

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

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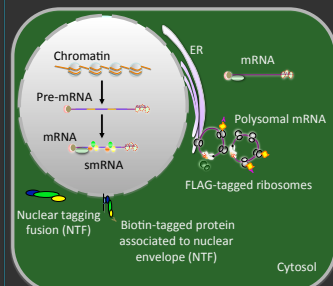
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(1) Introduction

INTACT and TRAP technologies developed in the model plant *Arabidopsis* 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 | <i>OshMA5</i> | 34 |
| Root hairs | <i>OsexPB5</i> | 7 |
| Meristematic endodermis | <i>AtSCR</i> | 18 |
| Endo/Exodermis | <i>OsLSI2</i> | 26 |
| Root vasculature | <i>OsNRPMP3</i> | 19 |
| Root meristem | <i>OsRSS1</i> | 40 |
| Root cortex | <i>OsCMZ</i> | 26 |
| Quiescent center | <i>OsQHB</i> | 17 |
| Shoot meristem | <i>OSH1</i> | 15 |
| Endodermis | <i>OsCASP</i> | 21 |
| Near-constitutive | <i>CaMV 35S</i> | 47 |
| Total | | 270 |

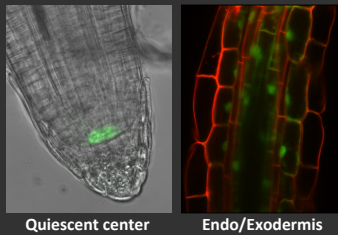
TRAP Construct



INTACT Construct



Confocal microscopy showing GFP-RPL18 for *pOsQHB* and *pOsLSI2* TRAP lines



(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



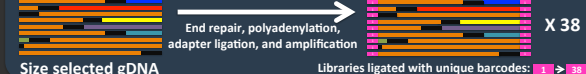
38 pools of gDNA from transgenic lines carrying constructs with different promoters



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



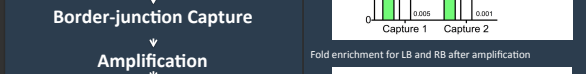
38 libraries for Illumina sequencing generated from 38 pools



Size selected gDNA



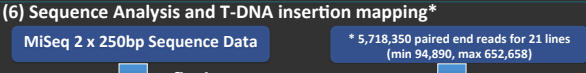
Libraries 1 - 38



Border-junction Capture Efficiency



Multiplexed



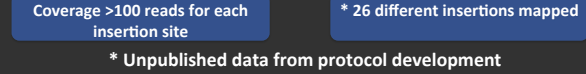
Border-junction Capture



Amplification



Multiplexed Library sequenced on Illumina MiSeq



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

MISeq 2 x 250bp Sequence Data



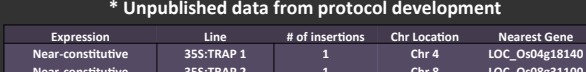
flash



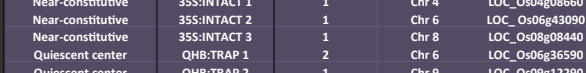
Overlapping paired-end reads



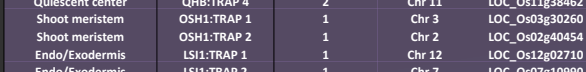
grep



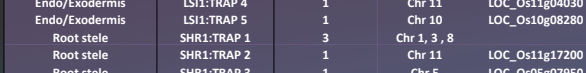
Filter reads containing LB RB sequences



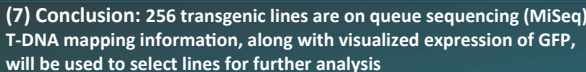
Bowtie2 - local



Map Border-gDNA junction reads to genome



Coverage >100 reads for each insertion site



* 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:

Velasco, J. A. et al. (2014). Genomic Scale, Cell-Specific Monitoring of Multiple Gene Regulation Terms. Annual Review of Plant Biology 64, 293-325.

Uppiger, E., Zampieri, E., Singh, S., and Branson, N. (2013). Time- and Cell-Specific Identification of T-DNA Insertion Sites Through Targeted Genomic Sequencing. PLoS ONE 8, e70512.

Velasco, J. A., et al. (2014). Agrobacterium T-DNA integration: mechanisms and models. Trends in Genetics 30, 370-380.

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