

Detailed Phloroglucinol Staining Protocol

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Solutions:

- 2% Phloroglucinol solution (1g phloroglucinol in 50ml 95% ethanol).
 - Keep in a bottle covered with foil to prevent exposure to light.
 - Can keep for months at RT.
 - Solution should be light yellow; if it turns brown than it's no longer any good.
- Concentrated HCl

Procedure:

- Collect fresh samples to be sectioned (do at least 3 biological replicates).
- Work on parafilm paper; add a couple of drops of H₂O so the samples don't dry out.
- Hand section sample directly in the water.
 - Work under a dissecting microscope and try to make sections paper thin.
 - Use a new, sharp double-edged razor blade.
 - Make approximately 20-30 sections so you'll have a good set to choose from.
- Transfer the samples using a soft brush (fine paint brush would work) into a small weigh boat containing 2ml of 2% phloroglucinol solution and leave it at RT for 3-5 mins (make sure 95% EtOH doesn't evaporate; if it does, add a little bit more directly to the weigh boat).
- Add 3-5 drops of concentrated HCl into the weigh boat
 - Takes 10-15 secs for the reaction to complete and the stain to show up clearly.
- Take pictures under the microscope immediately.
 - I take pictures of the samples directly in the weigh boat but if the samples are to be mounted on a slide then mount in 2% phloroglucinol + HCl solution and cover with a coverslip (can try mounting in 50% glycerol but I've never tried that).
 - Samples do not stay for very long because the EtOH evaporates making phloroglucinol precipitate. The solution and samples also turns light brown and crusty after about 10 mins of adding HCl.
- Repeat for the next biological replicate.