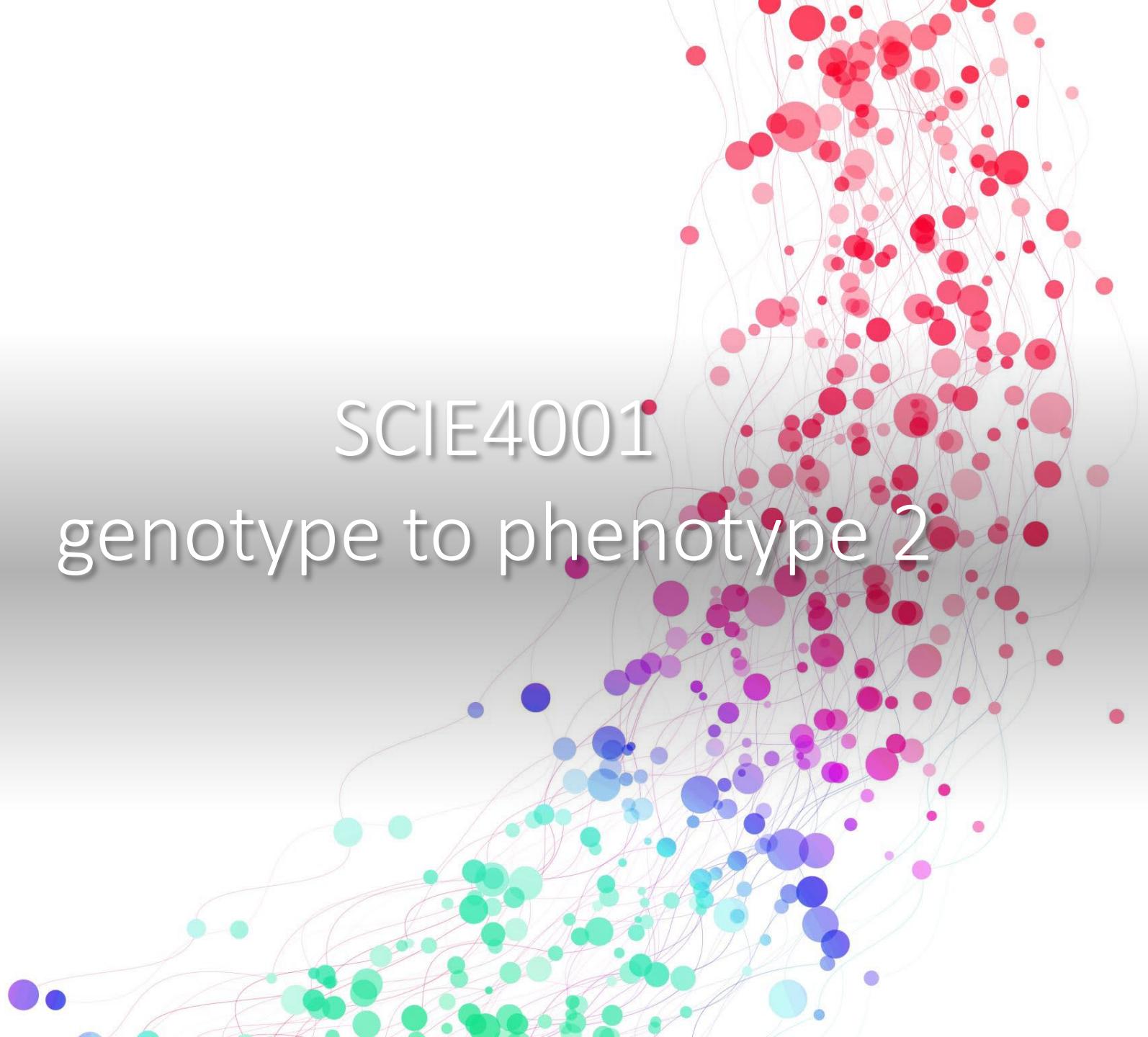


SCIE4001

genotype to phenotype 2



The examples of functional genomics will help to:

- Understand how targeted genetic approaches can be utilised to determine the function of a protein.
- Understand how plant phenotypes can be used to discover protein function.
- Understand the current research themes and approaches used in reverse and forward genetic screens in the model plant *Arabidopsis thaliana*
- Critically evaluate the pros/cons of various techniques and/or tools available for researchers today.

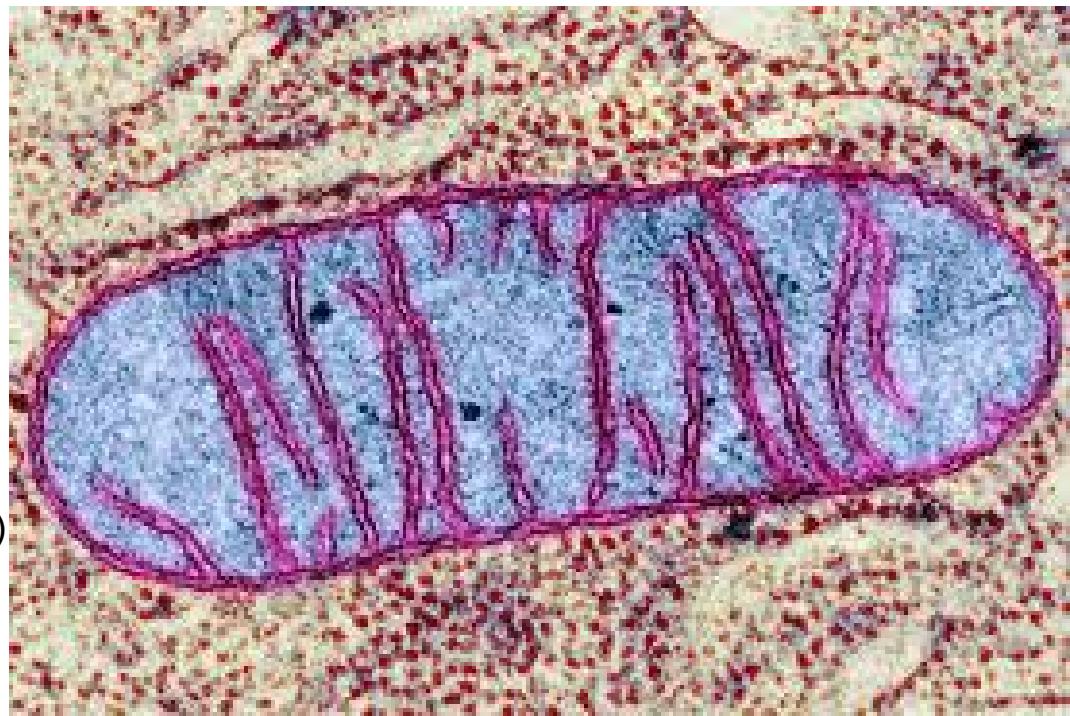
Arabidopsis thaliana as a model plant

Example 1. *RMB regulators of mitochondrial biogenesis*



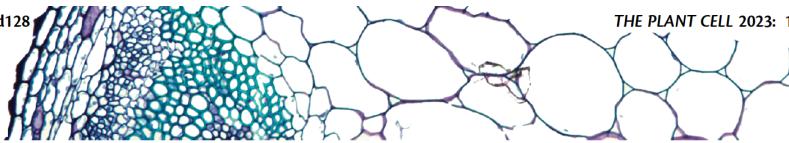
Dr Aneta Ivanova. Abi Ghifari (PhD candidate)

Murcha lab; regulators of mitochondrial biogenesis



<https://doi.org/10.1093/plcell/koad128>

THE
PLANT
CELL



THE PLANT CELL 2023; 1

FTSH PROTEASE 3 facilitates Complex I degradation through a direct interaction with the Complex I subunit PSST

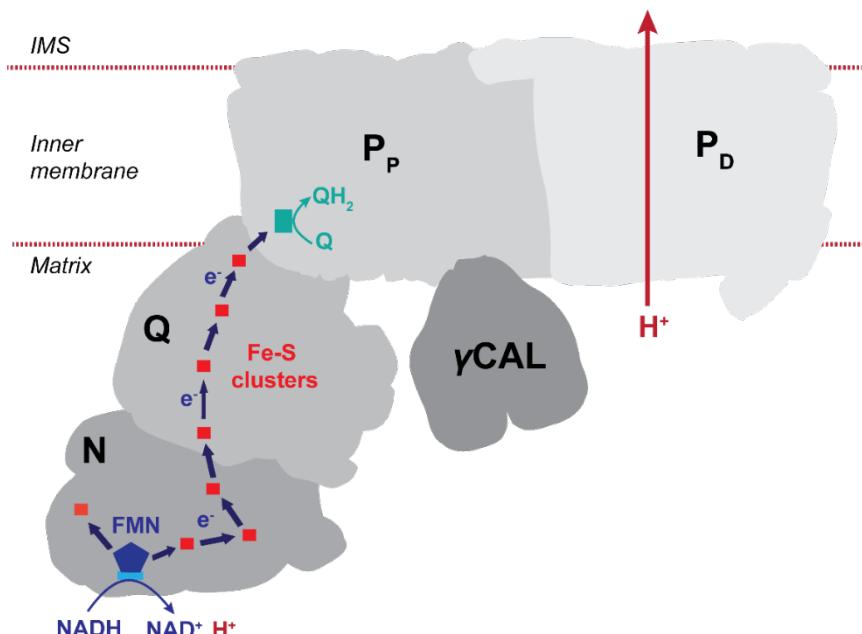
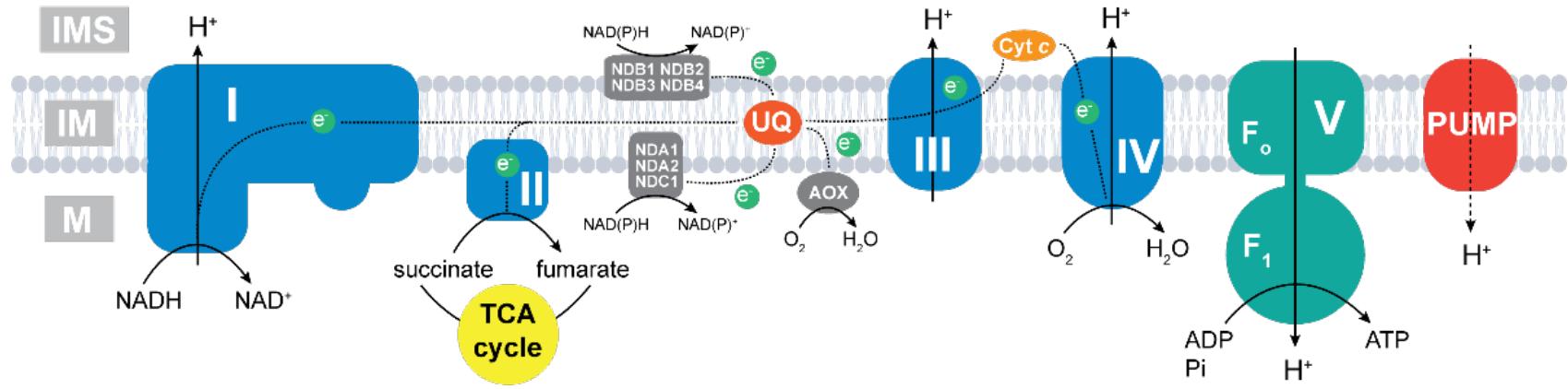
Abi S. Ghifari ¹ Aneta Ivanova ¹ Oliver Berkowitz ² James Whelan ³ and Monika W. Murcha

¹ School of Molecular Sciences & ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Perth, WA 6009, Australia

² Department of Animal, Plant and Soil Science, School of Life Science, ARC Centre of Excellence in Plant Energy Biology, La Trobe University, Bundoora, VIC 3086, Australia

³ College of Life Science, Zhejiang University, Hangzhou, Zhejiang 310058, PR China

Mitochondrial Complex I: the largest OXPHOS complex and first site of electron transfer

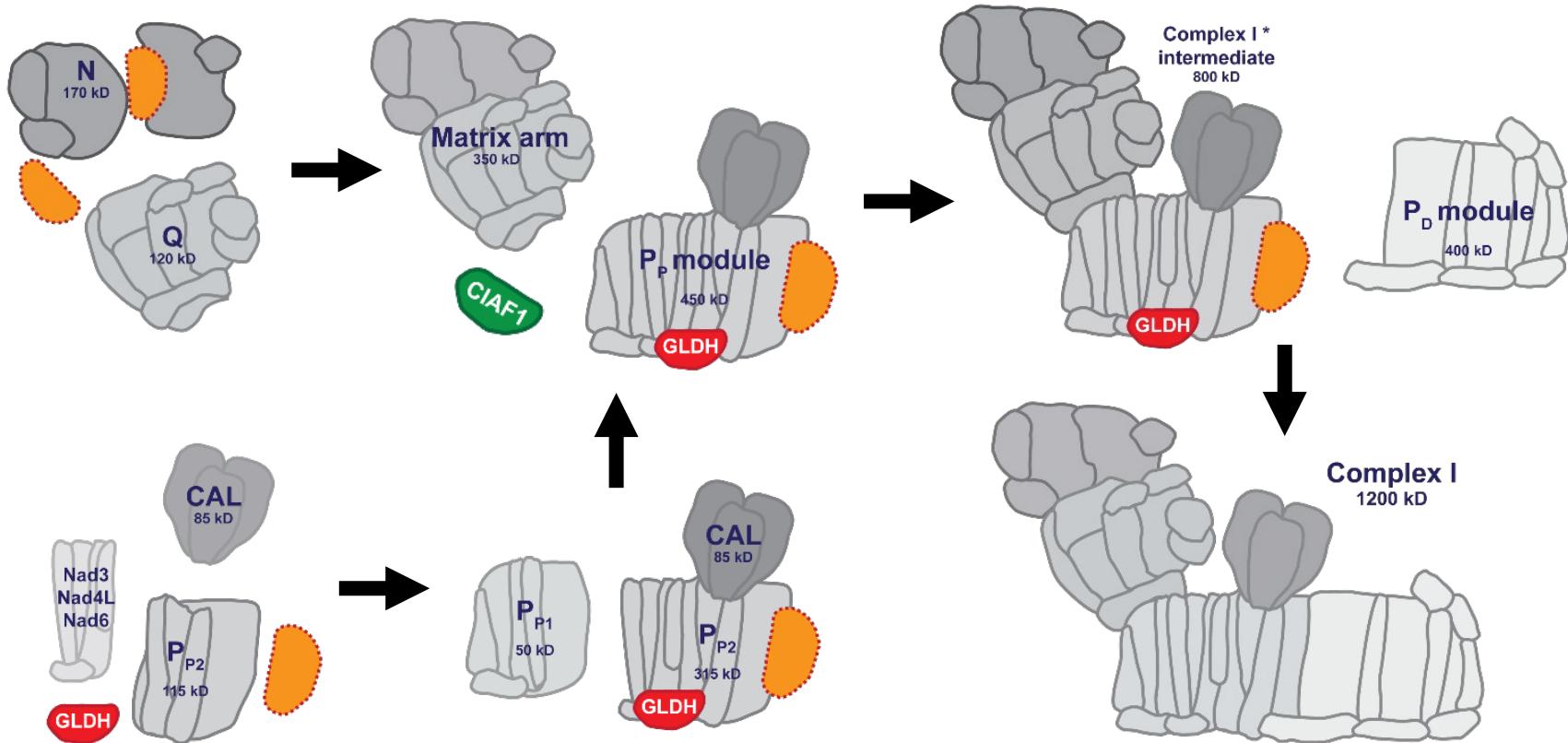


Largest OXPHOS complex:
47-51 subunits (plants)

Oxidative damage → high turnover rate (matrix arm)

Biogenesis (assembly) and degradation (disassembly)?

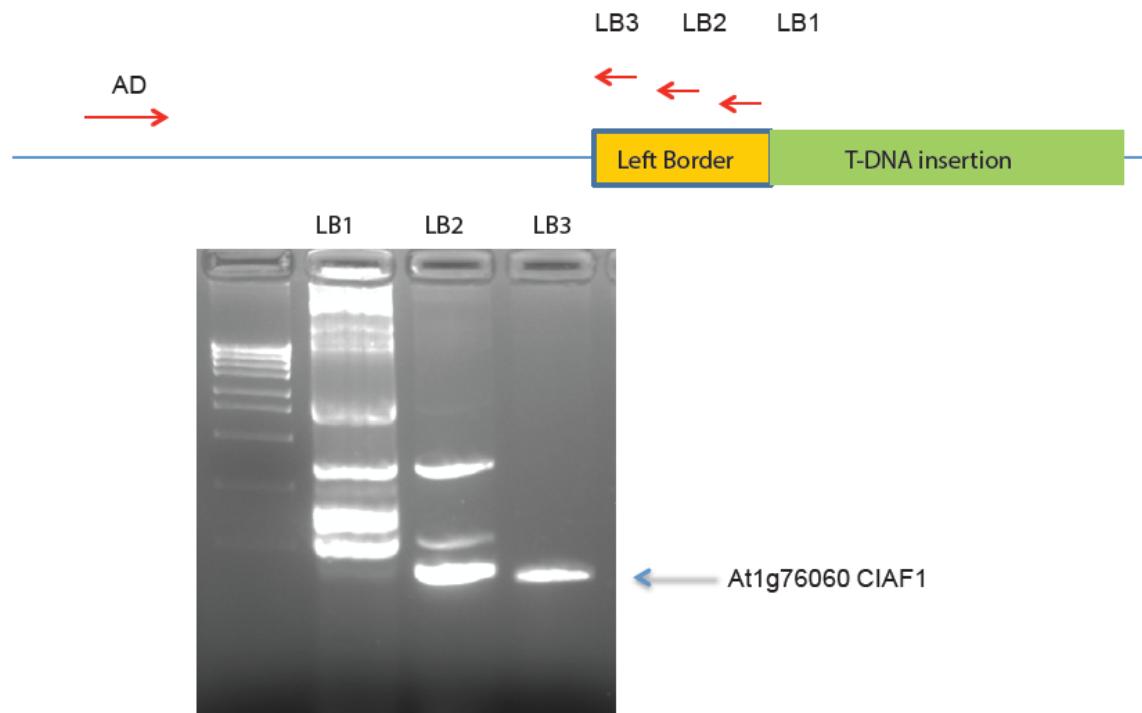
CIAF1 involved in the assembly of matrix arm NQ module to CI*



Ivanova et al. (2019) *Plant Physiology*
Ligas et al. (2019). *Plant Journal*

COMPLEX I ASSEMBLY FACTOR-1 (CIAF1) deletion causes growth delay associated with CI defect

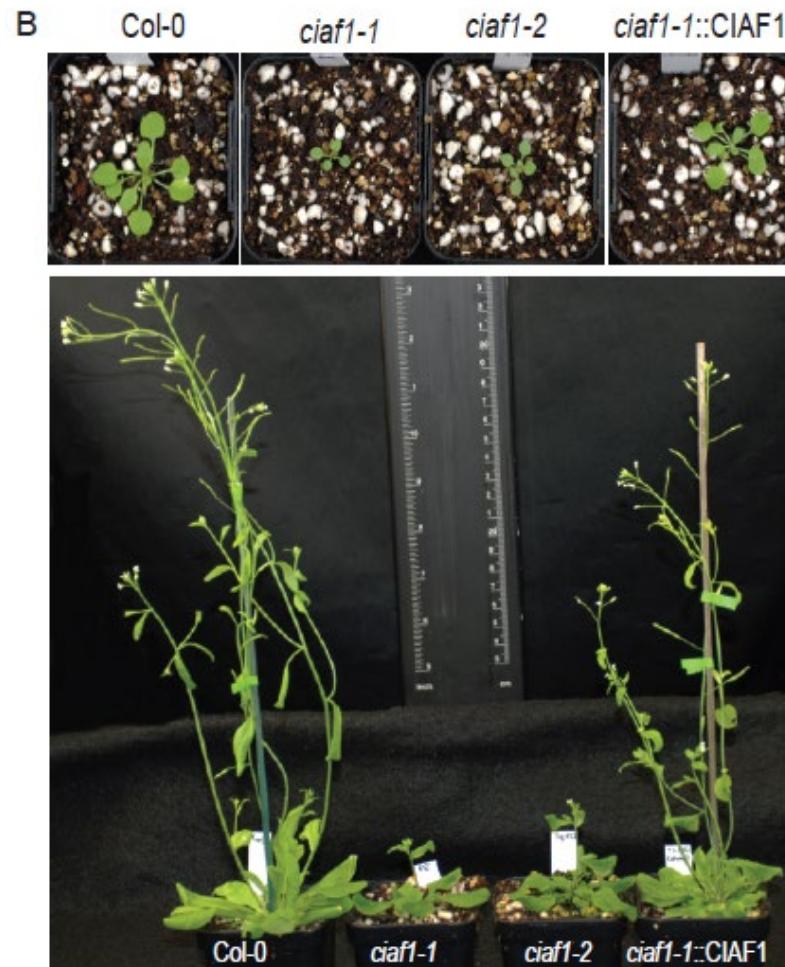
**T-DNA insertion mutant line of a Complex I assembly factor (*ciaf1*)
Was identified by TAIL PCR**



Supplemental Figure S1. TAIL PCR identifies an additional T-DNA insertion within At1g76060 in the T-DNA insertion line SALK_143656. Agarose gel showing the PCR products of the consecutive rounds of PCR using LB1 and AD, LB2 and AD primers and finally LB3 and AD primer sets. The final PCR product indicated with an arrow was analysed by Sanger sequencing.

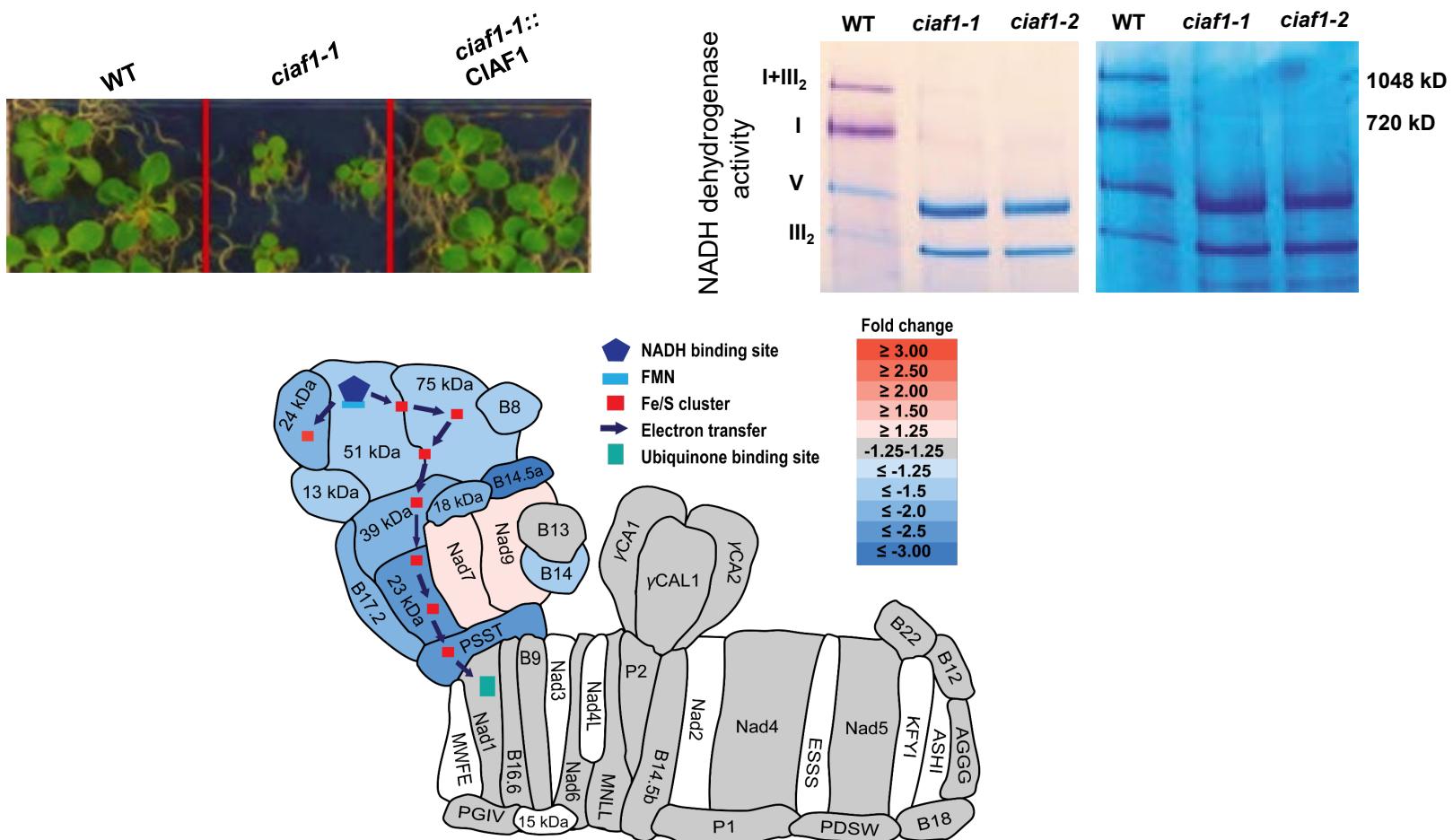
COMPLEX I ASSEMBLY FACTOR-1 (CIAF1) deletion causes growth delay associated with CI defect

T-DNA insertion mutant line of a Complex I assembly factor (*ciaf1*) was confirmed by complementation



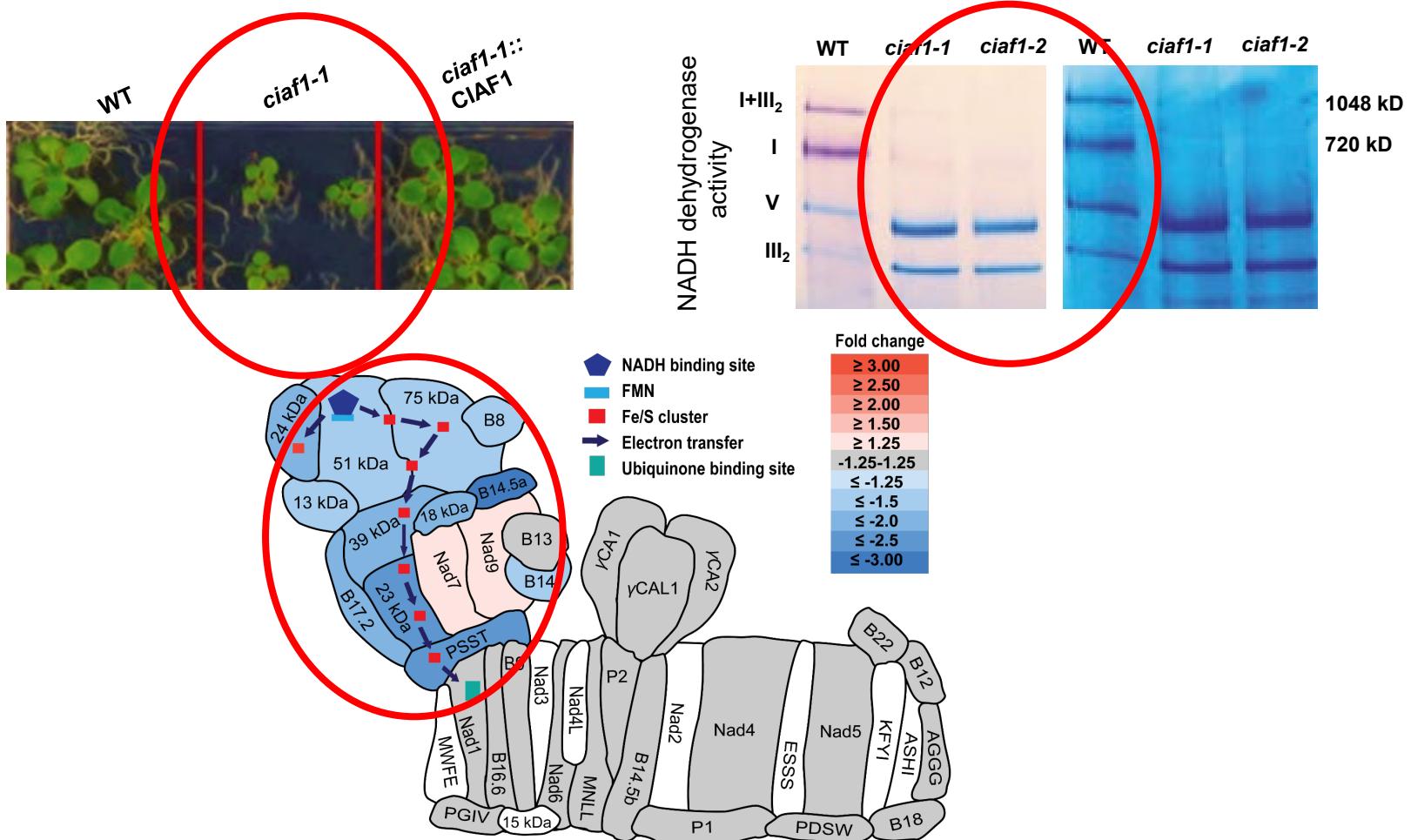
COMPLEX I ASSEMBLY FACTOR-1 (CIAF1) deletion causes growth delay associated with CI defect

*T-DNA insertion mutant line of a Complex I assembly factor (*ciaf1*) showed retarded growth and almost complete lack of complex I*



COMPLEX I ASSEMBLY FACTOR-1 (CIAF1) deletion causes growth delay associated with CI defect

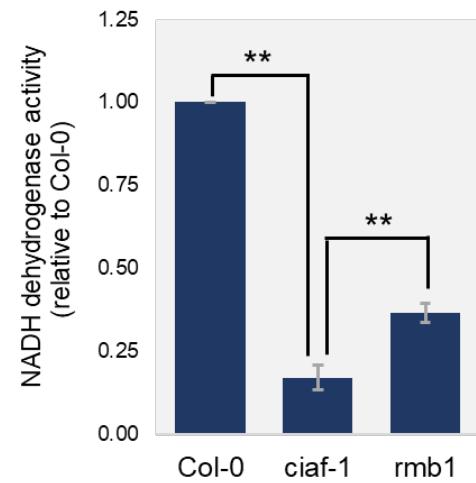
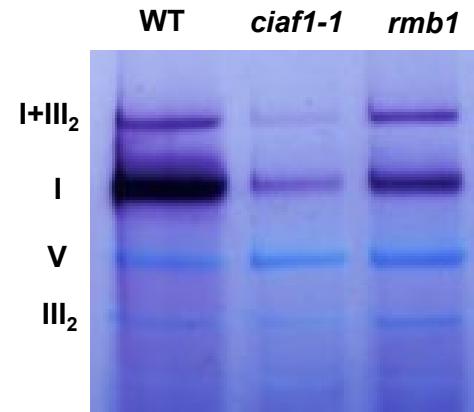
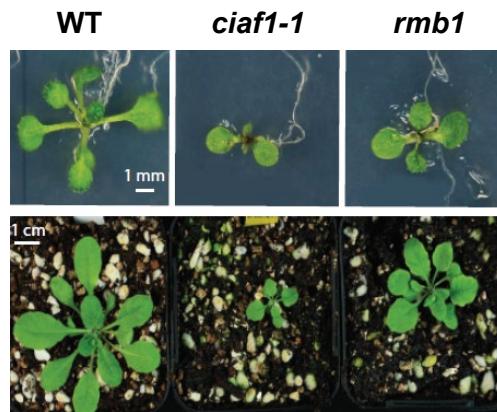
*T-DNA insertion mutant line of a Complex I assembly factor (*ciaf1*)
Provides for a genetic background to find restorers of CI assembly*



Forward genetics approach: random EMS mutagenesis

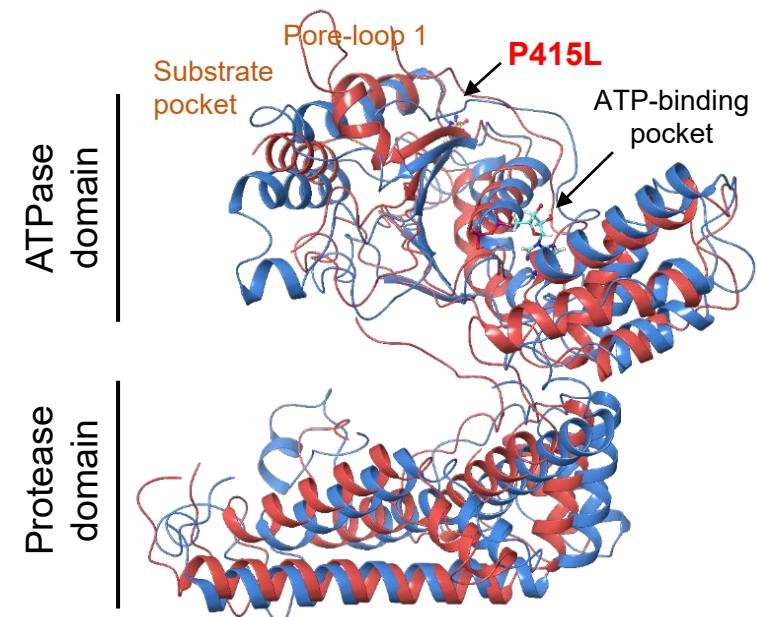
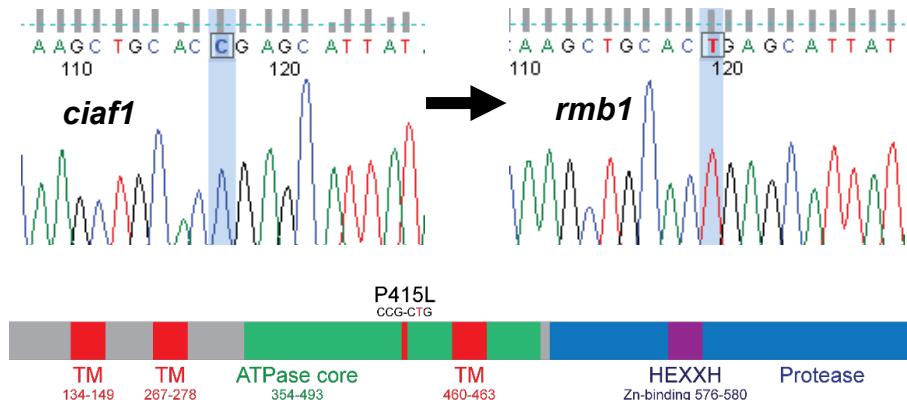


RMB1 growth restoration corresponds to Complex I restoration



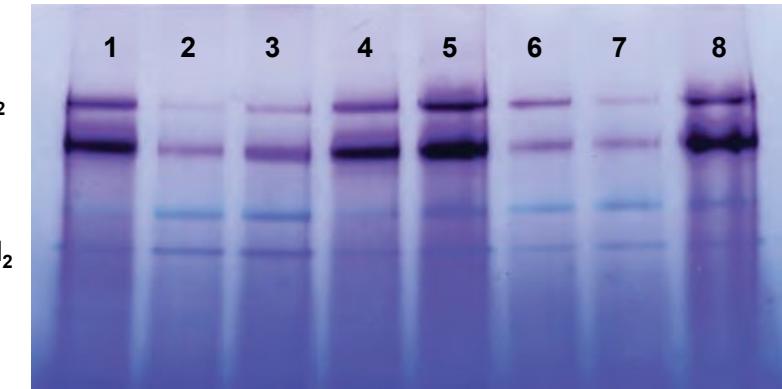
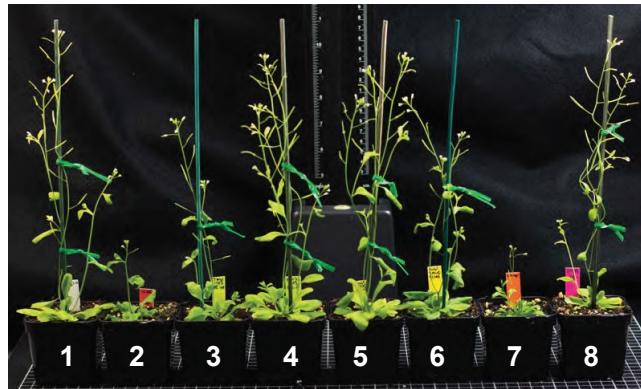
RMB1 has a single amino acid mutation in FTSH3 ATPase domain

FTSH3: a matrix-facing inner-membrane bound AAA+ protease



- ATPase-Associated with various Activity (AAA+) superfamily
- FTSH (AAA) family members found as hexamer
- Uses ATP to recognise and unfold substrate, translocate to proteolytic domain

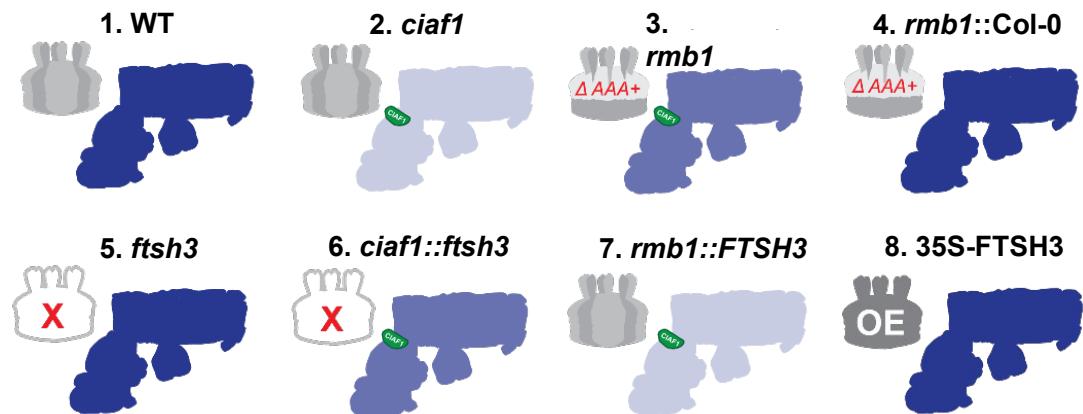
Single point mutation in FTSH3 AAA domain restore CI in *ciaf1*



↓
**Mitochondrial isolation
Complex solubilisation**

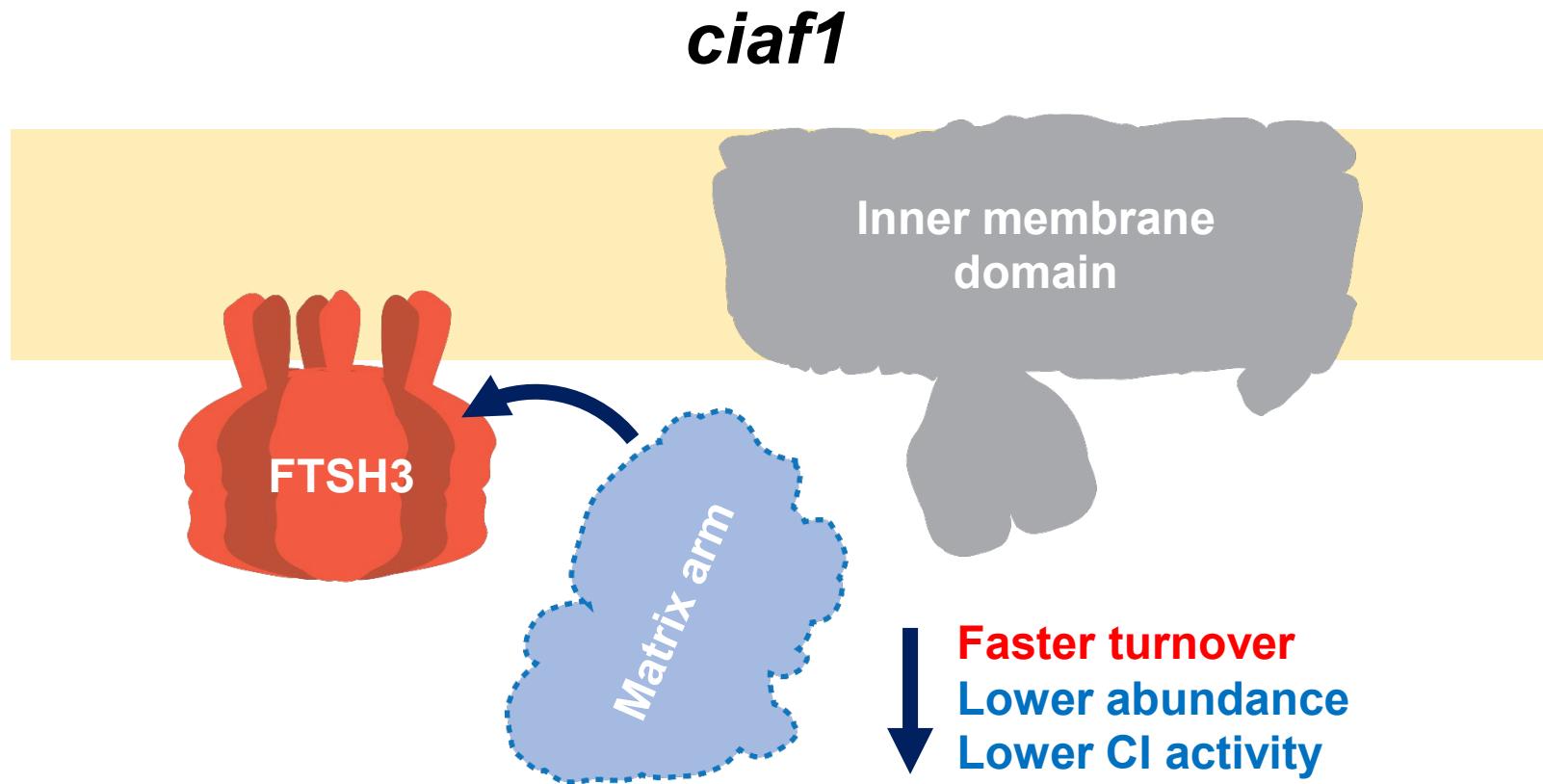
**Blue Native PAGE
Complex I (NADH dehydrogenase)
activity stain**

 NADH → NBTH₂ (diformazan)



Ivanova, Ghifari, Berkowitz, Whelan, Murcha (2021) *Plant Phys.*

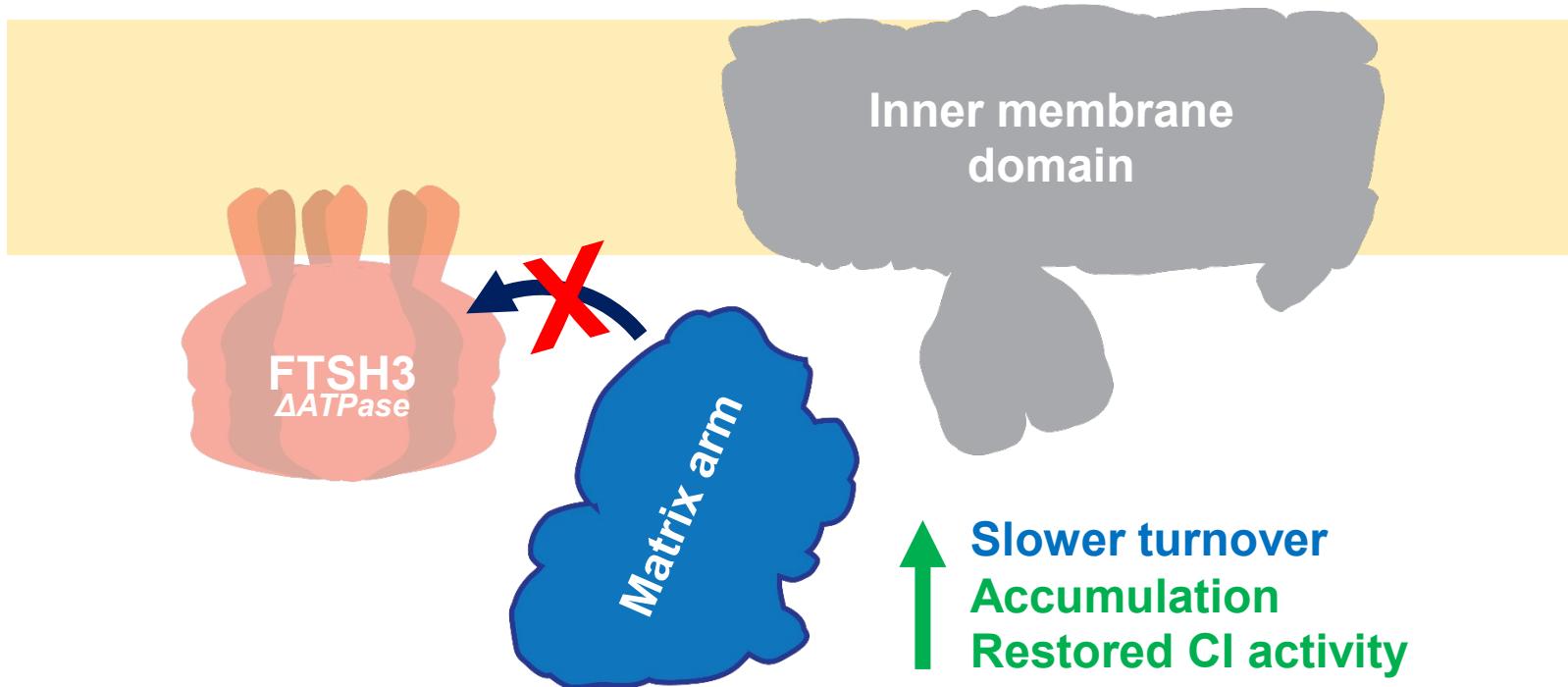
Possible mechanism



When CIAF1 is missing, NQ module unassembled and dissociated by FTSH3

Possible mechanism

rmb1 (FTSH3 Δ ATPase)

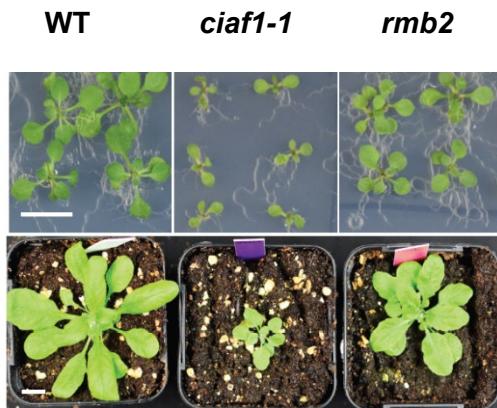


**Mutation in FTSH3 ATPase slows turnover and accumulates NQ module
→ restored Complex I activity**

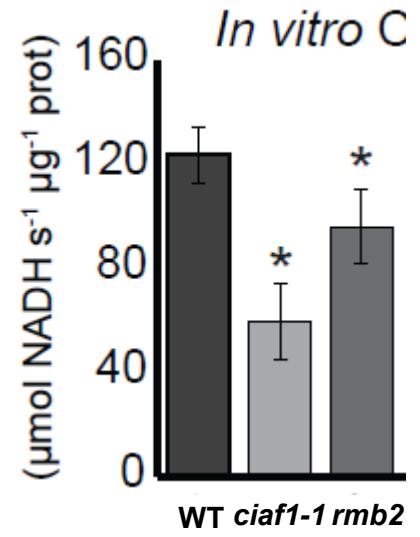
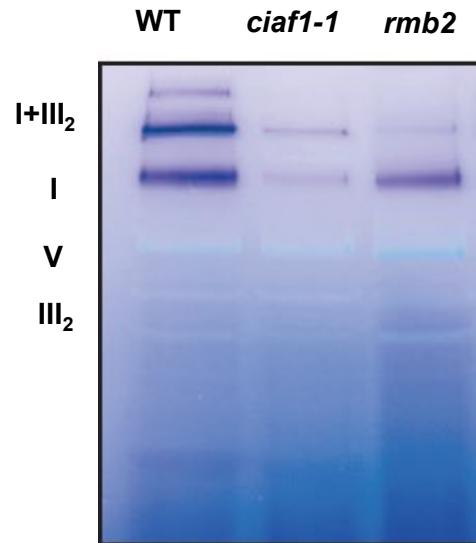
RMB2 a second EMS mutant of *ciaf1*!



Rmb2 growth restoration corresponds to Complex I restoration

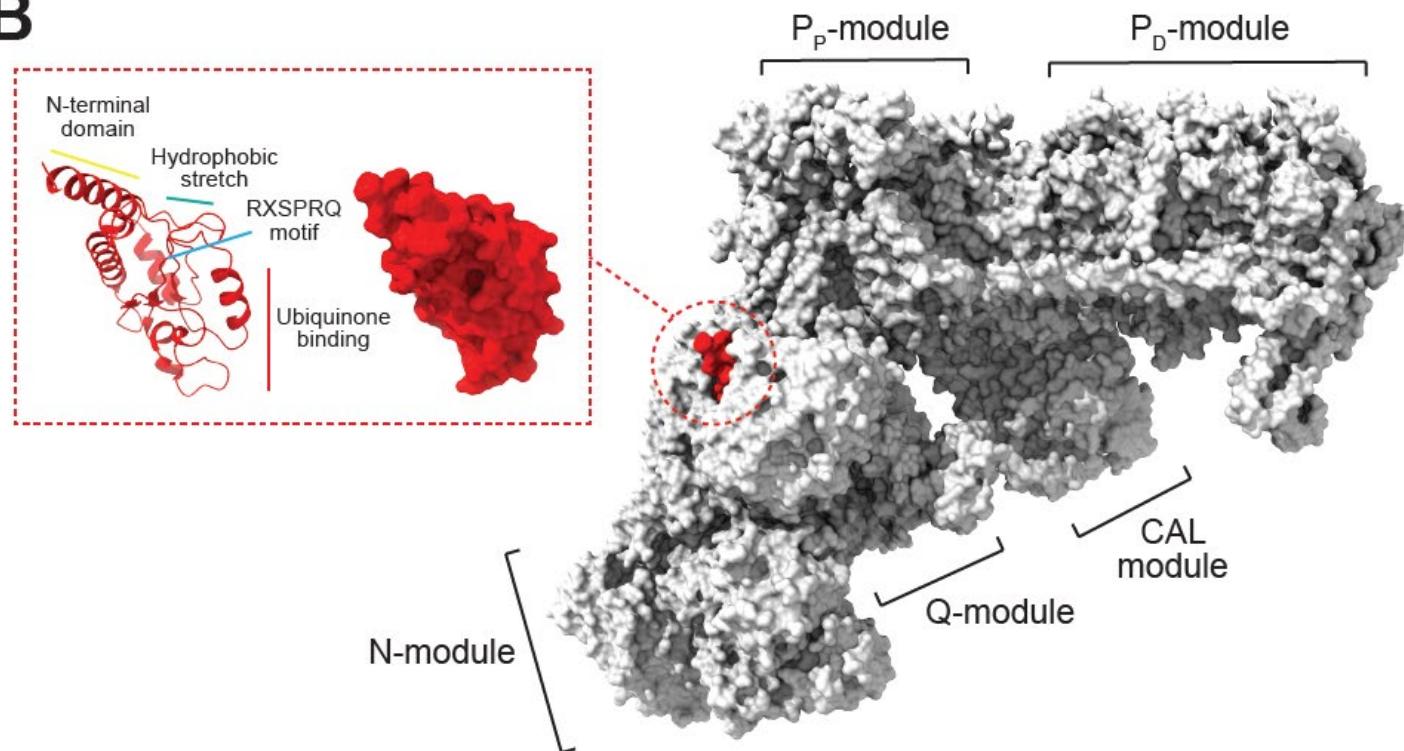


Ghofari et al. unpublished



RMB2 is a complex I subunit

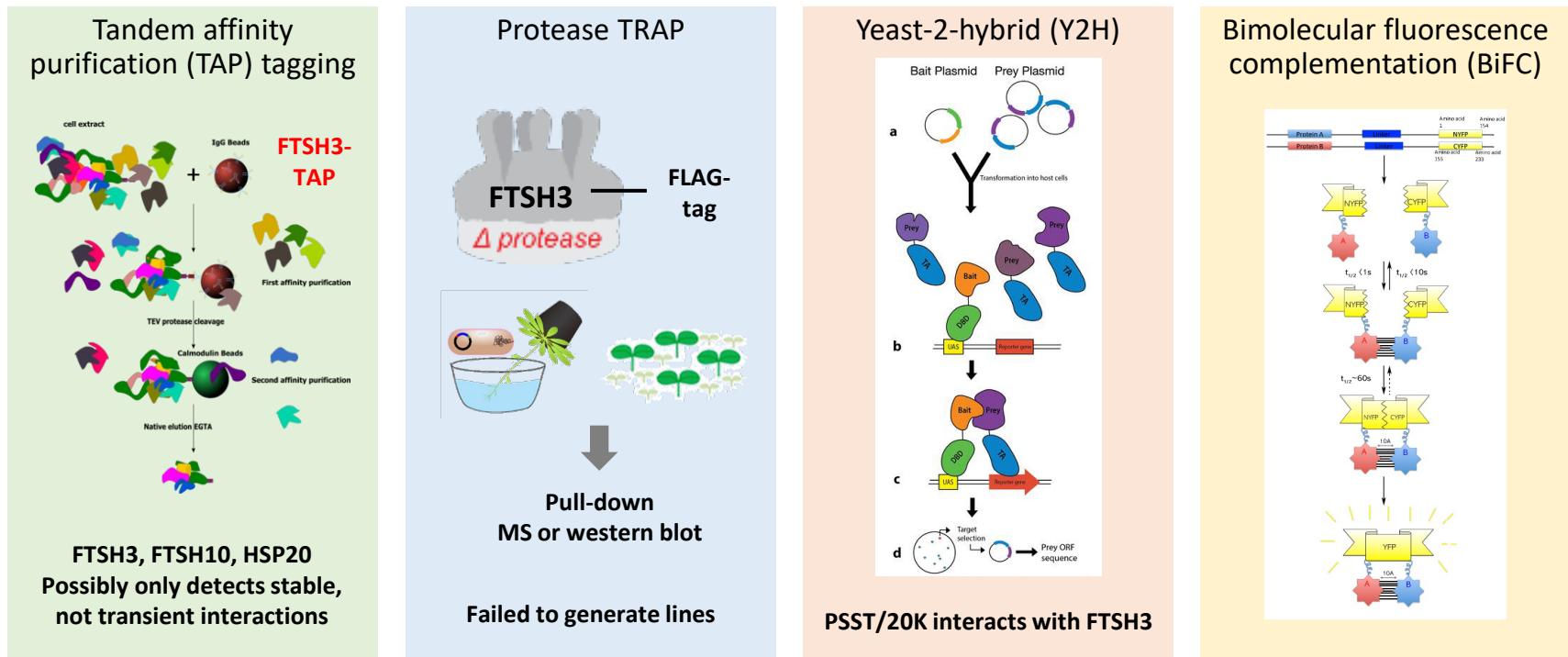
B



***rmb2* is a single aa mutation
within a Complex I subunit**

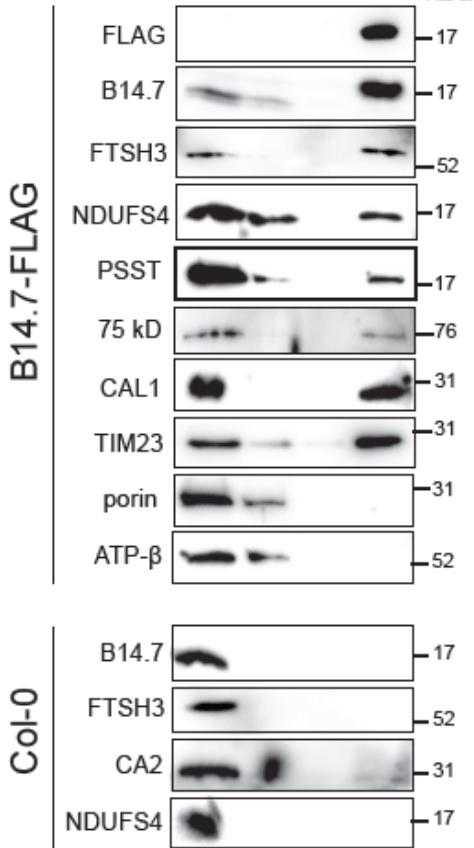
PSST as a possible FTSH3 substrate

Biomolecular approach: Protein-protein interaction

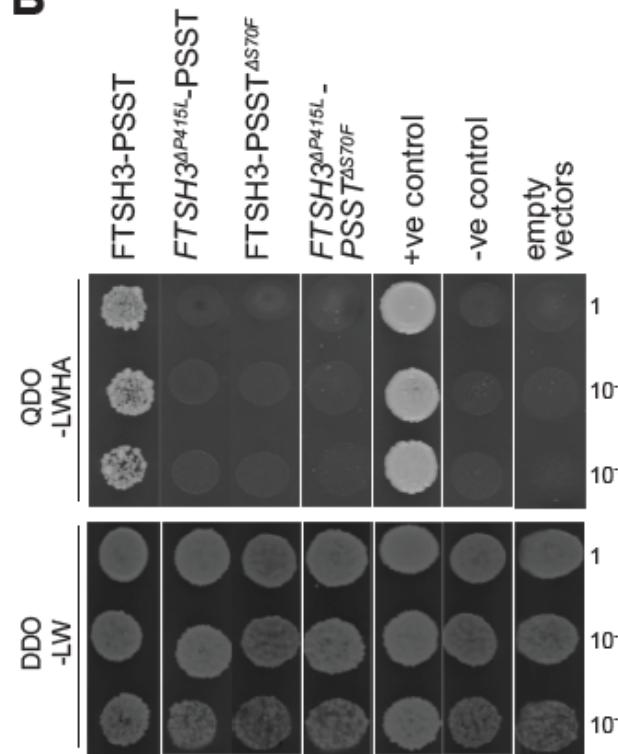


RMB2 interacts with RMB1 (FTSH3)

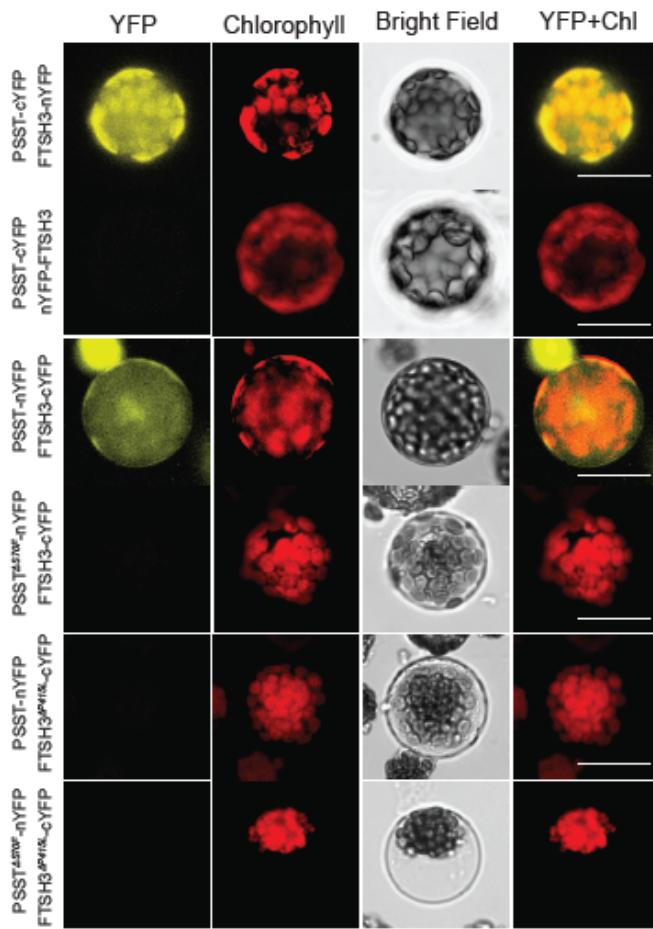
A



B



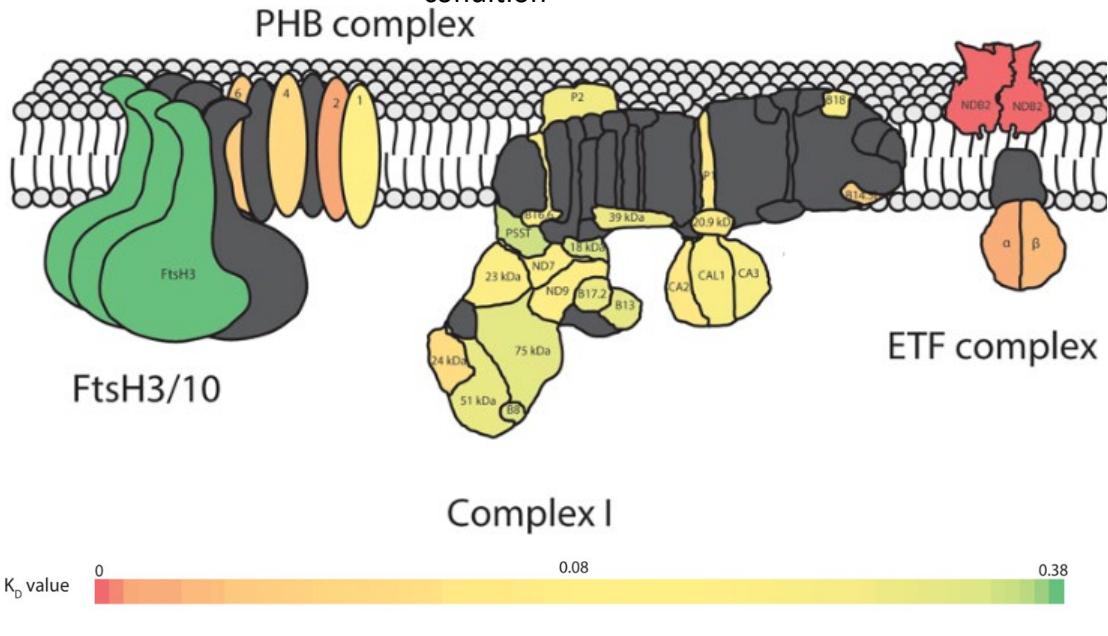
C



Impact on Complex I subunits turnover?

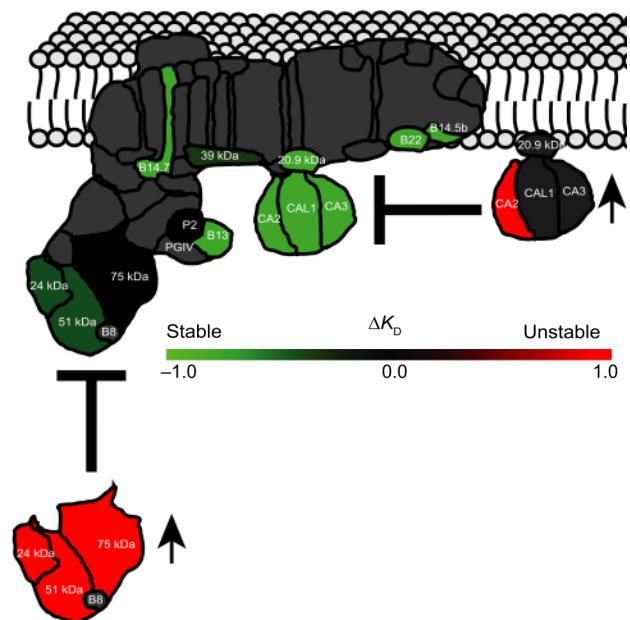
Approach: mass spectrometry proteomics using heavy nitrogen (^{15}N) labelling

FTSH3 and Complex I NQ module are rapidly turnover in native condition



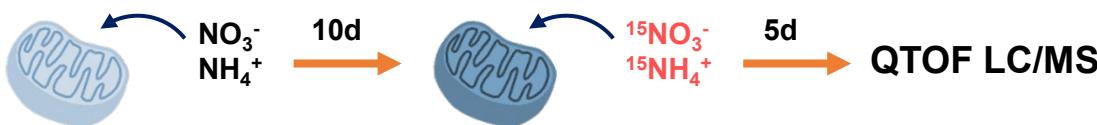
Nelson, Li, Jacoby, Millar (2013) *J Proteome Res*

Deletion of LON1 protease slows the turnover of non-assembled NQ & CAL modules

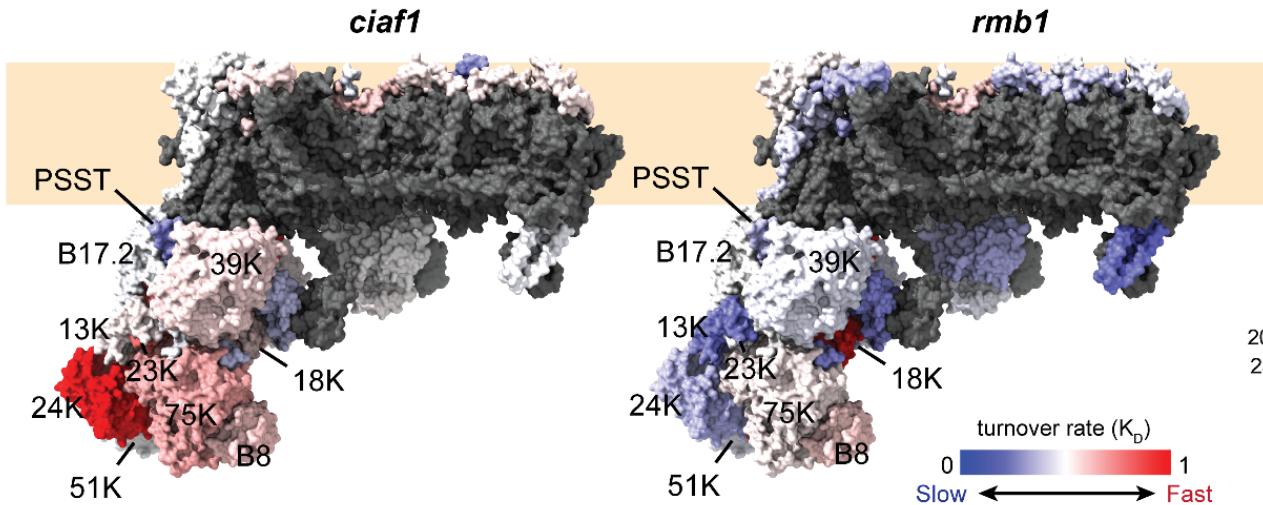


Li, et al. (2017) *Plant Journal*

Complex I subunit turnover rates



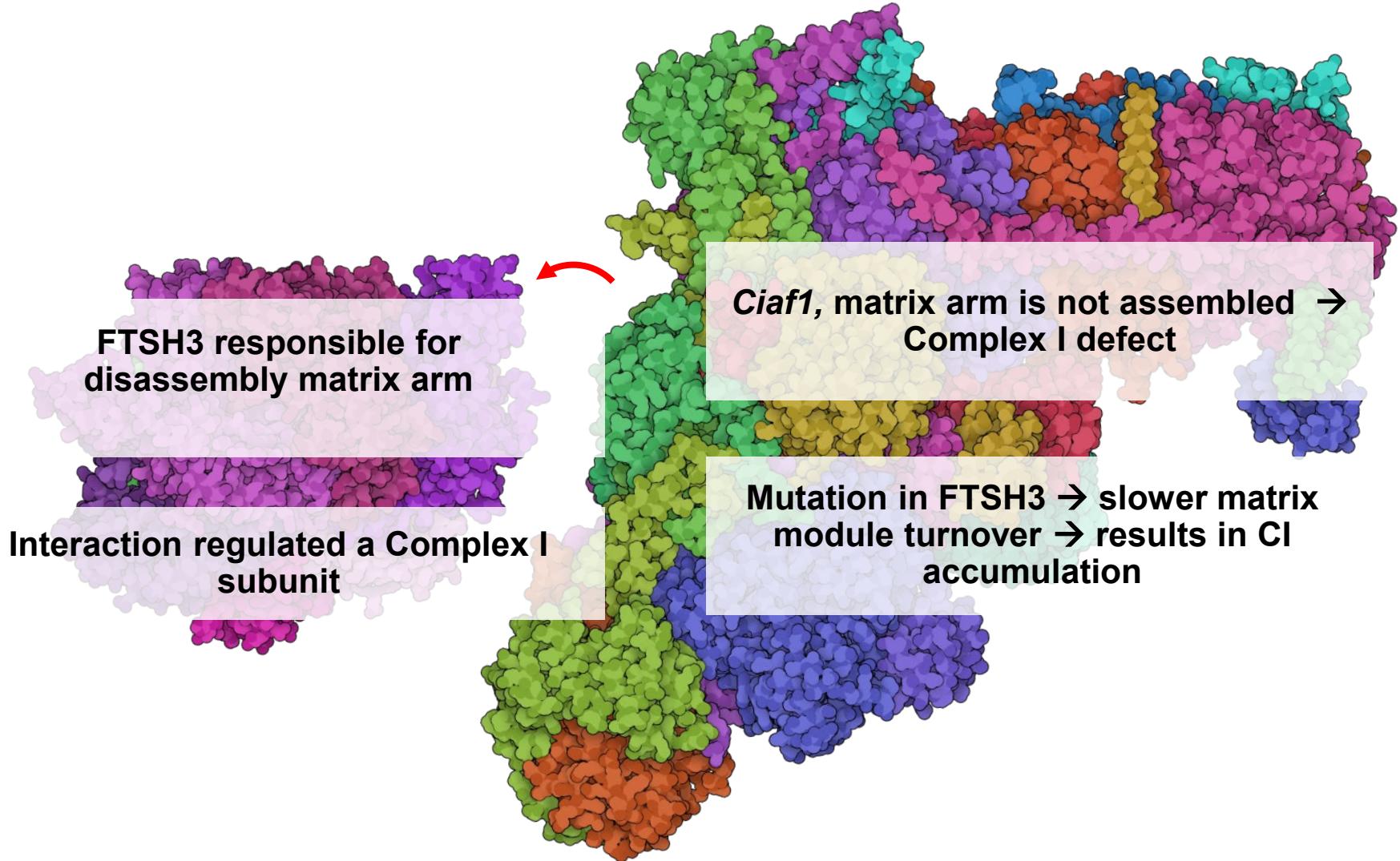
Li, et al. (2017) *Plant J.* 89(3): 458-471



	<i>ciaf1</i>	<i>rmb1</i>	ΔK_D		<i>ciaf1</i>	<i>rmb1</i>	ΔK_D		
N module	75K 0.61	0.49	-0.12	Nad7 0.81	0.83	0.02	Nad7 0.81	0.83	0.02
	51K 0.45	0.42	-0.03	Nad9 0.59	0.35	-0.25	Nad9 0.59	0.35	-0.25
	24K 0.91	0.32	-0.59	P1 0.56	0.55	-0.01	P1 0.56	0.55	-0.01
	18K 0.52	0.88	0.36	P2 0.47	0.43	-0.03	P2 0.47	0.43	-0.03
	13K 0.49	0.23	-0.26	PGIV-1 0.45	0.48	0.03	PGIV-1 0.45	0.48	0.03
B17.2	0.44	0.46	0.02	B16.6-2 0.52	0.31	-0.20	B16.6-2 0.52	0.31	-0.20
	B8 0.55	0.54	0.00	MWFE 0.45	0.38	-0.07	MWFE 0.45	0.38	-0.07
	B14.5a 0.47	0.43	-0.04	B9 0.38	0.35	-0.03	B9 0.38	0.35	-0.03
	B14 0.37	0.28	-0.09	ESSS-1 0.25	0.42	0.17	ESSS-1 0.25	0.42	0.17
	B13 0.51	0.39	-0.11	ESSS-2 0.25	0.42	0.17	ESSS-2 0.25	0.42	0.17
	39K 0.52	0.42	-0.10	PDSW-1 0.50	0.35	-0.15	PDSW-1 0.50	0.35	-0.15
	20K (PSST) 0.26	0.34	0.07	PDSW-2 0.45	0.38	-0.07	PDSW-2 0.45	0.38	-0.07
	23K (TYKY) 0.71	0.46	-0.25	B14.5b 0.42	0.42	0.01	B14.5b 0.42	0.42	0.01
	CA2 0.47	0.43	-0.03	B18 0.50	0.41	-0.09	B18 0.50	0.41	-0.09
CAL	CAL2 0.46	0.34	-0.12	B22 0.44	0.21	-0.23	B22 0.44	0.21	-0.23

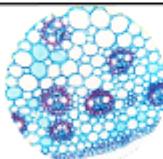
Matrix arm domain display a faster turnover rate in *ciaf1*
Mutation of FTSH3 ATPase slowed the turnover rate (more stable subunits)

Mechanism of Complex I turnover



Summary II:

- Having multiple mutants available to examine the role of a specific gene can be advantageous, as it enables the study of dosage effects on plant phenotype.
- Additionally, examining the function of a particular gene in various plant species can provide insight into whether a mutation yields distinct consequences, such as in dicots versus monocots.

MONOCOT	DICOT
Single Cotyledon 	Two Cotyledon 
Long Narrow Leaf Parallel Veins 	Broad Leaf Network of Veins 
Vascular Bundles Scattered 	Vascular Bundles in a Ring 
Floral Parts in Multiples of 3 	Floral Parts in Multiples of 4 or 5 

- Reverse genetics is an effective approach, as demonstrated by the success of the *Arabidopsis* T-DNA insertion collection.
- BUT! The generation of reverse genetic resources by insertion mutagenesis is labour- and time-consuming in other plant species.
- A major limitation of the T-DNA-based technique is the requirement for a highly efficient transformation system to generate a large number of transgenic lines.
Unfortunately, in many plant species, *Agrobacterium*-based transformation is either not yet developed or is not efficient enough to produce a sufficient number of T-DNA lines that would allow a genome-wide gene tagging.

Generation of genetic resources by using the mobility of (retro)transposons

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Genome-wide *LORE1* retrotransposon mutagenesis and high-throughput insertion detection in *Lotus japonicus*

Dorian Fabian Urbanski, Anna Małolepszy, Jens Stougaard, Stig Uggerhøj Andersen 

First published: 1 December 2011 Full publication history
DOI: 10.1111/j.1365-313X.2011.04827.x [View/save citation](#)
Cited by (CrossRef): 52 articles [Check for updates](#) | [Citation tools](#)

  (fax +45 86123178; e-mail sua@mb.au.dk).

Summary

Use of insertion mutants facilitates functional analysis of genes, but it has been difficult to identify a suitable mutagen and to establish large populations for reverse genetics in most plant species. The main challenge is developing efficient high-throughput procedures for both mutagenesis and identification of insertion sites. To date, only floral-dip T-DNA transformation of *Arabidopsis* has produced independent germinal insertions, thereby allowing generation of mutant populations from seeds of single plants. In addition, advances in insertion detection have been hampered by a lack of protocols, including software for automated data analysis, that take full advantage of high-throughput next-generation sequencing. We have addressed these challenges by developing the *FSTpoolit* protocol and software package, and here we demonstrate its efficacy by detecting 8935 *LORE1* insertions in 3744 *Lotus japonicus* plants. The identified insertions show that the endogenous *LORE1* retrotransposon is well suited for insertion mutagenesis due to homogenous gene targeting and exonic insertion preference. As *LORE1* transposition occurs in the germline, harvesting seeds from a single founder line and cultivating progeny generates a complete mutant population. This ease of *LORE1* mutagenesis, combined with the efficient *FSTpoolit* protocol, which exploits 2D pooling, Illumina sequencing and automated data analysis, allows highly cost-efficient development of a comprehensive reverse genetic resource.



[View issue TOC](#)
Volume 69, Issue 4
February 2012
Pages 731–741

[Plant Physiol.](#) 2013 Sep; 163(1): 21–29.

Published online 2013 Jul 29. doi: [10.1104/pp.113.221903](https://doi.org/10.1104/pp.113.221903)

PMCID: PMC3762642

Insertional Mutagenesis Using *Tnt1* Retrotransposon in Potato^{1,[OPEN]}

Saowapa Duangpan, Wenli Zhang, Yufang Wu, Shelley H. Jansky, and Jiming Jiang*

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ABSTRACT

Go to:

Insertional mutagenesis using transfer DNA or transposable elements, which is an important tool in functional genomics and is well established in several crops, has not been developed in potato (*Solanum tuberosum*). Here, we report the application of the tobacco (*Nicotiana tabacum*) *Tnt1* retrotransposon as an insertional mutagen in potato. The *Tnt1* retrotransposon was introduced into a highly homozygous and self-compatible clone, 523-3, of the diploid wild potato species *Solanum chacoense*. Transposition of the *Tnt1* elements introduced into 523-3 can be efficiently induced by tissue culture. *Tnt1* preferentially inserted into genic regions in the potato genome and the insertions were stable during sexual reproduction, making *Tnt1* an ideal mutagen in potato. Several distinct phenotypes associated with plant stature and leaf morphology were discovered in mutation screening from a total of 38 families derived from *Tnt1*-containing lines. We demonstrate that the insertional mutagenesis system based on *Tnt1* and the 523-3 clone can be expanded to the genome-wide level to potentially tag every gene in the potato genome.

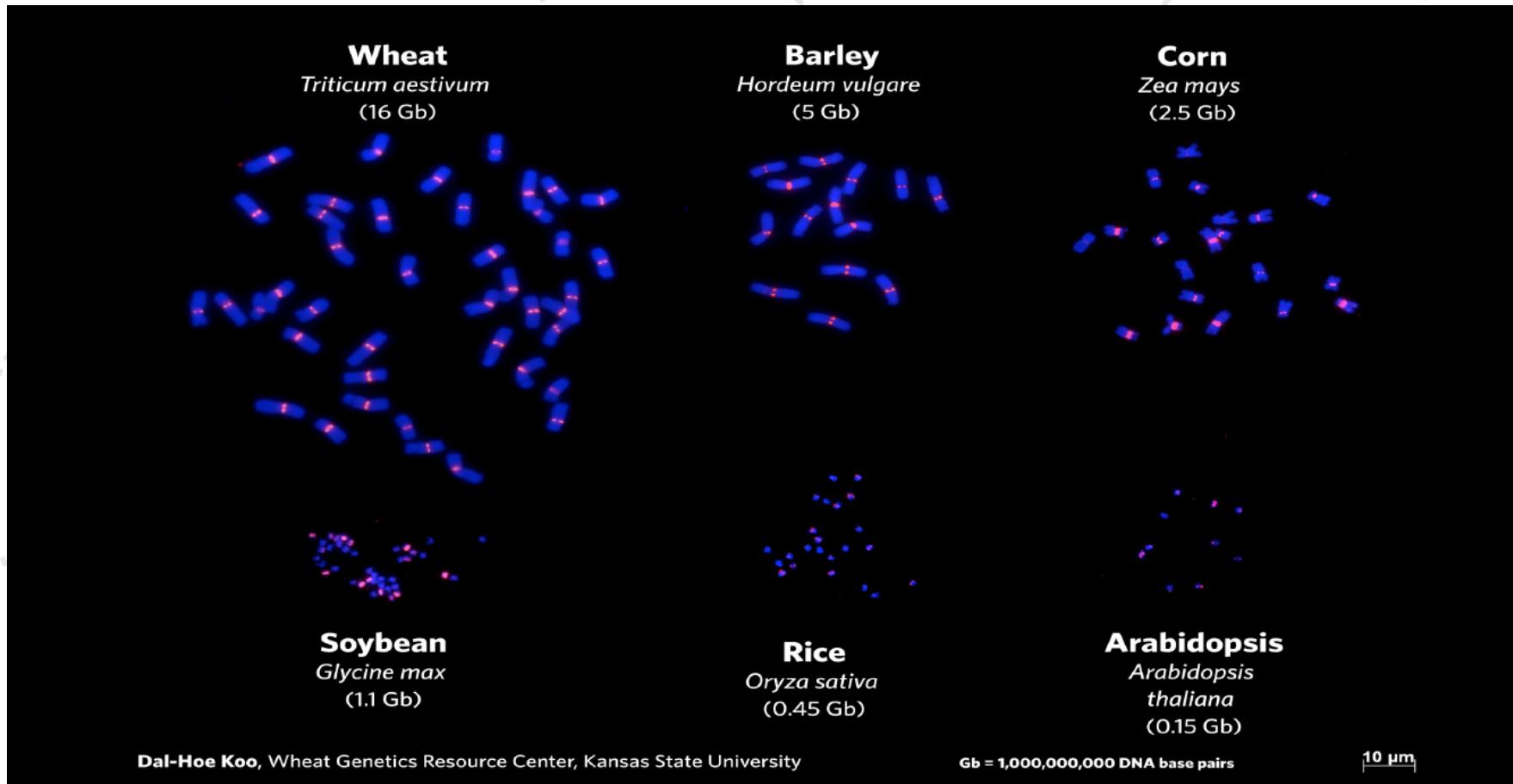
Mutant collections in plant species other than Arabidopsis

Comprehensive reverse genetic resources (e.g. T-DNA mutant collection), which have been key to understanding gene function in diploid model organisms, are missing in many staple crops like wheat and barley

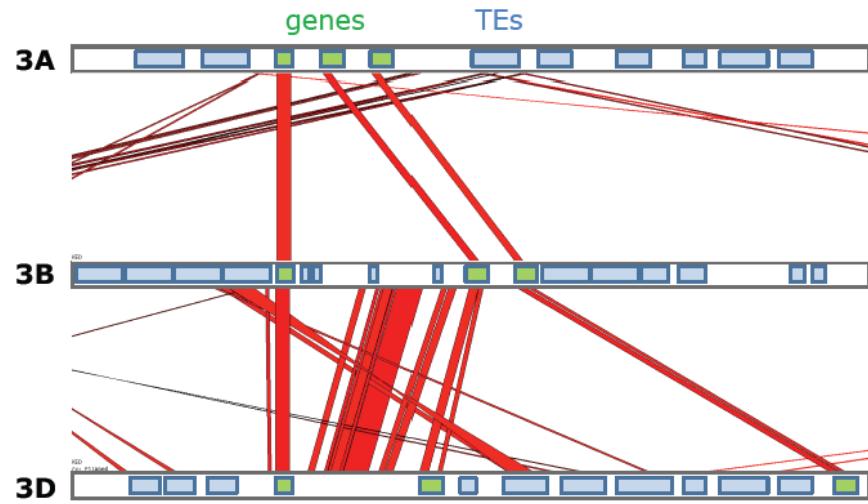
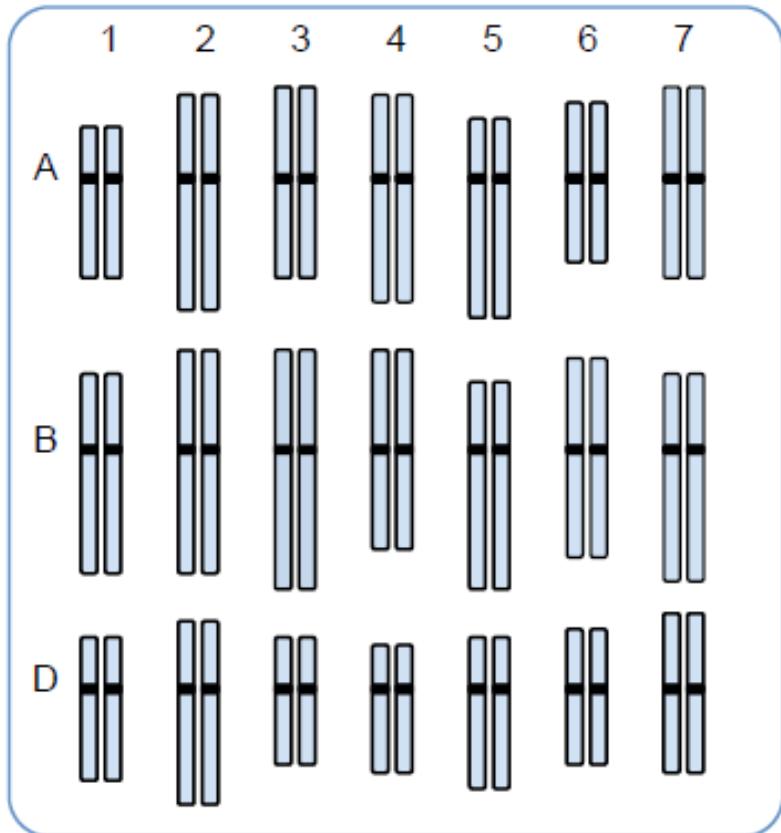
Why?

Mutant collections in plant species other than Arabidopsis

Unusually large and complex genomes of cereals



The complexity of wheat genome



Earlham Institute

TGAC 
The Genome Analysis Centre™

Frédéric Choulet
GDEC, INRA, UCA, Clermont-Ferrand, France

An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations

RESEARCH ARTICLE SUMMARY

WHEAT GENOME

Shifting the limits in wheat research and breeding using a fully annotated reference genome

International Wheat Genome Sequencing Consortium (IWGSC)*

INTRODUCTION: Wheat (*Triticum aestivum* L.) is the most widely cultivated crop on Earth, contributing about a fifth of the total calories consumed by humans. Consequently, wheat yields and production affect the global economy, and failed harvests can lead to social unrest. Breeders continuously strive to develop improved varieties by fine-tuning genetically complex yield and end-use quality parameters while maintaining stable yields and adapting the crop to regionally specific biotic and abiotic stresses.

RATIONALE: Breeding efforts are limited by insufficient knowledge and understanding of

wheat biology and the molecular basis of central agronomic traits. To meet the demands of human population growth, there is an urgent need for wheat research and breeding to accelerate genetic gain as well as to increase and protect wheat yield and quality traits. In other plant and animal species, access to a fully annotated and ordered genome sequence, including regulatory sequences and genome-diversity information, has promoted the development of systematic and more time-efficient approaches for the selection and understanding of important traits. Wheat has lagged behind, primarily owing to the challenges of assembling a genome that is more than five times as large

as the human genome, polyploid, and complex, containing more than 85% repetitive DNA. To provide a foundation for improvement through molecular breeding, in 2005 the International Wheat Genome Sequencing Consortium (IWGSC) set out to deliver a high-quality reference genome sequence.

RESULTS: A genome assembly representing 95% of the genome in the Chinese Spring reference sequence assembly was completed, giving access to 16 chromosomes, including the 21 wheat pseudochromosomes. This assembly includes 95% of tissue- and organ-specific coexpression data from a transcriptome atlas representing 100 tissues and organs. The dynamic

The tragic ripples of
an epic fraud pp. 636

Insect pest profits from
maize defenses pp. 642 & 694

Photoredox activation
of methane pp. 647 & 668

Science

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ROAD MAP FOR WHEAT

Ordered sequence will
speed research pp. 635, 661, & 662

Mutant collections in plant species other than Arabidopsis

Comprehensive reverse genetic resources (e.g. T-DNA mutant collection), which have been key to understanding gene function in diploid model organisms, are missing in many staple crops like wheat and barley

Why:

- complexity of the genomes (polyploidy -> wheat or big size -> barley) makes genome assemblies difficult, as a result no reference genome sequences available for e.g. wheat (but soon!) it's coming!
- long life cycle of wheat or barley slows down the studies ...

BUT! Researchers are looking for new solutions!



Uncovering hidden variation in polyploid wheat

Ksenia V. Krasileva^{a,b,c}, Hans A. Vasquez-Gross^a, Tyson Howell^a, Paul Bailey^c, Francine Paraiso^a, Leah Clissold^c, James Simmonds^d, Ricardo H. Ramirez-Gonzalez^{c,d}, Xiaodong Wang^a, Philippa Borrill^d, Christine Fosker^c, Sarah Ayling^c, Andrew L. Phillips^e, Cristobal Uauy^{d,1,2}, and Jorge Dubcovsky^{a,f,1,2}

^aDepartment of Plant Sciences, University of California, Davis, CA 95616; ^bThe Sainsbury Laboratory, Norwich NR4 7UH, United Kingdom; ^cThe Earlham Institute, Norwich NR4 7UG, United Kingdom; ^dJohn Innes Centre, Norwich NR4 7UH, United Kingdom; ^eRothamsted Research, Harpenden AL5 2JQ, United Kingdom; and ^fHoward Hughes Medical Institute, Chevy Chase, MD 20815

Contributed by Jorge Dubcovsky, December 20, 2016 (sent for review November 22, 2016; reviewed by Beat Keller and Joachim Messing)

Comprehensive reverse genetic resources, which have been key to understanding gene function in diploid model organisms, are missing in many polyploid crops. Young polyploid species such as wheat, which was domesticated less than 10,000 y ago, have high levels of sequence identity among subgenomes that mask the effects of recessive alleles. Such redundancy reduces the probability of selection of favorable mutations during natural or human selection, but also allows wheat to tolerate high densities of induced mutations. Here we exploited this property to sequence and catalog more than 10 million mutations in the protein-coding regions of 2,735 mutant lines of tetraploid and hexaploid wheat. We detected, on average, 2,705 and 5,351 mutations per tetraploid and hexaploid line, respectively, which resulted in 35–40 mutations per kb in each population. With these mutation densities, we identified an average of 23–24 missense and truncation alleles per gene, with at least one truncation or deleterious missense

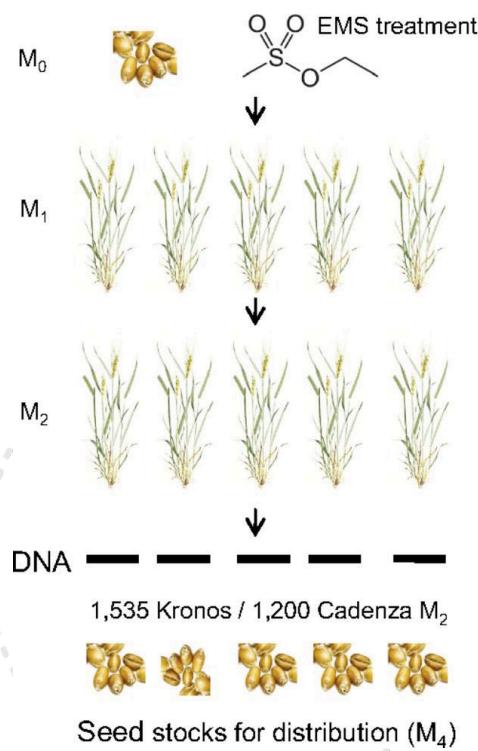
which entail the development and optimization of genome-specific primers for each target gene. A pilot study using three Cadenza lines with known mutations in the *GAI20α* gene and a small capture array including 1,846 genes demonstrated that exome capture (7) followed by sequencing was a viable strategy to identify mutations in wheat (8). Whole-genome resequencing of mutant lines also has been used for species with small genomes (9), but is a very expensive alternative for the large genomes of tetraploid (12 Gb) and hexaploid (17 Gb) wheat (10).

In this study, we describe the development of a wheat exome capture platform and its use to sequence the coding regions of 2,735 mutant lines. We characterized the obtained mutations, organized them in a public database including more than 10 million mutations, identified deleterious alleles for ~90% of the captured wheat genes, and discuss potential applications.

as soon as first draft of a genome arrives it is possible to generate mutant collections as it is possible to identify where in the genome (in which gene) the mutations are located (by using DNA-seq technologies)

TILLING (Target Induced Local Lesions in Genomes) mutant collection in wheat

a collection of EMS-mutants in wheat ...



EMS-treatment (to induce mutations) of a wheat accession

Ethyl methanesulfonate

DNA sequencing (to identify positions of the mutations)



Welcome to the *in silico* wheat Target Induced Local Lesions In Genome (TILLING) website

This resource consists of TILLING populations developed in tetraploid durum wheat cv 'Kronos' and hexaploid bread wheat cv 'Cadenza' as part of a joint project between the University of California Davis, Rothamsted Research, The Earlham Institute, and John Innes Centre.

We have re-sequenced the exome of 1,535 Kronos and 1,200 Cadenza mutants using Illumina next-generation sequencing, aligned this raw data to the IWGSC Chinese Spring chromosome arm survey sequence, identified mutations, and predicted their effects based on the protein annotation available at Ensembl Plants.

Search TILLING data

BLAST Scaffold

Population Cadenza Kronos Both

Type in list of search terms (scaffold, line or gene)

Search the database by:gene (eg. *Traes_1AL_9EC1E6FOC*;
Traes_1AL_9EC1E6FOC.1), scaffold (eg. *IWGSC_CSS_2AL_scaff_6343779*;
2AL_6343779) or, mutant line (eg. *Cadenza0250*)

Examples: *Traes_1AL_9EC1E6FOC*, *Traes_1AL_9EC1E6FOC.1*, *IWGSC_CSS_2AL_scaff_6343779*, *2AL_6343779*,
Cadenza0250

Paste query sequence(s) or drag file containing query sequence(s) in FASTA format here ...

EMS – chemical mutagen

Collection of EMS-mutants in tetraploid wheat



John Innes Centre
Unlocking Nature's Diversity

Earlham Institute
Decoding Living Systems



hhmi
Howard Hughes
Medical Institute



Export the displayed mutations for local analysis: Show lower quality mutations

Scaffold	C...	Line	Category	Pos	Chr	pos	rf	wt	mt	Het	Hom	WT cov	Mut cov	Gene	Consequence	cD...	CDS pos	Ami...	Cod...	SI...	sc...	Primer type
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2299	het5hom3	3,104	243,762,869	G	G	A	hom	0	9	Traes_2AL_0380ABB5D.1	5_prime_UTR_variant	502								
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos944	het5hom3	3,155	243,762,920	C	C	T	het	22	17	Traes_2AL_0380ABB5D.1	5_prime_UTR_variant	553								nonspecific
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2472	het5hom3	3,166	243,762,931	C	C	T	het	9	10	Traes_2AL_0380ABB5D.1	5_prime_UTR_variant	564								nonspecific
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos809	het5hom3	3,186	243,762,951	C	G	A	het	11	6	Traes_2AL_0380ABB5D.1	5_prime_UTR_variant	584								nonspecific
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2044	het5hom3	3,235	243,763,000	G	G	A	het	6	10	Traes_2AL_0380ABB5D.1	missense_variant	633	25	A/T	Gct/...	0.01			nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2477	het5hom3	3,241	243,763,006	C	C	T	hom	0	24	Traes_2AL_0380ABB5D.1	missense_variant	639	31	L/F	Ctc/...	0			nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos4520	het5hom3	3,241	243,763,006	C	C	T	hom	0	38	Traes_2AL_0380ABB5D.1	missense_variant	639	31	L/F	Ctc/...	0			nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos685	het5hom3	3,265	243,763,030	C	C	T	hom	0	60	Traes_2AL_0380ABB5D.1	missense_variant	663	55	L/F	Ctc/...	0.8			specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2414	het5hom3	3,288	243,763,053	G	G	A	hom	0	31	Traes_2AL_0380ABB5D.1	synonymous_variant	686	78		gaG/...					
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2569	het5hom3	3,337	243,763,102	C	C	T	het	16	23	Traes_2AL_0380ABB5D.1	intron_variant								specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos3921	het5hom3	3,337	243,763,102	C	C	T	het	19	14	Traes_2AL_0380ABB5D.1	intron_variant								specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos3633	het5hom3	3,408	243,763,173	C	C	T	het	21	19	Traes_2AL_0380ABB5D.1	intron_variant								specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos1253	het5hom3	3,501	243,763,266	G	G	A	het	3	5	Traes_2AL_0380ABB5D.1	intron_variant								nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos3840	het5hom3	3,896	243,763,661	G	G	A	hom	0	12	Traes_2AL_0380ABB5D.1	intron_variant								specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos708	het5hom3	3,948	243,763,713	C	C	T	het	7	12	Traes_2AL_0380ABB5D.1	intron_variant									
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos3898	het5hom3	4,002	243,763,767	G	G	A	het	28	32	Traes_2AL_0380ABB5D.1	intron_variant								nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos2423	het5hom3	4,044	243,763,809	G	G	A	het	49	45	Traes_2AL_0380ABB5D.1	splice_acceptor_variant								nonspecific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos499	het5hom3	4,059	243,763,824	C	C	T	hom	0	47	Traes_2AL_0380ABB5D.1	synonymous_variant	710	102		aaC/...				specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos3932	het5hom3	4,069	243,763,834	C	C	T	het	55	38	Traes_2AL_0380ABB5D.1	missense_variant	720	112	P/S	Cca/...	0			specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos229	het5hom3	4,074	243,763,839	C	C	T	het	25	37	Traes_2AL_0380ABB5D.1	synonymous_variant	725	117		ttC/t...				specific	
IWGSC_CSS_2AL_scaff_6343779	2A	Kronos1315	het5hom3	4,092	243,763,857	G	G	A	hom	0	43	Traes_2AL_0380ABB5D.1	synonymous_variant	743	135		ggG/...				specific	

Additional Resources

TILLING collections in other plant species:

TILLING (Target Induced Local Lesions in Genomes)

TILLING centres

Several TILLING centres exist over the world that focus on agriculturally important species:

- Rice – UC Davis (USA)
- Maize – Purdue University (USA)
- *Brassica rapa* – John Innes Centre (UK)
- Soybean – Southern Illinois University (USA)
- Lotus and Medicago – John Innes Centre (UK)
- Wheat – UC Davis (USA)
- Pea, Tomato - INRA (France)
- Tomato - University of Hyderabad (India)
- Arabidopsis – Fred Hutchinson Cancer Research

Reverse Genetics

RevGenUK is a TILLING service we offer to the plant science research community.

We find mutations in your favourite gene.

We offer a bespoke TILLING service on our populations of mutagenized model and crop species. You let us know what gene you would like to investigate and we will pick the optimum region to TILL, design the primers, carry out the TILLING and return to you a list of mutations. You can then pick the mutations you are interested in and request seeds of the lines bearing those mutations.

We also have populations of re-sequenced lines (with suffix RS) - search the database for mutations in your gene of interest.

The Reverse Genetics equipment and services at the John Innes Centre include:

- TILLING (service)
- ABI3730XL used for sequence confirmation
- Fragment Analyser (AATT) used for SNP detection
- Biomek NxP Liquid Handler (Beckman Coulter) for population development



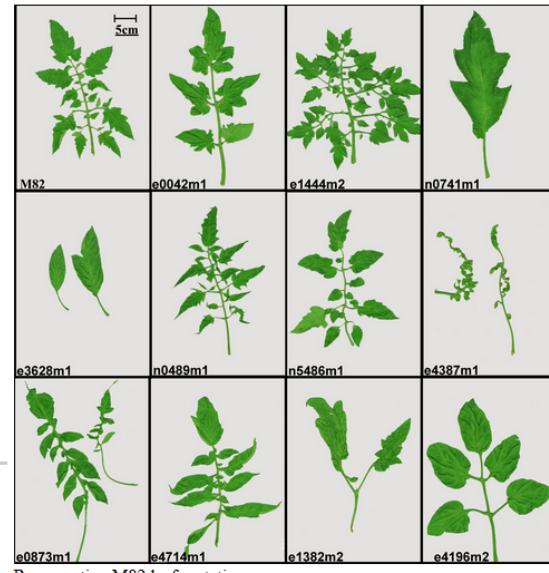
Cucumber (*Cucumis sativus* L.)

The cultivated cucumber (*Cucumis sativus* L.), an important species for the global food market, represents a model organism for investigations of organellar genetics, flower sex determination, fruit quality, virus resistance... Genetic and cultivation research of that species is conducted by many international groups including the International Cucurbit Genomics Initiative (ICUGI).

To elaborate tools to facilitate the development of cucurbitaceae research, we developed a **Beit Alpha cucumber** *in silico* forward and reverse genetics tool. Population was created with the collaboration of a private company BENCHBIO. We have created EMS-mutant population under controlled conditions.

Key features on the cucumber TILLING platform:

- ✓ 5000 M2 families
- ✓ Mutagen agent used : EMS (Ethyl methanesulfonate)
- ✓ Concentration used : 0.5% and 0.75%



Key features on the tomato TILLING platform:

- 'M82' population :
- ✓ 4759 M3 families mutagenised at 0.5% EMS
- ✓ More than 80 genes screened
- ✓ Mutation frequency : 1/700kb
- ✓ Phenotypic database: [Gene that make tomatoes](#) ↗

Mutant collections in plant species other than Arabidopsis

Comprehensive reverse genetic resources (e.g. T-DNA mutant collections like in Arabidopsis), which have been key to understanding gene function in diploid model organisms, are missing in many staple crops like wheat and barley.

Why:

- complexity of the genomes (polyploidy -> wheat or big size -> barley) makes genome assemblies difficult, as a result no reference genome sequences available for some plant species.
- long life cycle of wheat or barley slows down the studies
- money \$\$\$... to create and sustain a collection of T-DNA mutants like it has been done for Arabidopsis is very expensive and not applicable in some plant species... **TILLING collections are a good alternative**
- Other solutions: CRISPR/Cas9, RNAi lines

Generation of a Collection of Mutant Tomato Lines Using Pooled CRISPR Libraries¹

Thomas B. Jacobs,^{a,2,3} Ning Zhang,^a Dhruv Patel,^{a,4} and Gregory B. Martin^{a,b,2}

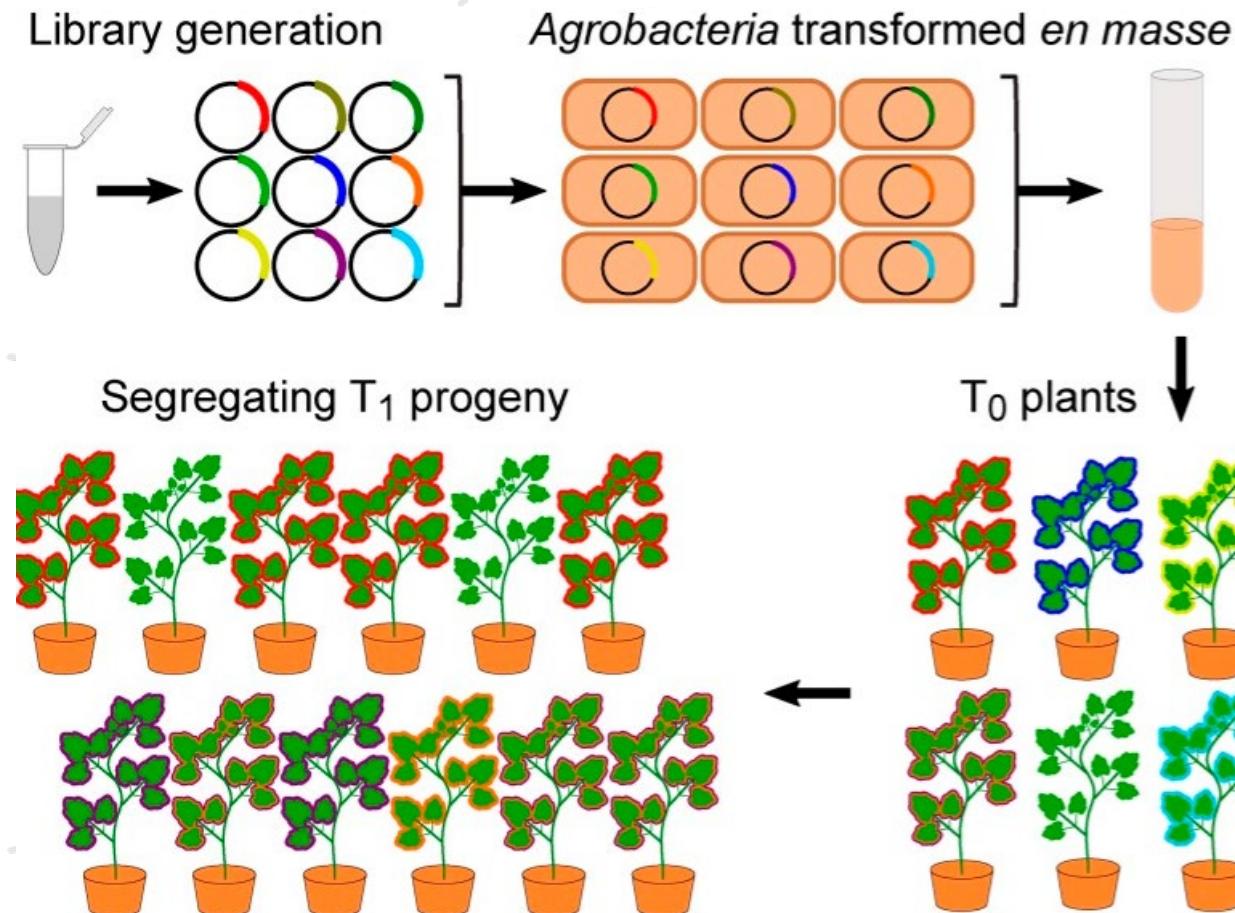


Figure 1. The concept of using a pooled CRISPR library screen in plants. A pool of CRISPR vectors is cloned, maintained as a library in *A. tumefaciens*, and transformed en masse into a plant system. The resulting T₀ plants will generally contain a single T-DNA insertion and a mutation in a single gene (colored outline). Identification of the target genes can be accomplished by sequencing the incorporated gRNA(s). Some plants will not be mutated (wild type; no outline), and ~20% of plants will contain multiple mutations due to multiple T-DNA insertions (multicolor outline). The T₀ plants will give rise to T₁ lines segregating for mutant and wild-type alleles and can be used in functional screens or to establish true-breeding mutant lines for future characterization.

Module 1 assessment

“tips and tricks to keep me happy”

- **Do not plagiarize**, if you are unsure see me (it will be checked for plagiarism)
- **Use references-** really, this is important
- **Write in paragraphs** –please don’t use dot points.
- **Your welcome to use figures**-cite the source and use a figure legend
- **Read the Ques and answer it.**
- Once you have answered it, read the question again.
- **Check for typos....**
- **keep your answers within the context of my lectures**-Extra brownie points
- **Stick to the word limits**

Any Questions feel free to see me, Rm 3.48 Bayliss building
Email: monika.murcha@uwa.edu.au

I am happy to provide feedback on your assessments in person if you are unsure of the LMS marking comments

Interested in doing a Masters project in my lab? Come and see me.



Please do the SPOT survey