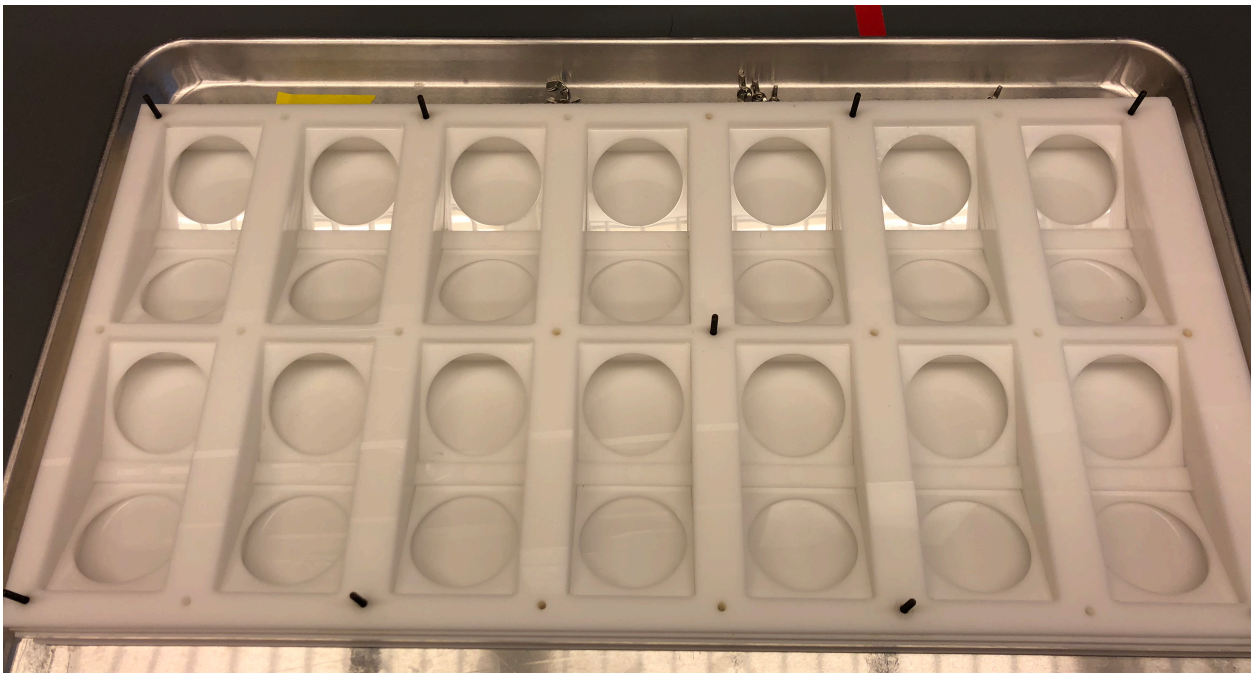
Single female *Ae. aegypti* egg-laying assays

Assay construction

See the required parts list at the bottom of this protocol for suitable sheets of acrylic. Using a laser cutter, cut each according to the the files in this repository and assemble by stacking in this order and securing with the screws and bolts. Note that there are more screw holes than necessary - we typically secure the outer edges with 4 screws and place at least 1 in the middle row to prevent bowing of the lid.

1. oviposition_assay_top_and_bottom.ai = 3mm white
2. floor-1x_6mm_white.ai = 6mm white
3. wells-3x_6mm_white.ai = 6mm white
4. wells-ledge-1x_6mm_white.ai = 6mm white
5. wells-3x_6mm_white.ai = 6mm white
6. wells-3x_6mm_white.ai = 6mm white
7. oviposition assay top and bottom.pdf = 3mm black

Also cut one sheet of 3mm white acrylic from the file petri_dish_holders_3mm_white.ai - this will result in 36 petri dish holders (you will need 28 for each tray). Add two of these holders to each well, diagonally so that the outer edge is supported by the ledge provided by piece #4.



A photograph of a correctly assembled tray (without the lid - piece 7) is above.

Rearing Mosquitoes

Hatching:
Hatch *Ae. aegypti* egg papers of the desired genotypes according to Vosshall lab SOP. Be extremely careful with mutant genotypes - change gloves after each pan, and wipe down the bench with 70% EtOH.

Thinning:
Two days after hatching, thin larvae into new pans according to Vosshall lab SOP. Rear at a density of ~500 per pan.

Feeding:
Check on larvae every day - feed according to need.

Picking pupae:
Pick male and female pupae when ready (~7 days after hatching) and divide into separate cages of mixed males and females - one cage per day of planned experiments. Add two sugar feeders (10% sucrose) and change every week to prevent mold and bacterial growth.

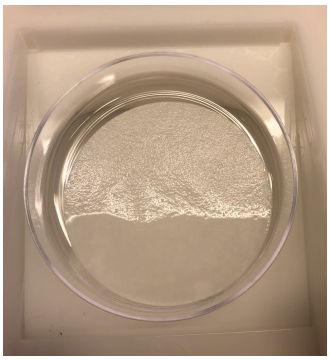
Egg-laying assay setup

We use mosquitoes that are between 5-10 days old at the time of blood-feeding (9-14 days old at the time of behavior). Blood-feeding occurs 96 hours before assays are setup.

Blood-feeding:
96 hours prior to assay, blood-feed mosquitoes. 12-24 hours post-blood-feeding, select fully engorged females in the cold room and return to a cage with ad libitum access to 10% sucrose.

Assay setup (preliminary), dry:
Can be done ahead of time - add a petri dish and a dry 47mm Whatman paper to each well of a tray (28 total). Put lid on (but do not screw down) to prevent accumulation of dust/debris.

Assay setup (day-of):
Immediately prior to starting assay, add 2mL of liquid to each well. A photo of a completed setup is to the right. Record the identity of the solution in each well on a datasheet, and, if doing a two-choice setup, alternate the position of the test solution and the control between wells.



Transfer the the sorted female mosquitoes to a paper cup with a netting lid. Place the cup on ice until the animals are knocked down (~ 5 minutes). Working quickly, transfer a anesthetized single female into each of the 14 chambers of the assay and secure the lid.

Transfer and leave in behavior room overnight (~18) hours, taking care not to spill the liquid in the dishes by jolting, tilting, or otherwise disturbing the tray.

Egg-laying assay takedown

Assay takedown:
Roll cart to cold room, leave for at least 45mins to fully anesthetize female mosquitoes.

Open lid, remove each female with forceps - note any wells with no eggs and escaped animals (or obviously dead animals).

Transfer the petri dishes to a tray for egg counting or imaging, keeping the correct order. Alternatively, if using an imaging/counting setup, you can immediately photograph and discard each tray. Be sure to freeze petri dishes for > 12 hours to kill all eggs.

Cleaning trays:
Spray entire surface of each tray with >70% ethanol, wipe down any residue, and allow to air dry. Store with lid on to prevent accumulation of debris and dust.

Parts list:

supplier	item #	description	# in pack/box	# needed per run, per tray
Fisher Scientific	09-805-342	whatman 47mm	100	28
Fisher Scientific	as-4050	petri dishes	500	28
			# in pack/box	**# needed to build each tray**
McMaster-Carr	8505K92	cast acrylic, white 12”x24”x1/4”	1	5
McMaster-Carr	8505K12	cast acrylic, white 12”x24”x1/8”	1	1
McMaster-Carr	8505K12	cast acrylic, black 12”x24”x1/8”	1	1
McMaster-Carr	92001A281	18-8 stainless steel wing nuts, 6-32	10	12
McMaster-Carr	91251A159	6-32 2” alloy steel socket head cap screw	50	12