BIOLOGY

ClearSee clearing method

Kurihara, Daisuke, Yoko Mizuta, Yoshikatsu Sato, and Tetsuya Higashiyama. 2015. "ClearSee: a Rapid Optical Clearing Reagent for Whole-Plant Fluorescence Imaging.." Development 142 (23): 4168–79. doi:10.1242/dev.127613.

Day 1

Prepare the ClearSee Solution:

	Final [ ]	For 100mL
Xylitiol	10%	10g
Na Deoxycholate	15%	15g
Urea	25%	25g
H_2O		up to 100mL

4% PFA = 4% Paraformaldehyde (diluted in 1X PBS)

PFA is keept in the lab fridge

PROTOCOL:

- Fix roots for 30 min in 4%PFA under vaccum

Use the vaccum pumb that is in the drawer under the Lab mac computer. Ask someone is you don't know how to use it

- Wash tissue 2x with 1x PBS (1 min each wash)

10x PBS recipe is in the ROCHE LAB-FAQs Book

- Add ClearSee Solution
- Incubate the samples for a minimun of 4 days at Room Temperature

Samples can be stored in the ClearSee solution for at least 5 months

- **Post staining**:

- Prepare Calcofluor White: 100mg/mL in ClearSee solution

- Incubate samples in **calcofluor** for 1h

If we need to stain the nuclei, we can use Hoechst 33342 (10ug/mL) in clearsee solution over night.

- Wash tissues with ClearSee for 1h
- Mount samples in ClearSee