DNA Libraries For Transgenic Marker Lines Of Tomato And Wild Relative

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1. Understanding tomato root development helps with future crop production

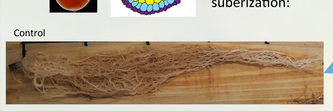

Solanum lycopersicum cv. M82

- *Solanum lycopersicum* var. M82: Cultivated tomato
- *Solanum pennellii* : Wild relative
- *S. pennellii* is drought tolerant and intercrossable with M82.
- Tomato has a sequenced genome


Solanum pennellii

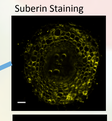
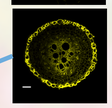

Tomato (M82) root development changes in response to 12-day drought stress, including cell-specific suberization:


Control



Drought

Suberin Staining

- Epidermis
- Exodermis
- Cortex
- Middle Cortex
- Endodermis
- Pericycle
- Phloem
- Procambium
- Xylem

2. Tomato marker resource has been created to study cell-specific gene expression

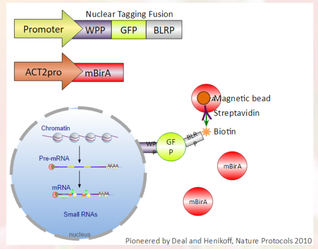
- Flooding and drought conditions elicit different responses in different cell types of roots.
- In order to understand the gene expression changes underlying these responses, 13 root cell type-specific marker lines were generated.
- A transfer DNA (T-DNA) was introduced into tomato genome using *A. rhizogenes* and it carries genes and a promoter that is expressed in specific cell types.
- The resulting marker expression provides useful tools to study cell types responses to drought and flooding.

Questions:

- Where have these T-DNAs been inserted into the genome?
- In which cells are the marker genes expressed?

3. Marker lines allow isolation of cell-specific nuclei and ribosomes

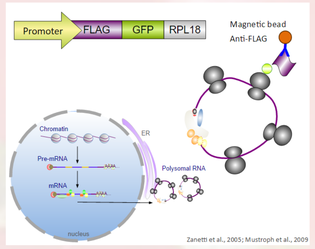
INTACT- Isolation of Nuclei TAgged in Cell Types



Pioneered by Deal and Henikoff, Nature Protocols 2010

T-DNA contains a construct with a nuclear tagging fusion under a cell-specific promoter. Its expression allows for magnetic pulldown of nuclei in specific cell types

TRAP- Translating Ribosome Affinity Purification



Zanetti et al., 2005; Mustroph et al., 2009

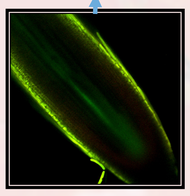
T-DNA contains a construct with a tagged ribosomal protein RPL18 under a cell-specific promoter. Its expression allows for magnetic pulldown of translating ribosomes

4. Marker expression patterns are confirmed

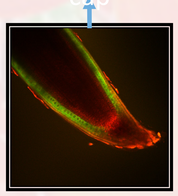
- GFP- Green Fluorescence protein excites under 488nm wavelength of light.
- Confocal microscope excites GFP allowing for visual confirmation that marker gene is expressed successfully in target cell type.

COR-IN-3 in *S. pennellii* is expressed in cortex cells

PH-IN-7 in M82 is expressed in phloem cells



EP-IN-9 in M82 is expressed in epidermis and lateral root cap



5. Insert-Sequencing allows identification of insert location in the genome

Completed:

1. Marker expression
2. DNA Extraction (130+)
3. DNA Pooling: 22 pools with up to 6 lines

COR-TR-2	WOX-TR-1	35S-TR-4	MCO-IN-1	MZ-TR-10	XY-IN-5
COR-TR-3	WOX-TR-2	35S-TR-5	MCO-IN-2	MZ-IN-7	PH-IN-4
COR-TR-4	WOX-TR-6	35S-TR-6	MCO-IN-3	MZ-IN-9	PH-IN-6
COR-TR-5	WOX-TR-8	35S-IN-1	MCO-IN-4	MZ-IN-10	PH-IN-7
COR-TR-6	WOX-IN-5	35S-IN-3	MCO-IN-6	MZ-TR-11	PH-IN-8
COR-TR-8	WOX-IN-6	35S-IN-5	MCO-IN-9	MZ-IN-11	XY-TR-1
COR-TR-10	WOX-IN-9	35S-TR-7	MCO-IN-10	XY-TR-2	EP-TR-2
COR-IN-1	WOX-IN-8	35S-TR-8	MCO-TR-1	XY-TR-6	EP-TR-7
COR-IN-2	WOX-IN-10	35S-TR-9	MCO-TR-2	XY-IN-1	EP-IN-11
COR-IN-7	EP-TR-1	35S-IN-2	MCO-IN-5	XY-IN-4	PH-IN-2
COR-IN-9	EP-IN-8	35S-IN-8	CYC-TR-7	EN-TR-1	XY-IN-7
COR-IN-10	EXO-TR-2	V-TR-11	CYC-TR-11	EN-TR-6	EP-IN-7
COR-TR-1	EXO-TR-11	V-TR-12	CYC-IN-3	EN-IN-1	XY-IN-9
COR-TR-9	EXO-IN-2	V-TR-13	CYC-IN-7	EN-IN-2	35S-TR-7
COR-TR-11	EXO-IN-4	V-IN-2	CYC-IN-8	EN-IN-7	35S-IN-4
COR-IN-11	EXO-IN-5	V-IN-8	CYC-IN-9	EN-IN-9	MCO-IN-1
COR-IN-12	EXO-IN-6	V-IN-9	CYC-IN-10	PH-TR-3	MCO-TR-4
COR-IN-13	EXO-IN-9	V-IN-7	CYC-IN-12	PH-TR-4	MCO-TR-6
COR-IN-14	ACT-TR-2	V-IN-8	MZ-TR-2	PH-TR-5	MCO-TR-8
COR-IN-15	ACT-TR-4	V-IN-10	MZ-TR-5	PH-TR-6	XY-TR-7
COR-IN-16	ACT-TR-6	MCO-TR-1	MZ-TR-7	PH-TR-8	WOX-IN-1
COR-IN-17	ACT-TR-9	MCO-TR-3	MZ-TR-8	PH-IN-1	EP-TR-13
COR-IN-18	ACT-TR-12	EN-TR-4			

4. DNA Fragmentation
5. Size selection to 400-500bp
6. Seq library generation
7. Library enrichment

6. Future goals

- With gene insertion location for each line PCR genotyping can be used to identify homozygous mutants.
- This allows for selection of true-breeding population of mutants that can be used in flooding and drought experiments and shared with the community.
- Identifying genes being upregulated in the nucleus and ribosomes during flooding and drought experiments.

7. Acknowledgements

I would like to thank Kaisa for mentoring me throughout this experiment and helping me develop as a researcher. The level of confidence Kaisa puts into me and my research efforts keep me motivated throughout the experiment . I would also like to thank Siobhan Brady for believing in me and my skills to go forward with this project and present what I have completed so far.