

## Plexiglass Hybridization Dish

Published date: Aug. 29, 2018

#### Technology description

Drosophila brains can be mounted on a 22x22 mm glass coverslip for efficient parallel processing of large numbers of unique samples via fluorescent in situ hybridization (FISH) or immunohistochemistry (Meissner et al., in preparation; Wu et al., 2016). FlyLight and Janelia Experimental Technology previously described a Drosophila Mounting T-Dish that aids the mounting .Most coverslip processing steps are carried out by moving coverslips between jars of solutions, but the 10 mL jar volume is too large for the hybridization step in FISH, which requires a high concentration of probes.

To address this problem, we designed an Inverted Coverslip Hybridization Chamber, based off of the Drosophila Mounting T-Dish design. Samples are mounted on the underside of the coverslip, which is held above the chamber on 0.5 mm spacers to each side, creating a 150 µL space for hybridization solution. The opening at the top allows easy application and removal of the coverslip. Minimal evaporation occurs for hours at hybridization temperatures when placed inside a humidified container. Chambers can be washed and reused indefinitely. Although designed for hybridization, the chambers should be suitable for any processing of coverslips with a small volume of solution.

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