UCLA/CIRM/TFS Pluripotent Stem Cell Course - Syllabus **November 2024**

Thermo Fisher Scientific - Stem Cells & Neurobiology
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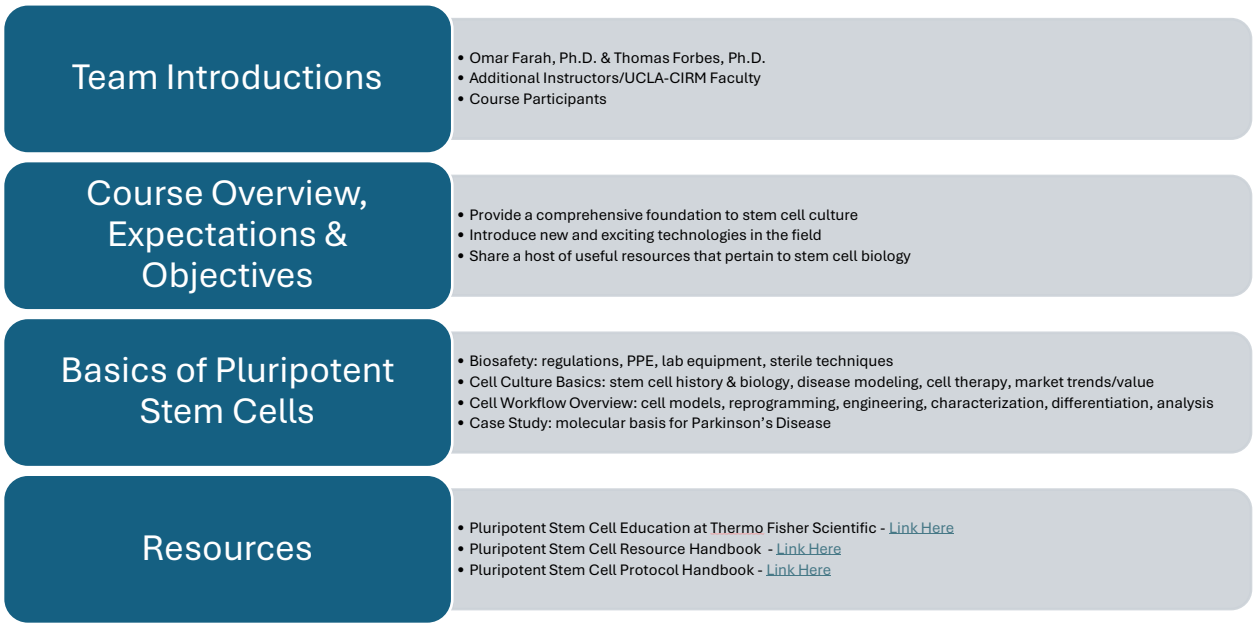
Course Structure
1. Introductory Lecture
2. Reprogramming Lecture
3. Feeder-Free hPSC Culture (basics) Lecture
4. Feeder-Free hPSC Culture (advanced) Lecture
5. Gene Editing Lecture
6. Suspension/3D Culture
7. Differentiation Lecture

Objectives
- Provide a comprehensive foundation to stem cell culture
- Introduce new and exciting technologies in the field
- Provide a host of useful resources that pertain to stem cell biology and offer insight to a career in this ever-evolving field

Lecture 1: Introduction to Pluripotent Stem Cells



Lecture 2: Stem Cell Reprogramming

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: Feeder-Free hPSC Culture Basics

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: Feeder-Free hPSC Culture Advanced

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

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: 3D Culture

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
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

Lecture 7: Stem Cell Differentiation

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
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