

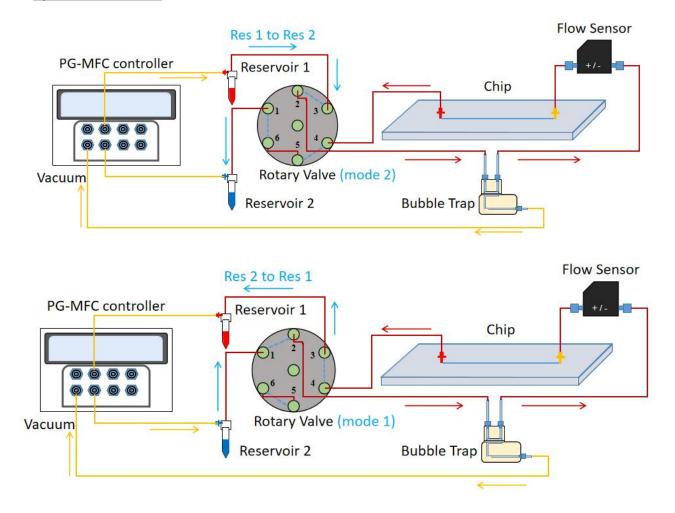
Recirculation System Setup and Operation

I. Introduction:

This document will detail how to assemble and operate a microfluidic recirculation system, which may be used to cycle fluid in a controlled, continuous, and unidirectional manner. It will cover:

- 1. How to connect the required components.
- 2. How to check for successful connection.
- 3. How to perform scripting to automate the system.

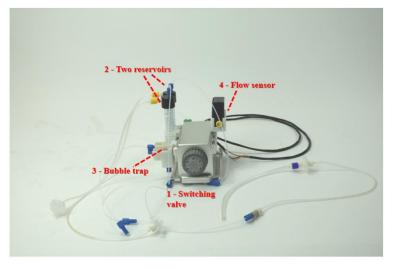
II. System Schematic:



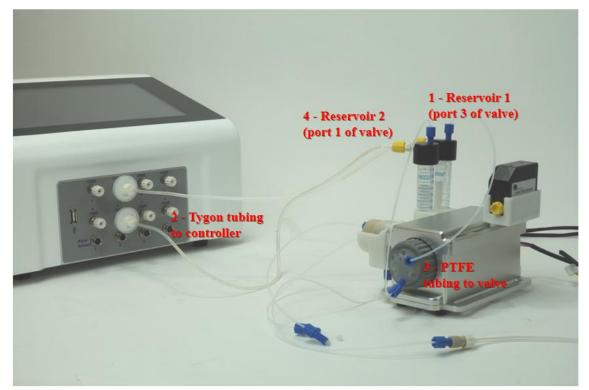


III. General Procedure:

i. <u>System Setup:</u>



- 1. Mount the rotary switching valve¹ with two 15 ml reservoir kits², a bubble trap³, and a flow sensor⁴.
- 2. Connect one reservoir¹ to the pressure controller at CH#3 and to the switching valve at port 3.
 - a) Tubing to the pressure controller should be Tygon² (gas).
 - b) Tubing to the valve should be PTFE³ (liquid).
 - c) From now on, this reservoir will be referred to as "reservoir 1".
- 3. Connect the other reservoir⁴ to the pressure controller at CH#4 and to the switching value at port 1.
 - a) Follow the same rules for tubing as step 2.
 - b) From now on, this reservoir will be referred to as "reservoir 2".



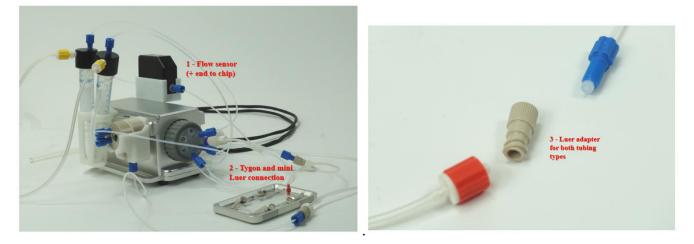
Address: 2176 Ringwood Ave. San Jose, CA, USA, 95131 Confidential



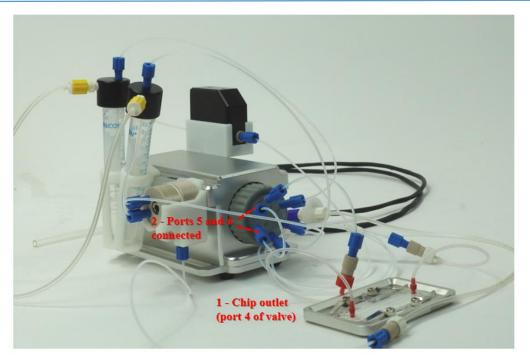
4. Using PTFE tubing, connect port 2 of the switching valve with one gas opening of the bubble trap¹. Connect the other gas opening of the bubble trap to the (-) end of the flow sensor, also with PTFE tubing.



- 5. Mount the reaction chamber chip. Connect its inlet to the (+) end of the flow sensor¹.
 - a) Connection type varies with chip; this guide will assume a mini-Luer chip.
 - b) Using a male mini-Luer connector, attach a short length of Tygon tubing to the chip².
 - c) Connect the free end of the Tygon tubing to a male Luer lock. Then connect this tubing to the PTFE tubing of the flow sensor with a female Luer-to-flat bottom adapter³





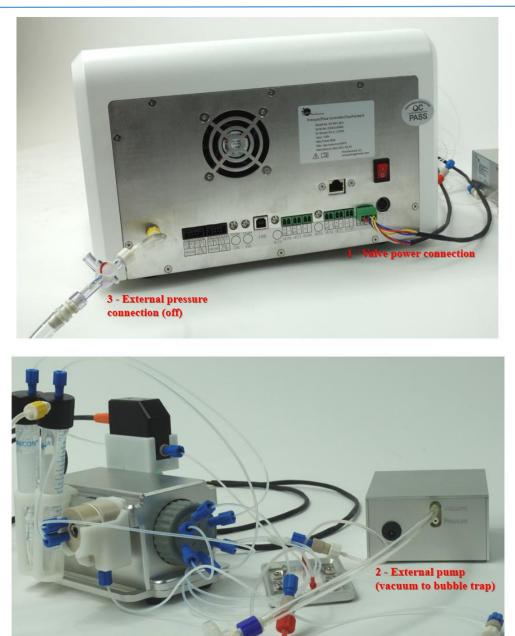


- 6. Connect the chip outlet to port 4 of the switching valve¹.
 - a) Use the same adapter setup as step 5.
- 7. Using PTFE tubing, connect ports 5 and 6 of the switching valve².



- 8. Connect the power cable of the flow sensor to the outlet on the pressure controller under CH#4. This ensures it is connected to the same source (2) as the reservoir channels¹.
- 9. If plugging the reservoirs into a different channel, ensure the power cable is still connected to the same source.





- 10. Connect the switching valve to the rear of the pressure controller with its 4-pin cable¹.
- 11. To enable active operation of the bubble trap, connect it to any channel of the pressure controller in source 1 or 3.

a) Make sure to add a filter before connection to prevent liquid entering the controller.

- 12. If using an external pump, connect the bubble trap to the vacuum port of the pump². Pressure control may still be left to the same controller however.
- 13. If using an external pressure source, connect its tubing to the rear of the pressure controller³.
 - a) Turn its valve's "off" section in the direction of the pressure source to keep it off, and perpendicular to this direction to turn it on.
- ii. System Connection Check:



1. Verify the flow sensor was recognized by the pressure controller.

tings List	Current Settings(Simple I	Mode)	
۸۲ About	- + × Default FlowControl		
Company Info: Precigenome.com Contact Info: info@precigenome.		мр	
Software Version 01.20.0701 Frimware Version	Load	Save Set as Defau	
F3.30a210701a F3.30a210701a Hardware Version	Import Export	Dele	te 🛛 🔀
V3.3 V3.3	Others		
Liquid Flow Meter 1: N.A. Liquid Flow Meter 2: OK PG-LFS-C Liquid Flow Meter 3: N.A.	430D ul/min Pressure Unit	⊙ kPa ◎ psi ○ mBai	r 🗠
Liquid Flow Meter 4: N.A.	Data Export	Software Update	0
	Import Data	Firmware Update	
Check OK	Flow Sensor Calibl	rating System Time	
	Self-Check	About	
	Lock Screen		
			Simple Mod

a) On the pressure controller, go to \mathbf{x} System Settings¹ > About² > Check³. Ensure that the flow sensor model is updated in the window⁴.

Flow Sensor Calibrating - +	× ent Settings(Simple Mode)		
dvanced properties for the flowmeters	afault FlowControl		
elect Device Source #2 PG-LFS-0430D v ul/min H20 device check			
elect Solvent H2O v current 2 read coefs	FlowControITEMP		
Coefficients	Load	Save Set as Default	
Custom Linear v	mport	Delete	
offset 0.0 3 Q_cal = a * Q + offset			
a 1.0	ers		M
nese settings change the behavior of the instruments and the measurement data. se them carefully. The properties are different for each instrument.	essure Unit O kPa	● psi ○ mBar	<u>.</u>
Save	Data Export	Software Update	0
	Import Data	Firmware Update	
	Flow Sensor Calibrating	System Time	
	Self-Check	About	\$ 2
	Self-Check Lock Screen	About	

- b) While still in System Settings, go to Flow Sensor Calibrating¹ > Select Solvent > H_2O^2 . Set the coefficients to Linear with offset = 0 and a (slope) = 1³.
- 2. Now verify the switching valve was recognized by the pressure controller.



	S	election Val	ves			3 Swit	ching Valv	es		
Valve #1	Valve #2	Valve #3	Valve #4	Disabled	Valve #1	Valve #2	Valve #3	Valve #4	Enabled	
~	~	~	~				-	-		
Disabled Init	NA.	NA.	NA.		NA.	Enabled Mode1	NA.	NA.		
urrent Status:	Valve #1	Port, 1			Current Status:	Valve #2 Mode		peration		
							- 1 	***	22	
	Port. 1	0 0	Port. 10			Port 1	0			
Port. 2	0		0	Port. 9	Port 2	0		0	Port 6	M
Port. 3	0	0	C	Port. 8						0
Port. 4	0		0	Port. 7	Port 3	C		0	Port 5	
	Port. 5	0 0	Port. 6				Po	rt 4		\$
				\$	otary Valve				\$	

a) On the pressure controller, go to Rotary Valve Settings¹ > Check Rotary Valve². If successful, the options under Switching Valves³ will now be colored instead of gray.



iii. System Scripting:

	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves	Status:Idle cur 0s.
Step	run:10.0		E	0	0	2:Close	0% Total 0:10: 32.0s.
p #1	run:1.0	DC P: 1.0 PSI	1	0		2:Mode 2	09:50:46 Flow control not available. 09:50:50 Flow control not available.
p #2	run:30.0	DC F: 50.0 ul/n	r			2:Mode 2	
p #3	run:1.0	DC P: 1.0 PSI		0	0	2:Mode 1	
p #4	run:30.0	DC F: 50.0 ul/n	r			2:Mode 1	
p #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	0	2:Mode 2	
p #6	run:1.0		0	0	0	2:Close	

2. Go to Advanced Scripting². If your desired script was already written, you may load it with

Load Script³ > [script name]. Otherwise, follow the rest of this procedure.



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Tim Ar Show or Hide Components	- + x	Status:Idle	
Image: source#1 Image: source#1 Image: source#1 Image: source#1 Image: source#2 Source#2	Time (sec) Source1 Profile Pump#1 Valve1 Valve2	Cur Os. 0% Total 0:10: 32.0s. 09:50:46 Flow control not available.	*
#2 run:30 Profile Pump#2 Valve#3 Valve#4 Source#3 Profile Pump#3 Valve#5 Valve#6	Source2 Profile Pump#2 Valve3		~
#3 run:1.(Source#4 #4 run:30 Profile Pump#4 Valve#7 Valve#8	Valve4 Up Source3 Profile Pump#3 Down Valve5		0
un:1. goto S loop :2 Selection Valves W Switching Valves	Valveő Source4 Profile Pump#4 Valve7		in a
run:1. Modules Motor	Valve8 Digital Out		

3. Click Script Settings¹ and uncheck all unused profiles, pumps, and valves. Also uncheck Digital Out². This will reduce the displayed options to just what is being used.

a) For the previously described setup, only pump 2 and valves 3 and 4³ should be turned on.

	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves		Status:Idle cur 0s.
InitStep	run:10.0	2 (E	0	0	2:Close		0% Total 0:10: 32.0s.
Step #1	run:1.0	DC P: 1.0 PSI		0		2:Mode 2		09:50:46 Flow control not available. 09:50:50 Flow control not available.
Step #2	run:30.0	DC F: 50.0 ul/n				2:Mode 2	Action - + ×	
Step #3	run:1.0	DC P: 1.0 PSI		0	0	2:Mode 1	a 💽	
Step #4	run:30.0	DC F: 50.0 ul/n				2:Mode 1	al	
Scep #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	0	2:Mode 2		
Step #6	run:1.0		0	0	0	2:Close		

4. InitStep: Turn off all valves and turn on the pump corresponding to the source of your channels. Set runtime to 10 sec and switching valve mode to Close. Do not set Source Profile.

- a) Press the symbol¹ to edit the selected step value.
- b) For the previously described setup, pump 2 should be turned on.



the symbol² \mathbb{E} instead of \mathbb{Q}

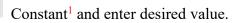
- c) Runtime should be kept this way to allow the switching valve to initialize.
- d) If using an external pump, make sure to check external when turning it on. This should display

1		2	×	Ø.	0		RecircTemp
	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves	Status:Idle
nitStep	run:10.0		Е	0	0	2:Close	III Rotary Valve Setup -+ × 6
Step #1	run:1.0	DC P: 1.0 PSI		0		2:Mode 2	Switching Valves Valve #1 Valve #2 Valve #3 Valve #4 Disabled Valve #1 Valve #2 Valve #3 Valve #4 Disabled
Step #2	run:30.0	DC F: 50.0 ul/r	r			2:Mode 2	NA. Mode 2 NA. NA. 2
itep #3	run:1.0	DC P: 1.0 PSI		0	0	2:Mode 1	Port 1 0 Port 6
Step #4	run:30.0	DC F: 50.0 ul/r	r			2:Mode 1	Port 3 O Port 5
step #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	0	2:Mode 2	Port 4
itep #6	run:1.0		0	0	0	2:Close	

e) To edit the switching valve mode, press the icon¹ so that it is colored. Toggle between active modes 1 and 2 by tapping the ports, or set to Close by toggling from Enabled to Disabled².

5. Step #1: Turn on valve 3 and set the source profile to 1 psi. Additionally, set runtime to 1 sec and switching valve to Mode 2.

a) To edit source profile pressure, set wave type to



b) PSI setting is kept low to prevent sudden spikes in pressure or flowrate when switching modes.



	•	-	-		0		RecircTemp	🍃 🔅	
	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves		Status:Idle	
InitStep	run:10.0		E	0	•	2:Close	JU Source 2 Pressure Profile - + ×	0% Total 0:10: 32.0s.	
Step #1	run:1.0	DC P: 1.0 PSI		0		2:Mode 2	Pressure Profile	09:50:46 Flow control not available. 09:50:50 Flow control not available.	
Step #2	run:30.0	DC F: 50.0 ul/n	r			2:Mode 2	V_I 1.0 V_h 3.3 period(sec) 0.1-65.5 10		
Step #3	run:1.0	DC P: 1.0 PSI		0	0	2:Mode 1	duty_cycle(%) 1 2		
Step #4	run:30.0	DC F: 50.0 ul/n	r			2:Mode 1			
Step #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	•	2:Mode 2			
Step #6	run:1.0	0	0	0	0	2:Close	🖌 🗶		

6. Step #2: Set source profile to desired flowrate and runtime to desired value. Keep switching valve at Mode 2.

a) To edit source flowrate, click the \bigvee symbol² to toggle from pressure to flowrate. The new

-	23	an s			0		RecircTemp	🍃 🔅	- Operation
	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves		Status:Idle	
InitStep	run:10.0		E	0	•	2:Close	Jr Source 2 FlowRate Profile - + × Wave Type	cur 0s. 0% Total 0:10: 32.0s.	
Step #1	run:1.0	DC P: 1.0 PSI		0		2:Mode 2	FlowRate Profile	09:50:46 Flow control not available. 09:50:50 Flow control not available.	
Step #2	run:30.0	DC F: 50.0 ul/r				2:Mode 2	flowrate 50.0 ul/min		1.00
Step #3	run:1.0	DC P: 1.0 PSI		•	0	2:Mode 1	3 🕡		0
Step #4	run:30.0	DC F: 50.0 ul/r	č.			2:Mode 1	PG-LF5-0430D -120 ul~120 ul H20 Linear Q_cal=1.0*Q+0.0		
Step #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	•	2:Mode 2			ð
Step #6	run:1.0		0	0	0	2:Close	🖌 🖌		W .

7. Step #3: Turn off valve 3 and turn on valve 4. Set source profile to 1 psi, runtime to 1 sec, and switching valve to Mode 1.

8. Step #4: Set source profile to desired flowrate and runtime to desired value. Keep switching valve at Mode 1.



9. Step #5: Turn off valve 4 and turn on valve 3. Set source profile to 1 psi, runtime to 1 sec, and switching valve to Mode 2. Additionally, adjust loop settings to desired value.

	•	-	-		0		RecircTemp		<u> </u>	י ש		00
	Time (sec)	Source2 Profile	Pump#2	Valve3	Valve4	Switching Valves					Status:Idle	
InitStep	run:10.0		E	•	0	2:Close					cur 0s. 0% Total 0:10: 1	
Step #1	run:1.0	DC		0		2:Mode 2	л	- + × ^{ntrol}				
	1011.1.0	P: 1.0 PSI		·			Run Time	1	1.0	Fixed 1	Time Period	
Step #2	run:30.0	DC F: 50.0 ul/n				2:Mode 2		2	3	4	back	
		2027					5	6	7	8	left	
Step #3	run:1.0	DC P: 1.0 PSI		0	0	2:Mode 1	9	0) [.	С	right	
Sten #4	run:30.0	DC	DC			2:Mode 1	Goto Step #		2]2		F
	run:30.0	F: 50.0 ul/n					Loop Count		9]3	1 (000)	
Step #5	run:1.0 goto Step loop :9	DC P: 1.0 PSI		0	0	2:Mode 2		~		×		
Step #6	run:1.0		0	0	0	2:Close						

- a) To implement a loop, edit the time setting for this step and toggle Loop¹. Enter the step to return to in Goto Step $\#^2$ and the desired Loop Count³ below.
- b) In this example, the loop returns to step 2 since its own step restores the system settings to match step 1.
- c) Since the first iteration of the loop is not accounted for in this setting, make sure to set loop count to 1 less than your total number of desired cycles. In this example, it is set to 9 for a total of 10 cycles.
- 10. Step #6: Turn off the pump and all valves. Set runtime to 1 sec and switching valve mode to Close.
- 11. When you are finished editing your script, press the symbol⁴ to save and apply your

changes. Press

Start⁵ to run the script.



IV. <u>Components Used:</u>

Name	Catalog Number	Website Link
Microfluidics flow/pressure control system	PG-MFC-8CH	Link
(8 channel)		
Microfluidic reservoir kit for 15 ml tube	PG-MRK-2P15ML	Link
(2 ports; tube)		
Microfluidic flow rate sensor	PG-LFS-2000	Link
(5000 µl/min full scale)		
Inline bubble trap	PG-BT-REC300UL	Link
(PEEK; 1/4"-28; 300 µl; EZMount version)		
SwitchEZ TM electric rotary/selector valve	PG-ROTV-6P-3W2P	Link
(2-position, 3-way, 6-port)		
Reaction chamber chip		Link
(4Device; Mini Luer; 50 μl volume; 350 μm depth)		
Microfluidic fitting connectors kit	KIT-MRK-MUNF-BARB	Link
(1/4"-28 to 1/16" OD * 3/32" ID; flangeless		
fittings; Luer barb; ferrule)		
PTFE tubing (1/16" OD * 0.012" ID)	TUB1-16-ID300-L50I	Link
(Use 1/32 ID for high; above for low)		
Luer Lock Fittings Kit for Pressure Controller	KIT-MFC-MLUR-MLUR	Link
Connection		
Tygon tubing		Link
(3/32" OD * 1/32" ID; Luer barb) - for chip		
Microfluidic fitting connectors kit		Link
(male Mini Luer; 1 mm OD)		
Microfluidic fitting connectors kit	FIT-FUNF-FLUR-PK-Q5	Link
(female Luer to 1/4"-28 flat bottom adapter)		