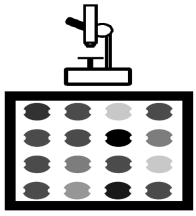
Nov 11, 2019 Version 1

Marchantia high throughput imaging in multiwell plates V.1



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol allows high throughput imaging of Marchantia gemmae, using a cheap setup made with broadly available lab equipment.
We used a transparent 384 wells plate filled with 1/2 strength Gamborg B5 media with 1.2% agar and placed a single gemma at the centre of the well. Gene frames and coverslips treated with anti-fog spray were used to cover (seal) the wells. This setup can be paired with automated imaging of samples.

Guidelines

Work under the flow hood to avoid gemmae contamination, but be aware that this causes the media to evaporate. To avoid it, try to cover the plate with a lid and leave it open for the shortest time possible while placing the gemmae.

Materials

MATERIALS

- Gamborg B5 medium including vitamins **Duchefa Biochemie Catalog #**G0210
- 384 well microplate clear flat bottom **greiner bio-one Catalog #**781186
- Gene Frame 1.7 × 2.8 cm **Thermo Scientific Catalog #**AB0578
- B-clean Anti-fog spray **Catalog #**B250
- Coverslip 22 × 50mm **Catalog #**0101142
- Agar capsule 1g **Melford Catalog #**1302310000

Troubleshooting

Before start

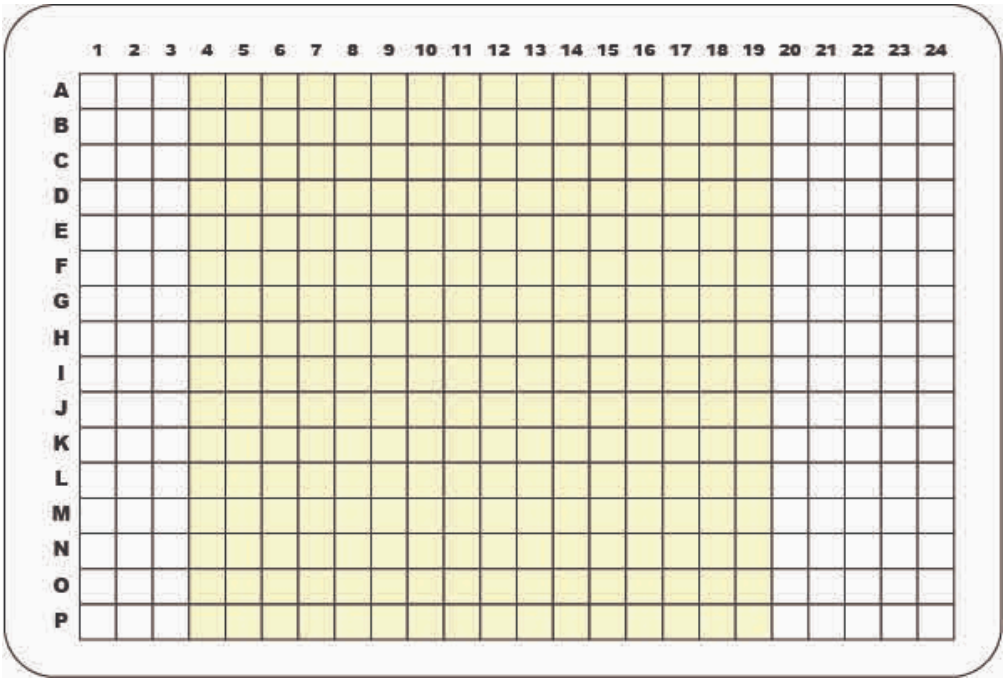
The set up we present in this protocol is optimised for our microscope stage travelling range and the size of available gene frames and coverslip. This does not mean that you cannot use larger areas of the plate.

Media preparation

1 Prepare 1/2 strength Gamborg media plus vitamin with 1.2% agar.

Plate set up

2 Pour the media into your selected wells, making sure they are filled up to the top and they not contain any air bubble inside.

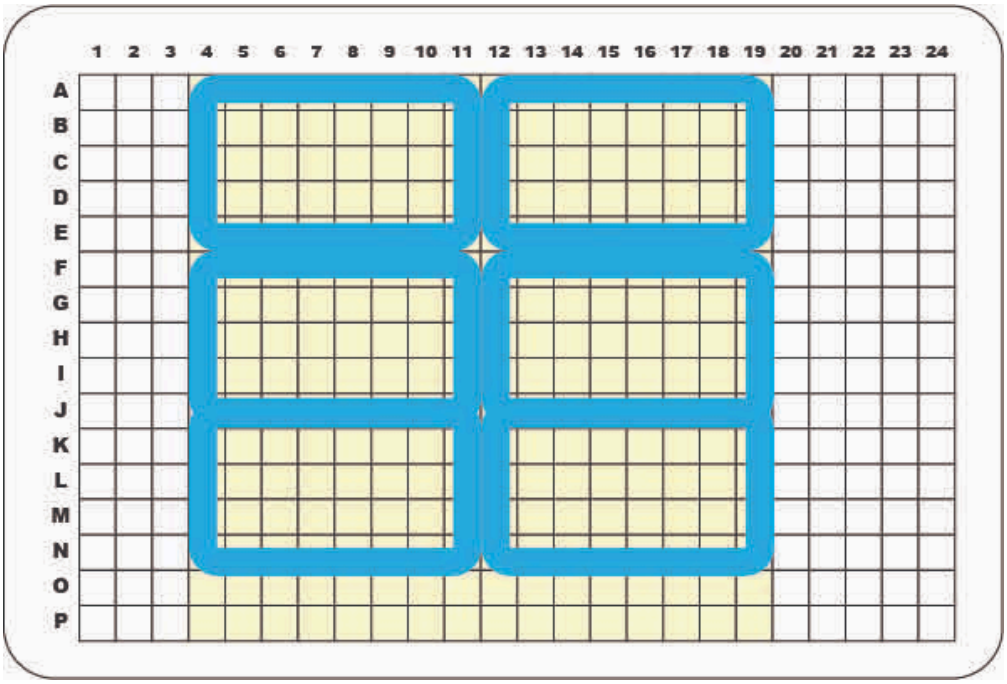


Note

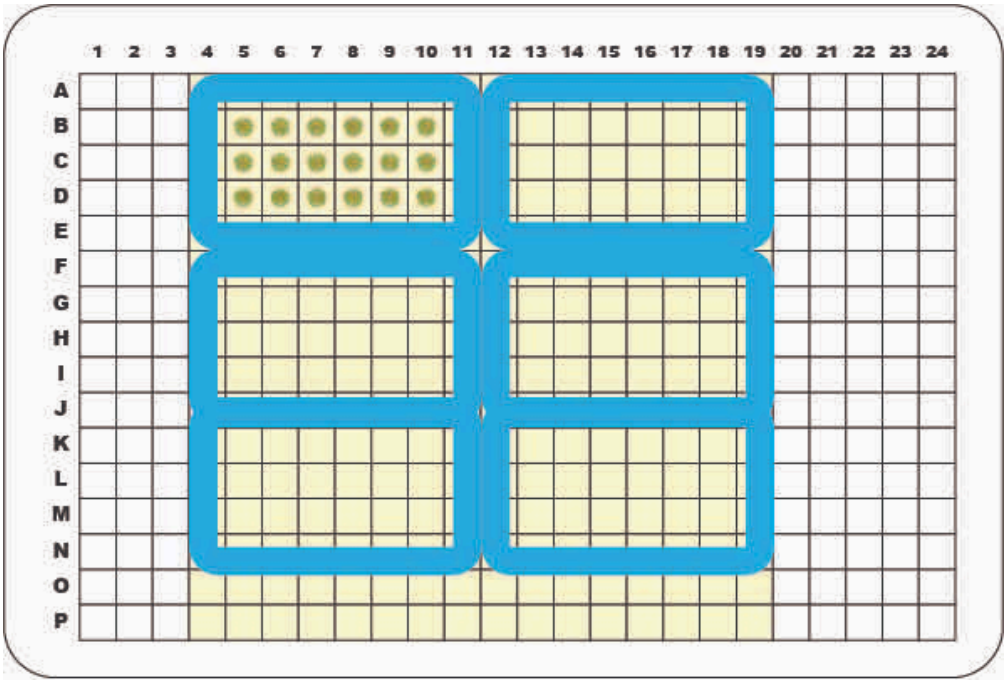
The number of wells to be filled depends on your requirements/ travelling range of the microscope stage.
We usually filled one more row/columns of wells around our samples to reduce media evaporation.

3 Let the media solidify and level it with a sterile scalple if it bulges out of the wells.

4 Place the gene frames to delimit the wells you are planning to image



5 Pick a single gemma from a gemma cup and place it at the centre of a well filled with media.



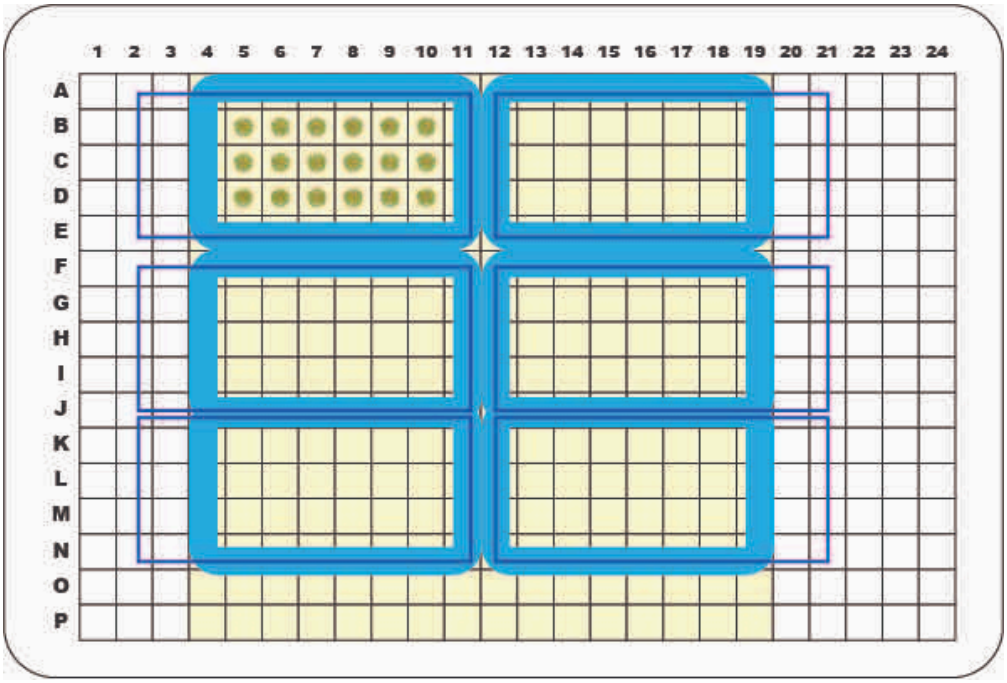
Note

Cover the plate with a lid to prevent media from evaporating when not adding gemmae.

6 Clean the cover glass with 70% EtOH and spray one side with the anti-fog spray.

7 Let the anti-fog solution evaporate and remove any residue with a lens tissue moving in one direction to avoid any visual artifacts.

8 Place the coverslip on the gene frame, making sure the anti-fog treated side is facing down.



9 The plate is now ready for imaging. Gemmae can be grown inside the plate up to 7 days.