



NimbleGen Chromatin Immunoprecipitation-on-chip (ChIP-chip) Microarrays

Sample Labeling Instructions

Outline

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

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

Protocol Information & Safety

Refer to the *NimbleGen Array's User's Guide: ChIP-chip Analysis* for protocol information and safety considerations.

Required Reagents/Consumables

| Component | Supplier | Package Size | Item Number |
|---|----------------------------|---------------------|-----------------------------|
| β -Mercaptoethanol | Sigma Aldrich | 25ml | M3148 |
| 0.5M EDTA | Sigma Aldrich | 100ml | E7889 |
| 100mM dNTPs | Invitrogen | 4 x 25 μ mol | 10297-018 |
| 1M MgCl ₂ | Sigma Aldrich | 100ml | M1028 |
| 1M Tris-HCl, pH 7.4 | Sigma Aldrich | 1 liter | T2663 |
| 5' Cy3- and Cy5-labeled Random Nonamers (9mer "Wobble") | TriLink Biotechnologies | 50 O.D. 200 O.D. | N46-0010-50 N46-0010-200 |
| 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/ μ l | M0212M |
| Water: reagent grade, ACS, nonsterile, type 1 | VWR | 2.5 gallon | RC915025 |

Sample Labeling

Pairs of samples intended for hybridization to the same array should be labeled in parallel using Cy3- and Cy5-Random Nonamers from the same lot. Roche NimbleGen recommends labeling experimental (IP) samples with Cy5 and control (input) samples with Cy3.

- 1 Refer to Chapter 2, Sample Preparation & QC, of the *NimbleGen User's Guide: ChIP-chip 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 | |
| Total | 15ml | |

| 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 | |
| Total | 500µl | |

| 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 ₂ | 12.5µl | |
| β-Mercaptoethanol | 1.75µl | |
| Total | 1ml | |

- 3 Dilute Cy3 and Cy5 dye-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 experimental (IP) and control (IP) samples in separate 0.2ml thin-walled PCR tubes.

| Component | 385K and 4x72K Arrays | | 2.1M Arrays | |
|-------------------|--------------------------|------------------------|-----------------------------|-----------------------------|
| | Experimental (IP) Sample | Control (Input) Sample | Experimental (IP) Sample | Control (Input) Sample |
| MedIP Sample | 1µg | 1µg | 1µg (in each of 3 tubes)* | 1µg (in each of 3 tubes)* |
| Cy3-labeled 9mers | | 40µl | | 40µl (per tube) |
| Cy5-labeled 9mers | 40µl | | 40µl (per tube) | |
| VWR water | To volume (80µl) | To volume (80µl) | To volume (80µl) (per tube) | To volume (80µl) (per tube) |
| Total | 80µl | 80µl | 80µl (per tube) | 80µl (per tube) |

* 2.1M arrays require three labeling reactions each of experimental (IP) and control (input) sample per slide. Therefore, for 2.1M arrays, assemble three separate 0.2ml thin-walled PCR tubes with each containing 1µg DNA for each experimental (IP) and control (input) sample. Experimental (IP) and control (input) sample pairs intended for hybridization to the same 2.1M array should be labeled in parallel.

- 5 Heat-denature samples in a thermocycler at 98°C for 10 minutes. Quick-chill in an ice-water bath for 2 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 |
| Total | 20µl |

- 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 | |
|------------------------------------|--------------------------|------------------------|
| | Experimental (IP) Sample | Control (Input) Sample |
| Reaction volume from step 5 | 80µl | 80µl |
| dNTP/Klenow Master Mix from step 6 | 20µl | 20µl |
| Total | 100µl | 100µl |

- 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 | |
|-----------------------------|--------------------------|------------------------|
| | Experimental (IP) Sample | Control (Input) Sample |
| Reaction volume from step 7 | 100µl | 100µl |
| Stop Solution (0.5M EDTA) | 10µl | 10µl |
| Total | 110µl | 110µl |

- 12 Add 5M NaCl to each tube.

| Component | All Array Formats | |
|------------------------------|--------------------------|------------------------|
| | Experimental (IP) Sample | Control (Input) Sample |
| Reaction volume from step 11 | 110µl | 110µl |
| 5M NaCl | 11.5µl | 11.5µl |
| Total | 121.5µl | 121.5µl |

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

| Component | All Array Formats | |
|------------------------------|--------------------------|------------------------|
| | Experimental (IP) Sample | Control (Input) Sample |
| Reaction volume from step 12 | 121.5µl | 121.5µl |
| Isopropanol | 110µl | 110µl |
| Total | 231.5µl | 231.5µl |

Note: Up to 3 reactions containing the same sample can be combined in a 1.5ml tube and precipitated together. If combined, be sure to scale the isopropanol volume appropriately.

- 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 (Cy3) or blue (Cy5) depending on the dye.
- 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 experimental (IP) sample and control (input) sample required for each hybridization per the following table and combine both experimental (IP) and control (input) samples in a 1.5ml tube:

| Sample Requirements | 385K Array | 4x72K Array | 2.1M Array |
|--------------------------|------------|-------------|------------|
| Experimental (IP) Sample | 6µg | 4µg | 34µg* |
| Control (Input) Sample | 6µg | 4µg | 34µg* |

* If the 34µg was not obtained from the 3 labeling reactions, the hybridization can be performed with as little as 24µg.

- 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: ChIP-chip 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/arrayssupport for contact information.



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