Gladstone-CIRM Shared Resources Laboratory

SYLLABUS

CIRM SRL Stem Cell Techniques Course

COURSE	MODULES AND DESCRIPTION
Short-term hands-on training	**I. Basic iPSC culture (3 days):** Trainees will learn iPSC culturing technique, including media preparation, plate coating, thawing, and counting iPSCs. Techniques such as media changes, single cell and clump passage, and cryopreservation will be covered. Trainees will also become familiar with iPSC colony morphology, proper passaging, distinguishing pluripotent from differentiated cells, and choosing suitable media and matrix. **II. iPSC reprogramming (4 days):** Training will include derivation of iPSC from fibroblasts and PBMCs. Topics include thawing and culturing of somatic cells, transfection with Yamanaka factors, iPSC colony picking, and expansion. Trainees will gain hands-on experience and knowledge in iPSC characterization. **III. iPSC gene editing (4 days):** Comprehensive training on CRISPR-Cas9 gene editing in iPSCs. Topics include guide design, ICE analysis, RNP delivery, clone picking and passaging in 96-well plates, DNA extraction, and clone analysis. Trainees will learn the fundamentals of gene editing and quality control. **IV. iPSC differentiation (4 days):** Trainees will learn principles of directed iPSC differentiation into cardiomyocytes and motor neurons. The training covers hands-on techniques and understanding differentiation progress. Topics include iPSC quality control, plate preparation, differentiation media preparation, and immunocytochemistry.
Long-term hands-on training	**I. Basic iPSC culture (2 weeks):** Training will cover media preparation, plate coating, thawing, passaging (single cell and clump), and freezing of human iPSCs, along with sterile tissue culture techniques. Trainees will learn to distinguish iPSC morphology and ways to characterize iPSCs. **II. iPSC reprogramming (4 weeks):** Trainees will learn two reprogramming methods: 1) Reprogramming from PBMCs using episomal methods and 2) reprogramming from fibroblasts using RNA vectors. The training includes culture of somatic cells, transfection with vectors containing Yamanaka factors, plating and maintenance of transfected cells, identification of newly formed iPSC colonies, picking iPSC colonies onto Matrigel-coated plates, and training on expanding and freezing iPSCs for viable recovery. **III. iPSC gene editing (4 weeks):** This training will focus on designing gRNA and donor templates, nucleofection of iPSCs with RNPs, plating of transfected cells, ICE analysis for gene editing, single cell cloning, DNA extraction in 96-well plates, PCR in 96-well plates, analysis of edited clones, and eventual expansion of clones. **IV. iPSC differentiation (4 weeks):** Training in directing iPSCs into cardiac and neural lineages. Topics encompass iPSC quality control, differentiation media preparation, differentiation method and analysis by immunocytochemistry.

Bioinformatics workshop	This workshop will include slide presentations rich in background and references, along with sample datasets and instructions for hands-on exercises. These will make up the bulk of the content for live workshop events as well as for self-paced online learning that is available at any time. We will also select a few of the most popular introductory workshops and prepare pre-recorded video tutorials that researchers can access at any time. In addition to scheduled workshop events, we will also accommodate project-based training, where we tailor content and examples for specific individuals and small groups. This approach helps to supplement generic bioinformatics training in cases where experimental designs are atypical or where trainees require more assistance.