Facile Mapping of T-DNA Insertion Sites for a Cell-type Gene Expression Toolbox of Rice

Joel A. Velasco[1,2], Mauricio A. Reynoso[3], Germain Pauluzzi[3] & Julia Bailey-Serres[3]

[1]: Center for Plant Cell Biology REU Site: Research Experience for Undergraduates in Next Generation Plant Cell Biology.
Department of Botany and Plant Sciences, University of California at Riverside

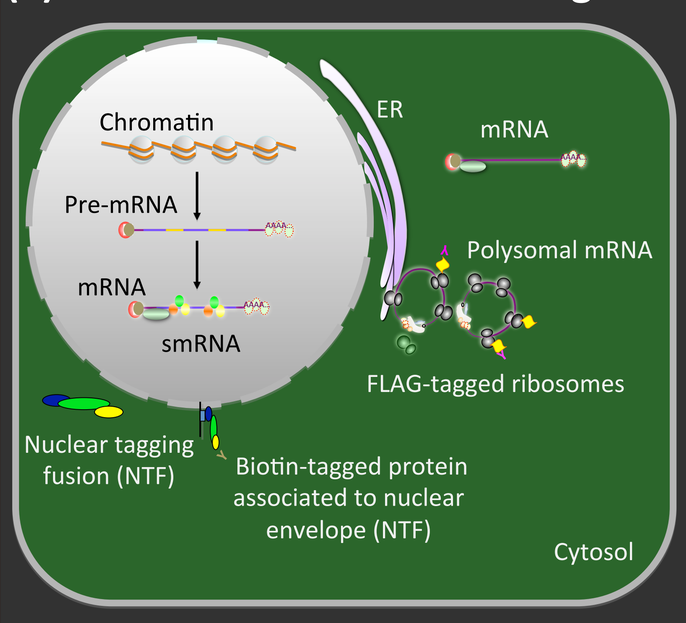
[2]: Department of Biological Sciences, Boise State University, Boise, ID

[3]: Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California at Riverside

(1) Introduction

INTACT and TRAP technologies developed in the model plant *Arabidops*is allow for cell-type specific gene expression analysis. To translate INTACT and TRAP to an important crop species, transgenic rice (*Oryza sativa*) lines containing the TRAP and INTACT constructs were developed. Shown here is a facile process of identifying the number and location of Transfer DNA (T-DNA) insertions containing the mentioned constructs, within genomic DNA (gDNA), of a transgenic collection. Knowledge of the insertion site and the location of INTACT /TRAP construct expression will be used to select the best lines for further use. This toolbox will be employed to study how physiology and development is perturbed by two major environmental threats: droughts and floods.

(2) TRAP and INTACT Technologies



Isolation of Nuclei Tagged in Specific Cell Types (INTACT)

- Access to nuclear chromatin and RNA
- Isolation of nuclei from specific cell types
- INTACT construct makes this possible

Translating Ribosome Affinity Purification (TRAP)

- Access to polysomal RNA
- Isolation of translating ribosomes from specific cell types
- TRAP construct makes this possible

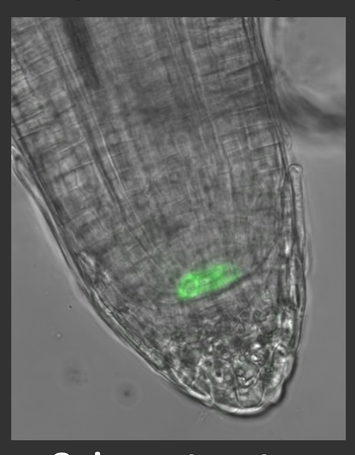
(3) Cell Types Targeted with Specific Promoters

Cell Type	Promoter	Lines
Pericycle	*OsHMA5*	34
Root hairs	*OsEXPB5*	7
Meristematic endodermis	*AtSCR*	18
Endo/Exodermis	*OsLSI2*	26
Root vasculature	*OsNRAMP3*	19
Root meristem	*OsRSS1*	40
Root cortex	*OsCMZ*	26
Quiescent center	*OsQHB*	17
Shoot meristem	*OSH1*	15
Endodermis	*OsCASP*	21
Near-constitutive	CaMV *35S*	47
	Total	270

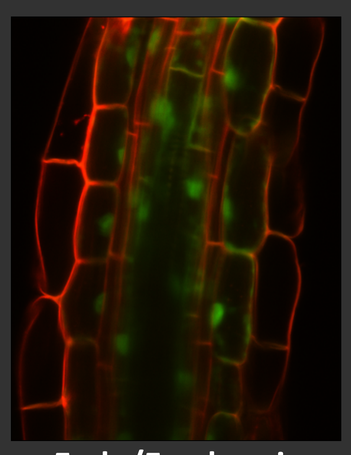
TRAP Construct

INTACT Construct

Confocal microscopy showing GFP-RPL18 for *pOsQHB* and pO*sLSI2* TRAP lines

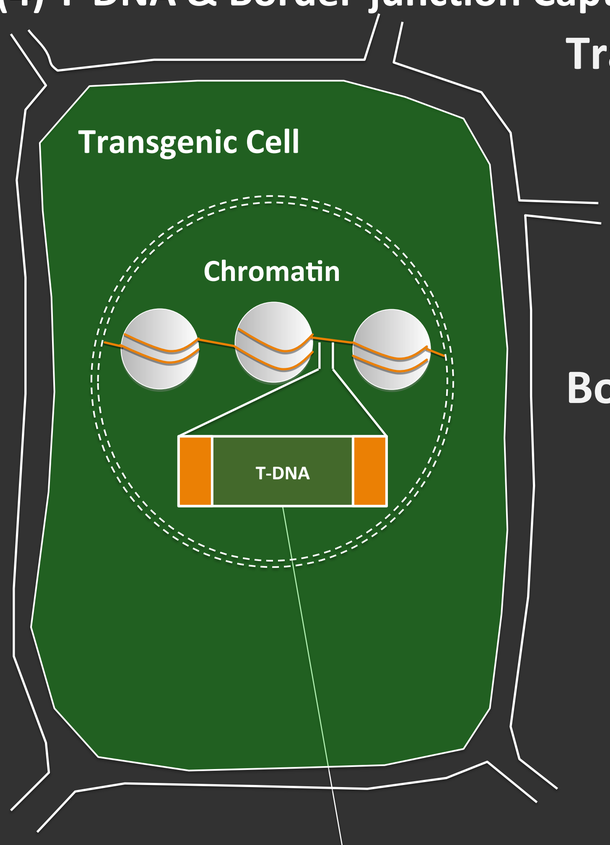


Quiescent center



Endo/Exodermis

(4) T-DNA & Border-junction Capture



Transfer DNA (T-DNA) insertion

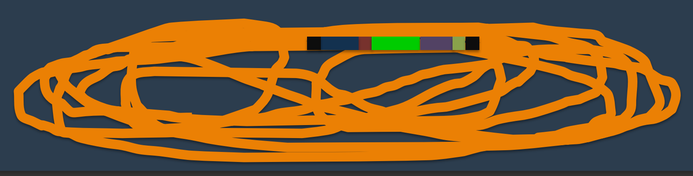
- Random
- Sometimes multiple
- Not always complete

Border-junction Capture

- Capture probes (▲) hybridized to border sequences
- Streptavidin beads bind capture probes

(5) Workflow

Genomic DNA (gDNA) was extracted from young leaf tissue of 270 transgenic rice lines



X 270 lines

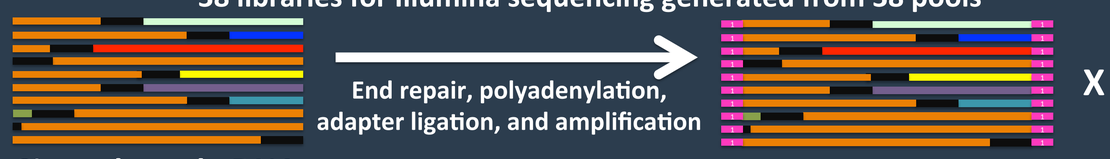
38 pools of gDNA from transgenic lines carrying constructs with different promoters

X 38

gDNA sheared by sonication, ~400bp fragments were selected using AMPure XP Beads

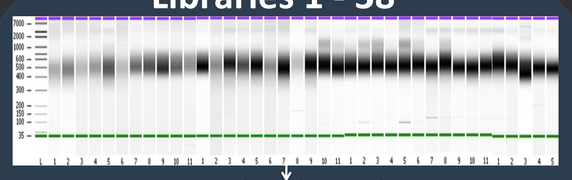
X 38

38 libraries for Illumina sequencing generated from 38 pools



X 38

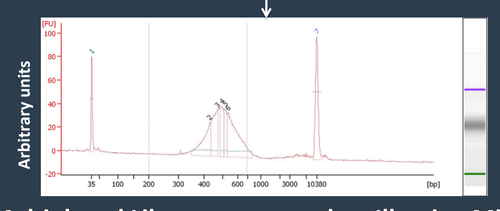
Libraries 1 - 38



Multiplexed

Border-junction Capture

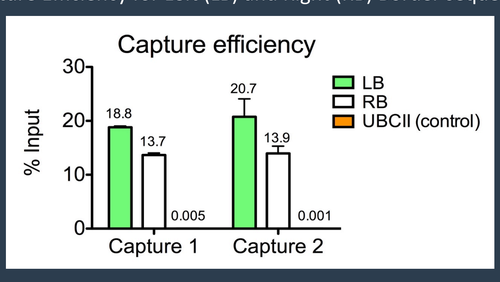
Amplification



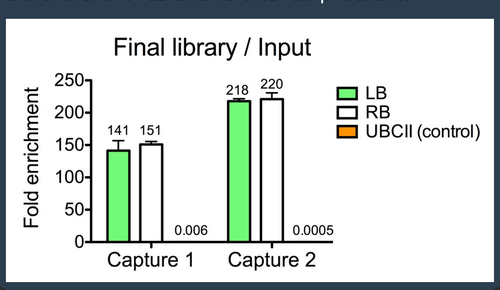
Multiplexed Library sequenced on Illumina MiSeq

Border-junction Capture Efficiency

Capture Efficiency for Left (LB) and Right (RB) Border sequences



Fold enrichment for LB and RB after amplification



(6) Sequence Analysis and T-DNA insertion mapping*

MiSeq 2 x 250bp Sequence Data

flash

Overlapping paired-end reads

grep

Filter reads containing LB RB sequences

Bowtie2 - local

Map Border–gDNA junction reads to genome

readGAligments, coverage, peaks

Coverage >100 reads for each insertion site

*** 5,718,350 paired end reads for 21 lines (min 94,890, max 652,658)**

*** 4,775,720 longer reads (390 bp) (83%, min 73,547, max 534,791)**

*** 229,856 border containing reads (4.8%, min 2079, max 31,885)**

*** 154,342 junction reads (67.1%, min 1,095, max 20,101**

*** 26 different insertions mapped**

*** Unpublished data from protocol development**

Expression	Line	# of insertions	Chr Location	Nearest Gene
Near-constitutive	35S:TRAP 1	1	Chr 4	LOC_Os04g18140
Near-constitutive	35S:TRAP 2	1	Chr 8	LOC_Os08g31100
Near-constitutive	35S:TRAP 3	1	Chr 4	LOC_Os04g55800
Near-constitutive	35S:INTACT 1	1	Chr 4	LOC_Os04g08660
Near-constitutive	35S:INTACT 2	1	Chr 6	LOC_ Os06g43090
Near-constitutive	35S:INTACT 3	1	Chr 8	LOC_Os08g08440
Quiescent center	QHB:TRAP 1	2	Chr 6	LOC_Os06g36590
Quiescent center	QHB:TRAP 2	1	Chr 9	LOC_Os09g12290
Quiescent center	QHB:TRAP 3	4	Chr 1, 2 , 3, 5	
Quiescent center	QHB:TRAP 4	2	Chr 11	LOC_Os11g38462
Shoot meristem	OSH1:TRAP 1	1	Chr 3	LOC_Os03g30260
Shoot meristem	OSH1:TRAP 2	1	Chr 2	LOC_Os02g40454
Endo/Exodermis	LSI1:TRAP 1	1	Chr 12	LOC_Os12g02710
Endo/Exodermis	LSI1:TRAP 2	1	Chr 7	LOC_Os07g10990
Endo/Exodermis	LSI1:TRAP 3	2	Chr 10, 11	
Endo/Exodermis	LSI1:TRAP 4	1	Chr 11	LOC_Os11g04030
Endo/Exodermis	LSI1:TRAP 5	1	Chr 10	LOC_Os10g08280
Root stele	SHR1:TRAP 1	3	Chr 1, 3 , 8	
Root stele	SHR1:TRAP 2	1	Chr 11	LOC_Os11g17200
Root stele	SHR1:TRAP 3	1	Chr 5	LOC_Os05g07950
Root stele	SHR1:TRAP 4	2	Chr 1	LOC_ Os01g57110

(7) Conclusion: 256 transgenic lines are on queue sequencing (MiSeq). T-DNA mapping information, along with visualized expression of GFP, will be used to select lines for further analysis

References:

Bailey-Serres, J. (2013). Microgenomics: Genome-Scale, Cell-Specific Monitoring of Multiple Gene Regulation Tiers. Annual Review of Plant Biology 64, 293–325.
Lepage, É., Zampini, É., Boyle, B., and Brisson, N. (2013). Time- and Cost-Efficient Identification of T-DNA Insertion Sites through Targeted Genomic Sequencing. PLoS ONE 8, e70912.
Tzfira, T., Li, J., Lacroix, B., and Citovsky, V. (2004). Agrobacterium T-DNA integration: molecules and models. Trends in Genetics 20, 375–383.

Contact info:
- joelvelasco84@gmail.com

This work is supported by:
- NSF REU - 1461297
- NSF DBI - 1238243