Automated 96-Well Plate Matrigel Coating

Purpose

This method is for Matrigel coating of 96-well glass bottom plates with an 1 hour incubation time, followed by the removal of the Matrigel and the addition of media.

Required Materials

- 96-well glass bottom plates (catalog # P96-1.5H-N, Cellvis)
- Flat bottom robotic reservoir (300mL): Thermo Scientific™ Nalgene™ Disposable Polypropylene Robotic Reservoirs (catalog# 12-565-572, Fisher Scientific)
- Matrigel (MG): Growth Factor Reduced (GFR) Matrigel® (catalog # 354230, Corning), diluted 1:30 in DMEM/F12 media
- DMEM/F12 media, phenol red-free (catalog # 11039-021, Gibco Life Technologies)
- Complete mTeSR1 media (catalog # 85850, STEMCELL Technologies) supplemented with 1% penicillin/streptomycin (catalog # 15140-122, Gibco Life Technologies)
- ROCK inhibitor (RI) [10mM] stock reconstituted in DMSO per manufacturer's instructions (Y-27632, catalog # 72308, STEMCELL™ Technologies)

Equipment

- Hamilton's MICROLAB® STAR Line workstation
- Cytomat™ 6002 D Series 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

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.

Matrigel Coating		**Aspiration**				**Dispense**			
		Channels	Tip size	Speed	Vol.	Channels	Tip size	Speed	Vol.
20 well	Matrigel	1-4	1000uL	250uL/sec	500uL	1-4	1000uL	150uL/sec	100uL/well

	mTeSR1	5-8	1000uL	250uL/sec	250uL	5-8	1000uL	150uL/sec	50uL/well
	Plate	1-4	1000uL	250uL/sec	100uL/well	1-4	1000uL	150uL/sec	500uL/waste
60 well	Matrigel	1-6	1000uL	250uL/sec	1000uL	1-6	1000uL	150uL/sec	100uL/well
	mTeSR1	4-6	1000uL	250uL/sec	500uL	4-6	1000uL	150uL/sec	50uL/well
	Plate	1-3	1000uL	250uL/sec	100uL/well	1-3	1000uL	150uL/sec	1000uL/waste

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

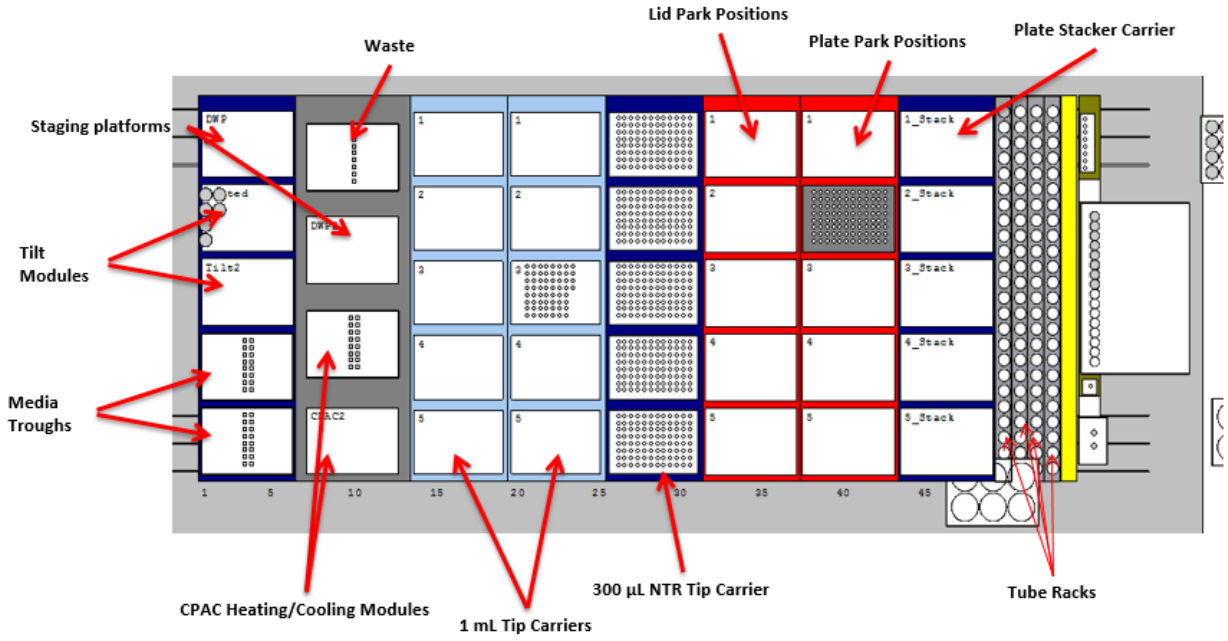

Figure 1. Hamilton STAR Deck Layout.

I. Matrigel Coating

1. The MG Coating method is selected for initialization in Hamilton Method Editor. The Method starts with the initialization of all necessary equipment and checks carrier presence. CPAC 1 is cooled to 4 ºC.

2. Place barcoded 96-well plates on the Plate Stacker Carrier (see Figure 1). Place a flat bottom robotic reservoir (300 mL) in CPAC1 location (see Figure 1).

3. Add enough (required volume plus an extra 10 mL to account for dead volume) 1:30 MG to the 300mL robotic reservoir located at CPAC1 position using a pipette aid and serological pipette. Volume necessary for one 96-well plate is about 10 mL per plate.

4. In the Hamilton Method Editor, the operator is first prompted to add the lot numbers for the MG and media added to the deck.

5. The operator is then prompted to select whether the plates to be MG coated are coming from the Cytomat 6002 or the Plate Stacker Carrier on the deck.

a. If plates are coming from the Cytomat, they are limited by its capacity.

b. If plates are coming from the stackers, the capacity is 25 plates per run.

6. The operator will also need to select plate type and number of plates to be treated.
7. 1 hour timer starts.
8. Plates move to the Plate Park Positions one plate at a time.

    a. If plates are coming out of the Cytomat 6002: with the STAR iSwap (robotic arm), the plates are moved from the transfer station platform to the Plate Park Positions, working in groups of no more than 5 plates staged on the deck at one time.

    b. If plates are coming from the Plate Stacker Carrier: with the STAR Core Gripper Paddles on Channels 7 and 8, the plates are moved from the Plate Stacker Carrier to the Plate Park Positions, working in groups of no more than 5 plates staged on the deck at one time.

9. Using the Core Gripper Paddles the plates are then de-lidded with the lids being placed at the Lid Park Positions.
10. The STAR moves to pick up 1000 µL tips using channels as indicated in Table 1.
11. The tipped channels then move to the media trough located at CPAC 1 to aspirate 1:30 MG (volume and speed in Table 1).
12. The channels then move to the de-lidded plates located at the Plate Park Positions and dispenses 100 µL per well across the designated wells.
13. Once the 5 or fewer plates are MG coated, the STAR will re-lid the plates using the Core Gripper Paddles.
14. Then, using the Core Gripper Paddles, the STAR will stack the plates in the Plate Stacker Carrier located on the deck for the remaining incubation time at room temperature.
15. If more plates are to be coated, they will be removed from the Cytomat or the next Plate Stacker Carrier location and the sequence is repeated.
16. After the 1-hour MG incubation time has ended, the plates are removed from the Plate Stacker Carrier in groups of 5 or fewer plates to the Plate Park Positions.

II. mTeSR1 + Rock Inhibitor (RI) Addition

17. The plates are then de-lidded again using the Core Gripper Paddles and the lids are placed in the Lid Park Positions.
18. Using the Core Gripper Paddles plates are moved one plate at a time to the Tilt Modules.
19. The Tilt Module is tilted 10º.

20. 1000 µL tips are picked up.
21. The first group of channels move to Media Trough Station 1 containing mTeSR1 media with Rock Inhibitor, and aspirate up media.
22. The second group of channels then go to the first column to aspirate up 100 µL/well of the 1:30 MG that has been incubating at RT.
23. The first group of channels are then moved to the first column and dispenses mTeSR1 + RI.
24. Steps 22- 23 are repeated to remove the 1:30 MG and to add the mTeSR1 + RI for the following columns.
25. The plate is returned to the flat (no tilt) orientation.
26. The second group of channels move to the liquid waste position and dispenses the MG. The tips are discarded.
27. Using the Core Gripper Paddles, the plate is returned to its pick-up location at the Plate Park Positions and the next plate is picked up and steps 18-26 are repeated.
28. After all plates in each group of 5 or fewer plates have been coated with 100 µL of MG and have received the final 50 µL of mTeSR1 + RI per well, the plates are re-lidded using the Core Gripper Paddles and moved from the Plate Park Positions to the Plate Stacker Carrier.
29. The plates will remain in the Plate Stacker Carrier until ready for seeding (within no more than 1 hour).