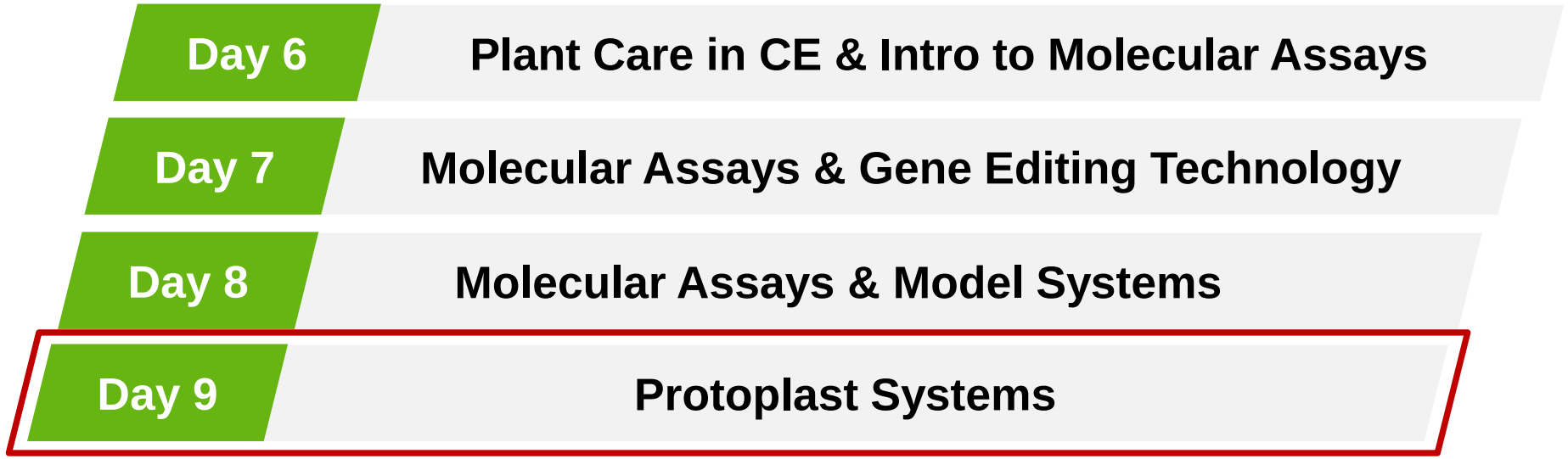
Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

July 2023

Bayer Russia Plant Biotechnology Conference:

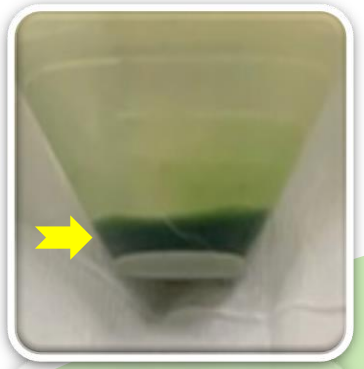


Protoplast Systems – Arabidopsis protoplast transformation

Bayer Russia Biotechnology Conference

July 2023

Protoplast isolation for transient assays



5. PEG delivery

4. *Protoplast collection*

DNA
RNA
Protein

3. Cellulase enzyme digestion

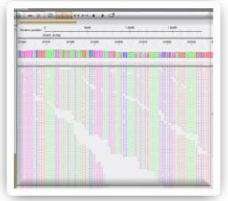
2. Leaves cutting

1. Arabidopsis plants

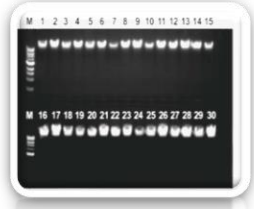
6. Secondary assays 2 - 72h post transformation

Localization, protein interaction & expression

ELISA & Reporter assays



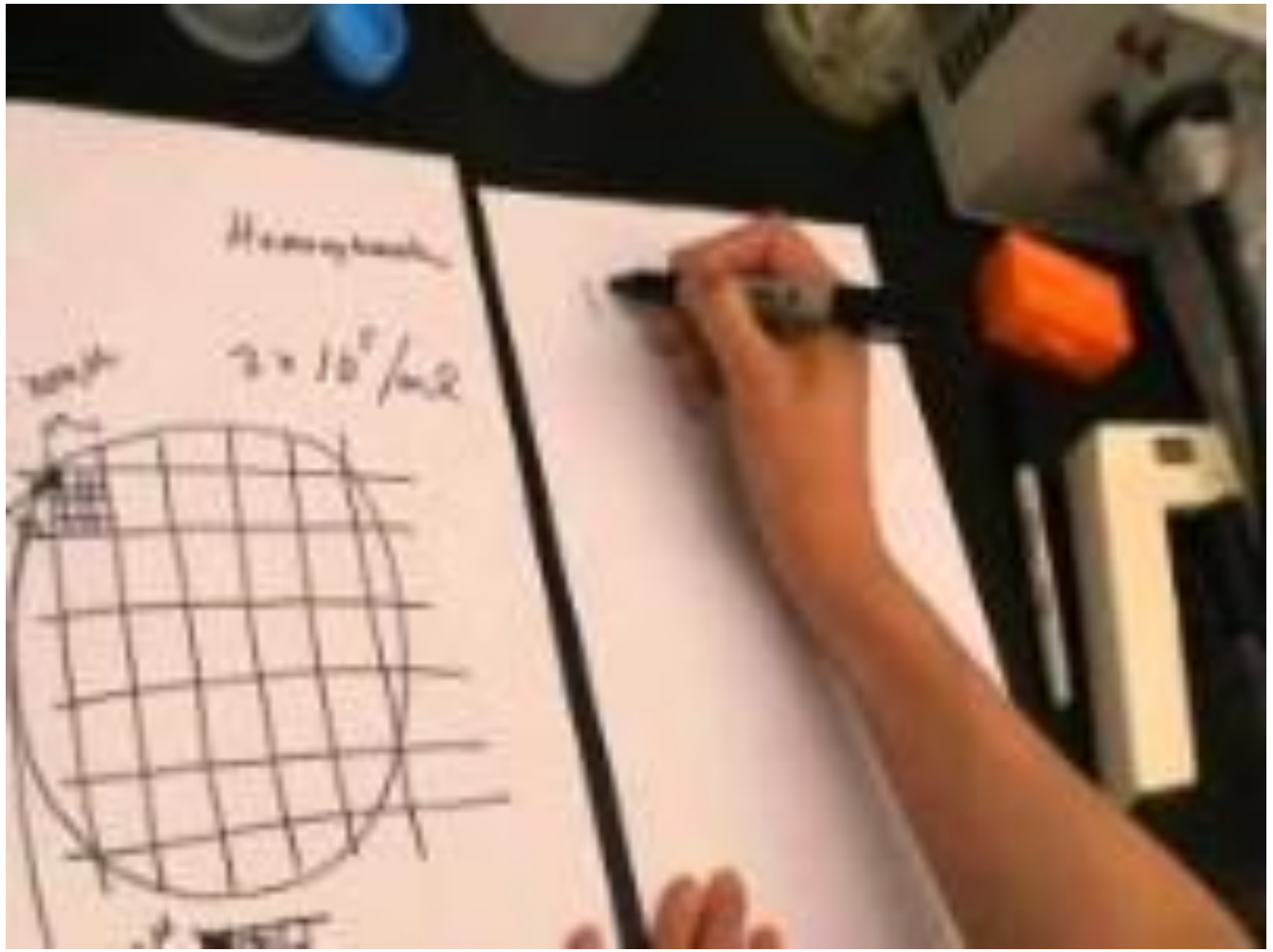
sequencing



PCR

How to Isolate Healthy Arabidopsis Leaf Protoplasts

ASPB2006 Protoplast Workshop Movie Jen Sheen's Lab - Harvard Medical School (22 minutes long)



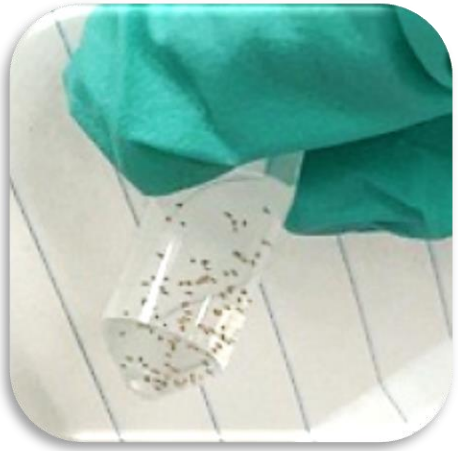
https://www.youtube.com/watch?v=5-xm1EoLrW4

ARABIDOPSIS PLANT GROWTH

PLANT SEEDS EVENLY

GROW IN LOW LIGHT

MAINTAIN CONSTANT GROWTH ENVIRONMENT



1. Seed Imbibition

2. Vernalization

3. Planting with dropper soil or Plate

4. Growth 3-4 weeks

PLANT GROWTH CONDITIONS

Metro-Mix 360 or Jiffy7 soil

low light (50–75 µE m^{-2} s^{-1})

10–13 h

23 °C

11–14 h

20 °C

40–65% relative humidity

ARABIDOPSIS LEAF CUTTING

SELECT UNIFORM LEAVES

DEVELOPMENTAL STAGE MATTERS!

⚠ SELECTING LEAVES TO CUT

- Select **dark green, healthy Arabidopsis leaves** prior to bolting stage
  - ❖ ***Youngest fully expanded leaves that are not touching soil or other leaves are ideal***
- Handle gently and cut leaves into **~1 mm strips**
  - ❖ ***maximize infiltration of enzymatic digestion*** *solution during vacuum infiltration*

SELECT LEAVES

SHARP, STERILE BLADES

CONSISTENT 1mm CUTS

Water
0.5 M Mannitol
10 mM MES
1% Cellulase R10
0.25% Macerozyme R10
10 mM CaCl2
20 mM KCl
0.1% BSA

COAT THOROUGHLY

ARABIDOPSIS CELL WALL DIGESTION

SUBMERGE LEAVES IN DIGESTION SOLUTION COMPLETELY

VACUUM INFILTRATION

CELL WALL DIGESTION

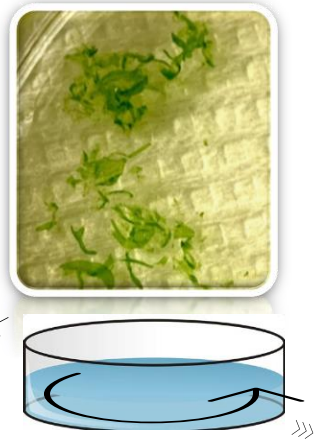
- Vacuum infiltrate
  - *Tissue should be **thoroughly coated** with enzyme digestion solution*
- **Cover with aluminum foil.** Digest at room temperature overnight (14-18 hours) in dark.
  - ❖ *Avoid **light exposure***

15-30 mins

14-18 h

PROTOPLAST RELEASE

Mesh Ordering Information
ELKO Filtering Co, LLC
Item # 03-60/42



40 RPM 2-3 Min

Filter
60 micron
mesh

W5

154mM NaCl
125mM CaCl2
5mM KCl
2mM MES
Water

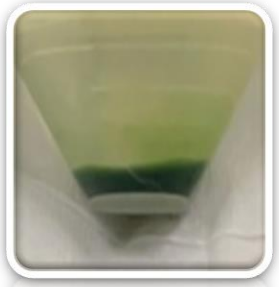
Rinse Remaining
tissue in petri dish

Filter

Centrifuge
150 x g

Remove Supernatant

Resuspend



WI

0.5M Mannitol
4mM MES
20mM KCl
Water

OBSERVE COLOR OF ENZYME SOLUTION

FILTER TO CLEAR DEBRIS

HANDLE GENTLY

Cell Counting in Hemacytometer

Add 10 ul of cells to hemacytometer

1- Count cells in each of the 4 quadrants
(TOTAL = A + B + C +D)

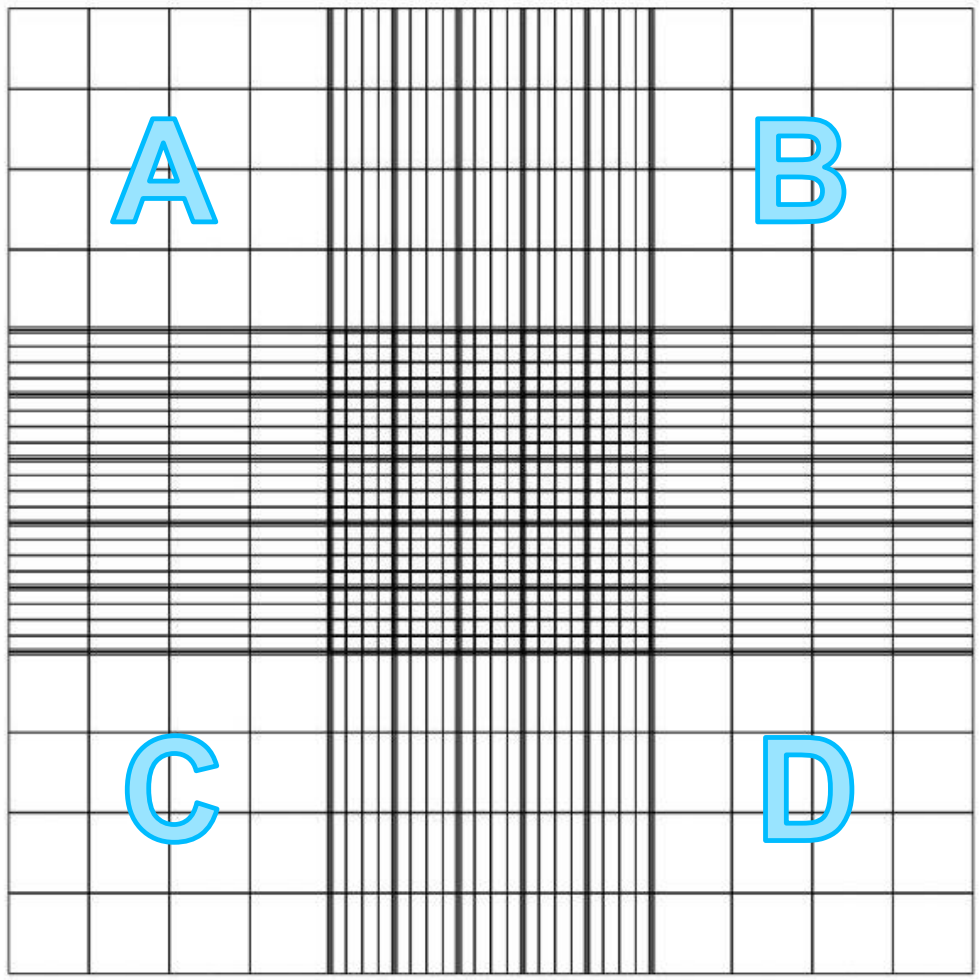
- IGNORE CELLS TOUCHING OUTER EDGES

2- Calculate Average number of cells (Total / 4) and multiply by 10^4 to obtain cell concentration per ml

3- Multiply cell concentration by total volume of cells

4 – Adjust cell concentration to 1 x 10^6 per ml

* If the cell count is above 4 x 10^6 or below 1 x 10^6 cells/ml, the count is inaccurate. Adjust the cell density and repeat the cell count.



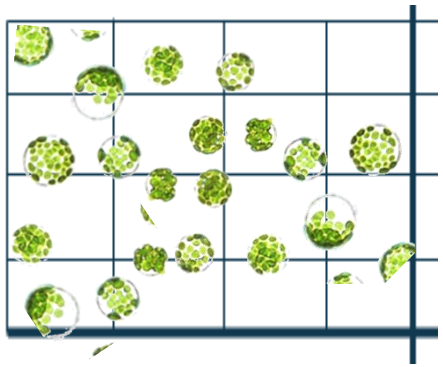
Example: If the calculated average (n) of cells in the four 1 mm corner squares of the hemacytometer is 30, then cells/ml = (n) x 10^4 (or) cells/ml = 30 x 10,000 = 300,000 cells/ml. Multiply by dilution factor and by total cell suspension volume obtained to get the protoplast yield.

ARABIDOPSIS PROTOPLAST PREP

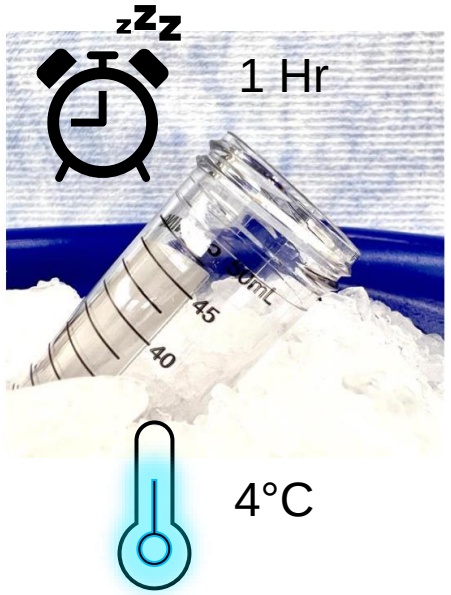
ALLOW CELLS TO REST

MINIMIZE TIME ON MMG MEDIA

- After counting the yield with hemacytometer, Rest cells 1 hour on ice
- Just before transformation, centrifuge 150 x g for 2-3 minutes. Remove the supernatant
- Resuspend the cells at 1 x 10^6 /mL in **MMg solution and keep on ice**



Count yield



Rest

150 x g for 2-3 minutes

MMG

0.4M Mannitol
15mM $MgCl_2$
4mM MES
Water

Resuspend at 1 x 10^6 /ml

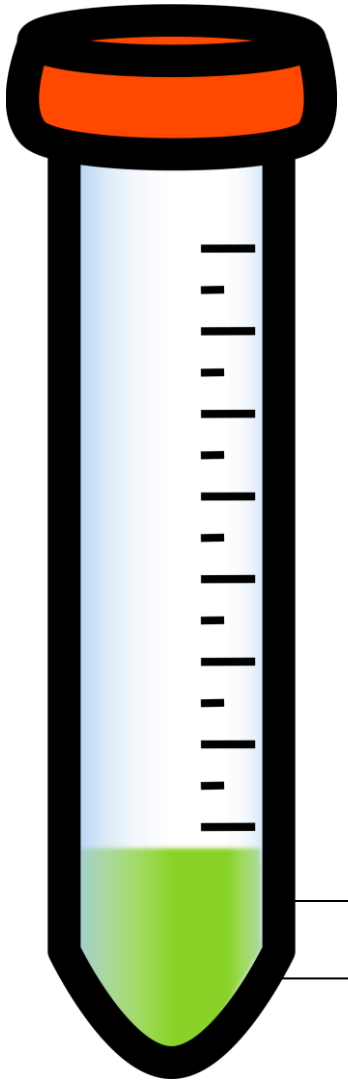
ARABIDOPSIS TRANSFORMATION

W5

154mM NaCl
125mM CaCl2
5mM KCl
2mM MES
Water

PEG

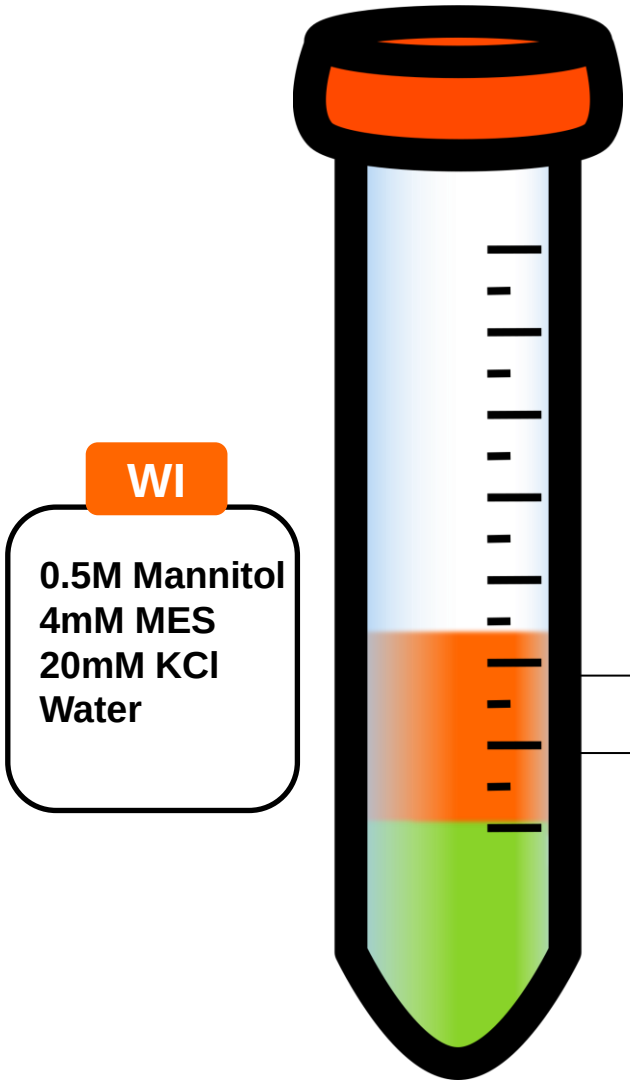
PEG 4000
Water
Mannitol
$Ca(NO_3)_2$



Centrifuge 2 mins 150 x g and remove supernatant

Cells & DNA

ARABIDOPSIS TRANSFORMATION

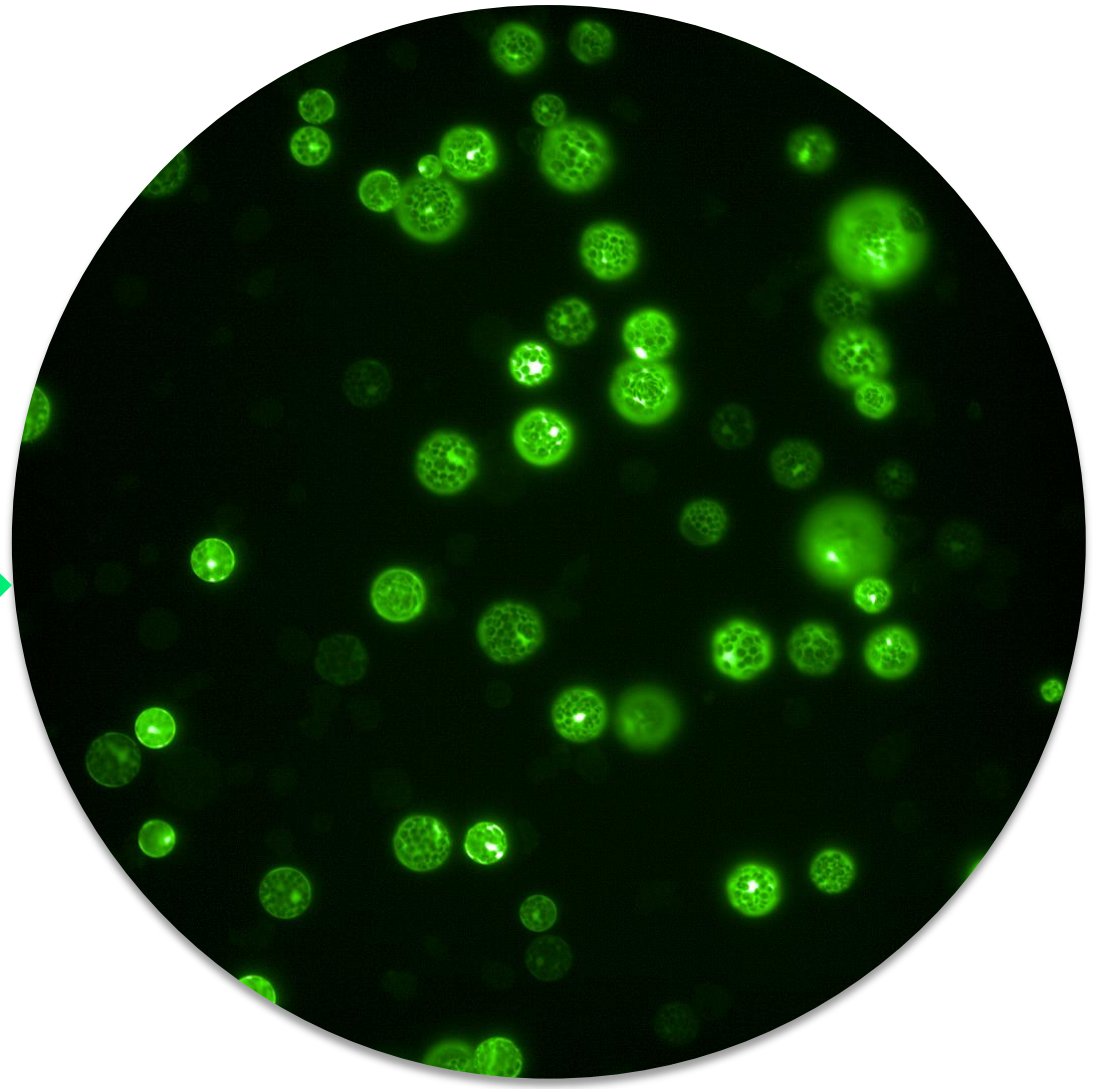


WI

0.5M Mannitol
4mM MES
20mM KCl
Water

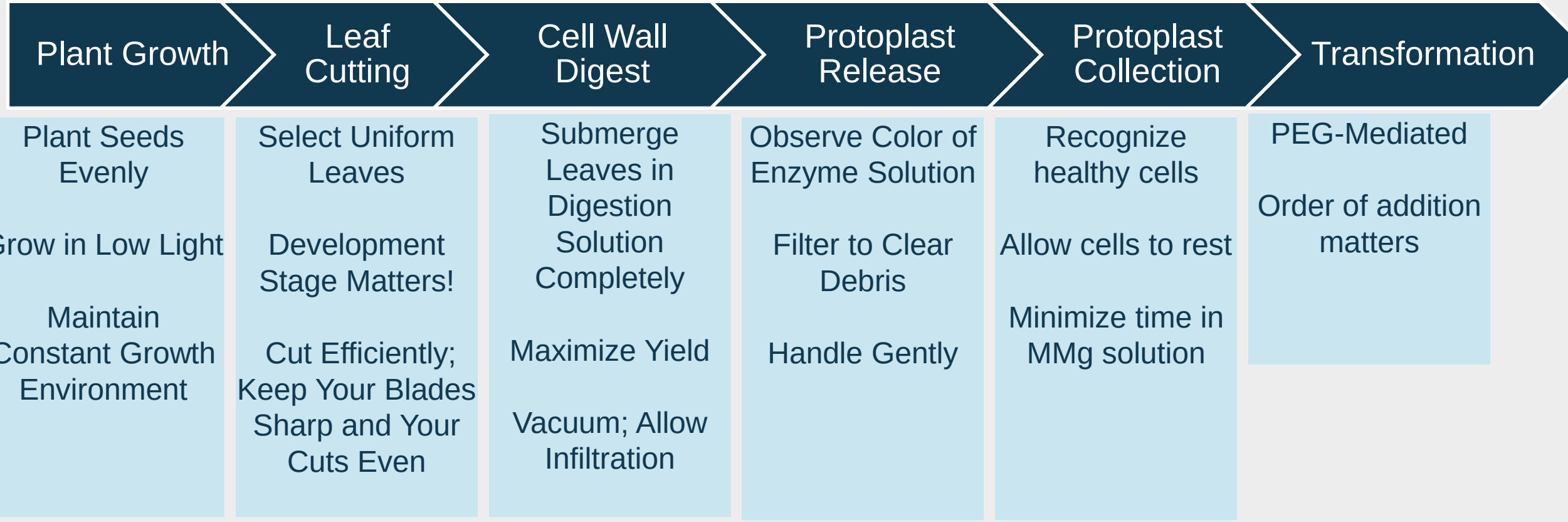
Add WI solution

4-18 h



Arabidopsis Protoplast Isolation and PEG-Mediated Transformation

Isolation Overview



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Thank you!

Any questions?