webtools

How can we explore the genes involved in **glucosinolate pathway** and **ER-body formation?**

Dedan Githae

Literature: Relationship between glucosinolate pathway and ER-body formation.

Commun Biol. 2020 Jan 14;3(1):21. doi: 10.1038/s42003-019-0739-1.

Endoplasmic reticulum-derived bodies enable a single-cell chemical defense in Brassicaceae plants.

Yamada K[1,2,3], Goto-Yamada S[4,5,6], Nakazaki A[6], Kunieda T[6,7,8], Kuwata K[9], Nagano AJ[10], Nishimura M[11,12], Hara-Nishimura I[13,14].

Author information

Abstract

Brassicaceae plants have a dual-cell type of chemical defense against herbivory. Here, we show a novel single-cell defense involving endoplasmic reticulum (ER)-derived organelles (ER bodies) and the vacuoles. We identify various glucosinolates as endogenous substrates of the ER-body β-glucosidases BGLU23 and BGLU21. Woodlice strongly prefer to eat seedlings of bglu23 bglu21 or a glucosinolate-deficient mutant over wild-type seedlings, confirming that the β-glucosidases have a role in chemical defense: production of toxic compounds upon organellar damage. Deficiency of the Brassicaceae-specific protein NAI2 prevents ER-body formation, which results in a loss of BGLU23 and a loss of resistance to woodlice. Hence, NAI2 that interacts with BGLU23 is essential for sequestering BGLU23 in ER bodies and preventing its degradation. Artificial expression of NAI2 and BGLU23 in non-Brassicaceae plants results in the formation of ER bodies, indicating that acquisition of NAI2 by Brassicaceae plants is a key step in developing their single-cell defense system.

Plant Physiol. 2019 Apr;179(4):1515-1524. doi: 10.1104/pp.18.00984. Epub 2019 Jan 29.

Leaf Endoplasmic Reticulum Bodies Identified in Arabidopsis Rosette Leaves Are Involved in Defense against Herbivory.

Nakazaki A[1], Yamada K[2], Kunieda T[3], Sugiyama R[4], Hirai MY[4], Tamura K[1], Hara-Nishimura I[3], Shimada T[5].

Author information

Abstract

ER bodies are endoplasmic reticulum (ER)-derived organelles specific to the order Brassicales and are thought to function in plant defense against insects and pathogens. ER bodies are generally classified into two types: constitutive ER bodies in the epidermal cells of wound-inducible ER bodies in rosette leaves. Herein, we reveal a third type of ER Arabidopsis (*Arabidopsis thaliana*) rosette leaves and designate them "leaf ERboc bodies). L-ER bodies constitutively occurred in specific cells of the rosette leaves: epidermal cells covering the midrib, and giant pavement cells. The distribution of L was closely associated with the expression profile of the basic helix-loop-helix trar NAI1, which is responsible for constitutive ER-body formation. L-ER bodies were observed in *nai1* mutant leaves, indicating that NAI1 is involved in L-ER body form Confocal imaging analysis revealed that L-ER bodies accumulated two types of β- PYK10, the constitutive ER-body β-glucosidase; and BETA-GLUCOSIDASE18 (B wound-inducible ER-body β-glucosidase. Combined with the absence of L-ER boc *bglu18 pyk10* mutant, these results indicate that BGLU18 and PYK10 are the majo of L-ER bodies. A subsequent feeding assay with the terrestrial isopod *Armadillidi* revealed that *bglu18 pyk10* leaves were severely damaged as a result of herbivor the *bglu18 pyk10* mutant was defective in the hydrolysis of 4-methoxyindol-3-ylme glucosinolate These results suggest that L-ER bodies are involved in the productic compound(s) from 4-methoxyindol-3-ylmethyl glucosinolate that protect Arabidops against herbivory attack.

Plant J. 2017 Jan;89(2):204-220. doi: 10.1111/tpj.13377. Epub 2016 Dec 19.

PYK10 myrosinase reveals a functional coordination between endoplasmic reticulum bodies and glucosinolates in Arabidopsis thaliana.

Nakano RT[1,2,3], Piślewska-Bednarek M[4], Yamada K[5], Edger PP[6], Miyahara M[3], Kondo M[5], Böttcher C[7], Mori M[8], Nishimura M[5], Schulze-Lefert P[1,2], Hara-Nishimura I[3], Bednarek P[4].

Author information

Abstract

The endoplasmic reticulum body (ER body) is an organelle derived from the ER that occurs in only three families of the order Brassicales and is suggested to be involved in plant defense. ER bodies in Arabidopsis thaliana contain large amounts of β-glucosidases, but the physiological functions of ER bodies and these enzymes remain largely unclear. Here we show that PYK10, the most abundant β-glucosidase in A. thaliana root ER bodies, hydrolyzes indole glucosinolates (IGs) in addition to the previously reported in vitro substrate scopolin. We found a striking co-expression between ER body-related genes (including PYK10), glucosinolate biosynthetic genes and the genes for so-called specifier proteins affecting the terminal products of myrosinase-mediated glucosinolate metabolism, indicating that these systems have been integrated into a common transcriptional network. Consistent with this, comparative metabolite profiling utilizing a number of A. thaliana relatives within Brassicaceae identified a clear phylogenetic co-occurrence between ER bodies and IGs, but not between ER bodies and scopolin. Collectively, our findings suggest a functional link between ER bodies and glucosinolate metabolism in planta. In addition, in silico three-dimensional modeling, combined with phylogenomic analysis, suggests that PYK10 represents a clade of 16 myrosinases that arose independently from the other well-documented class of six thioglucoside glucohydrolases. These findings provide deeper insights

Yamada K, Goto-Yamada S, Nakazaki A, et al. Endoplasmic reticulum-derived bodies enable a single-cell chemical defense in Brassicaceae plants. Commun Biol. 2020;3(1):21. Published 2020 Jan 14. doi:10.1038/s42003-019-0739-1

Akiko Nakazaki, Kenji Yamada, Tadashi Kunieda, Ryosuke Sugiyama, Masami Yokota Hirai, Kentaro Tamura, Ikuko Hara-Nishimura, Tomoo Shimada. Restricted Access Leaf Endoplasmic Reticulum Bodies Identified in Arabidopsis Rosette Leaves Are Involved in Defense against Herbivory. Plant Physiology Apr 2019, 179 (4) 1515-1524; DOI: 10.1104/pp.18.00984

Nakano, R. T., Piślewska-Bednarek, M., Yamada, K., Edger, P. P., Miyahara, M., Kondo, M., Böttcher, C., Mori, M., Nishimura, M., Schulze-Lefert, P., Hara-Nishimura, I., & Bednarek, P. (2017). PYK10 myrosinase reveals a functional coordination between endoplasmic reticulum bodies and glucosinolates in Arabidopsis thaliana. Plant Journal. https://doi.org/10.1111/tpj.13377

Gene Ontology (GO):
Vocabulary; Annotation & Tools for easy access to all aspects of Data

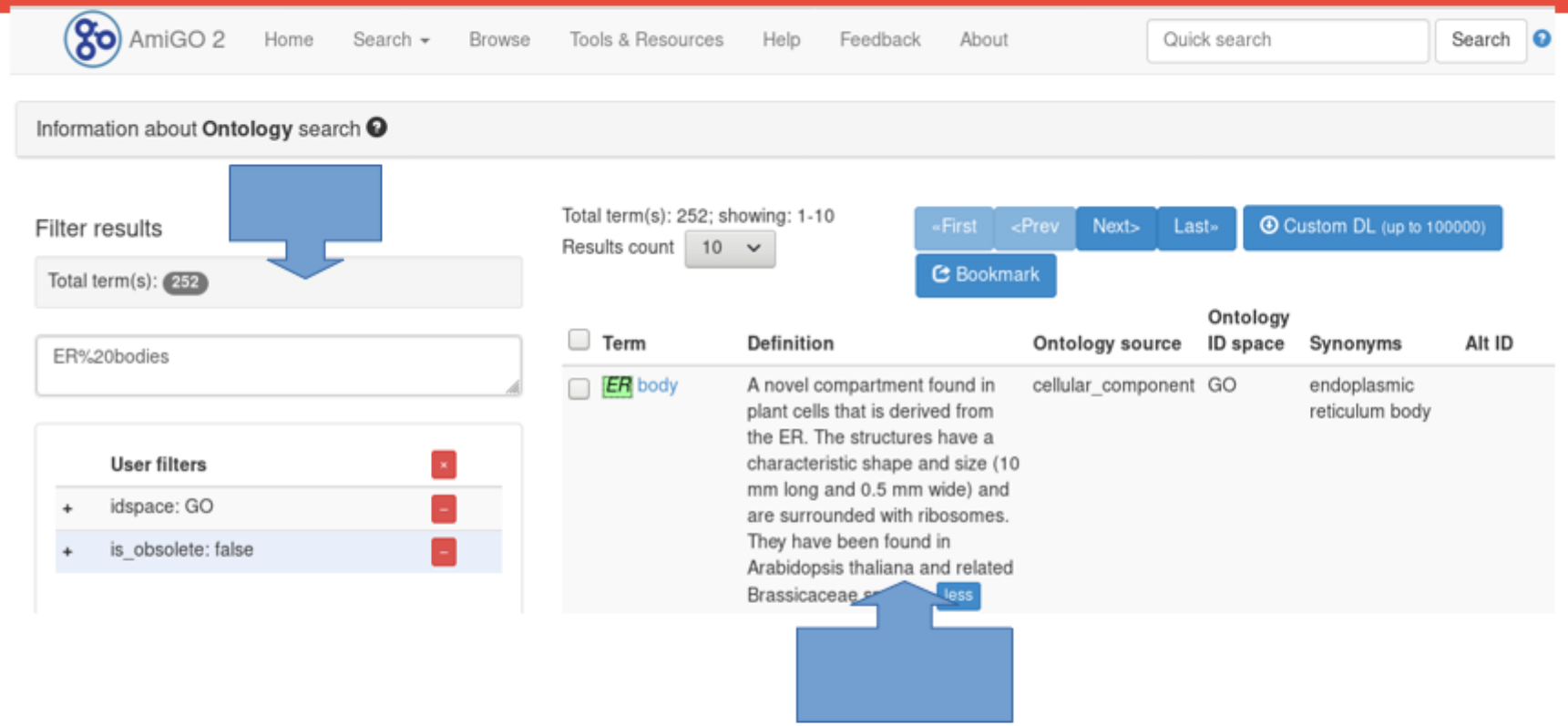
Ontologies usually consist of a set of classes (or terms or concepts) with relations that operate between them.

Molecular function: Molecular-level activities performed by gene products. Molecular function terms describe activities that occur at the molecular level, such as “catalysis” or “transport”.

Cellular component: The locations relative to cellular structures in which a gene product performs a function, either cellular compartments (e.g., mitochondrion), or stable macromolecular complexes of which they are parts (e.g., the ribosome).

Biological process: The larger processes, or ‘biological programs’ accomplished by multiple molecular activities e.g. of broad biological process terms are DNA repair or signal transduction

Gene Ontology (GO): Searching gene of interest for information and annotation



Searching for information on a gene of interest in the AMIGO database

KEGG: (Kyoto Encyclopedia of Genes and Genomes) Cross linked from Gene Ontology.

Links to cross-references files derived the Gene Ontology

Mapping

Enzyme Commission (EC) enzyme numbers
Enzyme Commission; contact: GO Ontology editors
Constructed and maintained in the GO ontology file by GO editorial staff
Citation: Hill DP, Davis AP, Richardson JE, Corradi JP, Ringwald M, Eppig JT, Blake JA. Program description: Strategies fo annotation of mammalian systems: implementing gene ontologies in mouse genome informatics. Genomics. May 200 [PMID:11374909 | doi:10.1006/geno.2001.6513]

KEGG pathways and reactions
Kyoto Encyclopaedia of Genes and Genomes
Constructed and maintained by Amelia Ireland and a script

MetaCyc pathways and reactions
MetaCyc; contact: GO Ontology editors
Constructed and maintained in the GO ontology file by GO editorial staff

MIPS FunCat
MIPS Functional Catalogue (FunCat)
Constructed by Michael Ashburner and Midori Harris

Reactome events and catalyst activities
Reactome
Constructed by Reactome curators and maintained in the GO ontology file by GO editorial staff

Rhea Annotated Reactions Database
Rhea
Constructed and maintained by Amelia Ireland and a script

EAWAG-BBD enzyme IDs
Swiss Federal Institute of Aquatic Science and Technology Biocatalysis/Biodegradation Database (EAWAG-BBD); contac GO Ontology editors
Maintained in the GO ontology file by GO editorial staff

Gene Ontology DB is crosslinked to other databases- eg enzyme classification; Metabolic Pathways etc

The Database is curated (creation of annotations on the basis of the data (for example data about gene products) contained in experimental reports, primarily as contained in the scientific literature published on the basis of the observation of corresponding instances.)

Following the link to the KEGG PATHWAY:

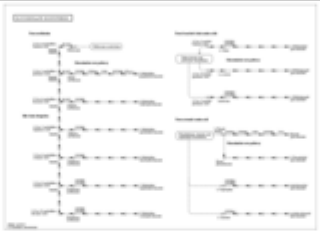
KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.

KEGG Database

Pathway Text Search

Number of entries in a page 20 Hide thumbnail

Items : 1 - 7 of 7

Entry	Thumbnail Image	Name	Description	Object	Legend
map00966		Glucosinolate biosynthesis	Glucosinolates are biologically active secondary metabolites found in Brassicaceae (mustard family) ...	...ucolesquerellin) C17252 (7-Methylthioheptyl glucosinolate) C17254 (8-Methylthiooctyl glucosinolate) ...	CYP79F1 CYP83A1 SUR1 UGT74B1 GLUCOSINOLATE BIOSYNTHESIS Methionine 2-Oxo-4-methylthio-butanoic acid...

KEGG

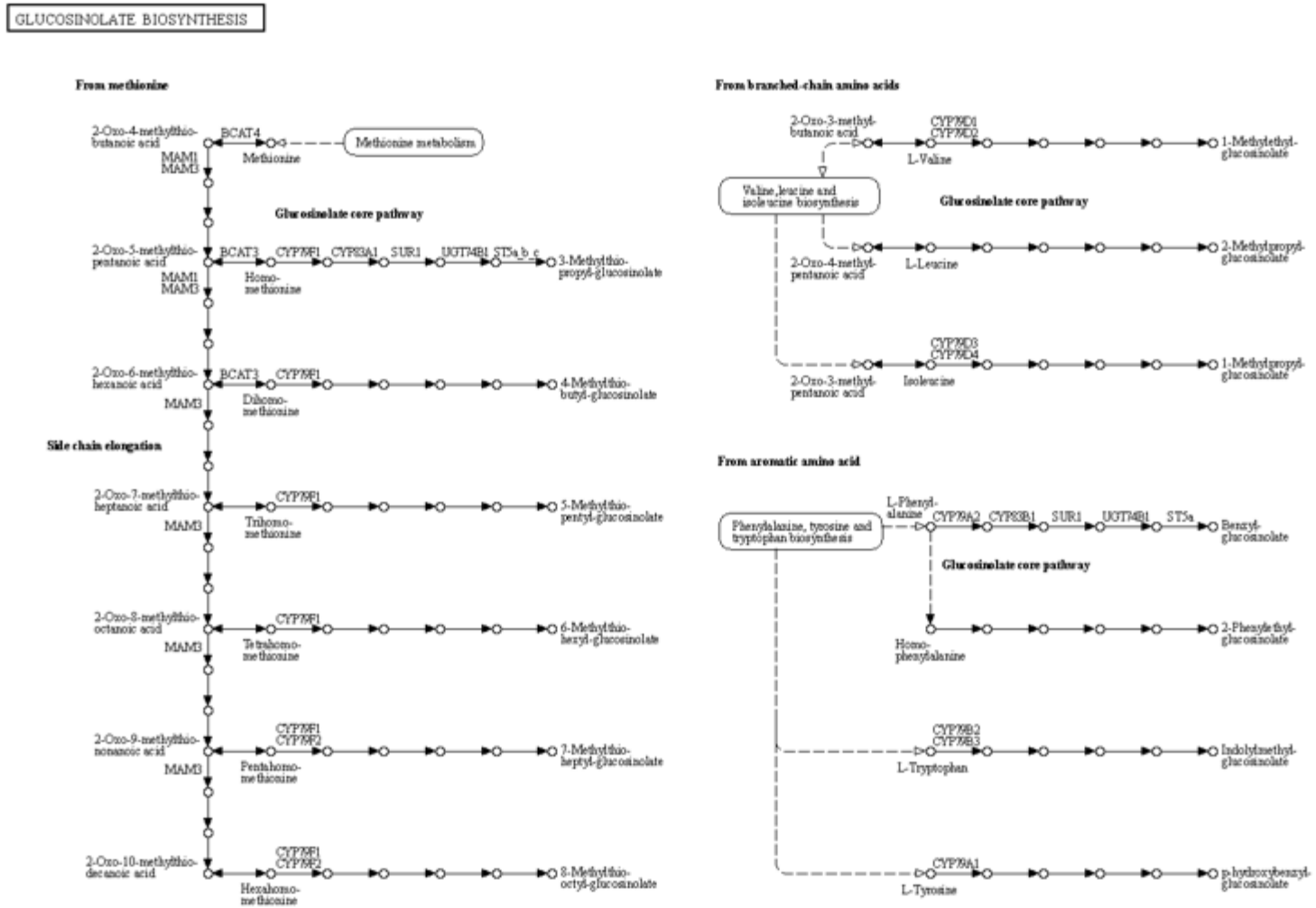
PATHWAY: map00966

Help

Entry	map00966 Pathway
Name	Glucosinolate biosynthesis
Description	Glucosinolates are biologically active secondary metabolites found in Brassicaceae (mustard family) and related families.These compounds are genetically variable within plant species and used as natural pesticides, such as against insect herbivores. All glucosinolates share a common structure consisting of a beta-thioglucose moiety, a sulfonated oxime moiety, and a variable aglycone side chain derived from an alpha-amino acid. Genes encoding glucosinolate biosynthetic enzymes have been identified in Arabidopsis thaliana by genetic polymorphisms and loss-of-function mutations. This map shows examples of side chain elongation in methionine-derived glucosinolates and the core pathway for biosynthesis of glucosinolates from amino acids.
Class	Metabolism; Biosynthesis of other secondary metabolites BRITE hierarchy
Pathway map	map00966 Glucosinolate biosynthesis

KEGG Pathway:

Vocabulary; Annotation & Tools for easy access to all aspects of Data



„Plant Omics” and statistics

Introduction to Bioinformatics in the plant world

Statistics in world of Molecular Biology

Multiple Hypothesis Testing and other signs that You might be addicted to p-value

Statistics used in Molecular Biology

Multiple hypothesis testing

- Simultaneous set of statistical inferences
- „Matters” in genomics and biological sciences in terms of speed of inference
- Adjustment of p-values for *m* hypothesis tests - controlling the Type I error rate
- Logarithmic grwoth of probability of False Positive discovery

Comparative statistics

- Often used in Biostatistics instead of multiple hypothesis testing
- Much more resistant to p-value hacking
- Treats every pair of hypothesis testing as stand alone example
- Post-hoc comparison of differences between tests – Honest Significant Differences e.g ***Tukey test***
- **Part of GCP for Biostatisticians**

Raised concern – Why do we still use significance testing?**

- You know before doing an experiment that there must be *some* difference that would show up given enough data. [1]
- Comparisons of hypotheses should be conditional on the data. There is a possibility that data was unlikely under null and alternative hypothesis for small p-values. [2]
- The most important question for science is the size of an effect, not whether the effect exists. [3]
- Statistical error (Type I Error) is only one component of real error, maybe a small component. Variability of data goes up with conduction of multiple experiments [4]
- Small *p*-values do not mean small probability of being wrong. In one review, 74% of studies with *p*-value 0.05 were found to be wrong. [5]

**https://www.researchgate.net/publication/317281157_P-value_and_Reduction_to_Absurdity

Sources

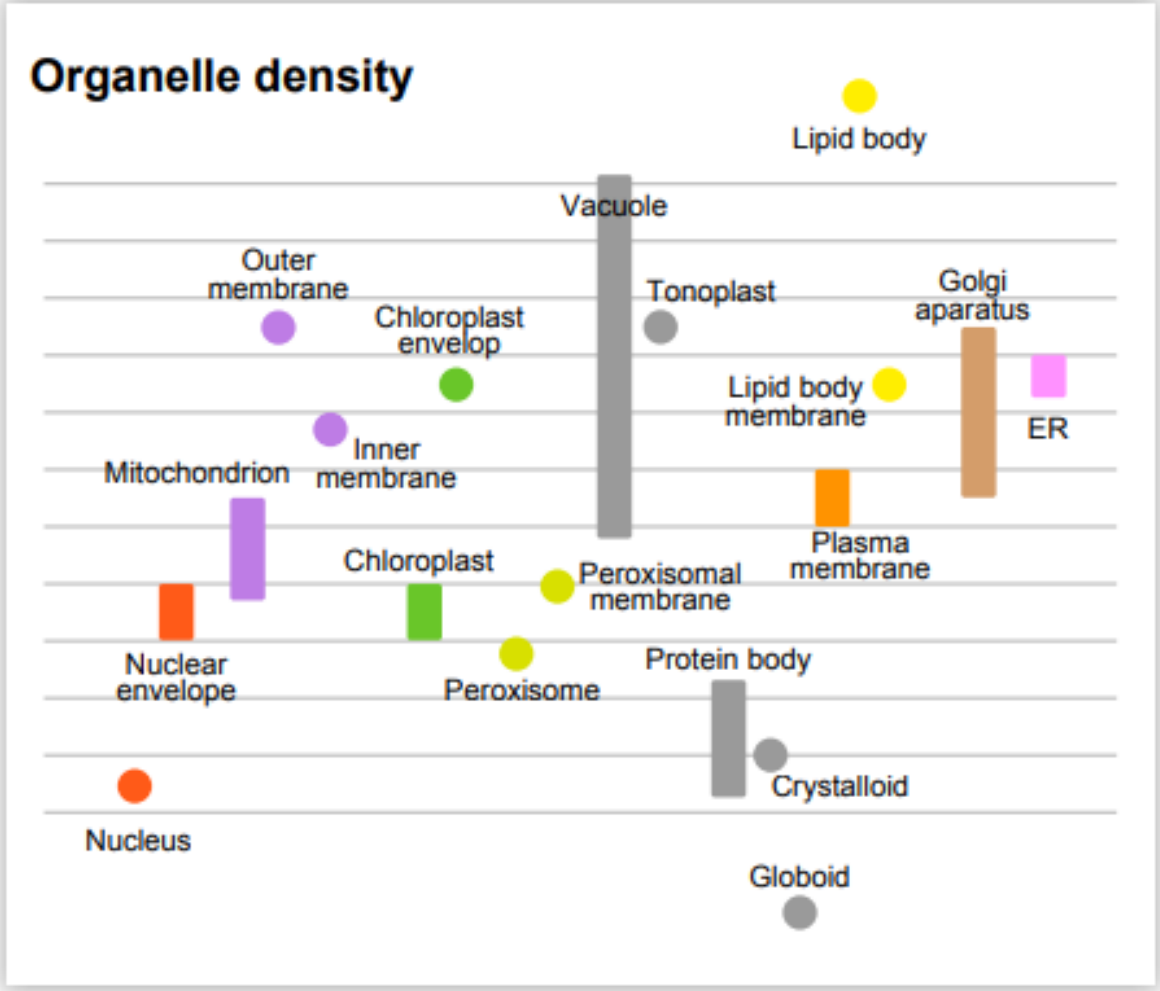
- [1] http://www.stat.columbia.edu/~cook/movabletype/archives/2004/12/type_1_type_2_t.html
- [2] http://www.stat.duke.edu/~berger/p-values.html
- [3] https://www.amazon.com/gp/product/0472050079/ref=as_li_ss_tl?ie=UTF8&linkCode=sl1&tag=theende-20&linkId=166f8beb56b9ec79d8ee911fe051fc9b&language=en_US
- [4] William Gosset (a.k.a *Student*) one of his conclusions about multiple hypothesis testing
- [5] https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0020124

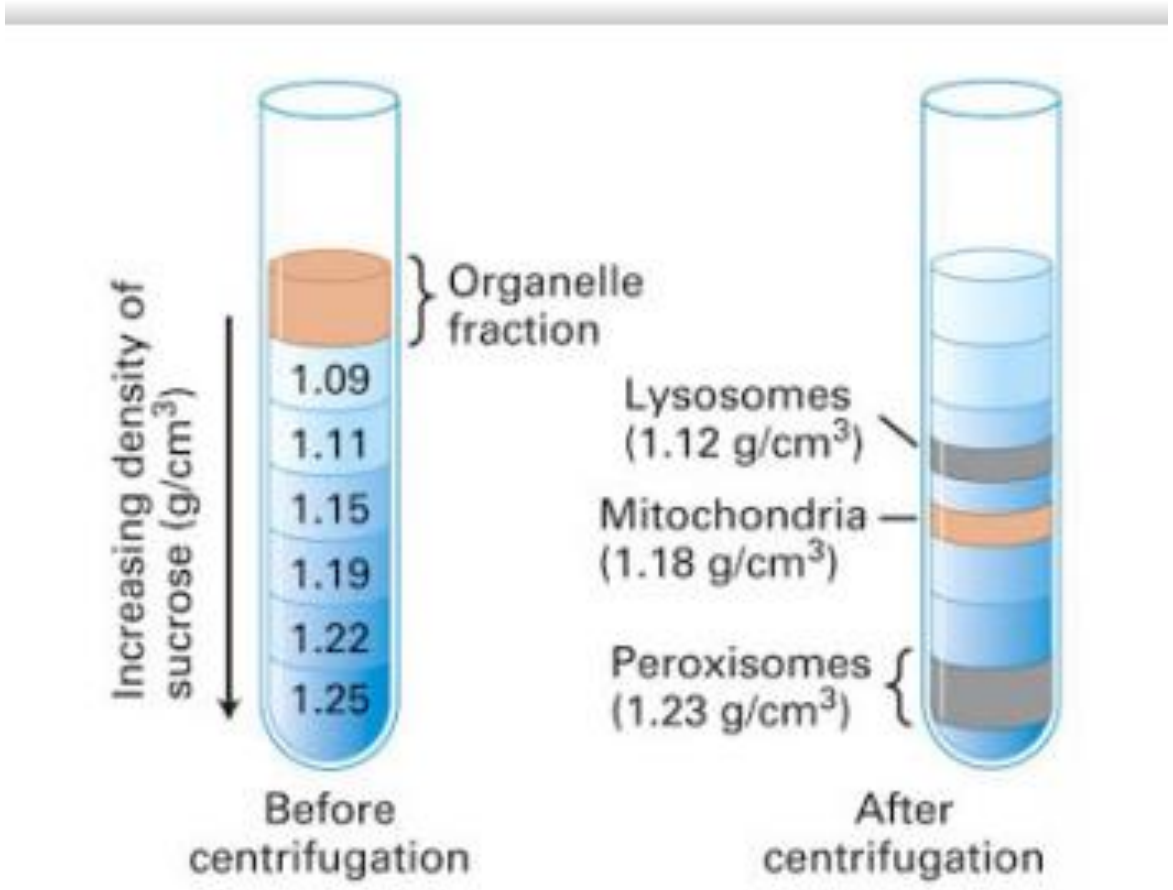
Raised question – What are the possible alternatives for NHST?

- Statistical tests based on Bayesian Theorem
- Cummulative statistical testing (e.g Mean Effect Size)
- Intuitive statistical testing (e.g Jaccard Distance)
- Bayesian and Non-Bayesian statistical inference (Machine Learning)
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1473027/

Plant organelle fractionation by density gradient centrifugation

Tutor: Shono Goto-Yamada and her collegue





Plant organelle fractionation by density gradient centrifugation

1. Sample preparation :

homogenization of tissue in chopping buffer

Cell strainer

→ **S** total fraction (for SDS PAGE)

Centrifugation 1500 g 4 C, 10 minutes – <u>debris and chloroplast sedimentation</u>

re-suspention of ppt → **S**

SUPERNATANT

S

Ultracentrifuge 100 000 g, 4 C, 20 minutes

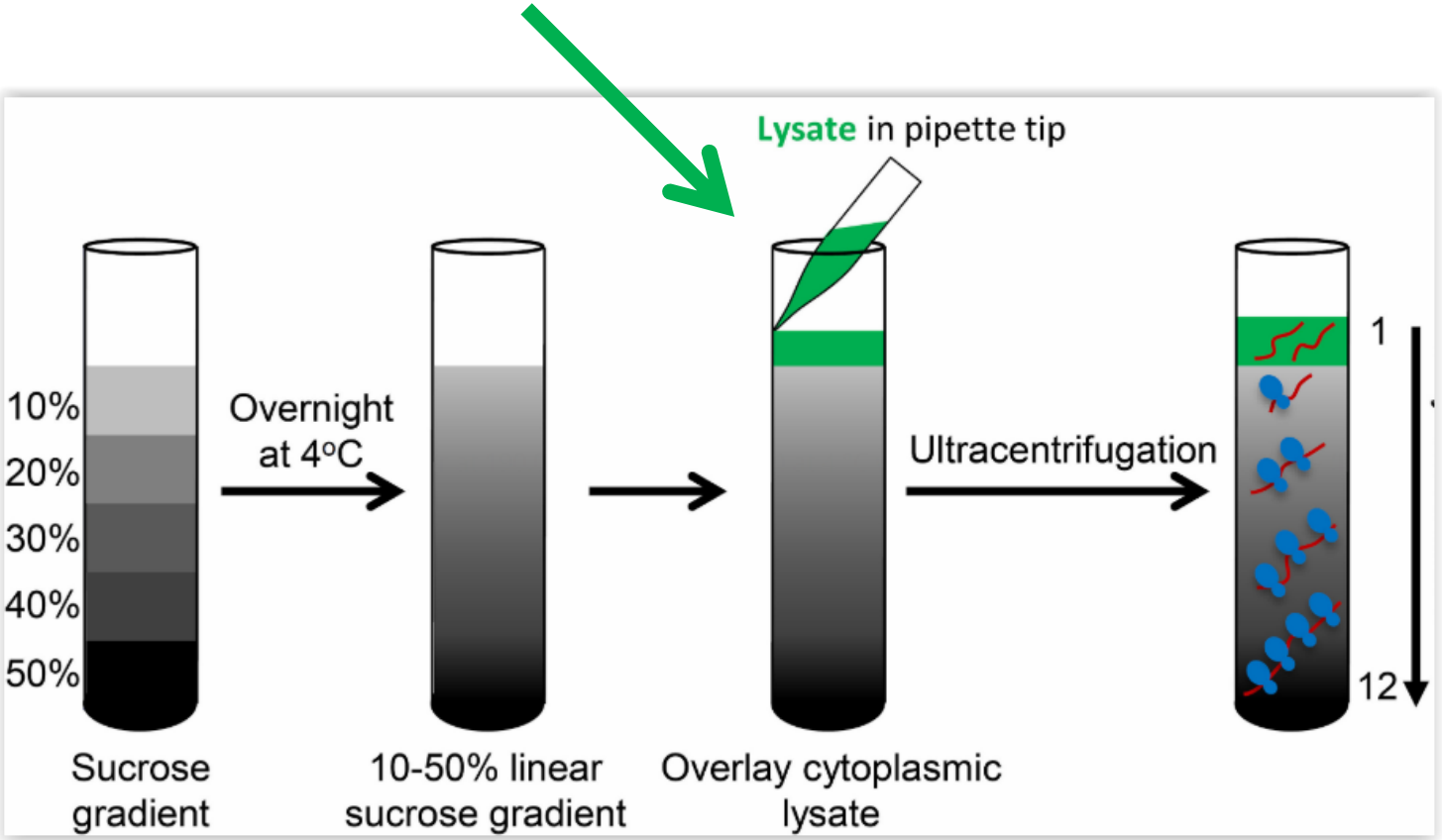
supernatant

PPT re-suspention →

applaying sample on the column

Plant organelle fractionation by density gradient centrifugation

2.Applying the sample on the top of 30-60% sucrose gradient



Ultracentrifugation
100 000 g, 4 C**, 2 hrs**

Plant organelle fractionation by density gradient centrifugation

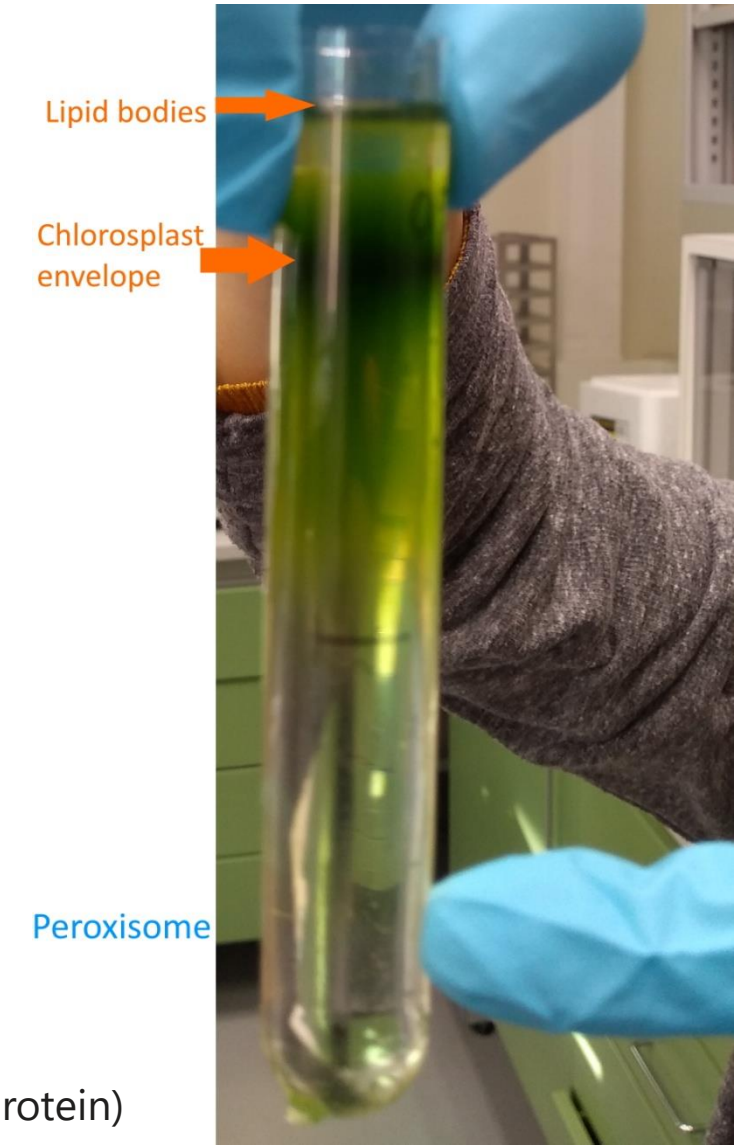
Fractionation on Piston Gradient Fractionator (BIOCOMP)

Collecting fractions in eppendorf tubes

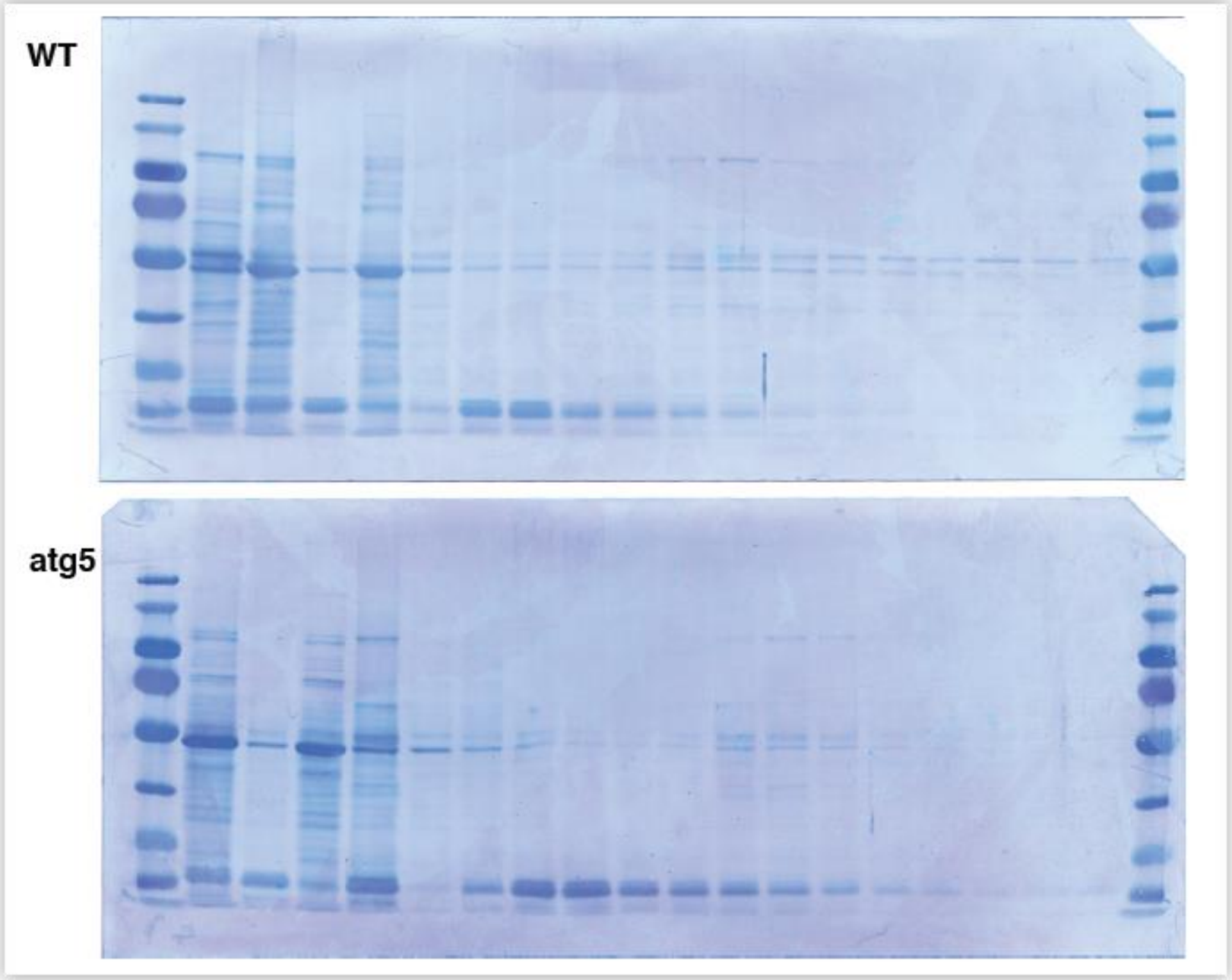
SDS PAGE

Western Blot

the antibody is anti-VDAC1 (mitochondrial membrane protein)

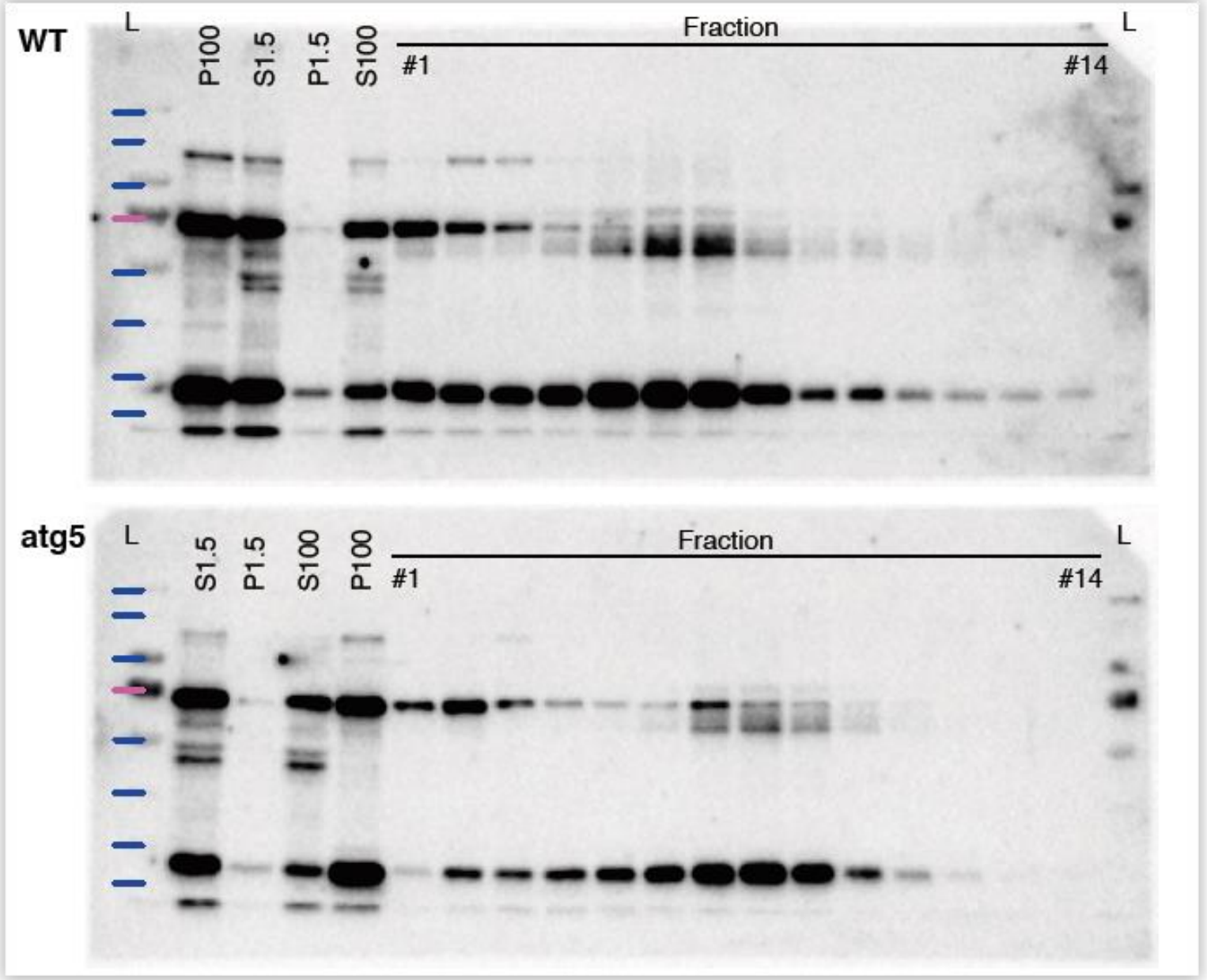


Plant organelle fractionation by density gradient centrifugation



SDS PAGE

Plant organelle fractionation by density gradient centrifugation



10% acryl amide gel
1st Ab: anti-VDAC1 (mitochondria), 1:4000 dilution (4°C, o/n)
2nd Ab: anti-rabbit-HRP, 1:4000 (RT, 40 min)

Western blot

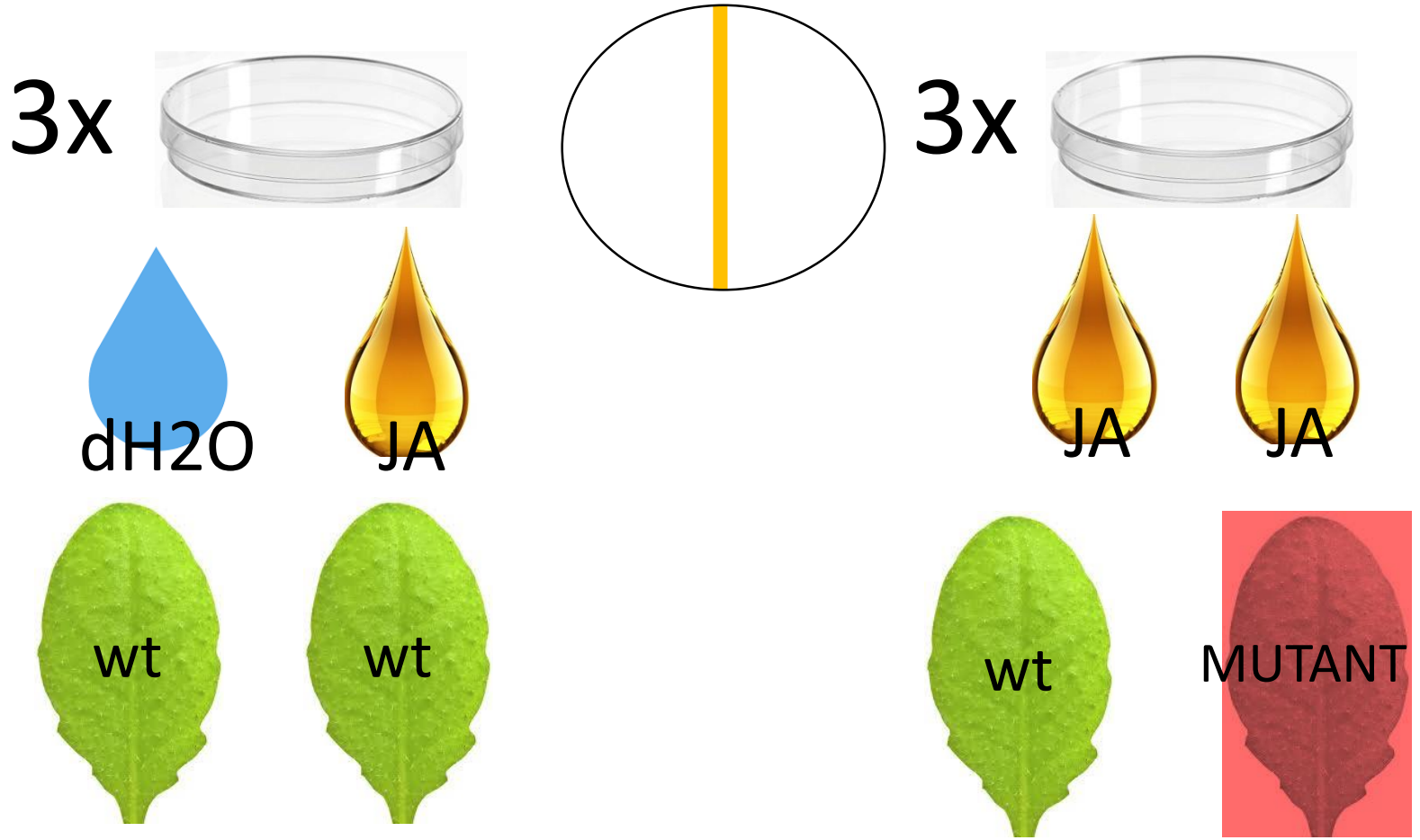
Plant organelle fractionation by density gradient centrifugation

Pill bug experiment
– feeding experiment on woodlouse

Tutor: Kaichiro Endo

Pill bug experiment – feeding experiment on woodlouse

1.



Pill bug experiment – feeding experiment on woodlouse

starved for 3 days woodlouse

1.

3x

dH2O

JA

wt

wt

incubated overnight

3x

JA

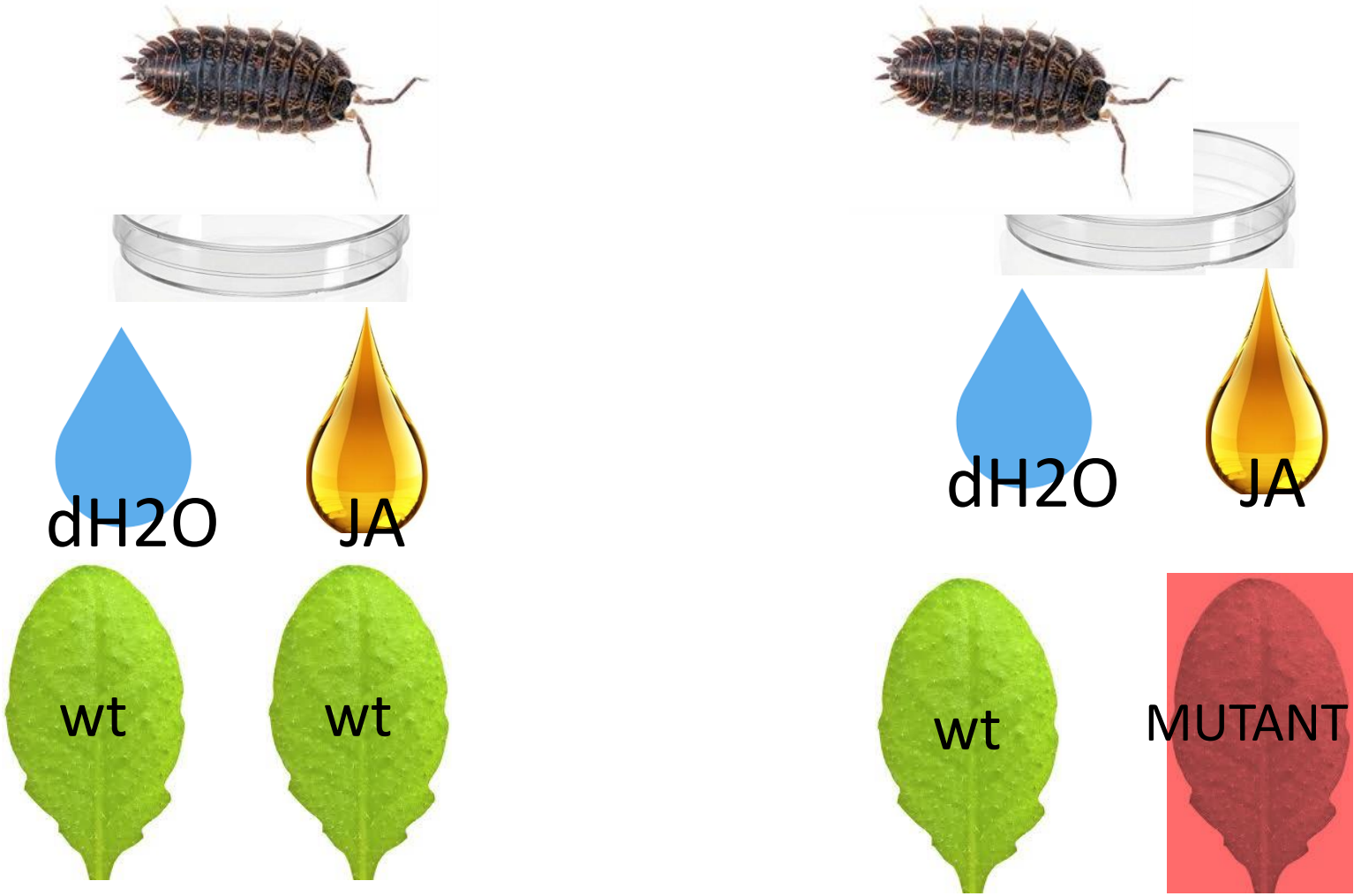
JA

wt

MUTANT

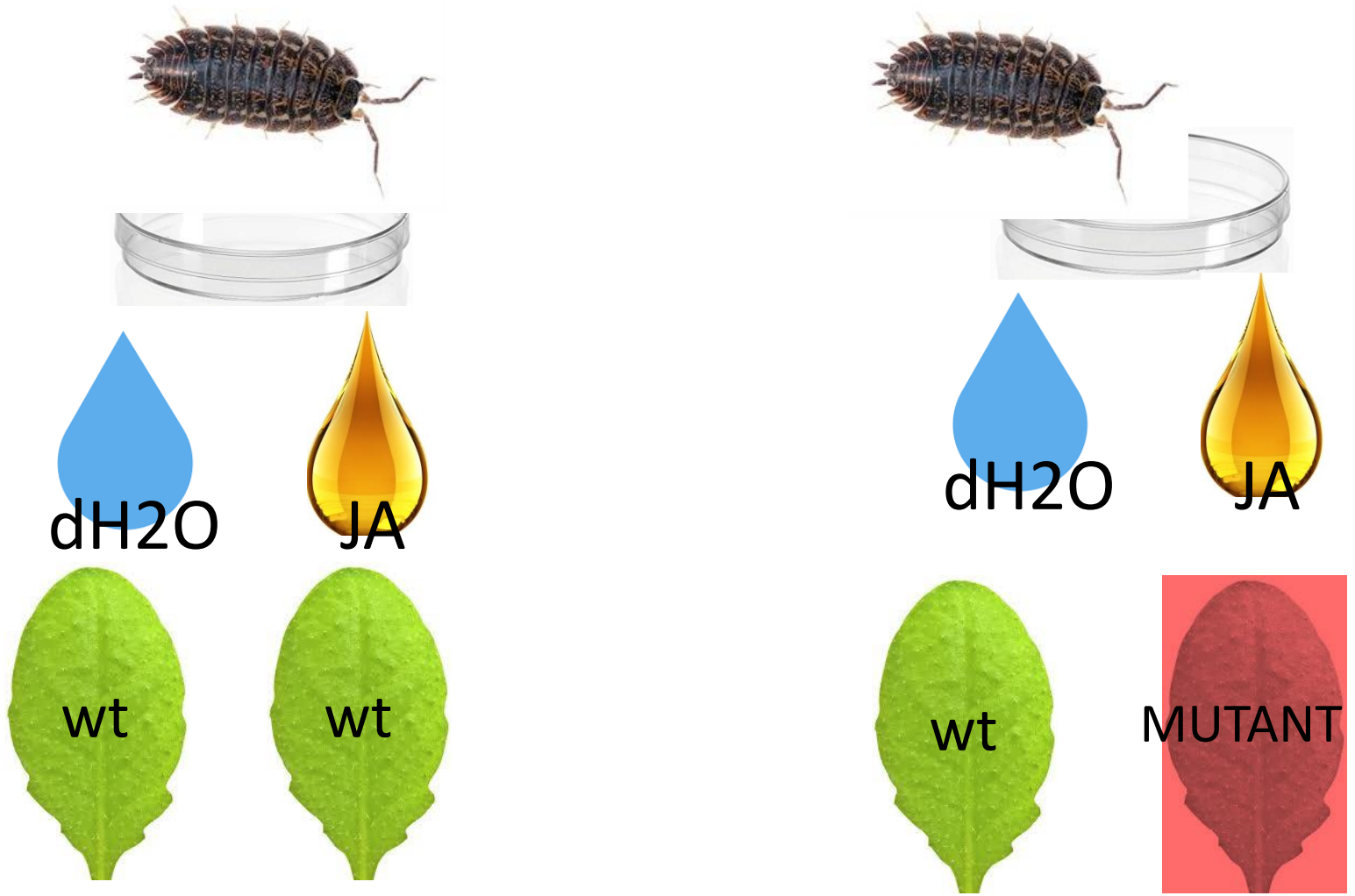
Pill bug experiment – feeding experiment on woodlouse

2.



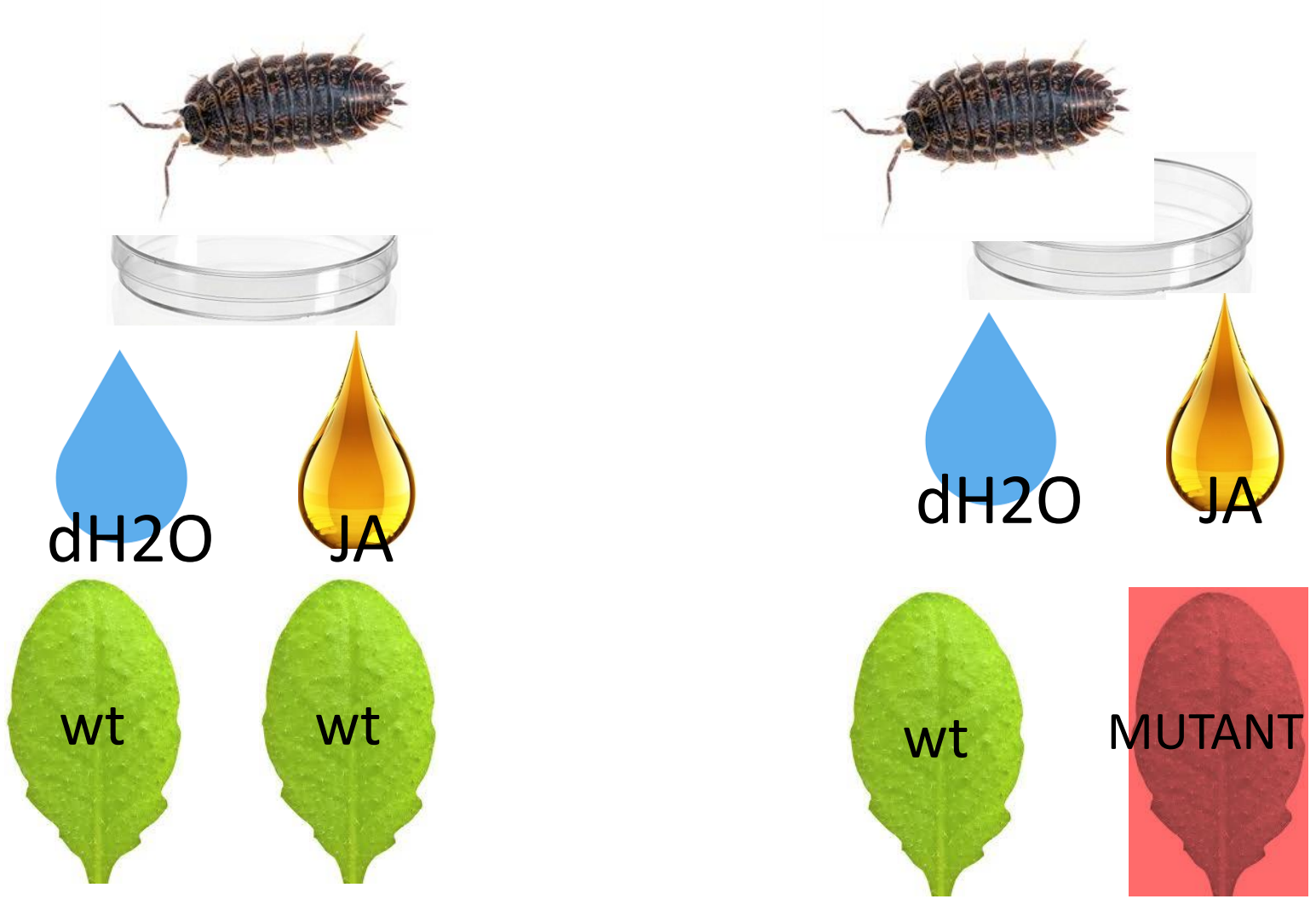
Pill bug experiment – feeding experiment on woodlouse

2.



Pill bug experiment – feeding experiment on woodlouse

2.

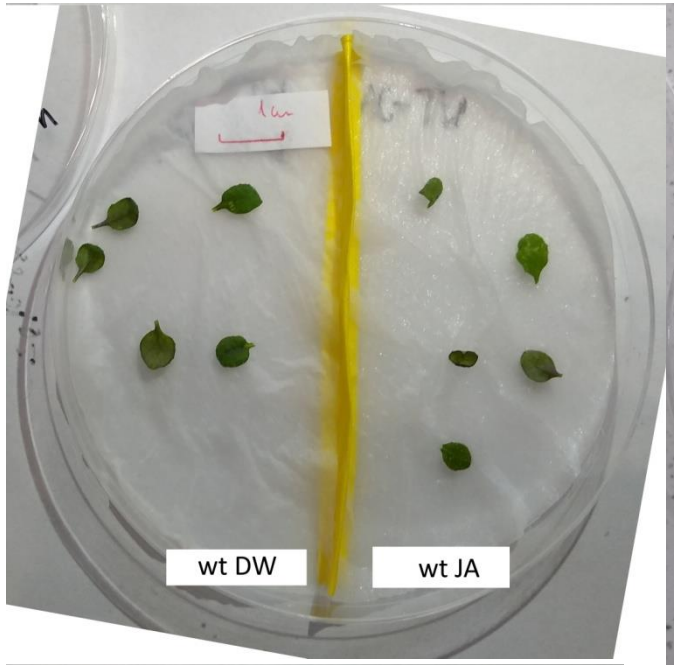


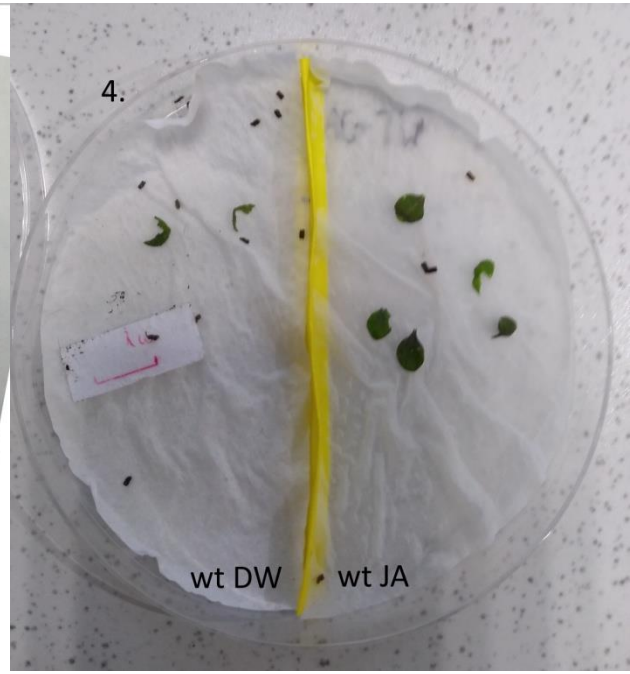
Pill bug experiment – feeding experiment on woodlouse

1. Calculate the surface area of leaves before and after treatment for every repilcate using IMAGEJ

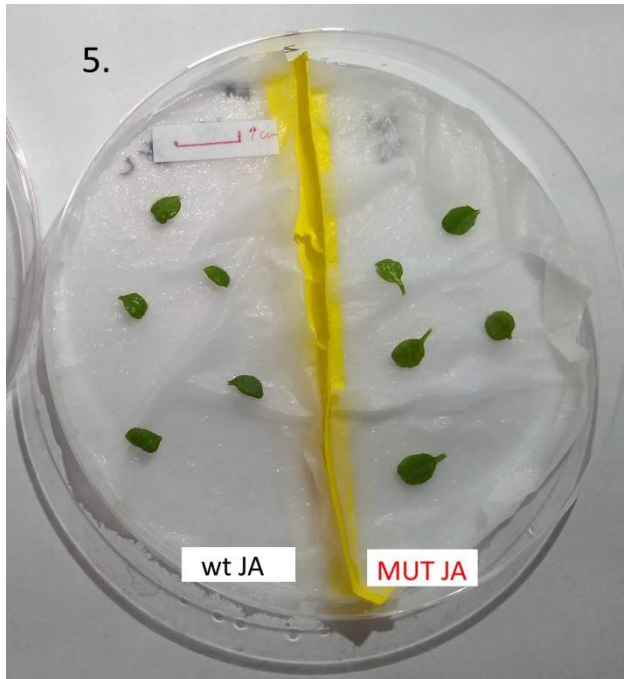
2. Comparison of results.

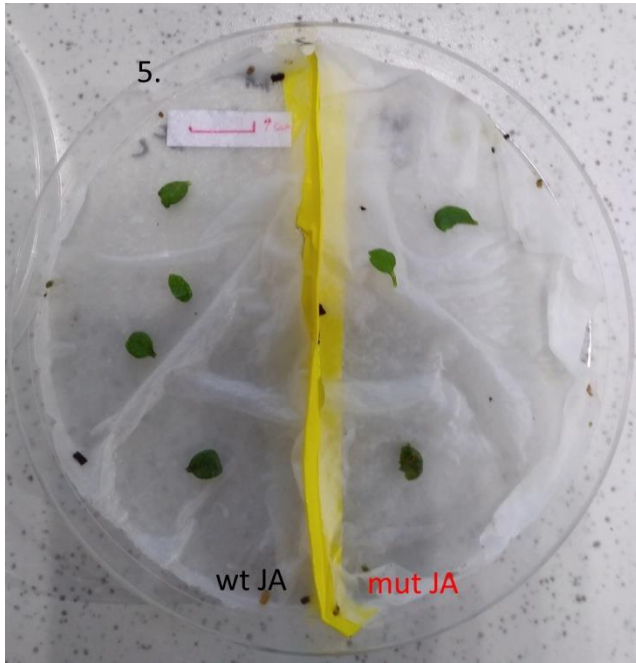
Before:



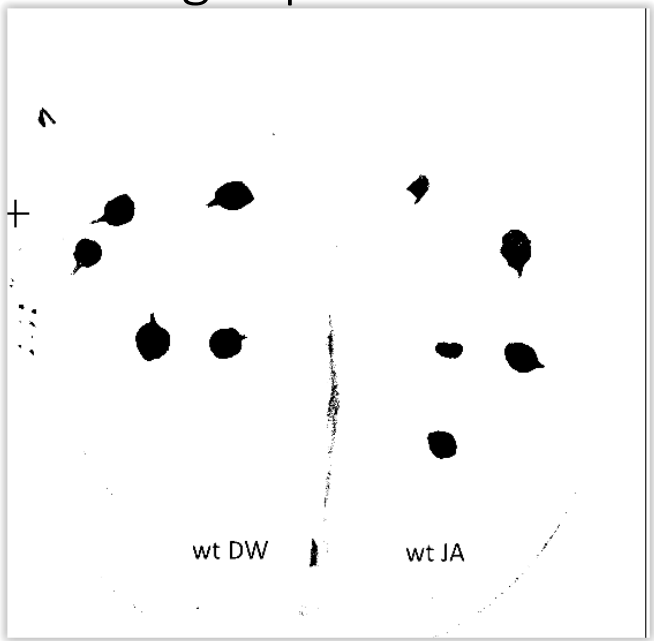


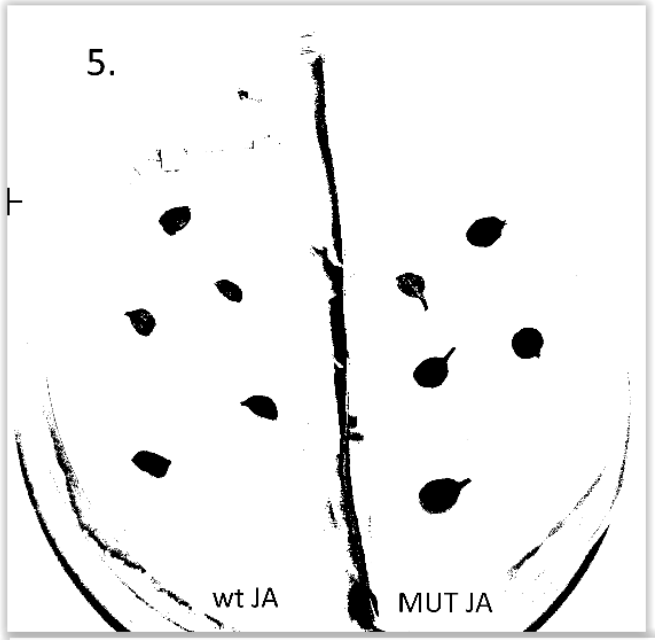
After:

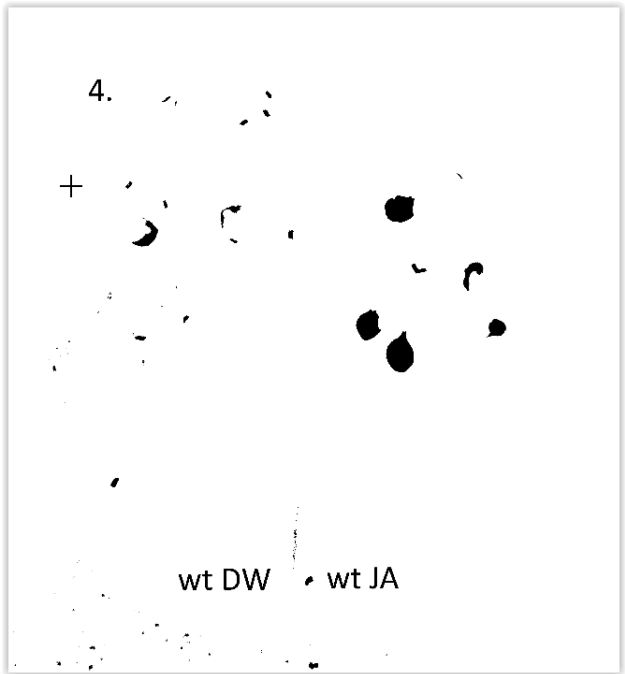


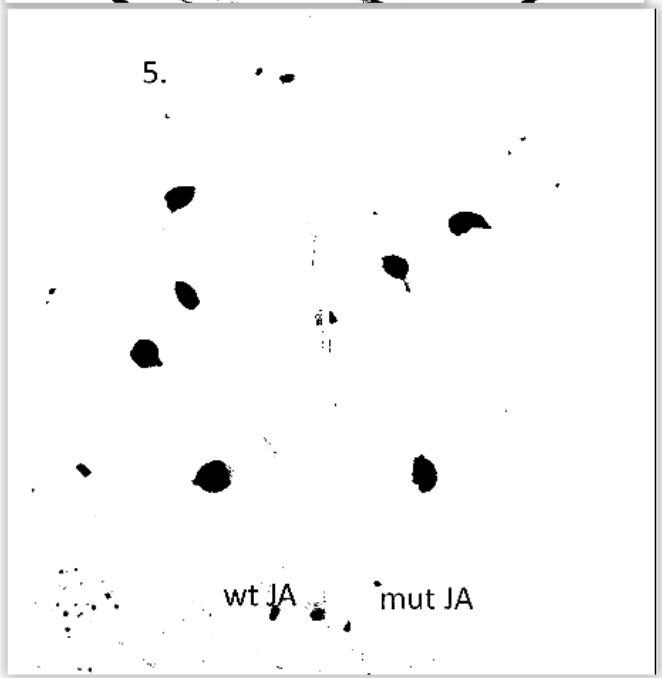


Pill bug experiment – feeding experiment on woodlouse









Pill bug experiment – feeding experiment on woodlouse

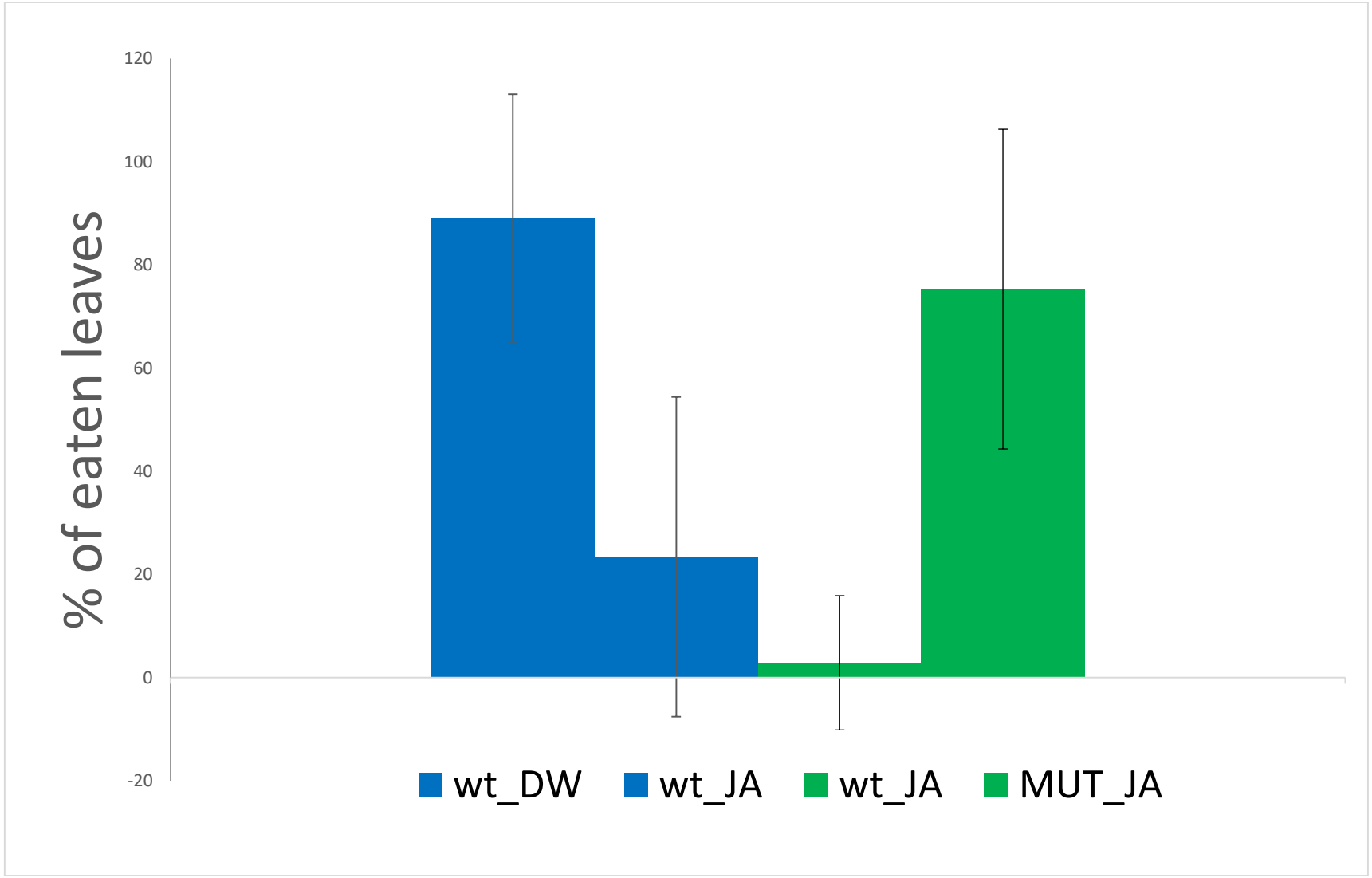
Results.

WT DW vs. WT JA

Woodlouse prefer to eat wild type incubated in water than incubated in jasmonic acid

WT JA vs. MUT JA

When exposed to leaves incubated in jasmonic acid only, woodlouse prefer to eat mutant plant



Fluorescence microscope-based determination of gene expression patterns in *Arabidopsis thaliana*

- ✓identified the expression pattern of TSA1 (ER body specific protein, accumulated mainly in inducible ER bodies)
- ✓identified β-glucosidases (BGLU) 19; 20; 21 and 22 (homologs to the ER body specific BGLU23/PYK10)
- ✓sterilized and sow the seeds

Fluorescence microscope-based determination of gene expression patterns in *Arabidopsis thaliana*

- plants constitutively expressing ER-localised GFP were genetically transformed with the 1000 bp long promoter (p) sequence genes fused to tdTOMATO (a red fluorescing protein)
- plants express GFPh in all cells, enabling the identification of ER-bodies
- cells that have a high activity of one of the genes show accumulation of tdTOMATO - red fluorescence

c1 - stands for red fluorescence; c2 – green fluorescence; c1+c2 - merged

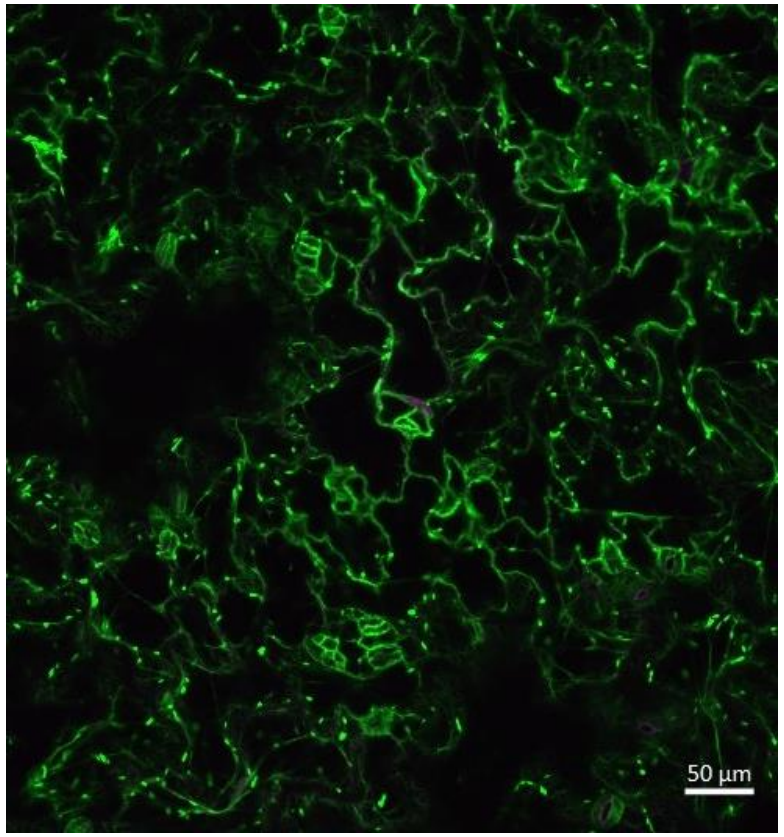


Fig 1.(A) PBGLU21-tdTOM; c2

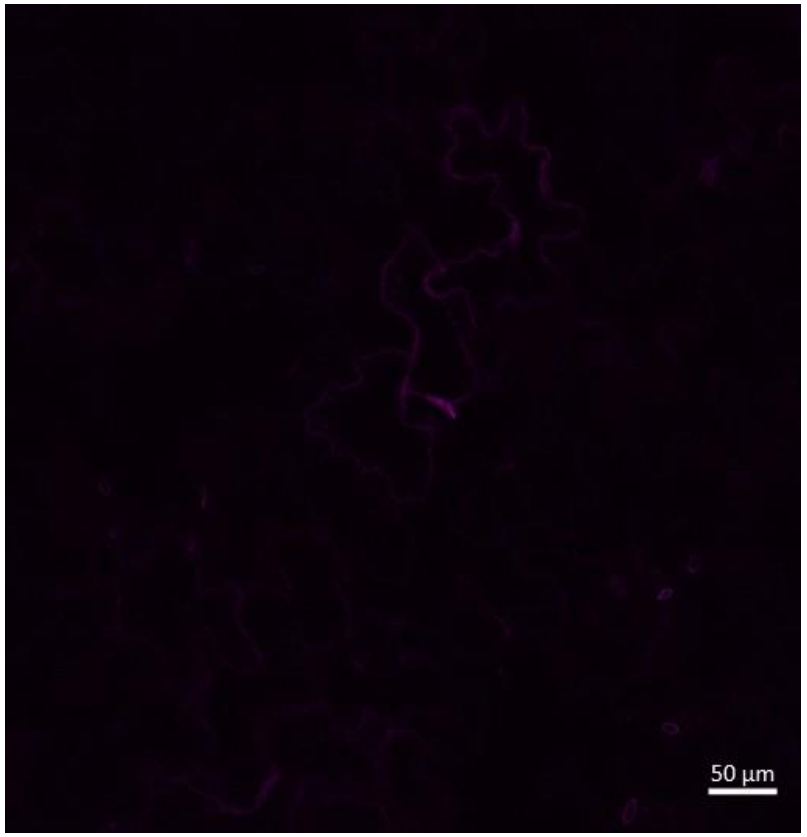


Fig 1.(B) PBGLU21-tdTOM; c1

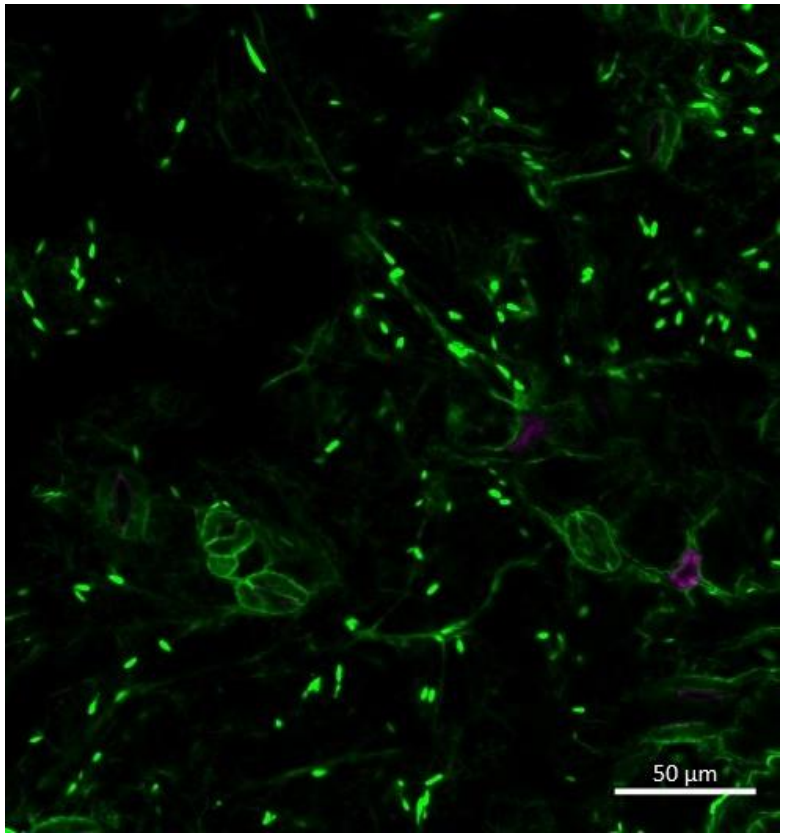


Fig 1.(C) PBGLU21-tdTOM; c1+c2

7 days old cotyledons

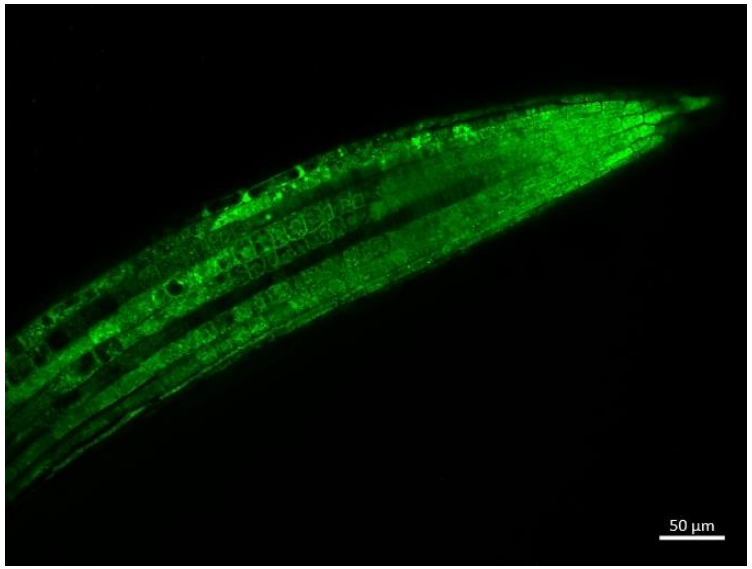


Fig.2(A) PBGLU21-tdTOM; c2

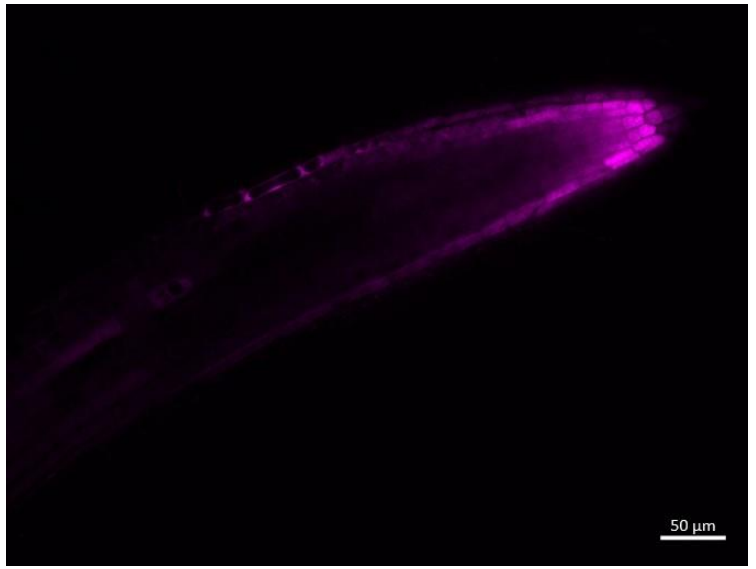


Fig.2(B) PBGLU21-tdTOM; c1

Fig.2(C) PBGLU21-tdTOM; c1+c2

7 days old plant root

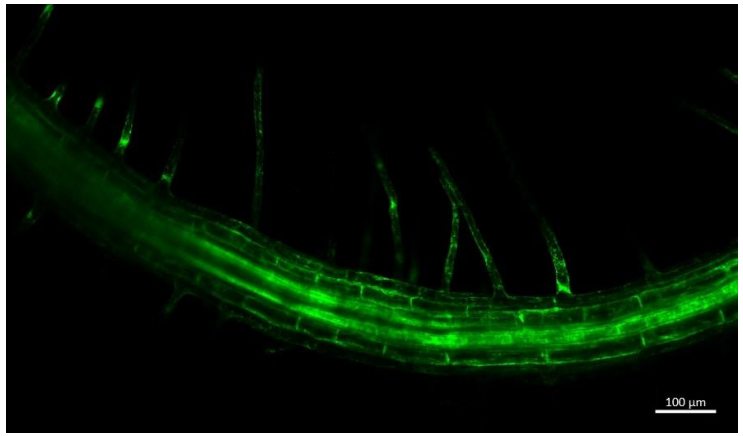


Fig.3(A) PBGLU22-tdTOM; c2

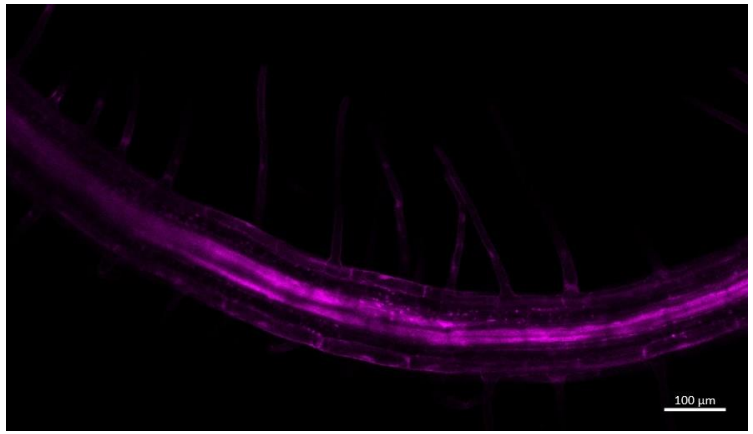


Fig.3(B) PBGLU22-tdTOM; c1

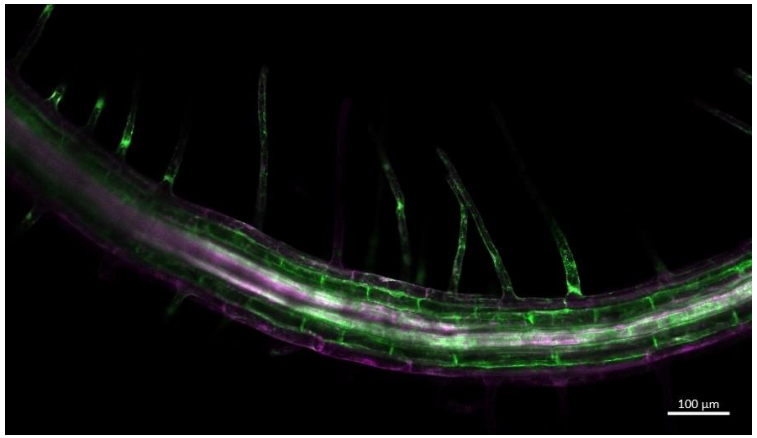


Fig.3(C) PBGLU22-tdTOM; c1+c2

7 days old plant root

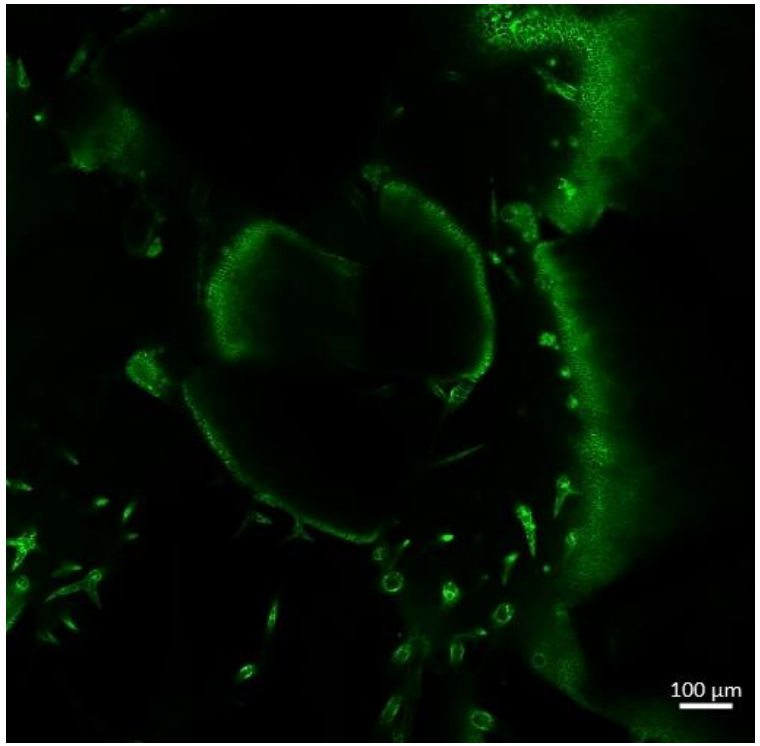


Fig.4(A) pTSA1-tdTOM; c2

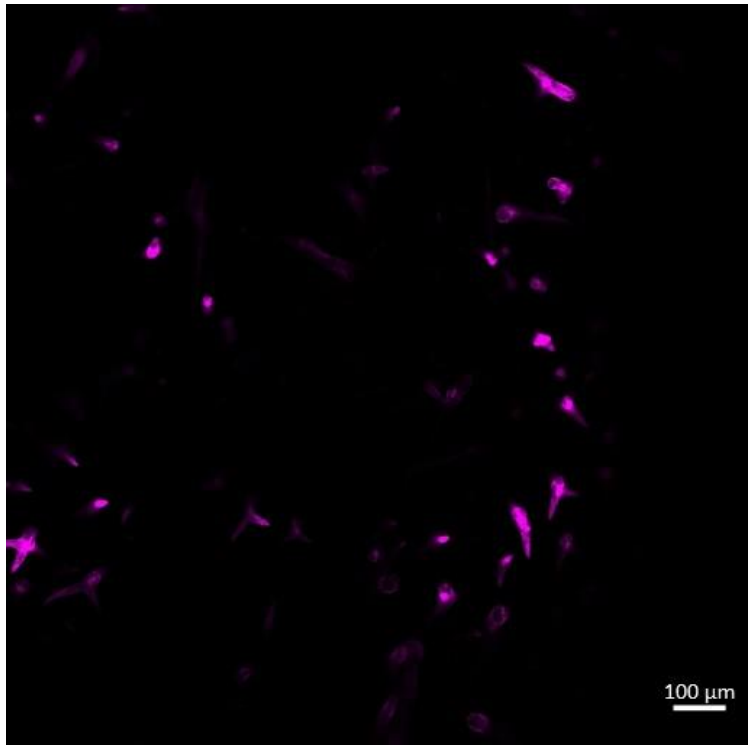


Fig.4(B) pTSA1-tdTOM; c1

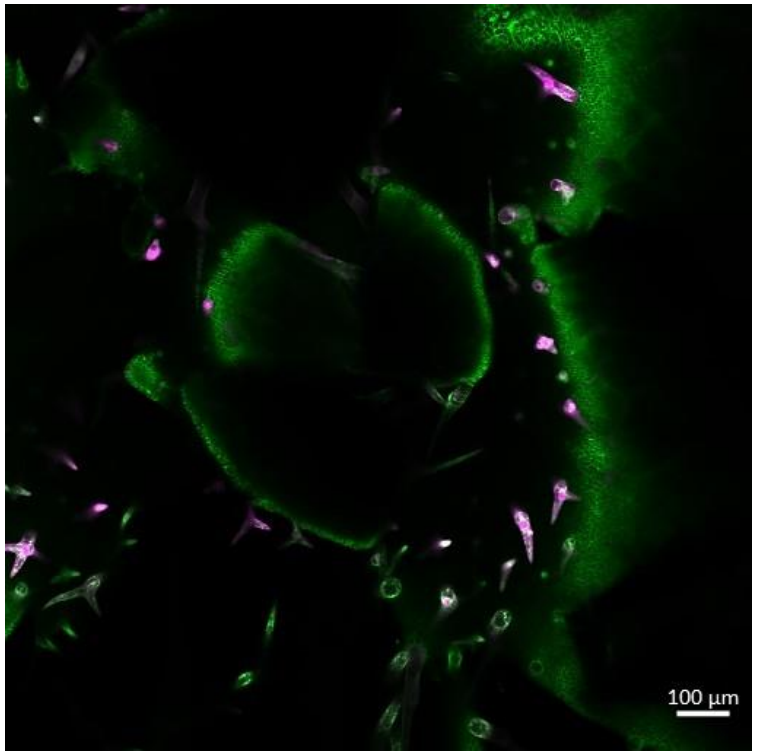


Fig.4(C) pTSA1-tdTOM; c1+c2

14 days old plant-central rosette

Fig.5(A) pTSA1-tdTOM; c1

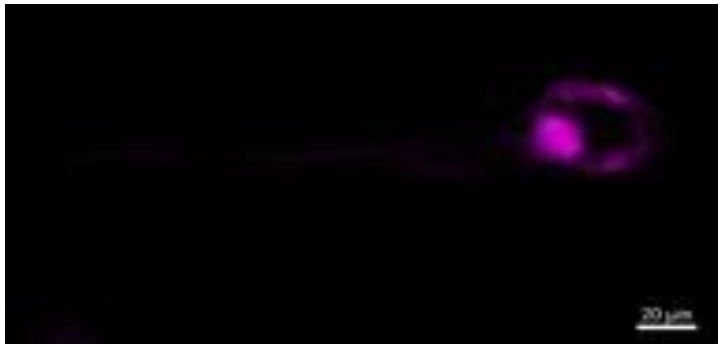


Fig.5(B) pTSA1-tdTOM; c1

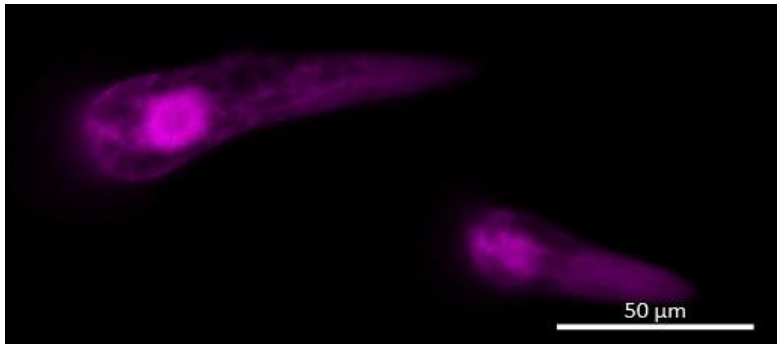
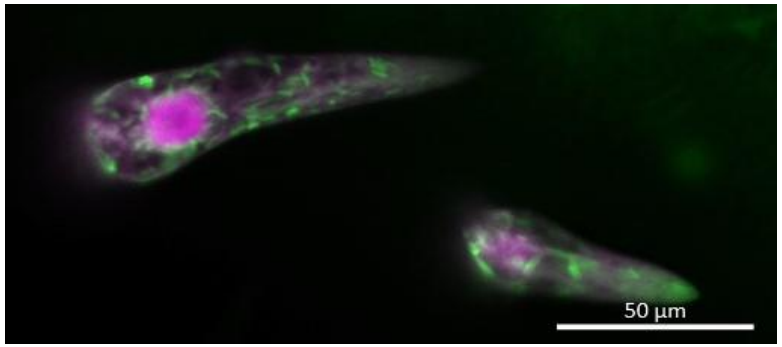


Fig.5(C) pTSA1-tdTOM; c1+c2



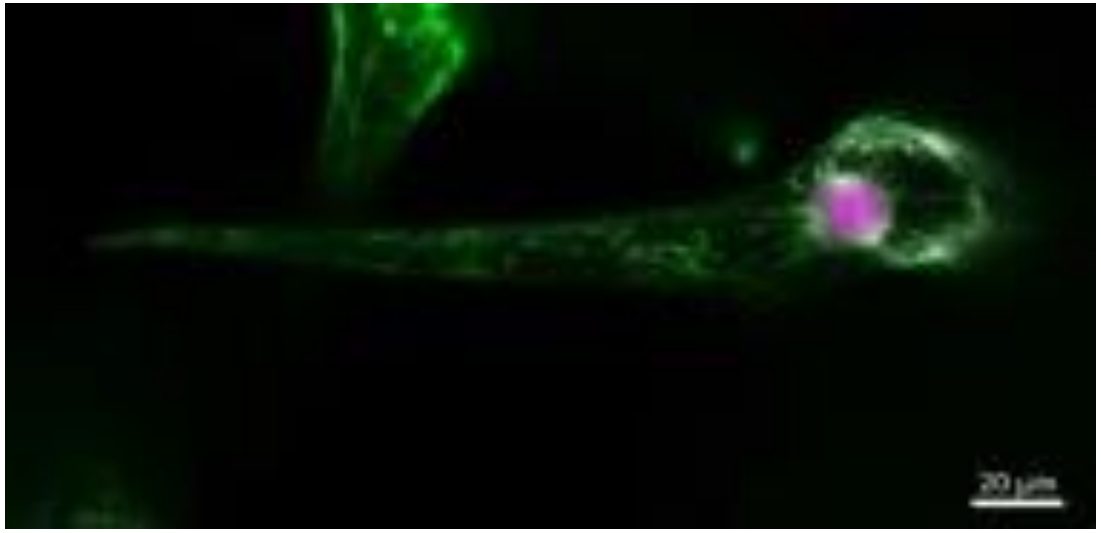


Fig.5(D) pTSA1-tdTOM; c1+c2

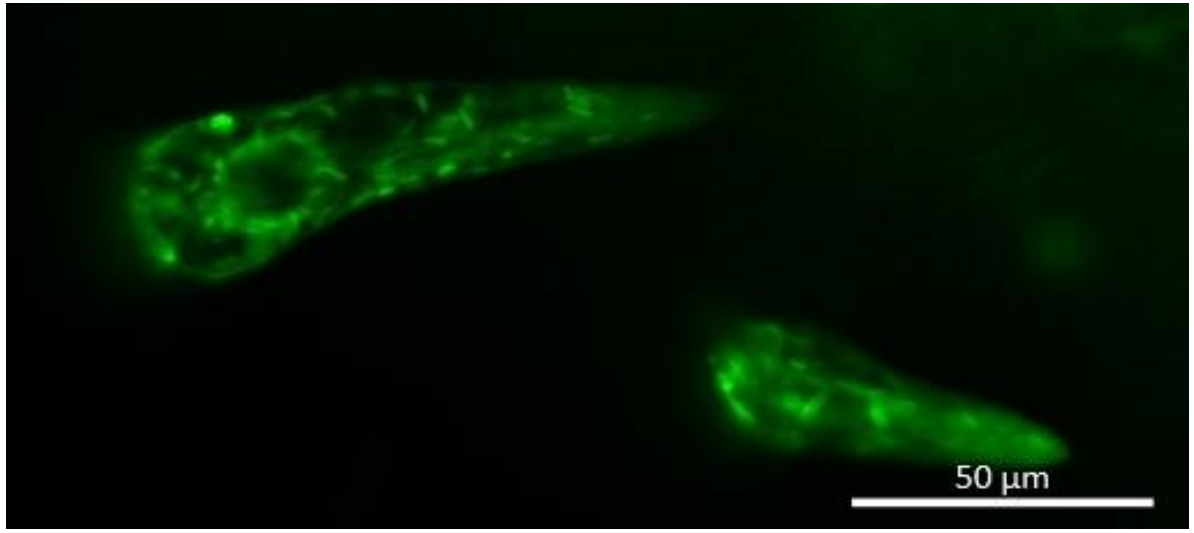


Fig.5(E) pTSA1-tdTOM; c2

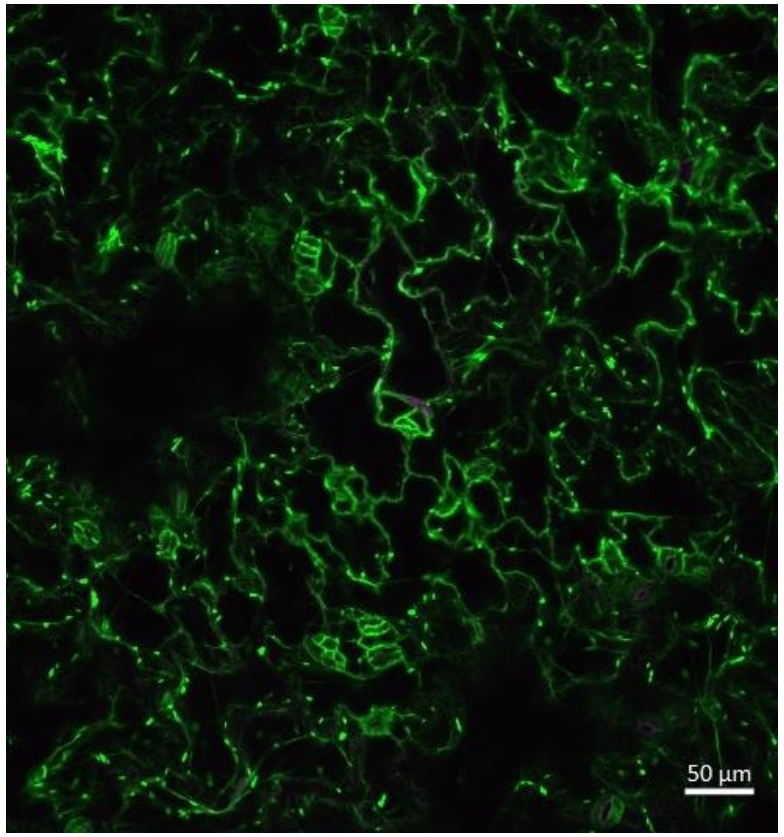


Fig 1.(A) PBGLU21-tdTOM; c2

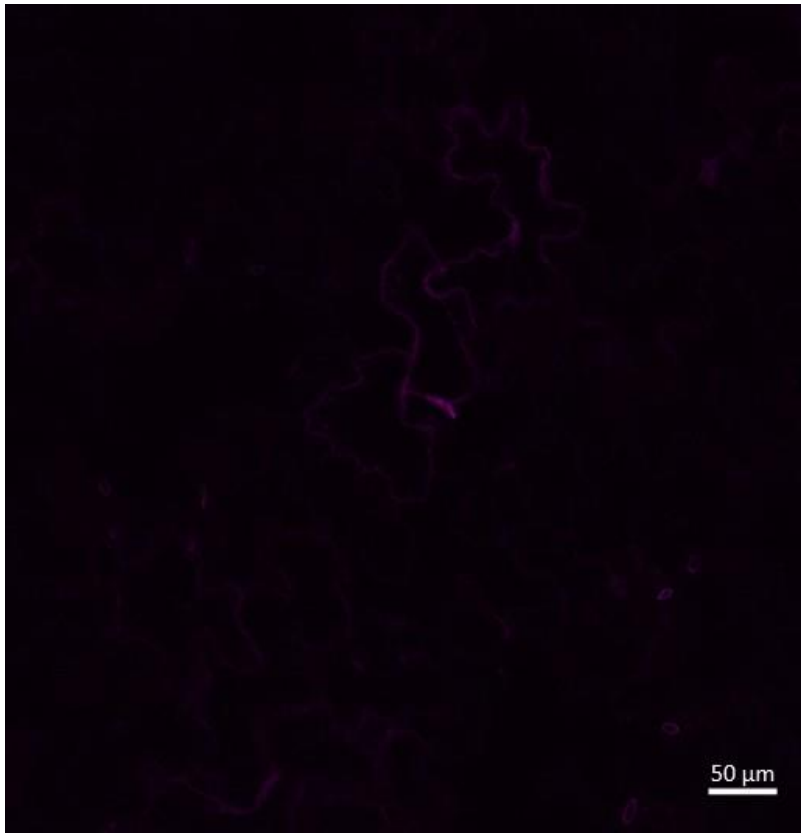


Fig 1.(B) PBGLU21-tdTOM; c1

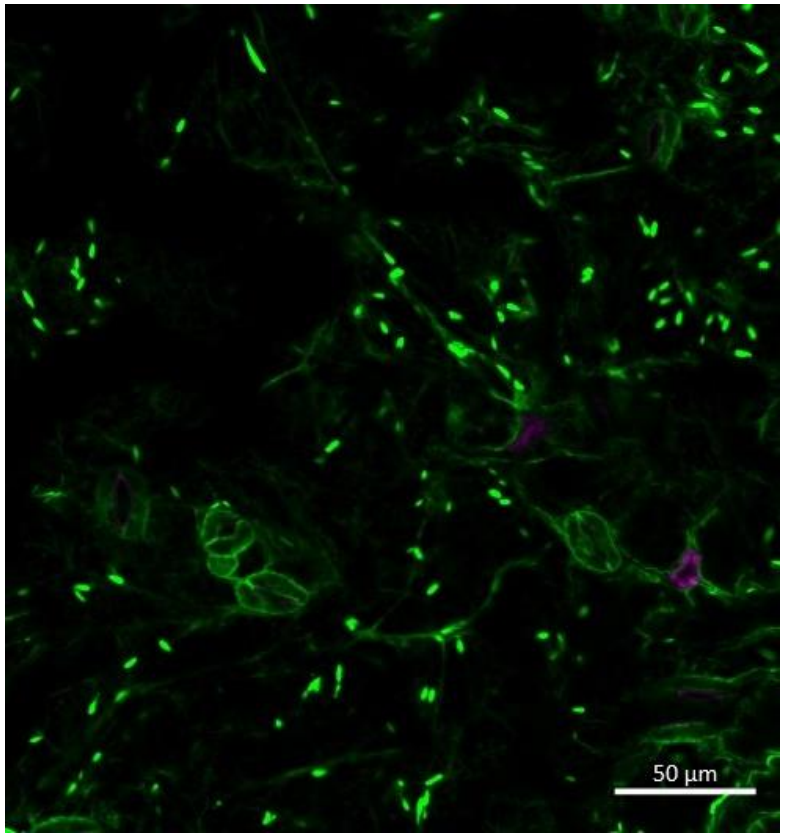


Fig 1.(C) PBGLU21-tdTOM; c1+c2

7 days old cotyledons

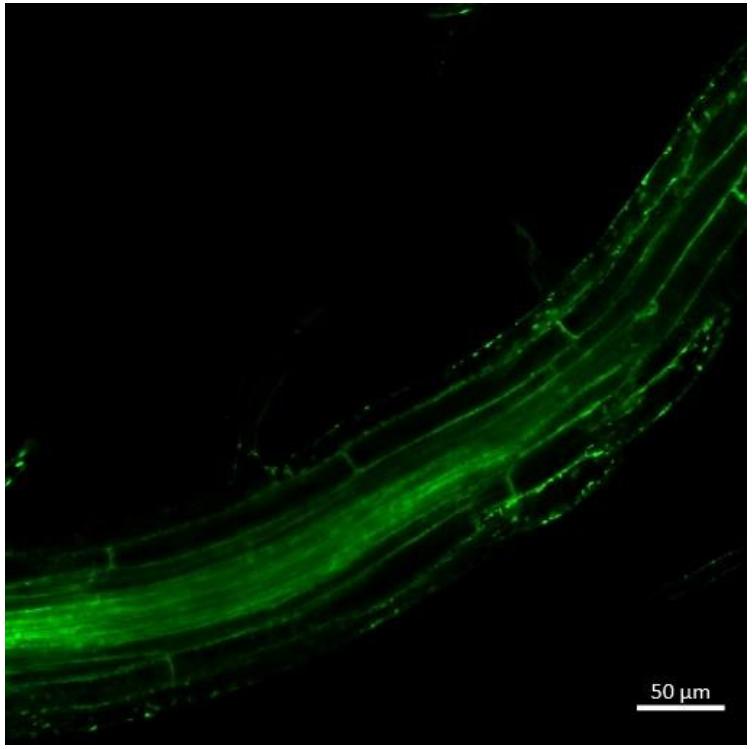


Fig.6(A) pTSA-tdTOM; c1

Fig.6(B) pTSA-tdTOM; c1

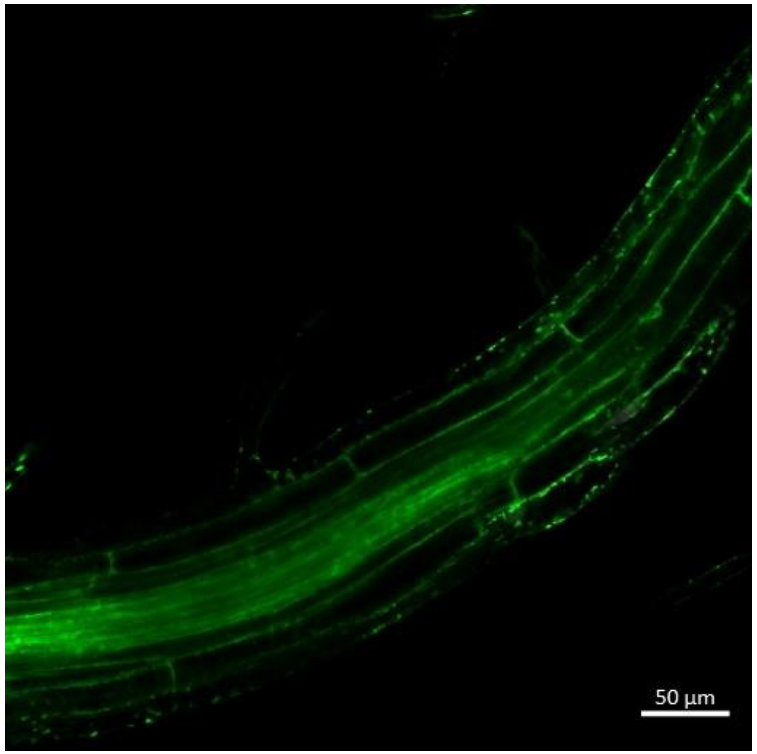


Fig.6(C) pTSA-tdTOM; c1+c2

14 days old plant root

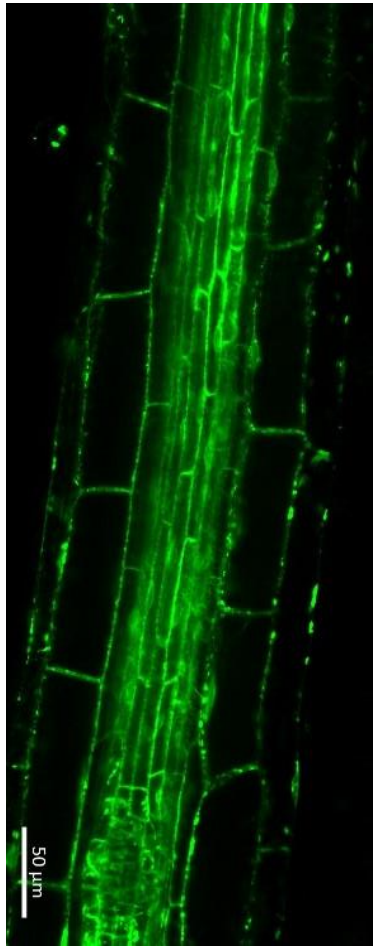


Fig.6(B) pTSA1-tdTOM; c2

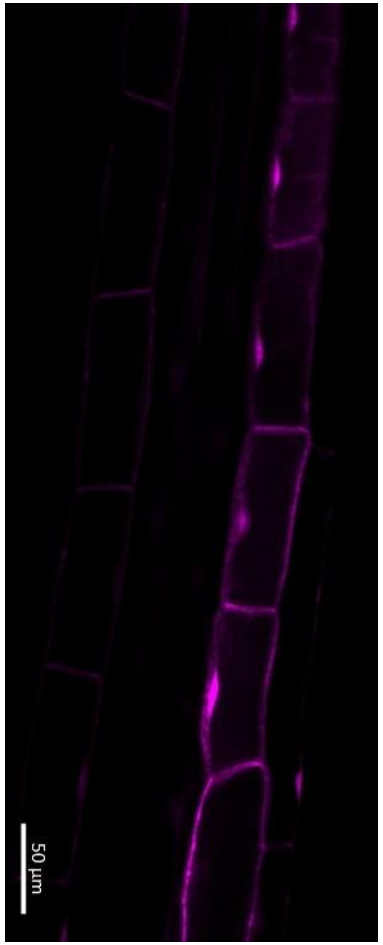


Fig.6(A) pTSA1-tdTOM; c1

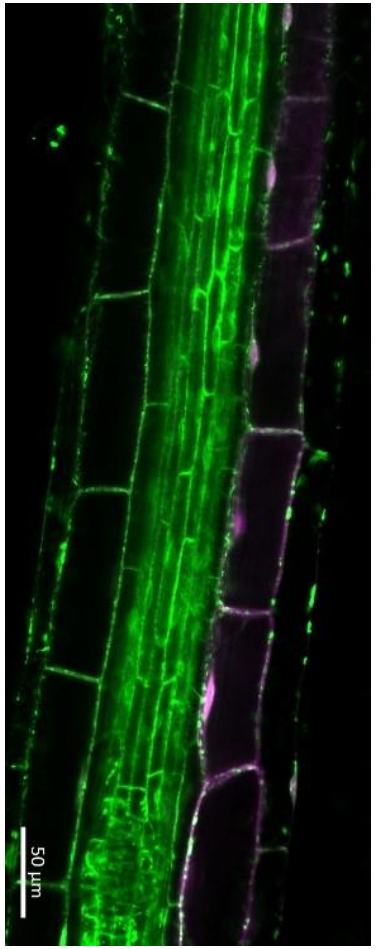


Fig.6(C) pTSA1-tdTOM; c1+c2

14 days old plant root treated with JA

Gene mapping

It is a group of various techniques used to identify the locus of genes (responsible for distinct features)

1. **Mutagenesis**
   - Chemical: EMS, MNG
   - Physical: gamma ray, fast neutron radiation
   - Biological: Transposon, T-DNA

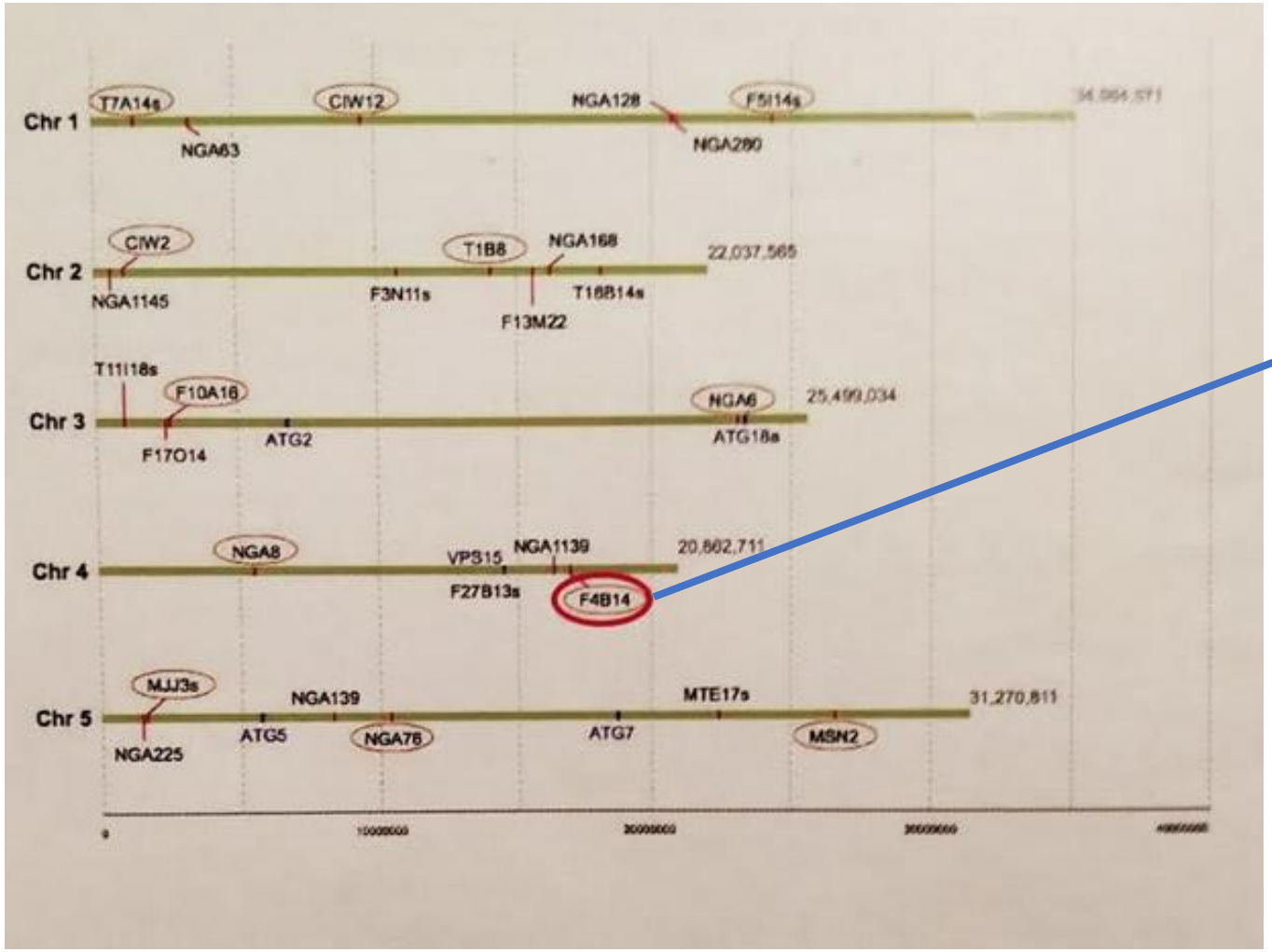
2. **Screening**

Looking for mutants with desired feature

3. **Gene identification**
   - Mapping with genetic markers
   - Flanking region sequencing (in case of T-DNA and transposon insertion mutants)
   - Whole genome sequencing

Gene mapping

Position of mapping markers on *Arabidopsis thaliana* genome between Columbia (Col) and Lansberg *erecta* (Ler)



Gene mapping – aim of experiment

Get to know which ecotype (Col vs. Ler) represents our plant.

Gene mapping

It is a group of various techniques used to identify the locus of genes (responsible for distinct features)

1. **Mutagenesis**
   - Chemical: EMS, MNG
   - Physical: gamma ray, fast neutron radia
   - Biological: Transposon, T-DNA

2. **Screening**

Looking for mutants with desired feature

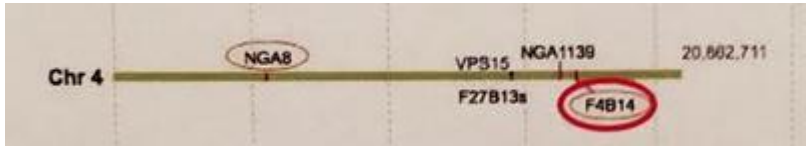
3. **Gene identification**
   - Mapping with genetic markers
   - Flanking region sequencing (in case of T-DNA and transposon insertion mutants)
   - Whole genome sequencing

Gene mapping

1. Isolation of genome DNA from *A. thaliana*

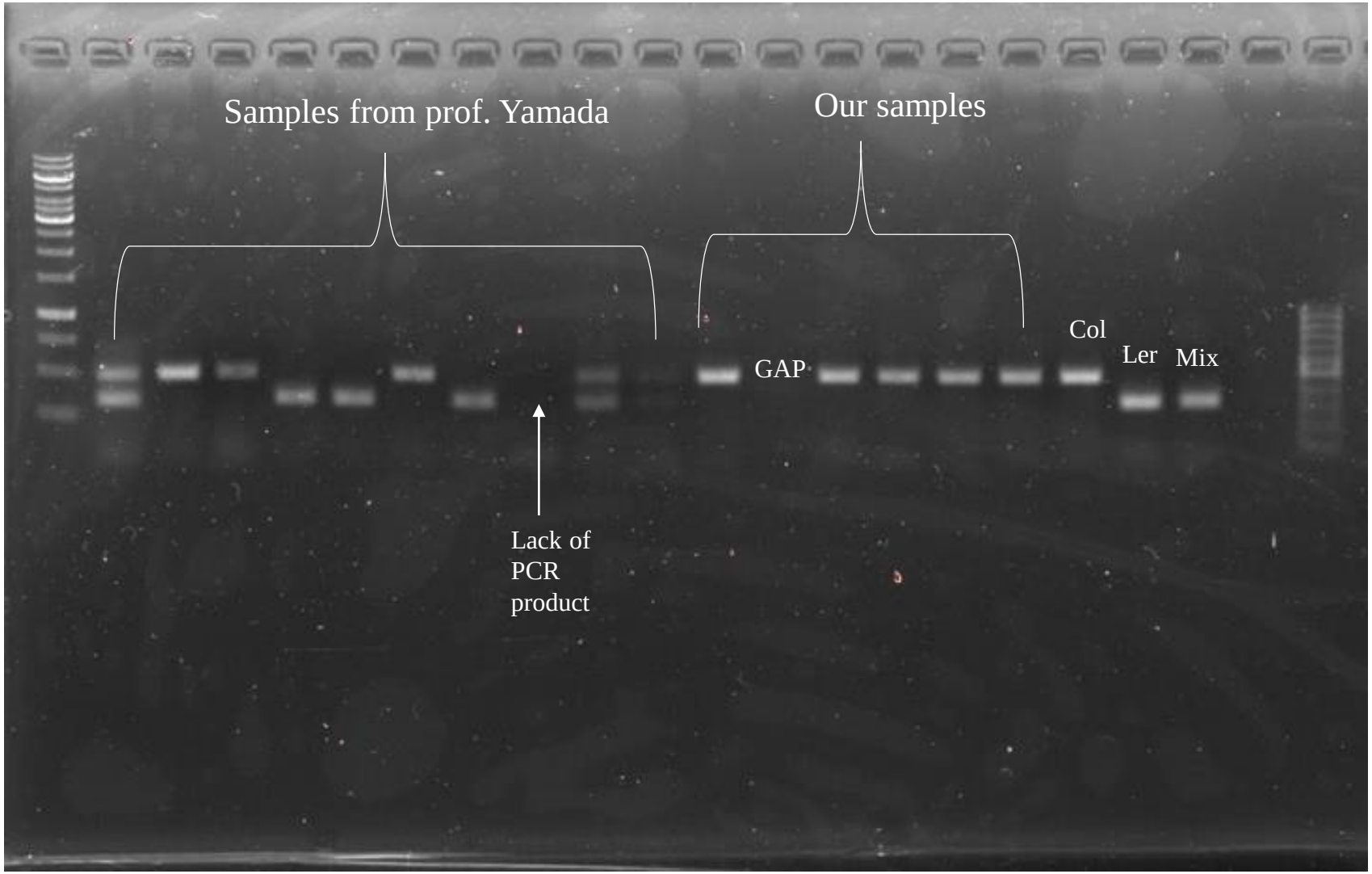
   Homogenization with phenol-chloroform extraction protocol

2. PCR reaction with specific primers



3. Agarose gel electrophoresis

Gene mapping - results

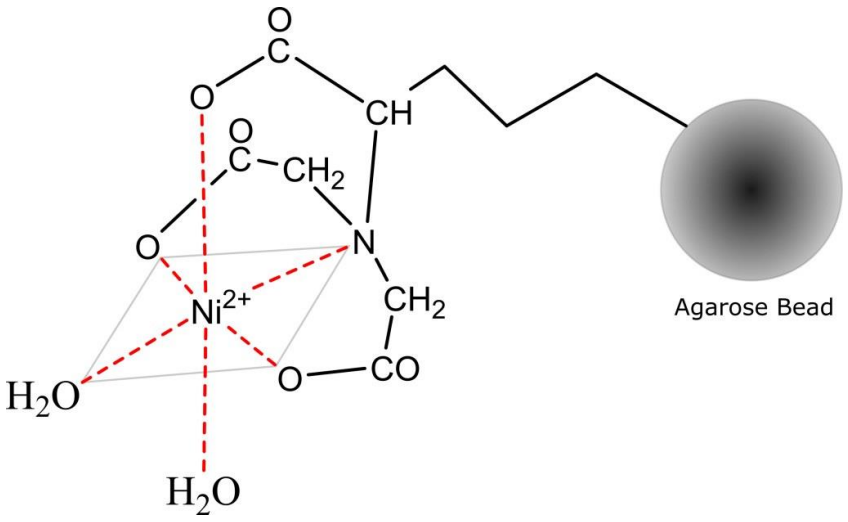


Purification of PYK10 from BY2 cells

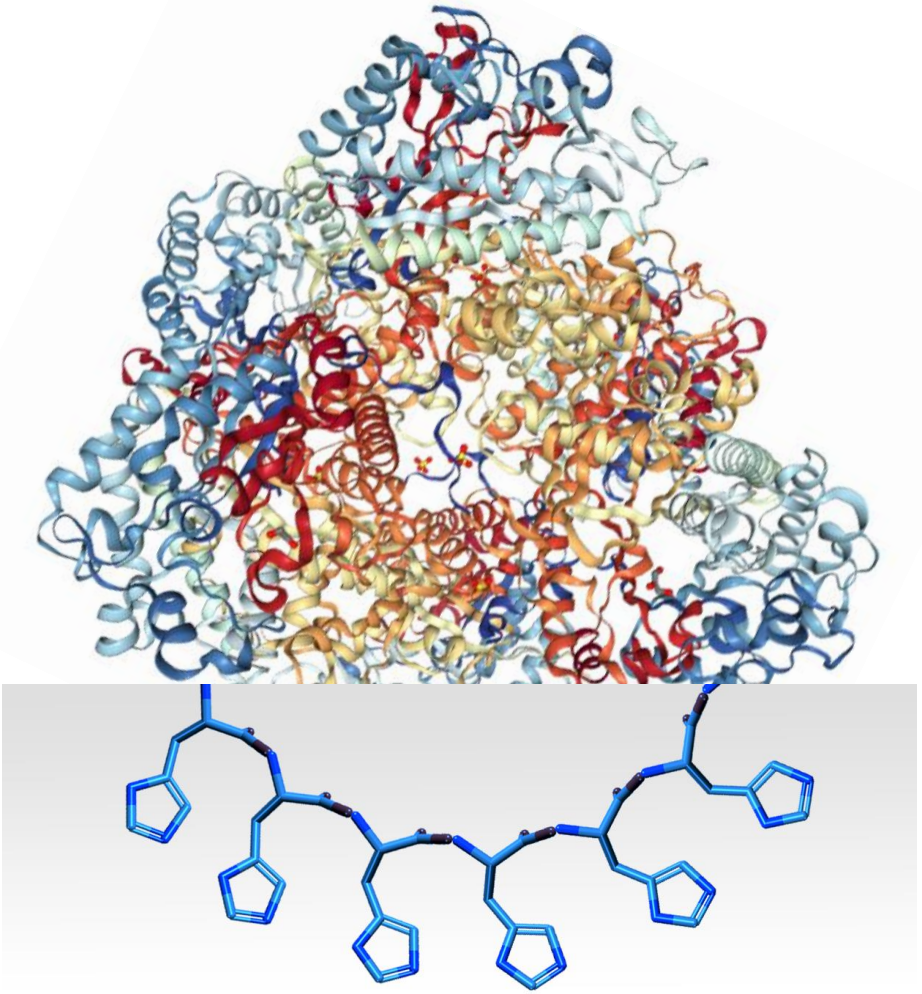
1. Lysis (Everything on ice or in liquid nitrogen)
   - TissueLyzer (30 s, frequency: 30/s)

2. Collection of supernatant by centrifugation

3. Incubation with Ni-NTA beads



Affinity



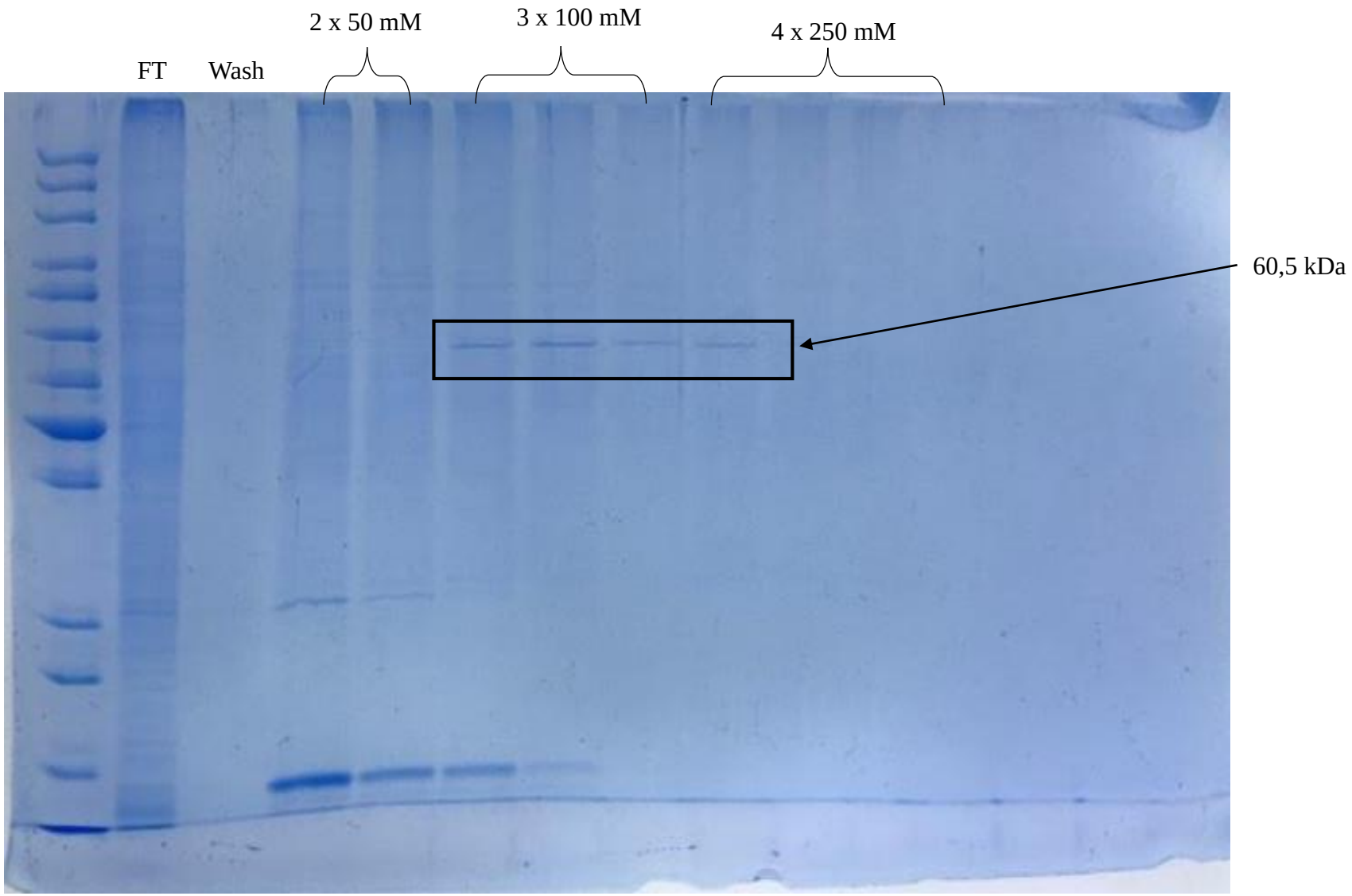
Source: 10.13140/RG.2.2.20211.53282; generon.co.uk; rcsb.org [3AIU]

Purification of PYK10 from BY2 cells

4. Washing of beads and elution with imidazole
    - 50 mM, 100 mM and 250 mM of imidazole

5. SDS-PAGE gel electrophoresis

Purification of PYK10 from BY2 cells



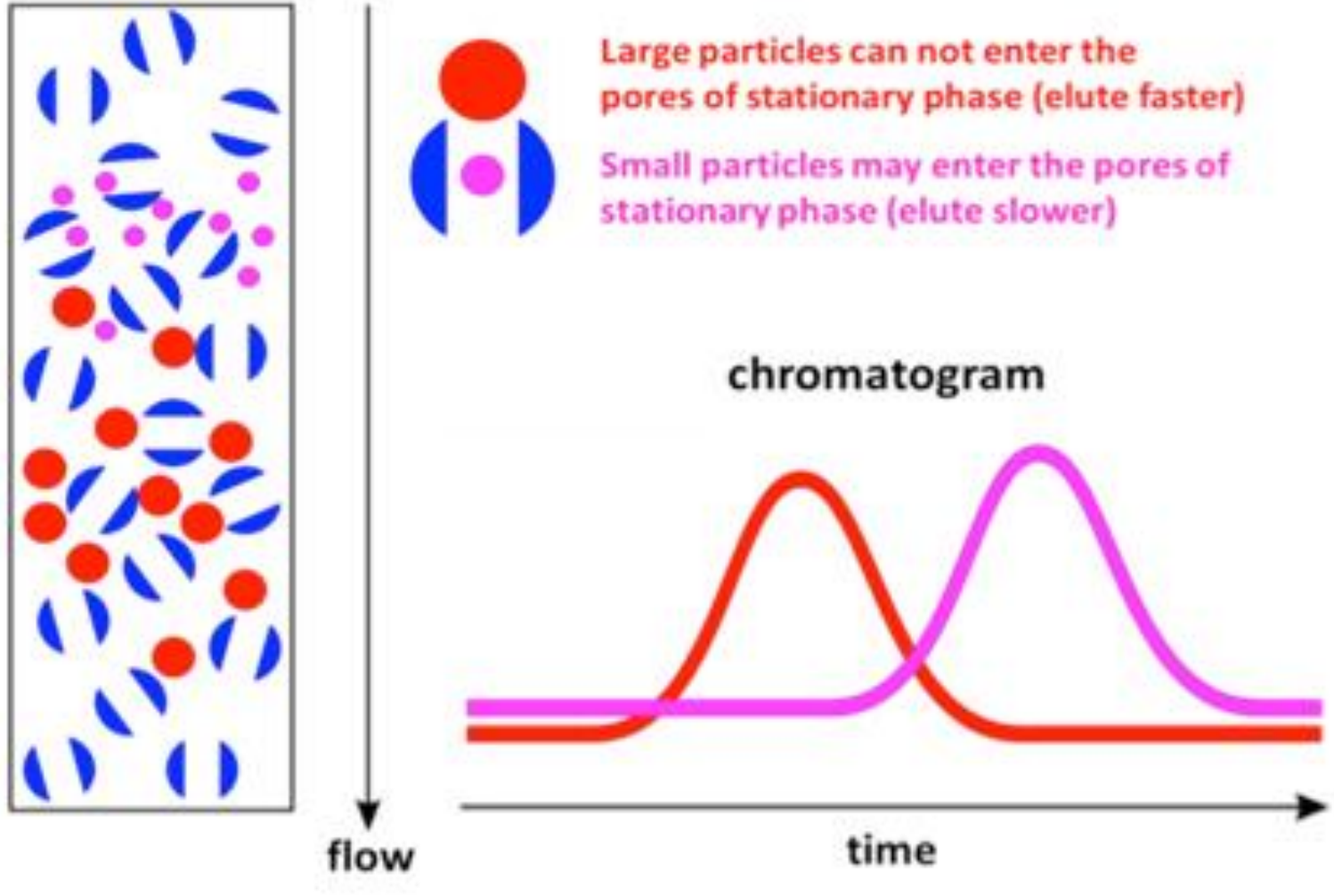
Purification of PYK10 from BY2 cells

4. Washing of beads and elution with imidazole
    - 50 mM, 100 mM and 250 mM of imidazole

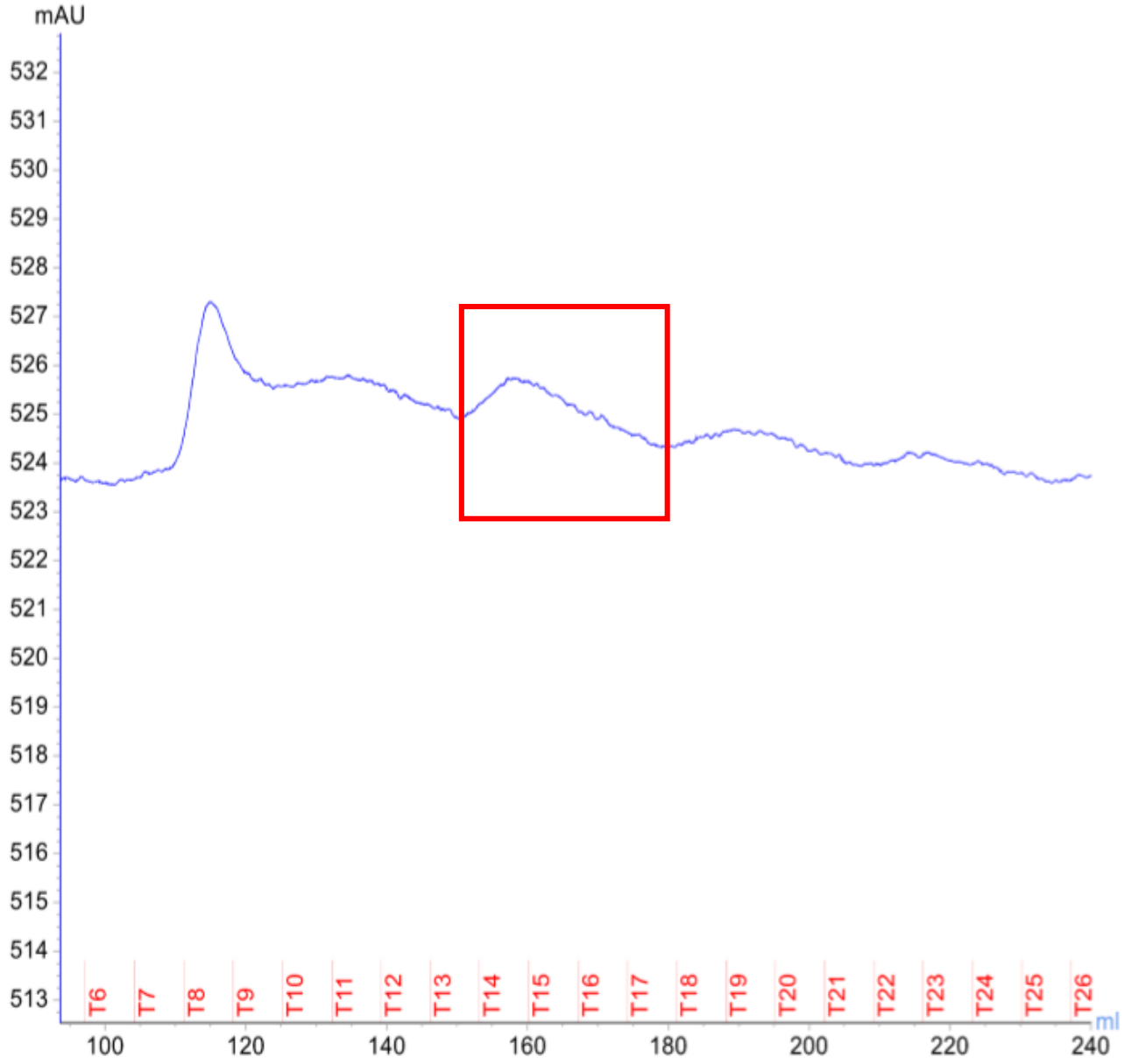
5. SDS-PAGE gel electrophoresis

6. Pooling the fractions with PYK10 and c

7. SEC (Size-exclusion chromatography)
    Superdex^{TM} 200



Purification of PYK10 from BY2 cells



Purification of PYK10 from BY2 cells

4. Washing of beads and elution with imidazole
    - 50 mM, 100 mM and 250 mM of imidazole

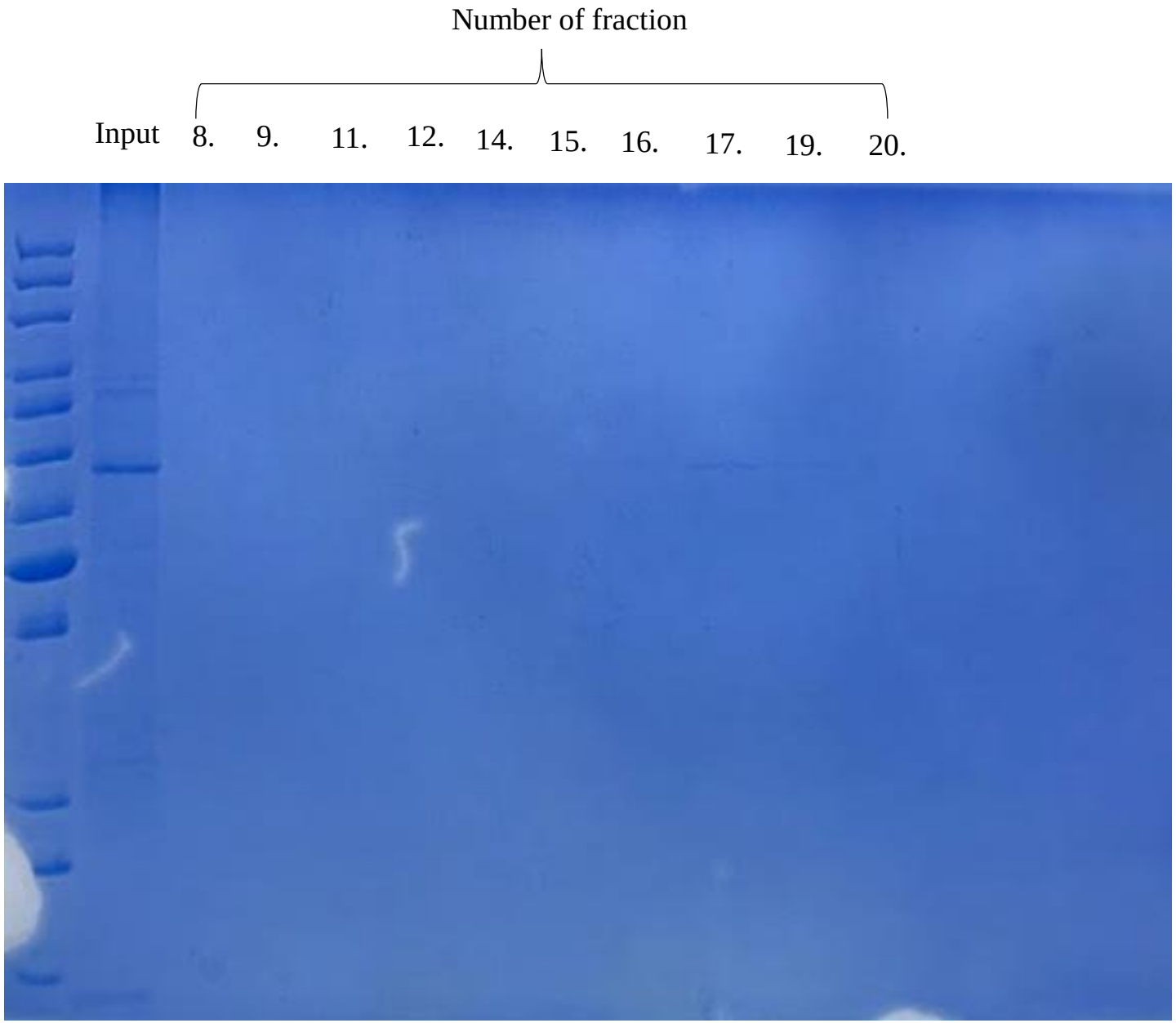
5. SDS-PAGE gel electrophoresis

6. Pooling the fractions with PYK10 and concentration to the volume < 5 ml

7. SEC (Size-exclusion chromatography)
    Superdex^{TM} 200

8. SDS-PAGE gel electrophoresis

Purification of PYK10 from BY2 cells



Purification of PYK10 from BY2 cells

4. Washing of beads and elution with imidazole
    - 50 mM, 100 mM and 250 mM of imidazole

5. SDS-PAGE gel electrophoresis

6. Pooling the fractions with PYK10 and concentration to the volume < 5 ml

7. SEC (Size-exclusion chromatography)
    Superdex^{TM} 200

8. SDS-PAGE gel electrophoresis

9. Pooling the fractions after SEC with PYK10

Finally we obtained 0,3 mg/ml of PYK10 from 300 ml liquid culture of BY2 callus.