



# NimbleGen Gene Expression Microarrays

## *Sample Labeling Instructions*

### Outline

This protocol describes the process for labeling cDNA before hybridization to NimbleGen Gene Expression arrays. It is intended for use by researchers who want to deplete existing stock of reagents before transitioning to the use of NimbleGen One-Color DNA Labeling Kits.

Use this protocol as a replacement for only Chapter 3, Sample Labeling, of the *NimbleGen Arrays User's Guide: Gene Expression Analysis*. After you complete sample labeling, follow the instructions in the other chapters of the user's guide for complete processing of Gene Expression arrays.

### Protocol Information & Safety

Refer to the *NimbleGen Array's User's Guide: Gene Expression Analysis* for protocol information and safety considerations.

### Required Reagents/Consumables

| Component                                      | Supplier                   | Package Size        | Item Number                 |
|--|----------------------------|---------------------|-----------------------------|
| $\beta$ -Mercaptoethanol                       | Sigma Aldrich              | 25ml                | M3148                       |
| 0.5M EDTA                                      | Sigma Aldrich              | 100ml               | E7889                       |
| 100mM dNTPs                                    | Invitrogen                 | 4 x 25 $\mu$ mol    | 10297-018                   |
| 1M MgCl <sub>2</sub>                           | Sigma Aldrich              | 100ml               | M1028                       |
| 1M Tris HCl, pH 7.4                            | Sigma Aldrich              | 1 liter             | T2663                       |
| 5' Cy3-labeled Random Nonamers (9mer "Wobble") | Trilink<br>Biotechnologies | 50 O.D.<br>200 O.D. | N46-0007-50<br>N46-0007-200 |
| 5M NaCl  | Sigma Aldrich              | 250ml               | 71386                       |
| Absolute Ethanol                               | Sigma Aldrich              | 500ml               | E702-3                      |
| Isopropanol                                    | Sigma Aldrich              | 500ml               | I-9516                      |
| Klenow Fragment 3' $\rightarrow$ 5' exo-       | NEB                        | 50U/ $\mu$ l        | M0212M                      |
| Water: reagent grade, ACS, nonsterile, type 1  | VWR                        | 2.5 gallon          | RC915025                    |

## Sample Labeling

- 1 Refer to Chapter 2, Sample Preparation & QC, of the *NimbleGen User's Guide: Gene Expression Analysis* for the sample amount required for the array format.

- 2 Prepare the following three solutions:

| 10X TE (100mM Tris-HCl, 10mM EDTA) | All Array Formats | Notes                              |
|------------------------------------|-------------------|------------------------------------|
| 1M Tris HCl, pH 7.4                | 1.5ml             | Mix and store at room temperature. |
| 0.5M EDTA                          | 0.3ml             |                                    |
| VWR water                          | 13.2ml            |                                    |
| <b>Total</b>                       | <b>15ml</b>       |                                    |

| 50X dNTP Mix | All Array Formats | Notes  |
|--------------|-------------------|--|
| VWR water    | 250µl             | Aliquot 50X dNTP mix into single-use amounts and freeze. Avoid freeze-thaw cycles, which can result in diminished yields. When in use, keep dNTPs on ice at all times. |
| 10X TE       | 50µl              |  |
| 100mM dATP   | 50µl              |  |
| 100mM dGTP   | 50µl              |  |
| 100mM dTTP   | 50µl              |  |
| 100mM dCTP   | 50µl              |  |
| <b>Total</b> | <b>500µl</b>      |  |

| Random Primer Buffer | All Array Formats | Notes   |
|----------------------|-------------------|---|
| VWR water            | 860.75µl          | Prepare fresh buffer each time primers are resuspended. 42µl of Random Primer Buffer is needed per O.D. of 9mer primer. |
| 1M Tris HCl, pH 7.4  | 125µl             |   |
| 1M MgCl <sub>2</sub> | 12.5µl            |   |
| β-Mercaptoethanol    | 1.75µl            |   |
| <b>Total</b>         | <b>1ml</b>        |   |

- 3 Dilute Cy3-labeled 9mers to 1 O.D./42µl Random Primer Buffer. Aliquot to 40µl individual reaction volumes in 0.2ml thin-walled PCR tubes and store at -20°C, protected from light.
- 4 Assemble the following components in separate 0.2ml thin-walled PCR tubes.

| Component         | All Array Formats |
|-------------------|-------------------|
| cDNA              | 1µg               |
| Cy3-labeled 9mers | 40µl              |
| VWR water         | To volume         |
| <b>Total</b>      | <b>80µl</b>       |

- 5 Heat-denature samples in a thermocycler at 98°C for 10 minutes. Quick-chill in an ice-water bath for 10 minutes.

**Important:** Quick-chilling after denaturation is critical for high-efficiency labeling.

- 6 Prepare the following dNTP/Klenow master mix for each sample prepared in step 5.

**Important:** Keep all reagents and dNTP/Klenow master mix on ice. Do not vortex after addition of Klenow.

| dNTP/Klenow Master Mix: Recipe per Sample | All Array Formats |
|---|-------------------|
| 50X dNTP mix                              | 10µl              |
| VWR water                                 | 8µl               |
| Klenow (50U/µl)                           | 2µl               |
| <b>Total</b>                              | <b>20µl</b>       |

- 7 Add 20µl of the dNTP/Klenow master mix prepared in step 6 to each of the denatured samples prepared in step 5. Keep on ice.

| Component                          | All Array Formats |
|------------------------------------|-------------------|
| Reaction volume from step 5        | 80µl              |
| dNTP/Klenow Master Mix from step 6 | 20µl              |
| <b>Total</b>                       | <b>100µl</b>      |

- 8 Mix well by pipetting up and down 10 times.

*Important: Do not vortex after addition of Klenow.*

- 9 Quick-spin to collect contents in bottom of the tube.  
 10 Incubate for 2 hours at 37°C in a thermocycler with heated lid, protected from light.  
 11 Stop the reaction by addition of the Stop Solution (0.5M EDTA).

| Component                   | All Array Formats |
|-----------------------------|-------------------|
| Reaction volume from step 7 | 100µl             |
| Stop Solution (0.5M EDTA)   | 10µl              |
| <b>Total</b>                | <b>110µl</b>      |

- 12 Add 5M NaCl to each tube.

| Component                    | All Array Formats |
|------------------------------|-------------------|
| Reaction volume from step 11 | 110µl             |
| 5M NaCl                      | 11.5µl            |
| <b>Total</b>                 | <b>121.5µl</b>    |

- 13 Vortex briefly, spin, and transfer the entire contents to a 1.5ml tube containing isopropanol.

| Component                    | All Array Formats |
|------------------------------|-------------------|
| Reaction volume from step 12 | 121.5µl           |
| Isopropanol                  | 110µl             |
| <b>Total</b>                 | <b>231.5µl</b>    |

- 14 Vortex well. Incubate for 10 minutes at room temperature, protected from light.  
 15 Centrifuge at 12,000 x g for 10 minutes. Remove supernatant with a pipette. Pellet should be pink.  
 16 Rinse pellet with 500µl 80% ice-cold ethanol. Dislodge pellet from tube wall by pipetting a few times.  
 17 Centrifuge at 12,000 x g for 2 minutes. Remove supernatant with a pipette.  
 18 Dry contents in a SpeedVac on low heat until dry (~5 minutes), protected from light.  
 19 **STOP POINT:** Proceed to step 20, or store labeled samples at -20°C (up to 1 month), protected from light.

- 20 Spin tubes briefly prior to opening. Rehydrate pellets in 25µl VWR water per reaction.
- 21 Vortex for 30 seconds and quick-spin to collect contents in bottom of the tube. Continue to vortex or let sit at room temperature, protected from light, for approximately 5 minutes or until the pellet is completely rehydrated, then vortex again and quick-spin.
- 22 Quantitate each sample using the following formula:

$$\text{Concentration } (\mu\text{g/ml}) = A_{260} \times 50 \times \text{Dilution Factor}$$

*Note: The NanoDrop user's manual specifies that in the Nucleic Acid module the maximum accurate reading is 3,700ng/µl, and in the Microarray module, the maximum accurate reading is 700ng/µl. Roche NimbleGen recommends using the spectrophotometer in the Nucleic Acid module. If sample concentration exceeds these values, dilute sample appropriately and re-quantitate.*

- 23 Based on the concentration, calculate the volume of Cy3-labeled cDNA sample required for each hybridization per the following table and aliquot in a 1.5ml tube:

| Sample Requirements*    | 385K Array            | 4x72K Array           | 12x135K Array         |
|-------------------------|-----------------------|-----------------------|-----------------------|
| Cy3-labeled cDNA Sample | Prok: 3µg<br>Euk: 6µg | Prok: 2µg<br>Euk: 4µg | Prok: 2µg<br>Euk: 4µg |

\* Prok = Prokaryote; Euk. = Eukaryote

- 24 Dry contents in a SpeedVac on low heat, protected from light.
- 25 **STOP POINT:** Proceed to Chapter 4, Hybridization & Washing, of the *NimbleGen Arrays User's Guide: Gene Expression Analysis*, or store labeled samples at -20°C (up to 1 month), protected from light.

## Technical Support

If you have questions, contact your Roche NimbleGen Account Manager or Roche Microarray Technical Support. Go to [www.nimblegen.com/arraysupport](http://www.nimblegen.com/arraysupport) for contact information.



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