CLEANING, PRIMING & STARTING PROCEDURES v1.2

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This document aim to give general recommendations for running an experiment using *Evoprog Machine (no valves)* already assembled. All the details regarding the assembling are collected in:

- Incubator_Procedures_Evoprog
- Lids_Procedures_Evoprog

The procedures are listed respecting a chronology description.
Last document section is dedicated to the pumps currently available.

After any experiment: CLEANING

1. Stop the pump/syringe and switch off heating and mixing systems;
2. Replace each chemostat bottles with sterile filled with 20mL of **NaOH 1M** (no magnet needed);

Proceed then with the cleaning and autoclaving of the chemostat bottles: leave them in water+10% bleach bath overnight. Then, rinse and autoclave.

3. Respecting sterile conditions, remove and replace the bottle of medium(*), with a reagent 1L bottle filled with **NaOH 0.5M**;

Proceed then with the cleaning and autoclaving of the Medium Bottle + Lid: fill completely the medium bottle with 10% bleach in H_2O. Using two 50mL syringes suck the bleach to fill the tubings as well. Leave it overnight and rinse + autoclave the morning after.

4. Start the pump at 15mL/h and leave it overnight;
5. After >14hrs, stop the pump and swap the NaOH with a 20% Ethanol 1L bottle for + 2hrs at 15mL/h;
6. Stop the pump and leave the system as it is.

(*)*We recommend to use a 5L media bottle for long-run experiment.*

Before any experiment: PRIMING (if the machine is already assembled/connected. If not, go to *STARTING Procedures*)

1. Respecting the sterile conditions, remove and replace the 20% Ethanol bottle con 1L bottle of sterile medium with double concentration of the antibiotics needed later for running the experiment;
2. Run the pump at 15mL/h for 2hrs;
3. Respecting the sterile conditions, replace unscrewing the tubing related to the medium bottle with the definitive medium bottle. Then, prime the system for 1hr at the experiment flow-rate;
4. Place the Medium bottle on a heater/stirrer plate at minimum temperature and speed equal to 2.

STARTING Procedures

General recommendations

- *Before every experiment, design the scheme of the connections, chemostats and cellstats needed. Modified the connections as required adding "Y junction" or any other tubings for inducer or co-culture etc…Use only sterile tubings/bottles and tools respecting as much as*

possible sterile working condition.

- *When replicas are needed, use the same ID tubings of the same shortest reasonable length. Avoid any narrow curve that could choke the tubes;*
- *Use 1.42mm ID tubings from all the connections downstream the chemostat. Wider, 2.06mm ID from the pump/syringe pump to the chemostat medium inlets;*
- *Keep the incubator in a higher position among the output tubings/luer;*
- *Clamp gently and similarly the tubings crossing the peristaltic pump cartridge (if used)*

Flow rates and volumes identification

1. Check if all the connections follow the scheme (one more time);
2. Fixed the flow-rate F (mL/min), determine the Cellstat Dilution rate D_c (**<30min**) or the Cellstat Volume V_c (mL).

i.e F= 15mL/h = 0.25mL/min => $\mathbf{V_c = D_c * F}$ (**<V_{ch}/4 mL**)

3. Fill a sterile bottle with the correspondent amount of volume in Ethanol 100% and add a small magnetic bar (8x3mm). Then, adjust the height of the "yellow" tube outlet needle. The tube mouth has to be positioned just under the surface of the liquid when the lid is tightly closed;

This operation will affect the accuracy of your experiment. Be very careful. When done, shake the bottle, the Ethanol will prevent any contamination introduced operating.

4. Start the rinsing and priming of the bioreactor (select the pump speed);
5. Adjust the temperature of both the heating systems (if required);

If the central-back chemostat holder (under the thermometer position) is not used, insert and leave a bottle for preventing any temperature fluctuation due to the fan/mixing flow.

STARTING THE EXPERIMENT (assuming the bioreactors already cleaned & primed)

1. When the priming ends and the temperature is stable, swap the primed chemostat bottles with the correspondent bottles filled with the volume of cell culture required and 1 magnet bar (15x4mm)

20mL < Chemostat Volume V_{ch}= 2*F **<38mL**

2. Start and check the mixing;

If the mix is not properly conducted, check the position of the bottle and then stop and re-start again the mixing.

3. After 2 hours, measure the OD and check again the temperatures and all the volumes into the bottles (*last check before adding the phage*);
4. Stop the pump and add the phage into each cellstat unscrewing the lid just for the time strictly needed and remaining as close as possible to the vessel for avoiding any undesired dripping from the chemostat.

20µL < Phage Volume < **200µL**

5. Start again the pump.