Affy SNP-typing protocol

Maitreya Dunham and Matt Brauer June 2005
From various Elizabeth Winzeler papers, the Affy manual, and the Enzo manual.

Use high-quality DNA.

Bring 10 ug DNA to 15.8 μl total volume in 10 mM Tris pH 8.
Add:

2 μl	10X one-phor-all buffer
1.2 μl	25 mM $CoCl_2$

Quickly add:

1 μl	0.15 U diluted DNase (1 μl DNase+5.7 μl 1X one-phor-all)

If doing several reactions, you may want to do them in batches.
Incubate 37C **exactly** 5 min.
Incubate 100C 15 min.

Run 1 μl on a 2% gel for 50 min at 50 V. Also run a similar amount of undigested DNA to check quality.
The DNA should be all in a smear ~50 bp.
If it is not, start again.

For labeling, use the BioArray Terminal Labeling Kit (Enzo 42630)

Add, at room temperature:

20 μl	5X reaction buffer
10 μl	10X $CoCl_2$
1 μl	Biotin-ddUTP
2 μl	Terminal Deoxynucleotide Transferase
48 μl	water to 100 μl

Incubate 37C 15 minutes to 1 hour.
Ice.
Stop with 5 μl 0.2 M EDTA.

Add:

27 μl	water

150 μl	2X hybe buffer
15 μl	10 mg/ml BSA
3 μl	10 mg/ml salmon sperm DNA
5 μl	3 nM control oligo B2

Incubate 99C 5 min.
While incubating, load array (see loading instructions below) with 200 μl 1X hybe buffer (see recipes) and prehybe in the oven for 10 min at 45C.
Incubate probe 45C 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.
Tape over septa to prevent leakage.
Put arrays in oven at 45C, 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.

Washes

Make all wash buffers before starting.

For each wash station, make about 0.5 L Wash A and Wash B.
For each array, make 1.2 ml (in two 600 μl aliquots) SAPE and 600 μl Antibody solution.

Log in to the Affy computer as maitreya with password affy123.
Follow directions for starting and priming the fluidics, starting on page 2.3.7 of the manual. (Make sure you use the instructions for the correct model of wash stations. A few little things change.)

Follow the directions for the washes, using the EukGE-WS2v4 protocol.

When done, remove the arrays from the fluidics station. Dab some whiteout on the septa to seal them. Store slides in the dark until they are scanned.

Scan.
Burn the data to CD.

Wash and shut down fluidics station.

Recipes

2X Hybe buffer
50 ml

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%)

water to 50 ml
Store 4C in dark.

Wash buffer A
1 L

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

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 4C in dark.

2X Stain Buffer
250 ml

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 4C in dark.

12X MES
100 ml

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 4C in dark. Discard if it turns yellow.

20X SSPE

3 M	NaCl
0.2 M	NaH_2PO_4
20 mM	EDTA

SAPE (aka stain 1 and 3)
1.2 ml

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

water to 1.2 ml
Make the day of use. Store 600 μl aliquots at 4C in dark until use.

Antibody solution (aka stain 2)
600 μl

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)
300 μl	2Xstain buffer (to 1X)

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