Design files for user-tested mold inserts

This document contains descriptions and schematics for 14 mold inserts that were designed to be used for a range of study organisms, including zebrafish, fruit fly, mouse, frog, annelid worm, amphipod, red flour beetle, acoel, and cricket. Each insert is compatible with the adjustable-height mounting tripod described elsewhere in this paper.

Components were designed using FreeCAD and Adobe Illustrator. Each mold component was made by cutting it from a sheet of 6.35 mm optically clear extruded acrylic (McMaster-Carr #8560k355). A laser cutter (Universal PLS 6.75) in engraving mode was used to generate mold inserts by removing acrylic in a pattern, leaving a grid of raised posts. When pieces needed to be combined, they were fused together in pairs using acrylic welding solvent (Weld-on 3 Assembly Adhesive, SciGrip #10799) (see Supplemental Materials S3 for details). Most of these mold inserts were designed to fit within a 35 mm diameter plastic petri dish with a 27 mm diameter glass coverslip bottom (VWR #89428-990). Many microscopes need a stage insert in order to stably hold a petri dish (e.g. Zeiss #432311-9901-000). In Configuration 1, the device can be used on a range of dish sizes; we tested it on 6 cm (VWR #25384-092) and 10 cm (VWR #25384-342) diameter plastic petri dishes.

To use these inserts with the OMMAwell device, simply attach slots to the back of the insert, following the procedure in step #7 of the assembly instructions in Supplemental Materials S3. Details for each insert are included below. Each schematic has been magnified for presentation and the key dimensions are given with each insert. For complete dimensions, please see the design files in Supplemental Materials S2. These are included in two formats: PDF and DXF. PDF files are readily viewed and edited by many common software packages. DXF is a file type that can be opened in a wide variety of drafting and vector graphics software packages, including AutoCAD, FreeCAD, Solidworks, SketchUp Pro, Adobe Illustrator, and CorelDRAW. Unlike PDF, DXF files contain precise length information. We hope readers will use these as a starting point to make their own inserts; if so we would be happy to hear about it. Each of these mold inserts can be made from 6 mm-thick acrylic sheet using a laser cutter. Alternatively, if a user prefers to fabricate them with a 3D printer, the user can adapt the included design files to make a 3D model of each component. The depth dimensions of each part is listed below. See Supplemental Materials S4 for a brief comparison of the two approaches.

Identifying wells: For some molds, there are symbols or numbers to identify columns or rows on the agarose wells. These provide the "coordinate address" of each well. In other molds, the arrangement of the molds themselves – that is, an arrangement without rotational symmetry – is sufficient information to uniquely identify each well.

Note: At the end of this document is a section that discusses considerations for using wells to hold specimens for injection.

Capitella teleta, 100-well

`Insert_Capitella_Injection_200x200um_100n`

Description: This mold layout was designed in collaboration Dr. Mark Martindale (University of Florida), Dr. Elaine Seaver (University of Florida), and Dr. Mansi Srivastava (Harvard University), for injecting the spherical eggs of marine invertebrates and acoel worms. Eggs are placed into the square wells and then injected with a pulled glass needle entering from the right. When an egg gets stuck on the needle, the user can move the needle tip into the large well on the left, and then slide the needle up and out through one of the thin troughs, freeing the egg from the needle. This mold been tested with eggs from the marine annelid worm *Capitella teleta*, but by adjusting the dimensions of the square microwells, this mold insert can easily by adapted to a wide range of species. To make this mold, cut on the **red** line, etch the **cyan** region to a depth of ~800 μm, and etch the **orange** regions to a depth of ~550 μm.

Dimensions: This insert generates agarose microwells that are 200 μm square, with a depth of 250 μm. The large well on the left has a depth of 800 μm. The outer margin of the insert is a 31 mm diameter circle to fit in a 35 x 10 mm plastic dish (Bio-One #627160) for injection.
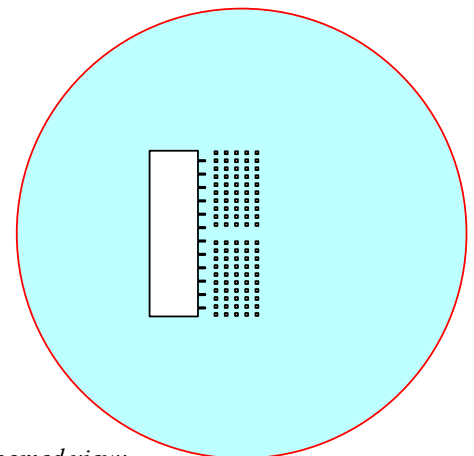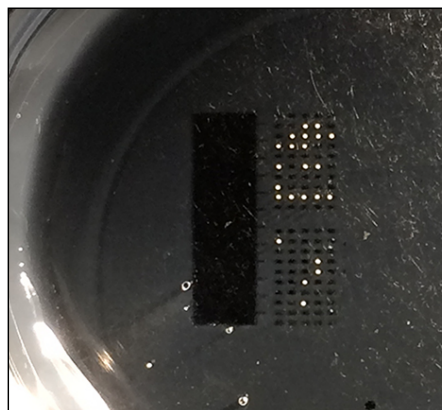

Zoomed view:
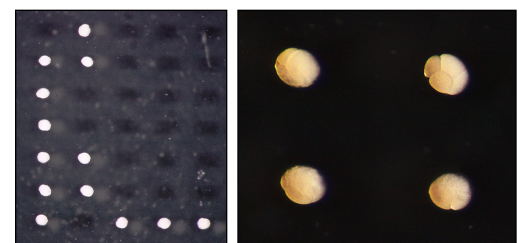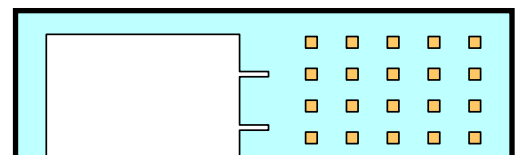
Photos by Elaine Seaver (University of Florida)

Danio rerio, chorions intact, 96-well

`Insert_Danio_1100x1100um_96n`

Description: This mold was designed for injecting and live-imaging zebrafish embryos with their chorion intact. To make this mold, cut on the **red** line, then etch the **cyan** region to a depth of ~1300 µm.

Dimensions: Each microwell is 1100 µm square, with a depth of 1100 µm. The outer margin of the insert is a circle with radius 35 mm to fit snugly into a glass-bottom dish for live-imaging with an inverted microscope and into the mold sheath (see Supplemental Materials 3). The wells are placed so that the embryos all land within the 27 mm diameter of the glass coverslip bottom (VWR #89428-990). The same is true for all mold inserts described in this document that have a rectangular notch.
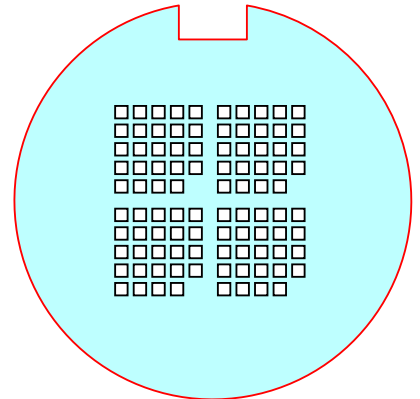
Danio rerio, chorions removed, 96-well

`Insert_Danio_800x800um_96n`

Description: This mold is used for live-imaging zebrafish embryos whose chorions have been removed. To make this mold, cut on the **red** line, then etch the **cyan** region to a depth of ~800 µm.

Dimensions: Each microwell is 800 µm square, with a depth of 1000 µm. The outer margin of the insert is a circle with radius 35 mm. It is compatible with the glass-bottom dishes and the mold sheath, as described above.
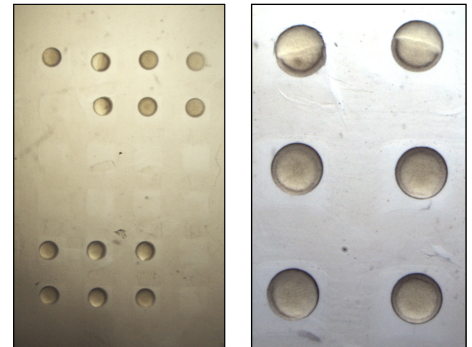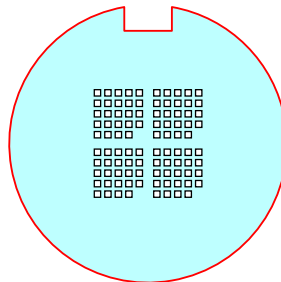
Photos by Megan Norris (Harvard University)

Danio rerio, embryos with growing tails, 47-well

`Insert_Danio_Lateral_Wells_47n`

Description: During embryogenesis, the growing tail of zebrafish embryos makes it difficult to keep them in place during long term live-imaging. To address this problem, this mold generates circular wells for the spherical yolks to settle into, and adjacent troughs for the elongating tails to enter. To make this mold, cut on the **red** line, etch the **cyan** region to a depth of ~500 µm, and etch the **orange** regions to a depth of ~250 µm. *Note:* at these depths, the embryos's midline is approximately flush with the upper surface of the agarose, meaning that the larva protrudes upwards, making this mold best suited for imaging on an upright microscope, but if the etchings were 2-fold deeper, it would be suitable for inverted microscopy as well.
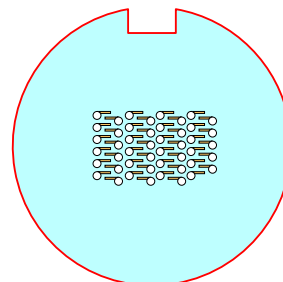
Zoomed view:
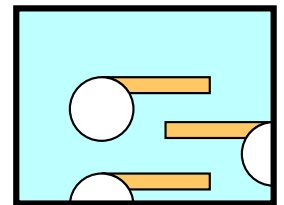
Dimensions: The circular portion of each microwell is 1 mm in diameter, with a depth of 0.5 mm. The tail trough is 1.7 mm long, 0.25 mm wide, and 0.25 mm deep. This mold is compatible with the mold sheath and the aforementioned glass-bottom dishes.

Danio rerio, injection troughs

`Insert_Danio_Injection_Troughs_10n`

Description: This mold makes troughs that can hold many zebrafish eggs for injection. Each trough has a stepped notch removed from one side so that the needle can enter the eggs without getting stuck in the agarose. To make this mold, cut on the **red** line, etch the **cyan** region to a depth of ~700 μm, and etch the **orange** regions to a depth of ~350 μm.

Dimensions: Each trough is 30 mm long and 0.7 mm deep at the bottom. A trough is 0.7 mm wide at the bottom, and 1.2 mm wide at the top. The outer margin of the insert is a rectangle 31.2 by 34.8 mm. This insert will not fit in the mold sheath, but once slots are attached to the back, it can be used with the adjustable-height tripod.
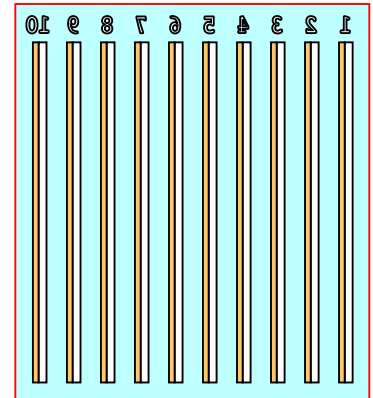
Drosophila melanogaster, 294-well

`Insert_Drosophila_220x500um_294n`

Description: This mold was designed for live-imaging arrays of fruit fly embryos. The etched shapes make it easier to navigate the array of microwells while using a microscope. To make this mold, cut on the **red** line, then etch the **cyan** region to a depth of ~240 μm.

Dimensions: Each microwell is 500 μm long, 220 μm wide, and 240 μm deep. The outer margin of the insert is a circle with radius 35 mm. It is compatible with glass-bottom dishes and the mold sheath.
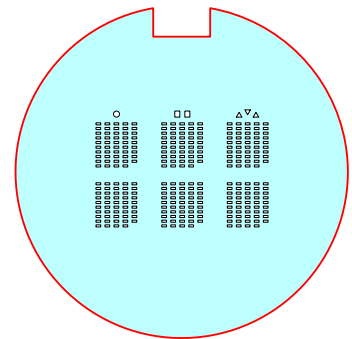
Eleutherodactylus coqui, 24-well

`Insert_Eleutherodactylus_4200x4200um_24n`

Description: This mold insert was designed to produce wells for injecting and live-imaging the embryos and larvae of the coquí frog. The channels in the agarose admit the tails of the larval frogs so that they can more easily be oriented by the user for microscopy. To make this mold, cut on **red** lines. Then use acrylic solvent (see Supplemental Materials S3 for details) or cyanoacrylate glue to attach the two pieces flat together, lining up the circular holes on the circular piece with the plus-sign-shaped holes on the rectangular piece. The holes allow air to enter and release the vacuum when removing the mold from the agarose.

Dimensions: Each well is 4.2 mm square; the channels connecting the wells are 1.5 mm wide. The array of wells is a square 32.2 mm to a side. The circular baseplate has a diameter of 47.8 mm. By varying the amount of agarose poured the user can adjust the depth of the wells.

Photos by Mara Laslo (Harvard University)
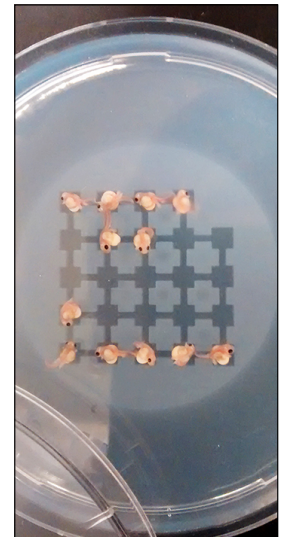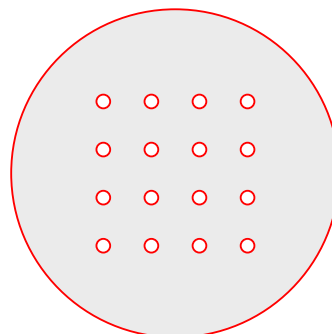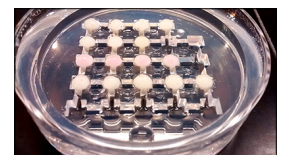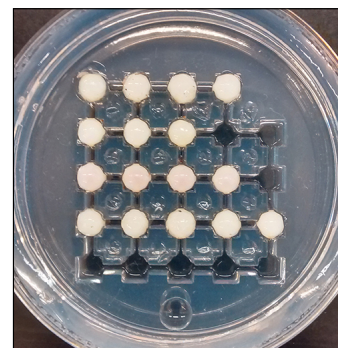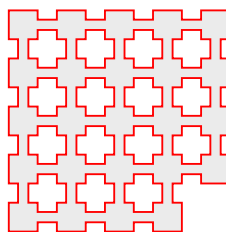
Gryllus bimaculatus, 120-well

`Insert_Gryllus_570x2930um_120n`

Description: This mold insert was designed to fit the maximum number of cricket embryos on a single glass-bottom dish. Cut on **red** lines, and etch the **cyan** region to a depth of ~650μm.

Dimensions: Each microwell is 2930μm long, 570μm wide, and 650 μm deep. The outer margin of the insert is a circle with radius 35 mm. It is compatible with glass-bottom dishes and the mold sheath.
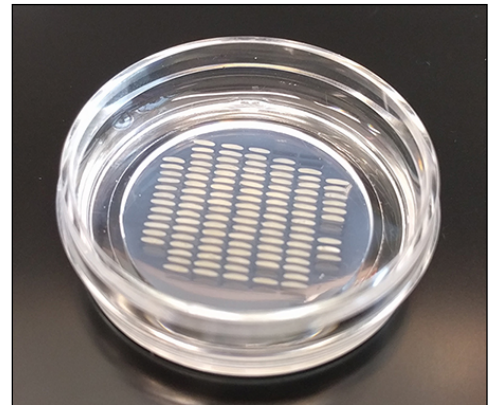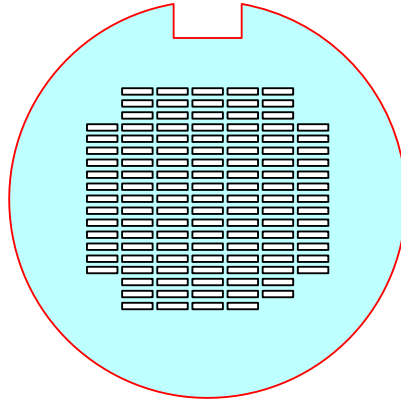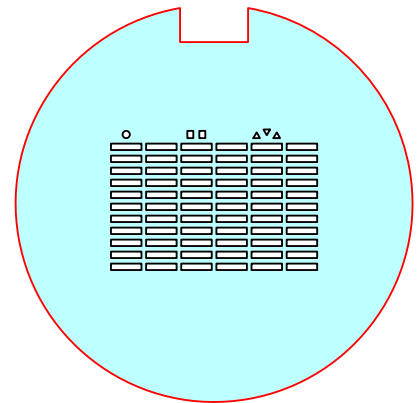

Gryllus bimaculatus, 66-well

`Insert_Gryllus_570x2930um_66n`

Description: This is similar to the previous mold insert, but with fewer wells and etched shapes to aid in the orientation on the microscope. Wells are arranged into a rectangle for time-efficient automated tiling. Cut on **red** lines, and etch the **cyan** region to a depth of ~650μm.

Dimensions: Same dimensions as previous insert.

Gryllus bimaculatus, 300-well

`Insert_Gryllus_Injection_570x2930um_300n`

Description: This mold insert is used for injecting large numbers of cricket eggs at once. Wells are arranged in groups of ten and labeled with an etched number. Cut on **red** lines, and etch the **cyan** region to a depth of ~750μm.

Dimensions: Each microwell is 2930μm long, 570μm wide, and 750 μm deep. Depending on the user's microinjection apparatus it may be helpful to adjust the depth of the wells.
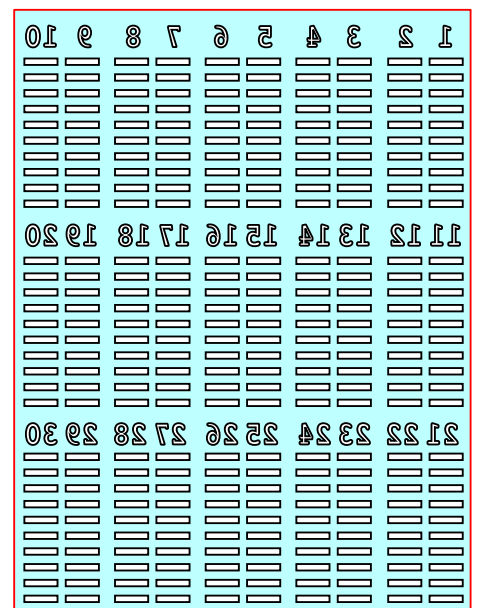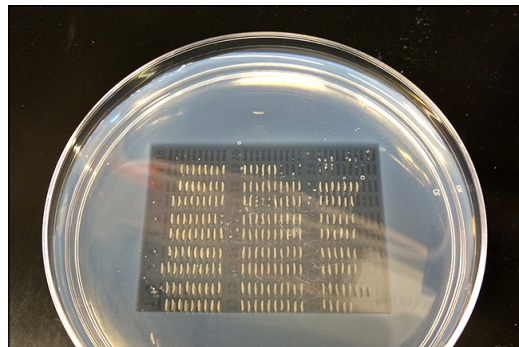

Photo by Taro Nakamura (Harvard University)

Hofstenia miamia, 100-well

`Insert_Hofstenia_Injection_275x275um_100n`

Description: This mold insert is quite similar to the *Capitella teleta* insert described at the beginning of this document; the only difference is that the wells are larger to fit the eggs of the acoel worm *Hofstenia miamia*. To make this mold, cut on the **red** line, etch the **cyan** region to a depth of ~800 μm, and etch the **orange** regions to a depth of ~500 μm.

Dimensions: The insert generates agarose microwells that are 275 μm square, with a depth of 300 μm. The large well on the left has a depth of 800 μm. The outer margin of the insert is a circle with radius 31 mm to fit in a 35 x 10 mm plastic dish (Bio-One #627160) for injection.
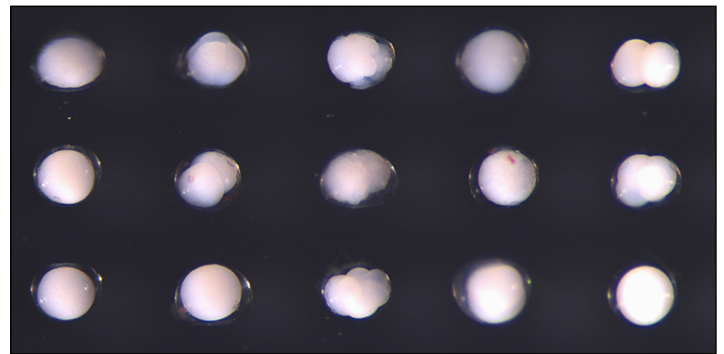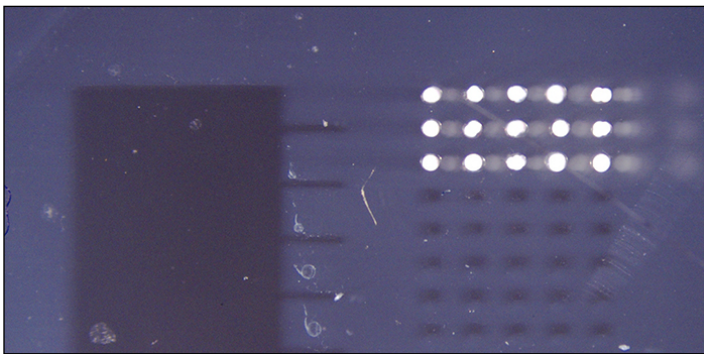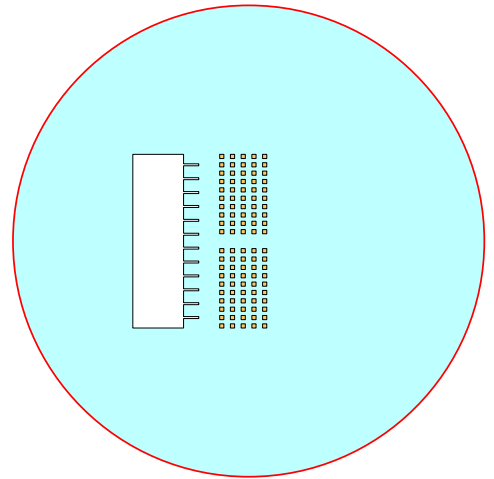

Photos by Andrew Gehrke (Harvard University)

## *Mus musculus,* neurospheres, 101-well

`Insert_Mus_Neurospheres_1000x1000um_101n`

Description: Neurospheres are clusters of cultured cells derived from neural stem cells. This mold insert was designed to make wells to hold neurospheres for imaging. Cut on **red** lines, and etch the **cyan** region to a depth of ~1000μm.

Dimensions: Each microwell is a cube 1 mm to a side. The insert is compatible with glass-bottom dishes and the mold sheath.
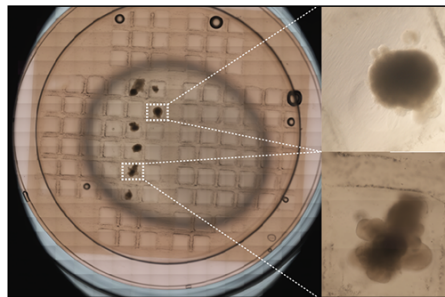
Photos by Richard Smith (Harvard Medical School)
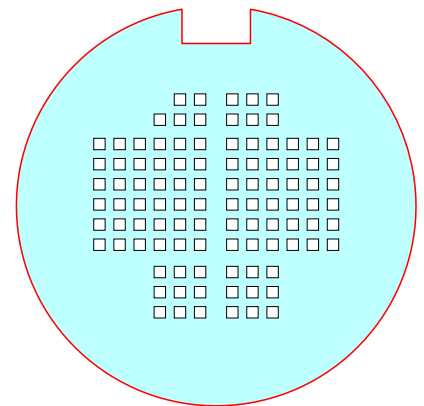
## *Parhyale hawaiensis,* 396-well

`Insert_Parhyale_round250um_396n`

Description: *Parhyale hawaiensis* is a marine amphipod crustacean, an emerging model system for the study of regeneration, germ cell specification, and limb differentiation. This mold insert was designed to make wells to hold its spherical embryos for injection and live-imaging. Cut on **red** lines, and etch the **cyan** region to a depth of ~300 μm.

Dimensions: Each microwell is 250 μm long, 250 μm wide, and 300 μm deep. The insert is compatible with glass-bottom dishes and the mold sheath.
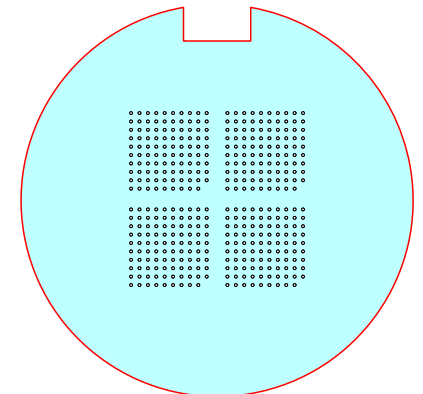
Tribolium castaneum, 147-well

`Insert_Tribolium_350x600um_147n`

Description: The red flour beetle is a pest of stored grain products and an emerging model system for a range of animal developmental processes. This mold was designed to produce wells that fit the embryos for timelapse live-imaging. Cut on **red** lines, and etch the **cyan** region to a depth of ~375 µm.

Dimensions: Each microwell is 600 µm long, 350 µm wide, and 375 µm deep. The insert is compatible with glass-bottom dishes and the mold sheath.
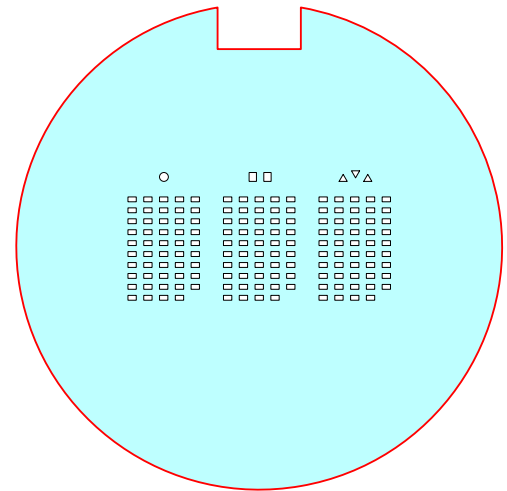
Considerations for using wells to hold specimens for injection

Several of the molds listed here have been used for successful injections, including the molds for zebrafish, cricket, coqui frog, three-banded panther worm, and annelid worm. When users test injection protocols for additional organisms, these molds and the usage described above my be helpful, but every species will have idiosyncratic challenges.

For instance, some embryos increase in size and/or become motile during development, which might necessitate a transfer from the injection wells to another vessel for subsequent development. Such transfers work effectively in crickets, amphipods, and coqui frogs. We have not tested mid-embryogenesis transfers for other species. Another potential concern is that eggs of some species might rotate under the injection needle. One possible solution for this is to adjust the shape of the well so that the egg can be jammed into a corner during injection.

Some users have also found it helpful to adjust the osmolarity of the acqueous medium in order to increase (or decrease) the swelling of specimens in the wells during injection.