Supporting Text

Bacterial Total RNA Isolation

Reagents

Stop Solution consisting of 5% buffer equilibrated phenol (pH 7.4) in ethanol (1), RNeasy Mini Kit and Shredders (Qiagen, Chatsworth, CA), DNase I Double Strength (dissolve the solid DNase I in 275 μl of RNase-free water) (Qiagen), DNase I (Amersham Catalogue no. 27-0514-01), RNA-guard (Amersham Catalogue no. 27-0816-01), One-Phor-All 10× Buffer (Amersham Catalogue no. 27-0901-02), DNase I Kit (Qiagen Catalogue no. 79254), TE buffer ((RNAse-free/10 mM Tris/1 mM EDTA, pH 8.0) containing 1 mg/ml lysozyme, RNase-free water, ethanol, and ice.

Cell Harvest

Grow bacterial cultures to desired density. Before harvest, label 2-ml centrifuge tubes, fill with 1/9th the sample volume of Stop Solution, and place on ice. For example, for a 1.7-ml culture, use 190 μl of Stop Solution. Rapidly transfer culture to tubes containing stop solution and cap and mix by inversion. Samples can sit on ice up to 20 min. Pellet cells at 4°C at maximum speed for 1 min in a microfuge. Discard supernatant. Freeze cell pellets in liquid nitrogen. Cells can be stored at -80°C or used immediately. As the cells thaw on ice, follow the RNeasy protocol for gram-negative bacteria.

QIAgen RNA Isolation

1. Important notes before starting:

(*i*) Do not overload column (see Qiagen guide).

(*ii*) Lysis Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.

(*iii*) Add 10 µl of 2 mercaptoethanol per 1 ml of Lysis Buffer RLT before use.

(*iv*) Wash Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%), as indicated on the bottle to obtain a working solution.

2. Loosen cell pellet by flicking the bottom of the tube. Resuspend cells in 100 µl of lysozyme-containing TE buffer (by flicking the tube; incubate for 3-5 min on the bench top).

3. Add 350 µl of Lysis Buffer RLT to the sample and vortex vigorously. Centrifuge for 2 min at maximum speed. Load supernatant to the QIAshredder spin column sitting in a 2-ml collection tube and centrifuge for 2 min at maximum speed. Transfer flow-through fraction from QIAshredder to a new tube (not supplied) without disturbing the cell-debris pellet in the collection tube.

4. Add 250 µl of ethanol (100%) to the lysate and mix well by pipetting. Do not centrifuge.

5. Apply sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy mini-spin column sitting in a 2-ml collection tube. Centrifuge for 15 seconds at maximum speed. Discard flow-through and reuse the collection tube.

6. Pipet 350 µl of Wash Buffer RW1 into the RNeasy column and centrifuge for 15 seconds at maximum speed to wash. Discard flow-through; keep the collection tube.

7. Add 10 µl of Qiagen DNase I (double strength) stock solution to 70 µl of DNase I Buffer RDD (supplied with the RNase-Free DNase I Set) in a microfuge tube. Mix by

gently inverting the tube (DNase I is especially sensitive to physical denaturation) and centrifuge briefly to collect residual liquid from the sides of the tube.

8. Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy silica membrane, and place on the bench top for 30 min.

9. Pipet 350 µl of Wash Buffer RW1 into the RNeasy column and leave on the bench top for 5 min.

10. Centrifuge for 15 seconds at maximum speed. Discard flow-through and collection tube.

11. Transfer the RNeasy column into a new 2-ml collection tube. Pipet 500 µl of Wash Buffer RPE onto the RNeasy column. Close the tube gently and centrifuge for 15 seconds at maximum speed to wash the column. Discard flow-through and reuse collection tube.

12. Add another 500 µl of Wash Buffer RPE to the RNeasy column. Close the tube and centrifuge for 2 min at maximum speed. Discard collection tube with flow-through.

13. Place the RNeasy column in a new 2-ml collection tube and centrifuge for 1 min at maximum speed.

14. To elute, transfer the RNeasy column to a new 1.5-ml collection tube. Pipet 30 µl of RNase-free water directly onto the silica-gel membrane and let it stand on the bench top for 1 min. Close the tube and centrifuge for 1 min at maximum speed to elute.

Second DNase I treatment

1. Pool eluates from four Qiagen columns (RNA amount should not exceed 100 µg = capacity of column).

2. For each pooled RNA sample (110-120 µl), add:

- 10 µl of 10 × One-Phor-All Buffer.

- 1.4 µl of RNA-guard.

- 5.0 µl (25 units) of Amersham DNase I based on 1.1 unit DNase I per microgram of cDNA fragment size 50-200 bases (depends on the titer calculated with cDNA fragmentation).

- Incubate at 37°C for 30 min.

3. Add 350 µl of Lysis Buffer RLT to the sample and mix.

4. Add 250 µl of 100% ethanol to the lysate and mix well by pipetting. Do not centrifuge.

5. Apply sample (usually 700 µl) to an RNeasy mini spin column in a 2-ml collection tube. Centrifuge for 15 seconds at maximum speed. Discard flow-through and collection tube.

6. Transfer the RNeasy column to a new 2-ml collection tube. Pipet 500 µl of Wash Buffer RPE into the RNeasy column. Close the tube gently and centrifuge for 15 seconds at maximum speed to wash the column. Discard flow-through and reuse collection tube.

7. Add another 500 µl of Wash Buffer RPE to the RNeasy column. Close the tube and centrifuge for 2 min at maximum speed. Discard collection tube with flow-through.

8. Place the RNeasy column in a new 2-ml collection tube and centrifuge for 1 min at maximum speed.

9. To elute, transfer the RNeasy column to a new 1.5-ml collection tube. Pipet 50 µl of RNase-free water directly onto the silica-gel membrane and let it stand on the bench top for 1 min. Close the tube and centrifuge for 1 min at maximum speed to elute.

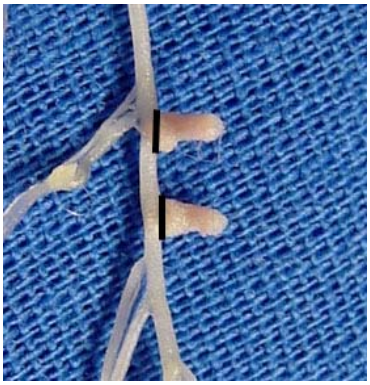
Nodule Total RNA Isolation

Reagents

Liquid nitrogen, dry ice, mortar and pestle, RNeasy Mini Kit and Shredders (Qiagen), DNase I (Qiagen) Double Strength (dissolve the solid DNase I in 275 µl of RNase-free water), DNase I (Amersham), RNA-guard (Amersham), One-Phor-All 10× Buffer (Amersham), DNase I Kit (Qiagen), TE buffer containing 1 mg/ml lysozyme, RNase-free water, ethanol, and ice.

Bacteroid Harvest

Grow plants as desired. At least 800 mg of nodules are needed for a single Affymetrix CHIP. Before harvest, label 50-ml centrifuge tubes and pack in dry ice. Periodically add more liquid nitrogen to the tube while harvesting. Keep tubes on dry ice until nodule harvest is completed, then cap and transfer to liquid nitrogen for transport. Tissue may be stored at -80°C or used immediately.



To excise nodule material, pinch the nodule with forceps at the nodule-root junction.

QIAgen RNA Isolation

1. Important notes before starting:

(*i*) Do not overload columns. We apply 800 mg of nodule tissue to six columns.

(*ii*) Lysis Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.

(*iii*) Add 10 µl of 2 mercaptoethanol per 1 ml of Lysis Buffer RLT before use.

(*iv*) Wash Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%), as indicated on the bottle, to obtain a working solution.

(*v*) Pack dry ice around the mortar; add liquid nitrogen and 800 mg of tissue. Grind the tissue to a fine powder with a pestle while adding liquid nitrogen as needed to keep the tissue frozen.

2. To the mortar, add 450 µl of Lysis Buffer RLT for each Qiagen tube used. Example: 800 mg of nodule tissue: 6 tubes × 450 µl = 2.7 ml of RLT. Add RLT to the fine powder in the mortar. The RLT buffer will freeze with the crushed tissue. Grind to a fine powder.

3. Remove mortar from the dry ice and grind as the tissue starts to thaw. The best RNA extraction occurs while the material is a half-frozen thick paste. Continue to grind until the tissue is thawed.

4. Load supernatant onto six QIAshredder spin columns and centrifuge for 2 min at maximum speed. Transfer flow-through from the QIAshredder to a new 2-ml tube without disturbing the cell-debris pellet in the collection tube.

5. Add 0.5 volume ethanol (100%) to the flow-through and mix well by pipetting. Do not centrifuge.

6. Apply sample (usually 650 µl), including any precipitate that may have formed, to an RNeasy mini-spin column. Centrifuge for 15 seconds at maximum speed. Discard flow-though and reuse the collection tube. If there is more than 700 µl of sample, add the rest to the RNeasy spin column and centrifuge again for 15 seconds. Discard this flow-through and keep the collection tube for the next step.

7. Pipet 350 µl of Wash Buffer RW1 onto the RNeasy column and centrifuge for 15 seconds at maximum speed to wash. Discard flow-through. Keep the collection tube.

8. Add 10 µl of Qiagen DNase I (double strength) stock solution to 70 µl of DNase I Buffer RDD (supplied with the RNase-Free DNase I Set) in a microfuge tube. Mix by gently inverting the tube (DNase I is especially sensitive to physical denaturation) and centrifuge briefly to collect residual liquid from the sides of the tube.

9. Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy silica membrane and place on the bench top for 30 min.

10. Pipet 350 µl of Wash Buffer RW1 onto the RNeasy column and leave on the bench top for 5 min.

11. Centrifuge for 15 seconds at maximum speed. Discard flow-through and collection tube.

12. Transfer the RNeasy column into a new 2-ml collection tube. Pipet 500 µl of Wash Buffer RPE onto the RNeasy column. Close the tube gently and centrifuge for 15 seconds at maximum speed to wash the column. Discard flow-through and reuse collection tube.

13. Add another 500 µl of Wash Buffer RPE to the RNeasy column. Close the tube and centrifuge for 2 min at maximum speed. Discard collection tube with flow-through.

14. Place the RNeasy column in a new 2-ml collection tube and centrifuge for 1 min at maximum speed.

15. To elute, transfer the RNeasy column to a new 1.5-ml collection tube. Pipet 50 µl of RNase-free water directly onto the silica-gel membrane and let it stand on the bench top for 1 min. Centrifuge for 1 min at maximum speed to elute.

Second DNase I Treatment

1. Pool eluates from Qiagen prep in 100-µl aliquots (RNA amount should not exceed 100 µg = capacity of column). Usually, when three tubes are pooled from the above RNA isolation, the volume is 150 µl.

For 150 µl of RNA, add:

- 15 µl of 10× One-Phor-All Buffer;
- 1.4 µl of RNA-guard (Amersham);
- 5.0 µl (25 units) of Amersham DNase I based on 1.1 unit DNase I/µg of cDNA fragment size 50-200 bases (depends on the titer calculated with cDNA fragmentation);
- Incubate at 37°C for 30 min.

2. Add 525 µl of Lysis Buffer RLT + 2 mercaptoethanol to the sample and mix.

3. Add 300 µl of 100% ethanol to the lysate and mix well by pipetting. Do not centrifuge.

4. Apply sample (usually 700 µl) to an RNeasy mini spin column in a 2-ml collection tube. Centrifuge for 15 seconds at maximum speed. Discard flow-through and collection tube. If there is more than 700 µl of sample, add the rest to RNeasy spin column and

centrifuge again for 15 seconds. Discard this flow-through and keep the collection tube for the next step.

5. Transfer the RNeasy column to a new 2-ml collection tube. Pipet 500 µl of Wash Buffer RPE into the RNeasy column. Close the tube gently and centrifuge for 15 seconds at maximum speed to wash the column. Discard flow-through and reuse collection tube.

6. Add another 500 µl of Wash Buffer RPE to the RNeasy column. Close the tube and centrifuge for 2 min at maximum speed. Discard collection tube with flow-through.

7. Place the RNeasy column in a new 2-ml collection tube and centrifuge for 1 min at maximum speed.

8. To elute, transfer the RNeasy column to a new 1.5-ml collection tube. Pipet 50 µl of RNase-free water directly onto the silica-gel membrane and let it stand on the bench top for 1 min. Close the tube and centrifuge for 1 min at maximum speed to elute.

Assay for Genomic DNA Contamination

To check for genomic DNA contamination in RNA preparations, carry out PCR with primers to an intergenic region of the genome. We use primers designed for the intergenic region adjacent to CtrA.

Primers

IG-5′ ATCCTGCTGCATCTTCAGCTCGCG 24 mer

IG-3′ TTATCCCGCTCGGGAACAGTAACC 24 mer

PCR

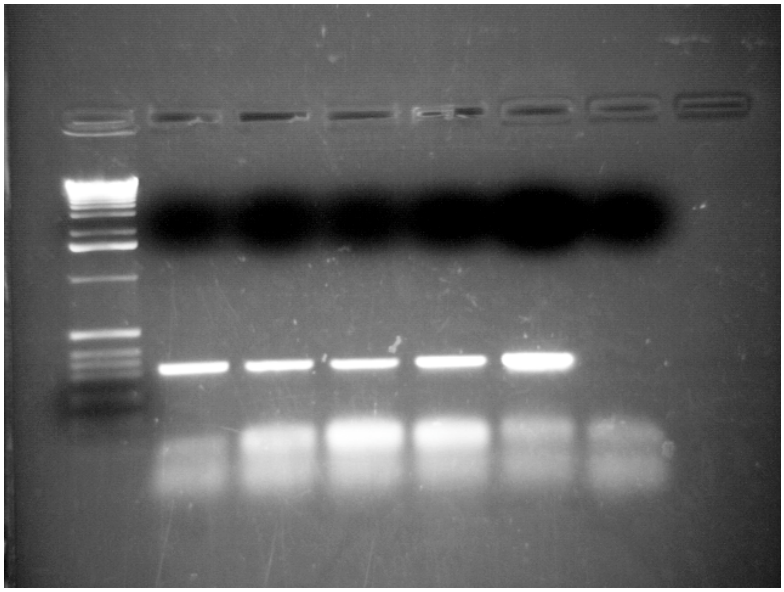
10 mM	dNTPs	0.5 µl
25 µM	primer 1	1.0 µl
25 µM	primer 2	1.0 µl
10×	Mg-free Buffer	2.5 µl
25 mM	$MgCl_2$	3.0 µl
0.5 µg	RNA	× µl
	Taq DNAPolymerase	0.5 µl
	H_2O	to 25 µl
	Total	25 µl

PCR settings

(*i*)	94°	4 min
(*ii*)	55°	30 sec
(*iii*)	72°	45 sec
(*iv*)	30 cycles	Go to step 2
(*v*)	72°	10 min
(*vi*)	4°	

Genomic DNA Contamination

Marker RNA1 RNA2 RNA3 RNA4 genomic, no DNA;



PCR using intergenic primers. 1.5% agarose gel in TBE; 10 µl of PCR reaction per lane.

DNase I Titer

Each lot no. of DNase I can vary greatly. To titer DNase I, determine the amount of DNase I needed to fragment 1 µg of cDNA to sizes between 50 and 200 bases. Choose between 0.2 and 1.1 DNase I units/µg cDNA to start.

1. Prepare the following reaction mix:

Fragmentation Reaction

Components	**Volume/amount**	**Concentration**
10× One Phor-All Buffer	5 µl	1×
cDNA	40 µl	3-7 µg
DNase I (Amersham)	× µl	× unit/µg of cDNA
Nuclease-free H_2O	Up to 50 µl	
Total volume	50 µl	

Note: The amount of DNase I depends on its titer. We usually start with 0.2 unit to 1.1 unit DNase I. Dilute DNase I to × unit/µl in 1× One Phor-All-Buffer. Use immediately and do not store.

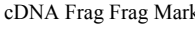
2. Incubate the reaction at 37°C for 10 min. Inactivate the enzyme at 98°C for 10 min.

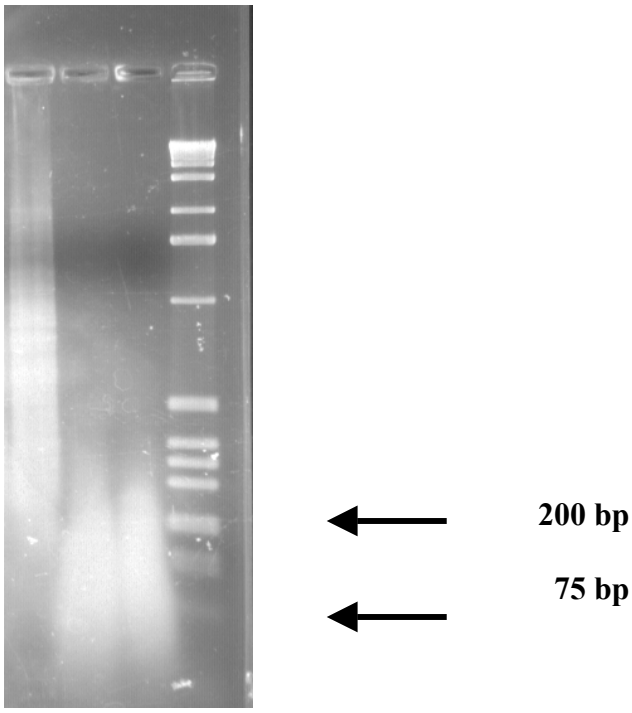
3. Add fragmented cDNA directly to the terminal labeling reaction. Alternatively, the cDNA can be stored at -20°C.

4. To examine fragmented cDNA, load 200 ng on a 1.5% agarose gel. Stain with SYBR gold (Molecular Probes) 20-40 min. Good results are obtained when the majority of the fragmented cDNA products migrate between 50 and 200 bp on nondenaturing agarose gels [we use Superladder-mid1 500/100 bp (GenSura Laboratories, San Diego) for size estimation].

5. SYBR Gold stain: To make a 1× staining solution, dilute SYBR Gold 10,000-fold in 1× TBE, pH 7-8.5. Add enough staining solution to cover the gel. Wrap container in foil and agitate gently (SYBR Gold stain is light-sensitive).

cDNA Before (lane 1) and After (lanes 2 and 3) Fragmentation





1.5% agarose TBE gel

cDNA synthesis and GeneChip hybridization.

Allow 30 µg of RNA per chip. Perform reactions in triplicate, using 10 µg of RNA in each.

1. Prepare the following mixture:

Primer Hybridization Mix

Components	**Volume/amount**	**Final concentration**
Total RNA	10 µg	0.33 µg
Random primers Hexamers (75 ng/µl) (Invitrogen)	10 µl	25 ng/µl
Nuclease-free H_2O	Up to 30 µl	

Total volume	30 µl	

2. Using a thermocycler, incubate the RNA-Primer mix at 70°C for 10 min followed by 25°C for 10 min and then chill to 4°C.

3. Prepare triplicates of the reaction mix for cDNA synthesis and add to the annealed RNA-primer mix.

cDNA Synthesis Reaction Mix

Components	**Volume/amount**	**Final concentration**
5× First Strand Buffer (Invitrogen)	12 µl	1×
100 mM DTT (Invitrogen)	6 µl	10 mM
10 mM dNTPs (Amersham)	3 µl	0.5 mM
SUPERase Inhibitor (20 unit/µl) (Ambion, Austin, TX)	1.5 µl	0.5 unit/µl
SuperScript III (Invitrogen) (200 unit/µl)	7.5 µl	25 unit/µl
Annealed RNA-Primer Mix	30 µl	
Total volume	60 µl	

4. Incubate the reaction at 25°C for 10 min, 37°C for 60 min, and 42°C for 60 min.

5. Inactivate the enzyme at 70°C for 10 min and hold at 4°C.

Removal of RNA

6. Add 20 µl of 1 N NaOH and incubate at 65°C for 30 min.

7. Add 20 µl of 1 N HCl to neutralize.

Purification and Quantitation of cDNA Synthesis Products

1. Use QIAquick Columns to clean up the cDNA synthesis product (for detailed protocol, see QIAquick PCR Purification Kit Protocols provided by the supplier). Elute the product with 40 µl of 1/2 strength Elution Buffer EB (supplied with QIAquick kit). Let EB stand on the filter for 1 min before spinning.

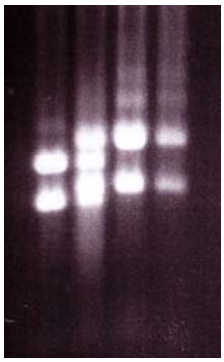
2. Speed Vac 10 min (without heat) to remove the residual ethanol that inhibits the fragmentation reaction.

3. Quantify the purified cDNA product by 260-nm absorbance (1.0 A_{260} unit = 33 µg/ml of single-strand DNA). Use 5 µl in 100 µl of water.

cDNA Fragmentation

Based on RNA Northern gels, we could predict what portion of RNA was plant and bacterial from each nodule.

1 2 3 4



(lane 1) Rm1021 RNA (bacteria)

(lane 2) Nodule RNA (bacteria and plant)

(lane 3) *Medicago truncatula* RNA (plant)

(lane 4) *M. truncatula* RNA (plant)

cDNA concentrations were adjusted for use with our GeneChip. Terminal labeling was increased for root nodules and plant roots to ensure excess label for these reactions.

CDNA	**Microgram cDNA for each GeneChip**	**Terminal labeling**
Bacterial cultures	4	1×
Root nodules	12	2×
Plant roots	8	2×

1. Prepare the following reaction mix:

Fragmentation Reaction

Components	**Volume/amount**	**Concentration**
10× One-Phor-All Buffer	5 µl	1×
cDNA	40 µl	4-12 µg
DNAse I (see Note)	× µl	× unit/µg of cDNA
Nuclease-free H_2O	Up to 50 µl	
Total volume	50 µl	

Note: The amount of DNase I depends on its titer. We usually start with 0.2 unit to 1.1 unit DNase I. Dilute DNase I to × unit/µl in 1× One Phor-All-Buffer. Use immediately and do not store.

2. Incubate the reaction at 37°C for 10 min. Inactivate the enzyme at 98°C for 10 min.

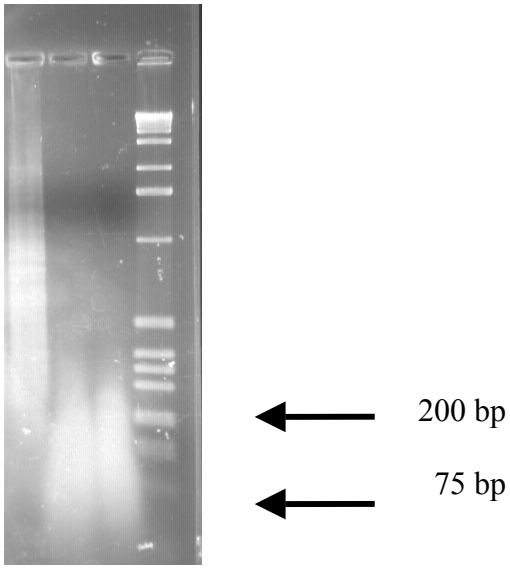
3. Add fragmented cDNA directly to the terminal labeling reaction. Alternatively, the cDNA can be stored at -20°C.

4. To examine fragmented cDNA, load 200 ng on a 1.5% agarose gel. Stain with SYBR Gold (Molecular Probes) 20-40 min. We get good results when the majority of the fragmented cDNA products migrate between 50 and 200 bp on nondenaturing agarose gels [we use Superladder-mid1 500 bp/100 bp (GenSura) for size estimation].

5. SYBR Gold stain: To make a 1× staining solution, dilute SYBR Gold 10,000-fold in 1× TBE, pH 7–8.5. Add enough staining solution to cover the gel. Wrap container in foil and agitate gently (SYBR Gold stain is light-sensitive).

cDNA Before (lane 1) and After (lanes 2 and 3) Fragmentation

cDNA Frag Frag Marker



Terminal Labeling

Use Enzo BioArray Terminal Labeling Kit with Biotin-ddUTP (Affymetrix) to label the 3′ termini of the fragmentation products.

1. Prepare the following reaction mix:

Terminal Label Reaction 1× (Free-Living Bacteria)

Components	Volume/amounts
5× Reaction Buffer	15.7 µl
10× $CoCl_2$	7.85 µl
Biotin-ddUTP	1 µl
Terminal deoxynucleotide transferase	2 µl
Fragmentation products (4 µg)	51.95 µl
Total volume	78.5 µl

Terminal Label Reaction 2× (Nodule and Plant)

Components	Volume/amounts
5× Reaction Buffer	15.7 µl
10× $CoCl_2$	15.7 µl
Biotin-ddUTP	2 µl
Terminal deoxynucleotide transferase	4 µl
Fragmentation products (8–12 µg)	41.1 µl
Total volume	78.5 µl

2. Incubate the reaction at 37°C for 60 min. Stop the reaction by adding 2 µl of 0.5 M EDTA.

3. The target is ready to be hybridized onto probe arrays, or it may be stored at -20°C.

Target Hybridization

1. Prepare the following solution mix:

Hybridization Cocktail for Single Probe Array

Components	Volume/amount	Final concentration
2× MES Hybridization Buffer	100 µl	1×
3 nM B2 Control	3.3 µl	50 pM
GeneChip Hybridization Control	10.0 µl of Never Freeze Thaw more than three times	1×
10 mg/ml herring sperm DNA	2.0 µl	0.1 mg/ml
50 mg/ml BSA	2.0 µl	0.5 mg/ml
100% DMSO	14.2 µl	7%
Fragmented labeled cDNA	78.5 µl	
Total volume	210 µl	

2. Equilibrate probe array to room temperature immediately before use.

3. Add the hybridization solution mix (210 µl) to the probe array.

4. Cover the septa with Tough Spots (USA Scientific, Ocala, FL)

5. Hybridize at 48°C, 60 rpm, for 16 h in an Affymetrix Hybridization Oven 640.

Probe Array Washing and Staining

Staining reagents are made according to Affymetrix protocol with volume adjustments.

Streptavidin Solution Mix

Components	Volume	Final concentration

2× MES stain buffer	300 µl	1×
50 mg/ml BSA	24 µl	2 mg/ml
1 mg/ml streptavidin	6 µl	10 µl/ml
DI H_2O	270 µl	--------
Total volume	600 µl	

Antibody Solution Mix

Components	Volume	Final concentration
2× MES Stain Buffer	300 µl	1×
50 mg/ml BSA	24 µl	2 mg/ml
10 mg/ml goat IgG	6 µl	0.1 mg/ml
0.5 mg/ml biotin antistreptavidin	6 µl	5 µg/ml
DI H_2O	264 µl	-------
Total volume	600 µl	

Streptavidin-phycoerythrin (SAPE) Solution Mix

Components	Volume	Final concentration
2× MES Stain Buffer	300 µl	1×
50 mg/ml BSA	24 µl	2 mg/ml
1 mg/ml streptavidin-phycoerythrin	6 µl	10 µg/ml
DI H_2O	270 µl	--------
Total volume	600 µl	

Reagents

12× MES stock (store at 4°C): For 1,000 ml: 70.4 g of MES free acid monohydrate, 193.3 g of MES sodium salt, 800 ml of molecular biology grade water. Adjust volume to 1,000 ml, pH 6.5–6.7; filter through a 0.2-µm filter.

2× hybridization buffer (store at 4°C, light sensitive): For 50 ml: 8.3 ml 12× MES stock, 17.7 ml of 5 M NaCl, 4.0 ml of 0.5 M EDTA, 0.1 ml of 10% Tween 20, 19.9 ml of molecular biology grade water.

Wash Buffer B (store at 4°C, light sensitive): For 1,000 ml: 83.3 ml of 12× MES stock buffer, 5.2 ml of 5 M NaCl, 1.0 ml of 10% Tween 20, 910.5 ml of molecular biology grade water. Filter through a 0.2-µm filter. Equilibrate to room temperature before each use.

Wash Buffer A (store at 4°C): For 1,000 ml: 300 ml of 20× standard saline phosphate/EDTA (SSPE) (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA), 1.0 ml of 10% Tween-20, 698 ml of molecular biology grade water. Filter through a 0.2-µm filter. Equilibrate to room temperature before each use.

2× Stain Buffer (store at 4°C, light sensitive): For 250 ml: 41.7 ml of 12× MES stock buffer, 92.5 ml of 5 M NaCl, 2.5 ml of 10% Tween-20, 112.8 ml of molecular biology grade water. Filter through a 0.2-µm filter.

10 mg/ml goat IgG stock (store at 4°C): Resuspend 50 mg in 5 ml of PBS,
1 mg/ml streptavidin solution (store at 4°C). Resuspend 5 mg in 5 ml of PBS.

Fluidics Protocol for Affymetrix Microarray Suite

Wash A1 recovery mixes	0
Wash A1 temperature (°C)	25
Number of wash A1 cycles	10
Mixes per wash A1 cycle	2
Wash B recovery mixes	0
Wash B temperature (°C)	48
Number of wash B cycles	4
Mixes per wash B cycle	15

Stain temperature, °C	25
First stain time (seconds)	600
Wash A2 recovery mixes	0
Wash A2 temperature	30
Number of wash A2 cycles	10
Mixes per wash A2 cycle	4
Second stain time (seconds)	600
Third stain time (seconds)	600
Wash A3 recovery mixes	0
Wash A3 temperature, °C	30
Number of wash A3 cycles	15
Mixes per wash A3 cycle	4
Holding temperature, °C	25

Fluidics Protocol for *Sinorhizobium meliloti* GeneChip

Post Hyb Wash 1	10 cycles of two mixes per cycle with Wash Buffer A at 25°C
Post Hyb Wash 2	Four cycles of 15 mixes per cycle with Wash Buffer B at 48°C
First stain	Stain the probe array for 600 seconds in streptavidin solution mix at 25°C
Poststain wash	10 cycles of 4 mixes per cycle with Wash Buffer A at 30°C
Second stain	Stain the probe array for 600 seconds in antibody solution mix at 25°C
Third stain	Stain the probe array for 600 seconds in SAPE solution at 25°C.
Final wash	15 cycles of 4 mixes/cycle with Wash Buffer A at 30°C

1. Bernstein, J. A., Khodursky, A. B., Lin, P.-H., Lin-Chao, S. & Cohen, S. N. (2002) *Proc. Natl. Acad. Sci. USA* **99,** p. 9697.