

The walking fly experiments usually consist of two rounds daily, one in the morning and one in the afternoon, and each is at least three hours. You must use female C1 flies from ATR bottles that are at least starved for 3 hours or else they won't respond to the LEDs. There are different procedures that must be completed to have the experiments run smoothly.

### **Making ATR**

There might be days where you're low on ATR, but there isn't anyone else available to make some.

1. Grab a small capsule of concentrated ATR from the mini freezer. The capsules are in a white box.
2. Place the capsule in your pocket so that it can melt as you are doing the next steps
3. Grab 3 empty falcon tubes (blue caps) in the black storage compartment
4. Go to the large fridge and grab the sugar water
  - a. Check for floaty things inside the bottle, if there are some present then this is a sign of mold and a new bottle needs to be made
5. Add around 10mL worth of sugar water in one falcon tube
6. Use the blue precise pipette to dispense the sugar water from the falcon tube into the other two falcon tubes. You should do this 4 times per falcon tube as the precise pipette is on 099 (990mL)
  - a. Use the pipette tips in a clear and blue box
7. Next use the yellow precise pipette to dispense the concentrated ATR once in each falcon tube as it is on 040 (40mL)
  - a. Use the pipette tips in a clear and yellow box
8. Store the new ATR in the freezer in the big fridge or use it fresh if you need it in that moment

\*The ATR made shouldn't be kept in the freezer for longer than a week\*

### **Prepping ATR bottles and making fresh ones for flies**

You need to make at least one fresh and empty ATR bottle and 1-2 bottles of ATR with C1 flies in them. These bottles must set for 48 hours before use so that the food can really soak the ATR and the flies can consume enough before experiments.

1. Grab the ATR from the freezer in the large fridge and melt it by either putting it in your pocket or rubbing the bottle between your hands
2. While it is melting, prep a bottle by putting a bit of yeast in it before squirting in the ATR
3. After it has melted, grab the precise pipette with a blue top and make sure that the numbers say 040 from top to bottom (this indicates 400mL will be taken) and stick a pipette micro tip at the end (they are in a clear and blue box)
4. Press down well when inserting the pipette into the pipette tip to make sure it doesn't fall off as you're grabbing ATR
5. To collect 400mL of ATR, make sure to press down the blue button until you feel the first sign of tension then insert it into the falcon tube with ATR completely and then let go of the button before pulling the pipette back up

6. Close the ATR bottle before grabbing your bottle of food with yeast without tilting the pipette or else the ATR will contaminate the rest of the pipette
7. Make sure the pipette tip is completely in the bottle of food before pressing down slowly and trying to cover the surface of the food as much as possible. Also, you can go past the first sign of tension and press down until it stops to make sure as much of the ATR is out of the pipette
8. Try to swirl the ATR around the bottle to cover the surface as much as possible before capping it and storing it inside one of the black boxes (it is best if ATR isn't exposed to light)
9. Repeat this until you have all the bottles you need then dispose of the pipette tip by pressing the white button to shoot out the pipette into the trashcan
  - a. I would recommend pressing down with the tip of your thumb facing away from the blue button and to the side so that when the button moves down you don't hit your finger and hurt it
10. If adding flies, just flip flies that are around 3-7 days old into the new ATR bottle and store it into the Walking Exp black box.
  - a. If you can't find flies that are that age just use whatever is available

\*The number of ATR bottles with flies you make is fully dependent on if you will have enough flies to be able to collect around 6-8 female flies per starvation vial for two days (4 experiments) \*

\*ATR bottles can last up to a week if needed but it is best if you use ones that are around 2 days old so try to make a new batch every Monday, Wednesday, and Friday\*

### **Starving Flies**

1. Grab two vials and one Kim Wipe
2. Rip the Kim Wipe in half and stuff each half into one vial and wet it enough so that the flies have water but not so much water to where they drown and die
3. Act as if you are restudding and counting flies with the cold block and light except use one of the ATR bottles instead of the regular bottles of flies.
4. After you have immobilized the flies, count around 8 female flies per vial
  - a. This is so that when running experiments you have room for error if you fail at putting a fly in the arena or if one is accidentally killed while transferring them into the arena, over time as you get used to putting flies in the arena you can reduce the number to like 5-6 flies per vial if you want but extra never hurts
5. Cap these vials with a Kim Wipe and put them with your ATR bottles in the Walking Exp black box.
6. One vial will be for the afternoon experiment (starved for a few hours) and one will be for the morning experiment (starved for around 24 hours)
7. Put any remaining flies on the cold block back into the bottle of ATR and food and put it back into the box

\*If you have leftover flies in your vial after placing them in the arena in your vials you can put them back into the bottle of ATR they came from (I do this in case I don't have flies in 48 hour ATR bottles and the next bottle won't be ready the next day for the afternoon experiment) \*

### **Running Experiments**

1. Use the cold block to immobilize your flies before putting them into the arena
2. As the flies are in the cold block, use the yellow L screw to unscrew the middle 11 screws on the blue area to remove the middle panel
3. After the panel is removed, make sure there isn't any debris in the channels or else the tracking system will pick up on those objects instead of the flies
  - a. You can also clean the channels and clear panel with ethanol before conducting your experiment. Also make sure it dries completely so that the flies aren't distracted by the smell of ethanol
4. Grab your flies from the cold block and tap the vial in each channel to let one fly out or use a paintbrush to move them
  - a. David made a suction tube for you with your name on it in orange tape, you can also use this as well to move flies if there are accidentally more than one per channel
  - b. Try to do this as quickly as possible so that they don't start moving and try to get out of the arena
5. Put the panel back on and screw all the screws, then turn on the air before starting the experiment
6. Turn off all the lights in the lab if nobody else is using the lab before moving on
7. In the computer to the left of the fume hood, if the terminal is not already open with the correct tabs, open the terminal and type in `roscore` then click enter. If they are already open, skip to step 8.
8. Hit Ctrl + T for a new tab and type in `roslaunch '/home/explorer/catkin_ws/src/pylon-ros-camera/pylon_camera/launch/pylon_camera_node.launch'` then click enter
9. Hit Ctrl + T and type in `cd ~/demo/demo_1/data` then click enter
10. In the same tab, type `roslaunch '/home/explorer/demo/demo_1/src/trigger_experiment_launcher.launch'` then hit enter, a live view feed should appear with the flies in view and there should be a red flash from the arena. In around 10 seconds the tracking system should kick in and start tracking the flies with big colorful circles and a trail behind the flies as they move.
  - a. The tracking might not be immediate but that's ok so just move onto the next step and the red LEDs should hopefully make the flies start moving
11. Hit Ctrl + T one more time, then type `rosbag record /WalkingArena` then click enter. Recording has started and now just wait around 3 hours before the next experiment or ending it.

\*If you want to retype one of the codes in the same tab without actually retyping, click the up arrow key to go through your most recent entries\*

\*If my instructions seem a little confusing, try asking David for clarification or just send me a message on slack and I'll do my best to reply as fast as possible\*

## Ending an Experiment

1. Click Ctrl + C on the `rosbag` tab and `roslaunch` tab with `~cd` to end the experiment.
2. You can click X on both of those tabs in step 1 and keep the other two tabs for future experiments
3. Click files and go demo --> demo\_1 --> data to find your recordings and data

4. After verifying that your data has successfully saved, rename the data folder to the current date and whether it was a morning or afternoon experiment in this format  
20240620\_afternoon
5. Add a new folder to the demo\_1 file named data for future experiments

If you have any questions about my instructions or if something happens and you don't now how to proceed, just text me on Slack or ask David if he's available.