Automated 96-Well Plate Seeding

Purpose

This method is for the seeding of iPS (induced pluripotent stem) cells in a 96-well glass bottom plate and store plate in the 37°C Cytomat 24.

Required Materials

- Cells suspended in phenol red mTeSR1 culture media as prepared under SOP "WTC culture v.1.5" in 15 mL conical tubes
- Matrigel-coated 96-well glass bottom plates with mTeSR1 + Rock inhibitor as prepared under SOP "Automated 96-Well Plate Matrigel Coating"

Equipment

- Hamilton's MICROLAB® STAR Line workstation
- Cytomat™ 24 C Automated Incubator
- 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 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.

Seeding		**Aspiration**				**Dispense**			
		Channels	Tip size	Speed	Vol.	Channels	Tip size	Speed	Vol.
20-well	Cell Suspension	1-4	1000uL	250uL/sec	500uL	1-4	1000uL	150uL/sec	100uL/well
60-well	Cell Suspension	1-6	1000uL	250uL/sec	1000uL	1-6	1000uL	150uL/sec	100uL/well

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

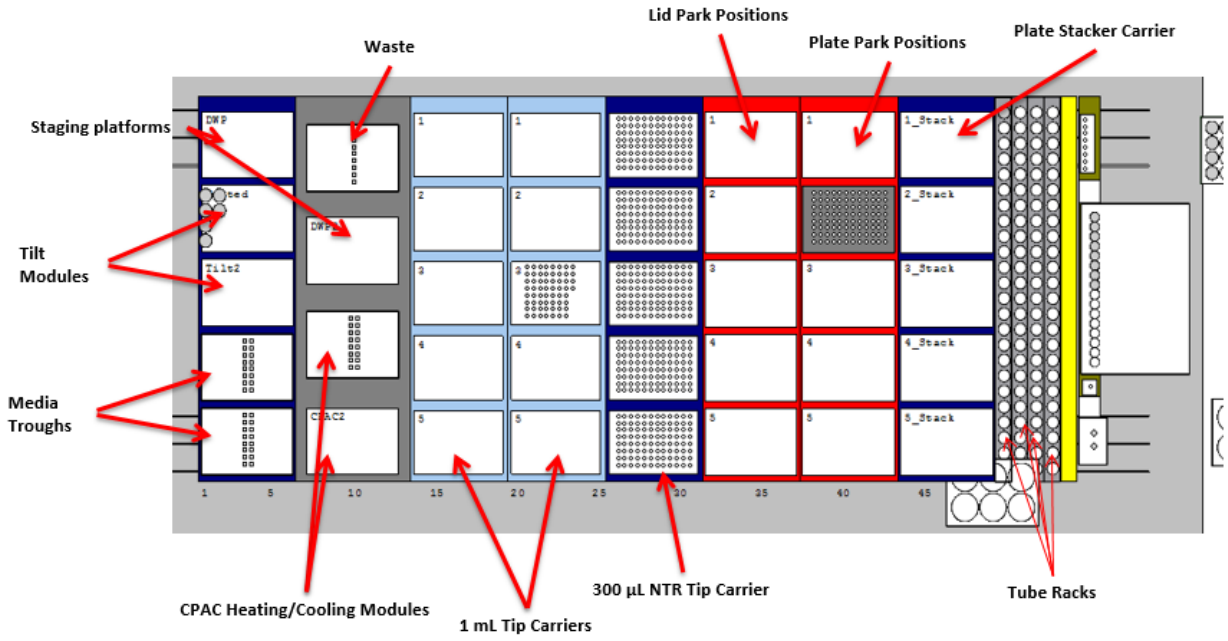

Figure 1. Hamilton STAR Deck Layout.

1. The Seeding method is selected for initialization in Hamilton Method Editor. Method starts with the initialization of all necessary equipment and checks carrier presence.

2. Load open-capped 15 mL conical tubes (one tube per row of seeding) for cell seeding into Tube Rack 1 in location indicated in Figure 1.

3. The operator is first prompted to enter the experiment number and cell lines to be seeded and their seeding concentration.

4. The operator is then prompted to select the plates coming from the Plate Stacker Carrier onto the deck. For plates coming from the Plate Stacker Carrier, the capacity is 25 plates per run. The operator will also need to select plate type and number of plates to be treated.

5. With the STAR Core Gripper Paddles on Channel 7 and 8, the plates are moved from the Plate Stacker Carrier to the Plate Park Positions one plate at a time, working in groups of no more than 5 plates staged on the deck at one time.

6. Using the Core Gripper Paddles, the lids of the plates are taken off and placed at the Lid Park Positions.

7. The STAR moves to pick up 1000 µL tips (refer to Table 1 for channels number recommended for different plate layouts).

8. The tipped channels move to the tubes located on the Tube Rack containing the cells to be seeded and are lowered 7 mm from the bottom of the tubes. Each channel aspirates up 900 µL at a speed of 250 µL/sec and does 1 complete mix cycle at a speed of 250 µL/sec (dispensing initial

aspirated volume and then aspirating up the same volume). This mix is only done on the first aspiration of each grouping of 5 plates.

9. The tipped channels then move to 1 mm below the surface of the liquid and dispenses the volume of liquid.
10. The channels then move to 10 mm below the surface of the liquid in each tube and aspirate up cell suspension across the channels.
11. Next, the channels move to the plate in the first position at the Plate Park Positions and dispense the 100 µL of cells suspended in media to each well across the designated wells of a 96-well plate.
12. The channels return to the tubes containing cells in suspension, aspirating up again the same volume of cell suspension per channel and move to the second plate in the next location at the Plate Park Positions until all plates in the first group of 5 or less have had cells delivered to all the designated wells.
13. The tips are discarded.
14. Using the Core Gripper Paddles the plates are re-lidded.
15. A 5-minute timer starts.
16. At the end of the timer the iSwap (robotic arm) will move the plates from the Plate Park Positions to the Cytomat 24 (37°C incubator) for storage.