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High throughput pipette-tip hydroponics for collecting salt stress samples for RNA-seq

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ABSTRACT

Growing on plants in hydroponics can take a lot of space, and thus failure to germinate is a risk that is usually taken into account but results in quite a lot of waste of space. Here, I describe a protocol for high-throughput *Arabidopsis* germination and early growth. The protocol described here is suitable for growing the *Arabidopsis* seedling for the 1st two weeks after germination. After this time period the seedlings should be transferred into the larger containers or harvested for the analysis.

GUIDELINES

The protocol described in here is not describing sterile growth conditions, however the materials should be prepared as cleanly as possible to avoid bacterial & fungal infections.

MATERIALS

NAME	CATALOG #	VENDOR
MES, free acid, monohydrate	MB0341.SIZE.25g	Bio Basic Inc.
Daishin agar	9002-18-0	Duchefa Biochemie
Potassium hydroxide	1050121000	Sigma Aldrich
Murashige & Skoog Basal Salts	MSP01-50LT	Caisson Labs

BEFORE STARTING

Make sure you have a lot of yellow pipette tips and preferably plenty of pipette tip boxes for 1 ml pipette tips.

Prepare seeds

24h

- 1 Sterilize the *Arabidopsis* seeds (no more than +/- 400 seeds) in 2ml Eppendorf tube with 1 ml of 50% household bleach (diluted with MQ water) - keep for 10-20 minutes
- 2 Wash the seeds with 2 ml of sterile MQ water - 5 to 8 times - preferably in the laminar hood
- 3 Incubate the seeds in MQ water in the dark at 4C at least overnight, but not longer than one week, to break the seed dormancy

- 4 Using the large and sturdy scissors - cut the yellow pipette tip so that you only keep the wider stud - as in the picture



The studed pipette tip - the bottom one is the desired cut of the pipette tip

- 5 Prepare Agar medium solution. Per 1 L use:

2.2 g Murashi Skoog nutrients

1 g MES buffer

adjust pH with KOH to 5.8

After adjusting pH add 10 g of Dashin Agar

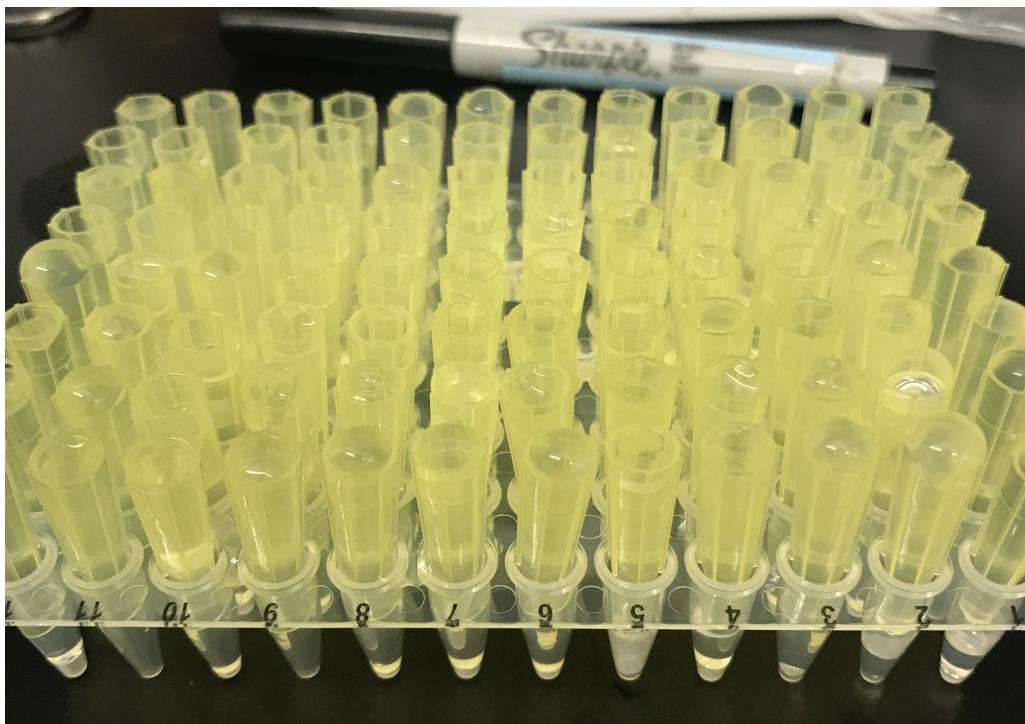
Autoclave the solution or melt the agar in the microwave.

You can store the nutrient agar solution on your benchtop, at room temperature, for about two weeks, or at 65 C cabinet for up to three days. Before using make sure that the solution is liquid by warming it up in the microwave

- Put the pipette studs into a clean PCR plate and add liquid nutrient agar solution so that it covers the bottom part - wait for the solution to solidify.

After the initial solidification of the media in the bottom part of the pipette tip - add more agar to fill the pipette tip

TIP: Make sure that your media forms a meniscus rather than a "bulb" - this will make it easier to place tiny Arabidopsis seeds without them sliding to the side



pipette studs in the final stage of filling them with nutrient agar solution

- Put the agar-filled pipette studs into the 1 ml tip box (they should be placed deeper into the box than in "yellow tip box"), fill the box using the liquid medium. Per 1L of liquid medium you need:

2.2 g Murashi Skoog nutrients

1 g MES buffer

adjust pH with KOH to 5.8

The above solution does not have to be autoclaved - unless you have a specific reason to do so.

- Using 1 ml pipette tip - drop the Arabidopsis seeds that underwent 24h cold treatment on top of the agar in the pipette studs. Place the pipette tip boxes in the growth chamber set at 18C, 12h light/dark cycle, 60% humidity for the seeds to germinate.

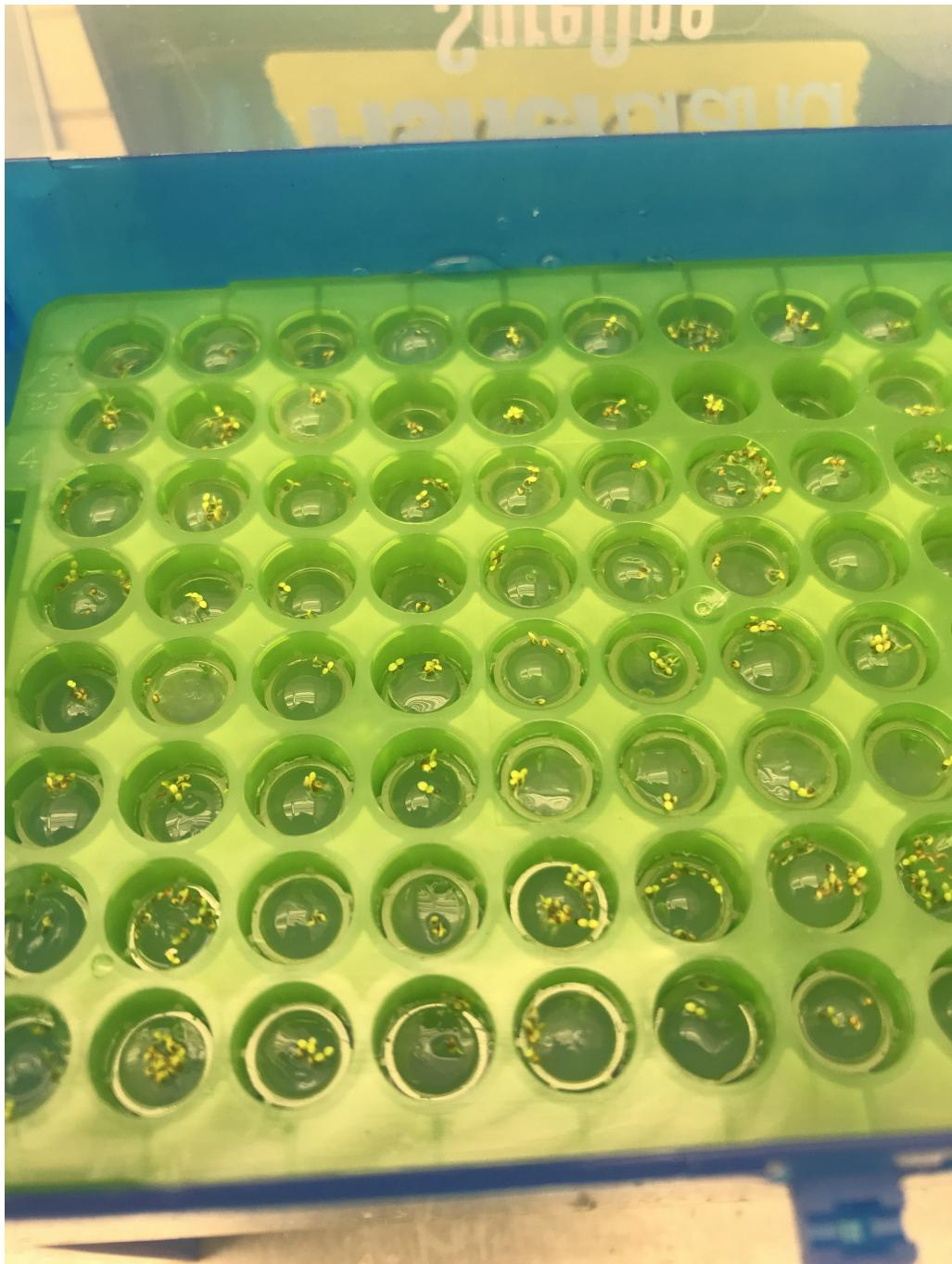


NOTE - it is important that the boxes are covered with the transparent lid - so the agar in the studs won't dry out



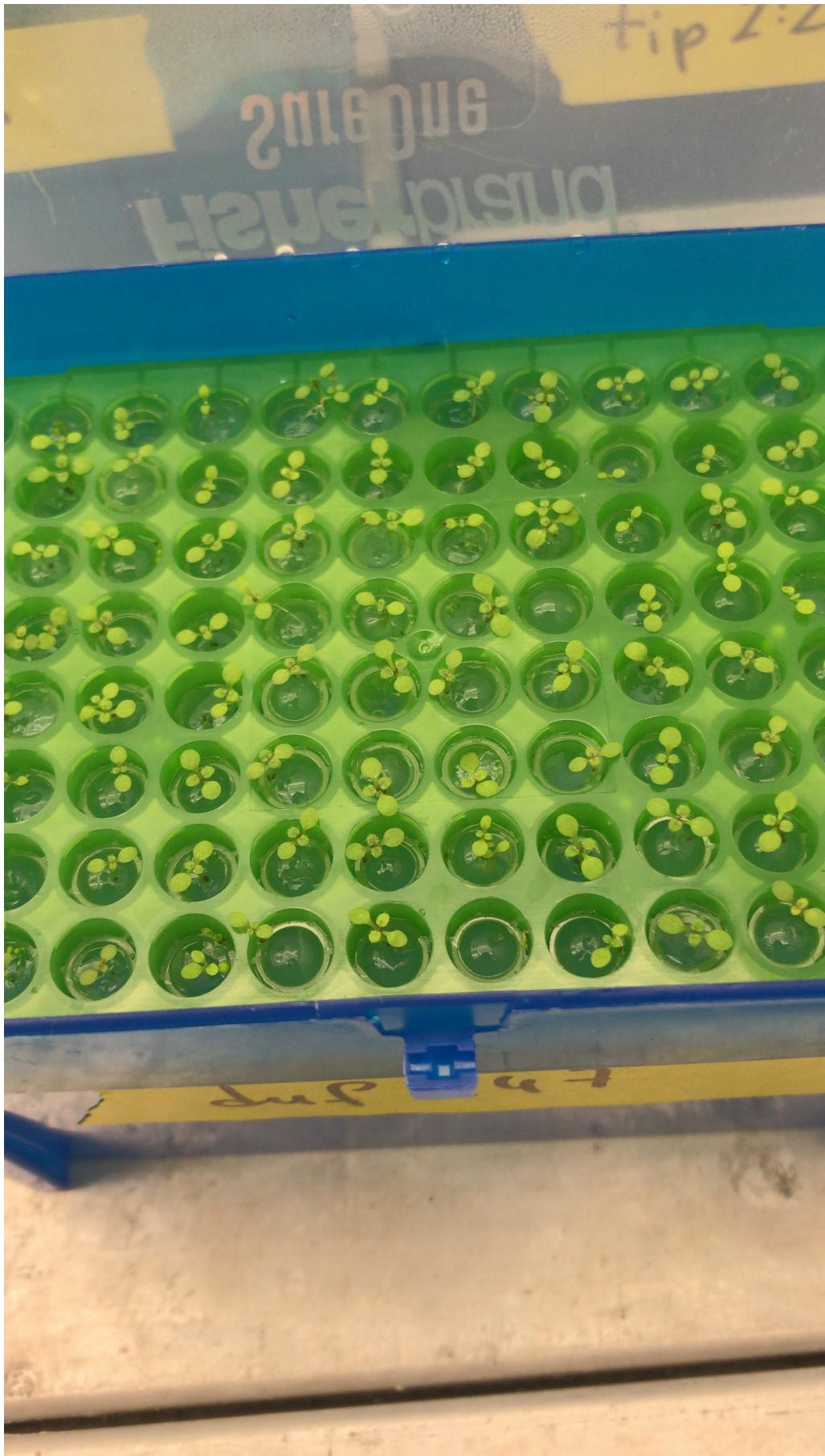
- 9 Make sure to check on the level of liquid media every day and replace the media completely every 5-7 days

Four days after placing the boxes into the growth room you should observe the germination of your seeds. I would advise you to "thin out" the seedlings at this stage - so you have only one germinating seedling per agar stud



Arabidopsis seedlings 4 days after germination

- 10 The roots should grow out of the agar studs between one and two weeks after germination. The level of media is adjusted as not to cover the studs completely, but rather be just below the plate holding the pipette tip studs



One week old seedlings of *Arabidopsis* in pipette-tip hydroponics



two weeks old seedlings of Arabidopsis

- 11 After two weeks of growth, the seedlings are transferred to larger hydroponics containers with 2L of solution per container



Two weeks old seedlings transferred to the larger Ara-ponics containers

- 12 For RNA-seq analysis, we add 100 mM NaCl to the liquid nutrient solution (as described above), when replacing the medium at 3 weeks after germination and harvest the shoot and root tissue to separate tubes for RNA isolation.

SUGGESTION: If you wish to treat your seedlings with salt stress, you could do it at earlier stage, as soon as the root will grow out of the agar stud

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