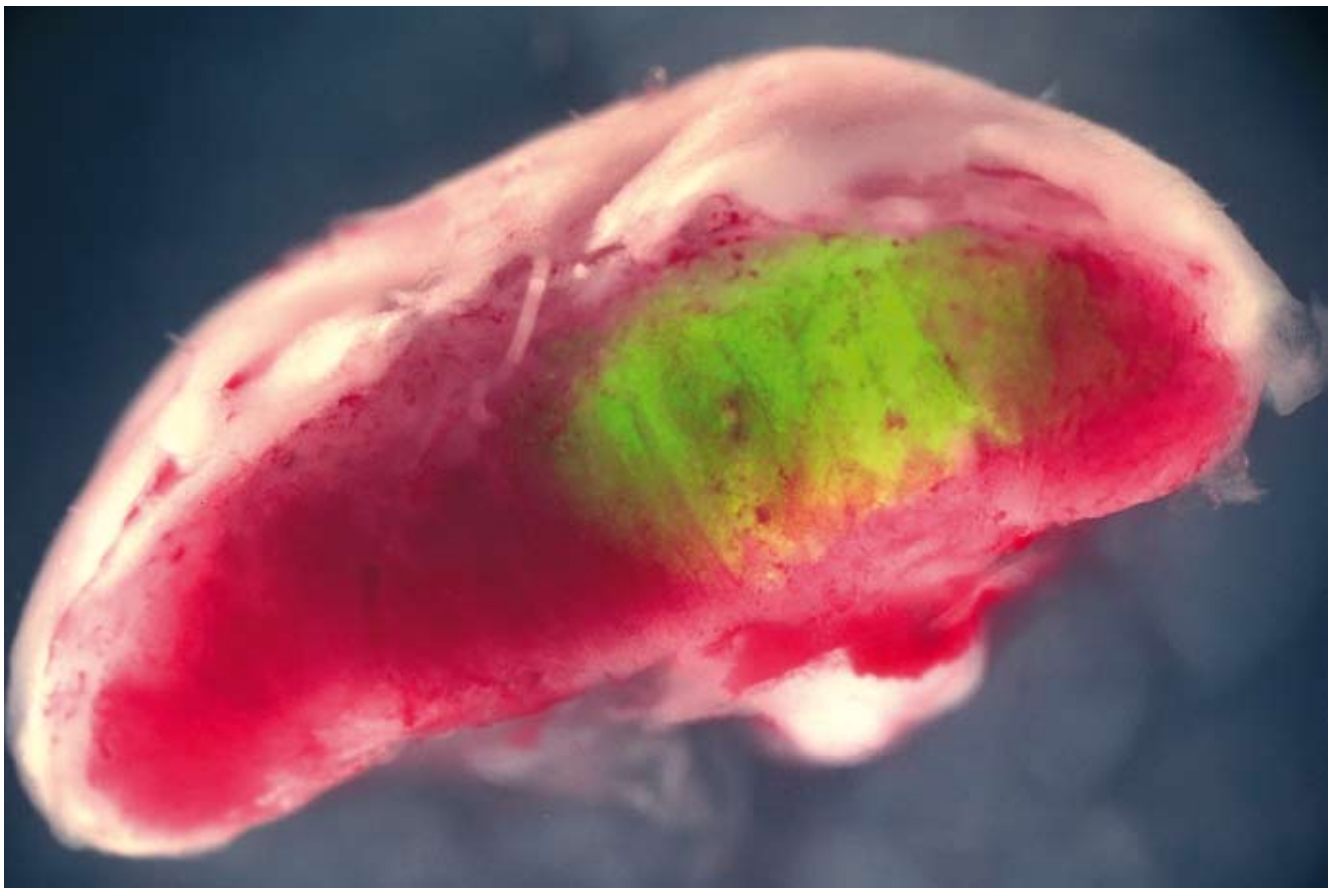


The next Big Thing – stem cell screening

STEM CELLS It's been less than a decade since Nobel laureate Shinya Yamanaka first reprogrammed human cells to return to an embryonic state. Now the first drugs based on human-cell models derived from induced pluripotent stem cells (IPSCS) are entering the clinic. Every major Big Pharma player has begun to create patient-specific cell models. It's a field with a lot of promise. Models that more accurately mimic human physiology are set to provide proof of safety and efficacy at the earliest stages of development – and that could in turn prevent costly clinical failures.

Picture: Caroline Kubaczka et al., Cell Stem Cell, doi: 10.1016/j.stem.2015.08.005

For decades, Big Pharma has been shaken by costly Phase III failures. The list of clinical programmes that have flopped – and racked up billions in losses along the way – is a long one. Especially in the area of neurological disease, where experts still face a screaming lack of both mechanistic understanding and appropriate disease models. The first tentative hopes that the situation would change, and that predictions on a drug's safety and efficacy in early preclinical drug development would grow less hazardous, were first raised after Yamanaka (Kyoto University) created human iPSCs (hiPSCs) for the first time in the lab in 2007. By expressing four factors (Oct3/4, Sox2, Klf4 and c-Myc) in skin cells, the biomedical researcher reprogrammed the adult cells, turning them back into stem cells that have the potential to become any cell in the body – and that can be cultured indefinitely. Just a year after his groundbreaking publication, the generation of motor neurons cultured from a child with inherited spinal muscular atrophy (Nature, doi: 10.1038/nature07677) fueled hopes that patient-derived hiPSCs might provide models to reflect what happens in diseased cells. If they lived up to their promise, drugmakers could potentially save major time and money by sourcing hiPSCs for cell-based drug discovery.

Making a splash in the industry

"Just eight years after the first hiPSCs were made in the lab, we've moved past the hope stage," says Simone Haupt, who heads bioengineering at Life & Brain in Bonn (Germany). Her company codeveloped the very first fully-automated hiPSC manufacturing platform for reprogramming and iPSC expansion. "Two years ago, we were still trying to draw attention from Big Pharma. Now it seems that hiPSC-based drug development has arrived in peoples' heads and markets," she adds. "At the moment, every Big Pharma company seems to have understood that when it comes to cellular testing systems for drug development and toxicity assessment, hiPSCs are the model of the future. Although proof-of-concept – market authorisation of a compound discovered by hiPSC screening – is still some way off, we are seeing more and more published evidence demonstrating that disease phenotypes generated from hiPSCs are actually relevant to disease." The first compounds developed with the help of hiPSC-derived cell screens have already reached stages of clinical development. Among them:

› GlaxoSmithKline has received FDA approval to launch a Phase II study for its epilepsy drug ezogabine (retigabine) in 192 patients with Amyotrophic Lateral Sclerosis (ALS). Using hiPSC-derived motor neurons, principal investigator Brian Wainger and his colleagues at Harvard Medical School previously demonstrated *in vitro* that ezogabine decreased neuron hyperexcitability – which is thought to cause ALS symptoms – by opening potassium channels.

› Data from a Phase I/IIa study presented in October by Roche licensor PTC Therapeutics suggest that the companies' spinal muscular dystrophy (SMA) candidate (RG7800) increases blood levels of the defective SMN1 protein. *In vitro* efficacy of the oral SMN2-splicing modifier was validated by a focus screen in a hiPSC-derived model. The trial has been on hold since May because a non-specified "adverse eye event" occured in a preclinical study. Roche announced that the effect has not been observed in humans, and that it will provide additional preclinical data to clarify the relevance of the event by the end of the year.

› Bristol-Myers Squibb has opened two Phase I trials with iPierian's anti-tau antibody IPN007, which was selected using the acquired company's induced pluripotent stem cell platform. Since December, BMS has been enrolling healthy volunteers for safety testing of the antibody, which has now been renamed (BMS-986168). In May, BMS also started recruiting patients with the orphan hereditary disease progressive supranuclear palsy (PSP) – one of the lesser-known tauopathies in which tau proteins aggregate in a patient's brain.

MARK POWERS
Senior Director, R&D Cell Biology
ThermoFisher Scientific

? Where do you see the main markets for hiPSCs?

! *hiPSCs represent an incredibly powerful technology. The ability to create virtually any cell type in the body drives many possibilities, ranging from the study of human biology in vitro to disease models for the discovery of novel therapeutics to the creation of cell-based therapies. While these possibilities also existed with ESCs, iPSCs have opened the door to the creation of personalised models or therapies, as well as the ability to direct key cellular and genomic characteristics.*

Changing the course of drug development

In addition to these pioneering clinical trials, early investments in the creation of iPSC banks aimed at delivering pluripotent cells of defined quality for pharma R&D or stem cell therapy began bearing fruit this autumn.

In September, Fujifilm's stem-cell arm Cellular Dynamics International (CDI) announced the launch of the world's largest public repository for research-grade iPSCs. The 2004 spin-out founded by US stem-cell pioneer James Thomson has been awarded US$16m by CIRM (Californian Institute for Regenerative Medicine) for the production of iPSC lines from 3,000 volunteers across 11 common diseases, and has so far manufactured 300 research-grade patient-derived lines. While undifferentiated, quality-

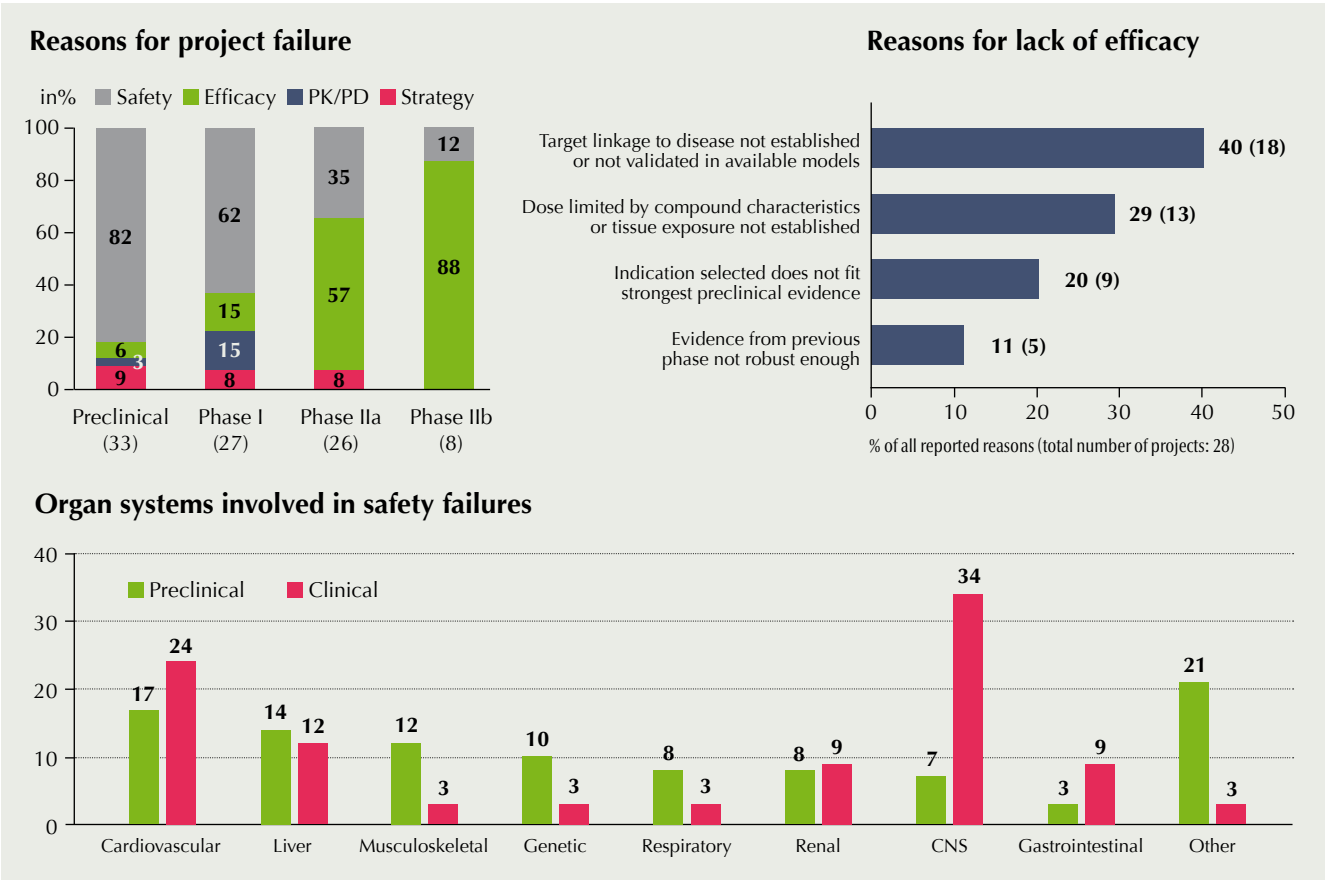
Picture: ThermoFisher Scientific

controlled iPSCs are distributed by the Coriell Institute for Medical Research, which also received US$10m in funding from CIRM, CDI offers differentiation into 12 iPSC-derived cell types for R&D and drug development. CIRM plans to make 750 high-quality iPSC lines available by February 2016 (see table).

Europe – hard on the heels of US-based competitors

Some analysts, however, believe that the CIRM approach has possible downsides, because as the owner of the cells, the institute is a third-party beneficiary of the agreement with Coriell and CDI. License fees claimed by CIRM for commercial applications involving iPSC lines from its bank, say critics, may limit both researcher access and exploitation.

The industry coordinator of the European Bank for Induced Pluripotent Stem Cells (EBiSC), Tim Allsopp from Pfizer subsidiary Neusentis, has a different take on the situation. His project kicked off last year with a total budget of €35m provided by the Innovative Medicines Initiative (IMI) and EFPIA group of pharma companies. While the mission of addressing the hunger in both Big Pharma and among independent researchers for quality-controlled, disease-relevant, research-grade iPSC lines, data and cell services is similar, "the operational model of EBiSC is not-for-profit, enabling easy access to qualified iPSC lines based on simple reagent transfer from a federation of iPSC depositors, via the bank." According to Allsopp, the current 26 EBiSC project partners are planning to set up a central production facility in Cambridge (UK) currently managed by Roslin Cells Ltd. and a mirror facility at the Fraunhofer IBMT (Germany). There, iPSCs "with known disease classification" from initially eight EU iPSC centres will be quality-controlled, expanded and frozen for further research with the help of state-of-the-art automation technology. The IMI project is designed to overcome the current heterogeneity in "iPSC quality, their generation, distribution and iPSC differentiation potential, as well as the bottleneck of supply of sufficient cell numbers for drug screening, target validation or patient stratification campaigns." In order to satisfy the specific needs of its commercial partners and researchers, EBiSC has now moved into a phase of deriving novel cell lines from patient populations of particular interest, using a harmonised ethics and legal governance framework in diseases of the nervous system, as well as cardio-



A five-year review of AstraZeneca's small molecule programmes has shown that high attrition rates in late clinical development stages due to lack of safety and efficacy have challenged pharmaceutical sector productivity. Cells that reflect human physiology better than existing models thus offer the potential to detect safety (particularly cardiomyocyte, CNS, liver and renal toxicity) and efficacy (targeted disease) issues early on in preclinical development.

Pictures: Cook et. al. Nature Reviews Drug Discovery, doi:10.1038/nrd4309

Active participants in the generation, differentiation and distribution of research-grade[1] and clinical grade[2] hiPSCs and progenitor cells

Company/Bank	Location	iPSC generation	iPSC distribution	Progenitor cells offered
Takara Bio Europe AB	Gothenburg	internally	no	cardiomyocytes[1], hepatocytes[1]
CIRM hPSC bank	San Francisco	Cellular Dynamics International, Madison, a Fujifilm Company	through Coriell Institute for Medical Research, fee required	through purchase from CDI[1,2]
EBiSC	Europe, Cambridge	currently eight centres across Europe led by Roslin Cells, Cambridge (UK)	ECACC, Porton Down (UK)	not currently
StemBancc	Europe, Basel, Oxford	Oxford, Newcastle, London	Birmingham	to project partners
HipSci	UK	Cambridge, Univ. Exeter, London	Cambridge Bioresource	not currently
NY Stem Cell Foundation	NY City	NA	NA	NA
RIKEN BioResource Center	Tsukuba	different suppliers	RIKEN BRC Cell Bank	not currently
CIRA, University of Kyoto	Kyoto	Hitachi healthy volunteers' iPSC panel CiRA patient-specific iPSC panel	RIKEN BRC Cell Bank	75 cell lines by 2020, GMP-grade HLA-matched progenitors for therapy

Source: BIOCOM

vascular, muscular and metabolic diseases. Experts estimate that these customised cells of particular interest make up about 20% of the iPSC bank.

According to Allsopp the bank "will eventually have the capacity to store 10,000 lines." EBiSC's main distribution stock at UK-based ECACC (European Collections of Cell Cultures) will be constantly refilled by new iPSCs coming in via EBiSC through ongoing and future major IMI, EU and internationally-funded programmes. "We have had tremendous interest from well-known charitable organisations who want to work with EBiSC," says Allsopp. The goal is to make the project a self-sustaining, not-for-profit resource by 2019 that can ensure the long-term supply of undifferentiated iPSCs, cell-line data and services, along with (likely) iPSC-derived cell progenitors in the mid-term. The advantages for the Big Pharma members of EFPIA that initiated the IMI public-private partnership with the European Commission are clear. Besides risk-sharing, EBiSC contributors Pfizer, Novo Nordisk, AstraZeneca, H. Lundbeck, Janssen Pharmaceutica, UCB Biopharma and two further undisclosed companies that are negotiating participation are to be granted access to iPSCs and cell-line data for project purposes for their role in contributing value to the lines destined for general distribution. "It's a resource initiated by Pharma for Pharma (and research)," as Haupt puts it. "Companies are investing in such projects because they want to use iPSCs in drug screening." Before they do, however, they'll have to clear a couple of technological hurdles.

> Highly penetrant monogenetic diseases are the low-hanging fruit. We have to look at them before progressing to idiopathic disorders.

"While quality control protocols for detecting karyotype aberrations have been established, the prediction of pluripotency is difficult, as none of the established tests will provide a definitive answer," explains Martin Graf from F. Hoffmann-LaRoche AG (Basel), a coordinator from another IMI project that has been funded with €55.6m. "Within the 12 work packages of the StemBANCC project, we don't want just to produce iPSCs, but also to identify phenotypes to acquire a better understanding of the underlying disease mechanisms," he explains. The project's 35 partners have one ultimate goal; to reprogramme three iPSC lines from 500 subjects who have donated skin biopsies, keratinocytes or blood samples and use them to study diabetes, diseases of the peripheral nervous system (PNS), central nervous system (CNS) and adverse drug effects. "So far, we have recruited 375 of the 500 patients, and by doubling our reprogramming capacity we will now be able to accomplish all of the foreseen reprogramming by the end of the project," according to Graf. "Our initial focus is to develop cellular models that are predictive for 120 monogenetic diseases. A secondary focus will be to develop models from idiopathic diseases like Alzheimer's, migraine, schizophrenia or Parkinson's. Here it will be challenging to understand the role of the multiple minor genetic changes that cause the onset of the disease." To generate more relevant control iPSC lines, a functional repair of defective genes in cell models for monogenetic diseases is carried out withCRISPR/Cas9-genome editing. Producing isogenic controls like this is an important validation for monogenetic diseases *in vitro*. Part of the genetic engineering is carried out by consortium partner AstraZeneca. Other Big Pharma partners in StemBANCC include AbbVie, Boehringer Ingelheim, Janssen Pharmaceutica, Eli Lilly, Merck KGaA, Novo Nordisk, Orion Corp, Pfizer UK and Sanofi-Aventis.

A current major technological bottleneck in assay development is the time it takes to differentiate iPSCs into functional cells. According to Graf, protocols have been established to reproducibly differentiate the cells into mature cortical and

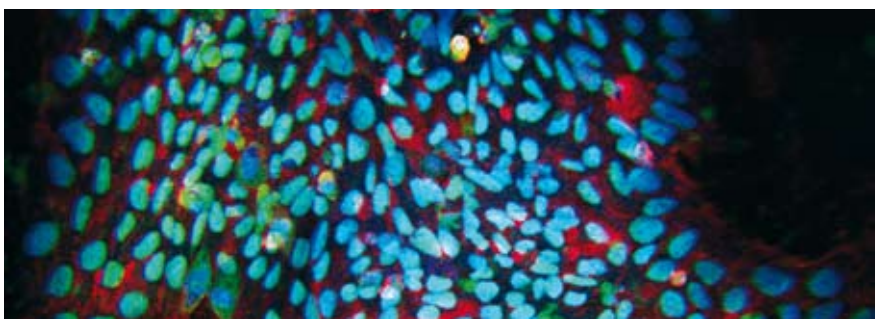
dopaminergic neurons or astrocytes in 384-well microtiter plates over a time span of six weeks. iPSC-derived assays might be very costly, Graf told EuroBiotech, but they "have the promise to be functionally more relevant." SOPs for insulin-producing beta-cells, podocytes and hepatocytes have also been established.

Another effort within StemBANCC is the development of preclinical assays aimed at predicting drug safety. Consortium members Carl-Frederik Mandenius (Linköping University) and Robert Zweigerdt (Hanover Medical School) recently developed a microfluidic device to detect the beating rate of 3D-grown cardiac bodies optically. With this technology, the researchers confirmed reported toxicities for doxorubicin, verapamil and quinidine. And another EBiSC member success story has just been published. Within the framework of the Detective Project, which is jointly funded by the European Commission and the European Cosmetics Association (Colipa), Cologne-based stem-cell researcher Jürgen Hescheler has identified about 50 biomarkers for repeated dose toxicity that are now under evaluation.

A fast-growing, emerging market

Allsopp predicts a not-for-profit market for hiPSC banking based on low-cost fees to access cell lines, although there might be a higher premium for generating custom-made disease models, screening assays and pharma R&D tools. Haupt confirms that the next market could be hiPSCs and cell progenitors from particularly interesting patient populations for pharma in-house research. New players and old are scrambling to update what they offer.

ThermoFisher Scientific VP R&D Mark Powers says that focus is "increasingly shifting towards how we can enable researchers to implement iPSC and their progeny in meaningful assays and therapies." Companies like CDI, GE Healthcare, Axiogenesis and Takara Bio Europe – which have long offered hiPSC- or hESC-derived cardiomyocytes, hepatocytes or renal cells for toxicity compound screening – are now being challenged by new players like Allele Biotech. The US-based firm announced in October that it would be offering banked cells for drug discovery programs, and that it was in the process of creating a bank of GMP-grade iPSC-derived progenitors for therapeutic use. According to CEO Jiwu Wang, his company "has established RNA-assisted differentiation protocols to generate cell types such as cardiomyocytes, skeleton muscle cells, satellite cells, adipocytes, brown fat cells, neurons, astrocytes, hepatocytes, lung epithelial cells nd mesenchymal stem cells....from banked hiPSCs…in many cases in less than a week". Additionally, the company offers "pathway-targeting assays for personalised drug testing and new drug development." Also in October, Newcastle University spin-out Newcells Biotech Ltd secured seed investment to build on products and services that use iPSCs for drug discovery and development.



In October, two teams presented new tools for testing iPSC differentiation potential. Atlas Regeneration and In Silico Medicine have transformed gene expression data into activation patterns of cellular signal pathways. Partners at the Fraunhofer IME and Scripps Institute used RNAseq and their Pluritest2 annotation tool.

Market estimates from Enal Razvi, the head of market specialist SelectBio, predict annual growth rates of over 20% – up from US$685m to US$1.1bn next year in the still small iPSC-based drug discovery and toxicology testing space. "Clinical translation of iPSCs is currently taking place from research towards clinical trials," Razvi believes, though he says there's still no market in the therapeutic space.

Next stop: RegMed

While the efforts being made by EBiSC or StemBANCC are clearly focused on iPSCs as drug discovery tools, Japanese iPSC pioneers are betting on banking iPSC-derived cells for therapeutic use. The goal of an ambitious, government-funded programme in Japan is to create 75 GMP-grade, hiPSC-derived cell types for allogenic transplantation at Yamanaka's Kyoto University that immune-match 90% of the Japanese population. However, a first clinical trial started in 2014 (wet AMD) was suspended after six mutations were discovered post-transplantation in a first patient. Therapeutic use therefore remains a dream, even though New York Stem Cell Foundation Director Mahendra Rao and scientists from CRO Lonza reported the GMP-compliant production of iPSCs in September. And Allele Biotech and German RheinCells have also announced intentions to start the GMP production of iPSC-derived cells.

Australia's Cynata Therapeutics Ltd. may have found a way around a major problem. iPSCs can be problematic as sourcing material for cell transplants because they can carry genetic or epigenetic variations that affect pluripotency and stability. According to Cynata CEO Ross Macdonald, the company is the first to set up GMP-compliant production of mesenchymal stem (MSCs) cells from iPSCs. Unlike iPSC-based transplants, "MSC-based therapeutics are currently being investigated in 350-400 clinical trials." Cynata's iPSC advantage is predictable cost and quality: "All other technologies rely on supply from multi donor-derived materials." ■

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Pictures: Salk Institute for Biological Studies