Automated Daily Feeding and Imaging of iPSCs

Purpose

This workflow is for the scheduled feeding and daily maintenance of iPSCs (induced pluripotent stem cells) in 6-well plate and 96-well plate format.

This workflow will also schedule the imaging of the selected plates on the Celigo cytometer.

Required materials

- Cells seeded in 6-well plates and/or 96-well plates according to the "Automated 96-Well Plate Seeding" SOP
- Complete mTeSR1 culture media: 400 mL basal media with provided 100 mL 5X supplement (catalog # 05850, STEMCELL™ Technologies) with added 5 mL (1% v/v) Penicillin/Streptomycin (catalog # 15140-122, Gibco). Refer to page 16 of the STEMCELL™ Technologies technical manual about preparation, storage and shelf life of this media.
- Media Trough: Single Well Reagent Reservoir with 8 Bottom Troughs, High Profile 290 mL Clear Sterile (catalog # RES-SW8-HP-S 10790-706, VWR)

Equipment

- Hamilton's MICROLAB® STAR Line workstation
- Cytomat™ 24 C Automated Incubator
- Cytomat™ 6002 D Series Automated Incubator
- Celigo Image Cytometer
- Venus Two software and additional packages:
    - Dynamic Scheduler (for optimized resource use)
    - TADM feature (for full traceability of the pipetting workflow)
    - DataBasePlus option (to use remote tracking servers)
    - Dynamic Liquid Classification plugin (for automatic liquid class selection)

Related SOPs

- WTC culture v.1.5
- Automated 96-Well Plate Seeding
- Automated 96-Well Plate Matrigel Coating

Methods

The following protocol is to be performed on the Hamilton's STAR robotic liquid handler, operated with Venus Two software. Note: specific channels are used for aspirating/dispensing to minimize the amount of dry time in the wells.

Feeding		Aspiration				Dispense			
		Channels	Tip size	Speed	Vol.	Channels	Tip size	Speed	Vol.
20-well	mTeSR1 Trough	5-8	1000uL	250uL/sec	800uL	5-8	1000uL	150uL/sec	150uL/well
	Plate	1-4	1000uL	250uL/sec	200uL/well	1-4	1000uL	150uL/sec	900uL/waste
60-well	mTeSR1 Trough	4-6	300uL	250uL/sec	300uL	4-6	300uL	150uL/sec	150uL/well
	Plate	1-3	300uL	250uL/sec	150uL/well	1-3	300uL	150uL/sec	300uL/waste

Table 1. Parameters used by the Hamilton's STAR for different plate layouts.

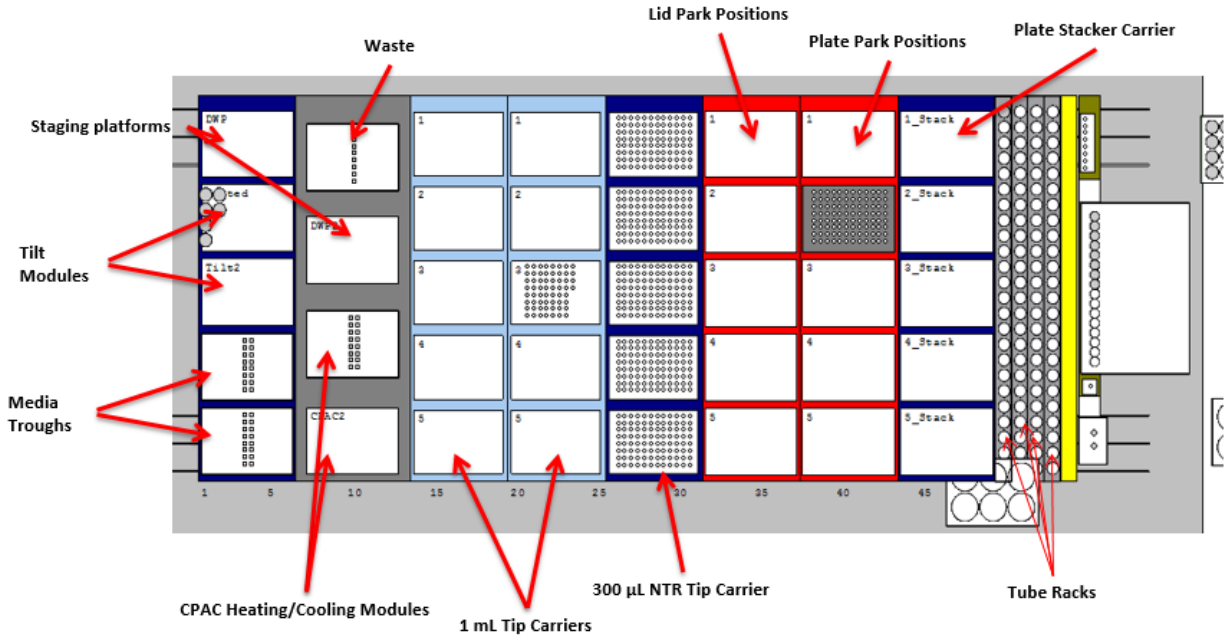

Figure 1. Hamilton STAR Deck Layout.

I. Plate Scanning by Celigo

1. The Feed Scan workflow is initiated in Hamilton Method Editor. The workflow starts with the setup of the experiment with the operator entering in the date and time to start the workflow along with the number of consecutive days that the workflow will be operating for.

2. The operator will be prompted to upload the worklist of plate ID's to be fed and/or imaged.

3. After the setup is complete the system will initialize and wait for the scheduled start time.

4. At the designated time, the Cytomat 6002 (4 °C automated refrigerator) will initialize and then retrieve the media designated for feeding that day and place the covered trough on the Cytomat platform to acclimate to room temperature for 2 hours.

5. While the media is acclimating, any plates designated to be imaged will be retrieved from the Cytomat 24 (37 °C incubator) and transferred across the STAR deck and loaded into the Celigo Cytometer one plate at a time to be imaged.

II. Plate Feeding

6. After all plates designated to be imaged have been imaged and approximately 2 hours after the media has been removed from the Cytomat 6002, the media trough is moved from the Cytomat Transfer Station to Media Fill Station 1 (see Figure 1). The Hamilton STAR then retrieves eight 300 µL tips across 8 channels and move them to the media trough located at Media Station1 and pierces the cover of the trough for subsequent, repeated access for feeding (see Figure 2).

Figure 2. Covered trough before piercing by the channels.

7. After media set up, the Cytomat 24 will deliver the first plate indicated in the worklist to the Cytomat 24 transfer station. The Hamilton STAR will then retrieve the plate from the transfer station platform using the iSwap (robotic arm) and delivers the plate to the Tilt Module 1.

8. The STAR then picks up the Core Gripper Paddles on Channels 7 and 8 to de-lid the plates.

9. The Tilt Module then tilts the plate 10°.

A) If 96-well plate format:

10. Tips of appropriate tip size are picked up on designated channels. Refer to Table 1 for recommended tip size and channel numbers for different plate layouts.

11. The first group of tipped channels will then move to the media trough located at Media Station 1 (see Figure 1) and will be lowered to aspirate up media. Refer to Table 1 for recommended channel numbers and media volume to be aspirated for different plate layouts.

12. The second group of tipped channels will move over to the plate located on the Tilt Module and aspirate up the old media. Immediately following the aspiration of old media, the first group of

channels dispenses new media to the wells and repeats this until all wells have been fed for the 20-well format. Tips are changed between columns for the 60-well format Refer to Table 1 for recommended channel numbers for aspiration and dispense.

13. The channels then move to the waste station to dispense the used media from the used channels and then discard the used tips to waste.

14. Steps 10-13 are repeated for the remaining rows for the entire plate.

B) If 6-well plate format:

10. 1000 µL tips are picked up on all 8 channels.

11. The first group of tipped Channels (5-8) will move to the media trough located at Media Station 1 and aspirate up 1000 µL/channel of media at 250 µL/sec

12. The second group of tipped Channels (1-4) then move over the plate on the Tilt Module and aspirates up the old mediaat 250 µL/sec. Immediately following the aspiration of old media, the first group of Channels (5-8) delivers 1000 µL at 150 µL/sec to the wells just aspirated from.

13. The channels then move to the waste station to dispense the used media and discard the used tips to waste.

14. Steps 10B-13B are repeated for the remaining columns for the entire plate.

III. Delivery of plates back to Cytomat for Incubation

15. The STAR returns the plate to the 0° position on the Tilt Module.

16. The plate is then re-lidded using the Core Gripper Paddles and the iSwap retrieves the plate from the Tilt Module 1 position and returns it to the Cytomat 24 transfer station, where the Cytomat then retrieves the plate and places in the incubator.

17. The Method repeats from Step 7 for all plates designated for feeding as indicated by the worklist. Once all plates have been fed, the STAR will de-initialize and the method will end.