

Two-choice Assay

assembly and use guide

De Obaldia_Vosshall, et al.

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https://github.com/VosshallLab/DeObaldia_Vosshall

Preface:

This guide discusses the elements that are necessary to make a Two-choice assay by adapting the Quattroport Assay: described in Basrur_Vosshall et al, 2020. Instructions for constructing the Quattroport assay are found at this Github link:

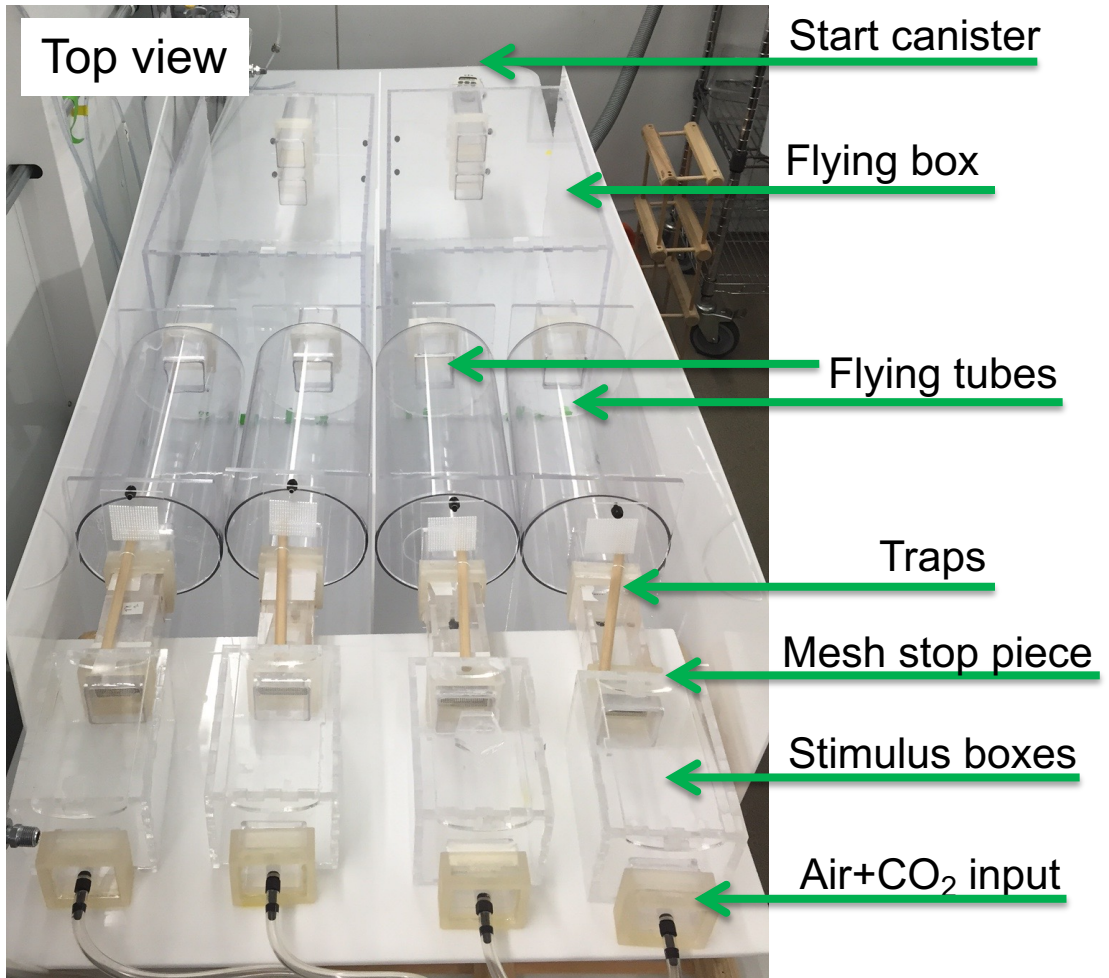
https://github.com/VosshallLab/Basrur_Vosshall2020

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Assay overview

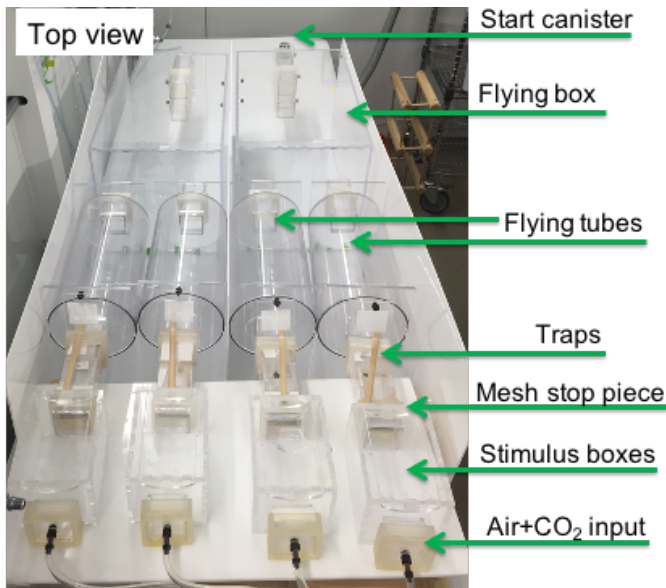
Two choice assay



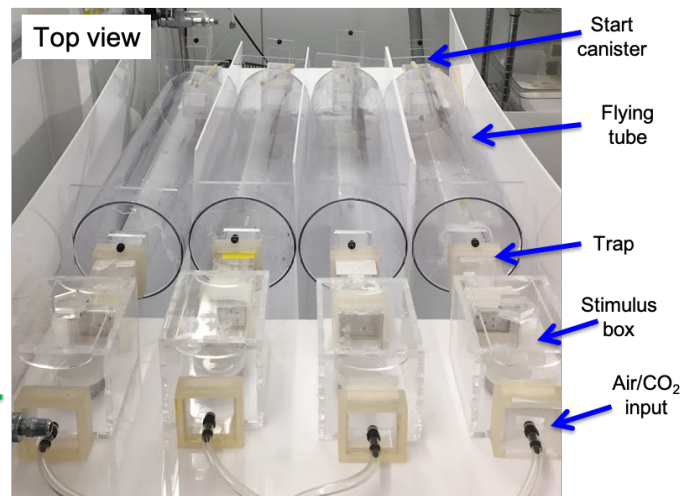
- The Two choice assay was made by adapting the Quattroport assay, a single-stimulus olfactometer assay (Basrur_Vosshall, 2020).
- Both assays use a manifold of 4 stimulus "ports", each of which can be used to test mosquito attraction to a single stimulus.
- In the case of the Two choice assay, we use 4 ports to perform 2 separate preference assays in parallel. Each preference assay tests mosquito preference between two stimuli, hence the name "Two choice" assay.
- The assay sits on a picnic table, and uses 2 wooden wine racks, covered with a white acrylic sheet) to elevate the stimulus boxes 5.75" above the table, so that they can connect with the flying tubes. The stands for the flying tubes sit on the table. The air flow setup sits on the table in front of the stimulus boxes. White acrylic sheets are used to block visual cues, as shown above (details on the Github site for Basrur et al).
- The Two choice assay is modular, and it is assembled for one day of use and then disassembled for cleaning at the end of the day (details in the Methods).

Two-choice assay vs Quattroport assay comparison

Two-choice assay



Single-stimulus assay “Quattroport”



- Parts that are common to both assays:
 - Start canisters
 - Stimulus boxes
 - 3D printed sliders and stops
 - All air/CO₂ supplies: flowmeter, tubing
 - Traps
- Parts that differ between the two assays:
 - The flying box is not used in the Quattroport assay, only the Two choice assay.
 - Flying tubes used in the Two choice assay are shorter than those used in the Quattroport (30.5 cm vs 61 cm).
 - Two start canisters (20 mosquitoes each) are used for each individual Two choice assay (vs. 1 start canister for each port of the Quattroport assay).

Assembly instructions

- Consult this Github site for Quattroport construction information:
https://github.com/VosshallLab/Basrur_Vosshall2020
- Follow instructions to make the needed Quattroport parts, including: 3D printed joins (stops and sliders), traps and sliding doors, stimulus boxes, stands for flying tubes, start canisters.
- Follow instructions to construct Quattroport air flow setup and tubing.
- Then, consult this document, and accompanying parts list (“Two choice assay parts list.xlsx”) to construct the flying box.
- The flying tubes for the Two-choice assay can be ordered to be 30.5 cm (1 ft.) long, 6" OD, 5-3/4" ID (McMaster-Carr catalog no.: 8585K58). You may need to sand the ends of these tubes with a deburring tool, in order to get them to fit into the acrylic stands (more detail in the Quattroport Guide PDF p. 8).

Flying box assembly

- Laser cut two flying boxes, as described on p. 7.
- Assemble the six sides of the flying box together and secure them with masking tape.
- Once all joints are tight, then use acrylic glue (Part no: 13417 on Parts list excel sheet) to bond them together.
- For each flying box, you will need to modify the two square walls by adding two short pieces of 2"x2" rectangular polycarbonate hard tubing (2.5" long).
- These pieces are used to connect parts of the assay together, using the 3D printed joins (see Basrur et al, 2020 Github site for details).
- These short polycarbonate pieces can be sanded with a deburring tool, to make them fit into the acrylic wall. Then, they can be glued with acrylic glue or super glue.

Laser cutting & 3D printing guide > Overview

3 PDF files are found here:

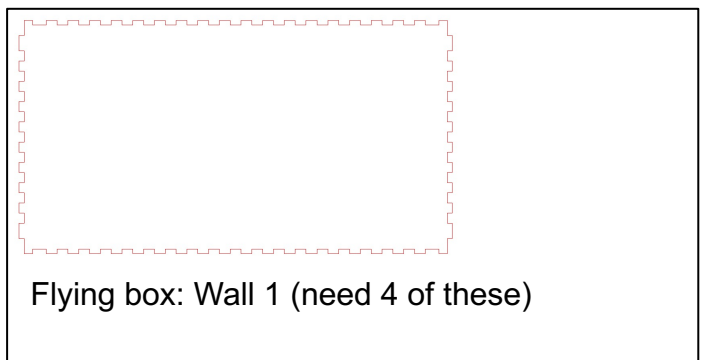
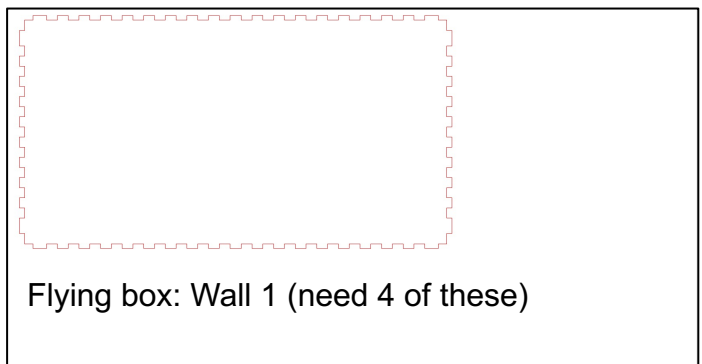
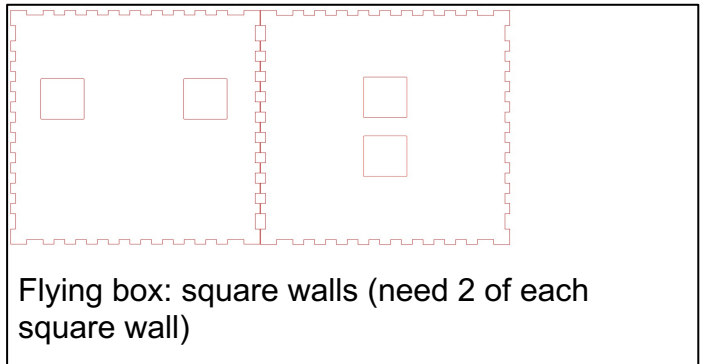
https://github.com/VosshallLab/DeObaldia_Vosshall2021

Laser cutting files

- 1/4" (6.35mm) clear acrylic sheets:
 - Flying_box_square_walls.pdf
 - Flying_box_Wall_1.pdf
 - Flying_box_Wall_2.pdf

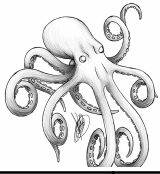
To make 2 choice boxes, you need 10 pieces of acrylic 12"x24"x1/4":

McMaster-Carr
Catalog #:
8560K355



Two choice assay operation checklist

- Set up 4 ports, check for snug fit of 3D printed joints, put parafilm/tape between trap and 3D printed parts, if needed to achieve snug fit. Keep trap doors open with wood dowel.
- Turn on air pump switch– Flow meter reads “15” for air. CO₂ flow meter reads: “0”. Check that the tripod is level.
- Attach the 3D printed slider piece of the 8 canisters of mosquitoes (n=20 females per canister) to the 4 starting positions of the assay (2 on each flying box). Make sure these are snugly attached, place parafilm between start position and 3D slider piece if needed. Insert wood dowel into circular hole on sliding door, THEN remove black screw, record room humidity and temperature. Acclimate mosquitoes for 10 minutes (with air only, No CO₂).
- To start trial, set timer for 5m30s. Start timer, turn on CO₂; the level on the right flow meter should be set to “30” to deliver 1% CO₂. Place stimuli in each of the 4 stimulus chambers.
- After 30s of CO₂ flow, remove wood dowels to allow sliding doors to open and release mosquitoes into the assay.
- After 5 minutes, turn off CO₂ off. Flow meter will read “0”. Close all 8 sliding doors, 4 in front of the traps, 4 in front of the star canisters, to keep mosquitoes in their position.
- Count mosquitoes in each section of the assay before vacuuming. Record the number of dead mosquitoes in the start canister. All mosquitoes that have left the start canister are considered “activated”, whether or not they entered the trap. Mosquitoes that entered the attraction trap are considered “attracted”.
 - If there are too many mosquitoes in the trap to count easily, label the trap with tape, and add a screw to secure the sliding door in the closed position. Then, carefully remove the trap from the assay (keeping the 3D stop and slider securely fitted on either end of the trap). Don’t forget to vacuum the mosquitoes out of the start canister and flying tube before removing the trap. Bring the trap into the cold room, and count the mosquitoes after they have been immobilized.



TWO-CHOICE

Experiment: _____
 Date: _____
 Hatch date: _____
 Starvation time: _____

Trial #	Port #	Stimulus	# in trap	# in fly tube	# In box	# in start
	1					
	2					
	3					
	4					

Notes:

Humidity	
Temp:	
Pressure:	

Trial #	Port #	Stimulus	# in trap	# in fly tube	# In box	# in start
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	3					
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