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 We use this protocol and it's working

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🌐 Chloral Hydrate Seed Clearing V.2

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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

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 START INSTRUCTIONS

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 4°C 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