Aim:

This protocol uses Klenow enzyme to incorporate a biotinylated nucleotide to label DNA. Sonicated DNA is used as a template. 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. The labeling method results in approximately a 7-fold amplification of DNA.

The hybridization protocol is a standard Affymetrix hybe and wash protocol.

Materials:

1. Genomic DNA – DNA must be good quality, DNA prepared using Qiagen Genomic Tips are preferred
2. Affy Yeast Tiling Arrays (Reverse) – can be purchased from the microarray facility
3. Invitrogen BioPrime Labeling Kit – Product 18094-011
4. Reagents for hybridization and washes (see below)

1. Preparing genomic DNA by sonication

   • Dilute 5µg of sample DNA in a total volume of 200uL dH2O
   • Sonicate DNA using program 4 on the Botstein sonicator (power=1, duration= 0.5s, total=30s)
   • Run 15uL on a 1% gel to confirm sonicated product is sufficiently fragments (median size of products ~600bp)
   • Concentrate DNA in a Zymo-5 column, add 1mL of DNA binding buffer to 200uL sonicated DNA and load 600uL (will have to do this twice as you will have 1.2mL, load one sample spin then load the other making sure to empty the tube between spins)
   • Elute in 25uL dH2O
   • Determine concentration using fluorimeter.

2. Labeling DNA using Invitrogen BioPrime Labeling

   • In 200ul PCR tube, add on ice:
     • Add 1000ng of labeled DNA in a total of 72uL dH2O
     • 60 uL 2.5X random primer solution
   • Denature by heating for 8min at 99°C in PCR block, fast ramp cool down to 4°C for 8min, centrifuge briefly. (Use BioPrime program on PCR machines)
   • Add: 15ul 10x dNTP mixture
     • Mix briefly
     • Add 3ul Klenow fragment
     • Mix gently but thoroughly by pippeting
   • Incubate at 25°C for 16hr (in PCR machine---use program 25for16)
   • Add 15ul stop buffer
   • Transfer the solution to 1.5ml Eppendorf tube, add:
     • 16ul 3M sodium acetate (pH5.2) – mix
     • 400ul cold ethanol
     • Mix by inverting the tube
   • Place at -20°C for 1-2h to precipitate DNA
   • Centrifuge at maximum for 10min to pellet DNA
3. Hybe Protocol (per Dunham Protocol with some modifications)

- Carefully remove the supernatant
- Add 500ul 80% cold ethanol – place at room temperature for 10min
- Centrifuge at maximum speed for 10min
- Carefully remove the supernatant
- Dry the pellet at room temp
- Resuspend the pellet in 50ul ddH2O (mix well, may have to disturb pellet with pipet tip to fully resuspend)
- Run 5ul on 2% agarose gel to confirm tightly distributed band around ~100bp
- Quantitate DNA using the fluorimeter
- Store at -20°C. (Label tube with DNA concentration and that it is a bioprime labeled sample)

| 150 µl | 2X hybe buffer (recipe at end of protocol) |
| 15 µl | 10 mg/ml BSA |
| 3 µl | 10 mg/ml salmon sperm DNA |
| 5 µl | 3 nM control oligo B2 |

Take tubes on ice downstairs to microarray facility.

- Incubate 99°C 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.
- Incubate probe 45°C 5 min.
- Spin 5 min.
- Remove the prehybe buffer from the array and load with 200 µl probe, avoiding any debris at the bottom of the tube.
- Cover septa with Tough Spots to prevent leakage.
- Put arrays in oven at 45°C, 60 RPM.
- Note time. Hybe 20 hours.

Loading the Array

Put a 200 µl filter tip in one of the septa as an air release. Pipet up your solution with a 200 µl filter tip. Holding the array so the air release is upward, puncture the other septum and slowly pipet to fill. Withdraw the tip. You should have a bubble in the array chamber. Remove the air release tip. It should not have any solution in it.

Place the array in the plastic holder tray. Snap it into the holders on the oven. Balance with another tray and trash arrays.

To remove sample, place a 200 ul filter tip in one of the septa as an air release. Pierce the other septa with a 200 ul filter tip and pipet out the solution.

Always have pipet tip with air release above the septa you are pipeting into!!

Entering New Experiments

Open GeneChip Operating Software on Affy Computer.

Go to File: New Experiment. Scan the barcode on the chip and enter in your sample name the following way: Lab_Initials_Date_Sample, for example: Bot_AW_20070804_FY4

Pull down Yeast for Sample Type and Tiling for Project Type. The experiment name should be the same as the sample name. Hit save and repeat for all arrays.

Washes

Make all wash buffers before starting. This includes Wash A, Wash B, SAPE and Antibody solution. Recipes are at the end of this protocol. Make sure you all have components made if necessary.

Turn on the wash station and open program on Affy Computer. (GeneChip Operating Software)

Prime the Wash Station using Prime450. Go to Run: Fluidics. Choose no probe array for sample. Choose Prime450 as your protocol. Repeat this for each module you are using. If you have 4 arrays, after entering the information for module 1, you may select all modules. Fill wash bottles with appropriate wash buffer and ensure that dH2O bottle is full before running the protocol. When ready, hit start.
After the station has been primed, set up the wash by choosing your sample from the pull down menu. Follow the directions for the washes, using the EukGE-WS2v4 protocol. Be sure to load the correct array into the correct spot on the wash station. Remove the ToughSpots from the septa before loading the arrays!

After loading the chips, turn on the Affy scanner. This gives the scanner plenty of time to warm up before you begin scanning.

When done, remove the arrays from the fluidics station but do NOT re-engage the washblock. Ensure there are NO bubbles in the array. If there are, place the chip back in the washblock and engage. Repeat this until there are no bubbles. Seal the septa with ToughSpots. Place the arrays in the scanner avoiding slot 1 (outlined in red – only use this slot if you are using manual mode)

Scan. Go to Run:Start Scanner. Keep the 1st box checked. Load arrays and click ok. (using the Autoloader)

Shut down fluidics station using Shutdown_450 protocol. Save any remaining wash buffer.

Recipes

**2X Hybe buffer**
50 ml

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3 ml</td>
<td>12X MES (to 200 mM)</td>
</tr>
<tr>
<td>17.7 ml</td>
<td>5 M NaCl (to 2M Na+)</td>
</tr>
<tr>
<td>4 ml</td>
<td>0.5 EDTA (to 40 mM)</td>
</tr>
<tr>
<td>100 µl</td>
<td>10% Tween-20 (to 0.02%)</td>
</tr>
</tbody>
</table>

water to 50 ml

Store 4°C in dark.

**Wash buffer A**
1 L

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 ml</td>
<td>20X SSPE (to 6X, i.e. 0.9 M NaCl, 60 mM NaH2PO4, 6 mM EDTA)</td>
</tr>
<tr>
<td>1 ml</td>
<td>10% Tween-20 (to 0.01%)</td>
</tr>
</tbody>
</table>

water to 1 L

Filter sterilize.

**Wash buffer B**
1 L

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>83.3 ml</td>
<td>12X MES (to 100 mM)</td>
</tr>
<tr>
<td>5.2 ml</td>
<td>5 M NaCl (to 0.1 M Na+)</td>
</tr>
<tr>
<td>1 ml</td>
<td>10% Tween-20 (to 0.01%)</td>
</tr>
</tbody>
</table>

water to 1 L

Filter sterilize.

Store 4°C in dark.

**2X Stain Buffer**
250 ml

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.7 ml</td>
<td>12X MES (to 200 mM)</td>
</tr>
<tr>
<td>92.5 ml</td>
<td>5 M NaCl (to 2 M Na+)</td>
</tr>
<tr>
<td>2.5 ml</td>
<td>10% Tween-20 (to 0.1%)</td>
</tr>
</tbody>
</table>

water to 250 ml

Filter sterilize.

Store 4°C in dark.

**12X MES**
100 ml

<table>
<thead>
<tr>
<th>Weight</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.461 g</td>
<td>MES hydrate (to 1.22 M MES)</td>
</tr>
<tr>
<td>19.33 g</td>
<td>MES sodium salt (to 0.89 M Na+)</td>
</tr>
</tbody>
</table>

water to 100 ml

Filter sterilize.
pH should be between 6.5 and 6.7.
Store 4C in dark. Discard if it turns yellow.

**20X SSPE (buy from amresco)**

<table>
<thead>
<tr>
<th>3 M</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 M</td>
<td>NaH2PO4</td>
</tr>
<tr>
<td>20 mM</td>
<td>EDTA</td>
</tr>
</tbody>
</table>

**SAPE (aka stain 1 and 3)**

1.2 ml

| 12 ¿l | 1 mg/ml | R-streptavidin phycoerythrin (to 10 ug/ml) |
| 240 ¿l | 10 mg/ml BSA (to 2 mg/ml) |
| 600 ¿l | 2X stain buffer (to 1X) |

water to 1.2 ml
Make the day of use. Store 600 ¿l aliquots at 4C in dark until use. You will have two tubes of this 600uL each.

**Antibody solution (aka stain 2)**

600 ¿l

| 6 ¿l | 10 mg/ml goat IgG (to 0.1 mg/ml) |
| 120 ¿l | 10 mg/ml BSA (to 2 mg/ml) |
| 3.6 ¿l | 0.5 mg/ml anti-streptavidin antibody (goat), biotinylated (to 3 ug/ml) |
| 300 ¿l | 2X stain buffer (to 1X) |

water to 600 ¿l
Make the day of use. Store 600 ¿l aliquots at 4C in dark until use.

**Data Upload**

### Exporting Your Data

To export files to your genomics account:

- Connect to your account by following the directions below. Open GCOS manager. Export the EPT, DAT and CEL files to your account.
  You can then add these files to the Puma DB.

To upload your files to PUMA:

- Either copy files from you genomic account to your incoming folder
  or
- Export directly to your incoming folder.

Once they are in your incoming folder, log into Puma and go to enter a new experiment into the database.

Select:

- Affymetrix as Print Technology
- Affymetrix tiling as Feature Extraction software
- Appropriate organism
  Click on enter a new experiment

Once here, enter all information in a way that you can easily recognize it later. No two experiments can have the same slide name.

Upload your experiments.

### Affymetrix Exporting and Archiving Directions:

All data will be exported and archived to \arrayfiles\arraydata, this server will contain three folders named Agilent, Affy, and People, all users will be given a subfolder in the People folder and this is where users will export and archive their data to. Please note that only you will have access to this folder, you may not share your folder with anyone.

Only array scans may be stored on this server. All other data is subject to deletion without prior notice. Data stored on this server will be archived within 12 months of its creation.
All files must be saved using the following convention:
First three letters of PI¿s lastname_Your Initials_Year Month Day_ your personal descriptive Example: BOT_DG_20061205_yb311 Botstein Lab, David Gresham, December 5, 2006, sample yb311

1. Make sure you are logged into the computer with your Princeton username and password.

2. Go to My Computer>Tools>Map Network Drive. Choose a drive letter from the drop down menu, and the folder is \arrayfiles\arraydata click Finish.

3. In the Start Menu, open the GCOS Manager, go to Tools> Set Data Path> choose your appropriate folder on the arrayfiles server.

4. In the Process Tab in the left field, under Samples> click Universal or Expression> double click the experiment you wish to export.

5. Right click on the experiment name in the right field, and click export.

6. Exporting will take around 3-8 minutes; you can tell that it is working because in the lower field it will say ¿running¿ when the export is done it will say ¿available¿.

7. After this is available you will double click on the experiment and DAT and/or CEL file will appear beneath it.

8. To archive these right click on them, and it will prompt you to choose where to archive to, you will choose your folder on the arrayfiles server. Click Archive. Archiving should take only 1-3 minutes it will say ¿available¿ when done, and the colored icon will turn gray. Remember to log out of the computer.