Course Syllabus:
Stem Cell Techniques: Generate Patient-Derived iPSC Lines

Course Overview and Objectives: This course consists of two modules: Module 1) Deriving iPSCs from Somatic Cells; and Module 2) Practical Techniques for Maintaining, Passaging, Cryopreserving, and Thawing Induced Pluripotent Stem Cells (iPSCs). Objectives include:

- Understand and master the process of somatic cells reprogramming into iPSCs
- Understand the different cell reprogramming approaches; advantages and disadvantages of each
- Assess and identify fully reprogrammed undifferentiated iPSCs colonies compared to partially reprogrammed iPSCs colonies
- Understand and apply the skills required to pick iPSCs
- Learn the strategies and tools for characterizing iPSCs clones

Course Duration: 15 days total
Module 1: Ten days, Monday through Friday, 1:00 pm to 5:00 pm
Module 2: Five days, Monday through Friday, 1:00 pm to 5:00 pm

Note: Enrollment may be limited to a max of eight attendees per class to ensure individualized attention and optimal learning outcomes. The syllabus is designed to provide a comprehensive overview of the course content and may be adjusted based on participant needs and advancements in the field.

Prerequisites: Participants must complete the web-based Introduction to Stem Cell Laboratory Techniques. Participants should have a solid foundation in cell biology, molecular biology, and basic cell culture techniques. Familiarity with stem cell biology concepts is beneficial but not required.

Module 1 Overview (Deriving iPSCs from Somatic Cells): This immersive 10-day course is designed to equip participants with the knowledge and practical skills required to derive induced pluripotent stem cells (iPSCs) from somatic cells. Participants will gain a deep understanding of the reprogramming process, molecular mechanisms, and technical nuances involved in generating iPSCs. The course combines hands-on laboratory sessions, interactive lectures, and discussions to ensure a comprehensive learning experience.

Day 1: Introduction to iPSCs and Reprogramming

- Overview of iPSCs: Historical context, significance, and applications
- Principles of cell reprogramming: Transcription factors and epigenetic modifications
- Ethical considerations and regulatory aspects of iPSC technology
- Laboratory safety protocols and guidelines

Day 2: Somatic Cell Selection and Preparation

- Choosing appropriate somatic cell sources for reprogramming
- Methods for somatic cell isolation and culture
- Characterization of somatic cells for reprogramming potential

Day 3: Reprogramming Techniques

- Overview of non-viral reprogramming methods (e.g., episomal vectors, mRNA, proteins)
- Benefits and challenges of non-viral techniques

- Introduction to viral vectors (retroviruses, lentiviruses): Mechanism and safety considerations
- Design and preparation of non-viral reprogramming components
- Evaluation and optimization of non-viral reprogramming conditions

Day 4: Reprogramming Somatic Cells into iPSCs
- Reprogramming somatic cells using episomal vectors
- Selection of reprogramming factors: Oct4, Sox2, Klf4, c-Myc, Nanog
- Experimental design and optimization of reprogramming protocols
- Monitoring and identifying iPSC colonies

Day 5: Molecular Monitoring of Reprogramming Progress:
- Detection of pluripotency markers: Gene expression analysis and immunocytochemistry
- Monitoring epigenetic changes during reprogramming: DNA methylation and histone modifications
- Time-course analysis of reprogramming dynamics
- Monitoring of colony shapes

Day 6: Culture and Expansion of Emerging iPSC Colonies
- Identification and picking of iPSC colonies
- Maintenance of emerging iPSC cultures: Media composition and passaging techniques
- Troubleshooting common challenges in iPSC colony culture

Day 7: iPSC Characterization and Validation:
- Pluripotency markers: SSEA-4, Oct4, Nanog, Tra-1-60, Tra-1-81
- Immunocytochemistry and flow cytometry for pluripotency assessment
- Assessing differentiation potential through embryoid body formation and tri-lineage differentiation

Day 8: Quality control and ensuring iPSC quality
- Molecular analysis: gene expression profiling and DNA methylation patterns
- Detection and management of genomic aberrations
- Detection of residual reprogramming factor

Day 9: iPSC Line Maintenance and Banking
- Long-term culture and expansion of validated iPSC lines
- Cryopreservation techniques and considerations for iPSC banking
- Documentation and quality control of iPSC lines

Day 10: Advanced Topics in iPSC Derivation and Application
- Genetic modification of iPSCs: Introduction to genome editing techniques
- Disease modeling using patient-specific iPSCs
- Drug screening and personalized medicine applications
- Current research trends and future directions in iPSC technology

Module 2 Overview (Practical Techniques for Maintaining, Passaging, Cryopreserving, and Thawing Induced Pluripotent Stem Cells (iPSCs)): This intensive one-week course is designed to provide participants with hands-on experience and in-depth knowledge of techniques for maintaining, passaging, cryopreserving, and thawing induced pluripotent stem cells (iPSCs). Participants will gain practical skills and theoretical understanding necessary for

successful iPSC culture and manipulation. The course includes laboratory sessions, lectures, discussions, and interactive activities to ensure comprehensive learning.

Day 1: Introduction to iPSCs and Basic Cell Culture Techniques

- Overview of iPSCs: Definition, history, and applications
- Introduction to aseptic technique and proper laboratory practices
- Cell culture environment and equipment setup
- Media preparation and sterilization techniques

Day 2: iPSC Culture and Maintenance

- Cell line selection and characterization
- Plating and seeding iPSCs: Optimal density and substrates
- Culture medium composition and supplementation
- Feeding and routine maintenance of iPSCs

Day 3: iPSC Passaging Techniques

- Cell detachment methods: Enzymatic vs. mechanical dissociation
- Calculation of cell density for passaging
- Passaging protocols for maintaining pluripotency and preventing differentiation
- Assessment of cell viability and growth after passaging

Day 4: Cryopreservation of iPSCs

- Introduction to cryopreservation principles and cryoprotectants
- Preparation of iPSCs for cryopreservation
- Cooling and freezing techniques: Controlled rate freezing vs. vitrification
- Long-term storage considerations and best practices

Day 5: Thawing and Recovery of Cryopreserved iPSCs

- Thawing protocols and strategies for successful recovery
- Viability assessment and post-thaw culture optimization
- Monitoring and troubleshooting post-thaw adaptation
- Strategies for minimizing differentiation and maintaining pluripotency post-thaw

Assessment Methods:

- Laboratory reports and practical assessment of lab skill
- Participation in class discussions and activities
- Study presentations on iPSC reprogramming experiments