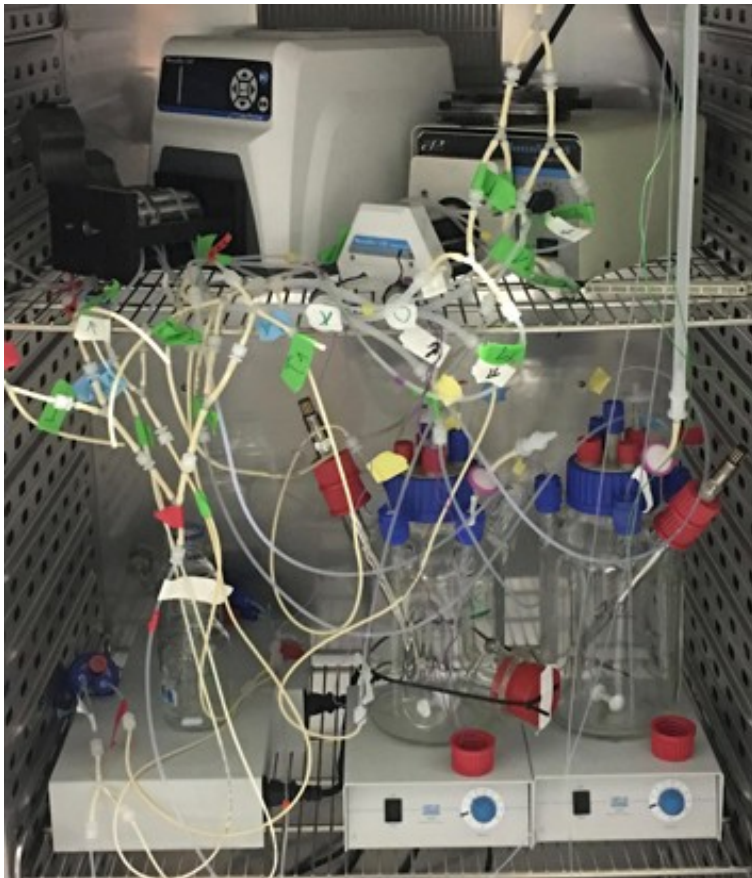


Instructions for manual PACE/PATHE setup using peristaltic pumps and large incubation chamber



**Heidelberg
PACE**

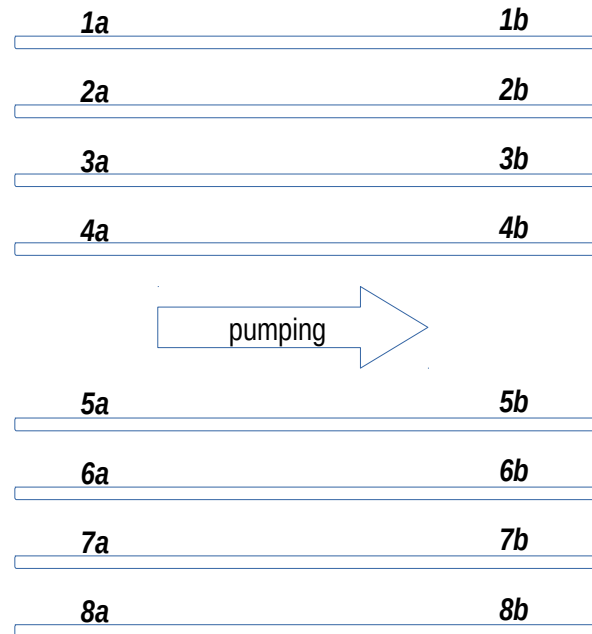
Innatrix Leaner/Meaner PACE

With diagram and
Instructions

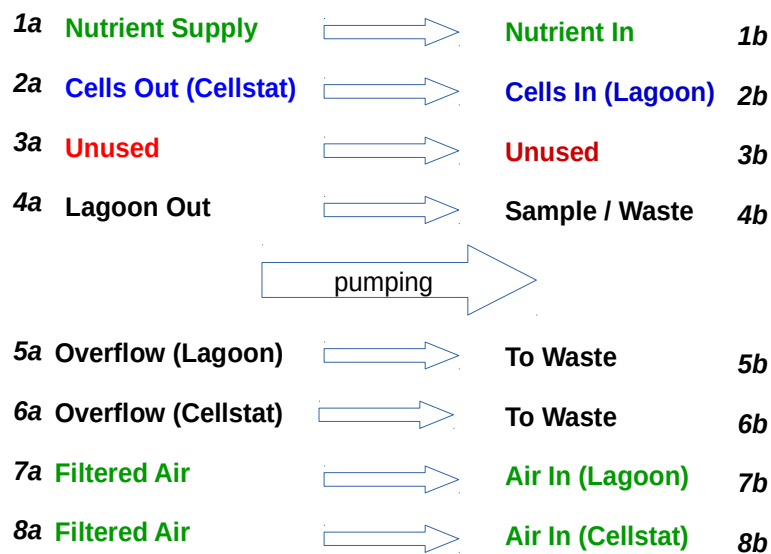


This setup uses seven (7) of the eight peristaltic pump channels. There may be hours between steps as they involve autoclaving, filling, and sterility testing. Filling steps can be accelerated by changing the pump speed.

Starting at the pump face and moving outward toward the observer, the pump channels are numbered 1-8. With the default mode of the pump moving liquid from left to right, we designate the ends of the tubes as [a] left/input and [b] right/output.



In the continuous evolution experiment, we make the following assignments.



Step 1: Sterilizing the Equipment

Autoclave vessels and place the [a] left/input ends of all pump tubes into an ethanol supply. Place [b] right/output ends of tubes into an ethanol receiver. Start pump.

Step 2: Initial Connections and Air Vents

With vessels still in the Autoclave, attach **new** 0.45 micron filters to the vent burets. Also install and verify **direction** of vents in Nutrient and Inducer vessels. Preferred air-flow direction in the 0.45 micron filters is into the luer connector and out through the nipple. Air **intake** is vital for Nutrient and Inducer supplies, while Air **exhaust** is required for CellStat and Lagoon. Each vessel must have either an intake or an exhaust filter.

Move vessels to incubator and place CellStat and Lagoon on stirring plates under pump.

Leave tubes 2a, 2b, and 4a in ethanol while removing other tubes from ethanol and connecting according to the labels in the diagram. Don't forget to insert **new** 0.45 micron filters into tubes 7a and 8a (Sterile Air **Intake**).

Allow CellStat to fill with Nutrient. Then, with heating, aeration, and mixing operational, remain in this state to verify CellStat sterility by turning the pump off (Note: this also turns aeration off, but if nothing is growing, the rate it isn't growing doesn't matter).

Step 3: Inoculate CellStat

Add host cells to CellStat and wait for growth to OD₆₀₀ of 0.4. Remove the buret air vent temporarily to inject cells. Pump is still off.

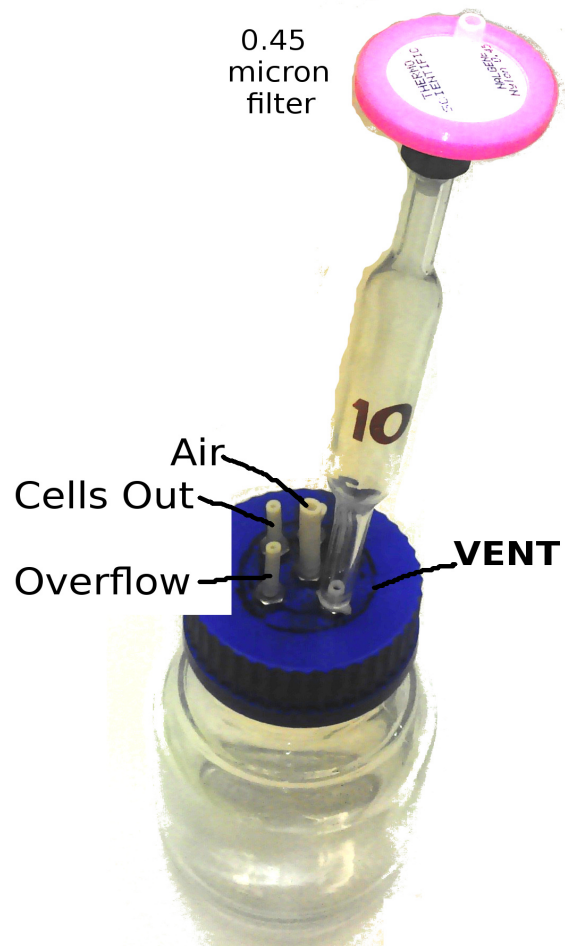
Step 4: Fill Lagoon

With CellStat at target volume, connect tubes 2a (**Cells Out / CellStat**) and 2b (**Cells In / Lagoon**) to begin filling Lagoon. Pump must be turned on.

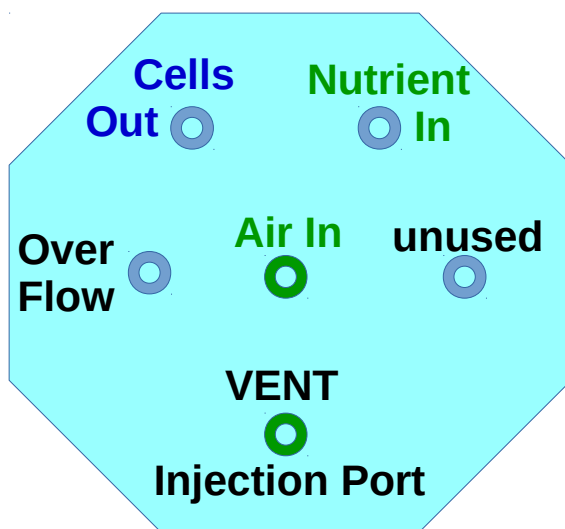
Step 5: Infect and Start Continuous Flow

With Lagoon at target volume, infect Lagoon with phage via buret/air filter vent port and connect 4a (**Lagoon Out**) while making sure that 4b is connected to the waste/sample collector. Connect **Inducer** from the secondary pump (not shown) which will be supplying additional chemicals to the Lagoon.

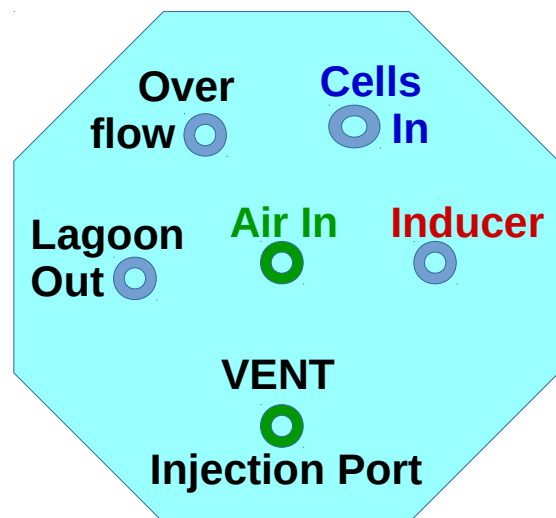
Right: CellStat vessel with new vent filter installed immediately after autoclaving



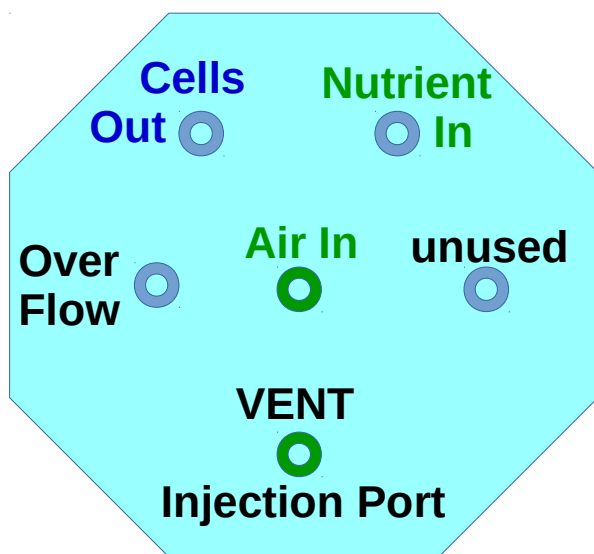
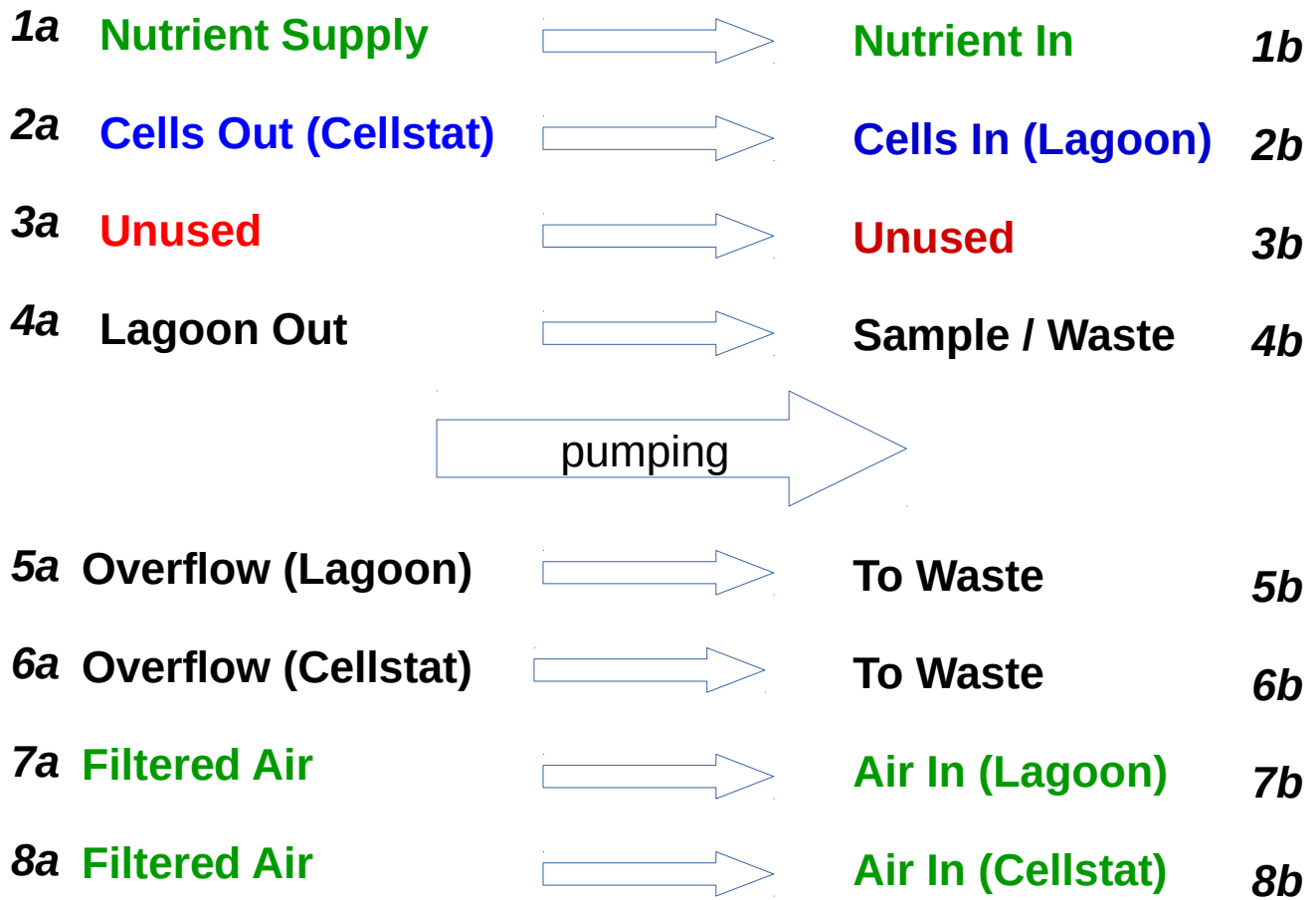
Below: Schematic showing ports in cap



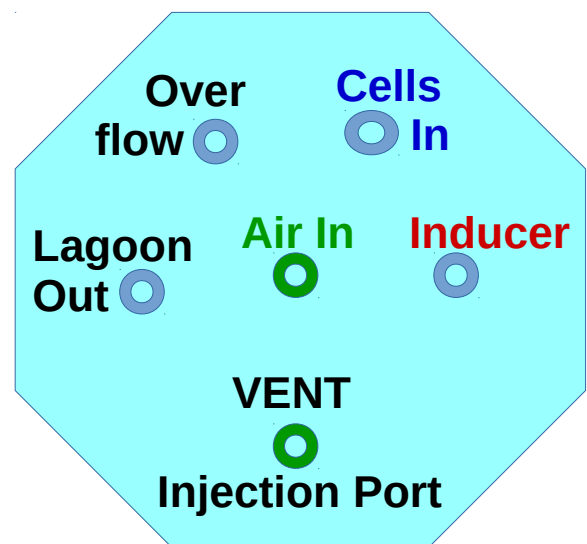
CellStat



Lagoon



CellStat



Lagoon