RNA-Seq Analysis Reveals Host Plant Transcriptomes in Response to Agrobacterium-mediated Transformation

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Background and Rationales

- *Agrobacterium*-mediated T-DNA transformation has become a predominant tool.
- However, many crop species are still difficult to be transformed using this approach.
- Genomics and genome editing demand for efficient T-DNA transfer in more crops.
- Studies on mechanisms of T-DNA transfer shall benefit crop transformation.
- Functions of most of the *Agrobacterium* genes in T-DNA transfer have been well studied.
- By contrast, our knowledge of plant genes involved in this process is very limited.
- This project has been undertaken to discover plant genes involved in T-DNA transfer.
- There have been transcriptomics studies previously using subjective cDNAs and microarrays.
- No study has used RNA-Seq to reveal whole plant transcriptomes in response to T-DNA transfer.
Advantages of RNA-Seq

- Allows discovery of complete transcripts and even splicing variants
- Doesn’t need prior knowledge of transcript sequences
- Is highly sensitive and enables to discover low abundant transcripts
- Is accurate, fast, and now becomes economically feasible
Experimental Approach

- *Agrobacterium* avirulent A136 and virulent At804 strains were used
- Mock inoculation was used as negative (blank) control
- AGROBEST (2014) was used for whole Arabidopsis seedling infection
- RNA samples were collected at 0, 3, 6, 12, 24, 48h post infection
- RNA-seq was performed at MU DNA Core Facility
- NextSeq 500 sequencer was used
- Sequences were trimmed and mtDNA and rDNA were removed
Time Course RNA-Seq: Comparison at each time point

0  3  6  12  24  48h pi

Mock  Negative (blank) control
Avirulent  Pathogen-Associated Molecular Pattern-Triggered Immunity (PTI)
Virulent  Elicitor-Triggered Immunity (ETI) + PTI
Results and Discussions
Verification of AGROBEST transformation system

A

At804

(Narasimhulu et al. 1996)

B

48 h pi

72 h pi

C

GUS activity (nmol 4MU/min/mg)

3 independent experiments = 1 RNA-seq replicate > 2 RNA-seq replicates
Number of differentially expressed genes (DEGs)

A

1010 DEGs
Up-regulated

1269 DEGs
Down-regulated

B

Numbers of DEGs

mock vs. A136 comparison
mock vs. At804 comparison
A136 vs. At804 comparison
Both mock vs. A136 and mock vs. At804 comparisons
Both mock vs. A136 and A136 vs. At804 comparisons
Both mock vs. At804 and A136 vs. At804 comparisons
All three comparisons

Upregulated
Downregulated
Three-dimensional scatterplots showing DEGs

log2-transformed FPKM (fragments per kilobase of transcript per million mapped reads) values

Scatterplot3d R package
K-means clustering

- mock
- A136
- At804

TrinityRNAseq
GO categories

log2-transformed FPKM (fragments per kilobase of transcript per million mapped reads) values

Gplots & RColorBrewer
Validation of Transcripts by qRT-PCR

Ribosomal L10 family
Cell cycle control
DNA methyltransferase
Cell wall organization and biogenesis
FLG22-induced receptor-like kinase 1
Arabidopsis NDR1/HIN1-LIKE 10
PEROXIDASE 33
Ethylene-responsive transcription factor 5

A-D, growth and development
E-H, defense
pEarleyGate202 for VirE2 and VirE3 Overexpression
AGROBEST

Leaf infiltration

Root assay

Root stable transformation
Responses of VirE2 and VirE3 overexpression lines to bacteria
Responses of VirE3 Overexpression lines (Courtesy raw data from Niu et al., 2015)
Conclusions

• We found a large number of plant genes in response to T-DNA transfer
• Growth and development gene expressions were repressed
• Defense response gene expressions were induced
• Vir proteins VirE2 and VirE3 may have additional role in enhancing defense
• Further study on individual genes and associated pathways are needed
Acknowledgements

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Mission

The goal of the Plant Transformation Core Facility at the University of Missouri is to enhance both basic and applied research in plant biology for the public by providing plant transformation services.

The facility is also expected to foster funding opportunities and national prominence for various research groups through our efforts in generation of transgenic events. The Core’s staff assists in developing transgenic approaches to address research questions.

We are making continued efforts in advancing transgenic technology to satisfy the needs of crop improvement and gene discovery.

As part of an educational role, the staff provides technical training in plant tissue culture and transformation for undergraduate and graduate students, post-doctoral researchers and other researchers.
## Service Pricing

### Stable Transformation Service:

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Internal</th>
<th>External</th>
<th>Number of events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine max</td>
<td>$1,560</td>
<td>$2,600</td>
<td>3-5</td>
</tr>
<tr>
<td>Zea mays inbred B104</td>
<td>$2,030</td>
<td>$4,065</td>
<td>3-5</td>
</tr>
<tr>
<td>Zea mays Hi-II</td>
<td>$2,240</td>
<td>$3,200</td>
<td>5-10</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>$2,420</td>
<td>$4,010</td>
<td>5-10</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>$2,600</td>
<td>$3,360</td>
<td>5-10</td>
</tr>
<tr>
<td>Panicum virgatum</td>
<td>$1,650</td>
<td>$2,925</td>
<td>5-10</td>
</tr>
</tbody>
</table>

Listed price is for per construct. The number of independent transgenic events produced with each construct is indicated in the last column. Each event will be carried to T1 seeds except for switchgrass which is carried to plantlets in culture vessels. Events that are carried to T0 seeding or earlier culture stages will be charged at a lower price.

For soybean (Glycine max) and maize inbred B104, a minimum of 3 events will be required for the service.

For remaining crop species, at least 5 events will be required for the service.

To request more events than 3-5 or 5-10, simply multiply the price by number of events needed to get an estimate of the cost.

Please contact Zhanyuan Zhang if you have questions regarding our pricing.
Thanks for Your Attention!