Emulsiflex C3 Homogenizer Protocol

Contact
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Please contact Annette Erbse or Nicole Kethley for training. It is fairly easy once you know what to watch out for, but can go very wrong if you don't!!

If you have questions or concerns or want to report a problem with the C3 homogenizer please contact either Annette or Nicole.

The most important thing:
Make sure you re-suspend your cells really well, in enough volume, and that there are absolutely no clumps!!

You Need

500 ml miliQ-H_2O, at least 250 ml 75% EtOH, 500ml buffer, your cell solution (at least 15 ml), an ice box to keep your samples cold, a large waste beaker, a serological 25 ml pipette and tissue paper for cleanup. If you have multiple samples you will need more buffer and H_2O for cleaning between the samples.

Cell Solution

Resuspend the cell pellet in your buffer. A ratio of **5:1 buffer (mL) to pellet (g) works well in most cases (for example 15 ml on 3 g cells).** Ensure an even suspension of your cells. It is important that there are no clumps left. They will clog the homogenizer. Pipette your cells up and down with a 10 ml pipette. Observe the solution flowing out to be sure there are no clumps left. Remember anything you can see by eye is way too big. Please be patient and make sure all clumps are **gone**. Consider running your sample through gauze to trap all particles or let it sit in a conical so that any remaining

clumps sink. Fill the solution carefully in once the homogenizer is ready leaving a few ml and the clumps at the bottom behind.

Running the Homogenizer:

Note:

Be careful and avoid trapping air bubbles in the system, they can block the homogenizer.

1. If the heat exchanger is installed turn on the cooling or place the sample loop on ice.
2. Turn on the Nitrogen flow to the homogenizer. You should just have to open the bottle. The regulator is set to the right pressure (- 100 to 120 psi).
3. Make sure the "**stop"** knob (red knob with arrows marked "STOP") on the top left corner is pushed in and that the gray air pressure regulator is loosened.
4. Turn the main power for the C3 on. The switch is on the back.
5. Unscrew the stainless steel cylinder cap from the top of the stainless steel cylinder.
6. The last user will have left the cylinder half full with 75% ethanol.
7. Place the end of the long white tube in an empty waste beaker.
8. Turn the red "STOP" knob clockwise so that it jumps out.
9. Push the green knob (start).
10. Run the ethanol through without pressure. Make sure not to introduce air. While the ethanol is pulsing out keep an eye on how much liquid is left in the cylinder, when 0.5 inches are left, add 50 ml H_2O before the cylinder is completely empty. Be careful not to introduce bubbles.
11. Run water through without applying pressure. While the water is pulsing out keep an eye on how much liquid is left in the cylinder, when 0.5 inches are left, add some more water (25 ml). This will clean traces of ethanol.
12. Keep an eye on the H_2O level in the cylinder, when 0.5 inches are left, add Buffer (at least 25 ml).

13. When half an inch of buffer is left in the cylinder stop the pumping by pushing the big red stop button.
14. Pour your cell solution in the cylinder.
15. Place the open end of the tubing into the cylinder making sure that it does not go all the way down but is hanging above your cell slurry. You can use the clip laying on top of the C3 to secure it. Just make sure to not pinch the tubing.
16. Hit the start button and wait till you cell suspension is coming out of the tube.
17. Turn the grey Air Pressure Regulator clockwise, until the bigger homogenizing pressure gauge on the right is pulsing (usually starts if the regulator pressure hits around 40 psi, if it does not start right away wait two seconds at 40 psi before you increase further).
18. Once the homogenizing pressure gauge on the right starts pulsing increase the pressure till it is pulsing around 15000 of E.Coli or at 22000 for Yeast. The homogenizing pressure might change over time keep an eye on it and adjust it to the desired level by rotating the grey knob.
19. Most cell solutions require 2-3 passes through the homogenizer.
20. You have two ways of achieving that.
    a. Transfer the tube into your collection beaker on ice and collect the homogenized cell suspension. Wait till there is half an inch of solution left and to empty the cell solution into the cylinder again. Repeat.
    b. You can alternatively leave the open end of the tubing in the cylinder. As a rule of thumb the C3 pushes solution through at a rate of 40ml/min independent of the pressure applied. So if you have 40 ml and let them circulate for 3 min you should have three passes. Since the tubing has additional volume I would add 10 ml to your volume. So if you added 40 ml of cells you should calculate with 50 ml of overall solution so you would need at least 3 min and 45 sec.
21. If the cells are homogenized and there is very little volume left (0.5 inches or less), add a few (10 or more) ml of buffer to get the last bit of suspension out.
22. Stop the homogenizer, loosen the grey Air Pressure Regulator.
23. Remove the sample funnel and wash it out.

24. Re-fasten it and fill in 100 ml water.
25. Run the water through the homogenizer. Do it for the most part without pressure. After the first few pulses increase the pressure until the homogenizing pressure gauge on the right is pulsing at about 5000 psi, keep there for 20 sec, release the pressure. Repeat the pressure increase and decrease one more time. This is to dislodge any small trapped particles.
26. If only little water is left (0.5 inches) add 100 ml 75% ethanol. Run ethanol through the system without pressure for a minute and then stop by pushing the red button. You want to leave the cylinder half full with 75% ethanol. Screw the cylinder cap back on.
27. Turn the air flow off.
28. Turn off the main power switch.
29. Clean up.

PROBLEMS:

Note: if you run into problems and the C3 stops working, get help. Either from Annette or Nicole or if you can't find either of them, from an experienced user in the Sousa/Batey labs. If you can't find anyone call the phone number of Avestin in the manual next to the C3. You will get a person on the phone right away and they are very good with helping.

The procedures below can be used but please do not use them on your own if you have never done it before.

1. If the C3 stops pulsing in the middle of a run you can use the long needle provided to very gently push on the ball valve in the cylinder. You should have been shown how to do this during training. Often that will fix it. If not get help.
2. The most common problem encountered, when users turn the grey knob and it has no effect on the pressure of the homogenizer. Usually that means that there is an air bubble somewhere. Press the "STOP" knob and shut off the air to the C3. Wait for the pressure build-up on the blue manifold to sink completely. If you have your buffered cells in the cylinder, carefully remove them with a transfer pipette. Fill the cylinder with water all the way, screw on the steel cap. Remove the black cover from the connection and attach the free blue tubing coming from

the air manifold to the silver end of the steel cap. Once it is secured, open the air flow again. The air pressure will push the bubble out of the C3. Once water runs though, detach the tube (it has an “easy snap connection”, make sure you know how to work that). Don't wait too long, you do not want to push all the water out otherwise you can introduce air again. You should now be ready to start again.

3. Really you will see the pressure build up normally but the liquid is flowing out constantly without any rhythm. That means there is something stuck in the valves. Please inform Annette or Nicole because the C3 will need some extensive work to fix this.
4. The same is true if you see that the C3 leaks fluid even if it is switched off.