PFGE arrays

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modification of the Invitrogen BioPrime array CGH kit and Agilent 60-mer oligo microarray processing

You can also use the labeling protocol with a homemade array hybe protocol, but use the entire amount of labeled band DNA in the probe.

DNA

Run PFGE as usual. Using double-wide lanes will give higher yields.
Excise band from gel.
Incubate in 1 ml water for 1 hour twice.
Replace water with 250 µl 1X HaeIII buffer (may need more depending on gel slice size). Add 5 µl HaeIII and digest at 37C overnight.
Gel purify using QIAQuick kit.
Nanodrop 1 ul (concentration is generally very low).
DNA can be stored –20C.

DNA labeling

DNA labeling is done with the Bio-Prime array CGH kit plus zymo columns. For reference, either use a wt PFGE plug that has not been run on a gel but has been processed as above, or use 1 µg HaeIII-digested wt genomic DNA.

Aliquot 21 µl gel-purified DNA into a new tube.
Add 20 µl 2.5X random primers solution.
Incubate 95C 5 min.
Ice 5 min.

Make a master mix for each dye:

1X

5 µl	10X dCTP nucleotide mix
3 µl	Cy dCTP
1 µl	Klenow

Add 9 µl to each reaction.
Mix gently.
Incubate 37C 2 hours.
Add 5 µl stop buffer.
Ice. The reaction can be stored –20C overnight if necessary.

Purify using the Zymo columns according to their instructions, except use 500 µl binding buffer for the first step.
Elute in 25 µl water.
Nanodrop 1 µl to check yield and dye incorporation.
Yield should be a few hundred ng with dye incorporation >20 pmol/µg.

Mix 100ng Cy3-labeled DNA and 100ng Cy5-labeled DNA.
Add water to bring the total volume to 44µl.
Add 11µl Agilent 10X Blocking Agent (prepare 10X Blocking Agent according to instructions that came with the tube).
For the next steps, only process 4 samples at a time so they don't rehybridize before assembling the array.
95°C for 5 minutes.
Cool @ room temperature for 5 minutes.
Add 55µl 2X Hi-RPM hybridization buffer, mix by pipetting.
Spin down.
Load 100µl onto the gasket slide:
Place a backing slide, Agilent side up, in a hybe chamber.
Pipet the whole volume of probe, avoiding bubbles, onto the center of one gasket area. Don't eject the last µl or two in order to avoid bubbles (only spare a couple of µl for this, otherwise the volume won't be enough and you'll get a dark spot in the middle of the array).
Spread it around as you pipet, but not too close to the gasket.
Do the same for the other gasket areas.
Remove the array from the box. The Agilent side is the Array side, and so should face down, onto the probe. Carefully lower the array over the gasket slide, keeping it flat.
Once the array is resting on the gasket slide, place the top of the hybe chamber, and slide the screw over the assembly. Tighten the screw all the way down, finger tight.
Look through the back of the chamber and rotate the slide. There should be one big bubble that moves freely. There may be one big bubble and a couple of little ones stuck to the sides. If they are small and isolated, don’t worry too much about them. You will probably do more harm than good trying to remove them. If they seem like they’ll interfere with the array, you can try knocking the array with the heel of your hand to dislodge them.
Put the array in the hybe oven. Make sure to balance the rotisserie.

Hybe 17 hrs. @ 65°C, 20RPM

Washing

Prepare your wash solutions. Be aware of array materials that may be for RNA only use.

Wash A (1 L)
add in this order:

700 ml	Water
300 ml	20X SSPE
0.25 ml	20% N-lauroylsarcosine

Filter. Shake to mix.

Wash B (1 L)
add in this order:

997 ml	Water
3 ml	20X SSPE
0.25 ml	20% N-lauroylsarcosine

Filter. Shake to mix.

Rinse the wash chambers, racks, and stirbars with water.
Set up:
two Wash A chambers, one with a rack and a stirbar on a stirplate.
one Wash B chamber with a stirbar on a stirplate.
one acetonitrile chamber with a stirbar on a stirplate.

For all stirring steps, the wash liquid should be visibly turbulent. Make sure the entire slide is submerged at all times.

Disassemble each hybe chamber one at a time. Use the plastic tweezers to gently wedge open the sandwich while submerged in Wash A. Transfer slide to the rack in the other Wash A chamber. Leave a gap between each slide and between the slides and the wall.
Once all the slides are in the rack, stir for 1 min.
Start stirring Wash B.
Transfer the rack into Wash B and stir for exactly 1 min. Don't worry about transferring some Wash A into Wash B.
Start stirring the acetonitrile.
Quickly transfer the rack into the acetonitrile, draining off some of the Wash B as you go.
Let stir 30 sec. Slowly and evenly pull the rack out of the acetonitrile. If you see droplets remaining on the slides, submerge them and try again.
Set the rack on a kimwipe.
Load the slides into scanning holders, Agilent side up and barcode sticking out, blotting excess acetonitrile if necessary. The scanner

scans through the back of the slide. Don't touch anywhere but the edges and the barcode.
Scan no more than 5 slides at a time to avoid ozone in the scanner.
You can reuse the wash buffers for more slides, covering when not in use and replacing the first Wash A for every batch.

Scanning

Open the Agilent scan control program. If the lasers refuse to warm up, power cycle the scanner.
Place the slides in the scanner, noting the slot numbers.
Select the appropriate slot numbers from the pulldown menus on the upper left.
Select the directory column and click edit values. Browse to find the directory you want to save in. Hit set. The column values should change.
Check the default preferences for the correct scanning area (61 x 21.6 mm), resolution (**5 µm**), laser power (100% each) and with the split and rotate box not checked.
Scan.
Open the scanned tif with the Agilent feature extraction software.
Process according to the appropriate protocol.
Check the visual results to make sure it looks ok. I usually check that it's aligned properly, and that the flagged spots make some sense.