BAC CGH LABELING AND HYBRIDIZATION PROTOCOL FOR MANUAL HYBRIDIZATIONS

BCA-1 CGH ARRAY

The following procedure details a direct labeling of 1 ug of genomic DNA.
We perform competitive hybridizations pairing the experimental sample (labeled with cy5 dCTP) to a pooled control of the same gender (labeled with cy3 dCTP).
URO341 contain 2 differents hyb area allowing user to perform a dye swap experiment.
The second competitive hybridization combined experimental sample (labeled with cy3 dCTP) to a pooled control of the same gender (labeled with cy5 dCTP).

Materials:

Bioarray CGH labeling system (Enzo)
Machery Nagel Kit (NucleoSpin Extract II Cat.No.740 609.50)
Human or Mouse Cot –1 (Invitrogen, Cat #'s 15279-011 and 18440-016 respectively)
Yeast tRNA (Invitrogen, Cat #15401-011)
Hybridization buffer (Ambion Hyb#3, cat# AM8863)
20X SSC (Invitrogen, Cat #15557-044)
10% SDS (Invitrogen, Cat#24730-020)
100% ETOH
PCR strip tubes
Thermocycler
Water bath
Slide carrier and staining dishes (CML, cat# BVITRI – PPL25L)
Hybridization chamber (Corning, Cat #2551)
25x32 inch Lifterslip (Erie Scientific, Cat #25X60I-2-4789)
Slide spinner (Arraygenomics)
Sonicateur (ELMA, cat# D-78224 Singen/Htw, HF 35kHz)

Prepare the following wash solutions:
0.1X SSC, 0.1% SDS (1000 ml per one day of experiments)
0.1X SSC (1000 ml per one day of experiments)

Labeling Procedure (Day 1)

1. Quantify test (patient) and reference (control) DNA samples using the QUBIT system (BR DNA Quant) or NANODROP.
2. Run your sample on a 0.8% agarose gel to check the DNA quality. You should not use degraded DNA
3. Prepare 2,4 µg of Test and Reference DNA in eppendorf tube (in 55 ul) and sonicate them in Elmasonic sonicator (12-13 sec)
4. Aliquot and load 200-300 ng of each DNA sample (5 ul) onto a 0.8% agarose gel and use gel electrophoresis to examine DNA quality and to confirm that the majority of the fragmented DNA is 600 bp and more (but no native DNA left)
5. Clean the DNA using the NucleospinExtract II: (**optional if the DNA quality control is passed)**
    a. Add 200 µl NT buffer / 100 µl of DNA to each tube and transfer to individual columns.
    b. Place columns in collection tubes and spin for 1 minute at 11000g.
    c. Add 600 µl of NT3 wash buffer to each column and spin for 1 minute at 11000g.
    d. Spin again for 2 minutes at 11000g.
    e. Transfer the columns to microcentrifuge tubes.
    f. Discard the collection tubes.
    g. Add 51 µl of sterile water to columns and allow to sit for 1 minute at RT.
    h. Spin the columns at 11000g for 1 minute.

---

6. Aliquot 25 µl (1.1ug each) of test DNA and 25 µl (1ug each) of reference DNA into a total of four microcentrifuge tubes for Cy5 and Cy3 labeling (**test Cy3, test Cy5, reference Cy3, reference Cy5**).
7. Add 20 µl of random primers (Vial 1, Enzo Kit) to each tube; vortex and spin.
8. Heat the tubes in a thermomixer or a waterbath set to 99^{o} C for **10 minutes**.
9. Place on ice for **5 minutes**, briefly centrifuge, and return to ice.
10. Add 5 µl of the appropriate cyanine dye-labelled nucleotide mix (Vial 2 or 3, Enzo Kit)
11. Add 1 µl of Klenow enzyme (Vial 4, Enzo Kit)
12. Mix gently and pulse spin the tubes.
13. Incubate the tubes in a heat-block (**no waterbath, only heat-block**) set to 37^{o} C for **4 hours** (dark).
14. Add 5 µl of Stop Buffer (Vial 5, Enzo Kit) to each tube; briefly vortex and pulse spin.
15. Place tubes on ice until combining.
16. Combine labeled DNA pairs (**test Cy3** with **reference Cy5**; **test Cy5** with **reference Cy3**).

Cleanup Procedure

1. Add 200 µl NT buffer / 100 µl of DNA to each tube and transfer to individual columns.
2. Place columns in collection tubes and spin for 1 minute at 11000g.
3. Add 600 µl of NT3 wash buffer to each column and spin for 1 minute at 11000g.
4. Spin again for 2 minutes at 11000g.
5. Transfer the columns to microcentrifuge tubes.
6. Discard the collection tubes.
7. Add 51 µl of sterile water to columns and allow to sit for 1 minute at RT.
8. Spin the columns at 11000g for 1 minute.

Precipitation Procedure

17. Add 50 µl of Cot-1 DNA (Roche) to each tube.
18. Add 1 Vol of the total volume (200 µl) of 0,3M sodium acétate (pH 5-8).
19. Add 2,5 Vol of the total volume (500 µl) of ice-cold ethanol.
20. Vortex the solution and incubate at -70°C for 30 minutes (dark).
21. Spin the tubes in microcentrifuge for 15 minutes at 11000g (dark) at 4-8 °C
22. Remove the supernatant with vacuum aspirator and spin again to complete supernatant aspiration.

Hybridization Procedure

Prepare the BAC slide for hybridization. Remove from the slide from storage in a dessicator and put in a UV crosslinker. Set the crosslinker to 350 mJ and crosslink slides

1. Resuspend 8 µl of ARNt yeast DNA at 50 ug/ul (Applied Biosystem)
2. Heat probe at 95C for **5 minutes**.
3. Add 16 ul of Ambion hybridization buffer
4. Heat at 37°C during **30 minutes**
6. Vortex for 10 seconds, spin down at 13K rpm for 30 seconds.
7. Add entire probe covering the array (see figure 1).
8. Place a clean 24x32 inch Lifterslip over the top of the slide covering the array (*See figure 1 : annexe*)
9. Add 20 ul of H2O to humidity ports.
10. Assemble the hybridization chamber and submerge it in a 55°C for **19 hours**

Post Hyb Wash and Scanning Protocol (Day 2)

Post Hyb Wash

1. Remove the coverslip by immerse and shake in 0.1X SSC, 0.1% SDS – 50 C
   Then proceed to the following washes :

   a) 0.1X SSC, 0.1% SDS – 50 C (**55sec** plunge(agitation), **55sec** allow to sit)
   b) 0.1X SSC, 0.1% SDS – 50 C (**1min** plunge(agitation), **55sec** allow to sit)

   c) 0.1X SSC – 50 C (**55sec** plunge(agitation), **55sec** allow to sit)
   d) 0.1X SSC – 50 C (**55sec** plunge(agitation), **55sec** allow to sit)

2. Quickly plunge the slide carrier into a room temperature 0.1X SSC-filled staining dish and then a 100 % ETOH-filled staining dish, place slides into 50 ml conical tubes.
3. Spin with our slide spinner few second
4. Store slides in dark until scanning.

Figure 1 : Hybridization area : Dye swap experiment