Double-Stranding Agilent Protein Binding Microarrays

Updated 2/12/14 - LA

Arrays can be double-stranded weeks in advance and stored in foiled slide box in desiccator.

1. Warm hybridization oven to 85°C and thaw reagents for primer annealing mix (protect Cy3-dUTP from light).

2. Prepare 900ul primer annealing/extension mix in 15mL falcon tube (screw cap is better than eppendorf, to avoid tube leaking when heated). Add Cy3-dUTP last and immediately cover the tube in foil.

90.0ul	10x Thermo Sequenase Reaction Buffer *(supplied w/ polymerase, recipe in original PBM paper)*
10.5ul	Agilent Primer 1 (100uM) *(IDT, 5'-CAGCACGGACAACGGAACACAGAC-3', 25nmol in 250ul, -20°C)*
14.7ul	dNTPs (10mM)
8.0ul	Thermo Sequenase Polymerase *(Affymetrix #785001KT, -20°C)*
775.3ul	Milli-Q H_2O (sterile)
1.5ul	Cy3-dUTP (1mM) *(Amersham #PA53022, -20°C)*
900.0ul	= 1 slide (max 3 at a time)

3. Prewarm primer annealing mix to 85°C for 20 minutes in hybridization oven - prop the tube up against rotisserie bar to avoid leakage at the caps.

4. Prewarm array slide, steel hybe chamber, single-well gasket slide (reusable), and annealing mixture for about 3 minutes at 85°C in hybe oven (don't let it get too hot). Place gasket slide face-up in lower piece of steel chamber and array slide face-up in upper piece of steel chamber to speed assembly.

5. Add 900ul of annealing mixture to gasket slide, seal with array slide and close chamber. Make sure this is done as quickly as possible to avoid cooling: Remove components from oven, pipette polymerase mix onto gasket slide, gently lower array onto gasket, close the steel chamber and screw tight, place back in hybridization oven.

6. Slowly bring down temperature of hybridization oven:

10 min at 85°C	*At each change point, lower the set temperature of the oven, then open the door and use it to "fan" the oven until it reaches the new temperature.*
10 min at 75°C	
10 min at 65°C	
90 min at 60°C	

7. While decreasing oven temperature, warm 1L of 0.01% TX-100/PBS ("Solution A") in 37°C water bath.

8. Disassemble gasket/array sandwich while submerged in 500ml Solution A in staining dish at 37°C: using plastic forceps, insert one tip between gasket coverslip and array slide, and twist to break the seal. Be careful not to let the forceps slip and scratch the array!

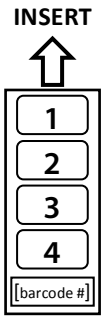
9. Wash array: Transfer array to new staining dish with rack and 500ml Solution A at 37°C. Stir 10 minutes with magnetic stir bar at ~600rpm in the dark under ice bucket.

10. Transfer array in rack to new staining dish with 500ml 1X PBS at room temperature. Stir 3 minutes at ~600rpm in the dark under ice bucket.

11. Remove slide from PBS slowly (tilted slightly downward) so that surface dries uniformly. Briefly spin slide to dry completely. Store in a dark (foil-covered) slide box in the desiccator.

Scanning Double-Stranded Arrays:

1. Turn on Axon 4300A scanner and open Gene Pix Pro software
    a. Allow 15 minutes for lasers to warm up
    b. Load slide with "Agilent" label facing down and barcoded end towards you
2. Laser Settings
    a. **Wavelength #1: 532nm**
    b. **Pixel Size: 5um**
3. Hardware settings
    a. **Wavelength #1: 532nm**
    b. **PMT Gain: 330**
    c. Power (%): 100
    d. **Filter: Standard Green (Cy3)**
    e. Lines to Average: 1
    f. Focus Position: 0um



Version number
251-60601 = v9
251-56811 = v11

4. Scan full slide
    a. Click "**>>**" button to preview scan (should see green scan)
    b. Drag in white side bars ("Scan Area") to outline all 4 arrays
    c. Click "**>**" for high-resolution scan (takes about 10 min)
        i. *To eject slide, open door on scanner*
5. Zoom in
    a. Should see dark control spots and slightly darker green spots (due to dUTPs in probe sequence)
    b. Brightness/contrast should be in upper 90's (click on "!" button to auto-adjust)
6. Save image
    a. Create a new folder for each slide with version number and slide number, Ex. "v11#417"
    b. Format file name as shown: [20130325_v11#417_Cy3_PMT330.tif]
7. Open Settings
    a. Open file "v11_15681.gps" gene pix settings file for version 11 slide (scan will show up red)
        i. v9 and v11 .gps files located in D: Scan Data > Llinas Lab > PBM Files
    b. Hit F8 to auto-align grid on all 4 arrays (if this does not work well, start over and only use F5)
    c. Zoom in on each array to check alignment (repeat as necessary until grid aligns with all spots)
        i. Use tool (arrow w/ square) to move and align grid
        ii. Use Feature Tool to manually align individual features
        iii. Press F5 to re-align all features on an array
    d. Zoom out to flag spots with dust
        i. Use Feature Tool to select spots and press "A" to flag them
        ii. Grid must be showing to flag a spot
    e. Repeat alignment and flagging for each array
8. Save Settings As
    a. Format file name as shown: [20130325_v11#417_Cy3.gps] and save in that slide's folder
    b. Click "Analyze" to generate table of data
9. Save Results – Selected Results Sheet
    a. Format file name as shown: [20130325_v11#417_Cy3_PMT330.gpr] and save in that slide's folder
    b. Select option to save each block as a separate .gpr file (will create 4 files total)