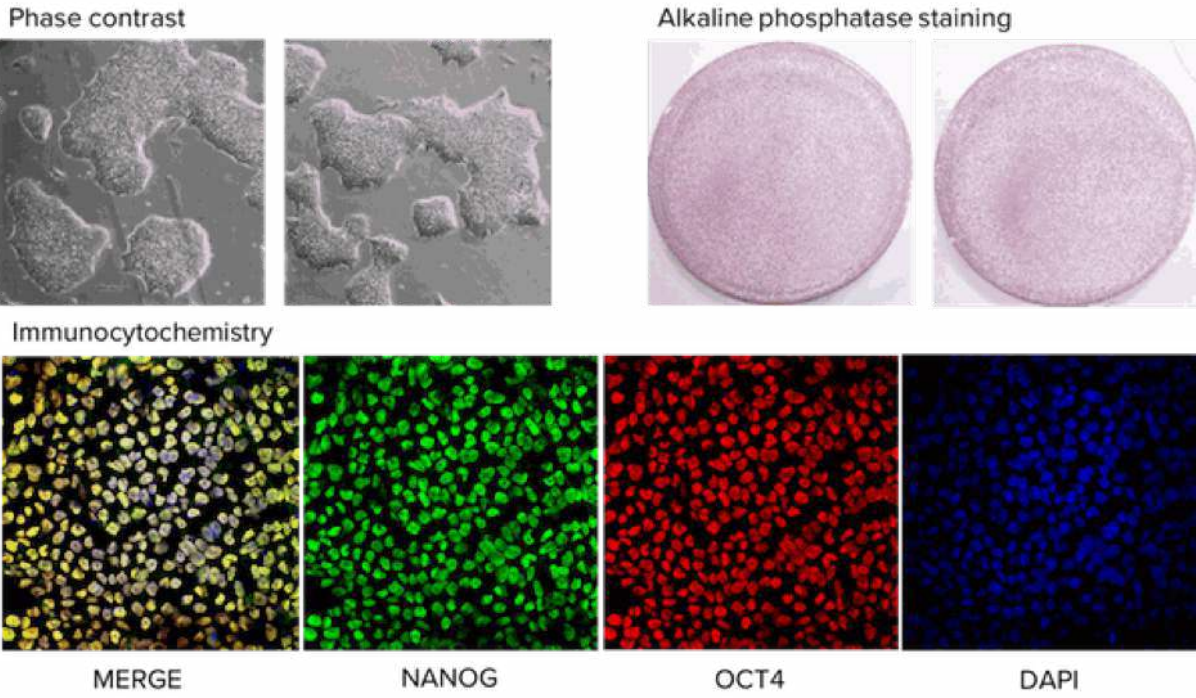
ASSESSING THE STABILITY AND DIFFERENTIATION OF iPSC CELL LINES

Once it has been established that an iPSC line is pluripotent, it is necessary to demonstrate that the cells can form tissues derived from the three germ layers of the embryo. If the differentiation test is performed *in vitro*, the cells are cultured in suspension until they form aggregates known as embryoid bodies. Pluripotent cells will spontaneously differentiate into cell types derived from the mesoderm, ectoderm or endoderm. During an *in vivo* differentiation test, the cells are injected into severe combined immunodeficient (SCID) mice. Pluripotent cells will proliferate and differentiate, ultimately forming a teratoma.

Prolonged culture of iPSC is associated with genetic abnormalities and so karyotype analysis is used to show

that new cell lines have maintained genetic stability. By arresting the cells in their metaphase and then staining, chromosomal abnormalities can be observed. DNA fingerprinting can also be used to investigate the genetic stability of a new cell line through the analysis of short tandem repeats (STRs), which will be unique to each cell line and allow for cell identification. This form of genetic assessment can add an additional level of confidence to conclusions drawn from karyotyping.

Bioinformatic methods for determining pluripotency potential, as analysed below.



CONCLUSION

A diverse range of assays and techniques are available to assess the pluripotency of a newly isolated iPSC line. Employing these methods will help you to assess this and ensure that the cell line can differentiate into tissue derived from the one of the three germ layers.

Stem Cell Differentiation

COST-EFFECTIVE SOLUTIONS

We provide cost-effective services to generate multiple cell types from your iPSCs. Our protocols are tailored for large scale production.

The majority of our culture conditions are fully defined in order to achieve both a high differentiation efficiency, and level of purity whilst retaining minimal lot-to-lot variation.

The turnaround time for directed differentiation is approximately 2 months. At least three frozen vials of iPS cells per sample will be needed. One vial will be thawed and tested for mycoplasma. We will start the differentiation process once the quarantine is completed.

WHAT WE CAN DO FOR YOU

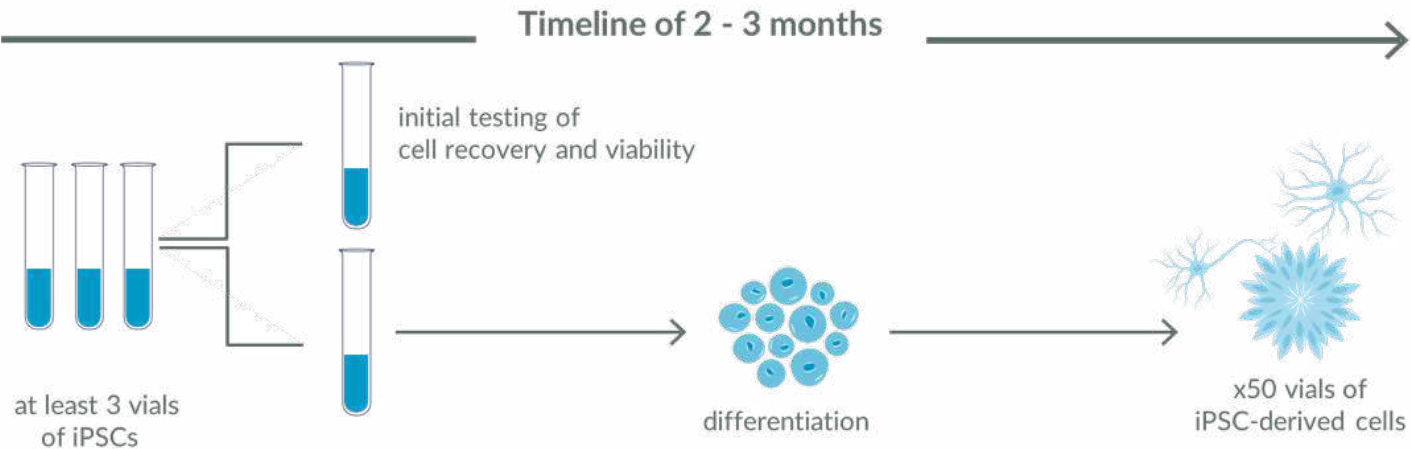
Our iPSC differentiation capabilities include:

✓ Directed differentiation of iPSCs to many cell types:

- Sensory neuron progenitors
- Motor neuron progenitors
- Astrocyte progenitors
- Mature astrocytes
- Dopaminergic neuron progenitors
- Ventricular cardiomyocytes
- Atrial cardiomyocytes
- Macrophages
- Cerebral cortical neural stem cells
- Cerebral cortical neurons

- ✓ Optional electrophysiological characterization of neurons and cardiomyocytes via multi-electrode array (MEA)
- ✓ Characterization of all iPSC-derived cells using immunocytochemistry to confirm the expression of lineage-specific markers
- ✓ Rigorous quality control checks to ensure all cells are free from contaminants and have achieved optimal post-thaw viability prior to shipping
- ✓ Ongoing technical support and a range of tailored media to ensure the optimal culture of all our iPSC-derived cells

OUR WORKFLOW



Differentiating iPSCs into Derivatives of the Embryonic Germ Layers

THE ORIGIN OF SOMATIC CELLS

Somatic cell reprogramming offers huge potential for disease modelling, drug discovery, drug development, regenerative medicine and cell therapy. Following the generation of iPSCs, various growth factors or small molecules can be used to drive the formation of a multiplicity of cell types. Optimization of these methods has been the focus of the many research groups endeavoring to develop robust and reproducible methods of differentiation. But where do these valuable career-changing cells originate from?

To answer this question, we must consider the germ layers. These are three distinct layers of cells produced during the gastrulation stage of mammalian embryonic development, which give rise to every organ and tissue within the body. All animals, including humans, form three germ layers, known as the ectoderm, endoderm and mesoderm. Different cell lineages evolve from each layer, resulting in mature somatic cells which perform organ- or tissue-specific functions. The iPSCs generated by reprogramming these mature somatic cells have the capacity to develop into cells from all three germ layers.

LAYERS OF COMPLEXITY

Now that we have the iPSCs, how do we encourage a pluripotent cell line into a specific tissue type derived from one of the three germ layers, ensuring that we maximize a cell's career potential? In this blog we will delve in and discuss how this is possible.

THE ECTODERM: POTENTIAL IS NOT ONLY SKIN DEEP

The ectoderm is the outermost of the three germ layers. It gives rise to many outer regions of the body such as the epidermis, hair, nails, mouth epithelium, cornea and olfactory epithelium. The central and peripheral nervous systems are also derived from the ectoderm.

Differentiation of iPSCs into the embryonic ectoderm and its derivatives has huge potential in wound healing. A 2015 study by Zhang et al differentiated iPSC into mesenchymal stem cells (MSC), which are well-documented as being key players in repairing and regenerating damaged tissue. MSC can develop into bone, cartilage, and fat cells, and are employed in many current stem cell therapies focused toward healing.

Following characterization by flow cytometry for surface markers including CD29, CD73, CD90, CD34, CD45, HLA-DR, the authors generated exosomes from the MSC. These were subsequently injected around wound sites in a rat model, where they were shown to facilitate wound healing through promotion of collagen synthesis and angiogenesis.

THE ENDODERM: FILLING THE VISCERA

The endoderm is the innermost germ layer, from which many of the internal linings of the body are derived. These include those of the gastrointestinal tract, lungs, liver and pancreas.

Cells derived from the endoderm offer therapeutic potential for conditions including diabetes and liver failure, yet the generation of such cells is technically challenging. At present, few protocols produce functionally relevant mature, adult cells. A 2014 study by Takeuchi et al showed promise in the generation of insulin producing cells (IPCs), which represent a valuable tool for cell therapy and drug discovery in diabetes. The authors established a robust method of producing mature iPCs via induction of DAZL-expressing (DE) cells and then PDX11 cells, which secreted insulin in response to glucose stimulation in 3D culture.

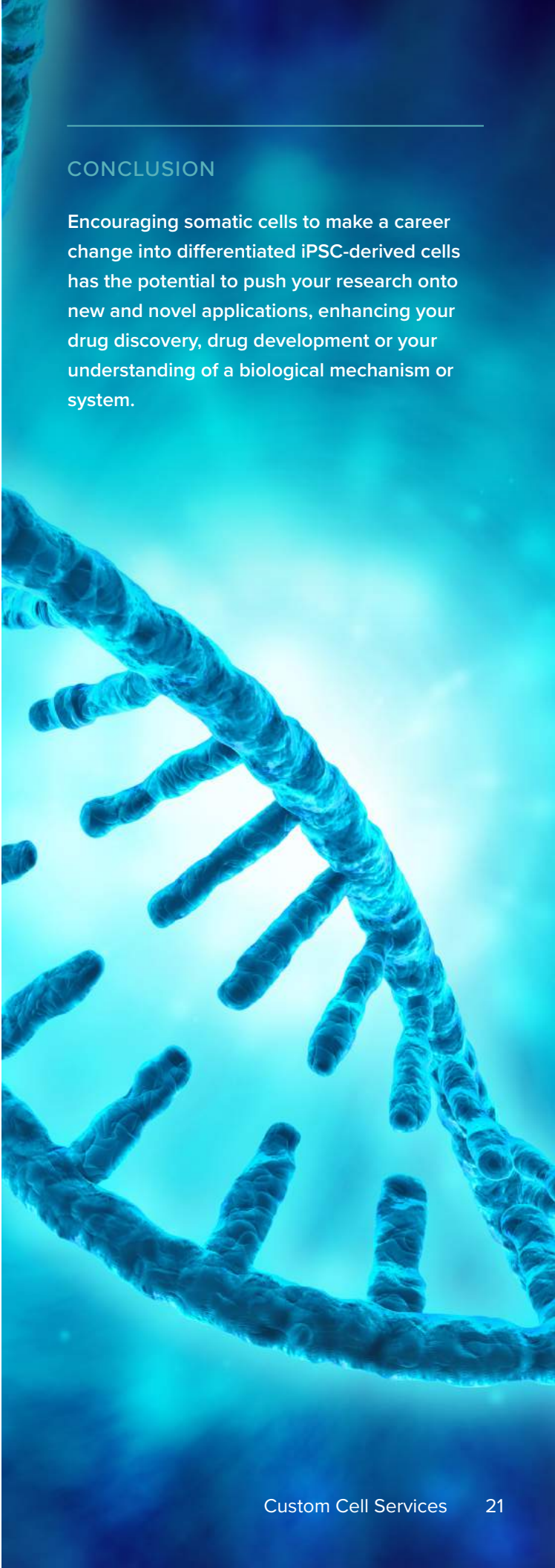
THE MESODERM: THE POTENTIAL WITHIN

The mesoderm lies between the ectoderm and the endoderm, and from this all other tissues of the body are formed. These include the dermis, heart, muscles, bones, bone marrow and the blood.

iPSC differentiation into cell types formed from the mesoderm has been studied across many research fields. Cardiomyocytes and kidney proximal tubules offer translational models for toxicity studies , while endothelial colony forming cells (ECFCs) are ideal for furthering our understanding of angiogenesis and tumor vascularization . A 2017 study byZuppinger et al demonstrated the translational potential of iPSC-derived cells by reporting that the proteins expressed byprimary cardiomyocytes matched those expressed by Axol's ventricular cardiomyocytes following ten days in culture . Axol's cardiomyocyte cells are a highly pure population, expressing a range of ventricular cardiomyocyte markers. Furthermore they exhibit appropriate action potential pharmacology of the three core cardiac ionic currents (I Na , I CaL , and I Kr) for physiologically relevant drug safety testing.

CONCLUSION

Encouraging somatic cells to make a career change into differentiated iPSC-derived cells has the potential to push your research onto new and novel applications, enhancing your drug discovery, drug development or your understanding of a biological mechanism or system.





Genome Editing

ACCURATE CELLULAR MODELS

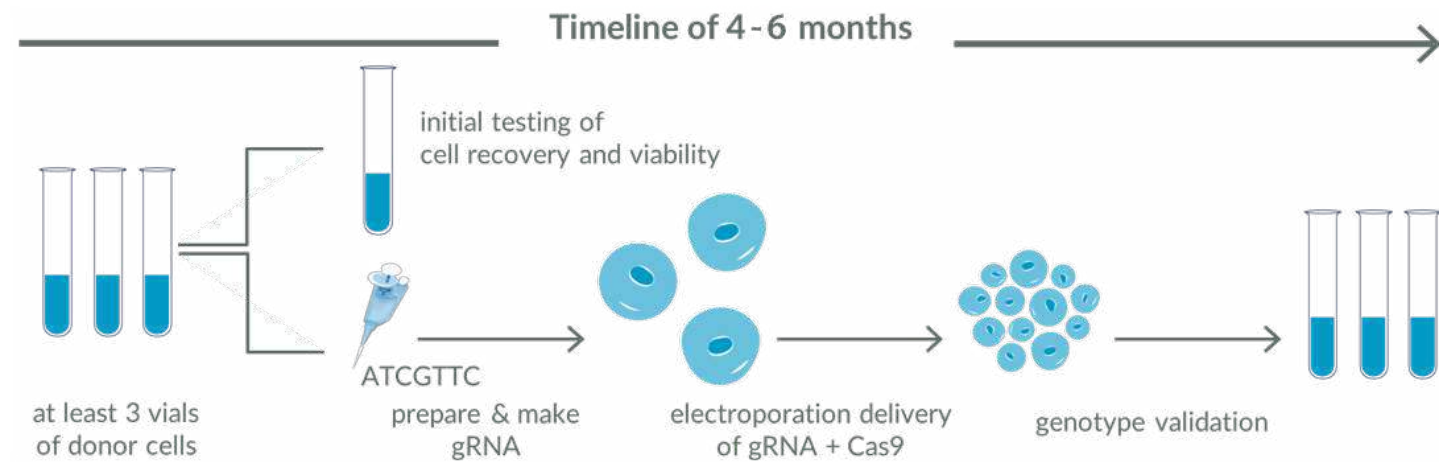
Genetically edited iPSC-derived models can help researchers better understand how specific genes contribute to disease pathogenesis at the molecular and cellular level in a tissue-specific nature.

Furthermore, they represent valuable platforms for identifying drug targets and studying drug mechanisms of action, paving the way for developing new therapeutic interventions for many genetic diseases that are currently untreatable. Our custom cellular reprogramming service and directed differentiation of iPSCs offers the possibility of generating many disease models from any genetic background.

By combining the power of CRISPR-Cas9 genome editing with our iPSC technology, we can create accurate cellular models of genetic conditions by introducing disease-causing mutations into healthy control cell lines.

We can also correct those mutations in genomes of patient-derived cells to provide the matched control cell line.

OUR WORKFLOW



WHAT WE CAN DO FOR YOU

Our custom iPSC genome editing service includes:

- ✓ Optimized and highly efficient gene editing workflow for iPSCs
- ✓ Screening a selection of gRNAs and resulting cell clones
- ✓ Sequence verification of genotypes engineered:
 - Homozygous and heterozygous knockouts
 - Homozygous and heterozygous point mutations
 - Tagged reporter cell lines
- ✓ Pluripotency assessment of gene-edited cell lines.

LET US KNOW

Do you have any questions about our cell genome editing services?

Our dedicated Services team can help you. Our custom iPSC genome editing services can be tailored to meet your experimental requirements, helping you to save time and resources.

Sequencing Services

Our Sequencing Services can help you access the latest technology to sequence Human samples, with any read type and read length you need. We can offer a variety of options to identify the best sequencing solution for you.

Our Sequencing Service includes:

✓ **Whole Genome Sequencing:**

Whole genome sequencing allows for the identification of the DNA sequence of the whole organism. Whole genome sequencing is performed using the shotgun sequencing method developed during the Human Genome Project launched in 1990 and complete in early 2000s. Libraries can be constructed either with PCR amplification or using a “PCR-free” method if enough material is available. PCR-free library construction is recommended to minimise possible bias that can be introduced during PCR amplification. Sequencing of the libraries is performed using 2x150bp paired end reads on an Illumina sequencing platform. The standard coverage targeted is 30X.

✓ **Whole Exome Sequencing:**

For projects which do not require information from the non-coding regions of the human genome, whole exome sequencing (WES) provides a great alternative. The library can be sequenced to your specifications required for your analysis.

✓ **RNA Sequencing:**

RNA sequencing / RNAseq can be used to gain insight into gene expression. RNA is first converted to complementary DNA (cDNA) before sequencing, this subsequent result reveals the relative abundance of RNA within the sample.

Axol offer a range of RNAseq services that includes:

- i. mRNA sequencing: Poly A enrichment is carried out using an oligo(dT) primer
- ii. Total RNA sequencing: First strand cDNA synthesis is carried out using random primers of which, subsequently, all the various RNAs can be generated (e.g. lncRNA, tRNA, mRNA, rRNA)
- iii. Ribosomal RNA depletion: Ribosomal RNA (rRNA) is removed following total RNAseq resulting in sequencing data only containing the complete transcriptome of coding and non-coding RNA species

✓ **Ready to Sequence Libraries:**

For researchers who need to turn constructed sequencing libraries into raw sequencing data, we offer ready to sequence libraries. Constructed libraries can be sent as FASTQ.gz analysis using any desired Illumina run configuration.

✓ **Amplicon Next Generation Sequencing:**

Next Generation Sequencing (NGS) allows a variety of samples to be analysed in parallel, eliminating the need for isolating or culturing a single sample prior to sequencing. Instead, diverse samples can be analysed in parallel to gain insight into heterogeneous starting material. Human material will undergo targeted sequencing of the 18S region, which are amplified using PCR.

✓ **Long Reads:**

When conducting NGS, long read lengths can be of benefit when researching highly repetitive genomes, as having a longer read length can be more of an advantage than base quality. The PacBio sequencing platforms offer read lengths of many 1,000s of bases in length using “Single Molecule, Real Time” chemistry. The platforms can get reads up to 40-60kb in length, and the Sequel offers a higher output and better value with read lengths that currently fall below 20kb.

WHAT WE CAN DO FOR YOU

Our Sequencing Service includes:

- ✓ **Design:** At Axol we can help you design your sequencing project from sample preparation to data analysis and delivery.
- ✓ **Sample Preparation:** As a human cell specialist, we are experienced in handling cells and extracting DNA and RNA. We can carry out DNA & RNA extraction, library construction and pooling plus full QC analysis.
- ✓ **Sequencing:** We give you access to the latest sequencing technology and the most up-to-date platforms including Illumina, PacBio and Ion Torrent, all with a fast turnaround time.
- ✓ **Data Analysis:** Your results are stored securely and can be transferred rapidly in a way which suits your requirements.

Human Cell and Tissue Sourcing

SOURCE OF CELLS AND TISSUES

Finding the right cells for your experiments can be difficult, especially when studying rare diseases or when specific donor inclusion/exclusion criteria are required.

On top of this, it can be particularly tough to source primary human cells and tissues, but these physiologically relevant cellular models can better reflect the biology of the tissue or disease you are studying.

So, let us find the cells and tissues for you.

By listening carefully to your needs, we can source biological specimens, such as blood and tissues, for a

wide range of clinical indications, including autoimmune, neurological, pulmonary, cardiovascular, skin, kidney and bladder disorders as well as liver cirrhosis.

We also specialize in sourcing primary cancer tissue for a broad range of cancers. Many of the primary cancer tissue samples are collected prior to treatment, so the tissue is treatment-naïve. This reduces potential unexpected variables when studying the effects of anti-cancer compounds on primary cancer cells.

All our biospecimens are:

- ✓ Anonymized
- ✓ Obtained with fully informed consent from the donor or the donor’s next-of-kin, with ethics committee or IRB approval
- ✓ Screened for human pathogens such as HIV and hepatitis viruses
- ✓ Provided as tissue samples either freshly frozen or formalin-fixed and paraffin-embedded.

WHAT WE CAN DO FOR YOU

Our custom cell and tissue sourcing services cover all the potential needs:

- ✓ Provide physiologically relevant cellular models that better reflect the biology of the tissue or disease you are studying
- ✓ Source specific donor cells and tissues (from more than 40 tissue types) from healthy donors and patients across a wide variety of disease indications
- ✓ Source specific donor cells and tissues from patients across more than 100 disease types
- ✓ Provide cells and tissues that meet all the data protection and ethical standards required in the UK.

LET US KNOW

If you have any questions about Custom Cell Services then please do not hesitate to ask.

Our dedicated Services team can help you. Our custom iPSC services can be tailored to meet your experimental requirements, helping you to save time and resources.

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