

## Genomic Hybridization onto Affymetrix S98 Yeast Arrays (2 day process)

Reagents are kept in either  $-20^{\circ}\text{C}$  (\*) or  $4^{\circ}\text{C}$  (\*\*).

### DAY 1

1. Take arrays out of  $4^{\circ}\text{C}$ ; allow to reach room temperature before use.

2. Fragment DNA:

-dilute DNase with water right before use.

#### **DNase Solution:**

	<b>X1</b>
10x One Phor All buffer**	5 $\mu\text{l}$
25 nM $\text{CoCl}_2$ *	3 $\mu\text{l}$
$\text{dH}_2\text{O}$	32 $\mu\text{l}$
DNase (0.05 units, stock is 1 unit)*	1.5 $\mu\text{l}$
DNA (1 $\mu\text{g}/\mu\text{l}$ )*	10 $\mu\text{l}$

Add DNA last and DNase before last. Vortex and spin down. Put in  $37^{\circ}\text{C}$  water bath for 5'. Don't let reaction stand in  $37^{\circ}\text{C}$  for more than 5' so as not to over fragment the DNA. If first time doing experiment, check fragmentation efficiency by running DNA on gel.

3. Put in  $100^{\circ}\text{C}$  plate for 15'. Let cool down on ice before adding terminal deoxynucleotidyl transferase (TDT) enzyme.

4. Add pre-hybridization buffer to arrays (1x MES\*\*) 200  $\mu\text{l}$  per array. To add solution to arrays, stab in a 1  $\mu\text{l}$  white tip into the top right hand corner red sponge, and pipette the solution into the red sponge in the lower left-hand corner. You should end up with a bubble. Make sure it moves around freely (shake chip if necessary). Put chips on a rotor, 60 rpm,  $45^{\circ}\text{C}$  or turn the arrays around every ~10-15 minutes.

5. Add to each reaction (51.5  $\mu\text{l}$ ):

terminal deoxynucleotidyl transferase (25 units/ $\mu\text{l}$ )*	1 $\mu\text{l}$
biotin-N6-ddATP (or is it now biotin-N11-ddATP, Perkin Elmer)*	1 $\mu\text{l}$

Incubate in  $37^{\circ}\text{C}$  water bath for 1 hour (try and keep to one hour).

6. Prepare a fresh aliquot of hybridization solution for each hybridization experiment and add to DNA at the following concentrations:

#### **Hybridization solution:**

Labeled DNA	52 $\mu\text{l}$
Herring sperm DNA (100 $\mu\text{g}/\mu\text{l}$ )*	3 $\mu\text{l}$
BSA (50 $\mu\text{g}/\mu\text{l}$ )*	3 $\mu\text{l}$
B2 control oligo*	4.3 $\mu\text{l}$
2x Hybridization Buffer (see below)**	130 $\mu\text{l}$
$\text{dH}_2\text{O}$	55.7 $\mu\text{l}$

7. Incubate in  $100^{\circ}\text{C}$  for 5', put on ice for 5'.

8. If you haven't put arrays (with pre-hybridization solution) on rotor yet do so now: 60 rpm, 45 °C. Make sure plastic trays are tightly attaches to rotor.

9. Spin down samples 15 krpm, 5'.

10. Remove pre-hybridization solution (keep array vertical to table and pipette should be vertical to array). Add 200  $\mu$ l hybridization solution with DNA to each array. Make sure bubble moves freely around. Shake array if necessary. **PUT TAPE ACROSS SPONGE OPENINGS ON BACK OF CHIPS TO PREVENT LEAKAGE AND DRYING OF CHIP.**

11. Put arrays on rotor, 60 rpm, 45 °C, ~20 hours (not recommended to go too much over 20 hr).

## **DAY2**

1. Prepare the two staining solutions according to Affymetrix's protocol. Mix well and divide to aliquots of 600  $\mu$ l in eppendorf tubes. Keep on ice until use. Keep solutions 1,3 with SAPE in dark by wrapping tube in silver foil.

### Stain Solution #1,3

	<u>1x</u>
Water	540 $\mu$ l
2x Stain Buffer**	600 $\mu$ l
BSA*	48 $\mu$ l
SAPE**	12 $\mu$ l
Total:	1200 $\mu$ l

### Stain Solution #2

	<u>1x</u>
Water	266.4 $\mu$ l
2x Stain Buffer**	300 $\mu$ l
BSA*	24 $\mu$ l
Goat IgG**	6 $\mu$ l
Biotinylated Ab**	3.6 $\mu$ l

2. At the Affymetrix fluidics station:

A) Turn on computer, open GCOS, open new experiment file for each chip:

File -> New Experiment

Fill in the following fields:

- Probe Array Type (e.g. YG-S98)
- Sample Name (e.g. Genomic DNA, general experiment name for all chips)
- Experiment info (will show up for each chip's scan)
- Experiment name (unique name for each chip)

B) Turn on fluidics stations; remove waste, fill bottles with water and buffers A and B, put tubes in appropriate bottles.

C) Turn on Scanner.

D) Prime fluidics stations: On the computer go to: Run -> Fluidics, choose:

Protocol: PRIME\_450 (450 is model of machine)

Choose 'All modules'; press 'Run'

Raise and put down needles into empty eppendorf tubes.

E) Remove hybridization solution from chips and pipette in 200  $\mu$ l buffer A. Wipe window of chip. Remove empty vials from station (keep them in box), add tubes with staining solutions 1,2,3 from left to right (solution 2 in middle), lower needle back into tubes. Insert chips into each module.

F) To run staining procedure, on computer:

Go to window opened through (Run -> Fluidics):

Change protocol to: EuKGE-WS2u4\_450 (for yeast S98 chips)

Choose a given module, choose an experiment name (Exp-1, Exp-2, etc.), and press 'Run'. Do for each experiment and module.

G) When protocol done running, take chips out, and make sure there are no bubbles in there. If so, remove liquid from chip and refill chip with buffer A completely, so that some liquid comes out of the small tip put in the red sponge in the upper right-hand corner of the chip. Wipe the chips' windows with Kimwipes. Put chips in dark place, such as a drawer. Chips will now be scanned.

H) Throw tubes from fluidics station away; put back empty tubes.

The station will then purge with water. If you are the last to use the station for the day need to shut down the station. To do so:

Go to computer: Run -> Fluidics

Change protocol to: SHUTDOWN

When machine done turn off station.

I) To scan chips: on computer go to: Run -> Start scanner

Choose Experiment: Exp-1

Put in chip into scanner

Press 'Run'

Once scan is done:

\* If no image appears on the screen press 'Grid'.

\* Check intensity range: click right button on mouse and choose appropriate option.

1-10,000 is good range.

\* Run analysis on chip: Run -> analysis

it will give you the mean chip intensity, the mean background intensity and more.

To run analysis following normalization of chips (the default is to scale the chips by 1):

Tools-> experiment settings, chose normalization

Run-> analysis

Drag all CEL files into white box on right

Press on Analyze button

To save the analysis information in text file: select all, save as txt file.

\* To see only your experiments go to Tools -> Filter

J) To rescan a chip you need to save a new experiment sheet.

Go to: Workflow (icons bottom left) -> use right button of mouse -> Advance to scan

K) Always save all data in a CAB and DTT format (as of now CAB is the only format Affymetrix accepts to run test on your data):

All programs -> Affymetrix -> Data transfer tool -> Transfer out -> DTT or CAB

L) Burn CD with hybridization data.

## **Solutions:**

### **2x Hybridization Buffer (50 ml)**

	<b><u>Volume (ml)</u></b>
12x MES Stock Buffer	8.3
2.5 M NaCl*	17.7
(this is half the recommended concentration by Affymetrix)	
0.5 M EDTA, pH8.0	4 From Ambion, p/n 9260G, 100 ml
10% Tween 20	0.1
Nuclease-free water	19.9
Total volume:	50

Final 1x concentrations: 100 mM MES, 0.45 M Na<sup>+</sup>, 20 mM EDTA, 0.01% Tween20.  
Store at 4 °C. Do not use if yellow.

\* The reduced salt concentration lowers the hybridization intensities away from saturation and brings the intensity range to a mean of ~1000-2000 units, which works well for our genetic mapping analysis.

### **12x MES Stock Buffer (1000 ml)**

MES free acid monohydrate	70.4 gr
MES Sodium Salt	193.3 gr
Nuclease Free water	800 ml

Mix and adjust to volume 1 lit, pH 6.5 to 6.7. Filter through 0.2 µm filter.

Final 1x concentration: 1.22 MES, 0.89 M Na<sup>+</sup>.  
Store at 4 °C. Do not use if yellow.

MES free acid monohydrate Ultra	M5287	Sigma	100 gr
MES Sodium Salt	M3885	Sigma	250 gr
Nuclease Free water	9932	Ambion 1 lit	

### **2x Stain Buffer**

	<b><u>Volume (ml)</u></b>
12x MES stock buffer (make from scratch)	41.7
5 M NaCl (Ambion 9759)	92.5
10 % Tween-20 (10 ml ampules; 6 ampules/package; Pierce Chemical 28320)	2.5
Nuclease Free water (Ambion 9932)	112.8
Total:	250 ml

Filter through 0.2 µm filter.

Final 1x concentration: 100 mM MES, 1 M Na<sup>+</sup>, 0.05 % Tween20

## **Reagents needed:**

10x One Phor All buffer PLUS	4 °C
25 nM CoCl <sub>2</sub> comes with the terminal deoxynucleotide transferase	-20 °C
DNase I, Amplification grade (1 unit/µl) Invitrogen, cat. no. 18068-015	-20 °C

terminal deoxynucleotidyl transferase (24000 units)\* Roche, cat. no. 3333547 -20 °C  
1-800-262-1640

biotin-N11-ddATP >95% Perkin Elmer NEL548001EA -20 °C  
1-800-762-4000 (they do not make biotin-N11-ddATP any more)

Herring sperm DNA Promega, P/N D1811 -20 °C

BSA, a solution of 50mg/ml from invitrogen P/N 15561-020 -20 °C

B2 control oligo -20 °C

Streptavidin R-phycoerythrin conjugated Molecular Probes S866 (1ml) (\$219)  
Invitrogen (800) 955- 6288  
S21388 dido but premium grade (\$418)

2x MES

Bought as powder:

Proteinase K - Boehringer, cat. no. 1000144

RNase A - I think we get it from Sigma

Zymolase - Seikagaku America Inc., cat. no. 120493