# Affymetrix Tiling Arrays

Updated: S. Silverman 6/2011
Updated by Mark Hickman (Nov 2009).
Updated by David Gresham and Alex Ward (June 2008).
This protocol is based on previous protocols developed by Maitreya Dunham, Matt Brauer, Justin Borevitz, Elizabeth Winzeler and Affymetrix.

**Aim:**
This protocol is used to identify SNPs compared to a reference genome (in this case, yeast strain FY3). Briefly, Klenow enzyme is used to incorporate a biotinylated nucleotide in sonicated genomic DNA. The labeling reaction is performed at 25°C, which is a nonpermissive temperature for Klenow and therefore limits its processivity. Labeled DNA is around 100bp in length and the labeling method results in approximately a 7-fold amplification of DNA. This labeled DNA is then hybridized to an Affymetrix yeast genome tiling array. The hybridization results are then processed by the SNP scanner program to identify peaks of non-hybridization, representing SNPs compared to the reference genome.

**Materials:**

1. Genomic DNA – DNA must be good quality, DNA prepared using Qiagen Genomic Tips is preferred (for enough DNA, use 50mL of saturated culture)
2. Affy Yeast Tiling Arrays (Reverse) – can be purchased from the microarray facility (~1 week before experiment).
3. Invitrogen BioPrime Labeling Kit – Product 18094-011
4. Reagents for hybridization and washes (see below).

**Preparing genomic DNA fragments by sonication**

1. Dilute 10 $\mu$g of sample genomic DNA in a total volume of 200$\mu$L $dH_2O$. (For all steps where it is required, measure DNA concentration with Qubit fluorimeter, which is more accurate.)
2. Sonicate DNA using program 4 on the Botstein sonicator (power=1, pulse duration= 0.5s, 0.5s off; total=30s)
3. Run 15$\mu$L on a 1% agarose gel to confirm sonicated product is sufficiently sonicated (median size of products ~600bp). You can run ~1 $\mu$g of non-sonicated genomic DNA for comparison.
4. Concentrate DNA in a Zymo-5 column: add 1mL of DNA binding buffer to 200$\mu$L sonicated DNA and mix. Load onto column 600$\mu$L and spin at max speed for 30 sec. Load the remaining ~600 $\mu$L onto column and spin for 1 min. Don't perform the wash step as this results in loss of DNA. Elute in 25uL $dH_2O$ (leave $dH_2O$ on column for 5 min to allow DNA to dissolve).

5. Determine DNA concentration.

**Labeling DNA using Invitrogen BioPrime kit**

1. In 200µl thin-walled PCR tube on ice, add:
    - 1000ng of sonicated DNA in a total of 72µl $dH_2O$
    - 60 µl of 2.5X random primer solution (from Bioprime kit)
2. Denature in PCR block by heating for 8min at 99°, and cooling down (fast ramp) to 4° for 8min (Use BioPrime program on PCR machines). Centrifuge briefly.
3. Add 5µL 10X dNTP mixture (from Bioprime kit) and mix briefly.
4. Add 3µL Klenow fragment and mix gently but thoroughly by pipeting.
5. Incubate at 25°C for 16hr and then hold at 4°C (Use program 25for16 in PCR machine)
6. Add 15µl stop buffer (from Bioprime kit).
7. Transfer the solution to 1.5ml Eppendorf tube and add:
    - 16µl 3M sodium acetate (pH5.2) – mix
    - 400µl cold ethanol
8. Mix by inverting the tube and place at -20°C for 1-2h to precipitate DNA
9. Centrifuge at maximum for 10min to pellet DNA and carefully pour off the supernatant.
10. Add 500ul 80% cold ethanol and place at room temperature for 10min.
11. Centrifuge at maximum speed for 10min and carefully remove the supernatant.
12. Spin the tube to bring all remaining supernatant to the bottom of the tube, and remove supernatant with a 200µL pipette.
13. Resuspend the pellet in 50µl $ddH_2O$ (mix well, may have to pipet up and down to fully resuspend; there will be some insoluble material).
14. Run 5µl on 2% agarose gel to confirm tightly distributed band around ~100bp.
15. Quantitate DNA and store at -20°C.

**Hybe Protocol (per Dunham Protocol with some modifications)**

1. Bring 3µg of labeled DNA to a total of 133µl with water.
2. Add: (BSA, Salmon sperm DNA and control oligo kept at -20C)
    - 150 µl 2X hybe buffer (recipe at end of protocol)
    - 15 µl 10 mg/ml BSA (stored at -20°C)
    - 3 µl 10 mg/ml salmon sperm DNA (stored at -20°C)
    - 5 µl 3 nM control oligo B2 (ordered from Affymetrix; see note at end, stored at -20°C)
3. Take tubes on ice downstairs to microarray facility.
4. Incubate 99°C for 5 min. While incubating, load array (see loading instructions below) with 200 µL 1x hybe buffer (see recipes) and prehybe array in the oven for 10 min at 45°C, 60rpm.
5. Incubate probe 45°C 5 min. (If there is no block or water bath set to this

temperature, you can put the tubes in the oven).
6. Spin the tubes 5 min.
7. Remove the prehybe buffer from the array (see Removing instructions below) and load with 200 µl probe, avoiding any debris at the bottom of the tube.
8. Cover septa with Tough Spots to prevent leakage, and put arrays in oven at 45°C, 60 RPM for 20 hours.
9. You can make the Wash Buffers A and B to be used the next day.

**Loading the Array (for above steps)**

1. Put a 200 µl filter tip in one of the septa as an air release.
2. Pipet up your solution from a tube with a 200 µl filter tip.
3. Holding the array so the air release is upward, puncture the other septum and slowly pipet in your solution to fill the chamber.
4. Withdraw the tip. You should have a bubble in the array chamber.
5. Remove the air release tip. It should not have any solution in it.
6. Place Tough-Spots on the septa.
7. Place the array in the plastic holder tray. Snap it into the holders in the oven. Balance with another tray.

**Removing solution from array (for above steps)**

1. Remove the Tough-Spots with forceps.
2. Place a 200 *µ*l filter tip in one of the septa as an air release. Pierce the other septum with a 200 ul filter tip attached to a pipet that is pressed down.
3. Keep the air release above the septum you are pipetting from, which will allow all the solution to accumulate at the bottom. Pipet out the solution.

**Washing (including antibody incubations)**

1. Make Wash A, Wash B, SAPE and antibody solutions (recipes below).
2. Turn on the Affymetix computer and log into Princeton using your Princeton ID and password. Open the GeneChip Operating software. Turn on Wash station.
3. Enter new experiments by first going to the File menu and choosing “New Experiment”.
4. Click the Barcode field and scan the barcode on the microarray.
5. Enter in your sample name the following way: Lab_Initials_Date_Sample (e.g., Bot_AW_20070804_FY4). Copy and paste the sample name into the Experiment name field.
6. For Sample Type field, choose “Yeast” from the pull-down list. For Project Type field, choose “Tiling” from the pull-down list. Press Save.
7. Repeat step 3-6 for each microarray.
8. Prime the wash station: Go to the Run menu in the GeneChip Operating software. Choose Fluidics. A window will open. Choose “no probe array” as the

sample. Choose “Prime450” as the protocol. This will set the parameters for each module (a module accommodates one microarray), but you can select all modules if you have 4 arrays. Fill wash bottles with the appropriate wash buffers and fill the $dH_2O$ bottle. Press Run and follow the instructions. The subsequent priming will take ~10 minutes.

9. After priming is finished, set up the parameters in the Fluidics window for each module (array): Choose your sample from the pull-down list. Select “EukGE-WS2v4” for the protocol.
10. Press Run for each module and follow the instructions in the window. Remove the ToughSpots from the septa before loading the array into the wash station, and be sure to load the correct array into the correct module. The subsequent washing will take ~1 hour and 20 minutes.
11. After loading the arrays into the wash station, turn on the Affymetrix scanner. This gives the scanner time to warm up before you begin scanning.
12. When the washing is completed, remove the arrays from the wash station but do NOT reengage the washblock. Ensure there are NO bubbles in the array. If there are, place the array back in the washblock and engage. Repeat this until there are no bubbles. Seal the septa with ToughSpots.
13. Place the arrays in the scanner, being sure to avoid slot 1 (which is outlined in red and should only be used if you are using manual mode).
14. Scan the arrays by going to the Run menu and choosing Start Scanner. A window will appear. Make sure the 1$^{st}$ box is checked. Press OK. Scanning will take ~2 hours for 4 arrays.
15. Shut down wash station using Shutdown_450 protocol for all modules. Save any remaining wash buffer.

**Data export and SNP scanner**

1. The Affymetrix program generated EPT, DAT and CEL files that are stored in a user folder named after your Princeton ID. The folder is located on the hard drive of the Affymetrix computer.
2. Export your data to \\arrayfiles\arraydata\people\XX where XX is your Princeton ID, using the following protocol: Make sure you are logged into the computer with your Princeton ID and password. Go to My Computer>Tools>Map Network Drive. Choose a drive letter from the drop down menu, and choose \\arrayfiles\arraydata as the folder. Click Finish. In the Start Menu, open the GCOS Manager, go to the Tools menu and choose Set Data Path. Choose \\arrayfiles\arraydata\people\XX as the folder. In the Process Tab in the left field, click Samples, click Universal or Expression, and then double click the experiment you wish to export. In the right field, the experiment will appear. Right click on the experiment name and select export. Exporting will take around 3-8 minutes; you can tell that it is working because in the lower field it will say “running”. Repeat the export for each experiment and they will be exported serially. When the exporting is finished, the lower field will say “available”. Once

finished, you can double click on the experiment in the right field, and the DAT and CEL files will appear beneath it. Log out of the computer. (Do not archive your data at this point. It will be done automatically later on, and if you do archive, the CEL file will not be in the correct format for the next steps.)

3. Establish account on rackett server and copy SNP_Scanner folder to your folder.
4. Copy CEL file from arrayfiles to rackett: From Mac finder, connect to server smb://arrayfiles/arraydata. Then open Fugu, a secure file transfer program. On the left part of the window, go to the top directory /. Open "Volumes". Then open "arraydata/people/XX", where XX is your Princeton ID. On the right part of the window in Fugu, open XX@rackett. The password for rackett is your genomics password. Open CEL folder and drag CEL files from left part (arrayfiles) to right part of window (rackett).
5. Run the SNP scanner script using the following steps: Open a terminal. ssh to your rackett folder using your genomics username and password. Copy the SNP_Scanner1_1 directory from Botlabshare to your directory on rackett using Fugu or any file transfer utility. Type "cd SNP*" and press Enter, which will change to that directory. Type command "./tiling_predict.sh FileName.CEL NO" and Press Enter. The script will take >2 hours to run. Leave the terminal window open. (Or you can run the script in the background by adding a space and then "&" without the quotes to the end of the command. You can then close the terminal window.) The script will create an SGR file in the folder "SNP_Scanner_1_1/results" and the name of this file is FileName.SGR. Also, the script will create text files in the folder "SNP_Scanner_1_1/Peaks" that will list the significant peaks identified by SNP scanner using a stringent threshold. (The reference genome is FY3, so all mutations identified will be relative to that strain.)
6. Copy the SGR file to your computer using Fugu. Download IGB 5.12 to your computer (this is an older version but works with the SNP scanner output.) Start IGB; it will run a script off the web. Open preferences in File menu, select Data Sources, make sure QuickLoad is turned on and "http://puma.princeton.edu/quickload_data/ " is in the QuickLoad URL field. Close window. Click QuickLoad tab and choose "Personal" as data access and "S_cerevisiae_Jul_2007" as genome. In file menu, select Open File and select your SGR files to open. They will take a few minutes to load. Using IGB, you can verify that the peaks identified by SNP scanner are specific/significant or you can identify peaks not picked up by SNP scanner.

## Recipes

**2X Hybe buffer (**50 ml total)
8.3 ml 12X MES (to 200 mM)
17.7 ml 5 M NaCl (to 2M Na+)
4 ml 0.5 EDTA (to 40 mM)
100 µl 10% Tween-20 (to 0.02%)

19.9 ml $dH_2O$
Store at 4°C in dark.

**Wash buffer A (**1 L total)
300 ml 20X SSPE (to 6X, i.e. 0.9 M NaCl, 60 mM $NaH_2PO_4$, 6 mM EDTA)
1 ml 10% Tween-20 (to 0.01%)
water to 1 L.
Filter sterilize.

**Wash buffer B (**1 L total)
83.3 ml 12X MES (to 100 mM)
5.2 ml 5 M NaCl (to 0.1 M Na+)
1 ml 10% Tween-20 (to 0.01%)
water to 1 L.
Filter sterilize.
Store 4°C in dark.

**2X Stain Buffer (**250 ml total)
41.7 ml 12X MES (to 200 mM)
92.5 ml 5 M NaCl (to 2 M Na+)
2.5 ml 10% Tween-20 (to 0.1%)
water to 250 ml
Filter sterilize.
Store 4°C in dark.

**12X MES (**100 ml total)
6.461 g MES hydrate (to 1.22 M MES)
19.33 g MES sodium salt (to 0.89 M Na+)
water to 100 ml
Filter sterilize.
pH should be between 6.5 and 6.7.
Store 4°C in dark. Discard if it turns yellow.

**20X SSPE** (buy from amresco)
3 M NaCl
0.2 M $NaH_2PO_4$
20 mM EDTA

**SAPE** (These will be stains 1 and 3. Make 1.2 mL per array.)
12 µl 1 mg/ml R-streptavidin phycoerythrin (to 10 ug/ml) (Invitrogen S-866)
240 µl 10 mg/ml BSA (to 2 mg/ml)
600 µl 2X stain buffer (to 1X)
348 µl water
Make the day of use in a 1.5 mL tube. Store in 600 µl aliquots at 4°C in dark until use.

600 µl will be used as stain 1, and 600 µl will be used as stain 3.

**Antibody solution** (This will be stain 2. Make 0.6 mL per array.)
6 µl 10 mg/ml goat IgG (to 0.1 mg/ml)
120 µl 10 mg/ml BSA (to 2 mg/ml)
3.6 µl 0.5 mg/ml anti-streptavidin antibody (goat), biotinylated (to 3 ug/ml) (Vector Labs BA-0500)
300 µl 2X stain buffer (to 1X)
170.4 µl water
Make the day of use in a 1.5 mL tube. Store in 600 µl aliquots at 4°C in dark until use. 600µl will be used as stain 2.

**Part #: 900301,** Control Oligo B2, 3nM

http://www.affymetrix.com/estore/browse/products.jsp?navMode=34000&productId=131541&navAction=jump&aId=productsNav#1_1

Use Control Oligo B2, 3 nM to provide alignment signals for image analysis software products.

The reagent is also included in the Hybridization Control Kit, part numbers 900454 and 900457.

**Goat IgG**

NC9538546 from ThermoFisher.

OR Non-immune Goat IgG (Cedar Lane Labs CLGT00, lot IG963R1). stock is 10mg/ml in 50 % glycerol, store at -20°C