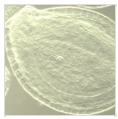

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Chloral Hydrate Seed Clearing V.2

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

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.

Prepare Hoyer's Solution

1 Hoyer's Solution: **19 g** gum Arabic, **12 g** glycerol, **250 g** Chloral Hydrate, **75 ml** Water.

10 ml Diluted Hoyer's solution: **7.5 ml** Hoyer's solution: **2.5 ml** 10% gum Arabic

Dissecting fruit and clearing

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

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

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

5 After dissection, pipette about **30 µl** 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.

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