Introductory course to human pluripotent stem cell culture, reprogramming, differentiation, and gene editing techniques

Syllabus

Course Description

We will provide foundational training on basic stem cell techniques, including human induced pluripotent stem cell (iPSC) reprogramming, human pluripotent stem cell (PSC) culture, gene editing, and differentiation. The introductory training is conducted virtually by UCLA faculty in collaboration with Thermo Fisher Scientific, a prominent member of the California biotech community and trusted provider of standardized reagents for stem cell modeling and regenerative medicine applications. This partnership leverages course materials successfully used and updated for over a decade by Thermo Fisher. UCLA Faculty will cover specific aspects of the course as neural differentiation (Dr. Michael Wells) and muscle differentiation (Dr. April Pyle) from human PSCs, and be available to answer questions (Dr. Kathrin Plath, Dr. Kitai Kim). The course gives a basic introduction to conducting human PSC-based research in their home laboratories. The course represents a component of the UCLA-CIRM Shared Resource Laboratory education activities (https://www.uclastemcellengineering.com/ucla-cirm-srl).

Course Objectives

Upon completion, participants will possess a robust knowledge of the following key stem cell laboratory techniques and new professional proficiencies:

- Best practices for proper aseptic tissue culture techniques and maintenance of lab equipment.
- Human somatic cell-to-iPSC reprogramming and identification of human iPSC colonies.
- Expansion and maintenance of human PSCs, including cryopreservation, thawing, and passaging of human PSCs, feeder-dependent and feeder-free passaging techniques.
- Human PSC differentiation into specific cell types, including neural and mesodermal lineages.
- Specific insights into the neural and muscle differentiation and gene editing services offered at the UCLA stem cell engineering core
- Techniques to characterize pluripotent stem cells and differentiated cell populations.
- Gene editing approaches in human PSCs, including tools and protocols for designing, delivering, screening, and clonal expansion of CRISPR-edited PSC lines and clones.
- Understand policy and regulation considerations and their effects on hPSC research.
- Outline considerations for clinical applications and use of human PSCs in regenerative medicine.
- Enhanced professional network through close interactions with teaching faculty, experts, and staff.

Course Format and Dates
7 virtual sessions (1.5 - 2 hours each), Mondays at 9 AM – spanning seven weeks from March 10, 2025 – April 21st, 2025. The specific lecture dates are listed below:

The individual sessions will consist of lectures by experienced stem cell biology scientists and educators from Thermo Fisher, with support from UCLA faculty. For a thorough learning experience, the lecture material will be paired with relevant e-learning resources and instructional videos.

Lecture 1:	Introduction to human PSCs	Monday 9 am, March 10th
Lecture 2:	Reprogramming to human iPSCs	Monday 9 am, March 17th
Lecture 3:	Basics of feeder-free human PSC culture	Monday 9 am, March 24th
Lecture 4:	Advanced feeder-free human PSC culture	Monday 9 am, March 31st
Lecture 5:	Gene Editing on human PSCs	Monday 9 am, April 7th
Lecture 6:	Mesodermal differentiation (w/ Dr. Pyle)	Monday 9 am, April 14th
Lecture 7:	Neural differentiation in 2D/3D (w/ Dr. Wells)	Monday 9 am, April 21st

Course Instructors

Thomas Forbes, PhD, Staff Scientist, Field Applications, Thermo Fisher Scientific thomas.forbes@thermofisher.com
Omar Farah, PhD, Manager, Field Applications, Thermo Fisher Scientific omar.farah@thermofisher.com
Kitai Kim, PhD, Director of the CIRM-UCLA Shared Resource Lab, knkim@mednet.ucla.edu
Kathrin Plath, PhD, PI of the CIRM-UCLA Shared Resource Lab, kplath@mednet.ucla.edu
April Pyle, PhD, Muscle Differentiation Expert, UCLA, apyle@mednet.ucla.edu
Michael Wells, PhD, Neural Differentiation Expert, UCLA, mwells@mednet.ucla.edu

Course Tuition
Free of charge through the collaboration with the Thermo Fisher Outreach program.

Course Prerequisites:
- Introductory courses in cell biology and molecular biology
- Basic understanding of laboratory safety protocols

Course Materials
Course materials, including the presentation slides, session recordings, detailed protocols, eLearning modules, how-to-videos, and step-by-step guides, will be made available to the trainees. Additionally, recommendations for relevant research articles, protocols, and books to further expand the understanding of the material will be provided where relevant.

In addition to the course material, networking and mentorship opportunities with the teaching instructors will be emphasized. All instructors are willing to support the trainees during and after the course is concluded, including being a sounding board for experimental approaches, potential collaborations, career development, etc.

Overview of the content of each lecture

Lecture 1: Introduction to Human Pluripotent Stem Cells

Team Introductions	• Omar Farah, Ph.D. & Thomas Forbes, Ph.D. • UCLA-CIRM SRL Faculty: Drs. Kim, Pyle, Wells and Plath • Course Participants
Course Overview, Expectations & Objectives	• Provide a comprehensive foundation to stem cell culture • Introduce new and exciting technologies in the field • Share a host of useful resources that pertain to stem cell biology
Basics of Pluripotent Stem Cells	• Biosafety: regulations, PPE, lab equipment, sterile techniques • Cell Culture Basics: stem cell history & biology, disease modeling, cell therapy, market trends/value • Cell Workflow Overview: cell models, reprogramming, engineering, characterization, differentiation, analysis • Case Study: molecular basis for Parkinson's Disease
Resources	• Pluripotent Stem Cell Education at Thermo Fisher Scientific - Link Here • Pluripotent Stem Cell Resource Handbook - Link Here • Pluripotent Stem Cell Protocol Handbook - Link Here

Lecture 2: Reprogramming to Human iPSCs

Introduction to Stem Cells & Reprogramming	• Stem cell basics review • Why are induced pluripotent stem cells a powerful tool? • Disease model generation • Common somatic cell sources for iPSC generation
Technologies for Reprogramming	• Methods of reprogramming • Integrative versus non-integrative technologies • Reprogramming method considerations: Safety, consistency, workload, and cell type • CytoTune sendai reprogramming • Episomal iPSC reprogramming
Reprogramming Workflows	• Fibroblast reprogramming on feeders • Fibroblast feeder-free reprogramming • Colony identification via EVOS live-cell imaging • Building capabilities to transition from RUO to translation • Cell therapy compliant xeno-free workflows
Resources	• eLearning Training Module: Generation of induced pluripotent stem cells - Link Here • CytoTune –iPS Sendai Reprogramming - Link Here • Epi5 Episomal iPSC Reprogramming - Link Here

Lecture 3: Basics of Feeder-Free Human PSC Culture

Quick Review	• Stem cell basics review • Types of stem cells • Stem cells & disease modeling workflow • Applications of stem cells
PSC Culture Formats	• Cell culture formats • Types of culture vessels • Adherent culture versus suspension culture • Expansion capability – PSC suspension culture vs. adherent culture
Intro to PSC Culture Systems	• What is required to grow human PSCs?: matrices, media, cells, passaging methods • Common culturing terminology • Estimate confluency • Feeder-dependent culture systems • Feeder free culture systems
Resources	• Training Video: selecting a matrix for PSC culture - Link Here • Training Video: passaging reagents for PSC culture - Link Here • eLearning Training Module: StemScale PSC Suspension Medium - Link Here

Lecture 4: Advanced feeder-free human PSC Culture

PSC Workflow Considerations	• Evolution of PSC culture systems • Feeder-dependent & feeder-free PSC media • Essential 8 Medium: fully-defined, feeder-free PSC culture • Applications of stem cells
PSCs in Feeder-Free Culture	• Cell characteristics • Observation of PSC colonies • Caring for PSCs • Strategies & tools for passaging cells • Expansion capability – PSC suspension culture vs. adherent culture • Cell culture automation
Routine Media Changes	• Maintenance of pluripotency • FGF2 stability • Essential 8 Flex & FGF2 stability • Cell culture challenges: inconsistent culture
Resources	• Optional Lecture: Optimizing stem cell workflows to build better disease models - Link Here • Training Video: how to cryopreserve PSCs Training Video - Link Here • eLearning Training Module: Thawing Pluripotent Stam Cells - Link Here

Lecture 5: Gene Editing on Human PSCs

Optimized Workflows	• Stem cells & disease modeling workflow • Cell models: an *in-vitro* approach to predict human biology • Disease model generation • Common somatic cell sources for iPSC generation • Stem cell characterization
Gene Editing Basics	• Define genome editing • Genome editing applications • Historical timeline of genome editing methods • Generation & application of modified PSCs • Strategies of genome editing
Designer Engineered Nucleases	• Genome editing with designer engineered nucleases • CRSPR-Cas9 • TALENs • Concerns about off-target effects • Improved tools for genome editing in stem cells • Case study: cardiomyocyte differentiation & dilated cardiomyopathy
Resources	• Landing Page: Gen Editing at Thermo Fisher Scientific - Link Here • eLearning Training Module: Gene Editing in Pluripotent Stem Cells - Link Here • Optional Lecture: Advanced Tools for Knock-In Genome Editing in iPSCs - Link Here

Lecture 6: Mesodermal differentiation (including a brief introduction to muscle differentiation by Dr. Pyle)

Hematopoietic Stem Cells	• Differentiating iPSCs into clinically relevant cell types • PSC to iHSC differentiation starting in StemScale • iHSC differentiation workflow: phenotyping • iHSC differentiation workflow: morphology • iHSC differentiation workflow: differentiation at scale • iHSC differentiation workflow: multipotency • iHSC differentiation workflow: cryopreservation
Natural Killer Cells	• Differentiating iPSCs for "off the shelf" therapy • PSC to iNK differentiation derived from CTS StemScale cultures • Protocol review: initiation of mesoderm induction > initiation of iHPCs > maturation of iHPCs > iNK induction • Phenotypic characterization during iNK differentiation • iNK enrichment in CTS NK-Xpander Medium • Activation/inhibition surface marker expression
Cardiomyocytes	• StemScale PSC to cardiac differentiation in 3D • Importance of starting spheroid size • Cardiac differentiation protocol • Small-scale test of Harvard method • Large-scale differentiation • Cardiac organoid imaging • Spontaneous cardiac activity
Skeletal Muscle	• Dr. Pyle will discuss the skeletal muscle differentiation approaches that will be offered in the UCLA-CIRM Shared Research Lab
Resources	• eLearning Module: CTS StemScale PSC Suspension Medium – Innovation in Stem Cell Suspension Culture - Link Here • Landing Page: CTS StemScale PSC Suspension Medium - Link Here • Landing Page: Stem Cell Differentiation at Thermo Fisher Scientific - Link Here • Landing Page: 3D Cell Culture Models at Thermo Fisher Scientific - Link Here

Lecture 7: Neural Differentiation in 2D and 3D (including a brief introduction to 2D neural differentiation methods by Dr. Wells)

Suspension Culture	• Model systems for human disorders • 3D culture introduction • Suspension culture introduction • StemScale PSC Suspension Culture Media System • PSC suspension culture vs. adherent culture
Organoid Culture	• Spheroids vs. organoids • Neural organoids • Unguided vs. guided protocols • Differentiation of PSCs into cerebral organoids • Generating mature forebrain specific cultures
2D/3D Dopaminergic Neuron Workflow Optimization	• Parkinson's Disease introduction • Dopaminergic neurons via 2D & 3D protocols • Dopaminergic organoid imaging with the Cellnsight CX7 LZR • Neuromelanin • Electrical activity following specification in 2D vs. 3D • Products across the neural organoid workflow
Stem cell-derived NGN2-accelerated Progenitor cells (SNaPs)	• Discussion of the SNaP differentiation methods for neural progenitors, neurons and astrocytes by Dr. Wells offered in the UCLA CIRM Shared Rescource Lab
Resources	• eLearning Training Module: StemScale PSC Suspension Medium - Link Here • eLearning Training Module: Getting started in 3D Cell Culture - Link Here • eLearning Training Module: Neural Organoid Generation from Pluripotent Stem Cells - Link Here • Training Video: How to Culture PSCs in Suspension Culture - Link Here • Optional Lecture: Differentiation of iPSCs in 3D: Leveraging Suspension Cultures for Scale & Efficiency - Link Here