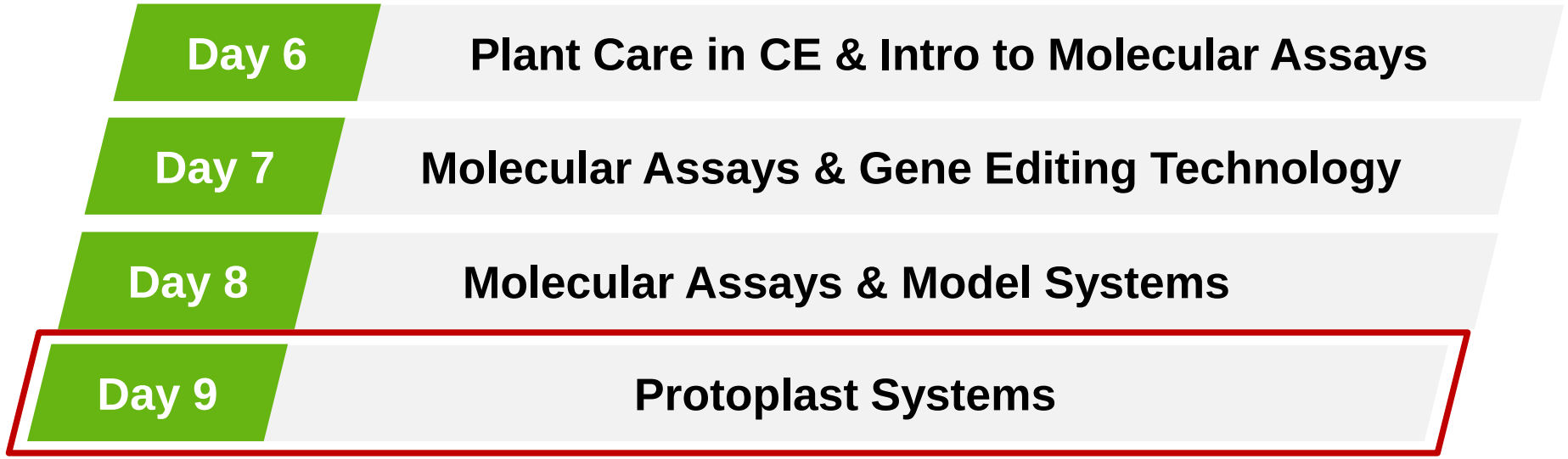
Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

July 2023 – week 2

Bayer Russia Plant Biotechnology Conference:

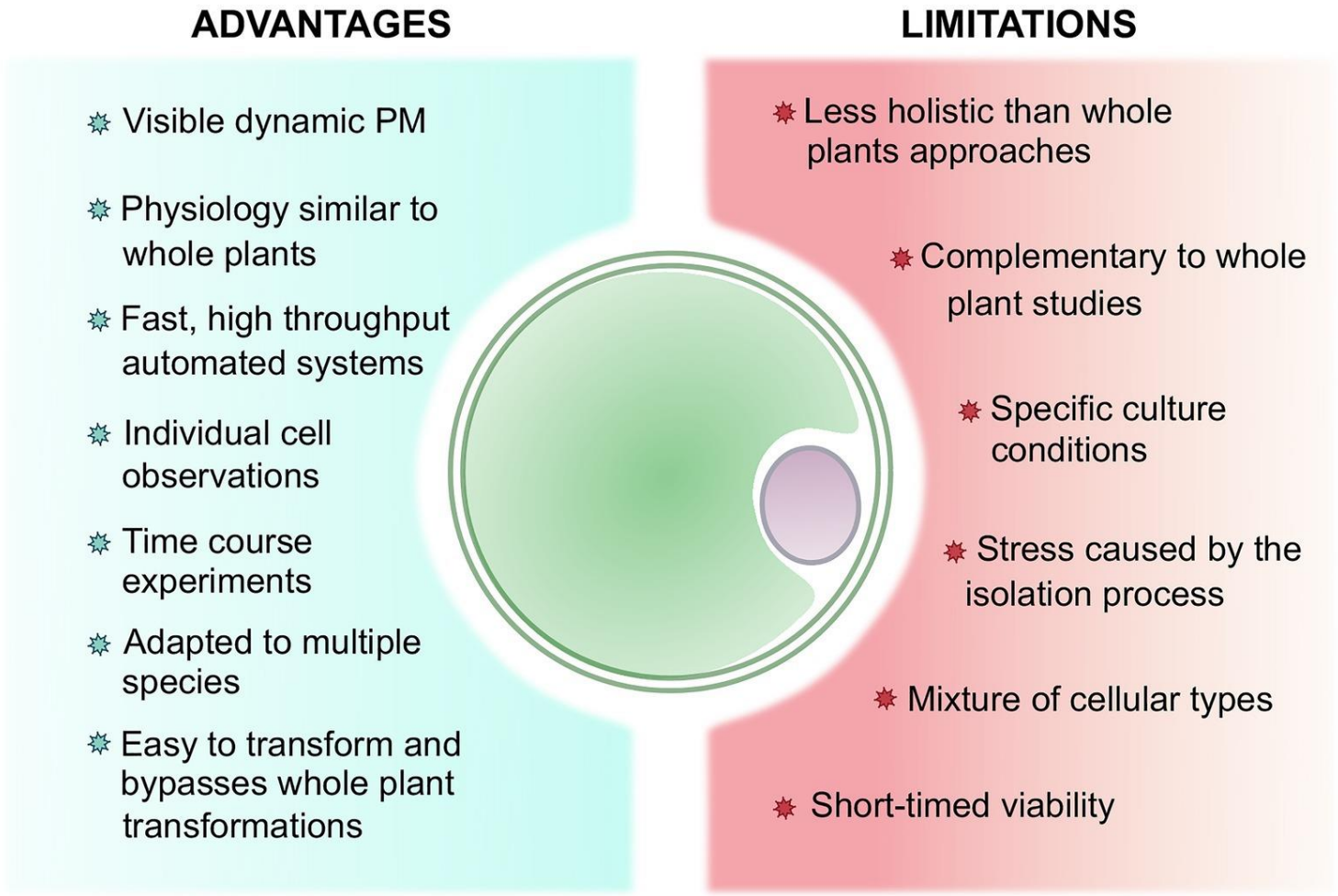


Protoplast Systems

Bayer Russia Biotechnology Conference

July 2023

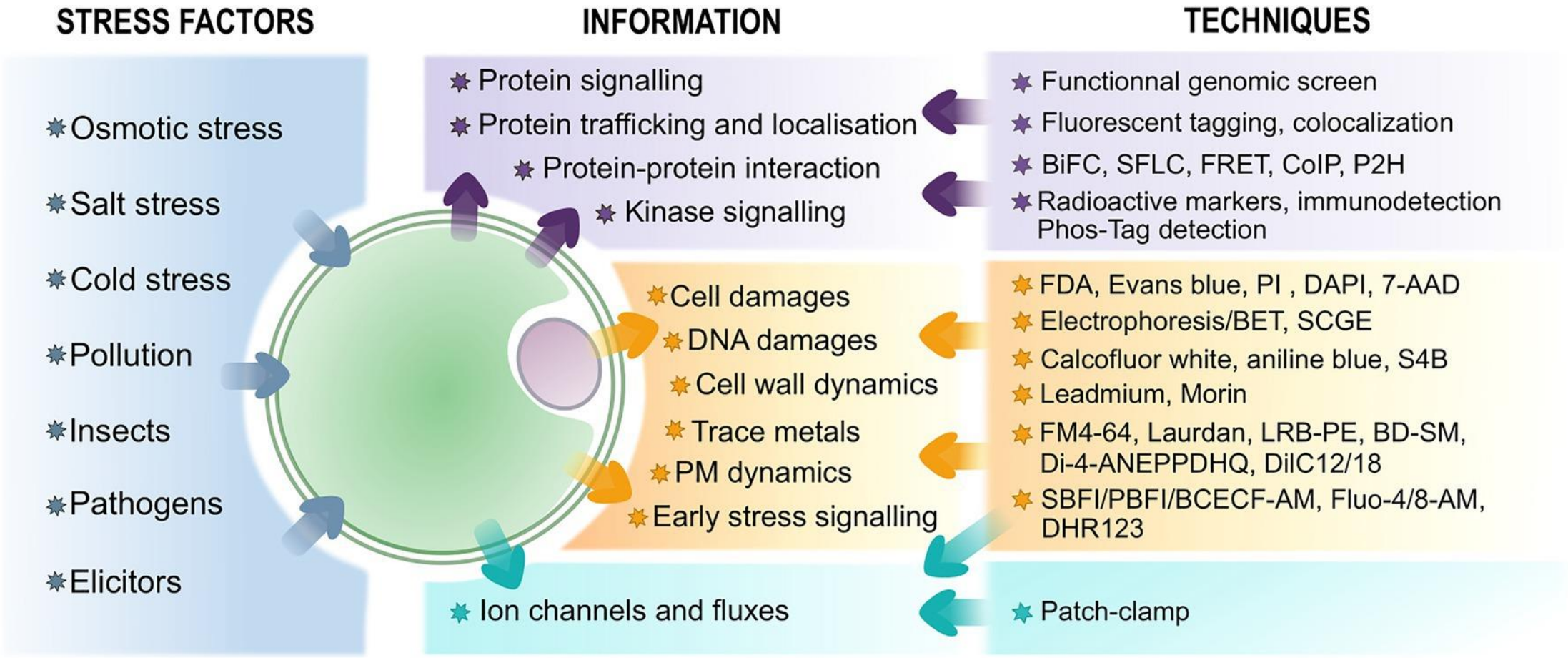
Plant Protoplasts as Tools



https://www.frontiersin.org/articles/10.3389/fpls.2021.749581/full

Protoplast: A Valuable Toolbox to Investigate Plant Stress Perception and Response. Frontiers in Plant Science (2021).

Plant Protoplasts: Common Techniques Applied



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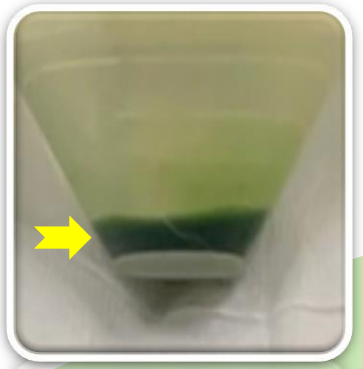
Protoplast: A Valuable Toolbox to Investigate Plant Stress Perception and Response. Frontiers in Plant Science (2021).

Protoplast isolation for transient assays



5. PEG delivery

4. Protoplast collection

DNA
RNA
Protein

3. Cellulase enzyme digestion

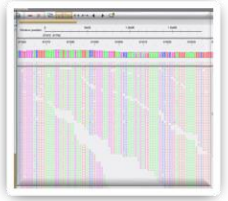
2. Leaves cutting

1. Arabidopsis plants

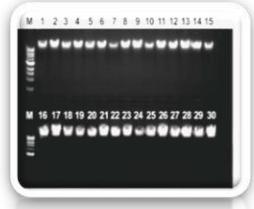
6. Secondary assays 2 - 72h post transformation

Localization, protein interaction & expression

ELISA & Reporter assays



sequencing



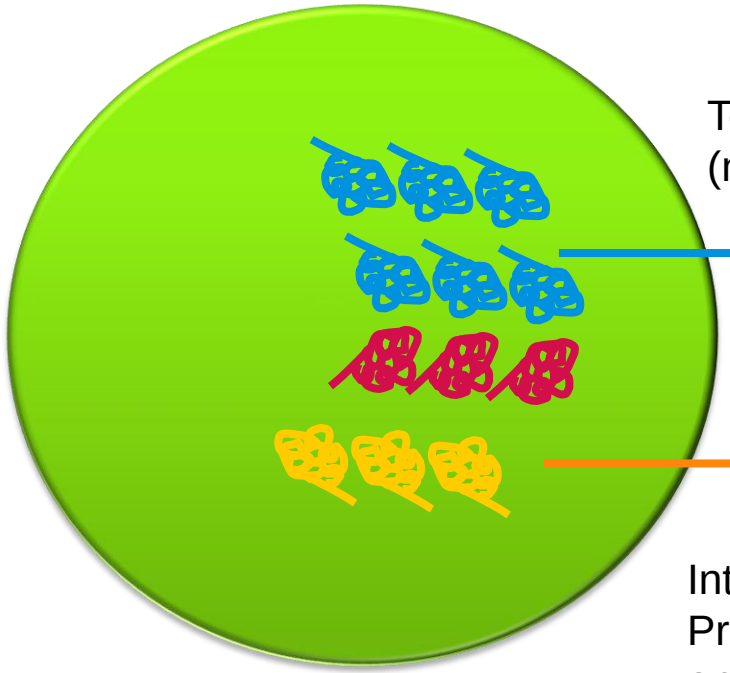
PCR

Transient Assay Setup

Generic co-transfection of plasmids & setup

DNA
Plasmids

- Test 1 Fluc (Promoter 1)
- Test 2 GUS (Promoter 2)
- Internal control Renilla Luc



Tested variants
(n=3)

Internal Controls:
Provide viability
and transformation
efficiency
information across
wells

Samples

Replicates suggested to include:

Test	GFP	No DNA
Promoters Fluc GUS Rluc	Standard	Background No Fluc No Rluc

Data Analysis Outline

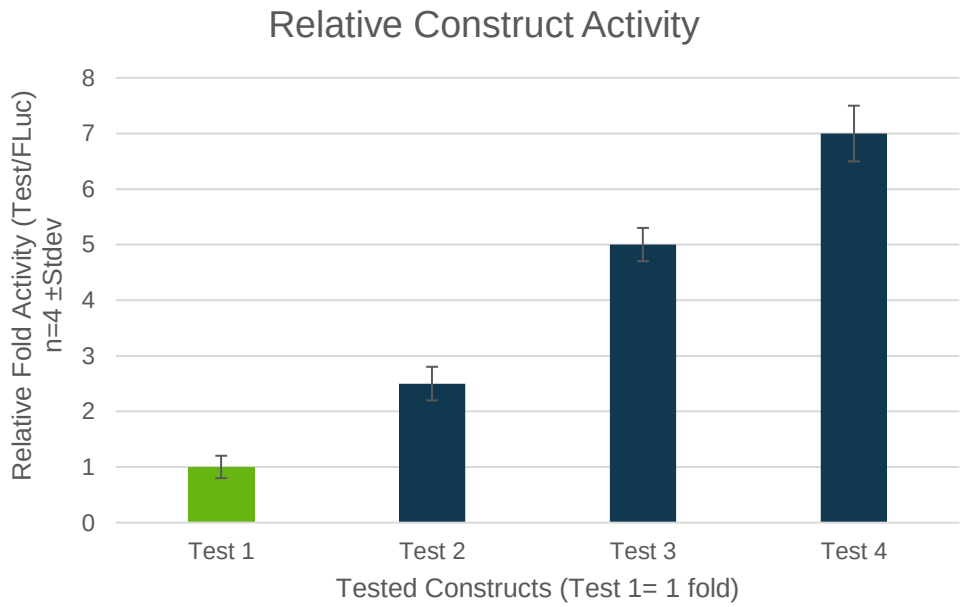
QC of controls and test samples + calculations for data analysis

Raw Data → Background Subtraction → Normalization → Fold Calculation → Graphs

Subtract mean of No DNA samples for each assay

Divide test values by Internal Control(s) to obtain ratio

Set mean ratio of 1 reference test construct to 1 fold or 100%. Remaining test constructs shown relative to 1 fold

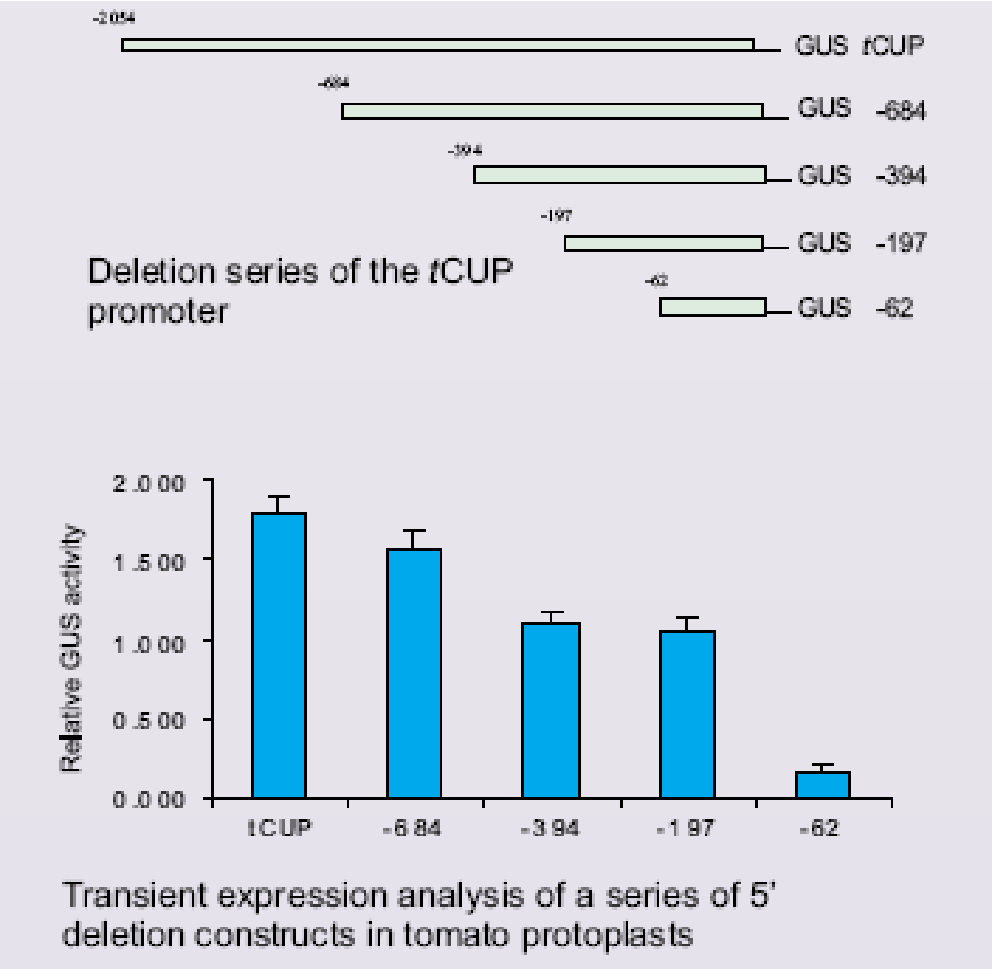


QC metrics:

- Outlier detection/removal

Comparison of Expression Elements in Protoplasts

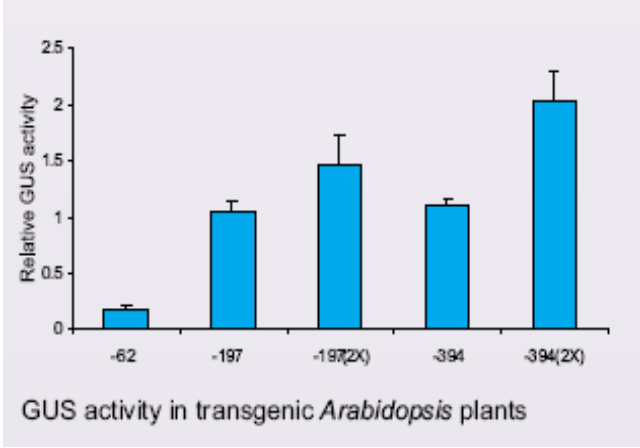
Transient expression profiles mimic plant expression profiles



Promoter deletion series

Relative Promoter strength assessment

Stable Plant Data



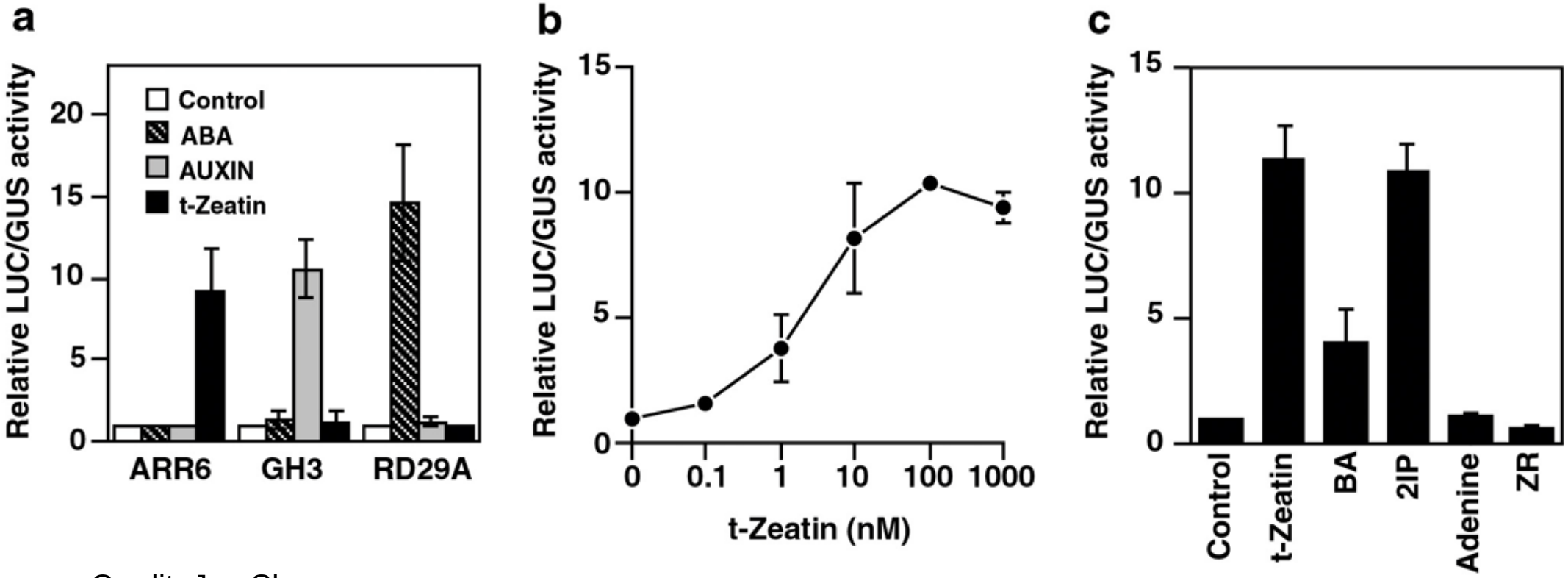
Useful reporter constructs for transient expression studies in *Arabidopsis* mesophyll protoplast assays

Nature Protocols **2**, 1565–1572 (2007) https://doi.org/10.1038/nprot.2007.199

Name	GenBank locus	*Arabidopsis* Biological Resource Center stock number	Promoter of interest (POI)	Use	References
pHBT-sGFP(S65T)-NOS	EF090408	CD3-911	35S derivative	Transfection control	19
pRD29A-LUC-NOS	EF090409	CD3-912	At5g52310	Abscisic acid response	5,13
pAtGH3-LUC-NOS	EF090410	CD3-913	At2g23710	Auxin response	5,13
pWRKY29-LUC-NOS	EF090411	CD3-914	At4g23550	Bacterial flg22 response	15
pGST6-LUC-NOS	EF090412	CD3-915	At2g47730	General stress response	5
pHSP18.2-LUC-NOS	EF090413	CD3-916	At5g59720	Heat response	13
pARR6-LUC-NOS	EF090414	CD3-917	At5g62920	Cytokinin response	13
pGCC1-LUC-NOS	EF090415	CD3-918	8xGCC box synthetic promoter	Ethylene response	12
pFRK1-LUC-NOS	EF090416	CD3-919	At2g19190	Bacterial flg22 response	16

Examples of Comparisons Used in Literature

Promoter activity in response to various hormone stimuli. A) Different promoters are shown in different color bars B) Dose response of a single promoter response C) One promoter response to various compounds tested.



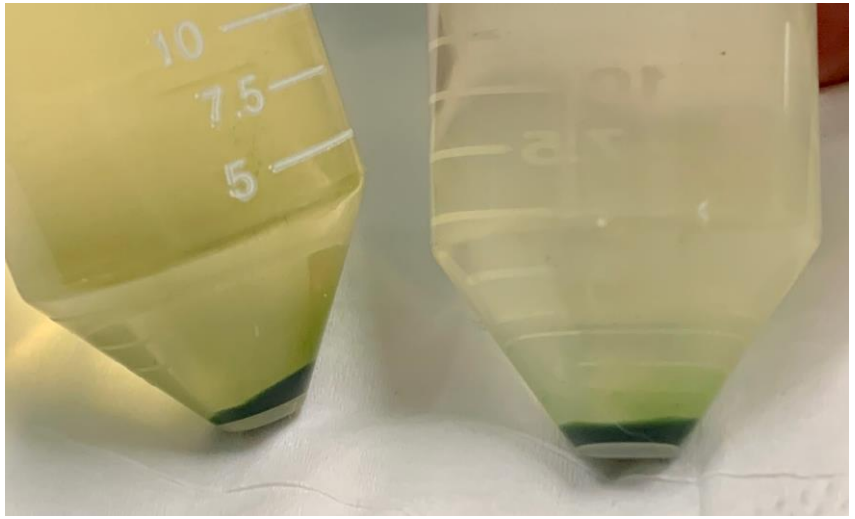
Credit: Jen Sheen

Arabidopsis Protoplast Isolation

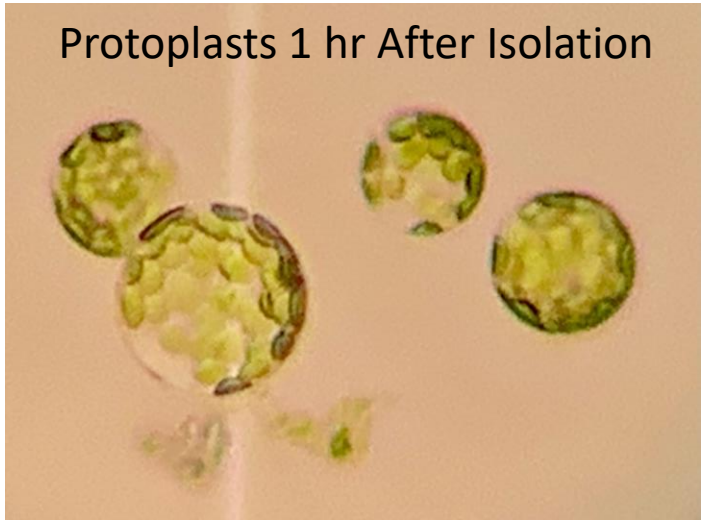
Before Digestion

After Digestion & Release

Cell Pellet After Filtration & Centrifugation

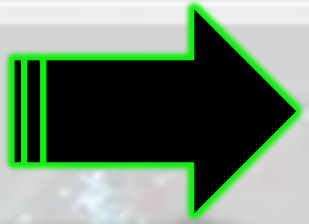


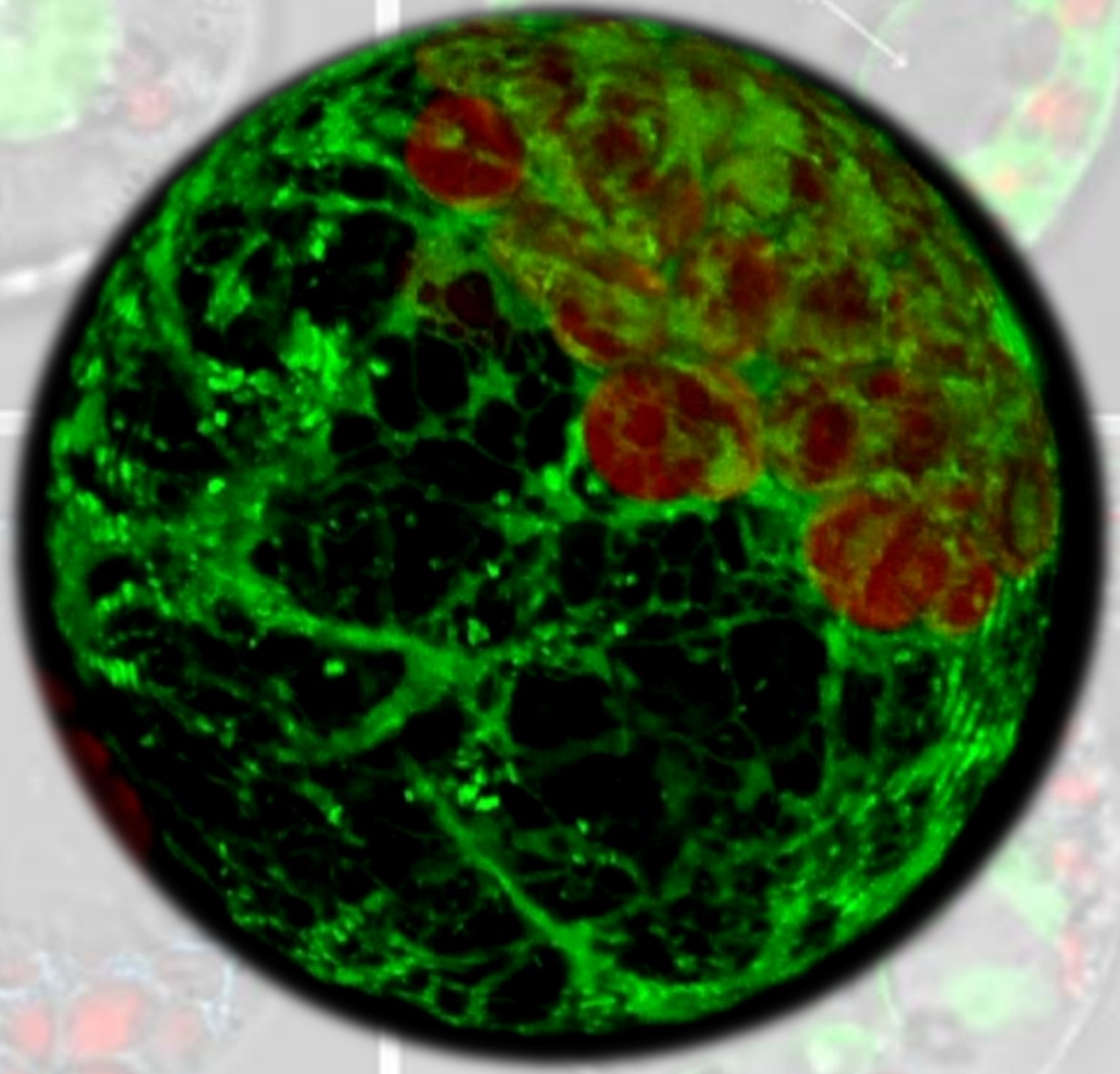
Protoplasts 1 hr After Isolation



Sub-cellular Localization

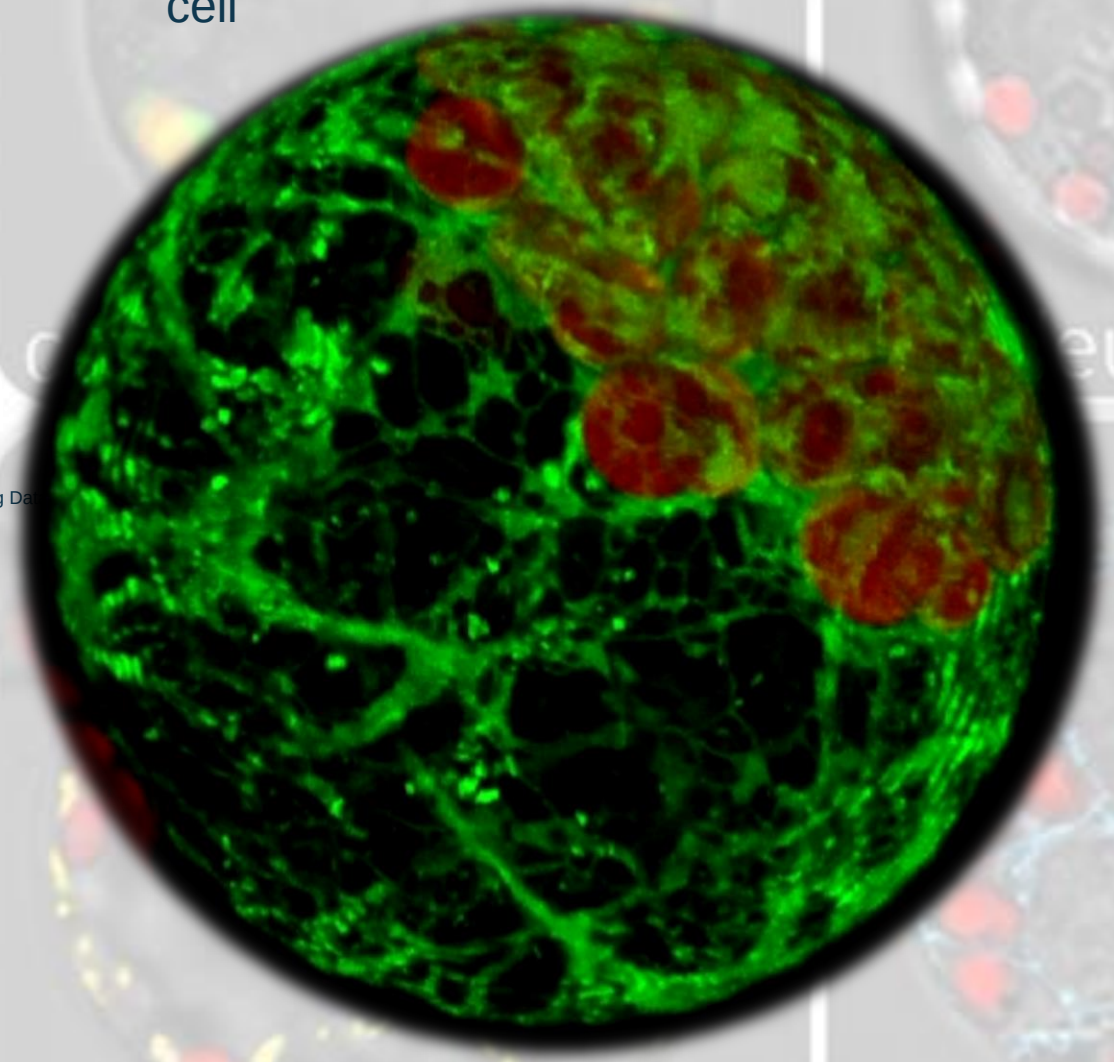
Fluorescent proteins allows us to study protein localization, signaling and protein-interactions in-vivo.

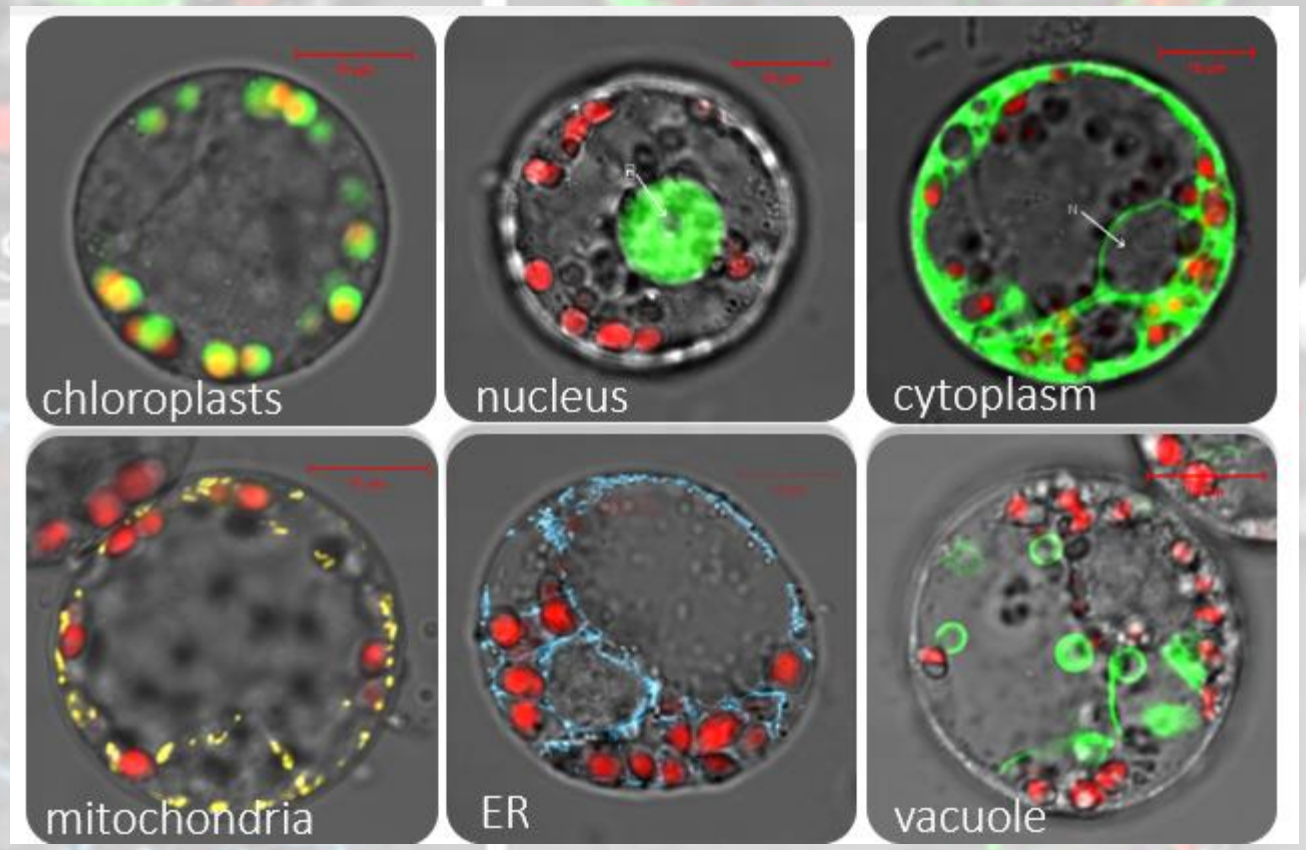




Sub-cellular Localization

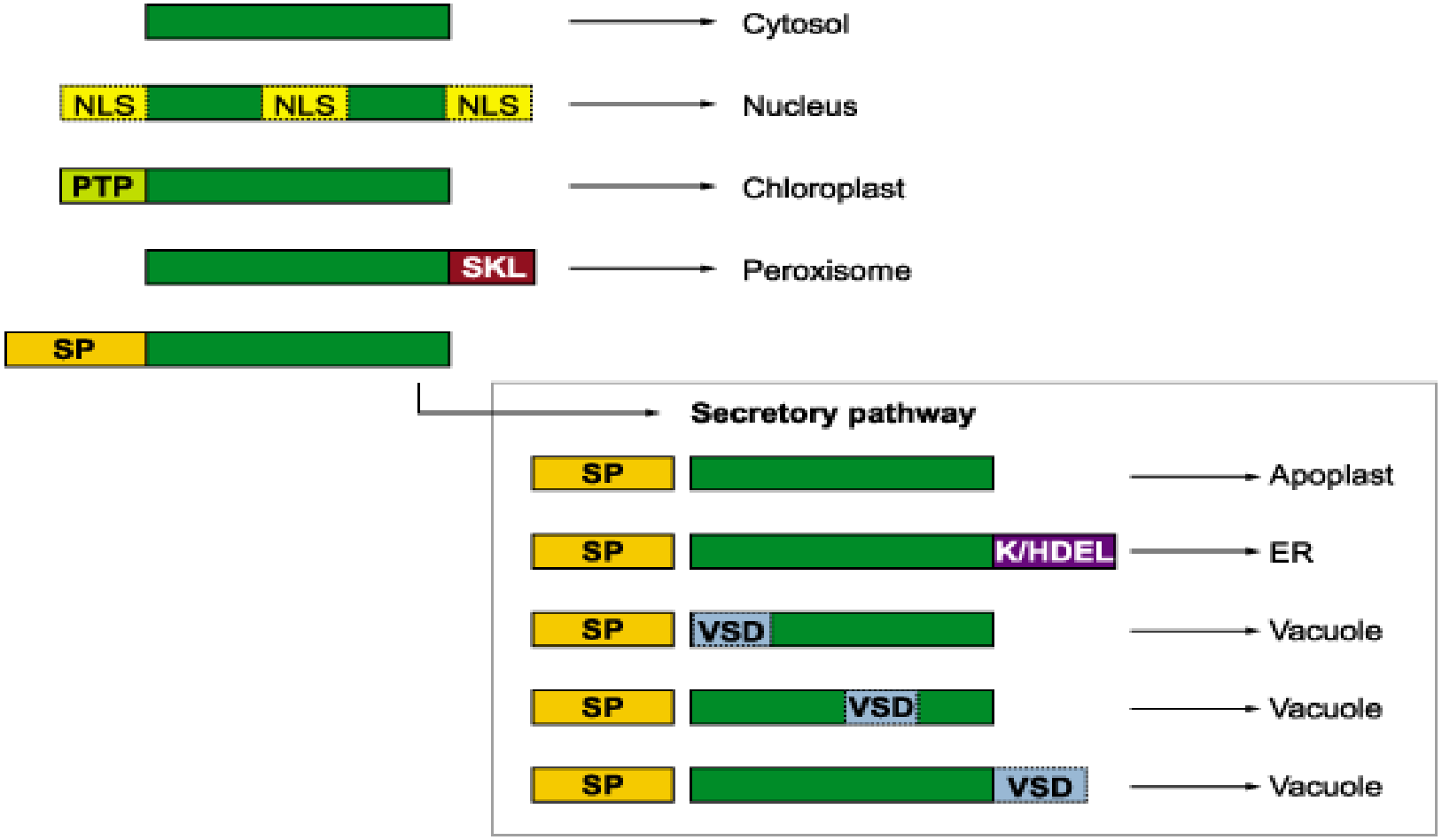
Different color fluorescent proteins can be used to tag multiple compartments or study co-localization in a cell





Signal Peptides & Localization Signals

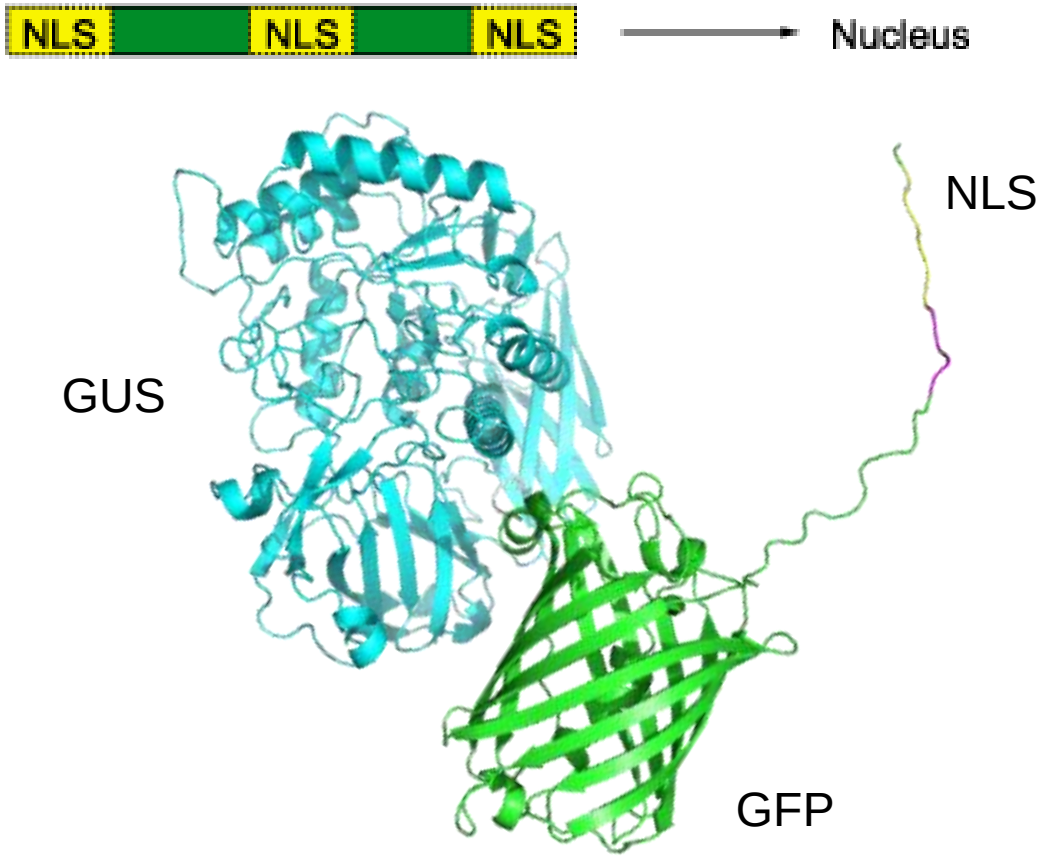
Localization of native proteins or chimeric protein designs

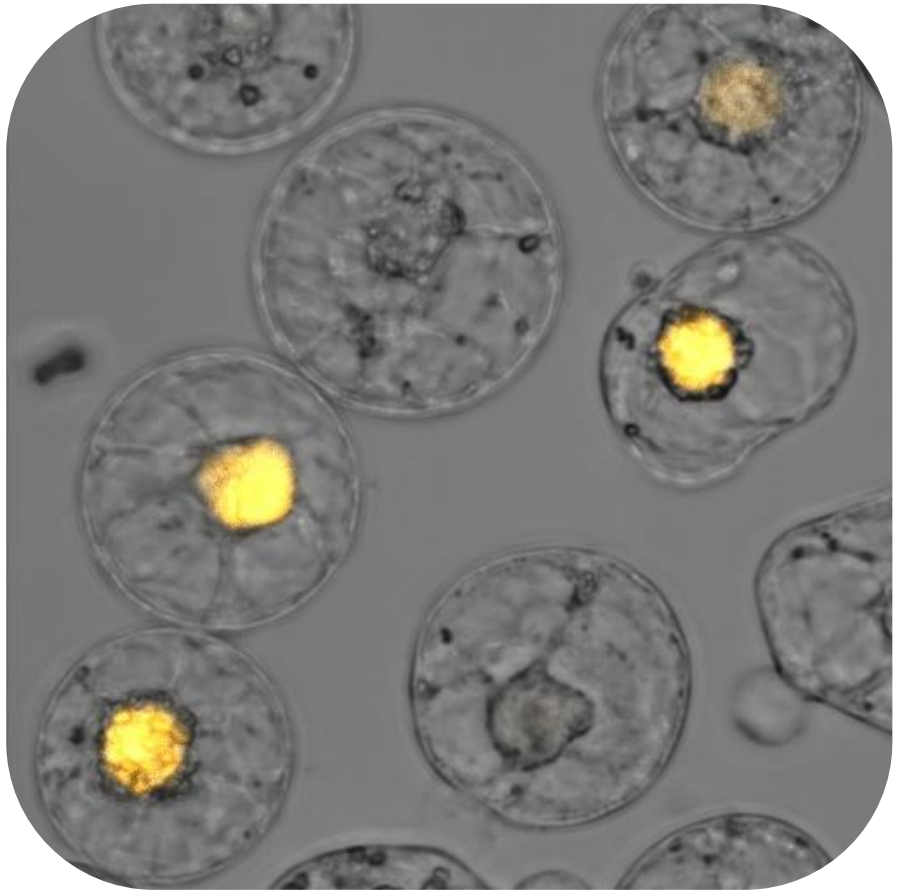


Plant Biotechnology Journal, Volume: 6, Issue: 7, Pages: 633-648, First published: 08 August 2008, DOI: (10.1111/j.1467-7652.2008.00344.x)

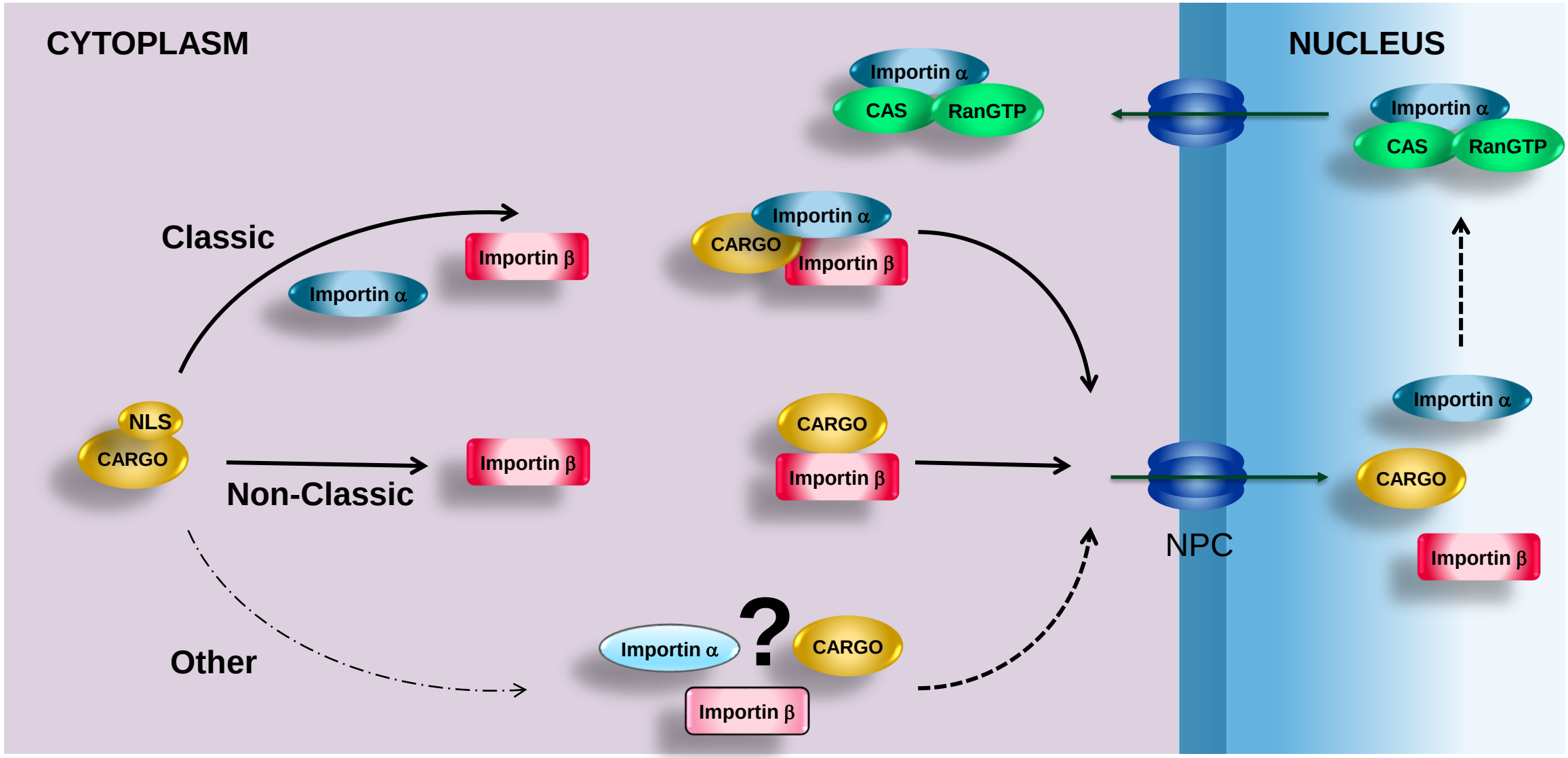
Nuclear Localization Signals (NLS)

Proteins of <70 KDa can passively diffuse into nuclei. NLS studies generally use protein fusions that require active import (>70 kDa).





Two major import pathways to the nuclei: Classic & Non-classic

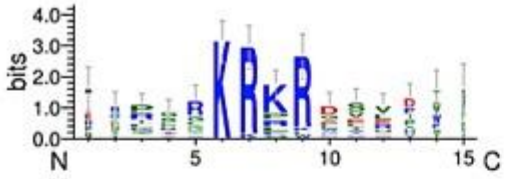


There are Six Classic NLS Classes

Class 1 & 2 are predominant across organisms, Class 3-5 vary by importin and tissue. Class 5 is plant specific

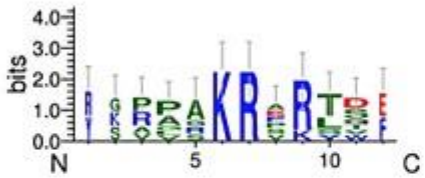
1

Class-1: KR(K/R)R, K(K/R)RK



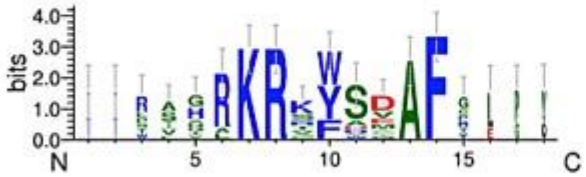
2

Class-2: (P/R)*XX*KR(^DE)(K/R)



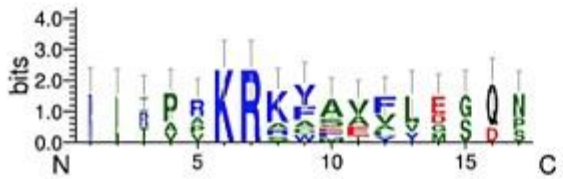
3

Class-3: KR*X*(W/F/Y)*XX*AF



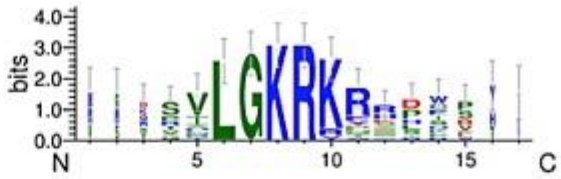
4

Class-4: (R/P)*XX*KR(K/R)(^DE)



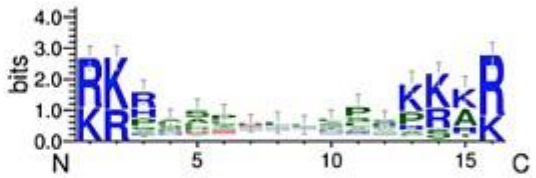
5

Class-5: LGKR(K/R)(W/F/Y)

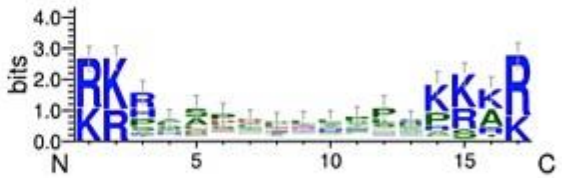


6

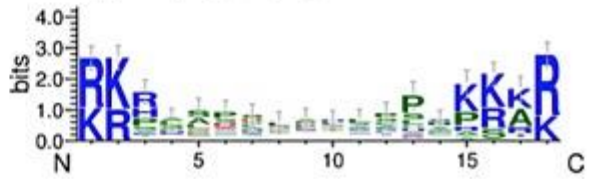
Class-6_{10}: KRX[10]K(KR)(KR)



Class-6_{11}: KRX[11]K(KR)(KR)



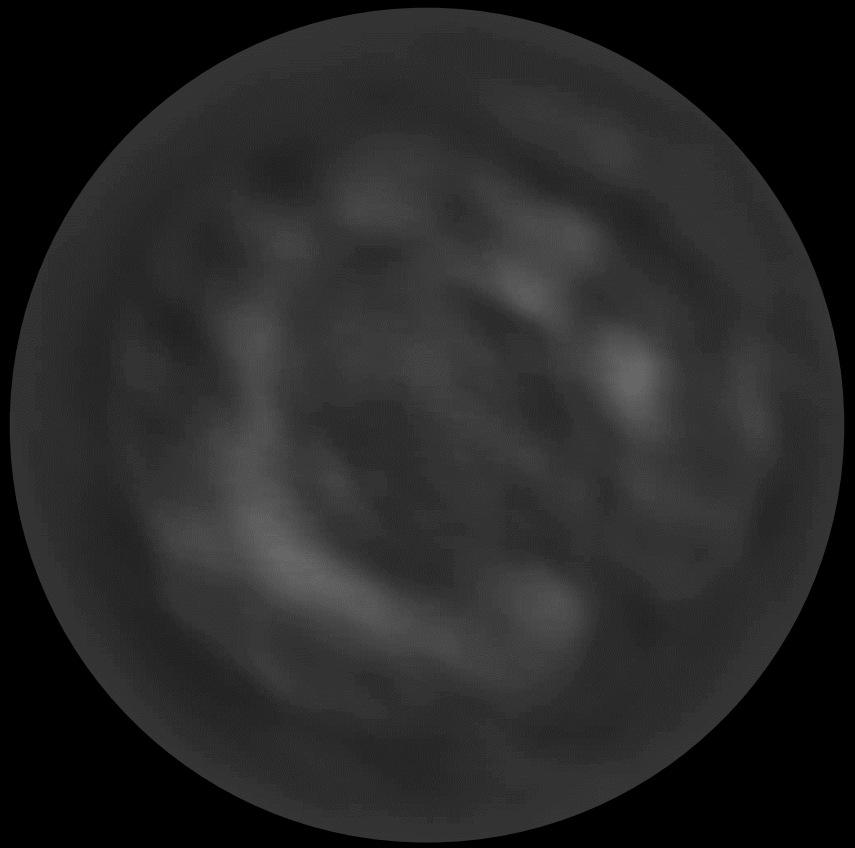
Class-6_{12}: KRX[12]K(KR)(KR)

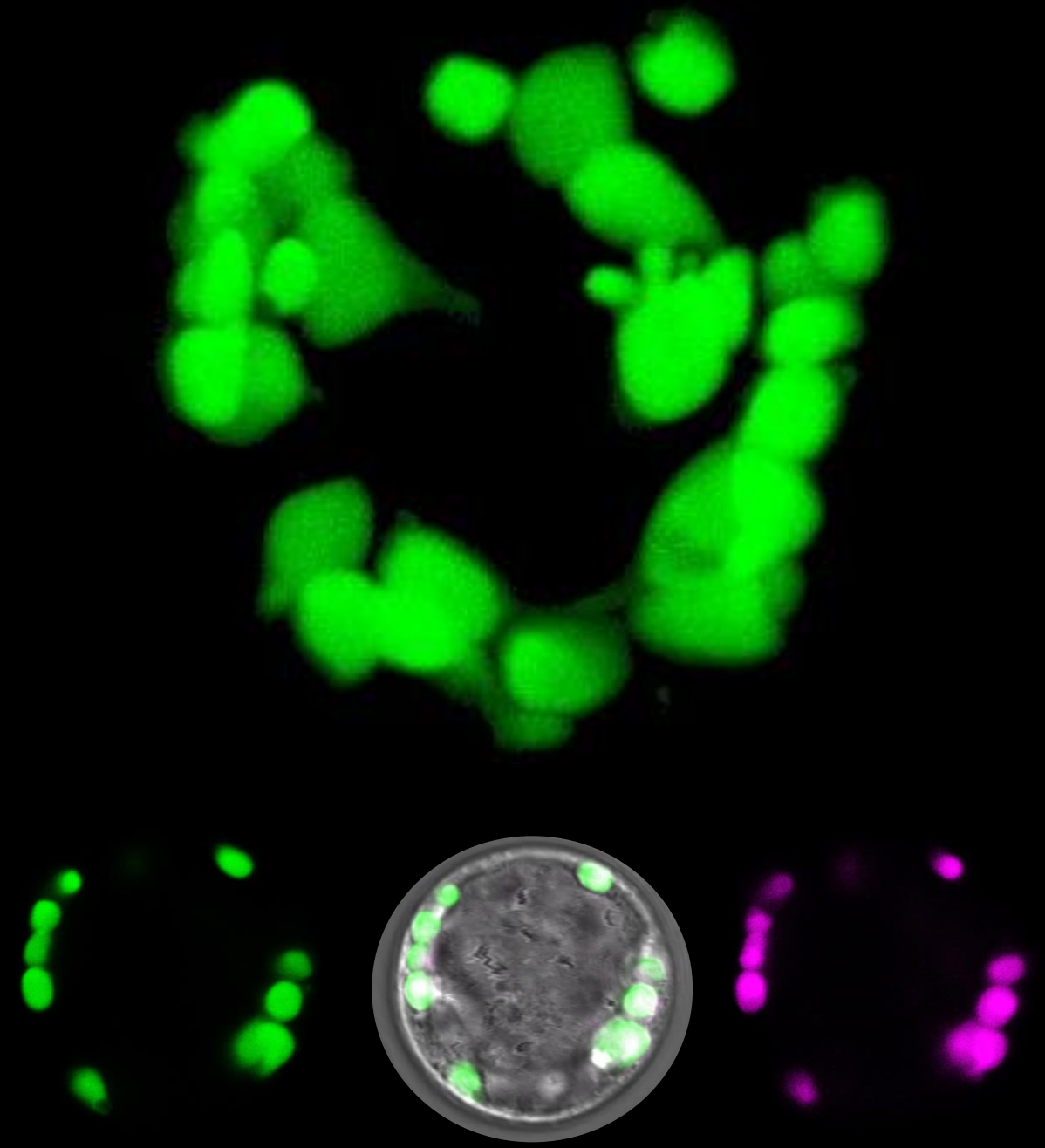


References:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707185/pdf/main.pdf

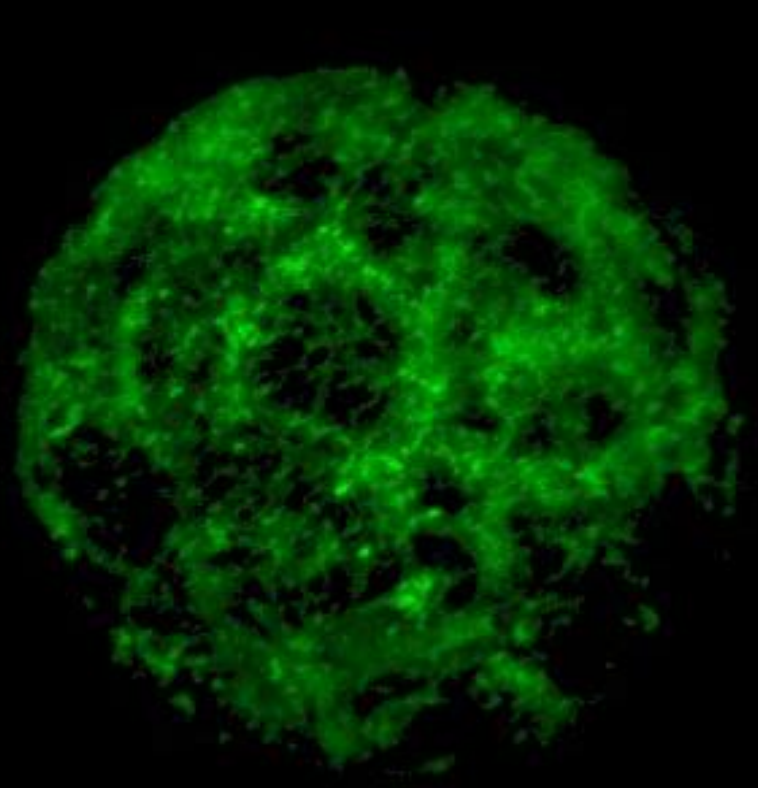
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4091121/

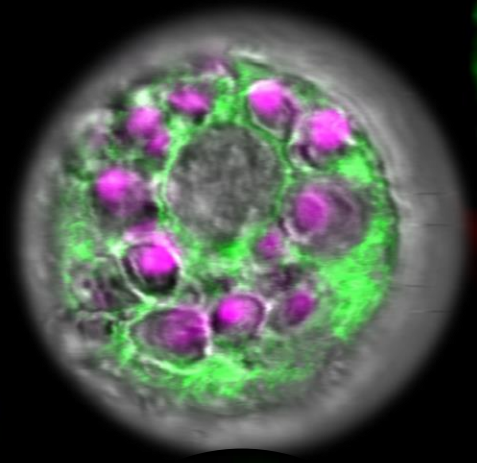
Plastid

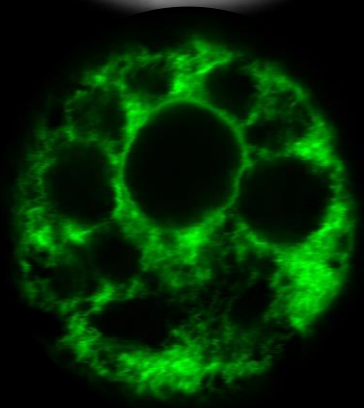


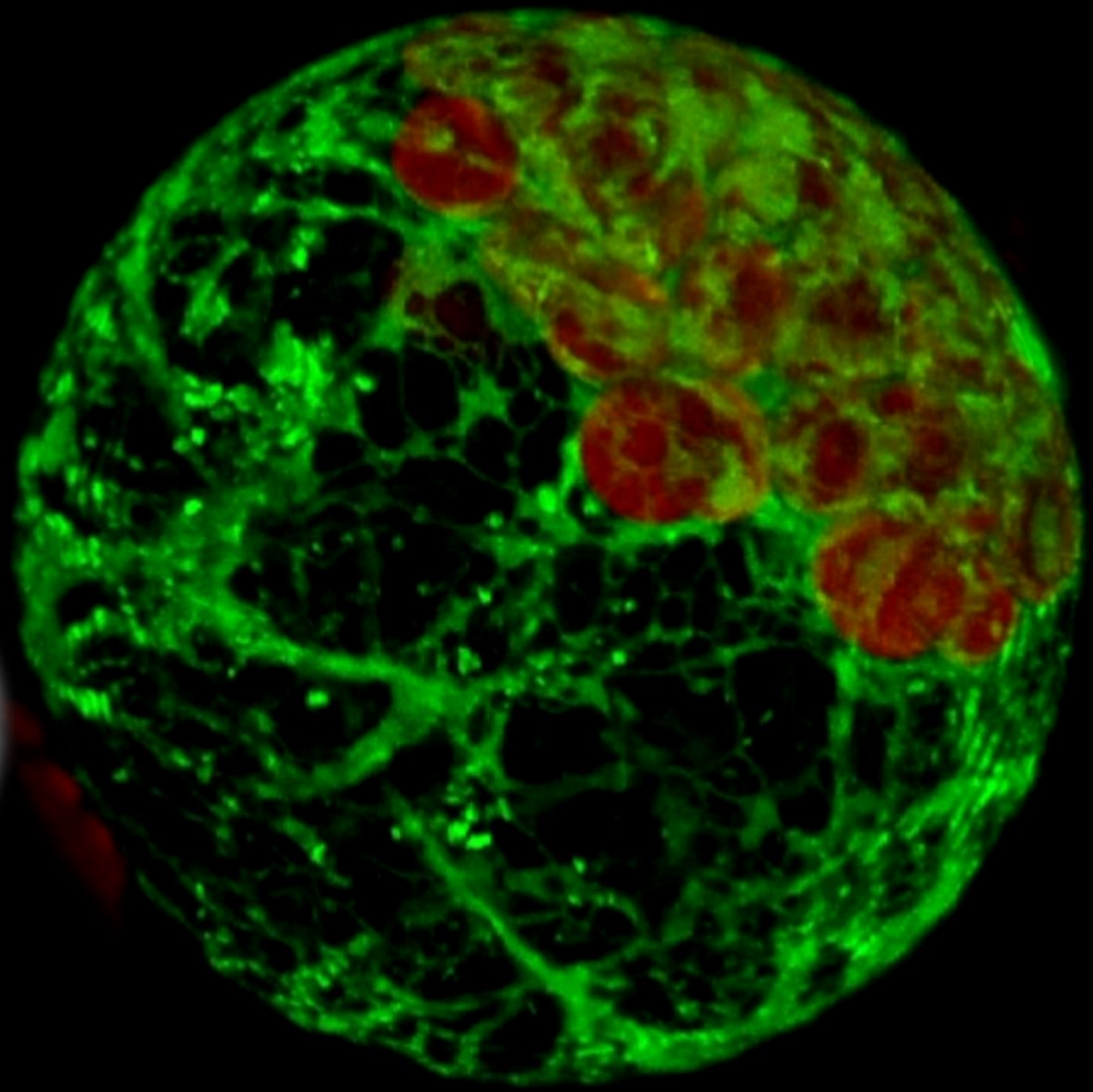


Endoplasmic Reticulum

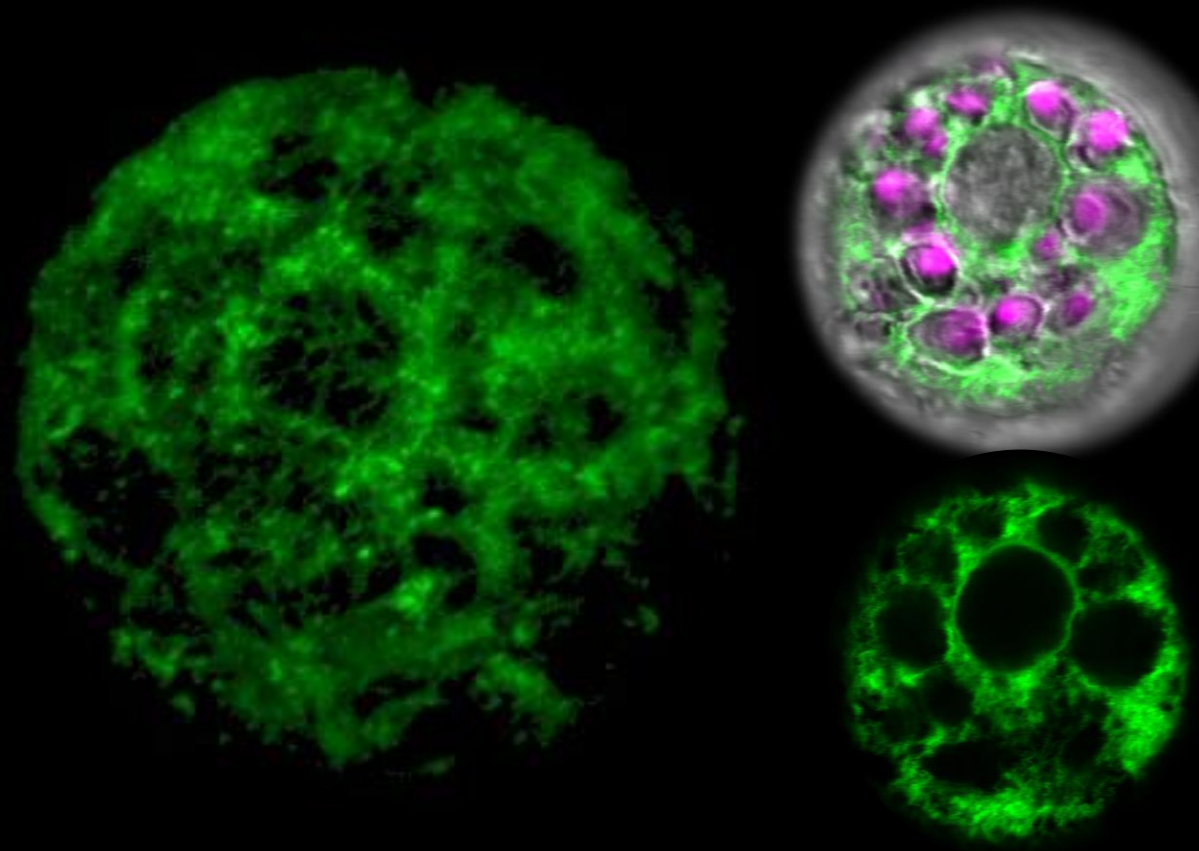


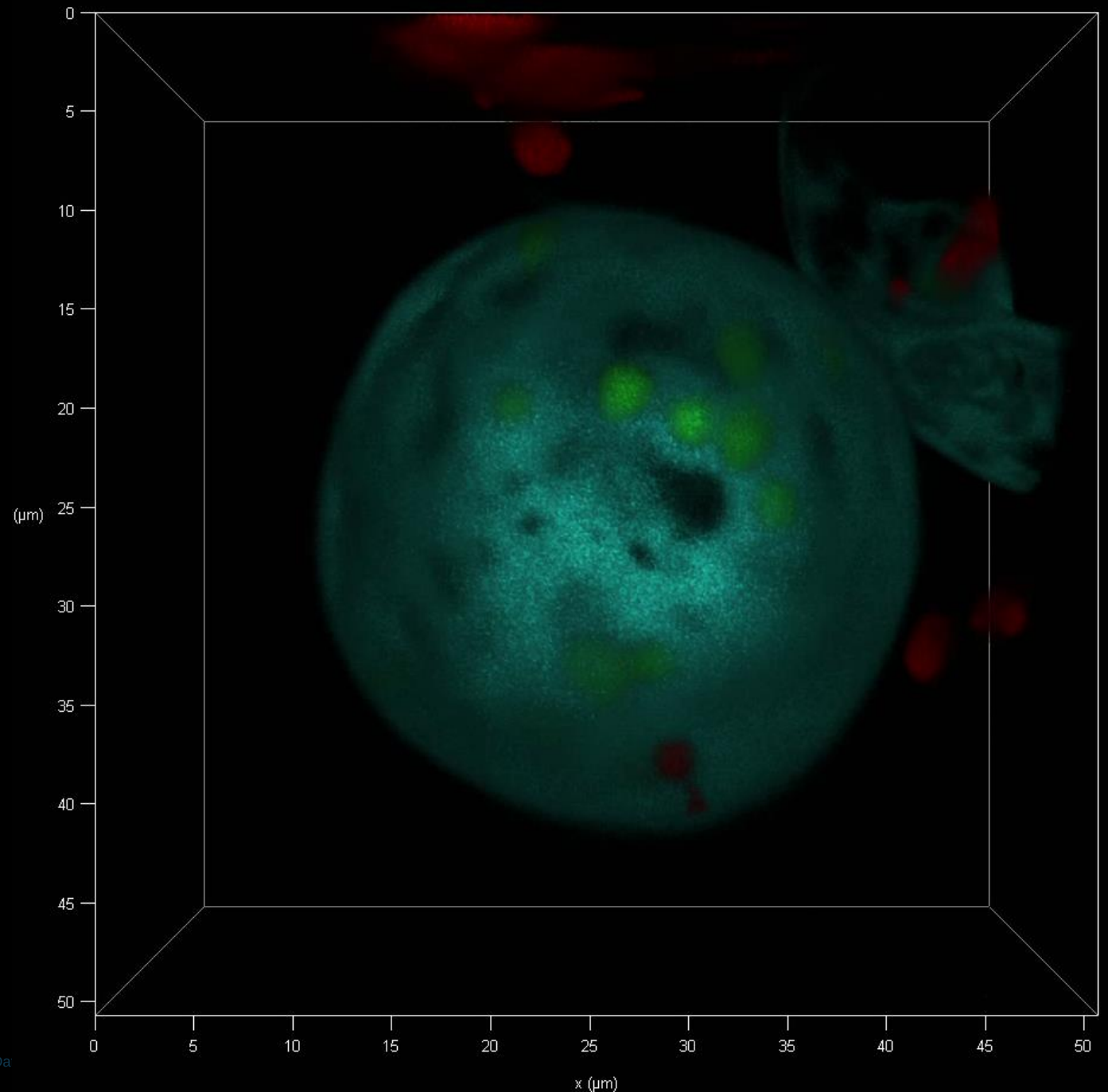






Endoplasmic Reticulum





Cyan – Cytoplasm
Yellow – Nucleus
Green – Chloroplasts
Red – Plastid autofluorescence

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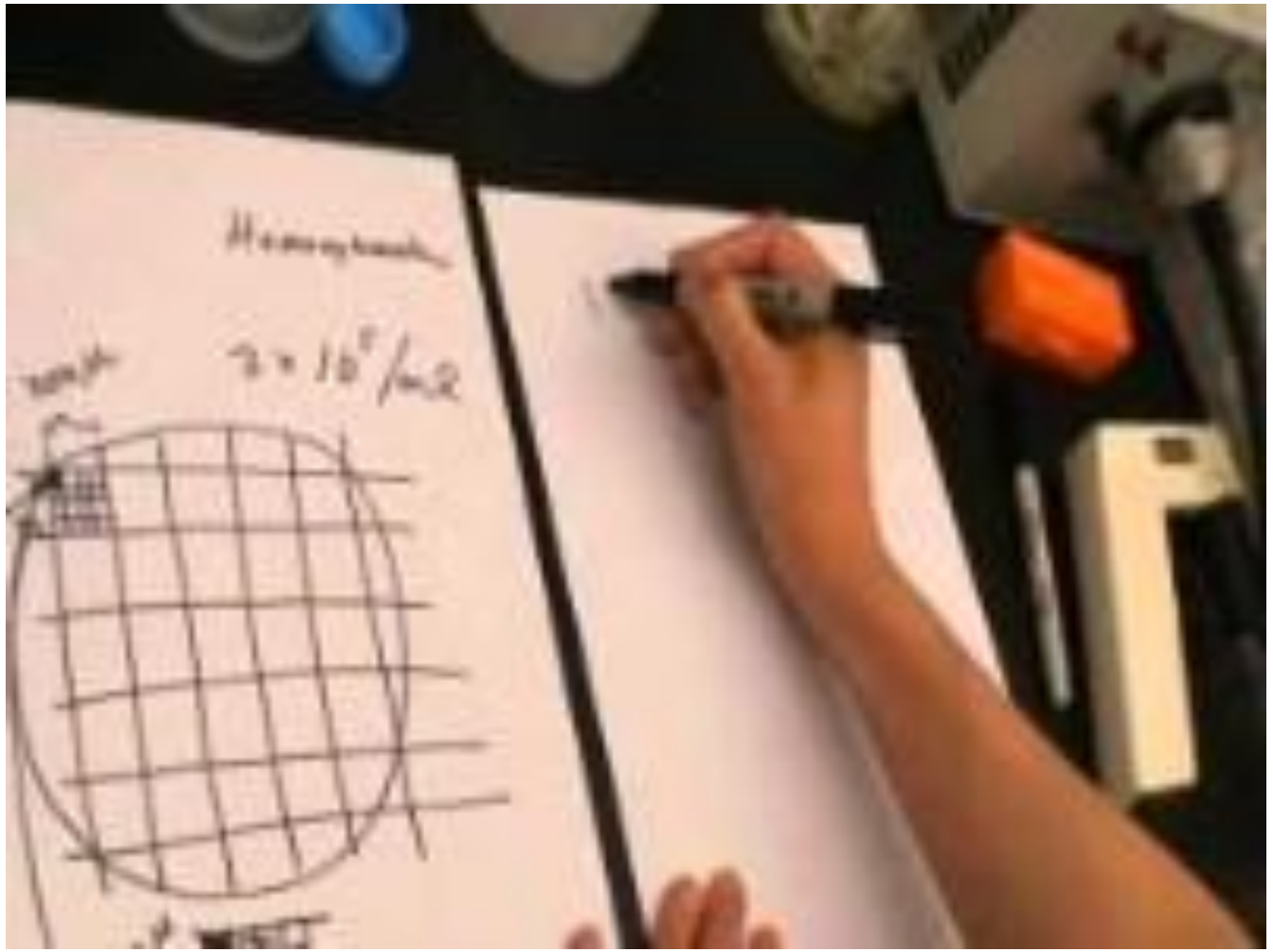
Thank you!

Any questions?

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