General spotter SOP (SCI)

Spotter operation

Nozzle mounting and removal

Have a healthy fear of the nozzles as they are expensive and easy to break. If one should break, order a back-up to replace the back-up.

To mount a nozzle: **please get trained on this before attempting!**

1. Remove the silver fingertights for each corner of the nozzle mount station, and pull it off the robot head.
2. Pull the clean plexiglass front off the nozzle station and remove the two black plastic holders.
3. Remove the nozzle from the glass tube and place it on the first nozzle station. Slide it into place.
4. Place bottom black plastic piece on, postioning the spring of the nozzle, then place second black plastic holder in place.
5. Slide on clear plexiglass cover.
6. Place nozzle mount station back on robot head and reattach fingertights.

To remove a nozzle:

1. Remove the silver fingertights for each corner of the nozzle mount station, and pull it off the robot head.
2. Pull the clean plexiglass front off the nozzle station and remove the two black plastic holders.
3. Remove the nozzle from the nozzle station by sliding and place in the glass tube.
4. Leave disassembled nozzle station pieces on cold chuck or reassemble and place back on robot head and reattach fingertights.

Spotter priming

1. Add previous system liquid (bottle in front of pump tower) to the wash bottle (bottle on floor).
2. Turn on spotter computer and start Scienion program. Turn on chiller (takes roughly an hour to cool cold chuck).
3. Filter 700mL of Millipore water through a Millipore Express Plus 0.22uM filter. Degas and sonicate for 30 minutes using the sonicating waterbath and the vacuum adaptor lid. This is your fresh system liquid.
4. Check that the waste bottle is empty and the wash bottle contains sufficient Millipore water.
5. Place the tubing from the robot pump tower into the system liquid and select Prime from the main menu, following the prompts from the program:
6. Connect flush bottle with finger tights to robot head, remove when prompted, and attach nozzle, using the first drop to leave the manifold to wash the tubing at the end of the fingertight before screwing it in. Align the nozzle from Nozzle Setup>Nozzle offset using the up and down arrows to the crosshair.

Quick Prime (without priming the wash station)

1. Align nozzle and move to wash station
2. Attach flush bottle
3. Pump 5000 uL (10000 uL if it is the beginning of the day and you have not primed yet) at 60 uL/s
4. Remove flush bottle
5. Pump 1000 uL at 12 uL/s. Attach nozzle, using the first drop to leave the manifold to wash the tubing at the end of the fingertight before screwing it in.
6. Run a Wash Flush Strong.
7. Check for a drop, if no drop appears, use AirEx and prime again.

Spotter maintenance

1. Keep spotter dust and fibre free
2. Use freshly sonicated and degassed water for all experiments.
3. Perform the **14 day wash** frequently to prevent Pseudomonas or other contamination of the system.
4. If the spotter will be inactive for more than 7 days, perform the 7 day purge.
5. After every reagent/sample spotting, perform a wash cycle (wash flush strong, SciClean wash tray 1, wash flush strong).
6. After completing spotting for the day, perform a Nozzle Removal wash, save settings, and exit the program before shutting down the spotter.
7. If libraries have unmapped reads (esepecailly if these reads BLAST Pseudomonas), perform a **Proclin wash.**

14 day wash

Each step of this procedure is prompted by a popup in the Scienion software. Each step takes a different amount of time so operator should stay in the clean room for the duration, with the exception of step 3 which takes 20 minutes. It is also good practice to empty the air conditioning drawer of water and rinse the wash and waste bottle with ethanol the day you perform the 14 day wash.

Before you start:

- Prepare 500mL of freshly filtered 70% Ethanol (350mL ethanol, 150mL Millipore water)
- Prepare 700mL of freshly filtered and degassed system liquid - note the wash procedure can be started with just the ethanol, with the system liquid sonicating during steps 1-4.
  Procedure:

1. In the Scienion program, go to Nozzle Setup > Do Task > 14_DayWashProcedure
2. Remove the nozzle and attach the flush bottle. Remove the system liquid bottle so that the tube will intake air. Click okay.
3. Connect the system with 70% ethanol. Click okay. This takes approximately 20 minutes.
4. Remove ethanol bottle and replace with system liquid. Click okay.
5. Remove system liquid bottle to allow system to flush with air. Click okay.
6. Reattach system liquid bottle. Click okay.
7. If the system will not be used for 5 days, remove system liquid and flush with air. If it will be used in 5 days, keep system liquid attached. Click okay. All remaining steps will take place with the fresh water bottle tubing.
8. Remove tubing from fresh water bottle and place in 70% ethanol. Click okay.
9. Place tubing back into the fresh water bottle. Click okay.

Proclin wash

Prepare 1 liter of Proclin wash solution.

The solution can be stored for up to 30 days at room temperature and used up to 3 times during this period. This is a useful protocol for cleaning the system if you see a high level of bacterial contamination in your libraries, but with regular maintenance, should not be necessary.

1. Prepare 50 ml of 10% Tween 20 by combining the following volumes. Add the water first.

- Laboratory-grade water (45 ml)
- Tween 20 (5 ml)

2. Place a stir bar in an empty carboy that is at least 1 liters.
3. Combine the following volumes in the carboy. Add the water first.

- Laboratory-grade water (150 ml)
- 10% Tween 20 (50 ml) made in previous step
- ProClin 300 (0.3 ml)

These volumes result in approximately 2.5% Tween 20 and 0.15% ProClin 300 solution.

4. Place the carboy onto a stir plate and stir until the solution is thoroughly mixed.

5. Add 1 liter laboratory-grade water to the solution.

These volumes result in approximately 0.5% Tween 20 and 0.03% ProClin 300 wash solution.

6. Continue stirring until the solution is thoroughly mixed.

7. Set aside in a closed container at room temperature until you are ready to fill or replenish reagent bottles and tubes with wash solution.

8. Pump Proclin wash solution through system liquid system (10mL), then wash system (250mL). Use to rinse millipore water carboy, then waste bottle. Discard down drain.

Wash Proclin from system

After washing the spotter with Proclin wash solution, follow with pumping 0.5% Tween, then water through the system:

1. Prepare 25mL of 10% Tween.

- Laboratory-grade water (22.5 ml)
- Tween 20 (2.5 ml)

2. Prepare 500mL 0.5% Tween.

- Laboratory-grade water (475 ml)
- 10% Tween 20 (25 ml) made in previous step

3. Pump 0.5% Tween through system liquid system (15mL), then wash system (250mL). Use to rinse millipore water carboy, then waste bottle. Discard down drain.

4. Pump water through system liquid system (25mL), then wash system (400mL). Use to rinse millipore water carboy, then waste bottle. Discard down drain.

Spot troubleshooting

Lost drop?

1. Dip in wash station, pump 1uL, immediately dip in wash station, then move to camera. Dispense 500 drop two times.
2. Failing that, pump out 500uL with continuous dispensing on.
3. If the nozzle is truely clogged, pumping will not help, but it will if there is a bubble in the system. Keep an eye on the syringe pump - if it is leaking, there is likely a clog.

Strange drop? Nozzle build-up?

1. Adjust voltage and pulse. Tailing satellite droplets can often be corrected by increasing the pulse. It can be worth spending the time to find stable droplet conditions (where the drop is stable up and down 3 on voltage and pulse) before spotting new reagents or using a new nozzle.
2. Wash cycle (wash flush strong, SciClean wash tray 1, wash flush strong).
3. Pump out 500uL with continuous dispensing on.
4. Especially with iffy cell spotting, can try a take-probe of Buffer G2, and hold the nozzle in EppiStation1 in a tube of G2 for a minute, then wash cycle as usual. This can remove build-up.
5. Home and tap the nozzle with the sterile fiber-free Q-tips (get trained on this)
6. Pump and clean the nozzle with the sterile fiber-free Q-tips (get trained on this)
7. Re-prime.

Clogged nozzle? (no drop, no pump stream)

1. Stop! Change nozzle.

Notes

- System liquid should always be prepared fresh before a spot run and be particle free.
- Do not overtighten the fingertights! Also, do not undertighten the fingertights! You can tell if it is undertightened because water will accumulate on the ledge below the fingertight. If this should happen, tighten it. Watch for this behaviour during spotting.