Transcript Profiling of the Anoxic Rice Coleoptile

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The MIAME Checklist

Experiment Design:

1. Type of experiment:

- Comparison of control (aerobic) vs. anoxic rice coleoptiles

2. Experimental factors:

- anoxia

3. The number of hybridizations performed in the experiment: 4

4. Hybridization design:

		Experiment	Experimental condition
1	*Oryza sativa*, CV. Nipponbare	1	air
2	*Oryza sativa*, CV. Nipponbare	2	anoxia
3	*Oryza sativa*, CV. Nipponbare	1	air
4	*Oryza sativa*, CV. Nipponbare	2	anoxia

5. Quality control steps taken: Electropheresis of fragmented cRNA

Samples used, extract preparation and labeling:

1. Biological sample:

- Coleoptiles from *Oryza sativa*, CV. Nipponbare

2. Manipulation of biological samples and protocols used:

- *Oryza sativa*, CV. Nipponbare grown in a growth chamber controlled at 28°C; germination for 4 days in the darkness since the imbibition of the seeds; Four days after germination, both the anoxic and the aerobic coleoptiles are close to their maximal length, and the aerobic coleoptile is large enough to allow a fast and clean removal of the primary leaf, avoiding the extraction of leaf mRNA that would otherwise contaminate coleoptile mRNA preparations.
- ONLY coleoptile tissue was used for the microarray experiment
- Darkness
- Sterile culture
- Water used as a medium

3. Protocol for preparing the hybridization extract:
   - RNA extraction with Ambion RNAqueous extraction kit (Ambion, Austin, Texas USA)

4. Labeling protocols:
   - First strand cDNA synthesis from 10 micrograms of total RNA using Ambion MessageAmp kit (Ambion, Austin, Texas USA)
   - Double strand cDNA synthesis using Ambion MessageAmp kit (Ambion, Austin, Texas USA)
   - Synthesis of biotin-labeled cRNA using Ambion MessageAmp kit (Ambion, Austin, Texas USA)
   - Purify labeled cRNA with Ambion MessageAmp kit (Ambion, Austin, Texas USA)
   - Fragmentation of labeled cRNA. 94°C, 35 min in Fragmentation Buffer (40 mM Tris acetate pH8.1, 100mM potassium acetate, 30mM magnesium acetate).
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Hybridization procedures and parameters:

1. Hybridization of fragmented cRNA to Arabidopsis Genome GeneChip array (Affymetrix)
   - 45°C, 16 hr hybridization in a Hybridization Oven model 640 (Affymetrix)
   - Hybridization Cocktail:
   - 50 µg/mL fragmented cRNA
   - 100 mM MES, 1M[Na+]
   - 20 mM EDTA, 0.01% Tween20 (Pierce, Rockford, IL)
   - 1X GeneChip Hybridization Control (Affymetrix)
   - 50 pM Control Oligonucleotide B2 (Affymetrix)

- 0.5 mg/mL acetylated BSA (Invitrogen)
- mg/mL herring sperm DNA (Promega, Madison WI)
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2. Washing and staining in a GeneChip Fluidics Station model 400 (Affymetrix)

- Wash Buffer A:
- 6X SSPE
- 0.01% Tween 20 (Pierce)
- Wash Buffer B:
- 100 mM MES, 1M[Na+]
- 0.01% Tween 20 (Pierce)
- SAPE solution:
- 100 mM MES, 1M[Na+]
- 0.05% Tween 20 (Pierce)
- 2 mg/mL acetylated BSA (Invitrogen)
- 10 µg/mL streptavidin phycoerythrin (Molecular Probes, Eugene, OR)
- Antibody solution:
- 100 mM MES, 1M[Na+]
- 0.05% Tween 20 (Pierce)
- 0.005% Antifoam 0-30 (Sigma)
- 2 mg/mL acetylated BSA (Invitrogen)
- 0.1 mg/mL goat IgG (Sigma)
- 3 µg/mL biotinylated anti-streptavidin antibody (Vector Laboratories, Burlingame,CA)
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- 10 cycles of 2 mixes/cycle with Wash Buffer A at 25°C
- 4 cycles of 15 mixes/cycle with Wash Buffer B at 50°C
- Stain the probe array for 10 min in SAPE solution at 25°C
- 10 cycles of 4 mixes/cycle with Wash Buffer A at 25°C
- Stain the probe array for 10 min in antibody solution at 25°C
- Stain the probe array for 10 min in SAPE solution at 25°C
- 15 cycles of 4 mixes/cycle with Wash Buffer A at 30°C
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Measurement data and specifications:

1. Scanning: GeneArray™ scanner manufactured by Hewlett-Packard http://www.affymetrix.com/products/instruments/specific/completeinst.affx
2. Image analysis: Microarray Suite 5.0 (Affymetrix)
3. Microarray analysis was performed using R/Bioconductor (Gentlemen *et al.,* 2004). Expression measures were obtained using GCRMA (Wu and Irizarry, 2005), a multiarray analysis method estimating probesets signals taking into account the physical affinities between probes and targets. Normalization was done by a quantiles method (Bolstad *et al.,* 2003). To reduce the number of non informative genes, two different filters were applied: the first removes the probesets presenting an Affymetrix Absent Call (A) for both the conditions; the second eliminates the probesets showing, as maximum signal in the two conditions, a value equal or less than the 95th percentile of the overall Absent calls signals distribution. This filtering yielded 21484 probesets. To identify a statistically reliable number of differentially expressed genes among the two conditions, a linear model was performed (Wettenhall *et al.,* 2004). For assessing differential expression, an empirical Bayesian method (Smyth, 2004) was used to moderate the standard error of the estimated log-fold changes. In order to control p-values in a context of multiple testing problem, a Benjamini-Hochberg's correction of the false discovery rate (Reiner *et al.,* 2003) was applied (adjusted P-value <= 0.01) leading to 3134 differentially expressed probesets. Microarray datasets were deposited in a public repository with open access (accession no. GSE6908, http://www.ncbi.nlm.nih.gov/projects/geo/).

Array Design:

Arabidopsis Rice GeneChip array (Affymetrix)