

# Plastic Bag Bioreactor Setup SOP

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## Materials

- 1x plastic bioreactor bag
- 3x 2UM air filters (Whatman Polycap 36AS)
- platinum cured silicone tubing (various lengths/amounts/sizes, detailed further)
  - for bioreactor connections: approx 6.5m of Masterflex #96410-25
  - for media/cell inoculation process: approx 4.5m of Masterflex # 96410-73



5x glass inserters

- 3x Tubing connector Y (PP 8MM, Novatech)
- 1x Tubing connector T (PP6mm, Novatech)
- 2x Series VFA Flowmeter (10L/min, Dwyer)
- Clamps (amounts vary)
- Zip ties (various sizes/amounts)



1x metal poking tool



1x bioreactor cage/holder (custom made from crab cage material)

- 1x scissors

- 1x pair clean lab gloves (per person working on the reactor)
- 1x roll electrical tape
- 1x spray bottle ethanol (70%)
- media filter(s)
  - this has been done multiple ways:
    - I. 2x 5uM filters & 1x 0.2uM filter
    - II. 1x stacked 0.2uM filter
- 40L MS media (standard protocol for scale up)
- 1x Easy-Load Masterflex Peristaltic pump (I/P 7602-30)
- various volume of inoculum from standard seed train processes
  - typical inoculum is 10% vol/vol to media added



1x "custom made" metal inserter

## Protocol

### a) Setting Up Tubing (Air Flow Connections)

- utilizing zip ties, attach a flow meter to each side of the bioreactor about 3/4 from the top, aligned with the holes at the bottom near the base
  - it is helpful to use one zip tie on the bottom of the flow meter, and one on the top
- attach elbow connectors to the flowmeters
- Cut and label tubing sections for bioreactor air flow according to Table 1

- utilizing Masterflex #96410-25 tubing
- tubing H varies in length because it will be the distance from the connector attached to tubing E to the air supply in the wall, and this distance will vary based upon where the bioreactor is placed within the room

Table 1. Tube Connections for Air Flow (Masterfle...

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	Tube	Approx. Length (cm)	Amount needed
1	A	20.32	2
2	B	105.41	2
3	C	22.86	4
4	D	50.8	2
5	E	8.89	1
6	F	25.4	1
7	G	68.58	1
8	H	varies	1

- set up the bioreactor tubing according to the following images and steps:



Figure 1. Image of the bioreactor cage and its connections from the back. Tubing C not pictured.





Figure 2. Image of the bioreactor cage and its connections from the side. As can be seen, each side mirrors the other. Tubing H, in the back, connects to the airflow in the warm room.

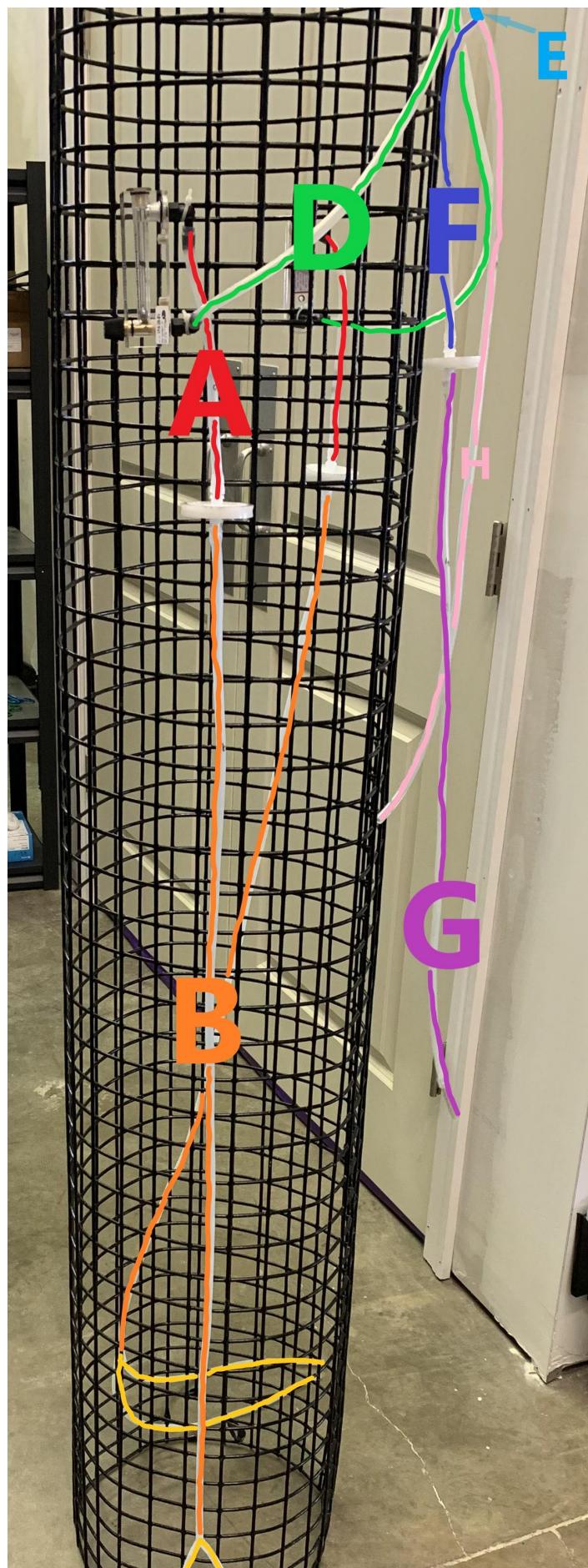
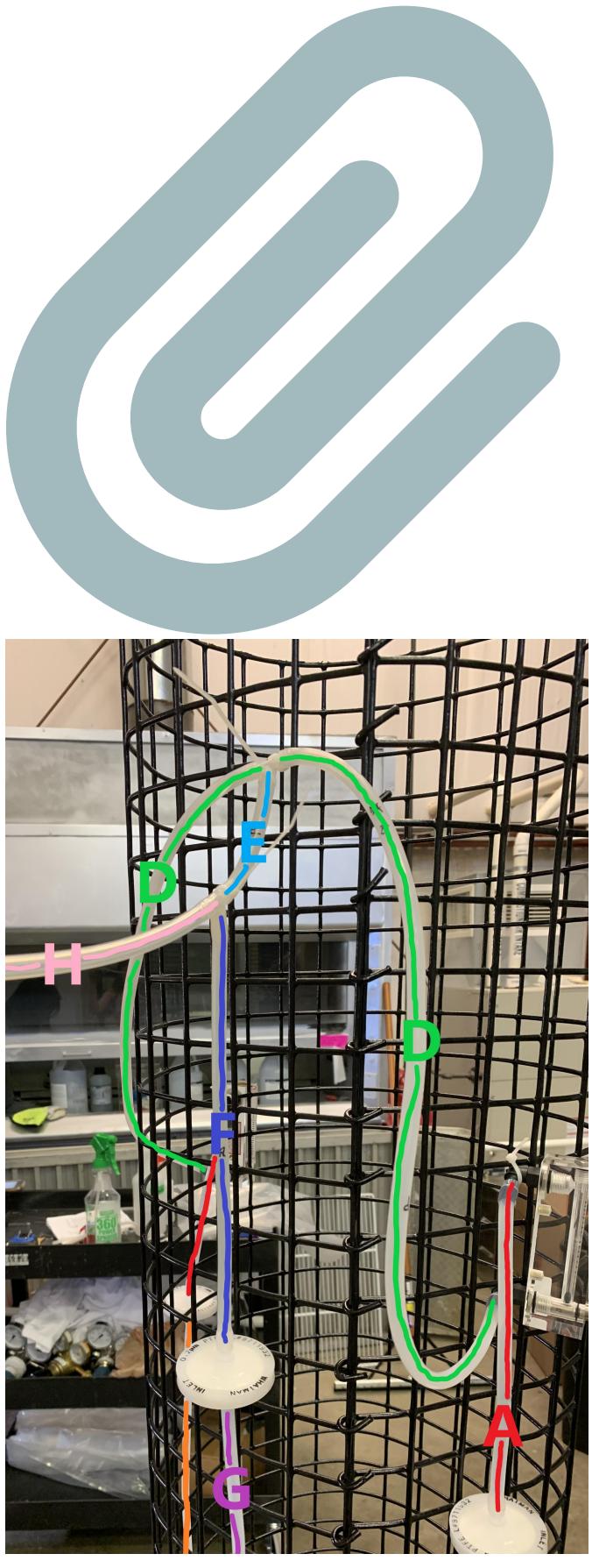




Figure 3. A more up close image of the bioreactor cage and its connections from the back. (tubings C not pictured).



- Tube A is connected to the top of the flowmeter, and to the top of the air filter. Tube B is attached to the other side of the filter, and to a Y connector. This Y connector is also attached to 2x Tube C's that are have glass inserters attached on the ends. This is repeated on the other side.
- Tube D is attached to the bottom part of the flowmeter and connected to a T connector on either side. The middle part of the T connector is connected to Tube E, which is then connected to another T connector. Tube F is connected to the middle part of the T connector. An air filter is attached to Tube F, and Tube G is attached after, below the inlet. A glass inserter should be placed on the end of Tube G.
- Tube H is connected to the downward facing part of the T connector, which connects to the air source. The length of Tube H depends on the bioreactor's distance from the air source.

**b) Setting Up Tubing (Media and Cells)**

- Cut and label tubing sections for media and cell inoculation according to Table 2
  - using Masterflex # 96410-73

**Table 2. Tube Connections for Media/Cells (Master...**

	Tubing #	Length (cm)	Amount
1	1	187.96	1
2	2	33.02	1
3	3	104.14	1
4	4	109.22	1



- set up tubing according to the following schematic:

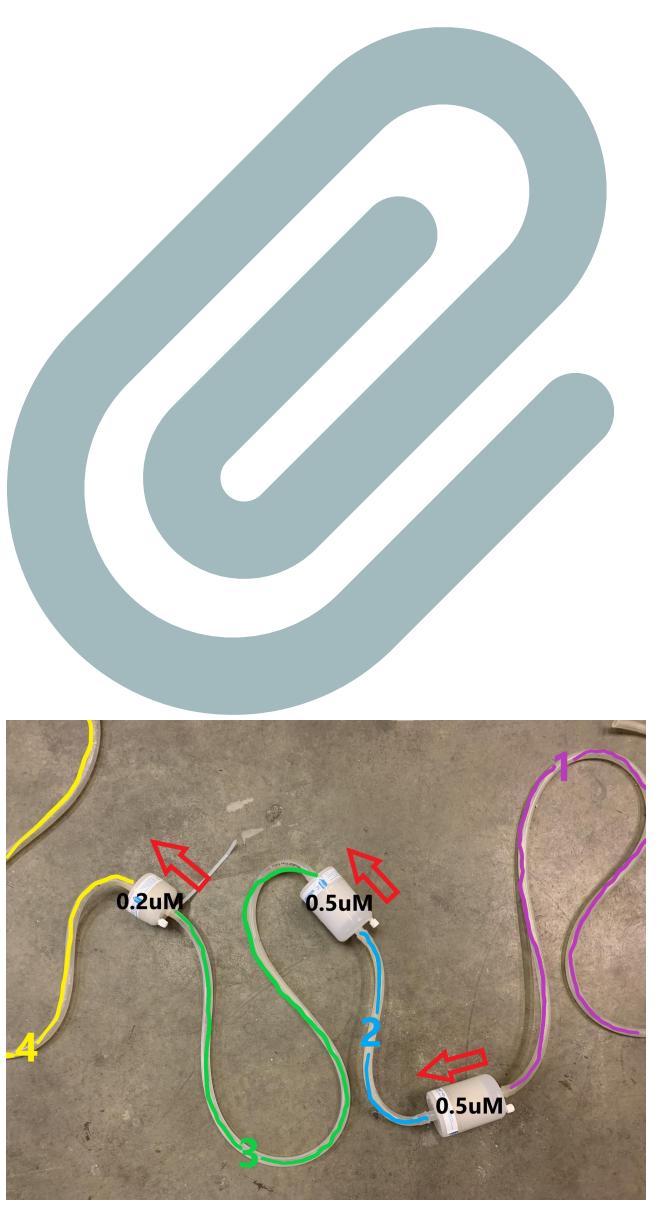


Figure 4. Red arrows show the direction of liquid flow. This flow direction is also labeled on the individual filters, so ensure the filters themselves are also oriented correctly.

- Add the custom metal inserter to the end of tube #4, making sure that the sharper, angled, end is facing out.

### c) Tubing Sterilization

- The 0.2μM Whatman air filters function to sterilize the air that is flowing through them which is very important for maintaining the overall sterility of the bag reactor and media/cultures inside of it. Therefore, it is important that all tubing downstream of these air filters be sterilized as not to re-contaminate the air that has passed through the sterilizing filters as it enters the tubing, and subsequently the bag reactors.
  - Remove filter and all downstream tubing and prep for autoclave
  - Keep all downstream tubings and glass inserters connected
    - 2x Filter connected to tube B with tube B and 2x tube Cs connected
      - Wrap filter and glass inserters with aluminum foil
    - 1x Filter connected to tube G
      - Wrap filter and glass inserter with aluminum foil



Figure 5. An example of tubing with a filter prepped for autoclave. This is done for both sides of the bioreactor.

- Sterilizing tubing utilized for media/cell inoculation
  - Keep all tubing connected as outlined previously, but wrap exposed ends (end of tube 4 with custom metal inserter and end of tube 1) with aluminum foil
- Place all tubing in autoclavable bins or on autoclavable trays, and run through 15-20 minute autoclave dry cycle to sterilize
- Wait until tubings have cooled to utilize

#### d) Setting Up Airflow

- Attach Tube H to the airflow in the wall, making sure the airflow is in the "OFF" position
- Reattach the filters/tubing to the metal cage according to Figures 1-3.
  - Put on clean lab gloves
  - Spray hands, tubings, and aluminum wrapped filters with 70% ethanol solution
  - Carefully unwrap the aluminum foil around the filter, and attach tubings to filters without touching the tips of the filters with fingers
    - Reference Figures 1-3 here as well
- Fold plastic bag in half vertically and insert into metal cage
  - Allow the two bottom corners to come out of the fold and over the base at the bottom of the cage, making sure that they align with the two openings at the bottom
    - It is helpful here to actually pull the two bottom corners out of these openings in preparation for inflation
- Spray hands, plastic bag, and metal poker tool with ethanol and create a small single hole at the top corner of the plastic bag, where it sticks out of the top of the cage, through only one layer of the bag
- Using tubing G, place glass inserter into this hole for airflow
  - Unwrap aluminum foil carefully and try not to touch the glass inserter with hands if possible
  - It can be beneficial for contamination control to spray the glass inserter with ethanol as well
- Turn on airflow and allow bag to inflate
- Once bag has become fully inflated, poke another hole at the top of the bioreactor bag to serve as the exit port for air to prevent the bag from overinflating
  - Spray hands, tool, and bag with ethanol to ensure best sterility before doing so
- Once inflated, find where the corners of the bag protrude from the holes at the bottom of the cage
- Spray the corner of the bag protruding from the cage with ethanol, and spray hands, metal tool, etc.
- Using the metal tool, gently poke a hole into the inflated bag, making sure not to poke through to the other side of the bag.
- While holding sterility, place glass inserter connected to tubing C into hole.
  - Again, unwrap the aluminum foil very carefully and ensure everything is sprayed with ethanol
- Repeat again on the same side and 2 more times on the other, for a total of 4x connections per bioreactor, 2x per side.



Figure 6. Image showing where the glass inserters are to be put into the bottom of the bag to inflate/aerate. Glass inserters are outlined in yellow.

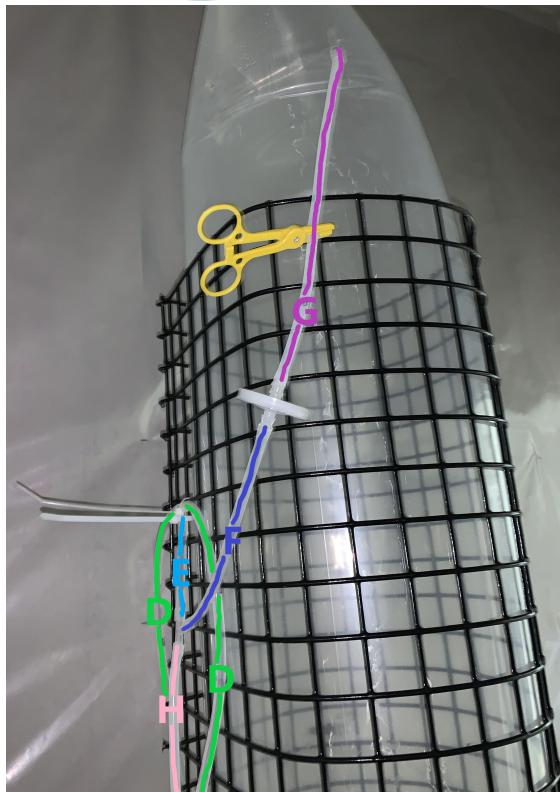


Figure 7. A side view of the top of an inflated bag with connections. The glass inserter connected to tubing G was inserted into the bag and the line was used for inflation. After the bag was inflated, the line from C is providing air to the bag, and G is no longer needed for inflation, so G is clamped to prevent overinflation of the bag as it runs.



Figure 8. The air control in the warm room in both the on and off position, with the H tubing connected.

#### e) Media Transfer

- Make insertion about 12 inches (30 cm) from the bottom of the bag with the custom made metal inserter attached to the media tubing, attaching the media tubing to the reactor bag
  - Sterilize bag, tools, hands, etc. with ethanol before insertion and be very careful when unwrapping metal inserter/tubing
- Place the other end of the tubing (#1, Figure 4) into the media bucket
- Place tubing (#4, Figure 4) in the peristaltic pump
- Turn on pump and pump the media in at a rate of 2000-4000L/min
  - This flow rate can be adjusted to accommodate the needs of the filters, media, bag, user, etc.
- Once all of the media has been pumped into the plastic bag, turn the pump off

#### f) Inoculum Transfer

- Remove the end of tube #4 (Figure 4) from the 0.2uM filter and spray thoroughly with 70% ethanol solution
- Carefully peel up the aluminum cover of inoculum flask
  - Typically, spraying the cover and body of the flask with 70% ethanol solution is best practice to prevent contamination here as well
- Place the end of tube #4 (Figure 4) into the flask of cells
- Close the aluminum foil cover over the tubing, trying to create an "airtight" environment
- Turn the pump on 2000L/min
  - Again, this flow rate can be adjusted to accommodate the needs of the tubing, bag, user, etc.
- Consistently manually agitate the flask/cells in an attempt to get all biomass into the bioreactor and avoid cells getting stuck at the bottom of the flask
- Repeat the process for as many flasks as are needed

- If possible, combine seed train flasks into larger flasks with some sterile media (inside a laminar flow or BSC!!) to prevent tubing from being transferred over and over again between flasks as this transfer of tubing outside of a sterile environment invites unwelcome contamination
- Turn off pump
- Clamp tubing close to entry
- Cut tubing close to clamp
- Alter airflow, using flowmeters and even wall air controllers, as needed for agitation
- Mark volume in bioreactor on side using sharpie and make note of the date