MIAME

1. Experimental design

1a) authors

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URL: http://www.fhcrc.org/labs/tsukiyama/supplemental-data/isw2locationanalyses/

1b) type of experiment

chromatin IP: starting material (input) vs. immunoprecipitate (IP)

1c) experimental variables

genetic variation

1d) multiple hybridizations, type:

Hybridization ID	Genotype	Target Label (Cy3)	Target Label (Cy5)	Array Type
MG966o-1a	ISW2-3FL	input	IP	ORF
MG966o-1b	ISW2-3FL	IP	input	ORF
MG966o-2a	ISW2-3FL	input	IP	ORF
MG966o-2b	ISW2-3FL	IP	input	ORF
MG966o-3a	ISW2-3FL	input	IP	ORF
MG966o-3b	ISW2-3FL	IP	input	ORF
MG1996o-1a	isw2-K215R-3FL	input	IP	ORF
MG1996o-1b	isw2-K215R-3FL	IP	input	ORF
MG1996o-2a	isw2-K215R-3FL	input	IP	ORF
MG1996o-2b	isw2-K215R-3FL	IP	input	ORF
MG1996o-3a	isw2-K215R-3FL	input	IP	ORF
MG1996o-3b	isw2-K215R-3FL	IP	input	ORF
MG966i-1a	ISW2-3FL	input	IP	Intergenic
MG966i-1b	ISW2-3FL	IP	input	Intergenic
MG966i-2a	ISW2-3FL	input	IP	Intergenic

MG966i-2b	ISW2-3FL	IP	input	Intergenic
MG966i-3a	ISW2-3FL	input	IP	Intergenic
MG966i-3b	ISW2-3FL	IP	input	Intergenic
MG1996i-1a	isw2-K215R-3FL	input	IP	Intergenic
MG1996i-1b	isw2-K215R-3FL	IP	input	Intergenic
MG1996i-2a	isw2-K215R-3FL	input	IP	Intergenic
MG1996i-2b	isw2-K215R-3FL	IP	input	Intergenic
MG1996i-3a	isw2-K215R-3FL	input	IP	Intergenic
MG1996i-3b	isw2-K215R-3FL	IP	input	Intergenic

1e) quality related indicators, quality control steps taken:

* biological replicates:

MG966-1, 2, and 3 are 3 independent cultures of the same strain (YTT966), grown in identical conditions.
MG1996-1, 2, and 3 are 3 independent cultures of the same strain (YTT966), grown in identical conditions.

* technical replicates:

dye-swap for all extracts.

*other:

Amp codes were assigned to mark spots on the arrays ORF produced by questionable PCR products. "N" = no detectable gel band; "M" = multiple gel bands; "O" = gel band corresponding to a fragment size different than predicted. If no code is given, a single gel band in the predicted size range was positively identified.

empty wells, blank wells, 3xSSC, Arabidopsis and bacterial genes, positive as well as negative control sequences.

1g) text description of the experiment

Haploid strains YTT966 (W303 *MAT**a*** ISW2-3FL) and YTT1996 (W303 *MAT**a*** isw2-K215R-3FL), were grown at 30°C in YEPD medium in log-phase up to OD_{600}=0.5. Cells were fixed with formaldehyde for 5 minutes. A partial purification of chromatin was performed from cell lysates and DNA was fragmented by sonication. α-FLAG-M2 antibody (Sigma) was used to immunoprecipitate Isw2p (wild-type or the K215R mutant). DNA from starting material (input) and Isw2p immunoprecipitation were subsequently purified.

2. Array design

2.1.Array copy

*Array design name

F.H.C.R.C. Yeast ORF

F.H.C.R.C. Yeast intergenic

2.2.a) array features

*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:

approx. 6300 (ORF); approx. 6500 (intergenic)

*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 or ~6400 intergenic-specific primer pairs (Research Genetics, Huntsville, AL), which were used to amplify either open reading frame (orf) or intergenic regions of the yeast genome, respectively. 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: MG966(i/o)-(1/2/3)(a/b)
MG1996(i/o)-(4/5/6)(a/b)

Nomenclature indicates strain number of sample (YTT966 or YTT1996), type of array (orf or intergenic), replicate identifier, and dye channels (a and b are dye swaps). The replicate identifier is the same for all arrays derived from the same initial chromatin sample. For example, arrays MG966i-1a, MG966i-1b, MG966o-1a, and MG966o-1b were all performed using samples derived from the same chromatin preparation.

Organism:

Saccharomyces cerevisiae

Cell source and type:

YTT966 and YTT1996

Development stage:

Haploid

Genetic variation:

YTT966: *MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-3FLAG-KanMX*
YTT1996: *MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2-K215R-3FLAG-KanMX*

In vivo treatment: none.

In vitro treatments:

Cells were grown at 30°C in YEPD medium

Separation technique: none.

3.b. preparation of hybridization extracts

*Description:

yeast chromatin immunoprecipitation:

Chromatin Preparation
1. At OD_{600}=0.5, add 30 mL fix solution to 300mL culture. Shake slowly at room temp (RT) for 5 min.
2. Add 54 mL 2.5M glycine to terminate fixation and shake 5 min at RT.
3. Pellet cells (5,000 rpm 5 min in JA-10 (Beckman)). Resuspend in 10 mL cold TBS and transfer to 14mL tube. Spin 3,000 rpm 5 min in J6 (Beckman). Repeat wash one additional time.
4. Resuspend cells in 500 μL Breaking Buffer. Transfer to 2 mL flat-bottomed microfuge tubes.
5. Add 500 μL of acid-washed glass beads (Sigma) to each tube. Vortex at 4°C 40 min at full speed.
6. Puncture a hole in the bottom of the tube with a needle and place in Falcon 2059 (14 mL snap cap tubes). Spin 1,000 rpm 2 min in J6 (Beckman) to recover lysates.
7. Add 1 mL FA buffer to lysates and transfer to 2 mL flat-bottomed microfuge tubes. Spin 14,000 rpm 1 min 4°C in microfuge and discard supernatant.
8. Resuspend pellet in 1 mL FA buffer. Repeat step (7) 2 more times to partially purify chromatin.

9. Resuspend pellet in 300 μL FA Buffer. Sonicate 12 x 15 sec on microtip setting 1 (Branson Sonifier 185) with 2 min intervals on ice between sonications.
10. Add 1.2 mL FA buffer. Centrifuge 14,000 rpm 30 min 4°C in microfuge. Rotate tubes and repeat spin. Transfer supernatant to siliconized Eppendorfs and store at -80°C.

Bead Prep

1. Pipet 20 μL ProteinG Dynabeads (Dynal)/sample into a siliconized Eppie. Concentrate beads on magnetic particle concentrator (MPC) and remove supernatant.
2. Add 1 mL 5 mg/mL IgG-free PBS-BSA. Rotate at RT 5'. Concentrate on MPC and remove supernatant.
3. Repeat wash step 2 additional times.
4. Add 100 μL 5 mg/mL IgG-free PBS-BSA/sample and resuspend beads. Add 4 mg α-FLAG-M2 antibody (Sigma)/sample and rotate at 4°C overnight.
5. Immediately prior to use, wash beads twice with 1 mL 5 mg/mL IgG-free PBS-BSA as in (2). Resuspend beads in 30 μL 5 mg/mL IgG-free PBS-BSA/sample and aliquot to siliconized Eppendorfs.

IP reaction

1. Thaw chromatin and pipet 300 μL into siliconized Eppendorf. Spin 14,000 rpm 15 min 4°C in microfuge. Filter supernatant through Ultrafree-MC 0.45 μm (Millipore) (14,000 rpm 1 min 4°C in microfuge). Save 20 μL filtrate for DNA ("input").
2. Remove IgG-free PBS-BSA from beads and add 200 μL filtrate to beads. Rotate 90 min at RT.
3. Concentrate on MPC. Aspirate off supernatant.
4. Add 1 mL FA Buffer and rotate 5 min at RT. Concentrate on MPC and aspirate off buffer.
5. Repeat step (4) wash 2 additional times with FA Buffer (3 total), twice with FA-HS Buffer, and once with RIPA Buffer.
6. Elution: Resuspend beads in 10 μL 5 mg/mL 3xFLAG peptide and
   then add 40μL RIPA Buffer. Shake beads for 30' at RT 1200 rpm on Eppendorf 5436 shaker.
   Recover eluate and repeat. Pool the 2 eluates (save 10μL for Western; remainder = "IP").
7. DNA purification:

"Input": Add 70 μL ddH20 and 90 μL 2x Stop Buffer
"IP": Add 90 μL 2x Stop Buffer
Add 20 μg glycogen to all tubes.
Incubate at 75°C 6 hours to overnight.
Add 20 μg/mL ProteinaseK and incubate at 55°C 3 hours to overnight.
Phenol extract once and phenol-chloroform extract once.
Ethanol precipitate and dry pellet.
Resuspend in 50 μL TE + RNaseA (10 μg RNaseA/100 μL TE) and incubate 30 min at 37°C. Purify on Qiaquick PCR Purification column (Qiagen). Elute in 50 μL EB.

Solutions

1. *Fix solution*: 11% formaldehyde, 0.1 M NaCl, 1 mM EDTA, 50 mM Hepes-KOH pH 7.6. Freshly prepared. Do not store in glass bottles.
2. *TBS*: 20 mM Tris pH 7.6, 150 mM NaCl.
3. *Breaking buffer*: 100 mM Tris pH 8.0, 20% glycerol, 1 mM PMSF (added fresh).
4. *FA buffer*: 50 mM Hepes-KOH pH 7.6, 150mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate. Add protease inhibitors just before use.
5. *FA-HS buffer*: same as FA buffer but contains 500 mM NaCl.

6. *RIPA buffer*: 10 mM Tris pH 8.0, 0.25 M LiCl, 0.5% NP40, 0.5% sodium deoxycholate, 1 mM EDTA.
7. *3XFLAG peptide* (Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys. 5 mg / ml stock in Buffer H 0.1.)
8. *2x Stop buffer*: 20 mM Tris pH8.0, 100 mM NaCl, 20 mM EDTA, 1% SDS.

3.c. labelling description:

*protocol: Ren *et al.* (2000) Science *290*, 2306-2309.
http://web.wi.mit.edu/young/research/genome/protocols.html

Blunting
1. Take 40 µL of "IP" DNA or 1 µL of 10x diluted "input" DNA sample. Add ddH_20 to 100µL on ice.
2. Add 12.2 µL Blunting Mix to each tube and incubate at 12°C for 20'.
1x Blunting Mix
11 µL 10x NEB Buffer 2
0. 5µL BSA (10mg/mL) (NEB)
0.5 µL dNTP mix (20 mM each)
0.2 µL T4 DNA polymerase (3U/µL) (NEB)
3. Put tubes on ice and add 12 µL glycogen mix. Vortex to mix.
1x Glycogen Mix
11.5 µL 3M NaOAc
0.5 µL glycogen (20mg/mL)
4. Add 120 µL phenol-chloroform. Vortex and spin 14,000 rpm 5 min RT in microfuge. Transfer 110µL to new Eppie.
5. Add 230 µL cold EtOH. Incubate 15 min at -20°C and spin 14,000 rpm 15 min 4°C in microfuge. Wash 2x cold 80% EtOH. Spin 14,000 rpm 2 min 4°C. Dry pellet and resuspend in 25 µL ddH_20 and put on ice.

Linker ligation
1. Add 25 µL of ligase mix to each tube and incubate at 16°C overnight.
1x Ligase Mix
8 µL ddH_20
10 µL 5x ligase buffer (Life Technologies)
6.7 µL annealed linkers (15µM)
0.5 µL T4 DNA ligase (Life Technologies)

Ligation-mediated PCR
1. Add 6 µL 3M NaOAc to DNA. Vortex and add 130 µL cold EtOH. . Incubate 15 min at -20°C and spin 14,000 rpm 15 min 4°C in microfuge. Wash 2x cold 80% EtOH. Spin 14,000 rpm 2 min 4°C. Dry pellet and resuspend in 25 µL ddH_20 and put on ice.
2. Add 15µL of PCR labeling mix to each tube:
1x PCR Labeling Mix
4µL 10x ThermoPol reaction buffer (NEB)
6.5µL ddH_20

2μL lowT mix (5mM each dATP, dCTP, dGTP; 2mM dTTP)
2μL Cy3-dUTP OR Cy5-dUTP (Amersham)
0.5μL oJW102 (100pmol/μL)

3. Transfer to chilled PCR tubes. Run PCR program.
   Step 1: 2 min 55°C
   Step 2: 5 min 72°C
   Step 3: 2 min 95°C
   Step 4: 30 sec 95°C
   Step 5: 30 sec 55°C
   Step 6: 1 min 72°C
   Step 7: Go to step 4 31 more times (Cy5) or 33 more times (Cy3)
   Step 8: 4 min 72°C
   Step 9: hold at 4°C
4. During step 1 of PCR, add 10μL of polymerase mix to each tube.
   1x Polymerase Mix
   8μL ddH_20
   1 μL 10x ThermoPol reaction buffer (NEB)
   1 μL Taq (Qiagen)
5. Run 5 μL each on 1.3% agarose minigel to check length.
6. Purify samples on Qiaquick PCR purification column. Elute in 80 μL EB. Spec entire sample at OD_{260} and OD_{550} (Cy3) and OD_{650} (Cy5). Quantitate specific activity of dye incorporation.
7. Pool 20 pmol of each dye.

Primer sequences:
   oJW102: GCGGTGACCCGGGAGATCTGAATTC
   oJW103: GAATTCAGATC
Annealed oligos:
Anneal 15μM each in 0.25M Tris-HCl pH 7.9. Place in 95°C heat block for 5 min. Transfer to 70°C block and allow to cool to RT. Transfer to 4°C overnight. Store at -20°C.

4. Hybridizations.

*hybridization 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.

*time, concentration, volume, temperature:

16h, 26 microliters at 63 degrees C

*Hybridization instrument:

Manual, TelChem hybridization 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 are available on this website. .gpr files are available upon request. Please contact Dr. Toshi Tsukiyama (ttsukiya@fhcrc.org).

5b2) image analysis information:

*image analysis software:

Axon GenePix Pro 3.0

6. Normalization procedure

Before further analysis, flagged spots and spots with signal to noise ratio < 3 were removed. For ORF arrays, spots with amp codes “M,” “N,” and “O” were removed. The ratio of IP/input was

calculated and log-transformed. Each array was median normalized, and dye swap pairs were averaged. The three biological replicates were then averaged. Cyber-T was used to generate Bayesian p-values. Datapoints were required in all 6 datasets, and a lower bound threshold was set at 1.5-fold. This ensured p-values less than 10^{-3}, which remain significant when compensating for multiple testing using false discovery rate methodologies.