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Bedinger Lab *In-vitro* liquid tomato pollen germination media protocol

Stock recipe

PGM	Stock Concentration	To make 5ml	10ml	20ml	30ml	40ml
PEG 4000	40%	3	6	12	18	24
Boric Acid	0.1%	0.5	1	2	3	4
Sucrose	40%	0.25	0.5	1	1.5	2
HEPES buffer	0.5M, pH6.0	0.2	0.4	0.8	1.2	1.6
$Ca(NO_3)_2 \cdot 4H_20$	0.1M	0.15	0.3	0.6	0.9	1.2
$MgSO_4 \cdot 7H_20$	2%	0.05	0.1	0.2	0.3	0.4
KNO_3	1% or 0.1M	0.05	0.1	0.2	0.3	0.4
H_2O	Double Distilled	0.8	1.6	3.2	4.8	5.6

1. Collect pollen from open and dehiscing flowers off a young tomato plant into 1.5 ml tubes with 1ml pollen germination media (PGM). Label the tube.
2. Mix well by inversion and minivortexing with a pollen buzzer. Record time as t_0.
3. Dilute pollen/PGM solution 1:10 into a total of 300µl of new PGM.
    a. I take 30µl of the pollen/PGM solution and add it to 270µl of PGM.
    b. Remix by vortexing.
    c. Use a cut tip to transfer.
4. After mixing aliquot 100µl of the diluted pollen/PGM into an individual well of a 96-well plate.
    a. We view on an inverted scope because the pollen settles to the bottom of the well and is easily viewed. For and upright scope you'll have to use slides or a shallow well plate.

Notes:

- The quality of the PEG can be an issue. If available try batches from a couple different powder stocks. Getting this into solution does take time.
- All my stock solutions are filter sterilized; PEG and Sucrose take the most time to filter because at 40% they are viscous.
- All stock solutions are kept at -20 C and the media is made fresh each time. It wouldn't be a problem to make some media and keep it stored frozen as well and use it when you need to.