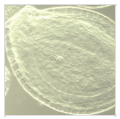

Dec 16, 2019

Chloral Hydrate Seed Clearing V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.baj6icre

Mimulus

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ABSTRACT

To characterize early seed development in Mimulus (1-5 days after pollination), we clear seeds with chloral hydrate and quickly obtain images of embryo and endosperm development using a Differential Interference Contrast Microscope.

MATERIALS TEXT

Hoyer's Solution: 19g gum Arabic, 12g glycerol, 250g Chloral Hydrate, 75ml Water.
Diluted Hoyer's solution: 3 parts Hoyer's solution: 1 part 10% gum Arabic.

Forceps, glass microscope slides, coverslip, access to Differential Interference Contrast Microscope.

SAFETY WARNINGS

Chloral Hydrate is DEA regulated and you either need a permit or access to a lab that has a permit with this substance.
This substance is also hazardous and acutely toxic. SDS: https://www.caymanchem.com/msdss/21843m.pdf

BEFORE STARTING

Prepare Hoyer's solution: 19g gum Arabic, 12g glycerol, 250g Chloral Hydrate, 75ml Water.

Prepare a diluted Hoyer's solution: 3 parts Hoyer's solution: 1 part 10% gum Arabic.

1 Emasculate a bud from some maternal plant. 2-3 days later pollinate by selfing/outcrossing or use an unfertilized fruit.

2 Remove the developing fruit 1-5 days after pollination or 2-3 days after emasculation. In Mimulus, this protocol is useful for capturing early seed development (0-5 days). After 5 days, the seed tissue thickens and becomes difficult for viewing.

3 Pipette 10uL of diluted Hoyer's solution onto a glass slide and dissect developing ovules from the fruit directly onto the glass slide using sharp forceps

4 After dissection, pipette 20-40uL of the diluted Hoyer's over the developing ovules and place a coverslip on top. Then, set the slide flat, upright in a 4ºC fridge.

5 Depending on how developed the ovules are, clear for at least 1 to 12 hours before viewing with a Differential Interference Contrast microscope.