Supplemental Materials

Molecular Biology of the Cell

Howard and Tansey
Legend to Supplemental Figure S1

Supplemental Figure S1. Nuclear localization of Gcn4 is not impacted by proteasome inhibition. GCN4-GFP HTB2-mCherry (GHY339) yeast were grown to log phase at 30°C in minimal media, treated with either DMSO or MG132 for one hour, and induced with SM for 1.5 hours. Samples were imaged using fluorescent microscopy to visualize (A) Gcn4-GFP and (B) Htb2-mCherry. (C) Overlay of Gcn4-GFP and Htb2-mCherry images. (D) Differential interference contrast (DIC) microscopy of corresponding fields in (A-C). Scale bars represent 10 µm. This image is a wider field view of that presented in Figure 2D.

Supplemental Figure S2. Proteasome inhibition reduces the ability of native, untagged, Gcn4 to bind the ARG1 UAS. (A) gcn4 (GHY004) and GCN4 (GHY010) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with a polyclonal antibody against Gcn4. Co-precipitating ARG1 promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. n=3. (B) GCN4 (GHY010) and GCN4-Myc (GHY021) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with an antibody against the Myc epitope. Co-precipitating ARG1 promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. n=4. Error bars represent SEM.

Supplemental Figure S3. Mutations in Gcn4 that modulate its ubiquitylation status. (A) Graphical representation of Gcn4 showing the functional domains of the protein (TAD, transcriptional activation domain, blue; DBD, DNA-binding domain, gray). The wild-type (WT) Gcn4 protein is represented on top, the 3T2S mutant (showing the location of five alanine substitution mutations) in the middle, and the lysine free, K0, mutant at the bottom. (B) GCN4-HA (GHY356) and 3T2S-GCN4-HA (GHY360) yeast carrying a copper-inducible His-Myc-Ubiquitin expression plasmid (pUB221) were grown to log phase at 30°C in minimal media and treated with 0.5 mM CuSO4 and either DMSO or 50 µM MG132 for one hour. Yeast were induced with 0.5 µg/ml SM, or a DMSO control, for an additional 1.5 hours, at which time protein lysates were collected under denaturing conditions. Ubiquitin-conjugates were captured by nickel-resin (Ni-NTA) chromatography, resolved by SDS-PAGE, and probed for HA-tagged Gcn4 protein by western blotting. A sample of the input material to the nickel resin was also probed for HA-tagged Gcn4. IB, immunoblot. A single Ub-conjugate of Gcn4 (arrow) persists in the 3T2S Gcn4 mutant. (C–D) GCN4 (GHY010) and 3T2S-GCN4 (GHY008) yeast were grown to log phase in minimal media and treated with either DMSO or 50 µM MG132 for one hour. Strains were then treated with 0.5 µg/ml SM, or DMSO control, for 1.5 hours, at which time RNA was collected and ARG1 (C) and HIS4 (D) mRNA levels quantified by RT-qPCR, relative to an ACT1 control. Relative mRNA levels were then normalized to the SM-induced, DMSO-treated, sample for each gene. Error bars represent SEM. n=3. (E) pup1–T30A pre3–T20A GCN4-HA (GHY356) and pup1–T30A pre3–T20A K0 GCN4-HA (GHY052) yeast carrying either empty vector or a copper-inducible His-Myc-Ubiquitin expression plasmid (pUB221) were grown to log phase at 30°C in minimal media and treated with 0.5 mM CuSO4 and 50 µM MG132 for one hour. Yeast were induced with 0.5 µg/ml SM for an additional 1.5 hours, at which time protein lysates were collected under denaturing conditions. Ubiquitin-conjugates were captured by nickel-resin (Ni-NTA) chromatography, resolved by SDS-PAGE, and probed for HA-tagged Gcn4 protein by western blotting. A sample of the input material to the nickel resin was also probed for HA-tagged Gcn4. IB, immunoblot. Ni-NTA pull-down material was also probed for total His-Myc-Ubiquitin. (F) GCN4-HA (GHY025) and K0 GCN4-HA (GHY052) yeast strains were grown to log phase at 30°C in minimal media and treated with either DMSO or MG132. After one hour, Gcn4 was induced with SM for 1.5 hours. At this time, ChIP was performed with either IgG or antibody against the HA epitope. Co-precipitating ARG1 promoter DNA was quantified by qPCR, expressed relative to the percentage of input DNA. n=3. Error bars represent SEM.
Supplemental Figure S4. Model. In this model, unmodified Gcn4 binds its cognate UAS element but the resulting complex is inactive for gene activation (OFF). Ubiquitylation of Gcn4 by the SCF^{Cdc4} complex converts Gcn4 into a state that is competent for gene activation (ON) but at the same time renders it a substrate for a Cdc48-containing complex. Cdc48 mediates stripping of Gcn4–Ub from DNA, allowing Gcn4 to be destroyed by the 26S proteasome. Although not shown in the figure, it is possible that Gcn4 could be deubiquitylated after extraction (recycled) and not destroyed. It is also possible that Gcn4 could be ubiquitylated before it encounters DNA, in which case the model still predicts that it would be stripped from promoters in a Cdc48-dependent manner.
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| Strain        | Genotype                                      | Origin                  |
|--------------|-----------------------------------------------|-------------------------|
| W303-1a      | _leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15_ | Patton et al., 1998     |
| MT670        | W303-1a _cdc34-2_                           | Patton et al., 1998     |
| MT668        | W303-1a _cdc4-1_                             | Patton et al., 1998     |
| GHY107       | MT668 _GCN4-HA::KAN_                         | This study              |
| HHY168       | MATα _tor1-1 can1-100 leu2-3,112 ura3-1 ade2-1 his3-11,15_ | Haruki et al., 2008     |
| GHY139       | HHY168 _pdr5::LEU2 GCN4-HA::KAN_             | This study              |
| GHY149       | HHY168 _pdr5::LEU2 GCN4-HA::KAN CDC34-FRB::HIS_ | This study              |
| GHY145       | HHY168 _pdr5::LEU2 GCN4-FRB::HIS_            | This study              |
| YUS5         | WCG4a _pup1-T30A pre3-T20A_                   | Heinemeyer et al., 1997 |
| GHY010       | YUS5 _GCN4_                                  | This study              |
| GHY025       | YUS5 _GCN4-3xHA::KAN_                        | This study              |
| GHY021       | YUS5 _GCN4-9xMYC::HIS3_                      | This study              |
| GHY339       | YUS5 _GCN4-yEGFP::KAN HTB2-mCherry::HIS3_     | This study              |
| GHY004       | YUS5 _gcn4::URA3_                            | This study              |
| GHY081       | YUS5 _GCN4 arg80::NAT_                       | This study              |
| GHY079       | YUS5 _gcn4::URA3 arg80::NAT_                 | This study              |
| GHY356       | YUS5 _GCN4-3xHA::KAN [pUB221]_               | This study              |
| RHY2455      | MATα _ura3–52 leu2–3,112_                    | Sato and Hampton, 2006  |
| RHY2457      | MATα _ura3–52 leu2–3,112 cdc48–3_            | Sato and Hampton, 2006  |
| GHY116       | RHY2455 _GCN4-3xHA::KAN_                     | This study              |
| GHY118       | RHY2457 _GCN4-3xHA::KAN_                     | This study              |
| GHY279       | RHY2457 _cdc48-3::CDC48_                     | This study              |
| GHY304       | RHY2455 _gal80::NAT_                        | This study              |
| GHY305       | RHY2457 _gal80::NAT_                        | This study              |
| GHY285       | YUS5 _GCN4 CDC48-3xMYC::HIS3_                | This study              |
| GHY287       | YUS5 _GCN4-3xHA::KAN CDC48-3xMYC::HIS3_      | This study              |
| GHY124       | YUS5 _K0 GCN4-3xHA::KAN CDC48_               | This study              |
| GHY293       | YUS5 _K0 GCN4-3xHA::KAN CDC48-3xMYC::HIS3_   | This study              |
| GHY008       | YUS5 _3T2S-GCN4_                             | This study              |
| GHY027       | YUS5 _3T2S-GCN4-HA::KAN_                     | This study              |
| GHY360       | YUS5 _3T2S-GCN4-HA::KAN [pUB221]_            | This study              |
| GHY052       | YUS5 _K0 GCN4-HA::KAN [pUB221]_              | This study              |
| Purpose | Name                  | Sequence                                      |
|---------|-----------------------|-----------------------------------------------|
| RT-qPCR | ACT1 F                | AGCCGTTCCTTGCTCTTGTACTCTTCC                   |
| RT-qPCR | ACT1 R                | AGGTAATTTTGGATGACGTGAGTA                     |
| RT-qPCR | ARG1 F                | GCCACCGTGTGTTGGTAGA                          |
| RT-qPCR | ARG1 R                | AGTCAATGGAGCTTGGTGCTGT                      |
| RT-qPCR | ARG4 F                | GTCAATCCAAACGAGAGGAGAT                      |
| RT-qPCR | ARG4 R                | ACCGTTGGGACTTTACAC                          |
| RT-qPCR | HIS4 F                | ACAACTGCCAGTGTTGGATA                        |
| RT-qPCR | HIS4 R                | TTGGACATTTTCAAGGGGTT                        |
| ChiP    | ARG1 Gcn4 BS2 F      | GCTGTCGCAACCTATTTCCA                        |
| ChiP    | ARG1 Gcn4 BS2 R      | TCAATCTGATCCAATGAAGATGA                     |
| ChiP    | ARG1 TATA F         | ATCTGAGCAGTTGCAGACCC                       |
| ChiP    | ARG1 TATA R         | AACTGTGGCGAAGAACAAGG                       |
| ChiP    | ARG1 ORF3 F       | CAAGCCACATTTCTTACAGAG                      |
| ChiP    | ARG1 ORF3 R       | ATCGAAGTCATATTCCACA                        |
| ChiP    | ARG4 Gcn4 BS F      | GCTTACTCATGGCGAGATCC                       |
| ChiP    | ARG4 Gcn4 BS R      | TTTCAATTTGCGCAGCTTTAT                      |
| ChiP    | ARG5 Gcn4 BS F      | TCCGAATGACTCAGTCTACATCA                    |
| ChiP    | ARG5 Gcn4 BS R      | GCGCGCAAGCTTTTATATG                        |
| ChiP    | ARO4 Gcn4 BS F      | CACCCGTGACTTTTACG                          |
| ChiP    | ARO4 Gcn4 BS R      | CGTCCCCGCACATTTTTT                        |
| ChiP    | CPA2 Gcn4 BS F      | GAGATAGGAACCTCCATGTCG                      |
| ChiP    | CPA2 Gcn4 BS R      | TGGCGAGAAATCTTATACAG                      |
| ChiP    | HIS4 Gcn4 BS F      | TGCACGCTGACTCAGT                          |
| ChiP    | HIS4 Gcn4 BS R      | TCAGAGATGCTAAGGACTTTT                      |
| ChiP    | HIS7 Gcn4 BS F      | GGCTATAGGATGTCATGAAAGAAAAA                |
| ChiP    | HIS7 Gcn4 BS R      | AACCTGATTGAGTAGTGCAGT                      |
| ChiP    | LEU3 Gcn4 BS F      | TCTAGCATTTCTAATCATCAGTGT                   |
| ChiP    | LEU3 Gcn4 BS R      | CTTCCGATCGAGAGAGGTT                       |
| ChiP    | LYS1 Gcn4 BS F      | TTTGGAATTTCCGTCTCAAC                      |
| ChiP    | LYS1 Gcn4 BS R      | ATCGTGTTTTCGGAGATG                        |
| ChiP    | SNZ1 Gcn4 BS F      | AGCCGGGTTTTTTCACTACT                      |
| ChiP    | SNZ1 Gcn4 BS R      | GTAACCTACGGTGCGGCAGA                      |
| ChiP    | THR4 Gcn4 BS F      | CAACGAGAATAGAAGAAGAATGAAGA                |
| ChiP    | THR4 Gcn4 BS R      | CCAAATGGAAAAATATAAGATACACAA                |
| MNase   | GAL1 NB F           | CCCACAACAACCTTCAATTACAG                    |
| MNase   | GAL1 NB R           | CGCTTCGCTGATATTACCC                       |
| MNase   | GAL1 NUB F          | CGGATTAGAAGCCGCCCA                        |
| MNase   | GAL1 NUB R          | ATCTTTATTTTGTCCGGACAGTCG                 |
| MNase   | ARG1 -820 F        | ACGTCCGCATGGAAGACCTA                      |
| MNase   | ARG1 -716 R2       | AAGAGGCAACAGGAAAGATCAGA                   |
| Purpose | Name    | Sequence                                                                 |
|---------|---------|--------------------------------------------------------------------------|
| MNase   | ARG1 -740 F2 | CTCTGATCTTTTCTTGGCCTTCTT                                               |
| MNase   | ARG1 -653 R2 | CTGTAGTAATGTTACTAGTAGTATGTTAGAACTTTGT                                  |
| MNase   | ARG1 -690 F3 | CACCAAGTTCTACAACCTACACTAGTAACTACATTACAGTT                               |
| MNase   | ARG1 -541 R3 | CGGTGATGTGATATGTAAGTGTAAACAGATAATAG                                    |
| MNase   | ARG1 -580 F4 | CCATTATACACGCTTTATACGTTTTACATATC                                      |
| MNase   | ARG1 -471 R4 | ATAGATAACAGAAAAAGTATTGACCGATA                                           |
| MNase   | ARG1 -557 F5 | TACATATCACACTACCGTTAATGGAAGA                                          |
| MNase   | ARG1 -510 F6 | ACAACAATTAAAAATCGCCATAACCTT                                            |
| MNase   | ARG1 -415 R6 | GGGCCATGTGGAGAATTACTG                                                   |
| MNase   | ARG1 -492 F7 | CATAACCTTTTTCTGTATCTATAGCCCTTTA                                       |
| MNase   | ARG1 -382 R7 | GTGACTAAACATAAGCCTTTAATCTGAG                                           |
| MNase   | ARG1 -469 F8 | GCCCTTTAAGCTGTTCTTCTCGAG                                               |
| MNase   | ARG1 -361 R8 | ATGACTGGAGAGCGGTCAGTAG                                                  |
| MNase   | ARG1 -444 F9 | TTTCACCTGCAGTAATTCTCCACAT                                               |
| MNase   | ARG1 -326 R9 | CCAATTCGCAATGAGTCACCTA                                                 |
| MNase   | ARG1 -410 F10 | CACTGAGATAGAGCGCTATGTTAGTC                                              |
| MNase   | ARG1 -297 R10 | AATAGGTTGGCGACAGCGGAA                                                   |
| MNase   | ARG1 -383 F11 | ACTACTGACCGCTCTCCAGTCAT                                                |
| MNase   | ARG1 -281 R11 | CGGCACCGTTAATGGAATAG                                                   |
| MNase   | ARG1 -348 F12 | TTAGTGACTCATGTCGATTAG                                                   |
| MNase   | ARG1 -256 R12 | CCTGCGCTTTAAATGACTCTTCTCATTAC                                         |
| MNase   | ARG1 -312 F13 | GCTGTCGCAACCTATTTCCATTA                                               |
| MNase   | ARG1 -211 R13 | ACGCAGTCATCAATGCTTCA                                                   |
| MNase   | ARG1 -290 F14 | ACGGTTGCCGTATGGAAGAG                                                  |
| MNase   | ARG1 -179 R14 | TCGCAACTGCTCAGATTACACTATCCT                                           |
| MNase   | ARG1 -231 F15 | GGATCAGATTGATGACTCGTA                                                   |
| MNase   | ARG1 -141 R15 | CCCATTAATATACATTGGAGACAGTG                                             |
| MNase   | ARG1 -207 F16 | GGCAGATAGTTGATATCTGAGCAGTTG                                          |
| MNase   | ARG1 -114 R16 | GCAAGAACAAGGGAGTACGAATG                                                |
| MNase   | ARG1 -170 F17 | CTGGCACTGTCTCATAATGATATTAAATGAG                                      |
| MNase   | ARG1 -76 R17 | AGACAAGAATAAAGAGAAGAGAGAGAGAGAGAGA                                     |
| MNase   | ARG1 -142 F18 | GGCATACATTGCATACCTCCTTGT                                               |
| MNase   | ARG1 -51 R18 | TTTGCCTTTATCGCTGCAAAATG                                               |
| MNase   | ARG1 -109 F19 | AGTTCCTCTCCTCTCCTCTTTACTCCTATGTC                                      |
| MNase   | ARG1 -15 R19 | TGTGATTCTTTTTGTATCCGTGTATATTAG                                       |
| MNase   | ARG1 -43 F20 | GCACCGATAAGAGACATTGGTCTCA                                              |
| MNase   | ARG1 +20 R20 | CAAAACCACTCCCTAGACATTATTATATTATG                                       |
| MNase   | ARG1 -35 F21 | CGGATAAAAAAGTATACACATAATTTG                                            |
| MNase   | ARG1 +60 R21 | CAAAATGACGGAGGATCTGAACATTTG                                             |
| Purpose | Name   | Sequence                  |
|---------|--------|---------------------------|
| MNase   | ARG1 -10 F22 | TGCATAAAAATAATGTCTAAGGGAAAAGTT |
| MNase   | ARG1 +79 R22  | CTTGGTCTAGTAGCCAAGCCAAA   |
| MNase   | ARG1 +21 F23  | TTTGGCTTTATCTGGTGTTTAGAT |
| MNase   | ARG1 +111 R23 | TACATTAGCCATGAAAGCTACAACCTTC |
| MNase   | ARG1 +33 F24  | TGGTGTTTAGATACCTCCGTCAAT   |
| MNase   | ARG1 +137 R24 | GCGGCACTGGAAATCTCTTCT     |
| MNase   | ARG1 +84 F25  | CGAAGTTGTAGCTTTTCATGGCTAAT |
| MNase   | ARG1 +170 R26 | TTGCAGGCAACCGATCCTCTCA    |
| MNase   | ARG1 +102 F26 | GGCTAATGTAGGGCAAGAAGAGAT  |
| MNase   | ARG1 +197 R26 | TCTTCACGACAAATCCACACAAAC  |
| MNase   | ARG1 +153 F27 | GAAGATCGGTGCCGTCAAGT       |
| MNase   | ARG1 +235 R27 | TGACCTGTAGCTGGGAATAAGAT    |
| MNase   | ARG1 +175 F28 | GTTTGTGTTGATTGTGTAAGAAGA   |
| MNase   | ARG1 +269 R28 | GTACCCCAACAGATAACGCTTTCTGT |
| MNase   | ARG1 +190 F29 | CGTGAAGATTTTTGGCAGGATATTCTATT |
| MNase   | ARG1 +291 R29 | AATAACAGGTCTTGGCAAAAGGTA   |
| MNase   | ARG1 +213 F30 | TCTATTCACGCTGTACAGGCTCA    |
| MNase   | ARG1 +312 R30 | GACGTCAATTTGGCTTTGG        |
| MNase   | ARG1 +234 F31 | GTACGAAGACGTTTATCTGGGTGTA  |
| MNase   | ARG1 +349 R31 | AACCATTGAGAGAGCCCGAAA      |
| MNase   | ARG1 +279 F32 | AAGACCTGTATTGGCAGGAAAAGCC  |
| MNase   | ARG1 +383 R32 | TCGAATCTGATTGCTTACGTTCTTT  |
| MNase   | ARG1 +325 F33 | GGCTGTTCGGGCTCTCT          |
| MNase   | ARG1 +426 R33 | TGTAATACACTTTAAGCTCTGGCTTCA |
| MNase   | ARG1 +341 F34 | CTCATGGTTGTACCGGTAAGATC    |
| MNase   | ARG1 +444 R34 | TTCAGGCAATTCTCCATGGGTGTA   |
| MNase   | ARG1 +381 F35 | CGAATTGTCATTCTTACGCTCTGAA  |
| MNase   | ARG1 +471 R35 | CTTTCTGCGCAATCTTTCTCG      |
| MNase   | ARG1 +411 F36 | CGTTAAGTGCTATACACATGGGAGATG |
| MNase   | ARG1 +506 R36 | GGAATACCCCTTTGTGCAGCATAG   |
| MNase   | HIS4 -757 F1  | TGTCGTAAGCCAAACTACAGA      |
| MNase   | HIS4 -679 R1  | TCAGGAATTCGACATCTTCC       |
| MNase   | HIS4 -707 F2  | TTTCATATAACACCAACTGGT      |
| MNase   | HIS4 -618 R2  | CAAATTGGTCTTCTATGTTGCGTA   |
| MNase   | HIS4 -679 F3  | CGAAGATGTCGAACTTCTGGA      |
| MNase   | HIS4 -586 R3  | GCGTTTTTAGCCCAACTTTG       |
| MNase   | HIS4 -640 F4  | AGCGAACATAGAAGACCAAATTT    |
| MNase   | HIS4 -550 R4  | TTTACTGAGCGAATCGTTATGC     |
| MNase   | HIS4 -598 F5  | GGCTAAAGAAGCGGAAACAAAT     |
| MNase   | HIS4 -493 R5  | CGATGAGGAATCTTGTGTTT       |
| Purpose | Name   | Sequence                              |
|---------|--------|---------------------------------------|
| MNase   | HIS4 -557 F6 | TCAGTAAGAATACCAAAATTTTGAGC          |
| MNase   | HIS4 -470 R6 | TCAGTAAAGAATACCAAAATTTTGAGC        |
| MNase   | HIS4 -509 F7 | CACCAAGATCCCTCATCGGAAG             |
| MNase   | HIS4 -412 R7 | TGTTTGTGCTTGGAGCCTGTGTT           |
| MNase   | HIS4 -469 F8 | AAAACTTGAAGAGGCTAATGAAAAAA        |
| MNase   | HIS4 -385 R8 | GTCGAAAATTGGCAACGATT            |
| MNase   | HIS4 -419 F9 | CACAAACAGCCGTGGAATC           |
| MNase   | HIS4 -328 R9 | ATCGCAATGCTCACACCCTC          |
| MNase   | HIS4 -381 F10 | CCTGCACCAAGTCGATACCAC       |
| MNase   | HIS4 -297 R10 | GGGGGCATTCTGCTGTATTA         |
| MNase   | HIS4 -342 F11 | TGTGACAGTGCAGCATGAGCTG          |
| MNase   | HIS4 -257 R11 | TCAGCTGCTAGAAGGAAGTCC          |
| MNase   | HIS4 -299 F12 | CCCATCAACACCTCTGACAAC         |
| MNase   | HIS4 -196 R12 | TCAGCTGCTAGGCTTTCTGAGC       |
| MNase   | HIS4 -257 F13 | AACTGACTCTAATAGTGACTCCGTTA     |
| MNase   | HIS4 -161 R13 | CCTTCTATATCGAATGACTGATAAAA   |
| MNase   | HIS4 -205 F14 | TGCACAGTGACTACGTTTTTTA        |
| MNase   | HIS4 -88 R14 | CGGAGTGAAATATACGTTCC        |
| MNase   | HIS4 -176 F15 | CATTCCATATAGAAGGTAAGAAAAAGGA     |
| MNase   | HIS4 -78 R15 | CAACACACATCGGAGGTAA          |
| MNase   | HIS4 -96 F16 | TCACCTCCGATGTGTTGTGTGT         |
| MNase   | HIS4+23 R16 | GGTAAGATCGGCAAACGTTT        |
| MNase   | HIS4 -56 F17 | GCACAACTGCGCTGTGTAAT         |
| MNase   | HIS4 +44 R17 | CATGAGCCAGATCATCAAT       |
| MNase   | HIS4 -6 F18 | CTGAAATAGGTTTTTGCCGATT        |
| MNase   | HIS4 +83 R18 | ACCTGACCAACAAGTGAACG         |
| MNase   | HIS4 +31 F19 | GCTCTGGCCCTATGGAATAG        |
| MNase   | HIS4 +140 R19 | TCCTCTTGGAGAACTGAGGA       |
| MNase   | HIS4 +71 F20 | TTGTTGTGTCAGGTACTTTTGGA     |
| MNase   | HIS4 +162 R20 | CAAAGCCCAACATGGAAC           |
| MNase   | HIS4 +120 R21 | TCTCCAGTTCCTCCAGAGAGA       |
| MNase   | HIS4 +218 R21 | CCGTTGTTCAGAAGGCAAT        |
| MNase   | HIS4 +158 F22 | CTTTTGTCCTTGCCAAAGTG          |
| MNase   | HIS4 +266 R22 | TGTTCGGCTGTTTAGCATC        |
| MNase   | HIS4 +202 R23 | GCCTTCTTGAAACAACGGGA          |
| MNase   | HIS4 +299 R23 | CGCTCCTTGGGATATTCAA        |
| MNase   | HIS4 +246 F24 | AGATGCTAAACACAGGCAACA        |
| MNase   | HIS4 +335 R24 | TGATTGGAGAAAACACCGTTTCA       |
| MNase   | HIS4 +285 F25 | TGTTAACAAAGGAGCGTGTGTT      |
| MNase   | HIS4 +375 R25 | CACAATTTATCTTGGCAAGAATTT     |
| Purpose | Name   | Sequence                          |
|---------|--------|-----------------------------------|
| MNase   | HIS4 +328 F26 | TCCAATCAATTCTGGTAACA            |
| MNase   | HIS4 +422 R26 | CCAAGCACTTGCTTTGCAAC          |
| MNase   | HIS4 +390 F27 | AAGCAAGGATATGTTGACCAAG        |
| MNase   | HIS4 +476 R27 | TGGTCGACACTAGGGTGTT         |
| MNase   | HIS4 +444 F28 | TGACGGTTATATACCACCCTAGTT   |
| MNase   | HIS4 +536 R28 | TCGATGGCCTTTGCTAGATT   |