UHN Microarray Centre

Genomic DNA Labelling Protocol for UHNMAC CpG Arrays

This protocol can be used with UHNMAC CpG-island (CGI) arrays.

The Human CGI 8.1K array contains 8,109 CpG-island clones, consolidated from the UHN's HCGI12K set and an independent 8.5K set obtained from Tim Huang at the Ohio State University (Columbus, OH). Redundant clones, clones with internal repeat sequences and clones mapping to chromosome M (Mitochondria) have been excluded. Compared to the HCGI12K array, the HCGI8.1K array has consistently lower signal intensities.

The Mouse CGI 4.6K array contains 4,642 CpG-island clones obtained from the Sanger Institute. This array set replaces the Mouse CGI 7.3K set.

Protocol Timeline:
Digestion of Genomic DNA and purification: 3.5 hr
Labelling digested genomic DNA: 3.5 hr
Purification of fluor-labelled genomic DNA: 0.4 hr
Hybridisation: 0.5 hr (set-up); overnight
Washing arrays: 1 hr

Digestion of Genomic DNA
1. To digest genomic DNA, combine the following reagents:

    5 µL 10X DpnII buffer
    2 µL DnpII enzyme (10U/ µL; NEB, cataloge # R0543S)
    5 µg genomic DNA
    Nuclease-free water to final volume of 50 µL

2. Incubate at 37°C for 3 hours.

Purification of digested genomic DNA with Qiagen QIAquick PCR Purification Columns
(Qiagen, catalogue # 28104)

1. Add 500 µL PB buffer (binding buffer) to each column.

2. Add digested DNA and mix.

3. Centrifuge each column at 20,800 x g (approximately 14,000 rpm on tabletop microcentrifuge) for 30 seconds in microcentrifuge; discard flow-through.

4. Add 600 µL PE buffer to each column and centrifuge at 20,800 x g for 30 seconds; discard flow-through. Repeat wash step twice more for a total of three washes.

5. Centrifuge each column at 20,800 x g for an additional 30 seconds to remove any remaining buffer at the tip of column; discard collection tube.

6. Transfer each column into a clean tube, add 10 µL of Sigma water per reaction and incubate at room temperature for 5 minutes.

7. Centrifuge at 20,800 x g for 1 minute to elute the purified DNA.

Labelling digested genomic DNA
(Mirus LabelIT® Nucleic Acid Labelling kit; Fisher Scientific, catalogue # Cy5-MIR 3725/Cy3-MIR 3625)

1. To label digested genomic DNA, combine the following:

5 µL 10X Mirus labeling A buffer
10 µL digested genomic DNA
1 µL Cy3/Cy5
1 µL Arabidopsis artificial transcript (5 ng/µL; plasmid from UHNMAC)
1 µL SpotReport™ Alien™ mRNA spike 1 and 2 (0.5 ng/µL; Stratagene, catalogue # 1-252551 & 2-252552)
Nuclease-free water to a final volume of 50 µL

2. Incubate reaction at 37°C for 3 hours.

Purification of fluor-labelled DNA using CyScribe™ GFX™ Purification kit
(GE Healthcare, catalogue # 27-9606-02)

Place the purification columns into the tubes provided, as described in the CyScribe™ GFX™ Purification kit manual. Samples that will be co-hybridised can be purified together. Please review the manual supplied by the manufacturer.

1. Add 500 µL of capture buffer to each column.

2. Transfer labelled genomic DNA product (approx. 100 µL) to the column, pipette up and down several times to mix.

3. Centrifuge at 13,800 x g for 30 seconds and discard flow-through.

4. Add 600 µL 80% ethanol and spin at 13,800 x g for 30 seconds and discard flow-through; repeat this step for a total of 3 washes.

5. Spin the column for an additional 30 seconds to ensure all ethanol is removed.

6. Transfer the GFX column to a fresh tube and add 60 µL of elution buffer (pre-warmed at 65°C) to the top of the glass fiber matrix.

7. Incubate the GFX column at room temperature for 5 minutes.

8. To elute purified labelled-cDNA, spin at 13,800 x g for 1 minute.

9. Use Speed Vac (heat setting on medium) to completely dry sample; be careful not to over-dry. Resuspend in 5 µL nuclease-free water.

Hybridisation
A prehybridisation step is not required.

1. Add 0.5 µL of Denaturation Buffer D1 (Mirus kit) to the labelled-DNA.

2. Incubate at room temperature for 5 minutes. Quickly chill on ice.

3. Add 0.55 µL Neutralization Buffer N1 (Mirus kit), mix well and incubate at room temperature for 5 minutes.

4. Prepare hybridisation solution:
   To each 100 µL of DIG Easy Hyb solution (Roche, catalogue # 1603558), add 5 µL of yeast tRNA (10 mg/ml; Invitrogen, catalogue # 15401-011) and 5 µg of species-specific Cot1 DNA (1 µg/ µL; Invitrogen, catalogue # 15279-011 (human), 18440-016 (mouse)). Incubate the mixture at 65°C for 2 minutes and cool to room temperature. Make enough solution for all hybridisations (estimate about 100 µL per slide).

5. Add the appropriate volume of the prepared hybridisation solution to each pooled pair of fluor-labelled cDNA (about 6 µL). Do NOT heat the samples at this point. The appropriate volumes of prepared DIG Easy Hyb solution (with yeast tRNA) are based on the size of the coverslip you are using.
   24x 60mm coverslip: 85 µL
   2 slides hybridised face-to-face (no coverslip): 85 µL

6. For the hybridisations involving the use of coverslips, the hybridisation mixture should be pipetted directly onto the coverslip. Place coverslip onto a reliable surface (the corner of a tip box works well) and add the hybridisation mixture. Lay the slide “array-side” down on top of the coverslip.

7. If you want to use two slides in a “face-to-face” hybridisation, coverslips are not required. The two slides which make up the pair should be faced together slightly offset to create a lip along one

edge. The barcodes on the arrays will create a small space between the slides. The hybridisation solution should be slowly and carefully applied along the lip. The hybridisation solution should evenly occupy the space between the slides and yield no bubbles. 85 µL is sufficient to cover the arrays.

8. Carefully place the slide(s) into hybridisation chamber(s) (VWR, catalogue # 48444-004). The hybridisation chambers that we use are plastic microscope slide boxes containing a small amount of DIG Easy Hyb solution in the bottom to keep a humid environment. Clean plain microscope slides are placed at every second or third slide position in the slide box to create rails or a platform onto which the hybridisation arrays can be placed. Each hybridisation chamber can hold two arrays. Carefully place the lid on the box and wrap with plastic wrap.

9. Incubate on a level surface in a 37 °C incubator overnight (about 16-18 hours).

Washing arrays

Use MilliQ water to prepare wash and rinse solutions.

1. Prepare wash solutions. Four staining dishes (Diamed, catalogue # E/S 258-4100-000) containing room temperature 1X SSC; three staining dishes containing 1X SSC/0.1% SDS pre-warmed to 50°C; one staining dish containing room temperature 0.1X SSC. Each staining dish holds about 250 mL.

2. Hold the array at the bar-code end with forceps and remove the coverslip by quickly but gently dipping the array in 1X SSC. The coverslip will slide off gently. For arrays used in a “face-to-face” hybridisation, carefully work the pair of arrays free of one another by immersing in 1X SSC and sliding the arrays past one another. It is critical that gloves be worn during this procedure.

3. Place the array into a staining rack and place into a staining dish with fresh 1X SSC.

4. When all of the arrays have been removed from the hybridisation chambers, wash for 3 sets of 15 minutes each at 50°C in staining dishes containing pre-warmed (50°C) 1X SSC/0.1% SDS. Gently agitate every 5 minutes.

5. To remove all trace SDS after the washes, rinse the arrays twice in room temperature 1X SSC (plunging 4-6 times each) and then rinse in 0.1X SSC.

6. Place the arrays in a slide box lined with blotting paper and spin slides dry at 80 x g for 5 minutes. Alternatively, slides can placed in a clean and dry 50 mL Falcon tube and spun at 80 x g for 5 minutes.

7. Store arrays in the dark until scanned. It is recommended that arrays be scanned as soon as possible after they are washed (at least within two days).

Reagent list and suggested suppliers

Reagent	Supplier	Product Code
DpnII restriction enzyme	NEB	R0543S
Qiagen PCR Columns	Qiagen	28104
Mirus Labelling Kit (Bufer A, D, N1)	Fisher Scientific	Cy5-MIR 3725 Cy3-MIR 3625
Spot Report Alien™ 1 and 2	Stratagene	#1-252551 #2-252552
DIG Easy Hyb solution	Roche	1 603 558
Yeast tRNA	Invitrogen	15401-011
Human Cot1 DNA	Invitrogen	15279-011
Mouse Cot1 DNA	Invitrogen	18440-016
CyScribe™GFX™ purification columns	GE Healthcare	27-9606-02
Staining Dishes	Diamed	E/S 258-4100-000
Slide Boxes	VWR	48444-004
Sigma Water	Sigma	W 4502
Artificial Arabidopsis transcript	UHN Microarray Centre	Arabidopsis plasmid