

Supplementary file 4. The MIAME Checklist

Experimental Design:

1. Type of experiment:

- Comparison of untreated vs. OPDA-, JA-, and MeJA-treated Arabidopsis.
- Comparison of control (no wounding) vs. wounded Arabidopsis.

2. Experimental factors:

- Time course (0 min, 30 min and 180 min)
- Treatment (OPDA, JA, MeJA, and wounding)

3. Number of hybridizations performed in the experiment: 16

4. Hybridization design:

	Label	Strain	Condition		Harvested	Tissue
			Growth	Treatment		
1	C1	wild-type	MS medium, 10 days	none	0 min	whole plants
	OP11	wild-type	MS medium, 10 days	OPDA	30 min	whole plants
2	C2	wild-type	MS medium, 10 days	none	0 min	whole plants
	OP12	wild-type	MS medium, 10 days	OPDA	180 min	whole plants
3	C3	wild-type	MS medium, 10 days	none	0 min	whole plants
	OP21	wild-type	MS medium, 10 days	OPDA	30 min	whole plants
4	C4	wild-type	MS medium, 10 days	none	0 min	whole plants
	OP22	wild-type	MS medium, 10 days	OPDA	180 min	whole plants
5	C5	wild-type	MS medium, 10 days	none	0 min	whole plants
	JA11	wild-type	MS medium, 10 days	JA	30 min	whole plants
6	C6	wild-type	MS medium, 10 days	none	0 min	whole plants
	JA12	wild-type	MS medium, 10 days	JA	180 min	whole plants
7	C7	wild-type	MS medium, 10 days	none	0 min	whole plants
	JA21	wild-type	MS medium, 10 days	JA	30 min	whole plants
8	C8	wild-type	MS medium, 10 days	none	0 min	whole plants
	JA22	wild-type	MS medium, 10 days	JA	180 min	whole plants
9	C9	wild-type	MS medium, 10 days	none	0 min	whole plants
	MA11	wild-type	MS medium, 10 days	MeJA	30 min	whole plants
10	C10	wild-type	MS medium, 10 days	none	0 min	whole plants
	MJ12	wild-type	MS medium, 10 days	MeJA	180 min	whole plants
11	C11	wild-type	MS medium, 10 days	none	0 min	whole plants
	MJ21	wild-type	MS medium, 10 days	MeJA	30 min	whole plants
12	C12	wild-type	MS medium, 10 days	none	0 min	whole plants
	MJ22	wild-type	MS medium, 10 days	MeJA	180 min	whole plants
13	C13	wild-type	MS medium, 21 days	none	0 min	Rosette leaf
	WOD11	wild-type	MS medium, 21 days	wounding	30 min	Rosette leaf
14	C14	wild-type	MS medium, 21 days	none	0 min	Rosette leaf
	WOD12	wild-type	MS medium, 21 days	wounding	180 min	Rosette leaf
15	C15	wild-type	MS medium, 21 days	none	0 min	Rosette leaf
	WOD21	wild-type	MS medium, 21 days	wounding	30 min	Rosette leaf
16	C16	wild-type	MS medium, 21 days	none	0 min	Rosette leaf
	WOD22	wild-type	MS medium, 21 days	wounding	180 min	Rosette leaf

5. Quality control steps taken: Confirmation of mRNA levels by RNA 6000 RNA Nano Assay (Agilent Technologies, Inc., U.S.A.)

Samples used, extract preparation and labeling:

1. The origin of the biological sample:

- *Arabidopsis thaliana* ecotype Columbia

2. Manipulation of biological samples and protocols used:

- *Arabidopsis thaliana* cultured in a growth chamber controlled at 22°C
- Continuous light
- Sterile culture
- MS medium (Murashige and Skoog, (1962) *Physiol. Plant.* 15, 493-497)

For comparison of untreated vs. OPDA-, JA-, and MeJA-treated *Arabidopsis*, liquid medium was used. On the other hand, solid medium was applied to compare wounded *Arabidopsis* to the control (no wounding).

3. Protocols or preparation of the hybridization extract:

- RNA extraction with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany)

4. Labeling Protocols:

- Double-stranded cDNA synthesis using Low RNA Input Linear Amplification Kit (Agilent Technologies, Inc.)
- Synthesis of Cy3-labeled (for treated sample) or Cy5-labeled (for untreated sample) cRNA by in vitro transcription using the Low RNA Input Linear Amplification Kit (Agilent Technologies, Inc.)
- Purification of labeled cRNA with the RNeasy column (Qiagen)
- Fragmentation of labeled cRNA: 60°C, 30 min using the In Situ Hybridization Kit Plus (Agilent Technologies, Inc.)

Hybridization procedures and parameters:

1. Hybridization of fragmented cRNA to Arabidopsis 2 Microarray (Agilent Technologies, Inc.)

- 60°C, 17 hr hybridization in a hybridization oven (Agilent Technologies, Inc.)
- Hybridization Cocktail
 - 1.0 µg fragmented cRNA labeled with Cy3 and Cy5
 - Hybridization Buffer (Agilent Technologies, Inc.)
 - Deposition Control (Operon Technologies, Inc., U.S.A.)
 - Human Cot-1 DNA (Invitrogen Corporation, U.S.A.)

2. Washing

- Wash Buffer 1
 - 6× SSPE (Agilent Technologies, Inc.)

0.005% N-lauroylsarcosine (Agilent Technologies, Inc.)

- Wash Buffer 2

0.06× SSPE

0.005% N-lauroylsarcosine

- Wash Buffer 3

Stabilization and Drying Solution (Agilent Technologies, Inc.)

- Wash Buffer 1, 1 min, room temperature

- Wash Buffer 2, 1 min, room temperature

- Wash Buffer 3, 30 sec, room temperature

Measurement data and specifications:

1. Scanning: DNA Microarray Scanner (Agilent Technologies, Inc.)

2. Image analysis: Agilent Feature Extraction software (Agilent Technologies, Inc.)

3. Fold expression of signal intensities (Relative Expression) were calculated from the following comparisons. Signal intensities of the probes and Relative Expression are presented in an Excel file (supplementary file 5).

A	OP11 vs. C1
B	OP12 vs. C2
C	OP21 vs. C3
D	OP22 vs. C4
E	JA11 vs. C5
F	JA12 vs. C6
G	JA21 vs. C7
H	JA22 vs. C8
I	MJ11 vs. C9
J	MJ12 vs. C10
K	MJ21 vs. C11
L	MJ22 vs. C12
M	WOD11 vs. C13
N	WOD12 vs. C14
O	WOD21 vs. C15
P	WOD22 vs. C16

Array Design:

Arabidopsis 2 oligo microarray (Agilent Technologies, Inc.)

The content of this microarray was derived from the ATH1 v. 3 database of The Institute for Genomic Research (TIGR) and represents 21,500 genes.