# MIAME

1. Experimental design

**1a) authors**

T.G. Fazzio
M.E. Gelbart
T. Tsukiyama
Fred Hutchinson Cancer Research Center
A1-175
1100 Fairview Ave N
Seattle, WA 98109, U.S.A.

URL: http://www.fhcrc.org/labs/tsukiyama/supplemental-data/H4basicpatch/

**1b) type of experiment**

mutant cells vs. wild type cells

**1c) experimental variables**

genetic variations

**1d) multiple hybridizations, type:**

genetic segregation

Relationships between samples, arrays and hybridizations:

Samples: YTT1781 (*MAT**a*** hht1-hhf1::HYG hht2-hhf2::NAT CEN-TRP1-HHT2-hhf2 R17A R19A)
YTT1948 (*MAT**a*** hht1-hhf1::HYG hht2-hhf2::NAT CEN-TRP1-HHT2-HHF2)

Arrays: (all from same type): A1 to A180.

**1e) hybridizations:**

| Hybridization ID | Target label (Cy3)* | Target label (Cy5)* |
|---|---|---|
| 1781-35a | mt | WT |
| 1781-35b | mt | WT |
| 1781-53a | WT | mt |
| 1781-53b | WT | mt |

*: WT and mt denote YTT1948 and YTT1781, respectively

**1f) quality related indicators, quality control steps taken:**

* technical replicates:

dye-swap for all extracts.

*other:

empty wells, blank wells, Arabidopsis and bacterial genes, positive as well as negative control sequences.

**1g) text description of the experiment**

Haploid strains YTT1781 (*MATa hhf2 R17A R19A*), isogenic to W303 and YTT1948 (*MATa HHF2*), a wild type control were grown at 30 degrees Celsius in complete synthetic C medium in log-phase up to a $OD_{660}$=0.7. RNA was prepared by acid phenol extraction.

2. Array design

**2.1.Array copy**

*Unique ID: arrays 1781-35a, 1781-35b, 1781-53a, 1781-53b.

*Array design name

F.H.C.R.C Yeast ORF v1.0

**2.2.a) array features**

*array design name:

F.H.C.R.C Yeast ORF v1.0

*platform type:

spotted

*array provider:

in-house FHCRC

*surface type:

glass

*surface type name:

in-house coated poly-lysine FHCRC slides

*physical dimensions of slides:

40 x 18 mm

*number of elements on the array:

6300 (approximately)

*reference system allowing to locate each element:

elements are spotted by blocks (16 blocks total) of elements. The blocks have referenced coordinates on the array.

*production protocol:

Yeast cDNA microarrays were constructed employing a set of ~6200 orf-specific PCR primer pairs (Research Genetics, Huntsville, AL), which were used to amplify each open reading frame (orf) of the yeast genome. Individual PCR products were verified as unique via gel electrophoresis and purified using ArrayIt$^{TM}$ 96-well PCR purification kits (TeleChem International, Sunnyvale, CA). Purified PCR products were mechanically "spotted" in 3X SSC (450 mM sodium cloride and 45 mM sodium citrate, pH 7.0) onto poly-lysine coated microscope slides using an OmniGrid high-precision robotic gridder (GeneMachines, San Carlo, CA).

**2.2.b) spot informations**

*simple or composite:

simple

*element type:

PCR products

*single or double stranded:

double

*spot dimension:

~130 micrometers

*generation protocol

Using the ResGen primer set and genomic DNA.

*attachment type:

electrostatically

**2.2.c) specific properties of each spot on the array**

*element type:

PCR products

*PCR primer information

The PCR primers used for generating the elements are identified by MIPS ORF names.

*approximate length:

up to 1Kb

3. Samples

**3.a. sample description**

ID: 1781-35a, 1781-35b, 1781-53a, 1781-53b.

Organism:

Saccharomyces cerevisiae

Cell source and type:

Strain YTT1781

Development stage:

Haploid

Genetic variation:

YTT1781: *MAT**a** hhf2 R17A R19A*
YTT1948: *MAT**a** HHF2*

In vivo treatment: none.

In vitro treatments:

Cells were grown at 30 degrees Celsius in C medium

Separation technique: none.

**3.b. preparation of hybridisation extracts**

*Description:

total RNA preparation from yeast cultures:

Harvest 50 ml cells at $OD_{660}$=0.7 (~10E7 cells/mL).
Spin 20mL culture, 2,500g, 4 degree C, 5 minutes. Discard supernatent.
Wash 1x with water, Snap-freeze in liquid nitrogen.
Add 400ul TES lysis buffer to frozen cell pellet.
Immediately add 400ul acid phenol that is prewarmed to 65 degree C. Vortex.
Incubate 65 degree C for 60 minutes with vortext at full speed every 5-10 minutes.
Incubate on ice 5 minutes.
Spin 14000g, 5 minutes, 4 degree C.
Transfer aqueous phase to fresh tube.
Add 400ul acid phenol. Vortex. Spin 14000g 5 min room-temperature.
Transfer aqueous phase to fresh tube.
Add 400ul Chloroform, vortex.
Spin 14000g, 5 minutes, room-temperature.
Transfer aqueous phase to fresh tube.
Ethanol precipitate RNA.
Dissolve RNA pellet in TE and take $OD_{260}$.

TES buffer:
10 mM Tris-HCl pH7.5
10 mM EDTA
0.5 % SDS

*extraction method: phenol-chloroform.
*total or messenger RNA:

total RNA

*amplification: none.

**3.c. labelling description:**

*protocol:

cDNA synthesis and labelling:

Reverse transcription performed on 30 micrograms of total RNA with oligo(dT)18 primer, SuperscriptII(invitrogen) enzyme and buffer, in presence of 25mM of each dATP, dCTP and dGTP, 15mM dTTP and 10mM amino-allyl-dUTP (Sigma). Incubation at 42 degree Celsius for 2 hours.
Add 10ul of NaOH and 10ul of 0.5M EDTA. Incubate 65 degree Celsius for 15 minutes.
Neutralize by adding 25ul 1M Tris pH7.4
Filter on Microcon-30 concentrator.
Dry on speed-vac, resuspend in water.
Cy3 and Cy5 Monoreactive dyes (Amersham) were resuspended in DMSO, activated by NaBicarbonate and incubated with the cDNA for one hour. The coupling reaction was quenched by addition of Hydroxylamine and incubation for 15 minutes. Labelled cDNA was then purified on QUIAquick PCR purification columns following QIAGEN protocol, final eluate was dried in speed-vac, resuspended in 18ul water. Add 3.6ul 20X SSC and 1.8ul of polyA (Roche) (10mg/mL). Filter through Millipores 0.45 micron spin membranes.

*amount of nucleic acids labelled:

30 micrograms of total RNA.

*label used:

Cy3, Cy5.

**4. Hybridisations.**

*ID:

1781-35a, 1781-35b, 1781-53a, 1781-53b.

*hybridisation buffer:

3X SSC + 10%SDS + 1 mg/mL poly(dA)

*blocking agent:

no prehybridization

*Slide blocking:

no prehybridization

*Probe blocking:

1mg/mL polydA during hybridization

*wash procedure:

wash1: 1X SSC, 0.03% SDS
wash2: 1X SSC dip slides 15 times
wash3: 0.2X SSC: shake slides 75rpm for 20minutes
wash4: 0.05X SSC: shake slides 75rpm for 10 minutes
spin-dry slides in centrifuge 50g for 5 minutes.

*quantity of labelled target used:

all material generated from 30 microgram total RNA

*time, concentration, volume, temperature:

16h, 26 microliters at 63 degrees C

*Hybridisation instrument:

Manual, TelChem hybridisation chambers in waterbath

**5. Measurements.**

5a1) image files :

not included.

5a2) scanning information:

*scanning harware:

Axon GenePix4000B

*scanning software:

Axon GenePix Pro 3.0

**5b1) image analysis output files:**

normalized datasets used in the manuscript are attached. The gpr files are available upon request. Contact Toshio Tsukiyama at ttsukiya@fhcrc.org

**5b2) image analysis information:**

*image analysis software:

Axon GenePix Pro 3.0

6. Normalisation procedure

The spots of bad amplification were identified by the FHCRC amp code and removed from the gpr files. The flagged spots after scan by GenePix were then removed. The median from 16 yeast genomic DNA spots were taken for each array, and used to divide the Cy3 and Cy5 signals for normalization. The signals from the mutant cell were divided by that of wild type cells from all 4 arrays to calculate the mean and SD.