Humboldt-CIRM Shared Resource Lab	STANDARD OPERATING PROCEDURE

Title: hPSC Maintenance
Purpose: To describe standard procedure for maintaining hPSCs
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Media and Supplies

Item	Purpose	Storage	Catalog Number	Supplier
mTeSR Plus	Primary maintenance culture media	2–8°C	1000276	STEMCELL
70% Ethanol (EtOH)	Sanitization of surfaces and materials	Room Temp		
Pen-Strep	Optional antibiotic for contamination prevention.	-20°C.	15-140-122.	Fisher
Conical Tubes	Aliquoting and warming media	Sterile	21008-936	VWR
6-well TC Plates	Advanced TC treated culture vessels.	Room temperature.	89131-688 (CS).	VWR
Serological Pipettes	Sterile liquid handling (10 ml)	Room Temp	76184-746	VWR
Inverted Microscope	Culture assessment (4x–20x)	Room Temp	LMI3PH2	Life Technologies
PPE (Gloves)	Nitrile Exam Gloves	Room Temp	99452683	Fastenal
Permanent Marker	Labeling culture vessels			

Maintenance of Cells

This protocol uses mTeSR Plus for the standard expansion and maintenance of undifferentiated hPSCs. This medium was selected as it is the most widely used in the field, it is easy to use and highly robust, and it contains stabilized FGF2 that allows for weekend-free feeding schedule. While alternative media may be substituted, these may require further optimization to ensure comparable results. Before beginning, ensure the mTeSR™ Plus medium has been previously prepared with supplement according to the manufacturer's instructions. Official Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus (See Section 6.0 for flexible feeding and weekend-free schedules).

Assessment (Daily): Evaluate culture health and confluency daily under a microscope (4x–20x magnification). Look for healthy, undifferentiated colonies characterized by highly compressed cells with a high nuclear-to-cytoplasmic ratio and clearly defined, smooth colony borders. Cultures require feeding if <70% confluent and are ready for passaging once they reach 70–80% confluency.

Preparation (Feeding/Passage Days Only): On scheduled maintenance days (Mon/Wed/Fri), calculate the total volume required for your specific vessel and aliquot this amount into a sterile conical tube (e.g., a 15 mL or 50 mL tube). Allow the medium to warm to room temperature.

Media Exchange and Incubation: When performing media changes, leave a small amount (100–200 µL) of conditioned medium in the well. This prevents the cell surface from drying out and maintains a baseline level of paracrine signaling factors. Add fresh, room-temperature mTeSR Plus and return the plate to a 37°C incubator.

Weekly Maintenance Schedule (for weekend-free culture):
To maintain healthy, undifferentiated cultures, follow the routine below using volumes specified for a 6-well plate (refer to Link for other vessels). **Note**: cell cultures are dynamic - if a weekend-free schedule becomes impractical e.g., due to specific experimental needs, rapid growth rates, or cell death, resume a standard every-other-day feeding regimen using 2.0 ml of medium.

1. **Monday Morning**: Perform a full media change (2.0 mL per well) if cells were passaged the previous Friday.

2. **Wednesday**: Perform a full media change (2.0 mL per well).

3. **Friday Late Afternoon Assessment:** Evaluate culture confluency. If confluency is low < 50%, you may proceed with feeding only (Option A). If cells are ≥ 50% confluent or rapidly growing, you must passage (Option B).

   Option A: Feeding Only. To skip two consecutive days (Saturday/Sunday), perform full media change, however, replace spent media with double the standard media volume (4.0 ml per well of a 6-well).

   Option B: Passaging. Perform clump passaging with ReLeSR and seed aggregates at a low-density (e.g., 1:10 split) into 4.0 ml of room temperature mTeSR Plus (no ROCK inhibitor). The double media volume and low-density split allows the culture to remain unattended until Monday.

   Note: This weekend-free schedule applies only to clump passaging. Single-cell dissociation requires a Saturday media change to remove ROCK inhibitor. Do not attempt a weekend skip with single-cell cultures.

Standard Maintenance Schedule (feeding on alternate days): Use this regimen if the weekend-free protocol is not utilized or if rapid growth prevents a two-day skip. Replace the medium with 2 ml of fresh mTeSR Plus every other day until the culture reaches 70–80% confluency. While cells can be fed at intervals shorter than 48 hours, they must never exceed the 48-hour window. For routine maintenance and expansion, use the clump passaging method with ReLeSR.