Experimental Setup: TGT LumeGen Bioreactor

1. Turn on Laptop

2. Turn on Growthworks Control box. Wait 15-20 seconds.

3. Start Growthworks Software located on desktop.

5. Click on "Pump" button.

6. Select tubing conversion factor based L/S 17

7. Type in desired flow rate (90mL/min)

8. Hit "Enter"

9. Click on "Start Pump"

10. Wait 10-20 seconds for pump to reach desired flow rate

11. Adjust restrictor valve so that the displacement reading is -0.37 +/- 0.050mm. Do not adjust the knob at the bottom of the stimulator flow pump as this is calibrated by TGT for a mean pressure of 90.

12. Turning the restrictor knob clockwise will increase the pressure and move the piston in the positive direction, turning the restrictor knob counterclockwise will decrease the pressure and move the piston in the negative direction

13. Click on the "Engage Mid Position" button on the pump control screen

14. The flow rate will oscillate around a flow value (usually the flow rate +/- 5ml/min)

15. If the flow rate is above the desired flow rate, slightly turn the restrictor knob counter clockwise (1/16th turn), and/or turn the control knob on the bottom of the stimulator clockwise (1/4 turn) and let the mid position controller reach equilibrium

16. If the flow rate is below the desired flow, do the opposite of #13

17. Exit out of the Pump Control panel

18. Click on the Define Press Wave button

19. Click on the Define Function Generation button

20. Enter in the desired pressure stimulation parameters. Make sure that Level 1 and Level 2 are **symmetric** (60 and 120) about the mean pressure (90). For example, a mean pressure of 50 mmHg would allow the user to run stimulation profiles of 60/40, 70/30, 80/20, 100/0, etc. **DO NOT** type in levels that are not symmetric. The motor **WILL** run out of stroke over time if the stimulation pressure levels are not symmetric about the mean pressure value and the experiment **WILL FAIL**.

21. Make sure that the displacement reading is near zero. If it is near zero, it is OK to override the waiting
times

22. Click on "Start Stimulation"

23. Scan time should be at least twice the period associated with your stimulation frequency (this ensures you will get at least 2 cycles of data for each scan the software records)

24. Scan Points should equate to at least 50/second of scan time

25. Minutes to first scan: Up to the experimenter, usually 0

26. Minutes between scans: Up to the experimenter, remember that taking data every 10 minutes can result in a significant number of data points

27. Click on "Start Acquisition" if data acquisition is desired, click on "No Acquisition" if you do not wish to record data for this experimental run.

28. Make sure that the time scale and y-scale are adjusted properly for the parameters that you have entered into your stimulation profile

29. Click on "Start"

30. Press Alt-F10 to bring up the Mid Position Controller panel

31. Use this panel to track the maximum and minimum flow rate speed limits that are being set by the software

32. Once the limits have been set and the displacement amplitude stays within +/- 1.5mm (**Takes 25minutes**) click on the "Set Drift Limits" button to ensure the long term stability of the experiment

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