Video Article

Technical Demonstration of Whole Genome Array Comparative Genomic Hybridization

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Abstract

Array comparative genomic hybridization (array CGH) is a method for detecting gains and losses of DNA segments or gene dosage in the genome [1]. Recent advances in this technology have enabled high resolution comparison of whole genomes for the identification of genetic alterations in cancer and other genetic diseases [2]. The Sub-Megabase Resolution Tiling-set array (or SMRT) array is comprised of a set of approximately thirty thousand overlapping bacterial artificial chromosome (BAC) clones that span the human genome in ~100 kilobase pair (kb) segments [2]. These BAC targets are individually synthesized and spotted in duplicate on a single glass slide [2-4]. Array CGH is based on the principle of competitive hybridization. Sample and reference DNA are differentially labeled with Cyanine-3 and Cyanine-5 fluorescent dyes, and co-hybridized to the array. After an incubation period the unbound samples are washed from the slide and the array is imaged. A freely available custom software package called SeeGH (www.flintbox.ca) is used to process the large volume of data collected - a single experiment generates 53,892 data points. SeeGH visualizes the log2 signal intensity ratio between the 2 samples at each BAC target which is vertically aligned with chromosomal position [5,6]. The SMRT array can detect alterations as small as 50 kb in size [7]. The SMRT array can detect a variety of DNA rearrangement events including DNA gains, losses, amplifications and homozygous deletions. A unique advantage of the SMRT array is that one can use DNA isolated from formalin fixed paraffin embedded samples. When combined with the low input requirements of unamplified DNA (25-100ng) this allows profiling of precious samples such as those produced by microdissection [7,8]. This is attributed to the large size of each BAC hybridization target that allows the binding of sufficient labeled samples to produce signals for detection. Another advantage of this platform is the tolerance of tissue heterogeneity, decreasing the need for tedious tissue microdissection [8]. This video protocol is a step-by-step tutorial from labeling the input DNA through to signal acquisition for the whole genome tiling path SMRT array.

Video Link

The video component of this article can be found at http://www.jove.com/video/870/

Protocol

PROBE LABELING

Note: limit exposure of Cye Dyes to light at all times (this can be achieved by working in a darkened area or by shielding the tubes with a cover such as aluminum foil)

1. Combine:

   (Setup 1 reaction tube for reference and 1 reaction tube for sample)
   - DNA (25-400 ng)
   - 5 µL of 5X random primers buffer (Final concentration: 5X Promega Klenow buffer and 7 µg/ µL random octamers)
   - Dilute to 17.0 µL total volume with distilled H_2O

2. Boil for 10 minutes at 100°C. Transfer immediately to ice for 1 minute.
3. Add 4 µL of 10X dNTP mix (2mM dATP, dGTP, dTTP, 1.2mM dCTP).
4. Add CyeDyes:
   - Add 2 µL (2 nmoles) of Cy-3 labeled dCTP to Sample DNA
   - Add 2 µL (2 nmoles) of Cy-5 labeled dCTP to Reference DNA (e.g., Novagen human genomic DNA)

5. Add 2.5 µL of Klenow (9 U/µL, Promega) and mix.
6. Incubate at 37°C overnight* (~18 hours).

*The overnight hybridization time can be adjusted between 14 to 24 hours without adversely affecting the labelling process to fit to a laboratory schedule.

SAMPLE CLEAN UP

(Combined probe clean-up and preparation for hybridization)

1. Using a Microcon YM-30 column:
   - Add 100 µL Cot-1 DNA (1µg/µL) to column. Do not touch membrane with pipette tip.
   - Pool the reactions (reference and sample) and add to column.
   - Place column in provided tube and spin at 13000 g for 10 minutes.
   - Add 200 µL distilled H_20 to membrane and repeat spin to wash.
   - Discard tube and add 45 µL hybridization solution: (Roche DIG Easy)

   Optional: add 5µL sheared herring sperm DNA (10µg/µL unit, Promega)

   - Invert Microcon, place in a new labeled tube, and spin at 3000 g for 3 minutes.

CALCULATION OF INCORPORATIONS

1. Remove 1.5 µL and measure Fluorophore incorporation using NanoDrop Spectrophotometer. Using your hybridization buffer as a blank (DIG Easy) follow the directions on the screen. (You can recover the sample from the pedestal, if necessary, after measurement.)

   (**Note:** Incorporations below 3.0 pmol/µL in either channel have shown variable results.)

2. Denature at 85°C for 10 minutes.
3. Place probe at 45°C for 30 minutes to 1 hour (allows Cot-1 DNA annealing.)

ARRAY HYBRIDIZATION

1. Place 44 µL probe solution onto the coverslip (22 mm x 60 mm) (Fisher Scientific). If available, place the coverslip on a slide warmer or heat block pre-warmed to 45°C to maintain temperature.
2. Align slide over coverslip and probe solution, lower one edge of the slide allowing contact with the hybridization solution. Continue to lower until the coverslip is attached to the slide with surface tension. Invert slide.
3. Place the slide into hybridization cassette (Telechem), pre-warmed to 45°C, and add 10 µL of water in the lower groove (to control humidity during hybridization).
4. Seal cassette and incubate for 36 - 40 hours at 45°C.

ARRAY WASHING

Note: All wash solutions are at pH 7.0

Removal of the slide from the hybridization cassette is critical. DIG Easy quickly crystallizes at room temperature. The slide should be immediately immersed and the cover slip removed in the wash solution.

1. Add approximately 60 mL of 0.1XSSC, 0.1%SDS (pH 7.0, 45°C) wash solution to a Coplin jar prior to opening the hybridization chamber.
2. Open chamber, add slide to wash solution and remove coverslip (it should slide off).
3. Wash the slide 3 times in 0.1XSSC + 0.1%SDS at 45°C for 1 minute each with agitation.
4. Rinse the slide 3 times in fresh 0.1XSSC*. There should be no residual bubbles from the SDS wash visible.

   *The slides can remain in the final wash solution until ready to centrifuge and scan (~15 minutes).

5. Centrifuge the slide in a 50 mL Falcon tubes at 700 g for 3 minutes.
6. Immediately scan the slides (signal intensities will diminish over time.)

Discussion

Poor quality DNA will not provide a good hybridization profile. It is essential to ensure that sample and reference DNA are free from contaminants such as phenol, RNA, salt, etc that may interfere with the random prime labeling step before starting a hybridization experiment. For example

the resuspension of DNA in Tris - EDTA (TE) instead of water is not recommended as high salt concentration can inhibit the labeling reaction. We recommend assaying DNA quality using the Nanodrop spectrophotometer to measure both the DNA quantity as well as overall shape of absorbance curve and the 260/280, 260/230 ratio.

Washing: Removing the slide from the hybridization cassette and transferring to the heated wash solution is a critical step in the hybridization process as the DIG Easy hybridization solution crystallizes very quickly. It is important that the wash solutions be at a neutral pH and the temperature of the wash solution is important for stringency while removing the unbound probe. After drying slides it is important to keep them in the dark if not scanning right away, or after scanning if you wish to rescan them at a later time.

Ozone: Ozone has been a worldwide concern when performing array CGH. Ozone levels above 5ppm have been shown to adversely affect the Cye dyes. Cye 5 is particularly sensitive to ozone degradation. Minimizing ozone exposure is key to preventing Cye dye degradation and loss of signal before and during scanning. Scanning at night for example, when environmental ozone is typically lower, is one possible option. Ozone free enclosures for automated slide washing and scanning and various chemical slide treatments are commercially available. Ozone resistant Cye dyes have also recently been developed.

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