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1. Experiment Design

1a) Goal

Determine changes in transcript levels between chromatin remodeling mutants grown in the presence or absence of methyl methanesulfonate (MMS)

1b) Description

Samples from *isw2* or *nhp10* single mutants were co-hybridized with an *isw2 nhp10* double mutant sample to the same microarray to determine changes in transcript levels specific to the double mutant.

1c) Keywords

Chromatin remodeling, *isw2*, *nhp10*, MMS

1d) Experimental Factors

Effect of gene knockouts on transcription
Effect of MMS treatment on transcription in gene knockouts

1e) Experiment design

Transcript levels of *isw2 nhp10* double mutants were compared directly to each single mutant (by co-hybridizing samples to the same array) to determine changes in gene expression specific to the double mutant. Samples were either from log-phase cells, or from MMS treated cells.

1f) Quality Control Steps

There are 3 biological replicates for each hybridization combination. Dye swaps were performed for each sample.

2. Samples, extract preparation, and labeling

2a) Organism

S.cerevisiae; W1588-4c background

2b) Genotypes

isw2
YTT1080 (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ isw2*::NatMX)

nhp10
YTT2060 (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ nhp10*::HphMX)

isw2 nhp10
YTT2109 (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ isw2*::NatMX *nhp10*::HphMX)

2c) Hybridizations

Hybridization ID	Target label (Cy5)	Target label (Cy3)	MMS treatment
i_v_in_1a	*isw2*	*isw2 nhp10*	-
i_v_in_1b	*isw2 nhp10*	*isw2*	-
i_v_in_2a	*isw2*	*isw2 nhp10*	-
i_v_in_2b	*isw2 nhp10*	*isw2*	-
i_v_in_3a	*isw2*	*isw2 nhp10*	-
i_v_in_3b	*isw2 nhp10*	*isw2*	-
i_v_in_MMS_1a	*isw2*	*isw2 nhp10*	MMS
i_v_in_MMS_1b	*isw2 nhp10*	*isw2*	MMS
i_v_in_MMS_2a	*isw2*	*isw2 nhp10*	MMS
i_v_in_MMS_2b	*isw2 nhp10*	*isw2*	MMS
i_v_in_MMS_3a	*isw2*	*isw2 nhp10*	MMS
i_v_in_MMS_3b	*isw2 nhp10*	*isw2*	MMS
n_v_in_1a	*nhp10*	*isw2 nhp10*	-
n_v_in_1b	*isw2 nhp10*	*nhp10*	-
n_v_in_2a	*nhp10*	*isw2 nhp10*	-
n_v_in_2b	*isw2 nhp10*	*nhp10*	-
n_v_in_3a	*nhp10*	*isw2 nhp10*	-
n_v_in_3b	*isw2 nhp10*	*nhp10*	-
n_v_in_MMS_1a	*nhp10*	*isw2 nhp10*	MMS
n_v_in_MMS_1b	*isw2 nhp10*	*nhp10*	MMS
n_v_in_MMS_2a	*nhp10*	*isw2 nhp10*	MMS
n_v_in_MMS_2b	*isw2 nhp10*	*nhp10*	MMS
n_v_in_MMS_3a	*nhp10*	*isw2 nhp10*	MMS
n_v_in_MMS_3b	*isw2 nhp10*	*nhp10*	MMS

2d) Sample treatment

MMS treatment: Samples were treated with 0.02% MMS for 2 hours.

2e) Preparation of Hybridization extract

Log phase cells were grown at 30°C YPD media to an optical density of 0.35 at 600nm; cultures were then split and MMS was then added to 0.02% to one half of the culture. After incubation for 2 more hours, cells were harvested for RNA preps.

Preparation of total RNA from yeast cultures:

- Harvest cells from 50 ml culture
- Spin 50mL culture, 2,500g, 4°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°C. Vortex.
- Incubate 65°C for 60 minutes with vortext at full speed every 5-10 minutes.
- Incubate on ice 5 minutes.
- Spin 14000g, 5 minutes, 4°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 OD260.

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

2f) Labeling

cDNA synthesis and labelling:
Reverse transcription was performed on 30 ug 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). Mix was Incubated at 42 degree Celsius for 2 hours; 10ul of NaOH and 10ul of 0.5M EDTA was added afterwards and incubated at 65°C for 15 minutes. Mix was neutralized by adding 25ul 1M Tris pH7.4. Unincorporated nucleotides were removed by filtration on a Microcon-30 concentrator. Samples were then dried in a speed-vac and then resuspended 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, and 3.6ul 20X SSC and 1.8ul of polyA (Roche) (10mg/mL) was added. Samples were filtered through Millipores 0.45 micron spin membranes.

amount of nucleic acids labelled:
30 ug of total RNA.

label used:
Cy3, Cy5.

3. Array Design

3a) Array name

FHCRC Yeast Amplicon v3.1, GEO Platform GPL1914

3b) array design

- 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™ 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).

3c) Spot information

- simple or composite:
  simple

- element type:
  PCR products

- single or double stranded:
  double

- spot dimension:
  ~130 micrometers

- generation protocol
  PCR using the ResGen primer set and genomic DNA

- attachment type
  electrostatic

3d) 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

4. Hybridization procedures and parameters

- 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.

- quantity of labelled target used:
  all material generated from 0.5 ug of EcoRI digested genomic DNA

- time, concentration, volume, temperature:
  16h, 26 microliters at 63°C

- Hybridization instrument:
  Manual, TelChem hybridization chambers in waterbath

5. Measurement data and specifications

4a) Scanning hardware
GenePix 4000B scanner (Molecular Devices)

4b) Image analysis
GenePix Pro v6.0 (Molecular Devices)

4b) Normalization

Normalized data File = isw2nhp10_exp_normalized.txt
Processed data File = isw2nhp10_exp_processed.txt

Data filtering, normalization, and processing were done as described[3] and are outlined below.

Data filtering: The raw values for the median fluorescence signal (for Cy5 and Cy3 channels) for each spot were filtered for signal quality; each spot used for further analysis had a diameter greater than 75um and a signal greater than 3 standard deviations above the background signal.

Normalization: Above data were then subjected to Lowess normalization using GeneSpring GX v7.3 (Agilent Technologies). The normalized signal was averaged for each dye swap pair. The average of ratios from all replicates was used as the final value.

Data processing: Normalized data were exported to Cyber-T[1] to assign Bayes *P* values to determine if the difference in transcript levels was significant. Changes were deemed significant by ranking the assigned *P* values and applying a false discovery rate algorithm[2]. The false discovery rate was set at 5%. The expression ratios that were deemed significant by the above tests, and had a $\log_2(\text{ratio}) \geq 0.585$, were used in further analyses.

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2. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289-300 (1995).
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