**SUPPLEMENTAL FIGURES AND LEGENDS**

**Figure S1. Comparison of cPrD-EYFP expression levels**
Yeast cells carrying expression plasmids for cPrD-EYFP fusion proteins were grown under inducing conditions for 24 h. Subsequently, cell lysates were prepared and analyzed by Western blotting using an anti-GFP antibody. Asterisks denote cPrDs with low expression levels that could only be detected with longer exposure times (data not shown). Endogenous Rnq1p was detected with a Rnq1p-specific antibody and served as a loading control.

**Figure S2. Fluorescence microscopy of cPrD-EYFP proteins**
cPrD-EYFP fusion proteins were expressed from a galactose-regulatable expression plasmid in yeast cells containing the [RNQ+] prion. Cells were subjected to fluorescence microscopy after 24 hours. The fluorescence of representative cells was recorded. Fluorescence images are shown together with DIC pictures (insets). Arrows point to aggregates in the yeast cytosol. Expression
levels of EYFP fusion proteins after 24 h were assessed by Western blotting (Figure S1).

**Figure S3. Most candidates tested positively in multiple assays**

A Venn diagram is shown to illustrate the degree of overlap between the different assays employed in this study. The blue circle represents the number of candidates that tested positively in the *in vitro* assembly assay, the green circle represents the Sup35C prion assay data, the grey circle the microscopy data and the red circle the SDD-AGE *in vivo* aggregation data after 48 h of expression.

**Figure S4. Using Sup35p for prion detection**

(A) Wild-type yeast cells were compared to *SUP35*-deleted yeast cells carrying a plasmid for expression of Sup35p or NM-Sup35C from the *SUP35* promoter. All three strains maintained the red colony color of the [psi-] state and the white colony color of the [PSI+] state in a stable manner. This indicates that translation termination is fully functional in the [psi-] state and that the [PSI+] prion can be stably propagated, despite plasmid-based expression and the presence of a linker between the NM and C domains.

(B) NM-Sup35C or (C) N-Sup35C fusion proteins were expressed from plasmids carrying different promoters (*SUP35*, *ADH1*, *TEF2* and *GPD*) to determine optimal conditions for the Sup35p-based prion assay. Expression of the fusion proteins was determined by Western blotting and detection with an anti-Sup35p antibody. The blot was stripped and reprobed with an anti-Rnq1p antibody to
confirm equal loading. Colony colors of the corresponding strains are shown for comparison.

**Figure S5. Candidate PrD-SUP35C strains display a variety of colony colors**

Colonies of 90 cPrD-Sup35C-expressing strains growing on YPD plates (see Supplemental Experimental Procedures for details of strain construction). [psi-] and [PSI+] cells are shown for comparison (upper left corner). Some cPrDs, such as Nrp1p, New1p, Lsm4p and Nsp1p showed colony color switching under these non-inducing conditions. The low basal activity of the cPrD-Sup35C fusions of Def1p, Nup116p, Psp2p, Nup100p, Ddr48p and Rbs1p (indicated by the white colony color) prevented their subsequent use in prion-induction and selection assays.

**Figure S6. Comparison of cPrD-Sup35C expression levels**

Candidate PrD-SUP35C strains (Figure S5) were grown to mid-log phase and analyzed for the expression of cPrD-Sup35C fusion proteins by Western Blotting and probing with a Sup35p-specific antibody. Endogenous Rnq1p was detected with an anti-Rnq1p-antibody and served as a loading control. Colony colors of the corresponding strains growing on YPD are shown for comparison.

**Figure S7. SDD-AGE of cPrD-SUP35C strains**

Candidate PrD-SUP35C strains (Figure S5) were grown to mid-log phase and analyzed by SDD-AGE and Western blotting with a Sup35p-specific antibody.
Colony colors of the corresponding strains growing on YPD are shown for comparison. Only the cPrDs of Rnq1p, Lsm4p, Nup100p and Nsp1p showed detectable SDS-resistant aggregation under these conditions. The aggregation of Rnq1p was by far the strongest, consistent with it being the only pre-existing prion in this strain background. Lysates from wild-type [psi-] and [PSI+] cells are shown for comparison.

**Figure S8. Induction of the prion state in cPrD-SUP35C strains**

Yeast strains expressing cPrD-Sup35C fusion proteins were transformed with galactose-regulatable plasmids coding for corresponding cPrD-EYFP protein chimeras. The transformants were grown in galactose-containing medium for 24 hours and were then plated on adenine-deficient medium (↑). The same strain grown under non-inducing conditions (raffinose-containing medium) served as a control. The candidates highlighted in red showed a detectable increase in the number of Ade+ colonies under inducing conditions.

**Figure S9. Comparison of [prd-c-] and [PrD-C+] strains on YPD**

Ade+ colonies from prion induction experiments (Figure S8) were re-streaked on YPD (bottom half of the plates). [prd-c-] cells (top half of the plates) and wild-type [psi-] and [PSI+] strains (plate in the top left corner) are shown for comparison.

**Figure S10. SDD-AGE and GdnHCl curing of additional [PrD-C+] strains**
(A) Cells lysates were prepared from [prd-c-] and [PrD-C+] strains and analyzed by SDD-AGE and Western Blotting. The cPrD-Sup35C fusion proteins were detected using an anti-Sup35p antibody. Colony colors of the corresponding [prd-c-] and [PrD-C+] strains growing on YPD are displayed above the SDD-AGE Western blots.

(B) [PrD-C+] strains were passaged three times on plates containing 5 mM GdnHCl and then spotted onto YPD plates (‘cured’). The corresponding [prd-c-] and [PrD-C+] strains are shown for comparison.

Figure S11. Plots of amino acid biases in core (A) and full-length (B) cPrDs

For each candidate the results from all four assays (see Table S2) were combined into a cumulative score. Only candidates that could be tested in all four assays were used for analysis. For the fluorescence microscopy and Sup35 prion assay results, positive candidates received 2 points and 0 points if they were negative. Results from SDD-AGE (48 hours of induction) and in vitro assembly were treated as follows: All candidates received points according to the scheme used in Table S2 (- = 0 points, + = 1 point, ++ = 2 points, +++ = 3 points). Therefore, the maximum combined score is: 2 + 2 + 3 + 3 = 10. The combined score (x-axis) was plotted against several other parameters on the y-axis. Parameters investigated were the relative frequencies of single amino acids or combinations of amino acids (percent X or percent XY), length of the cPrD, the number of occurrences of amphiphilicity patterns (count PHPH, HPHP, PHPHP and HPHPH, where H stands for a hydrophobic amino acid and P for a polar
amino acid according to the Kyte-Doolittle hydropathy index (Kyte and Doolittle, 1982)). The graphs of the form \( \log_2(\text{HP}/e\text{HP}) \) show the log of the actual counts of HP patterns divided by the expected counts given the amino acid composition and length (0.01 was added to the numerator and denominator to avoid infinities). The graphs "non P" and "mask P" examine the influence of proline abundance and spacing: "non P" shows the number of residues other than proline, and "mask P" shows the number of residues remaining when prolines and any intervening sequences of length <5 are removed. Spearman rank-correlations were computed using R, and p-values were computed by randomly permuting the gene labels 10000 times. For 95% of these random permutations, none of the 35 associated p-values was <0.002, so p-values <0.002 remain significant at the 0.05 level after accounting for the testing of multiple non-independent hypotheses.

**SUPPLEMENTAL TABLES AND LEGENDS**

Table S1 is provided as a separate PDF file. Tables S3 and S4 are provided as separate Excel sheet files.

**Table S1. Prediction of cPrDs in the yeast proteome**

Output format of the cPrD prediction algorithm for the top 179 prion candidates. The amino acid sequence of the candidate proteins are shown at the bottom in single letter code. The core region of the cPrDs is highlighted in orange and the extended region in pink. The top panel shows the probability of each residue
belonging to the HMM state “cPrD” (red) and “background” (black); the tracks “MAP” and “Vit” illustrate the Maximum a Posteriori and the Viterbi parses of the protein into these two states. The lower panel shows sliding averages over a window of width 60 of net charge (pink), hydropathy (blue), and predicted disorder (gray) as in FoldIndex, along with a sliding average based on cPrD amino acid propensities (red).

Table S2. Comparison of different aggregation and prion assays
Prion candidates are ranked according to their highest scoring core cPrDs (left column). Data from fluorescence microscopy, SDD-AGE analysis (after 48 hours of expression), Sup35C prion assay and in vitro assembly assay are shown to allow comparison of the results. N/A (not applicable) indicates that these experiments were not conducted for the following reasons: the cPrD could not be cloned (candidates shown in grey), were recalcitrant to purification (in vitro aggregation), expression levels were too low (microscopy and SDD-AGE) or the basal activity of the cPrD-Sup35C fusion protein was too low for selection. We used a simple scheme (-, +, ++, ++++) to categorize cPrDs depending on the extent and kinetics of aggregation. Candidates in red are considered to be most promising for further characterization (they either showed switching behavior or strong amyloid formation). Known prions are underlined.

Table S3. Oligonucleotides used in this study
Table S4. Yeast strains used in this study

Table S5. Comparison of median amino acid frequencies
Candidates PrDs were split into a positive (cPrDs that satisfied the criteria of the assay) and a negative (cPrDs that did not satisfy the criteria) set. The sequences of the complete PrDs and the core PrDs of the two groups were used to calculate the median amino acid frequencies. Amino acids are shown in the left column in single letter code. QN refers to the combined median frequency of Qs and Ns.

SUPPLEMENTAL RESULTS AND DISCUSSION

Pilot experiments using the Sup35p-based phenotype switching assay
Before we tested our cPrD library for switching behavior we conducted a set of control experiments to determine optimal conditions for the SUP35-based prion assay. First, we compared plasmid-expressed wild-type Sup35p to a Gateway® NM-SUP35C expression construct that carries a linker between NM and the C region (Figure S4A). This linker contains a stretch of glycines and prolines and a translated portion of the recombinogenic sites (see Supplemental Experimental Procedures for details). The linker-containing Sup35p was indistinguishable from wild-type Sup35p in all respects (Figure S4A). In a second set of control experiments we tested our recombination-based SUP35C fusion system with different promoters (Figure S4B). It turned out that too high expression levels
interfere with translation termination, probably by titrating out other factors that are important for translation termination.

The *SUP35* assay is based on a variety of previous studies that successfully used the Sup35p protein to test the prion character of cPrDs, including the PrDs of Rnq1p and New1p (Sondheimer and Lindquist, 2000; Osherovich and Weissman, 2001; Osherovich et al., 2004). In these studies, either the N or the NM domains of Sup35p were replaced by the cPrD to be tested and the resulting cells were then assayed for [*PSI*+]-like phenotypes. We generated Gateway® tagging plasmids for the expression of fusions to the MC or C domain of Sup35p. To test these plasmids, we inserted the N domain of Sup35p, resulting in fusion proteins with the linker located between the N and MC or the N and C domains of Sup35p. Only the fusion protein lacking the M domain showed sufficient translation termination activity (data not shown and Figure S4C), which lead us to focus our efforts on experiments with *SUP35C* tagging plasmids. The expression level of N-C Sup35p was lower than that of wild-type Sup35p (Figure S4C), but the overall translation termination activity seemed to be increased (as judged by the colony color). Notably, the colony color of the strain expressing N-C Sup35p from an *ADH1* promoter construct was comparable to that of wild-type Sup35p. Based on these results, we used an *ADH1*-promoter expression plasmid that enables fusions to the C domain for subsequent prion switching assays with the cPrD library.

We acknowledge that using the Sup35C domain rather than both the M and C domains may not detect some prions that, like Sup35, require a highly charged
domain to maintain a soluble non-prion state. However, prion proteins like Ure2p and Rnq1p do not contain a highly charged domain and thus arguably would be more closely mimicked by PrD fusions to the C domain only, as in our construct. Additionally, we did include the M domain in the *in vitro* assay, explained below, in part to increase our ability to detect prion properties using these diverse assays.

**[NEW1-C+] forms Hsp104p-independent prion particles**

Contrary to other [PrD-C+] strains, the [NEW1-C+] strain was not cured after passaging on GdnHCl-containing plates (Figure S10B). In agreement with this finding, we were able to induce the [NEW1-C+] prion in a HSP104-deleted strain (data not shown). We performed an SDD-AGE analysis on the Hsp104p-independent [NEW1-C+] strain and found that the pattern of aggregation was similar to that of the wild-type [NEW1-C+] strain (data not shown), indicating that the New1p PrD is able to undergo the necessary structural changes without assistance from the prion remodeling factor Hsp104p. These results do not agree with the findings of an earlier study (Osherovich and Weissman, 2001; Osherovich et al., 2004) that also investigated the prion properties of New1p using the Sup35p-based system. The fusion protein tested by Osherovich et al. gave rise to a prion state, [NU+], that could be cured by GdnHCl treatment. We noted a high spontaneous induction and loss rate for [NEW1-C+], suggesting that [NEW1-C+] has a high rate of nucleation and polymerization but an insufficient rate of aggregate shearing. In contrast, Osherovich et al. reported a much higher
stability for the [NU+] prion. We suggest that these differences arose because of the different constructs that were used. We fused the New1p PrD to the C domain of Sup35p, whereas Osherovich et al. used the MC domain. In addition, the sizes of the New1p PrDs used in each study were different. While the initial study focused on a region spanning amino acids 1-153, our prediction set the New1p PrD between amino acids 39-115. We think that additional sequences in the [NU+] prion could provide binding sites for Hsp104p that enable shearing of the prion particles and therefore more stable inheritance. Moreover, the M domain of Sup35p is important for stable inheritance of [PSI+] and could provide additional binding sites for Hsp104p (Liu et al., 2002).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Computational prion prediction
A hidden Markov Model (HMM) was used for identifying candidate prion domains. The HMM had two hidden states, PrD and background, and the output symbols were the 20 amino acids (AAs). The output probabilities for the PrD state were constructed based on the AA frequencies in the PrDs of Sup35p, Rnq1p, Ure2p, and New1p (Masison and Wickner, 1995; Edskes et al., 1999; Li and Lindquist, 2000; Santoso et al., 2000; Sondheimer and Lindquist, 2000; Osherovich and Weissman, 2001; King and Diaz-Avalos, 2004; Osherovich et al., 2004), each normalized to have 100 AA counts total (so that the longer PrDs would not have a disproportionate influence), along with 1 pseudocount for each
AA. The output probabilities for the background state were the AA frequencies for the entire *S. cerevisiae* proteome. The transition probability from PrD to background was set to 0.02 and the transition probability from background to PrD state was set to 0.001. Initial probabilities for PrD and background were set to 0.05 and 0.95, respectively.

Each *S. cerevisiae* protein was parsed using the Viterbi algorithm, and regions of at least 60 consecutive AAs classified in the cPrD state were considered as candidate prion domains. For each candidate prion domains we identified a core of 60 consecutive AAs for which the ratio of the probability of the sequence under the PrD AA frequencies relative to the “background” AA frequencies was maximized; we ordered the candidate prion domains according to this ratio of probabilities.

**Cloning procedures and vector construction**

We recently generated a set of Gateway® System cloning vectors that are derived from the commonly used pRS yeast shuttle vectors (see Alberti et al., 2007 and [http://www.addgene.org/](http://www.addgene.org/)). These pAG vectors have a Gateway® chloramphenicol/ccdB resistance cassette inserted into the single SmaI restriction site of each of the 24 pRS plasmids (Mumberg et al., 1995). The basic pAG vector set was modified to allow for the use of different promoters and C-terminal *SUP35* fusion tags. The coding sequences for the tags and the *SUP35* promoter were amplified from genomic S288C DNA using the primers listed in Table S3. The *TEF2* and *ADH1* promoter were derived from the pRS4xx series of
plasmids (Mumberg et al., 1995). To generate pAG4xxADH-ccdB, pAG4xxTEF-ccdB and pAG4xxSUP-ccdB (please see Alberti et al., 2007, for nomenclature), the GPD promoter sequence of pAG4xxGPD-ccdB was deleted through digestion with SpeI and SacI, followed by the insertion of the TEF2, ADH1 and SUP35 promoter sequences. A PCR product (see Table S3 for oligonucleotide sequences) coding for the Sup35C domain (amino acids 234-685) was cloned between the HindIII and XhoI sites producing pAG4xxADH-ccdB-SUP35C, pAG4xxGPD-ccdB-SUP35C, pAG4xx-TEF-ccdB-SUP35C and pAG4xxSUP-ccdB-SUP35C. The 5' oligonucleotides used for generating the SUP35C-tagging vectors contained a linker that translates into the sequence GGPGGG. This sequence was included to increase the flexibility and reduce any adverse effects of the PrDs on the translation termination function of Sup35C.

Multiple Gateway® compatible destination vectors were generated for expression of His-tag fusions in E. coli. pRH1 was generated from pAED4-SCNM-his7 (Osherovich et al., 2004) as follows. The NM ORF was cut out with HpaI and NdeI and replaced with the Gateway® cassette RfB (Invitrogen, CA). Quick-Change mutagenesis was then applied to remove the Shine-Dalgarno site and add a codon for tryptophan (Trp) prior to the 7xHis sequence, to allow for spectrophotometric quantitation of purified proteins. To create pRH2, an NdeI site was added to pRH1 just prior to the Trp codon, into which the M domain (amino acids 125 - 320 of Sup35p) was inserted. Finally, the NdeI sites were deleted. For N-terminal His-tagged proteins, pRH3 was generated by inserting the M
domain into the NdeI site of pDest17 (Invitrogen, CA), followed by deletion of the resulting 3' NdeI site. All vector modifications were verified by sequencing.

Candidate PrDs and other amyloidogenic gene fragments, such as the N and NM domains of Sup35p and the Q25, Q72 and Q103 length variants of huntingtin were amplified using a two step PCR with overlapping primer sets (see Table S3 for a list of the oligonucleotides used). A two step PCR was chosen as a cost-effective alternative that avoids the synthesis of very long oligonucleotides. In the first PCR, gene-specific primers were used to amplify the region of interest from genomic DNA of the yeast strain S288C or plasmid DNA. Universal primers (Table S3) were then used in the second round to attach the recombinogenic attB1 and attB2 sites at the 5' and 3' ends of the primary PCR product, respectively. Proof-reading Platinum Pfx DNA polymerase (Invitrogen, CA) was used in both reactions to guarantee accurate DNA synthesis. The correct amplification and integration of the DNA sequences into pDONR221 (Invitrogen, CA) was confirmed by sequencing. Several cPrDs contained a single cysteine at the N or C terminus to allow the incorporation of dyes in follow-up experiments. In addition, we added an N terminal serine residue to internal PrDs to increase PrD stability. Below is an illustration of the sequence features that were incorporated into the oligonucleotides to allow Gateway® recombination and dual expression in bacteria and yeast (the sequences overlapping in the gene-specific and the universal primers is underlined):
attB1 site                                    Shine-Dalgarno     Kozak

ACA AGT TTG TAC AAA AAA GCA GGC TTC GAA GGA GAT AAC AAA ATG --

attB2 site

AC CAC TTT GTA CAA GAA AGC TGG GTT --

Yeast strains and media

The yeast strains used in this study were derived from YJW509 (MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [psi-]; [pin-]) and YJW584 (MATa, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [psi-]; [PIN+]) (see Osherovich et al. (2004) for details on strain generation). A detailed list of the strains generated in this study is shown in Table S4. The media used were complete standard synthetic media or media lacking particular amino acids containing either 2 % D-glucose (SD), 2 % D-galactose (SGal), 2 % D-raffinose (SRaf) or a mix of raffinose and galactose (SRafGal, 1 % each). Plates used for prion curing contained 5 mM guanidine hydrochloride (GdnHCl). For determining drug-resistance prion phenotypes, the indicated concentrations of drugs were added to YPD media after autoclaving, prior to solidification.

A PCR-generated (Baudin et al., 1993; Wach et al., 1994; Goldstein and McCusker, 1999) deletion strategy was used to systematically replace a yeast open reading frame from its start to stop codon with a kanMX4 or hphMX4 module. 64 bp forward and 67 bp reverse primers were used to amplify the kanMX4 gene from pFA6-kanMX4 or the hphMX4 gene from pAG32 DNA. These
primers consisted of 45 bp gene-specific sequences and sequences that recognize regions in the kanMX and hphMX4 modules - a 19 bp sequence (CAGCTGAAGCTTCGTACGC) for the forward primer and a 22 bp sequence (GCATAGGCCACTAGTGGATCTG) for the reverse primer. A standard lithium-acetate transformation protocol (Gietz et al., 1992; Gietz et al., 1995) was used to introduce the gene disruption cassettes into yeast cells followed by selection of colonies on G418 (200 μg/ml) or Hygromycin (300 μg/ml) containing agar plates. The knockouts were confirmed using the KanB primer (Wach et al., 1994) which binds in the kanMX4 cassette and individual 20-22 bp primers several 100 bp upstream of the start codons of the candidate genes.

A PCR-based tagging strategy (for details see Wach et al., 1997 and http://depts.washington.edu/yeastrc/pages/plasmids.html) was used to integrate the coding sequences for Cerulean at the 3’ end of genes. 60 bp primers were used to amplify a Cerulean-hphMX4 cassette from pBS10 DNA. The oligonucleotides consisted of a 40 bp homology to the gene of interest and the sequence GGTCGACGGATCCCCGGG for the forward primer and ATCGATGAATTCGAGCTCG for the reverse primer.

The dan1::URA3 strain for studies of [MOT3+] was constructed as follows. A URA3-HIS5 cassette was amplified from a modified pUG27 (Gueldener et al., 2002) plasmid (bearing URA3 (-160 to +78 from pRS306) inserted between the HindIII and Sall sites), a kind gift from Sherwin Chan and Gerald Fink, Whitehead Institute for Biomedical Research, Cambridge, MA. Forward and reverse primers for amplification of the cassette contained 44 and 49 bp, respectively of
homology to the immediate upstream and downstream sequence at the *DAN1* ORF. The PCR product was gel-purified and transformed using the methodology described above, followed by selection on SD-his media and confirmation of correct integration using primers *dan1upseq* and *URA3-1-3*, which amplify across the 5’ integration junction.

**Fluorescence microscopy**

The library of 94 cPrDs was cloned into the vector pAG424GAL-ccdB-EYFP. Each of the resulting constructs was then introduced into the YJW584 strain. The cells were grown for 24 hours in galactose-containing medium and subjected to fluorescence microscopy using an Axiovert 200M microscope (Carl Zeiss, Jena, Germany). Fluorescence and DIC images were acquired and processed using Axiovision (Carl Zeiss, Jena, Germany) image analysis software. To evaluate the expression levels of the cPrD-EYFP fusions, cell lysates were prepared after 24 hours of expression and analyzed by Western blotting and detection with a GFP-specific antibody (Figure S1).

**In vitro aggregation assays**

In pilot experiments, we found that many cPrDs expressed with only a His-tag (without any additional solubilizing features) could not be completely solubilized prior to the assembly experiments. This was likely due to the absence of solubilizing elements normally found in the native full-length proteins. Prion proteins are able to switch between two states because of a balance between
insolubility – driven by the prion domain – and solubility – driven by non-prion domains. In the case of Sup35, this latter activity is largely provided by a highly charged, natively unfolded middle region, M, which is located adjacent to the prion domain. Thus, to more accurately mimic the solubility of the native proteins while still avoiding the necessity of a renaturing step after purification, we incorporated the M region between the cPrD and poly-His coding sequences. Most were purified with M and a poly-Histidine tag at the C-terminus, with a few exceptions, as explained below, due to expression difficulties. We successfully purified 91 of the 94 cPrDs under fully denaturing conditions, as have been employed for previously characterized prions.
Candidate PrDs were recombined into pRH2 and transformed into BL21AI (Invitrogen, CA), an *E. coli* strain optimized for toxic protein expression. Some cPrDs (Ksp1p, Wwm1p, Nup49p, Nup100p, and Med2p) were coexpressed with pRARE2 (EMD Biosciences, NJ) to increase expression levels. The Swi1p and Nup100p cPrDs were expressed in pRH1. Cbk1p, Def1p, and Psp2p PrDs were expressed in pRH3. 300 ml 2 x YT cultures were induced with 1 mM IPTG at an OD of ~ 0.4. After 3 hours, cells were sedimented and resuspended in lysis buffer (7 M GndHCl; 100 mM K$_2$HPO$_4$, pH 8.0; 5 mM imidazole; 300 mM NaCl; 5 mM 2-mercaptoethanol) buffer for 1 hr at RT. Lysates were then cleared for 20 min at 20,000 rcf and loaded onto a BioRobot8000 (Qiagen, CA) for purification using TALON® Superflow resin (Clontech, CA), according to the manufacturers’ instructions. Proteins were eluted (8 M urea; 100 mM NaOAc/HOAc, pH 4; 5 mM β-mecaptoethanol) and precipitated with 5 volumes of methanol.

For assembly reactions, methanol-precipitated proteins were resuspended in 10-50 µl of resuspension buffer (7 M GndHCl; 100 mM K$_2$HPO$_4$, pH 5.0; 300 mM NaCl, 5 mM EDTA, 5 mM TCEP). Protein concentrations were determined by measuring absorption at 280 nm using calculated extinction coefficients. Protein stocks were heated for 5 min at 95°C before being diluted to 20 µM in assembly buffer (5 mM K$_2$HPO$_4$, pH 6.6; 150 mM NaCl; 5 mM EDTA; 2 mM TCEP) plus 0.5 mM ThT. Assembly reactions were performed in black nonbinding microplates (Corning, NY) with 100 µl per well, with 1400 rpm agitation at 30°C in an iEMS incubator/shaker (Thermo Scientific). Fluorescence measurements (450 nm excitation, 482 nm emission) were made with a Sapphire
II plate reader (Tecan, NC). In order to minimize variation arising from sporadic sampling of very large fluorescent aggregates, values for each time point represent the averages of 10 readings taken over a five minute period.

After the final fluorescence time point, reactions were applied to nitrocellulose and cellulose acetate membranes as described by Boye-Harnasch and Cullin (Boye-Harnasch and Cullin, 2006). Ponceau S was used to detect immobilized proteins.

**Semi-denaturing detergent-agarose gel electrophoresis (SDD-AGE)**

A library of pAG424GAL-cPrD-EYFP constructs in YJW584 was used to investigate the amyloid propensities of cPrDs in vivo. The cPrD-EYFP proteins were expressed for 24 or 48 hours and then processed for SDD-AGE analysis.

Cells were harvested by centrifugation and resuspended in buffer A (50 mM Hepes, pH 7.5; 150 mM NaCl; 2.5 mM EDTA; 1 % (v/v) Triton X-100) containing 30 mM NEM, 1 mM PMSF and 1 x Complete Protease Inhibitor (Roche). Cells were lysed using glass beads and were briefly spun at 3,000 rcf to sediment cell debris. The protein concentrations of the cell lysate were adjusted and mixed with 4 x sample buffer (2 x TAE; 20 % (v/v) glycerol; 4 % (w/v) SDS; bromophenol blue). The samples were incubated at room temperature for 15 minutes and loaded onto a 1.8 % agarose gel containing 1 x TAE and 0.1 % SDS. The gel was run in running buffer (1 x TAE, 0.1 % SDS) at 50 V, followed by blotting onto Hybond-C membrane (Amersham Biosciences), as described in Halfmann and Lindquist (2008).
**SUP35-based prion assay**

We used *SUP35C* or *SUP35MC* tagging plasmids (see section 'cloning procedures and vector construction' for details) to generate an array of cPrD-Sup35C or cPrD-Sup35MC-expressing strains (see table S4 for a list of the strains). First, the strain YSR100 was transformed with a *cPrD-SUP35C* or a *cPrD-SUP35MC* expression plasmid. Then, a plasmid shuffle was performed by plating the transformants on 5-FOA. This produced a strain that expressed a particular cPrD-Sup35C/MC fusion protein as the only source of functional Sup35p. All strains were examined by Western blotting and SDD-AGE to evaluate expression and aggregation levels of the fusion proteins (see Figure S4, S6 and S7).

To induce the prion state, the *cPrD-SUP35C* strains were transformed with a corresponding pAG424GAL-cPrD-EYFP expression plasmid. The transformants were grown in SRafGal-Trp medium for 24 hours and then plated on YPD and SD-Ade plates with a cell number of 200 and 50,000 per plate, respectively. The same strains grown in SRaf-Trp served as a control. We compared the number of Ade\(^+\) colonies on plates with cells grown in raffinose/galactose-containing medium to plates with cells grown in raffinose. A greater number of colonies under inducing conditions suggested that expression of cPrD-EYFP induced a prion switch. In these cases Ade\(^+\) colonies were re-streaked on YPD plates and analyzed by SDD-AGE and curing on GdnHCl plates.
Fiber transformation

Stock Mot3PrD protein (from expression of MOT3 cPrD in pRH2) in resuspension buffer was diluted to 10 µM in 1 ml assembly buffer and rotated end-to-end at 20 rpm for 3 days. The protein was confirmed to have converted to SDS-resistant aggregates by SDS-PAGE analysis of samples incubated for ten minutes in sample buffer at either 23°C or 95°C, and further confirmed to have a fibrillar appearance by transmission electron microscopy. Transformation of dan1::URA3 yeast strains was performed according to (Tanaka and Weissman, 2006), except that DNA was omitted and URA+ ([MOT3+]) spheroplasts were selected directly on SD-ura plates containing 1 M sorbitol.

SUPPLEMENTAL REFERENCES


Supplemental Figure S1
Supplemental Figure S3
Supplemental Figure S9
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501 IIPSTNIVPQICTGYGNNOPQSPPTNSPOONPTGISYSQFQQQQQQPOYMONFQQQQPOYAOFFQQQQPOYTONYQQQQPOYTOHPQQQQQQQQQQQQQO
601 GYTPDOGVSILDL
CBK1

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1001 LGYRELIPSVITKHFDVDGLDEIANHTPLGSLSGGQLVKVVIAGAMNNFPLLVLDEPTNYLRDSLGAVALAIRDWSGGVVMISHNNEFVAGLCPEQW
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NRP1

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701  SNVGDNSAFNGFNSIRW
YEL007W

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1 MDIEPTFKGYIEDDALLILQATLDGLKLHIPRPRYPEIERPYLIVSGIFSIFVIEEISGIKRWTDGVSWPSRISGKFLYKELDKENAGSNANATSGS
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1 MSAQDYGNASKOSYSRPSAPPGYETASRGYAPSQQQNYPPPQOQQQOQPOYYQQPOYYQQHPQPOPYYQQQPASSNEDCLAGCLAGLCLC
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MED2

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201 AANNGAAFSPLITTRIQSQQAQPSDVMFNDLNSMDISMFSGLDSTGFDSTAFNATVEKGFDNDSGNNYNDINISSIEIINNNINISTSNLKHNNNNESN
301 KNNNGDEKNNNNEDNENNNSSEKNNNNNNNNNNNNDNDDNGNNNNNNNSGNDDNNNTTTNNDSSNKNNNAITGNDNENIVNNDLPTTIVSVNPGDNPADNGE
401 EYLTLNDNIDLIDWSTTGDNGELDLSGFINI
SLM1

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201 NVIKSIVYLTEIASIQDEIVRQQLRSLHAVQFPFFSIENQYQFSSQEDKSVQKFFLPGNGSIDLPTILNQYHESLASSASKASREL TDVPRLEDL
301 RRDIVKIKEIKSLQDSFKNSCSKELQQTKQMKFQEQLDARYSVPKQDPFLTKLALDRQIKKQLQEEENFLHEAFDNLETSG ALEKIVVM EIQNSLT
401 IYARRLGQEAQLVF DILISKLDGSFFNVDPQFEWDNIFS RDPNFLLP NLPMRTFKEIVYQFDPLTEI KSGFLERRS KFLSKYSKG YV LTPNFLHEF
501 K TADRKDLV PMSLALSCT VTEHRS KNNSTSSPNTSGSDAKFVLHAKQNGIIRR GHNVFKAD SY ESMMSWF DNKIL TSTSNIQD KYK FIT QKL NLN S
601 DGFKL TNNHT SINKYS QLSNA NSTMVEND DINS NYSYGSTV PT KLDNQ TN STS MLSL PD INDSEL QDQPV PI YI QTP I NDFKS
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1 101 201 301 401 501 601 701 801

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501 601 701 801

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201  SLTSSTSN ASSSYNO TFEYILPSSTAASTNLSSSSNNMSMHTNPATATSTSDLINDLPVGGTPSSSLISDLHSPPTVTSFLPASQTLLMSSTTTSSIGTN
301  INPPQHSPSSQREDFSTAPVMSSSSASLMNSSLGLGNHNMVSSSSQPASSRPFGIWNTDMSVWS
1  MSSDEEDFNDIYGDDKPTTTEEVKKEEEQNKAGSTQLDQLAALQALSSLSNKNNPNSNNSNQDTSSSKODGTANDKEGSNEDTKEKQESA
101 TSANANANASSAGPSGLPWEQLQOTMSOFQPQSPQPQQPQVOTKKEERSKADLSKESCMMGGLNWDTEDNREYFGYGTVDLKimKDPATGRSR
201 GFGFSLFKEKPSSVDEVVKIQHILDGKVDPKRAIPEQDKTGKIFVGGIGPDVFKEEFPKDFSFQWGTIIDAQLMLDQMGTQQSRGFGFVTYDSADAVDRV
301 CQKFDIFKDKIEIKRAEPRHMGGSNNGNNGNMRGGNFGQDFQNYQNPNMGGYNNPMQAMTDYYQKMOEYYQQMQKGTGMMDXTOMQ
401 QOMOQAMMMPGFAMPNAMTLNPDQODSNATOGSPAPSDDNNKSNVOTIGNTSNTDSGPPINLPNGPKGPSZYNDDHNSGYGNYRDRGDRDRDNRD
501 RDYNHRSGGHRRNNGGRCRGGYYGRNNGYHPYNR
YNL208W

1    MSANEFYSQQGQYNNQDRGTPPNGQYGDNGPNGERGLFSTIVGSAAYAGSKVSNHSHKLSGVLGAIGGAFLANKISDERKEHQQEOYGN
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1 MSASLINRSLTNIRTELDFLKGSNVISNDVYDQINKSLPAKWDPANAPRNASPASLEYVEALYQFDPODDLDGLKPDKDVQLEKLSPEWYKGSCNGRT
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201 GAGASIGSDIVNNIF
MOT3

1  MNADHLLOOOOBOOHOOOHOOOHOOOHOOOHHTILONVSNNTNGSDL1ASQPFNTTVSSKDRVMVNSGARELPMLHQQOYIVPYYOYT2NNS
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- background
- PrD
- net charge
- hydropathy
- foldindex
- prd score
EPL1

MAP

Vit

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background

net charge

hydropathy

foldindex

prd score

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HRR25

MAP
Vit

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background PrD

net charge hydropathy foldindex prd score

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MAP
Vit

net charge
hydropathy
foldindex
prd score

background
PrD

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MAP
Vit

net charge
hydropathy
foldindex
prd score

background
PrD

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MAP
Vit

net charge
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PDR1

background

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distance

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301 RVTPMGTTTVNNISPSGRGTDAQELGSFPESDSNGVQPSNVPMVPSVYQSENOQQQLPPLLTSGLPSIQPEMPAHQIPSQSLVPQEDA
401 KKNDVEFSQAIYVKIKTRFAQDIYKHFLEILTQYREQKPINEVYAQVTHLFQANPDLLEDFKFLPDSASANOOQVAOAOQQHEAOAMHAO
501 AQOAOAQAQAOQVQQQQFLYPASGVYHPKSNRGIROQNLPRIGESRESPDRNGSTLYEAYODQHMOQPPFHMPLPSIVQHPNVMHGIANENPPLSDLR
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801 NOQEEELLKEIEERHEYDYMIESNLRTIPQLELTVKQLENMTEKANFKLPGLHTSMTYIIKYVKRYKDKRFEIEHALHPEVATPVLKRLKQ
901 KDEEWRRAQREWNVKRELEQKVFKSLDLHGLTQKQADKLLLTTQELISEISSIKVDQTNKKIHWLTPKPSQQLDFDFPDKNIFYIDILCLADFITHTT
1001 AYSPNDKRLKDLKLYFILFSISFEKIEEESLYHKNVSESSGGSDGSISIASKRPFYQPSQEMSLLDILHRSRYQKLKRSDDGKVPQSEPPEEPINT
1101 IEEELLIDEEAKNNWLTGNNVEEANQSIIQONISFTPNNFANTYIFFRHWFTITYLLEIKQMNERTKEINTRTSTVFAKDLDDLSSQSEMGLDFVG
1201 EDAUKQVRLSLRLINGDLEHQFEESLORAYNNKAKFLYTIKVTQSLVKAHTLMTDAKTAEIMALFVKDRNASTSSAKDIYRLQVRSHMSNTENM
1301 FRIEFDKRTLHVS1QIYLADDLTLKEDKWKYYVTSAHPEGHEKHLKIPFLERLIEFGQIDGTEVDEEFSPEGISVSTLKIQIPIYQL
1401 YLPSQKVRDPTLFWKRTMDNKTOKSCHKQKUHLCKLPQGNYRNLNRDEQKIYQKWKWTNY KPSMKRSGYOGDGTFSTQTGKVTQOCGNYL
MAP
Vit

background
PrD

net charge
foldindex
prd score

NSP1

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201 MLNSSQQA TVMDNILSILNDDVLYTKLKTIEITLNTTPFISPPPLPAIAISPIPNRDTQILNIDSVFSSSIPITNDPENTDNLLYQNWSPQPHSIPISQPIYD
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401 LNKSNONFVNNTITHPENGPTSATSTSTSSNGTPSSNSSNMPKSLTDPKLLKNIMWLKSLRLHYSDALSGTPWIELIYLDDETLLEKKGVLALGARR
501 KLLKAGFIVIDYKERDLIDRSAY
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401 O1RG1YKT1FEEI1Q1KEE11ARQOQ1E1DE1A1V1DE1K1Q1E1IK1FT1Q1SS1V1AST1RNL1MA1Q1DA1ERAGM1NT1L1GM1L1GH1Q1SE1Q1L1N1VE1G1L1DM1LK1V1QN1V1A1DE1K1V1A1EL1KL1N1
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LSB1

1  MSASLVNRSKNRNELEFLKESNVISGDIFELINSKLPEKWGDNQRSPQNADTEEVEALYDFEAQDGDSLKITGDKIQVLEKISPWDYRGKSNNKIG
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201 AQPQOSGASIAFKSFGLGNAIFGAGSAIGSDIVNSIF
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1 MSNLLNKFADKLHGDHDERYEEDNDDQTPORHEKQOREFRNOSGKAPYGEENQGNPQPGQPNLGGNTQFGGDFOOTDYGATGGTYTOT
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SLF1

0 100 200 300 400
MAP Vit

0 1
net charge
hydropathy
foldindex
prd score

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201 ANMAANISFPNYQARPEYVIPNASHWLNNNSRNSYKQLYKFROQQYYNNNYQOLOTYPYYSMEPIFKIESIKNQIEFYFSEEKLKDLEFDRSKFKK
301 ANDGFIPMSLIGFYSRMNLSLGDPNILASMSKLQHKTENHLEIALGSIEGAQKNMADDFNPLENYFIRRENWAEYAMESNFDENDDETEKYNIEKL
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DHH1

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201 LSRDFKTIIEQILSLPPTHQSSLFSATFPLTVKEMVKHLHKYPSEINLMEELTLKGITQYYAFVEERQKHLHZINLTSKIQNAIFCNSTNRVELLA
301 KKTIDLGYSCYSHARMKQQERNKFHEFRQGKVRTLVCSDLLTRGIDIQAVNVVINFDFPKTAETYLHRIGRSRGFRGHLGLAINLINWNDRFNLYKIEQ
401 ELGTEIAIPATIDKSLYVAENETVVPFPIEQ0SYHQQAIPOOOLPSQQOFAIPPOOHHPQFVPPSHOQQQAYPPQMOEPPQQGGYQQOEHFMMAPP
501 QSRQOY
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101 LLDIEEMLDLPYAFNYHFDNGIFRGLAFANFTPPEETTVITSNLKGEISGRKLVEYKMLPQAERERIREEKREKQLEEQRHSSNLSDLSSK

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301 NDPTDLEIYSQLLFLKDREKYYEAYLAPMGISASHKRIINVCYSLGLVEYDPRFIIIRKILDHANLQSHLQQQGOMTSAHIQPNSSTGSGMRNSQSY

401 TSLQAHAAAAANISNOAVNNNSNNTINNSNGNNVIIINNSASSTPKISSOOGFSMOPILTSPKMNTHHSSQYNSAADQPOQPOPOQOQNOVOSAAQO

501 QQSFLRQQATLTPSLRIPSSGYSANHYQINSVNPILRNSQISPEPNSOLPINSOLTLOAOQPAOQOPQVPVAYONASLSSQOLYILNGPSANSQOSQILLE

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NUP145

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**201** ASITGGLGNNNASFPRGETTGTMSTNPYGINISNVPMAVADMPRSTISSSLSDVNGKSDAEPKIENRRRTYSFSSVSIGNAPLNASQSSLVSRLSTRL
**301** KATQKSTPNEIFSPSYSKPLNGAGAPLVDFFSSKMTSLAPNENIFQFQNFNFLSSQRADLTLELRKLKDSNRAAKKLLLSGPTAITKKHMQDE
**401** QDSSENEPIANADSVTNIKRKENRDNLDNYLNGEQSNLNYDQGENTLQHEKSSSFYWCSPSPEQLERLSLKLQALAASNVIDGRGYYCITFQHDV
**501** DLTAFTKSFREELFGIKVIPFRSSKTVEYVPEDEATKPMIGHGLNVPAIITLENVYPDVKKTSDKMDTKTFAFQVFVDKRLRSMREMNYISYNPPGTWTF
**601** KYNHFISWGLVNEEADIEDLLSKQEDGEOQPRLKVRLAQSKPSOEDKEVILKTDFGTGTSGLDSDVEEKAYPEPDSLSDFAEGEAASPKLVDSDKDWE
**701** QLLAGSSLSRFATSKEFDGFCQNEIDLLFSECNDEIDNALKLMKFRRFTPASYTFAFKSTGSMLTDDIVGKSVISKRLPLEQRKFLFDDVDYLDKEI
**801** EKVTIEARKSNPYQISESSLFLFEDYMEKTSDDYNWLSSLILFDPVSYYPYKTNDQVKMALLKRRHQRLTSWIVSRS1GPEIEEEIKRINSSNEIEQI
**901** FLYLLNDVVRASKLAIIESKNGHLSVLISYLSGDPRIRDLAEQLQKWSTGGCSIDKNISKIYKLLGSFPEGLFLKELESEFSLWCLLNTLCLYQI
**1001** DEYSLESLVQSHLDKFLPSLPYDPIGVIFQLYAANENTEKLYKEVRQRTNALDVQCFWYLIQTLRFNGTRVFSKETSDEATFAFAAQLEFAQLHGHSLFVS
**1101** CFLNNDKAEDTIKLRVMREITLRLRSTNDDLRLKIPSLQIFNAQAQLDKRIDGEYNLYEVEQLNISGSSYDLAMEAVTLGPRLLLSSPYNiolKTL
**1201** REILNEFPSERDKWSVINVTFLVEYKLVLNDVETQETIDSLSGMKIFYQHKCREVAACNVMSQEVSKILEKNNPSIGDSKAKLELPLGQPEKA
**1301** YLRGEFAQDLMKCTYKI

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**MAP**

**Vit**

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**0** 50 150 250 350 450 550 650 750 850 950 1050 1150 1250

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-1 0 1

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**background**

**PrD**

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**net charge**

**hydrophathy**

**foldindex**

**prd score**

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201 GRHQQEEEELFQEMIEISNVIKFNQITMVLDASI QAAEQQSKAFKESSDFGAII LTKMDGHARGGGAISAVAAINTPI IFIGTGEEHIHDELFSPKSFIS
301 KLLGIGDIE SLEFQLQTVSNKEDAKATMENIQKGFTLDFKQMQT I MKGPSNIQAQM PGMNSNMNQVGEEETSQKMKKMVYVLDMSMTKEELESDG
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501 MNMFGGGGGMGGMGGMPDMNEMMKMMQDPQOOMAKQFGM:
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301  DDPLENMFGDYSNEPFTNFDEFGDLDAVFF
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SET1

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301 PPPATYCFIDVSQSSIKSGLLATTINTLLQLDSDPHDERTRISILCDVNAIHYFKIPLDSENNEEADQINMMDIADELFPLPRPSMVVSLKACR
401 QNIELTLKTIPQIFQSNLITNFALGPSAYHLLGGVGGKIVVSGTLPNLIGKLQRRNESGVVNTSKETQLLSCQDSFYKNTIDCSKQTVIDLF
501 LASEDYMDVAMLNSLRSFTAGQTHYPFGHGKIPDIEVFSTEFAKHISMDFCMETVMRARGSTGLRMSRFYGHFFRFRSLDCAFSTMPRDQSYLFEVN
601 DESIMAACYVQAVVLSLNNQQRIRRITLAMPTEESLAEVYASADQLAIAASFYNSKAVEKALNSSLDDARVLIKSVQDIILATYKKEIVVSNTAGGAP
701 LRLCANLRMFPITTSLTHMAFSGIVPSDHASALNNLESPLKYLIKNIYPDVYSLHDMADEAGLPVQTEDGEATSTIVLPQPINATSSLFERYGLY
801 LIDNGNELFLWMSGGDAPALVFDFVGTQDIFDIPIGKQEIPVVENEFNQVRNIINQLRNVHDVITYQSLYIVRGASLSEPVINHASAREVATHRLWASS
901 TLVEDKILNNESSYREFLQIMKARISK
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201 SFPSAIFNDTSRLNKSNSGMKRFSSLSSQSRFSDFPSNDSPSLQEQQQQQQQPQQOQPQOHSTOKHQPOQQCNQQVNNSS1PLSSSOQVIGLHSNHSHQ
301 DLSTVESTIQTSQIGKSLRDNTEINEKIGTSQIPSSINGYMSTQPSPTPTLEWGNQSAQHGSSFFLPASAASTAIAPTSNTSANANASSNNGANNG
401 ANOALSASSQOPMMOIGNNTNITSNLSLPPPYGLMSSQQOHISNVMNTSDMNITPOKONRFMOOPQPEHMYPVNOSNTPCVPPARLSSRNSHKNNST
501 TSLSSNITGSASISQADLSSLARIAPPANPADQNPCNNTLYVGNLSDATEQELRQLFSQEGFRRLSFRKNTTSNGHSHGPMCFVEFDDVSFAHAL
601 ELYGRQLPRTVSSKGGRSLFSKNNPLGVGPNSRRGGSNPNPVNMMLSSYNVNHKIN

MAP Vit

0 50 100 150 200 250 300 350 400 450 500 550 600 650

0 -1

net charge
hydropathy
foldindex
prd score

background

PrD
1 MSFRPGE5GSSRGGGGRGGRGRRGSRGGARAGGRSGRRGGGGGFRGGRGSGGGSSRGGARGGSRGGFRRGRRGGRGSRGGGARGGSGGRRGAAGGARGG
201 HRPGRELISMAKKRPNIIPIDARHPQKYRMLIGMVDCVFADVAQPQDQARIIALNSHMFLMKDQGGVVISIKANCIDSTVDAYFAREVQKLREERIKP
301 LEQTLTLEYRDHCIVGVRMSLKK

NOPI

MAP
Vit

PrD score

net charge
hydrophathy
foldindex
prd score

background
YIF1

MAP
Vit

background
PrD

net charge
hydropathy
foldindex
prd score

1  MSYNPYAYATSFONGVNDRESHTPOOQPMPQTPRNTPVPNGQGNANMNANVNGSGGGFPIFDPRGMSAFQGLGSAIFSGNFGQDNDNFDQFQETVKATANAAG
101 ISTYFQVSTRVINKKLILVPLNLGNTKNUVRIMDSGNFLPPRDDVNSPDMYMPIGLVTVIILWNTQQGLKGSFNPEDLYYKLSSTLAFVCLDLII
201 LKLGLYLLIDSKIPSFLVELLCYVGYKVFPLILAQLLTNVMPFNLNILIKFYLFIAFGVFLLRSVKFNLLSRSGAEDDDIHVSISKSTVKKCNYFLV
301 YGFIWQNVLMWLMG
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SR\text{VNMGVAPPPSTVIYQ}Q\text{PESAYRNDAKNTFYDEVKIP}S\text{NEVYELEEDFDEKQKE}KTRQKQK\text{E}RNKEGR\text{SPSRVAPLVYE}E\text{ENFEGSSPOQYD}

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\text{PIN2}
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201 TGMASKGFGKFTNSDEQQLALEMOQVFLNIGRAIKYVGPT...CQQQHVSGNNDDYNRSRSSSLNNENVSRSFLSKGQSLNGNNNNMGFKNHNSQFTYPVQQ
301 QPSLNHTDPNTTVIGGLSSLVTEDELRAFYQPFGTIVYVKIPVGGCGFVQYVDRLSAEAAIAMQGFPINRSVRLSWGRSAKQTALLQQAAMLSNS
401 LOVQQOPGLOQPNYGYIPSSTCEAPVLPDNVSSTMLPGCQILYNPSPYANANGLSNNFSFYSNNATNTQATSSLADTSMDLGTGGQQVIMQGSE
501 AVVNSTNAMLNRLEGNGFMSGFA
MSN1

MAP Vit

net charge
hydropathy
foldindex
prd score

1  MASNQHIGASNLNENAILTNRVAELERRMSMFEGIFHALSNRLDLHKKYDVVNSQQQINELTAFLSTLLNDQQRHAEILSEKLSGTLHGVSATSIS
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201 LEFYSANPVDGGQGSGILYISSNSSOSSKQSPNLKKSVPNHEAVESNAQESVPTFEEEQYTEKTLKRLRKIVCTRPFEFIKSPHSVEVWKEYTVEGNG
301 QPSIRKMEALYQTAWRDPAVNKRYSRRKVLWKAIQTLGNYSLNYVVEILENSRYVNDKQKVQPIGWLCHSHSHIPETLK
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101  WSYGHPSMIYPYQMSQMPYFPQPSQPPQ:PSVGTPLSTPSPEGNTFTDSSADSDMTSTKYYRPFPMLTSPNBFPNWVKNYIKFLQNSNLGGI
201  IPTVNGKPVQITDDELFLYNTFQIFAPSQFLPTWVDILSVYTDIMKSIEKMQSDTQEANDIVTLANLQNGSTPDATFETKVTNIIDRLNNN
301  GIHNNKVCQLMRGLSEYKFLRYTRHRHLNMTVAELFDHIIAIYEEQQGRNRSKPYRRNPSEKNDRSYNTTTPKVIARNPQKTNNSKSKTARA
401  HNVSTSNNSPSTDNSISKSTTEPIQLNNKHDLHLRPETY
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1  MESQQLSQHSHISHGSACASVTSKEVHTQDPLDVSAKTEECEKASTKANSQQTTPASSAVPENPHHASPTAOSSHSPONGPYPOOCMMTONOANPSG
101  WSFYGHPSMIPYTPYQMSMYFPGPQSQPQPSVGVTPSTPSPESGNTFTDSSADSDMTSTKVIYRPPMLTSPDNFWKVTVIKFLQNSNLGGI
201  IPTVNGKPVQITDDELFTLYNFTQIFAPSQFLPTWVDIILSVDYTDIMKILSIEKMQSDTQEANDIVTLANLQYNSTPDAFETKVTNIIDRLNNN
301  GIHINNKVACQLMRGLSHEYKFRLYTRHRHLNMTVAELFDIIHAIYEEQQGSRNSKPNYRRNLSEKNDERSYTNTTKPKVIARNPKTNNSKSKTARA
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YJR028W

MAP

Vit

foldindex

hydropathy

net charge

PrD background

PrD score

1  MESQQLSQHSHISHGSACASVTSKEVHTNQDPLDVSASKTEECESKASTKANSSQQTTPASSAVPENPHHASPOTAOSHSPONGPYPOOCMMTONQANPSG
101 WSFYGHPSMIPYTPQMSMYFPPGQPSQFPOQPSVGTPLSTPSPEGNTFTDSSADSMTSTKKYVRRPPLTMIPDNPFWVKTYIKFLQSNLGGI
201 IPTVNGKPVQITDDELFLYNTQIFAPSQFLPTWVDILSVDYTDIMKILSKSIEKMQSDTQEANDIVTLANLQNGSTPADAETFKTNIIIDRLNNN
301 GIHNNKVAQCLIMRGLSEYKFLRYTRHRHLNMTVAELFDLHAIYEEQOQGSRNSKPYRRNPSDEKNSRSYTNTPKVIARNPQKTNNSKSKTARA
401 HNVSTNNSPSTDNDSISKSTTEPIQLNNKHDHELHPETY
1 MEQQLSQHSPISHGSACASVTSKEVTNQDPDLSASKTEECEKASTKANSQVTTPASSAVPENPHHASPOATOSHSPONGPYPOOOCMTONOANPSG
101 WSFYGHPSMIPYTPMQMPYFPYPQPSFQQIO:PSVGTPLSTPSPEGNTFTDSSSADSMTSTKYYRPPPLTSPNDTPNWVKTYIKFLQNSNLGGI
201 IPTVNGKPRQITDDELTFLYNTFQIFAPSQFLPTWVDILSVDYTDIMKILSKIESKMQSDTQEANDIVTLANLQYNGSTPAFETKVTNIIDRLNNN
301 GIHNNKVACQLMRGLSGEYKFLRHYTRHRHLNMTVAELFLDIAIYEEQQGSRNSKPNYRRNPSDEKNSRSYNTTTPKVIARNPQKTNNNSKSKTARA
401 HNVSTSNNSPSTDNDSISKSTTEPIQLNKHDLLHRPETY
FIP1

MAP
Vit

1 MSSSEDEDDKFLYGSDSELALPSKRSRDDEADAGASSNPDIVKRQKFDSVPVEETPATARDRSDESDIYSDSSDDSDSDELVIIISLGPDPTRLDAKLLD
101 SYSTAATSSKDVISVATDVSNTITKTSERLITEGEANQGVATTVKATESDGNVPKAMTGSIDLDKEGIFDSVGITTIDPEVLKEKPRQPGANLSDY
201 FNYGFNEFTWMEYLRQEKLOQDYNPRRILMGLLLSQCGLNSANDTDSNLGNTIDINNIVNLNMSNLNSNGNSMSGTPENPPAPPMPHFSEPFLPMFG
301 SFPPFPMPGMMPMNQFPONGONQNSK
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YMNL284C–A

**MAP**

**Vit**

- **net charge**
- **hydrophathy**
- **foldindex**
- **prd score**

**Foldindex**

**Net Charge**

**Hydropathy**

**Predicted prD Score**

1. MESQQLSQHSPISHGSACASVTSKEVHTNQDPLDVSASKIQEYDKASTKANSQQTTPASSAVPENPHASPOTAOSHSPONGPYQQOCMMTONOANPSG
2. WSFYGRPSMIPYTPQMSPMYFPGHSQFPQ: PSSVGTPLSTPSPEGNTFTDSSSADSDMTSTKKYVRPPMLTSPNDLNWVKTYIKFLQNSNLGDI
3. IPTATRKAHRQMDTDLDTFLCHTFQLFAPSSQFLPTWVKDSLADYTDIMKILSKSINKMQSDTQEVIDTITTLATHLHYNGSTFADEFAEVTNILDRLNNN
4. GIPINNKVACQFMRGLSHEYKFLRYARHYIHMTVADLSDIHSMYEEQESKRKSTYRRNPSDEKKDSRTYTNTTKPKSITRNSQKPNNSQSRTARA
5. HNVSTSNNSGPDLNDSLIRGSTTEPIOLKNKHDHLHLRPGTY
GAR1

MAP
Vit

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1 MNEIFGYGNKKSHDOLLOESNOSMQAOOQLSNBRISOLDTOIAO1NFQLONIOKNIQNSNKKOPSRLKQALKILNKRQLENMKDSLDSQWSMTQAQLT
101 NDNLQNTMITINALQTNNAQYQYGINIDKLQDMQDEMLDLIEQGDELQEVLAMNNNSGELDDISDAELDAELDALEAQLQEDFTLPSENLSLGNDFSYL
201 LGANAPPAFIDEPNLDTEDKNALESQA
SGV1

MAP
Vit

1  MSDGSPAVLPKTEFNKYKIGVKSTPAIQRDAKTNLTYIKLRKRSSEKVKYGCTVFQNVHREDEKLGQTFGEVYKGIHLETQRQVAMKKIIIVSVEKDLF
101 PITAQREITILKRLNKHNIKLIEMVYDHSPITNAASSNLHKSFLMILPYMVADLSGVLHNPRINLEMCDIKNMMLQ1LEGNYIHCAKFMRDIKTAN
201 LIDIDHNGVKLADFGLARLYGCPPNLKYPGGAGSGAKYTVVTVRIVYRPELVLGDKQYTTAVD1WGVGCVFEEFFEKPI1QLQGKTDIDQGHHVIFKLLG
301 TPTEEDWAVARYLPGAELTTTNYKPTLREPKGKYLSETGLDFLQDLLDYPYKRTAMSAKHHWFKEDPLPSEKILPTEESHEADIKRYKEEMHSLS
401 QRVPTAPRIGHEVKGESPVKNLGAIPRGPKDDASFLPPSNVLAKPPPSKIRELHONPRPYHVNSGYAKTAIPPPAAPAGVNRYGPNNSSRNNRFSGN
501 STAPNNSRNPVNRFHPETNVSSKYNKVPPLLCFSQRYQGNSNESRSRYKNSPDNSRSRYHNPRYVNPETNFNROPOKYSROESNAPIKNYNPSNGSRNMAKG
601 NHQGSKPSHPQFP1PSQGOQHGCLETKPIEKKNGSFKDERAKPDESEKFQNSDIADLY
DBP1

MAP

Vit

0 50 100 150 200 250 300 350 400 450 500 550 600

-1 0 1

background

PrD

net charge

hydropathy

foldindex

prd score

1 MADLPQVSNLSINNKEENGGGGKSSYVPPHLSRGKPSFERSTPKQEDKVTGDFRRAGRTQNGGFFGESKEFNGGTSAANYRGGSSNYKSSGNRWB
101 VNGKHIPFKNAKLEAELFGVHDDPDISSGIFKFDNYDNPVDAGSKDVPPEPLDFSSPPLDEMMENIKLASFTKPTPVQYSIPIVTGRDLMACAT
201 GSGKTGGFLFLFTELFRSGPSVPEKAQSFYSRKYPSALVAPLTRLATQPFEEARKFTYRSWVRPCVYGGAPIGNQMRREVDRGCDLLVATFGRRLND
301 LLLERGKVSLANIKYLVVLDEADRMGFEPOQIRHVEEDMPSVENVQTLMSATFVPVDIQLANDFLDNYIFLSVGRVGSTSENITQRILYVDMDKKS
401 ALLDLSAEHKGLTLIFVETKRMADQLTDFLIMQNFKATAHGVRTQAERERALSAFKANVDILVATAVAARGLDIPNVTHVNYDLPSDIIDDYVHRIG
501 RTGRAGNTGVATSFNSNNQNIVKGLMEILNEANQEVPTFLSDLRRONSRGGGTKGGGFNSRNNGSRYRKJHGNGSFSTRPRNTGTSNWGSIIGGF
601 RNDNEKNGYGNSNASW
YBL005W-A

1  MESQQLSQHSPHFHSACASVTSKEVQTQDDLIDASKTEECEKVSTQANSOAPTTPPSSAVPENHHHASPOAATVPLPGPYPOORMMTQOANISG
101 WPVYGHPSIMPYPPQMSMPYAPPGAQSOFTPQVYGTHLNTSOPEGNSFPDSSSANSNMTSTNQHRPPILTPNDFLWNVKIIKFLQNSNLGD
201 IPTATRAVRQMTDELFLCHTFLFAPLPSLPPWVDILSVDYTDIMKILSKSINKMQQSTDTEVNDTTTTLHYNGSTPADAFAEVTNLDRLNN
301 GIPINNKACQFIMRGLSGGEYKFLPYARHRCIHMTVADLFSDIHSMYEEQESKRNKSTYRRPSDEKDSRTYTNNTTKPSSITRSQKPNNSQSRTARA
401 HNVSTFNNSPGPNDLIRGSTTEPIQLKTHDLHLRPGTY
YMR045C

1  MESQQLSQHSPISHGSACASVTSKEVOTTQDPLDISASKTEECEKVSTQANSQOPTPLSSAVPENHHASPOAAQYPLPONGYPQPOFRMMTQOANISG
101 WPVYGHSPIMYPYYCQQSPMYAPPQGQSFQTYPQIVGTTHLTPSPESGNSFDSSAKSNMSTNQHVRPPPILTSPENDLWNVYIYIFLQNSNLGDI
201 IPTATRKVRQMTDDETFLCHTFQAPSFLQFPLFWKDILSVDYTDIMKILSKNKMQSDTEVNDITTALTLHNGSTPADFAEAVTNILDRLN
301 GIPINNKVACFIMRGLSKEYKFLRYARHRCIHMTVADLFSDIHSYEEQESKRNKHRRSPSDEKDSRTYNTTKPSTIRNSQPKNNSQRTARA
401 HNVSTFNNSPGPNDLIRGSTTEIQKLKTHDLHLQELTESTYNHTNHSDKLPGLHLLDSGASRTLRSAHIIHASASNSPDINVDQAKRNINPIAIG
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601 MLAHANATIRYSLKNNTITYFESDWDWSSAIDYQCPCDLCIGKSTHRHIKGSRKLYQNSYEPFQYHLTDIFGVHNLPKASAPSIFISFTETDKFRWY
701 YPLHDRREDSDLVTITILAFIKNQFQASVLVIQMDRSGEYTNRTLKFLEKNGITPCYTTADSRAHGVAELNRTLLDCCRTQCSGSLPNHLWFSAIR
801 EFSIVRNSLASPKSKSARQHAGLALDISTLLEPQGQPVHNPNSKIHPRGYAPHLPNRSPNSYGIIYLYLPSLKKTVDNTNYLQGKESRLQFN
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1001 KKRSTPQISDIESTGSNGMRDLVPLLAPMPSQNTSHKSSSKDFRSDDSYDNTNVHTNPISSTGTTNKTVPQSTEOETEKKIHRSPSIDTSS
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1201 MRSLEPPRSKKRLIAAVKASIKPFTTLRYDEATYNDKEKEKYIEAYHKEVNLKLMKTVTDKYYDRKREIDPKRINSMFLINRKRDTGHKA
1301 RFVARGDIQHPDYTEGMSQNTVHYALMTSLSLADVNYITQDISSAYLADIEKELYIRPPPLGMDKLRLLKKSGLQSGANWYETIKSYL
1401 YQSSKTEFQDCYFNDNTHCYDQYLUFQNNKVRDML KCYDVTKLW GDEPBRDQAM O SLWVOSCKKINH VINOTKDEQK NYD

MAP
Vit

net charge
hydrophathy
foldindex
PrD score
YMR046C

1  MESQQLSQHSPISHGSACASVTSKEVQTTODPLDISASKTEECEKVTQANSQQPTTPLSSAVPENHHHASPOAOQVPLPONGPYPOORMNTQOANISG
101 WPVYGHPSIMPPPYQMSPMYAPPQOSQFTOPYQVGTHLNTSPESGNSFFDSSSAKSNMTSTNQHRFPPILTSPNDLNWVKYIKJLQNSNLGDI
201 IPTATRAVRQMTDELTFLCHTFQLFASPQFLPPWVDILSVDYTDIMKILSKSINKMQSDDTEVNDITTLKATLIHYNGSTPADAFAEAVTNILDRLNNN
301 GIPINNVACQFIRMGLSJEYKFLRYARHRCIHMTVADLFSDIHSMYEEQQESKRNKSTHRRPSDEKDSRTYNTTPKSKITRNSQKPNNSQSRTARA
401 HNVSTFNNSPGPNDLIRGSTIPEIQLKNTDNLHLRPGTY
MSFTEQKEIALEILSKDKHEFYEILKVKATDSEI IKYAYKLAIKLHPDKNSHPKAGEAEFKVINRAFEVLSNEEKRIYDRIGRDPDDROMPSRGAAS
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\begin{verbatim}
1 MDVGSCSVGNNPLAQLHKHTQUNKLSQFNOKNNGLNESPLOGTNKPGISEAFISNVNAISQENMANMQRFINGEPLIDDKRMIEIGPSSGRLPPFNSVH
101 SLQTSANPTIQGVNISHWSSQSFOGSSN1ORNADTGNSEKAWRGSTTASSRFQYPNTMMNNYAYASMNSLSGRSLQSPAFMNOQOSGSRKGVNEQEC
201 COPWTDQFEKLEKEVSLENLDINJNEIKEENVSEVEQNKPETVEKEEGVYGDQYQSFQEVWDISHKDAEEVLPSELVNDNLGEDLYKLGGVRGNIE
301 YAFQSNYFNNPNAYKIGCLLMENQALSEAAAFEEAVKEKPHDVDAWLRLGLVQTQNEKELNGISALEECLKLDPKNLEAMKTLAISYINEGYDMSA
401 ETMLDKWAETKYPFESRIQKQDDKFQKEKGFTHIDMNAHITKQFLQLANNLSTIDP EIQLCLGLLFFYTKDDFDKTIDCFEASLRVNPNDELMWNLGAS
501 LANSNRSEAIQAYHRALQLKPFSVFRARYNLAVSSMNIGCFKEAAGYLLSVELSMEVNTNKKGDVGSSLLNTYNDTVEITLKRVFIAMNRRDLQEVKPG
601 MDLKRFKGFESF
\end{verbatim}
MUM2

1  MNYMAYDPOHSLETSENVLAFPHOOSEQOALYESGERNDARPGIMNLTGOASKMNNSMLPOGSSASPLTGOHSILNSTTNFMPPSMNTYNQVPOQA
101 SMRNLTNHNNIMNGATANDYWLDPMNMTNNKDTNGNPNDSSMSMNTKTSINFSTAFKNSFVPFHVNTALSNNVNSNEMNSNKDDRMEEWLEVELQIK
201 ESQIESLENEIQRKKIFNEGLNYKQNEHKYEKENCHIPQTFELPFASLEVIFRKLSSSLHACEKLAETKENLESILTAALNPTNSVTKYGRYDAESIA
301 HKMVVRLENLTENKEKMAKMLAYGRSKETIQIELQLAKKENLEREKIASLEAHLASKESSKEDVAN
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101 QRPPPATAEYNDVFNTNSQQLPSEHQYNVPSYPLPSINVIQTPPEIHNGSQTMAPIERPFFNENDYNNRNRSRSPIASSSDGYADOEARPILE
201 QPNMNSGNIPOYHDQPGYNNGYHGLQAKDYDDPEGGYIDQRGDDYQINSYLGRENEMVDPYENDRSLHTMERREYLDSSRPNVGKEELDSV
301 KSYSHRDGEYDDDRDEYDDLNTIDKLQFQQNGPVASSSSSIESDIIVSNDLTANKRSGTEIRFKLWNGFVDPSISKTLLDQYA
401 TTENANTLPNEFKFMRYAVCTEPNLAENFTVRQLYLTFTREFELMLVTVMYNEDHILLGRTLKGMNDVKYMVKKNSSTWGPDAWIKTVICIND
501 GRSKINERSLLASSLSCGYQDGFAQDEINEKVMHVEYHTTMITNISESEVESLECNQGTVIPQLLFCLKEQNQKINSHRWAFEGFALLRPNIVTL
601 LDAGTMGKQSIYQLWREFRNPVGGACGEIRTDGLKRFVKLNFLVSQNFYKMSNILDKTESNFGFIVLPQAFSAYRFEAVRQFGFKQFYEIM
701 ENEGFIFSSMNALEDR1LCFEVVTKNCWILKCRSYASTDPERSVPEFLQRRRVLNGSFASVYFCHFYRVWSGSHIGNRKLLTVEFFYLFF
801 NTLISWFLSSFFLFRLTVSIALAYHASFNVLSVFYLWYICLSTFLSLNGKPSTEKFYVLTCVFAVMMIYMIFCSIFMSVKSQFNILKDNDT
901 SFEGLITTEAFRDIVISLGSTCYLISISSLQYFHLMTSFIQYILLSPYINLNIYAFCHVDSWGTKAMANPLGKINTTEDGTKFMEVLVSSS
1001 IQANYDKYLKNDFDFFKSTPSERTPESYDEKKTGGYANVRSLVIFFWITNFIIAVVLETTGGIYAMKSIAMDSTDDLETAKAEIPLMTSKASFYNV
1101 ILWLVALSIRFIGCSYIMVRFFKKTFR
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MAP
Vit

0 50 100 150 200 250 300 350 400 450 500 550 600

-1 0 1

net charge
hydropathy
foldindex
prd score

1 MSVTPSDELAIQATSESIPNGDLDPIALEISDVLRSRRVPNKDSMRCIKKRILNTADNPNTQLSSWLTNICVKNGGTPFIKEICSREFMDTMEHV
101 ILREDSNEELSELVKTIYLLEYVAFKNDQLNYVAHVDKLISRGKIKFPEKLTLSNPTAMFSDKIPADWIDSDACMIESKKFSLLNRKHHCRCGTVFC
201 QEHSNSIPLPDLGIYEPVRCDSCFEDYLDKRRHDSKSSKHKHRKRRKKDRDYSTFEDEEELIRKAIELSLKESRNSASSEPIVPVVESKNEVRQIEEE
301 EEDPLKAAIQESLREAEAKLRERQKASRQMOPQNQSPQOPQHSDLSDDEEKSDYFAMSVEKMKSRPLNEILEDSKLQNLQRVFASKARLNYYAL
401 NDKAQKYNTLIEMNGKISEIMNIYDRLLEQOIQSNLSSOYTLPQPVPSDPNYLTVVQPAESYSQTPPLQLSSHOYKQPQDVRQOSVKANSSPTTI
501 DHLKTDVPHAQQKPSHVELAPSDPPYPKKEAEDGTQAVQDEESSSTQESRFPYVPVENGETSINKRPQGITRYDFPTVPARKFVQPESTVPLPAS
601 SSEIPKEERPPSPQELLIEL
1  MVKETKLYDLLGVPSANEQELKKGYRKAALKYHDPKPTGDTEKFKEISEAFELNDPQKREYDQYGLEAARSGGPSFGP7GGAGGAGGAGFP7GGAGGF
101 SGGMAPSNEDAFNI7FSQFFGGSSPFGGADDGFGSFSSYPSG7GAGMGGMPPGGMGGMHGGMMPGGRASSSTYPEETVQLNLPV5LEDLFVVGKK5
201 FKIGRKGPHGASEKTQIDIQ7LPWG77KGATKITY7QGDY7NPQTGRRRKLQFV7QEKSHP7NFKRDGDLI7LPLSFKESLLGF7SK7QTIDGRTL7LPLSR7
301 QPVQPSQTSTYPQGMPTPKNFSQRGLIVKYKVD7P4ISLNDA7QRAIDENF
YDR170W-A

1  MESQQLQSHPISHGSAVSATKEVQTQDPLDIAASKTEECKVFTQANSQOPTTPPSAAVPEEHHASPOAAQVPLPONGPYPQORMTPOQANISG
101 WPVYGHPSIMPYPQMQMPSMYAPGAQQFTQYQVGTHLNTPSPEGNSFDSSASAKSNMTSTNQHRPPPLTSPNDFLNWVYIKFLQNSNLDI
201 IPTATRKAVRQMDTDELFLCHTFQLFALSQPLPTWAVDILSVDYTDMKILSKSINKMQSDTQEVDINTTLANLHYNGLSTPADAFAEAVTNILDRKNN
301 GIPINNVACQFIHRGLSYEKFRLYARHRYIHMTVADLFSDIHSMYEEQQESTRKNSTYRRSPSDEKDSRTYNTTKPSTIRNQKPNNSQSRTARA
401 HNVSTSNFPGDPDLRSTSTTIQLKHDLHLRPGTY
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TFRKiKGDKKN
HMS1

MAP
Vit

0 50 100 150 200 250 300 350 400

background
PrD

net charge
hydropathy
fold index
prd score

1 MPNFQKFSGSSDGNSVMNDLGKVAIKVFDCRSAQDGSEEQNVNVTNQMYLMFQSNNYNVPNPYNTEDLGSQGPPTHAYYAPFQHPHLQPVPPVY
101 KNNTYATDQYSDDSSFPNSHGTPVIDSNYNDALASIPTTTTGSTMINTDNGNTIDSEYIDNMEVSSEENENIDNVKQTDLKSSLLSAASIVK
201 KEQLSFENFLPLSKTESPLVTADIEKSSLNLENIDNASMSFKLKTSPIRKHFHVPKKRITVRGRVSHNIEKKYRSNINDKIEQOLLRTVPILRVAY
301 KKCNDLPITSRDLADLDGLEPATKLNASILTKSIEYICHLERKCLQQLSLANQHLSNDTDRDSFVHLTEFSQPLSDNNSSEQVOQOKTRSCORORORORQO
401 OPLHNIQYNIPHONGLMSGTNNSHDMDFNNAGDF
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1  EASEQYRAIAELQETLKPLALVEKIVTPCQELLLKIITYIKMATKRNMHKDLDRHLNTYNKHEKKKEPTAKDEERLYKAQAQVEVAQQEYDYNDL
101 LKTQLPILSFLEAFVKPLFSIFYFMQLNIFYTNRLQDMKIPYFDLNSDIVESYIAKKNVEEQTDLTIHFKLGYSKAKLEMTRRKYGVATAGSP
201 VSGASSGAGYDPAATATSTPTGYYGAQKHAAQAQPAAYQTAAGTAAAVGTAAGAAGAQVPGTYFQYAAPQPSPPLTGLGQOQPQQQGPEPA
301 YSNPLTSPVATPAAVAAAPGVETVTLADYQAAGDLSFPAGAVIEIVQRTPDVNEWWTGRYNGQQGVFPQNYVQLKNK
401
Vit

Net charge
Hydropathy
Fold index
PrD score

1  MSSSSLRVLIAIGNPNILFYTSRFQLAKNIDTHVNDSKSCQFEIETEYYKDRFELENHTSIEHLTEALSSKSEAVFDIIMSAPLQELSSLASKL
101 TSIIDSNTKIFLESSGFIQLEPFVKLSMESPHVNFVSILDLDIRQIGPNHKHFPSAKENTIYLGESKSSTEKYSSGVITLLTTFEKLFKLSNIK
201 NLCNFSSIEFLSQWKLAISRICFDPLLIMEQENPSDLQQIIAKPLISGLVTEIITVAKTMGARLNSHDNENLLSWKNSYHSTNKPPALVYHF
301 QTTPLNIDILLQTILLADDGFKTPYLEFLYSVLSQFRLNSGKSKFWIRSDEKTQILQQLQSOKNESALQITSLQQGIIKLRQELLMQAKQM
401 TNELEKHOVALKAQAOAQAQSAQATSIEALTPTEATQSĐTNEYKTAGTQLRDEMDALYSVYNBSPVRSPPPPPVSSQIQMNSPSLSSQTFGEN
501 NGTNDKLLQERELQLRKKELELINQERELEFKRALVQOREFNNNSNNSIPPPKKEPTQLQOSANVRSNSRGMHTNGAMSQPAASAGNFVDPIS
601 SPSPLQQPOSVO____PFHSHSIKPTSRKRNNSNPNIGNPSSINMSDFGRIPPNSSQOTRLNSMTPHSSIVNONRLRSSQSSKKNLMPHATNPNN
701 PSNNHVPQFRQFSSTMTIEVTNNNNKVNNSSNPDISTNSVVHANMQFTNTNNNTSTVDINDPKNIAPPPTTSVASSTPTLSSSQMANMASPSTDN
801 GDNEEKNGKKRFGFLKKNKSKK
MSASKEIAALIVNYSSIVEKKEISEDGAESLNVAMDCISEAFGFERAEVSGLGKFQHLADILNSASRVPESNNKDDAEVEINIPEDDAETKA
KAEDLKMQGNKAMANKDYELAINKYTEAIKVLTNAIYAYNRAAHAHSLKEYDQAVKDAEAISIDPSYFRGYSRLGFAKYAQGKPEEAEYKWELIDIE
GDNATEAMKDYESAUKKEQSLNLKTVPEQSRADVDA
SOGASAGGLPDLGSLLCGLGLMLMNPOLMQAQKMSNFGAMQNIQKMMQDESTQVAE
GFASGGTPNLSIIMNNPAIRNMGNLFGGAGAOSTDEPDNENKQ
MAELSEQQNLSSINDNENGYVPPLRKGKPRSRARNSNESSNNNGGGYNNGGRGGSFSSNRRGGYNGGGFNGGNGGSNGRSNGRSGRWIDGKHVPAPRNE
KAEIAIFGPEDPNFQSGGNGDNYDDIPVDASGDVPDPITEFTSPPLDLLENIKLARFTKPTVPQKYSVPIVANGRDLMACAQTGSKGTGFLFPV
LSESFKTPQPSQGFSFQRYKPYTAVIMAPTRELATQIFDEAKKFTYRSVKACVYYGGSPIGNQLREIERGCDLVLATPGRLNLDEERGKISLANV
KYLVLEDADRMLDMGFEPQIRHIVEDCDMPVGERTQLMFSAITFPADIQHLARDFLSYIFLSVGRVGSNITQKVLYVENQDKSAALDLSSASTD
GL
LTILFVETKRMADQLTLDFIMQNFRTAIHDRTQSERERALAAFRSGAATLVLVATAVAARGLDIHPNVTHVINYDLPSDVDDYVHRIGRTGRANGNTGLAT
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SSWW
YMR002W

MAP
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0 50 100 150

-1 0 1

background
PrD

net charge
hydropathy
foldindex
prd score

1  MARSRGSSRPISRSRPQTRSAStMAAPVHPQQQQQPNAYSHPPAAAGAOTQPFGMFAQMASTAAGVAVGSTIGHTLGAGITGMFSGSGSDAPVEQQON
101  MANTSGQOTQDQIGRTCEIDARNFTRCLDENNGNFQICDYYLOQLKACQAASQY
Gene-specific oligonucleotides for cPrD amplification

**GenID**  | **GeneName**  | **Forward primer**  | **Reverse primer**
---|---|---|---
YBR289W | SNAP1 | AGGAGATAACAAAATGTCTCAATTTGCTGCCAAGCAGCG | AGGAGATAACAAAATGTGTCAACAGCAGCCGCAGC
YCR44C | SMY3 | AGGAGATAACAAAATGTCTCAAAATGTTCCCAACCAACAATATCCTAAGATG | AGGAGATAACAAAATGTGTCAATAATTTTCTAATACCTCTATAAATGACAACGACTC
YDR170W | YPR013C | AGGAGATAACAAAATGTCTGGGTTTTCCAACAACAACAATAAACAGTAC | AGGAGATAACAAAATGTCTCAGCCGTTGAATTATCAGGATCAATATCAAC
YK143C | BAI2 | AGGAGATAACAAAATGTCTCAAGTTCAAATGCAACTAAGGCAGG | AGGAGATAACAAAATGTGTCAGGCGCAATTTACGAACCAATC
YML251C | YMR260C | AGGAGATAACAAAATGTCTCAACAACATAAATAATAATATCAATAGCACCAAGAACGG | AGGAGATAACAAAATGTCTAACAACATAAATAATAATATCAATAGCACCAAGAACGG
YOL051C | YDL032C | AGGAGATAACAAAATGTCTAACAACATAAATAATAATATCAATAGCACCAAGAACGG | AGGAGATAACAAAATGTCTAACAACATAAATAATAATATCAATAGCACCAAGAACGG
YOR392W | YNL016W | AGGAGATAACAAAATGTGTAACCAAAACCTCAATCCGAAACAAATACAAAG | AGGAGATAACAAAATGTGTCAGCAGCAATACGCCATGG
YFL024C | YMR173W | AGGAGATAACAAAATGTCTCAATCACCAGCACAGCCCC | AGGAGATAACAAAATGTCTCAATCACCAGCACAGCCCC
YMR201W | YMR011W | AGGAGATAACAAAATGTCTGAATAATCAGCCGCAGGGTAC | AGGAGATAACAAAATGTCTGAATAATCAGCCGCAGGGTAC
YGR206 | YEL059C | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG
YOR094C | YEL021W | AGGAGATAACAAAATGCTTAACTACTTCAACAATCAACAAGTGTTG | AGGAGATAACAAAATGCTTAACTACTTCAACAATCAACAAGTGTTG
YMR024C | YMR256C | AGGAGATAACAAAATGGTGGTTTCTTATAACAATAATAATAACAATAACAATAAC | AGGAGATAACAAAATGGTGGTTTCTTATAACAATAATAATAACAATAACAATAAC
YMR019W | YMR307W | AGGAGATAACAAAATGGTGGTTTCTTATAACAATAATAATAACAATAACAATAAC | AGGAGATAACAAAATGGTGGTTTCTTATAACAATAATAATAACAATAACAATAAC
YDR106C | YMR140W | AGGAGATAACAAAATGTCTCTGTGATGAGGTTGAAAGTGTGCGGC | AGGAGATAACAAAATGTCTCTGTGATGAGGTTGAAAGTGTGCGGC
YDR381W | YMR147W | AGGAGATAACAAAATGCACTCTTCCTCCCTTCAACATGACCATGCAAC | AGGAGATAACAAAATGCACTCTTCCTCCCTTCAACATGACCATGCAAC
YKL088C | YMR070W | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG
YML050C | YML050C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YMR115C | YDR008W | AGGAGATAACAAAATGCTTAACTACTTCAACAATCAACAAGTGTTG | AGGAGATAACAAAATGCTTAACTACTTCAACAATCAACAAGTGTTG
YGR253C | YFL002C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YMR062C | YDR248C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR106C | YMR140W | AGGAGATAACAAAATGCACTCTTCCTCCCTTCAACATGACCATGCAAC | AGGAGATAACAAAATGCACTCTTCCTCCCTTCAACATGACCATGCAAC
YDL035C | YMR147W | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG | AGGAGATAACAAAATGTTTGGCAACAATAGACCAATGTTTG
YMR320C | YMR320C | AGGAGATAACAAAATGAAATGCGGACCATCACCTG | AGGAGATAACAAAATGAAATGCGGACCATCACCTG
YMR147W | YMR147W | AGGAGATAACAAAATGAAATGCGGACCATCACCTG | AGGAGATAACAAAATGAAATGCGGACCATCACCTG
YPL184C | YMR173W | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YOR094C | YMR173W | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YOR215W | YOR215W | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR022C | YDR022C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YHR111C | YHR111C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YRP042C | YRP042C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR131C | YDR131C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YOR111C | YOR111C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR131C | YDR131C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YOR111C | YOR111C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR131C | YDR131C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YOR111C | YOR111C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
YDR131C | YDR131C | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC | AGGAGATAACAAAATGTCTTCTGGAAATATGGGTGCGTTCC
067Nde1WF
CTTGTAACAAGTGTTGAGAAC CATATG TGG CATCATACACCACCCACCATTAAG
Forward primer for addition of Nde1 site to pRH1

067Nde1WR
CTTAATGGTGGTGATGGTGATGATGCCACATATGGTTATCAACCACTTTGTAC
Reverse primer for addition of Nde1 site to pRH1

Nde1Mf
GATACA CATATG TCTTTGAACGACTTTCAAAAG
Forward primer for insertion of Sup35M at Nde1

068delNde1for
GATACAATGGTGGTGGATGATGCCACATATGGATTACCACTTTGTAC
Reverse primer for insertion of Sup35M at Nde1

068delNde1rev
GATACAATGGTGGTGGATGATGCCACATATGGATTACCACTTTGTAC
Reverse primer for insertion of Sup35M at Nde1

068delNde1for2
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Reverse primer for insertion of Sup35M at Nde1

068delNde1rev2
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Reverse primer for insertion of Sup35M at Nde1

068delNde1for
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Reverse primer for insertion of Sup35M at Nde1

068delNde1rev
GATACAATGGTGGTGGATGATGCCACATATGGATTACCACTTTGTAC
Reverse primer for insertion of Sup35M at Nde1

dest1NdelNde1for
GACGAAGTTGTTAACGATCGTACTACCATCACC
Forward primer for removal of 5' Nde1 site from pRH2

dest1NdelNde1rev
GACGAAGTTGTTAACGATCGTACTACCATCACC
Forward primer for removal of 5' Nde1 site from pRH2

3dan1p-ura3
TTTTTACATTTAAAGTGGTACATGTTACATTTATTTAACTTTAAAAGTGTAGCATACTAAATATATACCCCAAGTA ATGTCGAAAGCTACATATAAGGAAC
Forward primer for removal of 3' Nde1 site from pRH2

3dan1p-ura3
TTTTTACATTTAAAGTGGTACATGTTACATTTATTTAACTTTAAAAGTGTAGCATACTAAATATATACCCCAAGTA ATGTCGAAAGCTACATATAAGGAAC
Forward primer for removal of 3' Nde1 site from pRH2

dan1upseq
AAGTTTCATGTTTCCTGCGC
Used with URA3-1-3 to confirm Ura3-His5 integration at DAN1

URA3-1-3
GCGGCTTAACTGTCGCCCTCC
Used with dan1upseq to confirm Ura3-His5 integration at DAN1
### Yeast strains generated

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
<th>Construction</th>
</tr>
</thead>
<tbody>
<tr>
<td>YRS099</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; ura3-1; ade1-14; can1-100</td>
<td>Osherovich et al., 2004</td>
</tr>
<tr>
<td>YRS100</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-SLT2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS101</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-PDR1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
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<tr>
<td>YRS102</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-RGT1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS103</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-NUP49PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS104</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-SNF2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS105</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-SNK1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS106</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-CBX1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS107</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-SUP35PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS108</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-PUL2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS109</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-PAP2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS110</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-PUP2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS111</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-PUP3PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS112</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-RLM1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS113</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-YAP1801PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS114</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-MED2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS115</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-GTS1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS116</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-UPC2PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS117</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-TAF12PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS118</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-MCA1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
<tr>
<td>YRS119</td>
<td>MATa, leu2-3,112; his3-11,15; trp1-1; can1-100; [psi-]; [PIN+]; sup35::KanMX4; pAG415ADH-NEW1PrD-SUP35C</td>
<td>plasmid shuffle using YRS100</td>
</tr>
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MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-NM-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415SUP-NM-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-NMR022CPrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-SWI1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-NRP1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-PUF2PrD-SUP35C

Δhis3

Δα

ΔΔ

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; KSP1::Cerulean-HphMX4; pAG415ADH-KSP1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; PGD1::Cerulean-HphMX4; pAG415ADH-PGD1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-GLN3PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-SIN3PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-JSN1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-SSD1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-AZF1PrD-SUP35C

MATα, leu2-3,112; his3-11,-15; trp1-1; ura3-1; ade1-14; can1-100; [ψ-]; [PIN+]; sup35::KanMX4; pAG415ADH-SLA2PrD-SUP35C

ψ-  

PSI+

ψ-

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

CBK1PrD-C+

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