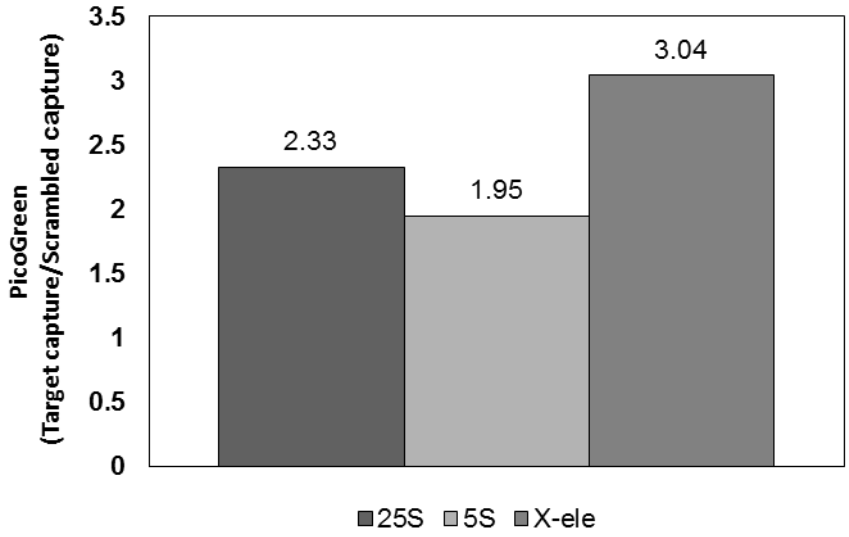
Supplementary Information

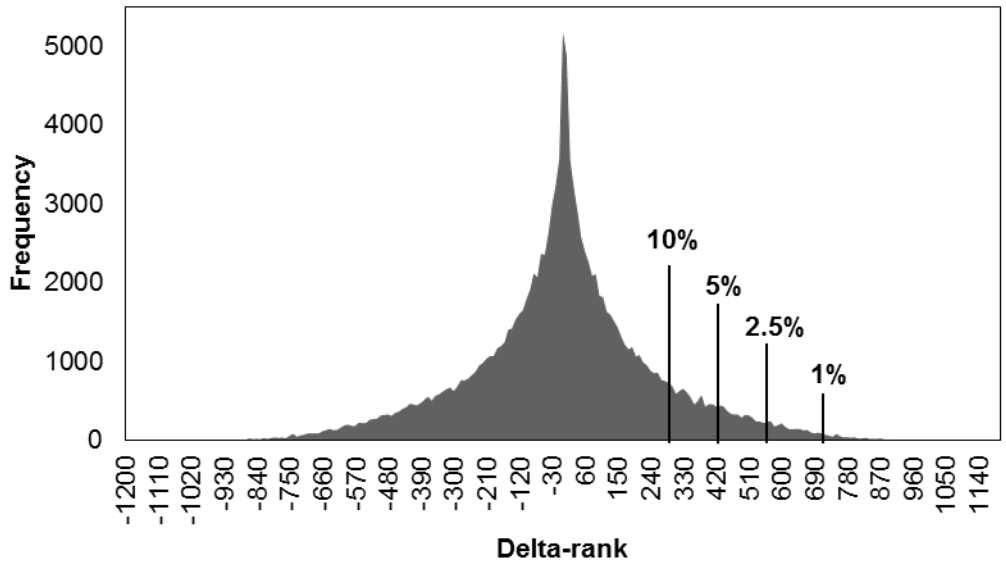
Discovery of chromatin-associated proteins via sequence-specific capture and mass spectrometric protein identification in *Saccharomyces cerevisiae*

Julia Kennedy-Darling[1], Hector Guillen-Ahlers [2], Michael R Shortreed[1], Mark Scalf[1], Brian Frey[1], ,Christina Kendziorski[3,5], Michael Olivier[2], Audrey P Gasch[4,5], Lloyd M Smith[4,5,*]

[1] Department of Chemistry, University of Wisconsin, Madison, WI
[2] Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX
[3] Department of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI
[4] Laboratory of Genetics, University of Wisconsin, Madison, WI
[5] Genome Center of Wisconsin, Madison, WI
* Corresponding Author



Supplementary Figure 1. DNA capture specificity measured by PicoGreen fluorescence of total DNA captured. HyCCAPP experiments were performed with four different capture oligonucleotides: 1) 25S rDNA, 2) 5S rDNA, 3) X-element and 4) scrambled. The total amount of captured DNA was measured with PicoGreen fluorescence. The ratios of the amount of DNA captured from each of the target loci divided by that captured by the scrambled oligonucleotide control are plotted. This ratio corresponds to the specificity of hybridization capture in the HyCCAPP experiments.



Supplementary Figure 2. Yeast-lysate delta rank distribution for bioinformatics analysis threshold determination. The eight biological replicates of yeast lysate generated for HyCCAPP experiments were combined in all 70 possible pair-wise comparisons of averaged groups of four and delta-ranks were calculated for each iteration (A). In total, 129,952 delta-ranks were calculated and are plotted in the histogram. For each yeast lysate:yeast lysate comparison, threshold values for the delta-rank of the protein corresponding to 1% of each sample, 2.5%, 5% and 10% were determined. These ranks were averaged across all 70 combinations and are graphically depicted above with each threshold designation.

Capture probes (5'-3')				
target	sequence	Tm(◦C)	GC%	length
25S rDNA	CTGAACTTAAGCATATCAATAAGCGGAGGA	58.2	40.0	30
5S rDNA	TTAACGGAAACGCAGGTGATATGAGGGCAG	63.3	50.0	30
X-element	TAGAATATTTTTATGTTTAGGTGATTTTAG	49.3	20.0	30
GAL1-10-1	CTTCTGGGATAGGAATCATATTTGGGAATC	56.9	40.0	30
GAL1-10-2	CTCTAATAAAACGACAGTTCCCAAAATGTT	56.1	33.3	30
GAL1-10-3	TGGCTACAGAATCATAAGTTGAATTCGACA	58.0	36.7	30
GAL1-10-4	AGCACCACCTGTAACCAAAACAATTTTAGA	58.9	36.7	30
GAL1-10-5	TTATCTTAGCCTAAAAAAACCTTCTCTTTG	54.2	30.0	30
GAL1-10-6	ACAGCCCTCCGACGGAAGACTCTCCTCCGT	69.4	63.3	30
GAL1-10-7	GGATGATAATGCGATTAGTTTTTTAGCCTT	56.1	33.3	30
GAL1-10-8	CATAACCACTTTAACTAATACTTTCAACAT	52.6	26.7	30
GAL1-10-9	TTGGCCGAAAAGTGCCCGAGCATAATTAAG	62.6	46.7	30
GAL1-10-10	GTGAACATATTGATTATTGTGACTTCTCGG	56.0	36.7	30
Scrambled	GCGCAAGCGCACCCCTCTGCGGGTCATATA	69.7	63.3	30

Supplementary Table 1. Capture oligonucleotide sequences. The DNA sequences for the 13 capture oligonucleotides used in the HyCCAPP experiments are listed. All capture oligonucleotides were ordered from IDT and include a 5' desthiobiotin modification ((N-desthiobiotinyl-3-aminopropyl)-triethyleneglycol). The Tms were calculated using the IDT OligoAnalyzer (http://www.idtdna.com/ANALYZER/Applications/OligoAnalyzer/default.aspx) using default parameters (50mM [Na^+] and 250nM [oligo]).

X-element qPCR assay			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	GCCGCCGAATGAGATATAG	58.3	---
Right	CGGTTTATACCCTGTGCCAT	59.71	---
Probe	CCCATAAAGCCCACGATTATCCACA	68.47	5'FAM - 3'TAMRA
25S rDNA qPCR assay			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	TTAGTAACGGCGAGTGAAGC	58.2	---
Right	CAAAGTTGCCCTCTCCAAAT	59.17	---
Probe	TCTGGTACCTTCGGTGCCCGA	69.22	5'FAM - 3'TAMRA
5S rDNA qPCR assay			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	GTGCATTGTGATGTGGAGAA	58.02	---
Right	CTACCTCTGCATGCCACCTA	58.9	---
Probe	CCGACCAACTTTCATGTTCTGTTTCG	68.55	5'FAM - 3'TAMRA
***GAL10* qPCR assay**			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	CAACCTCATAGAAGGGAATGTG	58.58	---
Right	TGTTGCTGATAACCTGTCGAA	59.33	---
Probe	TGCTTGGTCAAGACCTCTAACCTGGC	69.45	5'FAM - 3'TAMRA
***GAL1* qPCR assay**			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	TGCCCGAGCATAATTAAGAA	58.4	---
Right	TACCAGGCGATCTAGCAACA	59.44	---
Probe	AAATCCGGTTTAGCATCATAAGCGCTT	68.12	5'FAM - 3'TAMRA
***INO1* qPCR assay**			
Position	Sequence (5’-3’)	Tm	Fluorophore/quencher
Left	GCAGCAGCATCTATCTTGGA	59.13	---
Right	GGTTTGTCCCGACTTGAGAT	58.99	---
Probe	AGCCAGCTGAACCAAGCCGG	69.14	5'FAM - 3'TAMRA

Supplementary Table 2. qPCR assay sequences. The DNA sequences for the five qPCR assays used in the HyCCAPP experiments are listed. All qPCR assays were ordered from IDT. The assays were designed using the GenScript Real Time Primer Design Tool (https://www.genscript.com/ssl-bin/app/primer). The FAM modification at the probe 5’ termini was 6-Carboxyfluorescein, and the TAMRA modification at the 3’ termini was 5(6)-Carboxytetramethylrhodamine dT.

GO Molecular Function (1646 categories)	
Category	**p-value**
oxidoreductase activity [GO:0016491]	<1e-14
catalytic activity [GO:0003824]	<1e-14
nucleotide binding [GO:0000166]	1.142E-09
ATP binding [GO:0005524]	2.708E-08
binding [GO:0005488]	8.817E-08
transferase activity [GO:0016740]	9.418E-08
pyridoxal phosphate binding [GO:0030170]	3.149E-07
FMN binding [GO:0010181]	0.000002023
metallopeptidase activity [GO:0008237]	0.000002875
GO Biological Process (2062 categories)	
Category	**p-value**
oxidation-reduction process [GO:0055114]	<1e-14
metabolic process [GO:0008152]	1.843E-14
cellular amino acid biosynthetic process [GO:0008652]	3.899E-12
tricarboxylic acid cycle [GO:0006099]	0.000003996
GO Cellular Component (625 categories)	
Category	**p-value**
cytoplasm [GO:0005737]	<1e-14
proteasome complex [GO:0000502]	4.756E-12
mitochondrion [GO:0005739]	9.353E-12
proteasome storage granule [GO:0034515]	5.47E-09
cytosol [GO:0005829]	0.00000372
proteasome regulatory particle, lid subcomplex [GO:0008541]	0.000003872
proteasome regulatory particle, base subcomplex [GO:0008540]	0.000009837
chaperonin-containing T-complex [GO:0005832]	0.000009837
MIPS Functional Classification (459 categories)	
Category	**p-value**
electron transport [20.01.15]	4.777E-12
protein processing (proteolytic) [14.07.11]	1.238E-09
sugar, glucoside, polyol and carboxylate catabolism [01.05.02.07]	0.000001144
electron transport and membrane-associated energy conservation [02.11]	0.000001309
biosynthesis of serine [01.01.09.02.01]	0.000003027
C-compound and carbohydrate metabolism [01.05]	0.000003862
proteasomal degradation (ubiquitin/proteasomal pathway) [14.13.01.01]	0.000004746
tricarboxylic-acid pathway (citrate cycle, Krebs cycle, TCA cycle) [02.10]	0.000006001
NAD/NADP binding [16.21.07]	0.000006063

MIPS Phenotypes (142 categories)	
Category	**p-value**
Elongated cell and bud morphologies [52.10.10]	0.00005411
Canavanine sensitivity [92.05.10]	0.00006062
MIPS Subcellular Localization (48 categories)	
Category	**p-value**
mitochondria [755]	<1e-14
cytoplasm [725]	<1e-14
ER [735]	4.496E-11
ER membrane [735.01]	2.332E-07
nuclear envelope [750.01]	0.000002597
mitochondrial inner membrane [755.05]	0.00000521

Supplementary Table 3. Complete list of GO enrichment for proteins that have a greater relative abundance in yeast lysate than in captured material. Proteins on the left end of each delta-rank distribution for the four loci were combined and analyzed using FunSpec and are listed. There were 40 total GO terms enriched with a Bonferroni corrected p-value less than 1 x 10^{-4}.

Protein	Locus	Target qPCR (AB/no AB)	Target qPCR AB/off-target qPCR AB
Bdf1	*GAL1-10*	18.2*	0.4
Spt20	*GAL1-10*	1.3	0.5
Sth1	*GAL1-10*	5.3*	0.7
Zeo1	*GAL1-10*	3.7	0.7
Bre1	25S rDNA	3.0	1.2
Ctf4	25S rDNA	22.0	1.5
Ku70	25S rDNA	2.1	2.0
Rtf2	25S rDNA	729.3*	2.1
Sof1	25S rDNA	130.9	21.8
Nop53	25S rDNA	33.6*	2.4
Utp5	25S rDNA	880.1*	63.0
Utp13	25S rDNA	257.4*	35.9
Cdc25	25S rDNA	1.9*	3.2
Ifh1	25S rDNA	3.27*	5.6
Pde2	25S rDNA	5.60*	2.2
Sak1	25S rDNA	3.4	0.4
Sch9	25S rDNA	1.9	0.5

Supplementary Table 4. ChIP-qPCR antibody/no-antibody and ChIP-qPCR target qPCR antibody/off-target qPCR antibody ratios demonstrate enrichment of TAP-tagged proteins at target DNA. For each ChIP-qPCR performed, both a no antibody control and an off-target qPCR assay were used as controls. The ratios of the qPCR signal from the antibody pull-downs relative to the no antibody controls are shown in the third column. A qPCR assay measuring the *INO1* gene was used as the off-target control and the ratios of the target qPCR assays to the off-target qPCR assays are shown in the fourth column. The first four proteins (Bdf1, Spt20, Sth1 and Zeo1) were from the *GAL1-10* locus-specific HyCCAPP list, whereas the other 13 proteins (Bre1, Ctf4, Ku70, Rtf2, Sof1, Nop53, Utp5, Utp13, Cdc25, Ifh1, Pde2, Sak1 and Sch9) were from the 25S rDNA locus-specific HyCCAPP list. (*indicates a p-vale<0.05)