Mar 13, 2025 Version 2

Assembly and sample collection in the MetaFlowTrain V.2

Version 1 is forked from Assembly and sample collection in the MetaFlowTrain

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Guillaume Chesneau[1]

[1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany.

Guillaume Chesneau
Max Planck Institute for Plant Breeding Research

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We use this protocol and it's working

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Abstract

The following protocol is for the preparation of a MetaFlowTrain experiment. It provides the required materials, and the procedure. We also have a video protocol to assist you in handling the MetaFlowTrain
>>Video Protocol<<

We welcome all input to improve the system and are eager to craft new adapters/microchambers tailored to your scientific needs.

Materials

METAFLOWTRAIN 3D PRINTED MATERIALS

Certain materials need to be 3D printed before starting the experiments. The following objects can be printed in advance and stored in a dark, dry area. The quantity of 3D-printed items can be adjusted according to the specific number of pieces required.

All the files below in the '.form' format can be directly uploaded to a 3D printer interface (e.g., Preform, Formlabs).

- **Biomed Ambers Resin (Formlabs)**

- Microchambers (Model: *MetaFlowTrain_microchambers_60.form*)

MetaFlowTrain_microchambers_60.... 7.8MB

- Connectors (Model: *MetaFlowTrain_Connectors.form*)

MetaFlowTrain_Connectors.form 281.7KB

- Duran bottle adapters (Model: *Duran_Bottle_Cap.form*)

Duran_Bottle_Cap.form 4.1MB

- Falcon 15ml adapters (Model: *15mL_Falcon_Cap.form*)

15mL_Falcon_Cap.form 716.2KB

- **Clear Resin (Formlabs)**

- Rack for 1 microchamber train (Model: Rack_1_Microchamber.form)

Rack_1_Microchamber.form 3.1MB

- Racks for 2 microchambers train (Model: Rack_2_Microchamber.form)

Rack_2_Microchambers.form 1.3MB

In supplementary, we provide all the .stl file.

- Microchambers (Model: *MetaFlowTrain_microchamber.stl*)

MetaFlowTrain_microchamber.stl 906.9KB

- Connectors (Model: *MetaFlowTrain_Connector.stl*)

MetaFlowTrain_Connector.stl 539.6KB

- Duran bottle adapters (Model: Duran_Bottle_Cap.*stl*)

Duran_Botlle_Cap.stl 1.4MB

- Falcon 15ml adapters (Model: *15mL_Falcon_Cap.stl*)

15mL_Falcon_Cap.stl 1.8MB

- Rack for 1 microchamber train (Model: Rack_1_Microchamber.stl)

Rack_1_Microchamber.stl 583.6KB

- Racks for 2 microchambers train (Model: Rack_2_Microchamber.stl)

Rack_2_Microchambers.stl 926.7KB

OTHER METAFLOWTRAIN MATERIALS

- **Peristaltic pump**

- (LG-BT100-1L-A-EU/DG-24-A, Darwin Microfluidics)

- **Syringe filters**

- 0,22 µm Spritzenvorsatzfilter, PVDF (steril), blau, Durchmesser: ⌀33 mm (Starlab, E4780-1221)

- **Biocompatible Tape**

- Biocompatible tape (Polyolefin Diagnostic Tape, Nr 9793R, 3M)

- **Hoses**

- 3-stop Platinum-cured Silicone Tubing: SE-TUB-SIL-SSS-1∗1 (Darwin microfluidics)
- Platinum-cured Silicone Tubing (15m): SE-TUB-SIL-1∗1 (Darwin microfluidics)
- Tygon E-LFL Pump Tubing (7.5m): SA-AVX42007, (Darwin Microfluidics)

- **Silicon rings**

- RS PRO O-Ring Silikon, Innen-Ø 3.68mm / Außen-Ø 9/32Zoll, Stärke 1.78mm, 1 Beutel mit 50 Stück
- RS PRO O-Ring FKM, Innen-Ø 2mm / Außen-Ø 4mm, Stärke 1mm

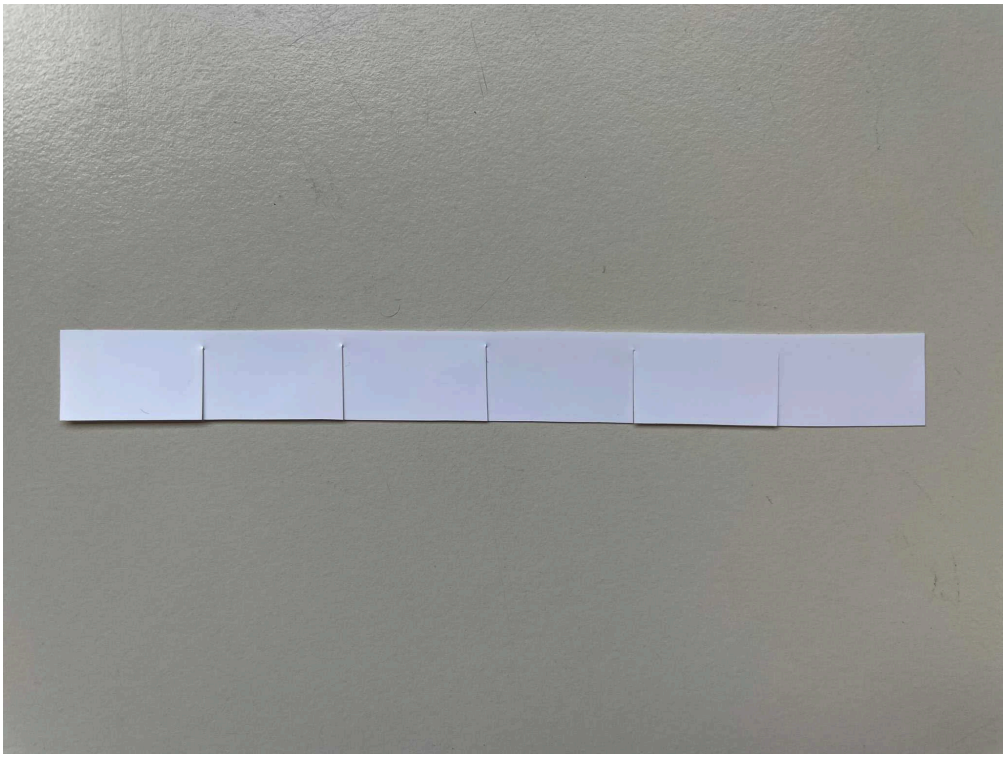
Procedure

1 **DAY 1 : PREPARE MATERIAL AND BUILD MICROCHAMBER TRAINS**

1.1 **1.1 PREPARE MATERIAL :** 1h

- **1.1.1 Cut 1cm-wide strips of biocompatible tape (Polyolefin Diagnostic Tape, Nr 9793R, 3M) with precuts every 2cm (Figure 1) and store it in a container (e.g. glass beaker).**

Comment: This tape cannot be autoclaved, which is not a problem since *the sticky side is sterile.* however, *we will still UV sterilize the surfaces later to maintain a sterile environment while handling the MetaFlowTrain system.*



- **Figure 1:** Pre-cut biocompatible tape (Polyolefin Diagnostic Tape, No. 9793R, 3M)

- **1.1.2 Prepare and sterilize your media.**

Tip: ensure your media is already in the container you will use for your MetaFlowTrain experiment.

- **1.1.3 Sterilize the necessary number of microchambers and connectors.**

Comment: All 3D-printed pieces can be autoclaved for sterilization, as the resin is autoclavable.
Tip: Place the microchamber end connectors in separate boxes to help keep the bench organized during building. Boxes on the *video protocol: 0:28.*

- **1.1.4 Sterilize one box to store the opened sterile filters during the building of microchamber trains.**

Comment: Box on the video protocol: 0:25-0:31.

- **1.1.5 Sterilize boxes to store the final built microchamber trains**

Comment: Box on the video protocol: 0:28.

- **1.1.6 Assemble the 15 ml Falcon tube adapters by connecting the tubing (Tygon E-LFL Pump Tubing: SA-AVX42007 from Darwin Microfluidics). Place the assembled 15 ml Falcon tube adapters (Figure 2) in a box (*Box on the video protocol: 4:07)* and sterilize them by autoclaving.**

Comment : The length of the tubing can vary depending on your specific setup but should be consistent (e.g., 12 cm for 15mL Falcon tube adapters)

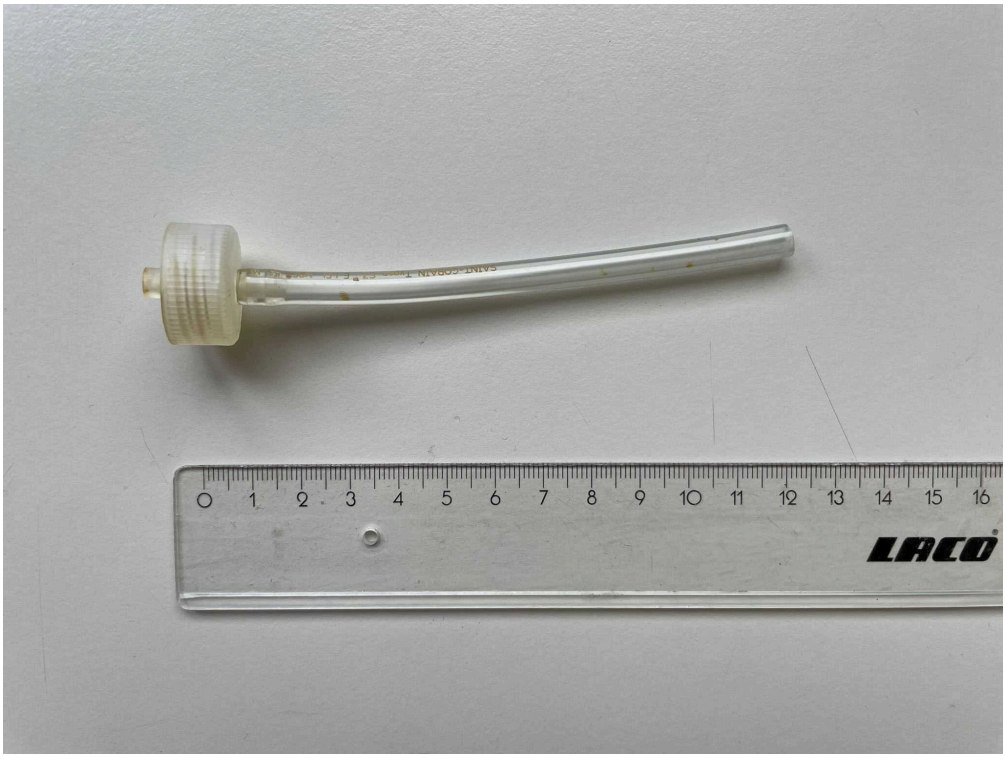


Figure 2: 15 mL Falcon tube adapter

- **1.1.7 Prepare the Duran bottle adapters by connecting the tubing (Platinum-cured Silicone Tubing: SE-TUB-SIL-1*1, Darwin Microfluidics). Place the assembled Duran**

bottle adapters (Figure 3) in a box (*Box on the video protocol: 2:24)* and sterilize (autoclave) them.

Comment : The length of the tubing should be 22 cm for a 500 mL Duran bottle, but adjust it based on the type of bottle/Flacon you're using to ensure it touches the bottom of the bottle.

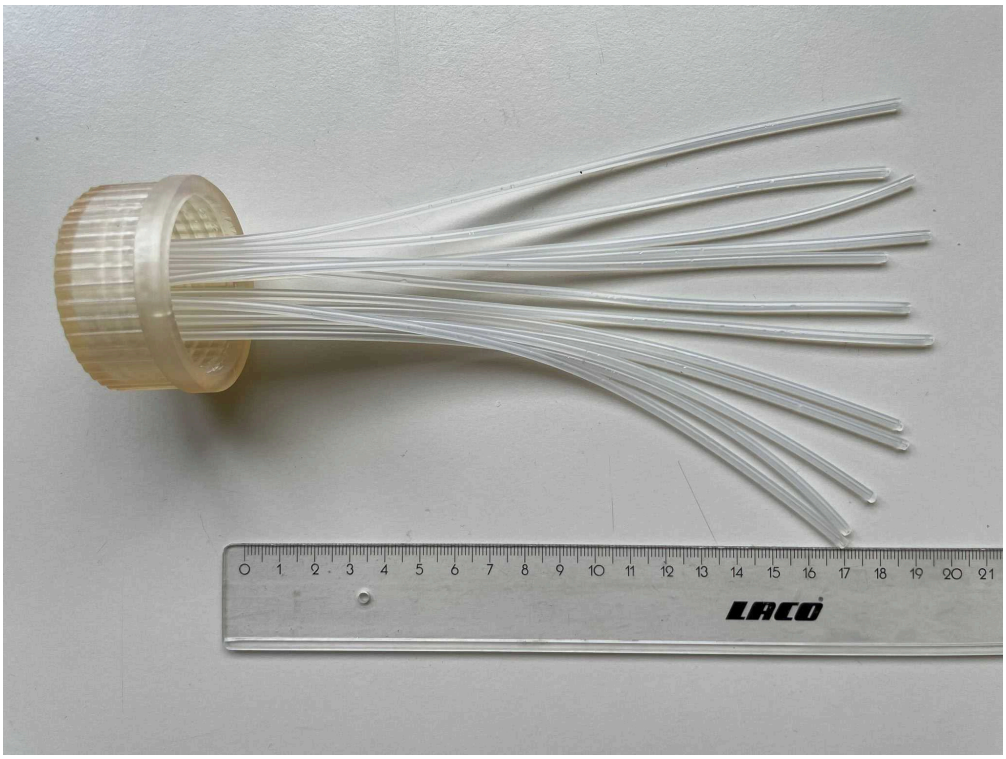


Figure 3 : Duran bottle adapter

- **1.1.8 Place silicon rings in a container and sterilize (autoclave).**

Comment: Beaker on the video protocol: 0:40.

- **1.1.9 (Additional) Place FKM rings in a container and sterilize (autoclave). FKM rings are necessary to ensure complete absence of leaks. See Troubleshooting 4.2 (Figure 8)**

1.2 **1.2. BUILDING MICROCHAMBER TRAINS (Video: 00:21:12) Video Protocol:**

1h

Comment before starting: *to ensure a complete absence of potential leaks, we recommend adding three autoclaved FKM rings to the male connector of the microchambers. This is not provided in the protocol video, they must be added in the*

male connector of the microchambers (video : 0:56 and 1:12) before plugging the filters in. Please see troubleshooting (4.2, figure 8) for details.

- **1.2.1 Surface sterilize all equipment with Bacillol and expose it to UV light for 15-30 minutes. Items to be surface ster**

ilized include boxes with autoclaved microchambers and connectors, silicon rings, box for opened filters, and box for the final trains.
Comment: This process ensures the sterility of your working environment.

- **1.2.2 Unpack the filters and place them in a sterile box.**

Comment: (Video : 0:25-0:30).

- **1.2.3 Put a silicon ring in the connector and screw it to a syringe filter, ensuring a tight fit!!**

Comment: You should see pressure on the silicone ring (Video : 0:51-0:55).

- **1.2.4 Attach the microchamber, put a silicon ring in each female connector of the microchamber and connect it to another syringe filter (Figure 4)**

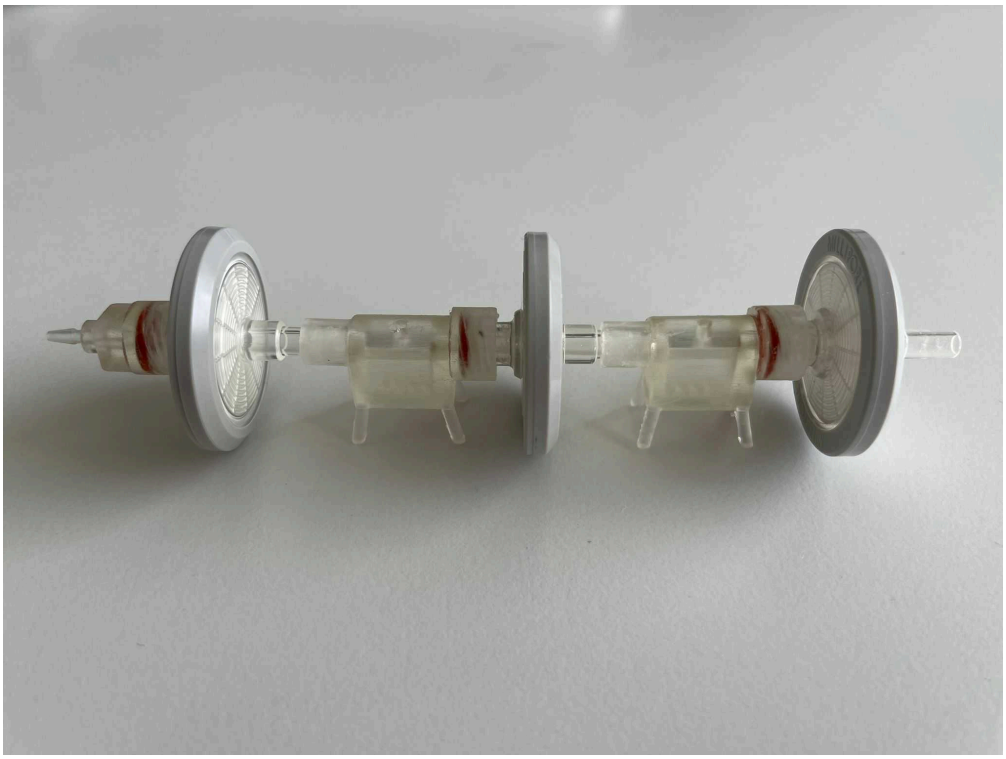
Comment: (Video : 0:57-1:04).

- **1.2.5 Place the completed trains in a sterile box**

Comment: (Video : 1:04).

- **1.2.6 You can repeat this step if you need a train of more than 1 microchamber.**

Comment: (Video : 1:05-1:26).



- **Figure 4 :** Completed train composed of two microchambers

Note

WARNING: Maintain a sterile environment throughout your experiment. Wash your gloves frequently to ensure sterility.

2 DAY 2 : METAFLOWTRAIN EXPERIMENT

2.1 2.1 PREPARE YOUR MICROBIAL INOCULUM

This step depends on your protocol.
We inoculate with 40uL of bacteria OD600, or 40uL of fungi, 2mg.mL.
Comment : The final volume in the microchamber is 400uL. Inoculating 40uL in the microchamber leads to a dilution to the tenth of your inoculum.

2.2 2.2 PUMP TUBE CONNECTION AND STERILIZATION (Video: 01:38:00) Video Protocol: 1h

- **2.2.1 Expose the pre-cut biocompatible tape (3M®, Figure 1) to UV light for at least 5 minutes on each side, then return it to sterile container.**

- **2.2.2 Pump tubes sterilization:**

a. Place the peristaltic pump on a clean bench.

b. Install the tubing (3-stop Platinum-cured Silicone Tubing: SE-TUB-SIL-SSS-1*1 (Darwin microfluidics) and tighten them to 7 notches.
Tip: can be done the day before (Video : 1:42-1:48).

c. Run 70% ethanol through the pump for 15minutes at speed 500uL.min. Then discard the Ethanol.
Comment : (Video : 1:48-1:59).

d. ***Run 500mL of water to clean the Ethanol, and empty the system.***
Comment : (Video : 2:02-2:17).
Tip : Always keep the tips of your tubing in a sterile container (e.g., a sterile beaker) to prevent contamination from touching your bench.

e. Carefully place the Duran bottle adapter (Figure 3) on your bottle filled with media and wrap it with Parafim or Micropore tape.
Comment : (Video : 2:20-2:42).

Note

WARNING : Don't tighten the Duran bottle adapter too much; you must keep the cap loose, and you might break the connectors.

f. Connect all the pump's tubing to the Duran bottle adapter.
Comment : (Video : 2:45-2:58).
Tip : If needed you can use a flat tweezers to help you adapting the tubing to the Duran cap adapter.

2.3 **2.3 MICROCHAMBER INOCULATION (Video: 03:00:00) Video Protocol:** 1h

- **2.3.1 Position your trains on racks (Figure 5)**

Comment : (Video : 3:03-3:10).

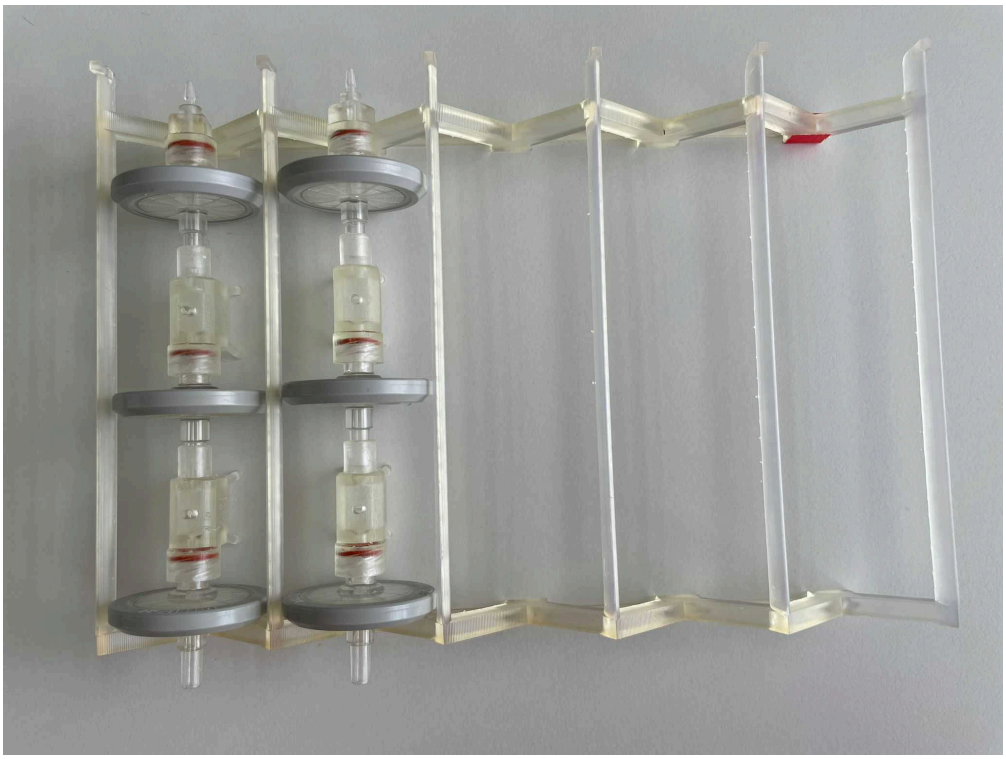


Figure 5: Example of two trains composed of two microchambers on a rack.

- **2.3.2 Add 40ul of the strain into your microchambers (your inoculum will be diluted 10 times in the microchamber).**

Comment : (Video : 3:11-3:23).

- **2.3.3 Seal the microchamber with biocompatible tape (3M®, Figure 1).**

Comment : (Video : 3:26-3:48).
Tip: Securely seal the tape with a PCR plate sealer to prevent leaks.

2.4 **2.4. CONNECT THE METAFLOWTRAIN (Video: 03:50:00) Video Protocol:** 1h

- **2.4.1 Connect the trains to the pump's tubes (3-stop Platinum-cured Silicone Tubing: SE-TUB-SIL-SSS-1*1 (Darwin microfluidics))**

Comment : (Video : 3:54-4:06).

- **2.4.2 Connect the tubes with the 15mL Falcon adapter to the end of the train**

Comment : (Video : 4:07-4:24).

- **2.4.3 Repeat these steps for the 24 channels of your pump.**

- **2.4.4 Screw 15mL Falcon tubes to the Falcon tube adapters, one per train (24 per pump).**

Comment : (Video : 4:07-4:24).

Note

WARNING: Don't tighten *them too much; you must keep the cap a bit loose.*

- **2.4.5 You're now ready to remove your pump from the clean bench and start your experiment !**

Comment: We are using a 90 cm x 60 cm plate with two handles to move the entire system from the clean bench to our growth chamber.

2.5 **2.5. START THE PUMP** 5m

- **2.5.1 Once all your pumps are prepared and settled in your growth chamber, fill the media at a high speed (e.g., 200 µL/min) until it reaches the first filters in the trains.**

- **2.5.2 Next, adjust the flow rate to a maximum of 20-50 µL/min to fill the microchambers.**

Comment: It's crucial to fill the microchambers slowly to avoid flushing your microbial inoculum into the filters.

- **2.5.3 When the liquid begins to exit the final filter in your train, set the pumps to your experimental flow rate (e.g., minimum flow rate = 7.349 µL/min).**

Tip: You can visually confirm that the microchambers are filling. Similarly, observe the filters; they will change color from dry (whiteish) to wet (grayish) as they become saturated. If a train fails to fill, abandon it and continue with your experiment.

- **2.5.4 Wait for the desired duration of the experiment.**

Figure 6: Example of a final MetaFlowTrain setup. This setup includes trains consisting of two microchambers, with four pumps running in parallel, resulting in 72 samples. Note that the MetaFlowTrain racks can be stacked to save space

Cleaning microchambers

3

- **3.1 After finishing your experiment, collect the microchambers and connectors in separate glass containers.**
- **3.2 Remove all the tape from the top of the microchambers.**
- **3.3 Fill the glass containers with water until all the parts are fully submerged.**
- **3.4 Place the glass containers in a sonicator for 15 minutes.**
- **3.5 Thoroughly wash the parts using a wash bottle with water, for microchambers, ensure the inside is thoroughly cleaned from the top hole.**
- **3.6 Let them dry completely.**
- **3.7 Store them dry in a box, in the dark, at room temperature until the next use.**

Note

WARNING: Carefully check for any cracks on your microchambers, as multiple autoclaving cycles can cause the resin to crack, especially if the microchambers are not properly dried before autoclaving.

TROUBLESHOOTING

4

- **4.1 Leaks on top of the microchambers:**

This is often caused by poor print quality of the microchambers, likely due to scratches or imperfections on their surface. Potential causes include:
1. Errors during the 3D printing process.
2. Insufficient drying of 3D prints before curing or autoclaving, which can cause cracks during heating and damage the prints.
3. Damage during removal of the prints from the support or platform.

Cracks on the microchambers can compromise their permeability. Refer to your printer's instructions and best practices to ensure high-quality prints.

- **4.2 Leaks between microchambers:**

On the screwed side: Leaks are typically caused by a loose connection. Ensure that the silicone rings are visibly compressed when screwing the microchambers or connectors with the syringe filters. While excessive force is not required, you should notice slight compression of the silicone ring to ensure a proper seal.

On the plugged side: Leaks may occur after multiple uses due to slight deformations of the microchambers. To prevent this issue, add 3 FKM rings (see Materials) inside the microchambers before inserting the filter. The filter will compress the FKM rings, ensuring a tight and secure seal. See the image below for reference (**Figure 8**).

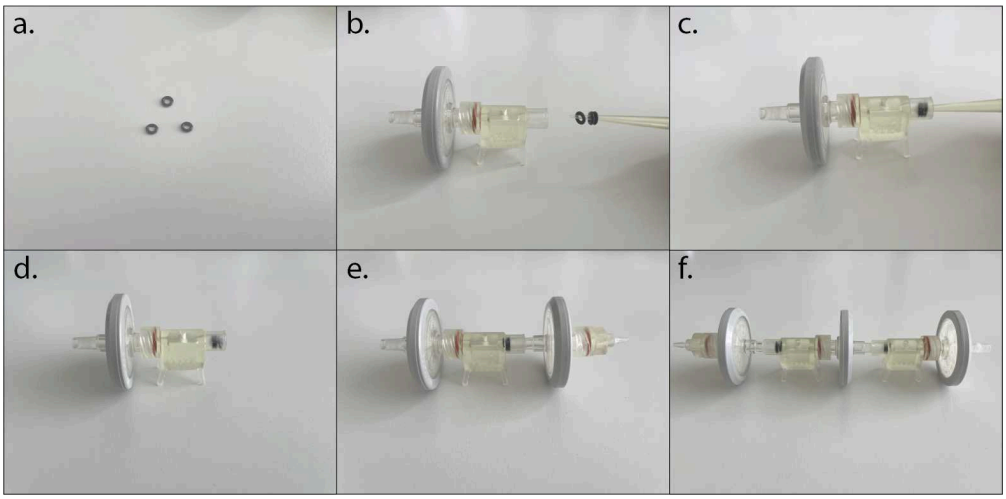


Figure 8: Adaptation of the MetaFlowTrain with FKM Rings. **a.** Three FKM rings (see Materials for details). **b.** Use a tip to pick up three FKM rings. **c-d.** Place the rings inside the microchamber. **e.** Insert the filter into the microchamber. **f.** Repeat as needed.

- **4.3 No media flowing into the hoses from the bottle:**

This may be caused by a loose connection to the bottle adapter. Check that no air is entering the connection, and ensure the hoses are fully plugged into the adapter. Also, inspect the adapter for any leftover 3D printing supports that could create air gaps and compromise the connection. In general, this issue often arises from poor print quality or improper cleaning/removal of supports from the print.

- **4.4 Air in the top of the microchambers:**

This is a common occurrence, as some air naturally passes through the MetaFlowTrain system. It's important to remove this air to ensure the microchambers are filled with liquid, especially before harvesting. To remove the air, tilt the microchambers to guide the air bubble toward the chamber's outlet. You can simply tilt the entire rack, and you'll notice the bubble moving toward the outlet. After a few minutes, the flow rate should push the air bubble out on its own.