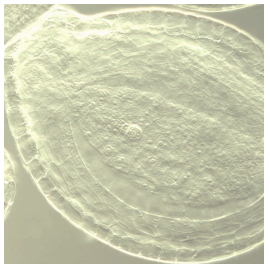
Dec 16, 2019 Version 1

Chloral Hydrate Seed Clearing V.1



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Protocol status: Working
We use this protocol in our group and it is working.

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Keywords: Chloral Hydrate, Seed Clearing, Embryo, Endosperm, early seed development in mimulus, seeds with chloral hydrate, chloral hydrate seed clearing, early seed development, images of embryo, pollination, embryo, chloral hydrate, days after pollination, seed, differential interference contrast microscope, mimulus, endosperm development

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.

Troubleshooting

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

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.