Genetic and epigenetic control of the efficiency and fidelity of cross-species prion transmission

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SUPPORTING INFORMATION

Supplemental materials and methods

De novo [*PSI*+] induction

De novo induction of [*PSI*+] by transient overproduction of Sup35 or Sup35N was achieved in the *S. cerevisiae* [*psi*-] cells bearing another QN-rich prion, [*PIN*+] (see Chernoff *et al*., 2002). We have previously shown that *de novo* induction by protein overproduction is observed even in the combinations of very distantly related inducer and inducee Sup35 proteins (Chen *et al.*, 2007; Chernoff *et al.*, 2000). To induce [*PSI*+], yeast [*psi*-] cultures bearing the chimeric or mutant *SUP35* constructs (see Table S1) were transformed with plasmids containing either the same construct (in most cases), or

SUP35 / *SUP35N* from one of the parental species under the galactose inducible promoter (P_{GAL}). Resulting transformants were grown on galactose media selective for the plasmid and were then transferred to glucose containing medium lacking adenine in order to detect newly induced [PSI^+] isolates. To confirm the prion nature of the Ade^+ colonies, they were checked for curability in the presence of guanidine hydrochloride (see Chernoff *et al.*, 2002). Each chimeric construct was induced into a [PSI^+] state by overproduction of either identical construct, or at least one of the parental constructs (data not shown).

Plasmids expressing *S. paradoxus*, *S. bayanus* or chimeric *SUP35* genes under the control of galactose-inducible (P_{GAL}) promoter were constructed by cutting respective *SUP35* ORF from a shuttle vector constructed as described below on Fig. S1, or in our previous paper (Chen *et al.*, 2007) and inserting it into a yeast expression vector next to P_{GAL} promoter, followed by moving the resulting P_{GAL}-*SUP35* cassette to the centromeric *TRP1* vector pFL39 (Bonneaud *et al.*, 1991) in case of *S. paradoxus* and *S. bayanus* *SUP35*, or the centromeric *LEU2* vector pRS315 (Sikorski & Hieter, 1989) in all other cases. Yeast plasmid pFL39GAL-SUP35N expressing *S. cerevisiae SUP35N* from the galactose-inducible (P_{GAL}) promoter was described earlier (Borchsenius *et al.*, 2001).

Details of protein analysis

Protein isolation from yeast and centrifugation analysis at 39 000 *g* were in accordance with the previously published protocol (Chen *et al.,* 2007) using the

centrifuge TL-100 (Beckman Coulter, Fullerton, CA), except that one tablet of Roche (Mannheim, Germany) Complete proteinase inhibitor mixture and 1 mM N-ethylmaleimide were added to the lysis buffer to inhibit proteolysis.

The "boiled gel" analysis followed the published protocol (Kushnirov *et al*., 2006). Specifically, protein samples prepared as described were mixed with SDS-containing sample buffer up to a final concentration of 2% SDS, and run on a 10% polyacrylamide gel (without a stacking gel) until the bromophenol blue dye reached the half-way point (usually 1 hr). Then electrophoresis was stopped, and wells of the gel were filled with acrylamide. After the newly added acrylamide was allowed to solidify, the gel, in a plastic bag, was placed into the boiling water bath for about 10 minutes. Due to boiling of the gel, SDS-resistant polymers that initially accumulated in the wells were now destroyed by boiling and capable of entering the gel. After cooling the gel, electrophoresis was resumed and continued until the dye front had left the gel, followed by Western blotting and reaction to antibodies as described below.

Western blotting and reaction to antibodies

Following electrophoresis, protein samples were transferred to Hybond ECL (GE Healthcare) membrane using the SD Semi-Dry Transfer Cell (Bio-Rad), at 200 mAmps for 60 min in transfer solution containing 20% methanol and 10mM CAPs, pH 11.0). Resulting blots were blocked with 5% milk in TBS-Tween, and reacted to the primary antibody, specific to Sup35C (a gift from D. Bedwell, University of Alabama at Birmingham, Birmingham, AL), followed by reaction to the secondary anti-rabbit HRP conjugate, for 1 hr. Detection was performed according to Amersham ECL Western Blotting Detection Reagents (GE Healthcare).

Table S1. Plasmids used in this study

Plasmid name	Yeast marker	Promoter	Origin of *SUP35* domains					References
			SUP35N			*SUP35M*	*SUP35C*	
			Module I	Module II	Module III			
pBC105	*URA3*	P_{SUP35}	*Sc*	*Sp*	*Sp*	*Sc*	*Sc*	This study
pBC107	*URA3*	P_{SUP35}	*Sb*	*Sc*	*Sc*	*Sc*	*Sc*	This study
pBC109	*URA3*	P_{SUP35}	*Sp*	*Sc*	*Sc*	*Sc*	*Sc*	This study
pBC110	*URA3*	P_{SUP35}	*Sc*	*Sb*	*Sb*	*Sc*	*Sc*	This study
pBC111	*URA3*	P_{SUP35}	*Sc*	*Sb+1*	*Sb*	*Sc*	*Sc*	This study
pKB102	*URA3*	P_{SUP35}	*Sc*	*Sb+1*	*Sc*	*Sc*	*Sc*	This study
pBC112	*URA3*	P_{SUP35}	*Sc*	*Sc*	*Sb*	*Sc*	*Sc*	This study
pBC113	*URA3*	P_{SUP35}	*Sp(S12N)*	*Sc*	*Sc*	*Sc*	*Sc*	This study
pBC114	*URA3*	P_{SUP35}	*Sc(N12S)*	*Sc*	*Sc*	*Sc*	*Sc*	This study
pBC106	*URA3*	P_{SUP35}	*Sp*	*Sb*	*Sb*	*Sc*	*Sc*	This study
pBC108	*URA3*	P_{SUP35}	*Sb*	*Sp*	*Sp*	*Sc*	*Sc*	This study
pBC103[1]	*URA3*	P_{SUP35}	*Sp*	*Sp*	*Sp*	*Sc*	*Sc*	Chen *et al*., 2007
pBC104[2]	*URA3*	P_{SUP35}	*Sb*	*Sb*	*Sb*	*Sc*	*Sc*	Chen *et al*., 2007
pBC102[3]	*URA3*	P_{SUP35}	*Sc*	*Sc*	*Sc*	*Sc*	*Sc*	Chen *et al.*, 2007
pGN100	*URA3*	P_{SUP35}	*Sc*	*Sb(P50Y)*	*Sb*	*Sc*	*Sc*	This study
pGN102	*URA3*	P_{SUP35}	*Sc*	*Sc(Y49P)*	*Sc*	*Sc*	*Sc*	This study
pGN103	*URA3*	P_{SUP35}	*Sb*	*Sb(P50Y)*	*Sb*	*Sc*	*Sc*	This study
pKB103	*URA3*	P_{SUP35}	*Sb*	*Sc*	*Sb*	*Sc*	*Sc*	This study
pKB100	*URA3*	P_{SUP35}	*Sc*	*Sb*	*Sc*	*Sc*	*Sc*	This study
pKB101	*URA3*	P_{SUP35}	*Sp(S12N)*	*Sp*	*Sp*	*Sc*	*Sc*	This study
pBC100[4]	*URA3*	P_{SUP35}	*Sp*	*Sp*	*Sp*	*Sp*	*Sp*	Chen *et al*., 2007
pBC101[5]	*URA3*	P_{SUP35}	*Sb*	*Sb*	*Sb*	*Sb*	*Sb*	Chen *et al*., 2007
pASB2	*LEU2*	P_{SUP35}	*Sc*	*Sc*	*Sc*	*Sc*	*Sc*	Borshsenius *et al*., 2001
pmCUP1MCSC	*URA3*	P_{CUP1}	---	---	---	*Sc*	*Sc*	Chen *et al*., 2007

[1] Also called p316-PS-SUP35NSP-MCSC
[2] Also called p316-PS-SUP35NSB-MCSC
[3] Also called p316-PS-SUP35NSC-MCSC
[4] Also called p316-PS-SUP35SP
[5] Also called p316-PS-SUP35SB

Primers used for constructing the plasmids are listed in Table S2.

Table S2. Primers used in this study

Name	Sequence	Usage
SUP35-PAR-F	5'-TATC GGATCCCTAGCAACAAT GTCGGATTCA-3'	Forward primer for module I of *Sc*, contains *Bam*HI site
SP-S12N-R	5' CACGGCCACCTTGTGGATTGA 3'	Reverse primer for module II *Sb* and includes the *Pfl*MI site
SB-Insertion-R	5'-TACCACGGCCACCTTGTGGGTTGA ATTGCTGTTGGTAACCGCCTTGAGGATTGTA CTGTTGATAGCCGCCTTGAGCGTTGTATTGT TGTTGGTAACCTGCTTCCGGG-3'	Reverse primer for module II *Sb* which adds 1 OR and includes the *Pfl*MI site
SP-S12N-F#2	5'-TATC GGATCCCTAGCAACAATGTC GG ATTCAAACCAAGGTAACAATCAGCAAAACTA CCAGCAATACGGCCAAAACTCT-3'	Forward primer for module I *Sp* which includes the mutation S12N and *Bam*HI site
NSC-R-BglII-SacI	5'-AGTC GAGCTCAGATCTACCTTGAGAC TGTGGTTGGA-3'	Reverse primer for module III *Sc* which includes the *Bgl*II and *Sac*I site
NSC-MCSC(N12S)	5'-AGCAGGATCCCTAGCAACAATGTC GGATTCAAACCAAGGCAACAATCAGCAAAG CTACCAGCAATACAGCCAGAA-3'	Forward primer for module I *Sc* which includes the N12S mutation and *Bam*HI site
NSB(P50Y)-F	5'- TATC AAGCTTACAATGCTCAAGCCCAACAAC CTGCAGGTGGCTATTACCAAAACTACCAAG GTTACGCTGGCTACCAACA-3'	Forward primer for module I *Sc* which includes the P50Y mutation and *Hind*III site
Sup35Rev 517	5'-CTTCCTCTTTCTTATCAG-3'	Reverse primer for module III *Sc* and *Sb*
NSC(Y49P)-F	5'-TATCAAGCTTACAATGCTCAAGCCC AACCTGCAGGTGGGTACTACCAAAATC CCCAAGGTTATTCTGGGTACCAACA-3'	Forward primer for module I *Sc* which includes the Y49P mutation and *Hind*III site

Restriction sites are underlined

Table S3. Results of direct shuffle for the strong [*PSI*$^+$] strain

PrD shuffled in	Shuffle results		
	[*PSI*$^+$] (%)	Standardized error %	Total number
Sc-Sc-Sc	196 (99%)	(0.7)	198
Sp-Sp-Sp	95 (93%)	(2.5)	102
Sp-Sc-Sc	75 (88%)	(3.5)	85
Sc-Sp-Sp	52 (95%)	(3.1)	55
Sb-Sb-Sb	15 (12%)	(2.8)	130
Sb-Sc-Sc	83 (98%)	(1.7)	85
Sc-Sb-Sb	3 (4%)	(2.4)	67
Sc-Sb-Sc	1 (3%)	(3.0)	33
Sb-Sc-Sb	35 (100%)	(1.7)	35
Sc-Sc-Sb	77 (100%)	(1.1)	77
Sc-Sb+1-Sb	4 (11%)	(5.1)	37
Sc-Sb+1-Sc	15 (45%)	(8.7)	33
Sb-Sp-Sp	34 (89%)	(5.1)	38
Sp-Sb-Sb	3 (6%)	(3.4)	49
Sp(S12N)-Sp-Sp	63 (98%)	(1.6)	64
Sp(S12N)-Sc-Sc	71 (99%)	(1.4)	72
Sc(N12S)-Sc-Sc	33 (57%)	(6.5)	58
Sb-Sb(P50Y)-Sb	13 (65%)	(10.7)	20
Sc-Sb(P50Y)-Sb	7 (30%)	(9.6)	23
Sc-Sc(Y49P)-Sc	21 (75%)	(8.2)	28

Direct shuffle was performed in the strain GT256-23C as shown on Fig. 1E.
PrD stands for prion domain.

Table S4. Results of cytoduction for the strong [*PSI*$^+$] strain

PrD for [*psi*$^-$] recipient strain	Cytoduction results	
	[*PSI*$^+$] (%)	Total numbers
Sc-Sc-Sc	96 (99%)	97
Sp-Sp-Sp	79 (80%)	99
Sp-Sc-Sc	48 (81%)	59
Sc-Sp-Sp	51 (94%)	54
Sb-Sb-Sb	4 (5%)	83
Sb-Sc-Sc	48 (81%)	59
Sc-Sb-Sb	0 (0%)	68
Sc-Sb-Sc	2 (6%)	32
Sc-Sb+1-Sb	0 (0%)	58
Sb-Sp-Sp	51 (59%)	87
Sp-Sb-Sb	8 (9%)	86

Strain GT256-23C was used as a donor for cytoduction, performed as shown in Fig. 1F.

Table S5. Results of direct shuffle for the weak [PSI^+] strain

PrD shuffled in	Shuffle results		
	[PSI^+] (%)	Standardized error %	Total number
Sc-Sc-Sc	100 (92%)	(2.6)	109
Sp-Sp-Sp	16 (27%)	(5.7)	60
Sp-Sc-Sc	26 (39%)	(6.0)	67
Sc-Sp-Sp	30 (86%)	(5.9)	35
Sb-Sb-Sb	24 (26%)	(4.6)	92
Sb-Sc-Sc	39 (83%)	(5.5)	47
Sc-Sb-Sb	10 (15%)	(4.5)	65
Sc-Sb-Sc	1 (2%)	(2.5)	64
Sb-Sc-Sb	12 (67%)	(11.1)	18
Sc-Sc-Sb	22 (88%)	(6.5)	25
Sc-Sb+1-Sb	24 (51%)	(7.3)	47
Sc-Sb+1-Sc	21 (58%)	(8.2)	36
Sb-Sp-Sp	19 (100%)	(2.3)	19
Sp-Sb-Sb	0 (0%)	(1.6)	37
Sp(S12N)-Sp-Sp	26 (58%)	(7.4)	45
Sp(S12N)-Sc-Sc	52 (76%)	(5.2)	68
Sc(N12S)-Sc-Sc	76 (70%)	(4.4)	109
Sb-Sb(P50Y)-Sb	20 (48%)	(7.7)	42
Sc-Sb(P50Y)-Sb	28 (80%)	(6.8)	35
Sc-Sc(Y49P)-Sc	35 (95%)	(3.7)	37

Direct shuffle was performed in the strain GT988-1A as shown on Fig. 1E.

Table S6. Results of cytoduction for the weak [*PSI*$^+$] strain

PrD for [*psi*$^-$] recipient strain	Cytoduction results	
	[*PSI*$^+$] (%)	Total numbers
Sc-Sc-Sc	87 (68%)	128
Sp-Sp-Sp	8 (10%)	81
Sp-Sc-Sc	18 (21%)	87
Sc-Sp-Sp	79 (93%)	85
Sb-Sb-Sb	48 (60%)	80
Sb-Sp-Sp	88 (79%)	111
Sp-Sb-Sb	2 (2%)	92

GT988-1A was used as a donor strain for cytoduction, performed as shown on Fig. 1F.

Table S7. Results of reverse shuffle for the strong [*PSI*$^+$] strain

PrD shuffled out	Reverse shuffle results			
	Number of isolates tested	[*PSI*$^+$] (%)	Standardized error (%)	Number tested
Sc-Sc-Sc	5	174 (100%)	(0.8)	174
Sp-Sp-Sp	4	56 (100%)	(1.3)	56
Sp-Sc-Sc	4	39 (87%)	(5.1)	45
Sc-Sp-Sp	4	56 (100%)	(1.3)	56
Sb-Sb-Sb	4	76 (45%)	(3.8)	170
Sb-Sc-Sc	2	130 (100%)	(0.9)	130
Sc-Sb-Sb	3	9 (56%)	(12.4)	16
Sc-Sb-Sc	1	16 (76%)	(9.3)	21
Sc-Sc-Sb	3	63 (98%)	(1.6)	64
Sp(S12N)-Sp-Sp	4	50 (96%)	(2.7)	52
Sp(S12N)-Sc-Sc	3	42 (100%)	(1.5)	42
Sc(N12S)-Sc-Sc	4	36 (82%)	(0.6)	44
Sb-Sb(P50Y)-Sb	2	7 (13%)	(4.5)	55
Sc-Sc(Y49P)-Sc	2	49 (98%)	(2.0)	50

[*PSI*$^+$] isolates obtained by direct shuffle (Fig. 1E and Table S3) were used in the reverse shuffle to *S. cerevisiae SUP35*. Several independent isolates were analyzed per each construct in most cases, as indicated. Total numbers are shown.

Table S8. Results of the reverse shuffle for the weak [*PSI*$^+$] strain

PrD shuffled out	Reverse shuffle results			
	Number of isolates tested	[*PSI*$^+$] (%)	Standardized error %	Number tested
Sc-Sc-Sc	2	30 (100%)	(1.8)	30
Sp-Sp-Sp	3	31 (100%)	(1.8)	31
Sp-Sc-Sc	1	30 (100%)	(1.8)	30
Sb-Sb-Sb	1	8 (73%)	(13.4)	11
Sc-Sc-Sb	1	11 (100%)	(3.0)	11
Sp(S12N)-Sp-Sp	3	17 (89%)	(7.2)	19
Sp(S12N)-Sc-Sc	2	47 (98%)	(2.1)	48
Sc(N12S)-Sc-Sc	3	29 (85%)	(6.1)	34

[*PSI*$^+$] isolates obtained by direct shuffle (Fig. 1E and Table S5) were used in the reverse shuffle to *S. cerevisiae SUP35*. Several independent isolates were analyzed per each construct in most cases, as indicated. Total numbers are shown.

Table S9. Mitotic stability of the representative Sup35 prion isolates generated by control and chimeric Sup35 proteins

S. cerevisiae strain background	PrD modules (I-II-III)	Number of isolates	% [*psi*$^-$]	Number of colonies per isolate
Strong	Sc-Sc-Sc	3	0	50-90
	Sp-Sp-Sp	4	0	33-98
	Sp-Sc-Sc	3	0	32-78
	Sc-Sp-Sp	2	0	50-80
	Sb-Sb-Sb	2	0	44-50
		1	17.9	39
		1	56.7	60
	Sb-Sp-Sp	3	0	35-64
		1	35.0	40
Weak	Sc-Sc-Sc	3	0	89-106
		1	0.6	161
	Sp-Sp-Sp	1	0	75
		2	1.4-1.9	53-69
		1	80.0	50
	Sp-Sc-Sc	1	0	50
	Sb-Sb-Sb	1	0	98
		1	68.8	32
		1	90	50
	Sb-Sp-Sp	2	1.2-3.7	54-81

Independent [*PSI*$^+$] isolates obtained from direct shuffle (see Figs. 1E, S3, and S5) in either a strong or weak *S. cerevisiae* [*PSI*$^+$] strain background were incubated for 3 passages (from 20 to 40 cell generations) on YPD, streaked out on YPD, and individual colonies were analyzed. The numbers of [*PSI*$^+$] and [*psi*$^-$] colonies are shown in Table. Mosaic colonies (usually rare in stable [*PSI*$^+$] isolates) were counted as [*PSI*$^+$]. Strain background refers to the stringency of the prion strain from which shuffle has started, either strong (GT256-23C) or weak (GT988-1A).

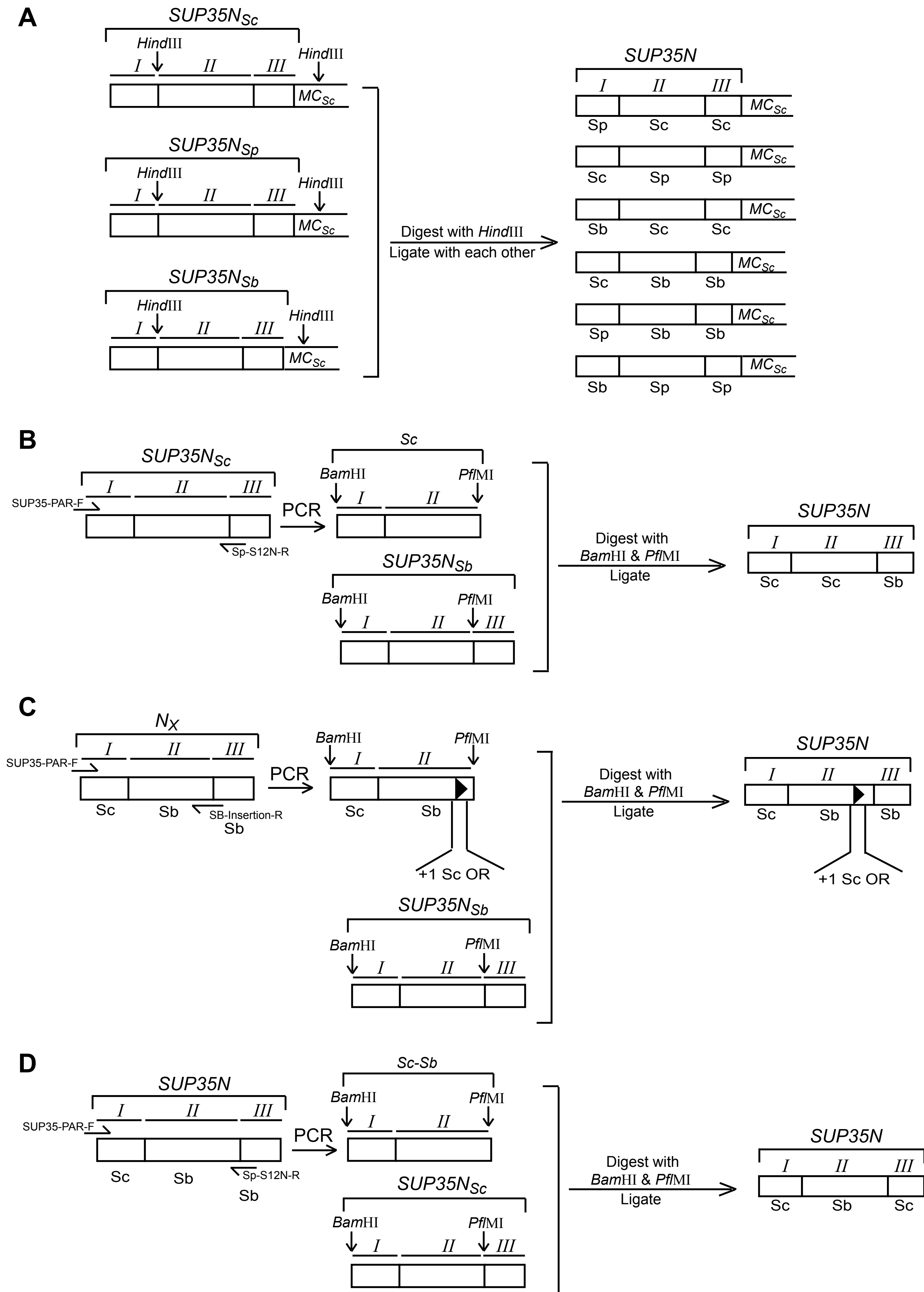

Figure S1. Construction of the *SUP35* genes with chimeric *SUP35N*.
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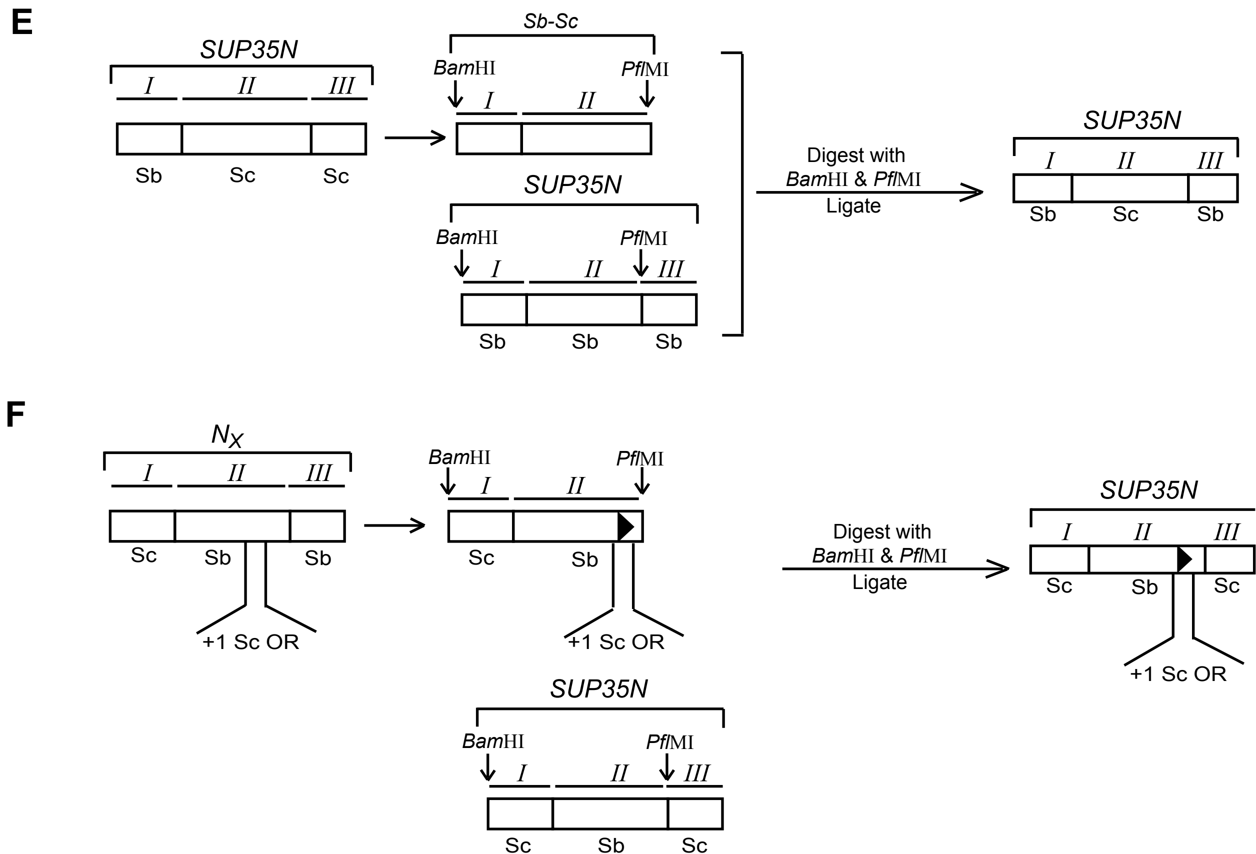


Figure S1. Construction of the *SUP35* genes with chimeric *SUP35N* domains. Designations Sc, Sp and Sb refer to *S. cerevisiae*, *S. paradoxus* and *S. bayanus*, respectively. Designations *N, M* and *C* refer to *SUP35N*, *SUP35M* and *SUP35C* domains, respectively. Modules of *SUP35N* domain (see Fig. 1B and D) are designated by Roman numerals. (A) Plasmids pBC102, pBC103 and pBC104 (Table S1) were digested with *Hin*dIII, and the 0.3 kb fragments containing modules II and III of different origins in conjunction with the small portion of *S. cerevisiae SUP35M* region were exchanged between plasmids. (B) To construct the plasmid bearing modules I and II of *S. cerevisiae* in conjunction with module III of *S. bayanus*, the *S. cerevisiae* fragment corresponding to modules I and II has been PCR-amplified with primers SUP35-PAR-F and SP-S12N-R (Table S2), containing *Bam*HI and *Pfl*MI sites, cut with *Bam*HI and *Pfl*MI and inserted into plasmid pBC102 at the same sites with the *Bam*HI site upstream of the *SUP35* ORF and cut with the same enzymes. (C) In order to generate a plasmid bearing *S. cerevisiae* module I in conjunction with modules II and III of *S. bayanus* with an extra repetitive unit of *S. cerevisiae* added to the ORs region, the fragment encompassing module I of *S. cerevisiae* and module II of *S. bayanus* was PCR-amplified from the *S. cerevisiae* / *S. bayanus* chimeric construct generated as described above with primers SUP35-PAR-F and SB-Insertion-R (Table S2), respectively, including the region with *Bam*HI site ahead of the ORF, and the region before (and including) *Pfl*MI site with an artificially added sequence corresponding to one *S. cerevisiae* repetitive unit. This fragment was inserted into the plasmid pBC102 cut with *Bam*HI and *Pfl*MI. (D) In order to generate a plasmid bearing *S. cerevisiae* modules I and III flanking module II of *S. bayanus*, the fragment encompassing module I of *S. cerevisiae* and module II and III of *S. bayanus* was PCR-amplified from the *S. cerevisiae* / *S. bayanus* chimeric construct generated as described above with primers SUP35-PAR-F and SP-S12N-R (Table S2), containing *Bam*HI and *Pfl*MI sites, cut with *Bam*HI and *Pfl*MI and inserted into plasmid pBC102 at the same sites with the *BamHI* site upstream of the *SUP35* ORF and cut with the same enzymes. (E) In order to generate a plasmid bearing *S. bayanus* modules I and III flanking module II of *S. cerevisiae,* the fragment encompassing module I and II was cut from plasmid PBC107 with *Bam*HI and *Pfl*MI and inserted into plasmid pBC104 at the same sites with the *Bam*HI site upstream of the *SUP35* ORF and cut with the same enzymes. (F) In order to generate a plasmid bearing *S. cerevisiae* module I and III in conjunction with modules II of *S. bayanus* with an extra repetitive unit of *S. cerevisiae* added to the ORs region, the fragment encompassing module I of *S. cerevisiae* and module II of *S. bayanus* with the extra repetitive unit was cut from plasmid pBC111 with *Bam*HI and *Pfl*MI and inserted into plasmid pKB100 at the same sites with the *Bam*HI site upstream of the *SUP35* ORF and cut with the same enzymes.

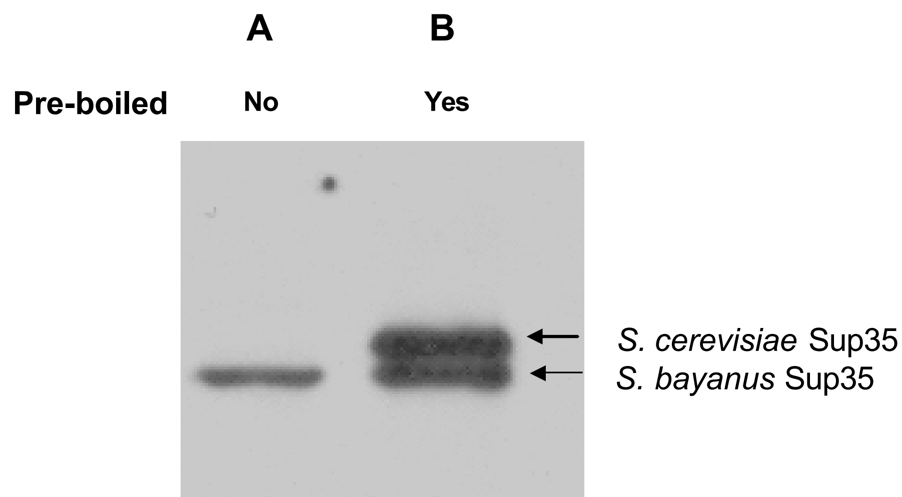


Figure S2: Identification of the non-polymerized *S. bayanus* protein associated with *S. cerevisiae* prion aggregates. Protein aggregates were precipitated from strong *S. cerevisiae* [*PSI*$^+$] strain expressing *S. bayanus SUP35* protein as shown on Fig. 2A, and analysed by electrophoresis in the 6% SDS polyacrylamide gel, that enables us to separate *S. cerevisiae* and *S. bayanus* Sup35 proteins by sizes. Electrophoresis was followed by Western and reaction to Sup35 antibody. Only Sup35 protein of lower molecular weight, corresponding to *S. bayanus* Sup35, is capable of entering the gel without boiling (lane A), indicated that this protein is present in a non-polymerised form. Higher molecular weight fraction of Sup35 protein, corresponding to the *S. cerevisiae* Sup35 protein, enters gel only is sample is pre-boiled, confirming that this protein is present in the SDS-resistant polymerized form (lane B). Increase in intensity of the lower molecular weight band in lane B indicates that a fraction of *S. bayanus* protein is also polymerized.

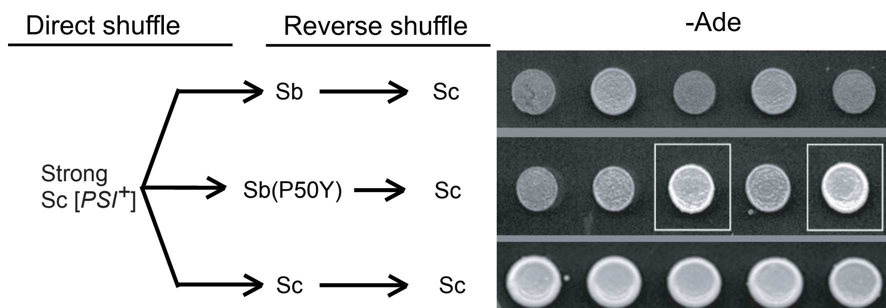


Figure S3: Variability in [*PSI*$^+$] stringency after propagation through a protein with *S. bayanus* PrD. Shuffle was performed starting from strong *S. cerevisiae* [*PSI*$^+$] strain GT256-23C, as shown on Fig. 1E and Fig. 4A. Designations Sc and Sb refer to *S. cerevisiae* protein and protein with *S. bayanus* PrD, respectively. P50Y refers to a mutation at amino acid position 50 of the *S. bayanus SUP35* sequence. All colonies shown on figure originate from reverse shuffle and contain only *S. cerevisiae* Sup35 protein. Prion derivatives that come from reverse shuffle via *S. bayanus* PrD show variable stringencies of suppression (as measured by intensity of growth on -Ade medium), in contrast to the control isolates propagated only through *S. cerevisiae* protein that are always homogenous. Remarkably, some prion variants originated from reverse shuffle through Sb (P50Y) protein (denoted by squares) match *S. cerevisiae* strong [*PSI*$^+$] by stringency, while prion variants originated from reverse shuffle through Sb protein never do.

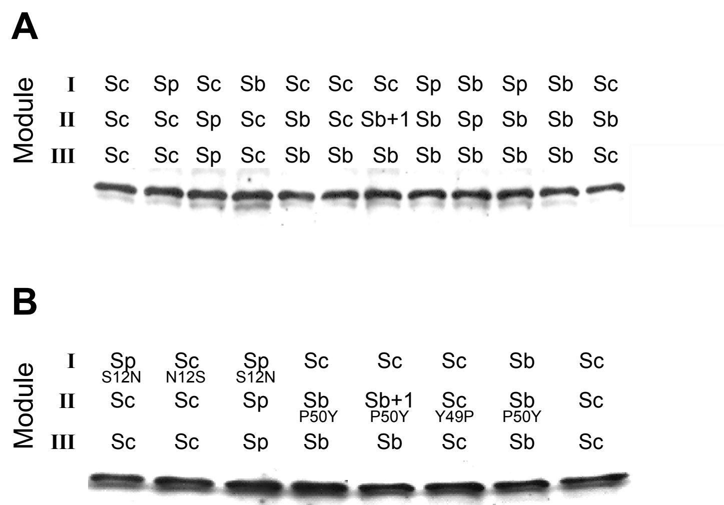
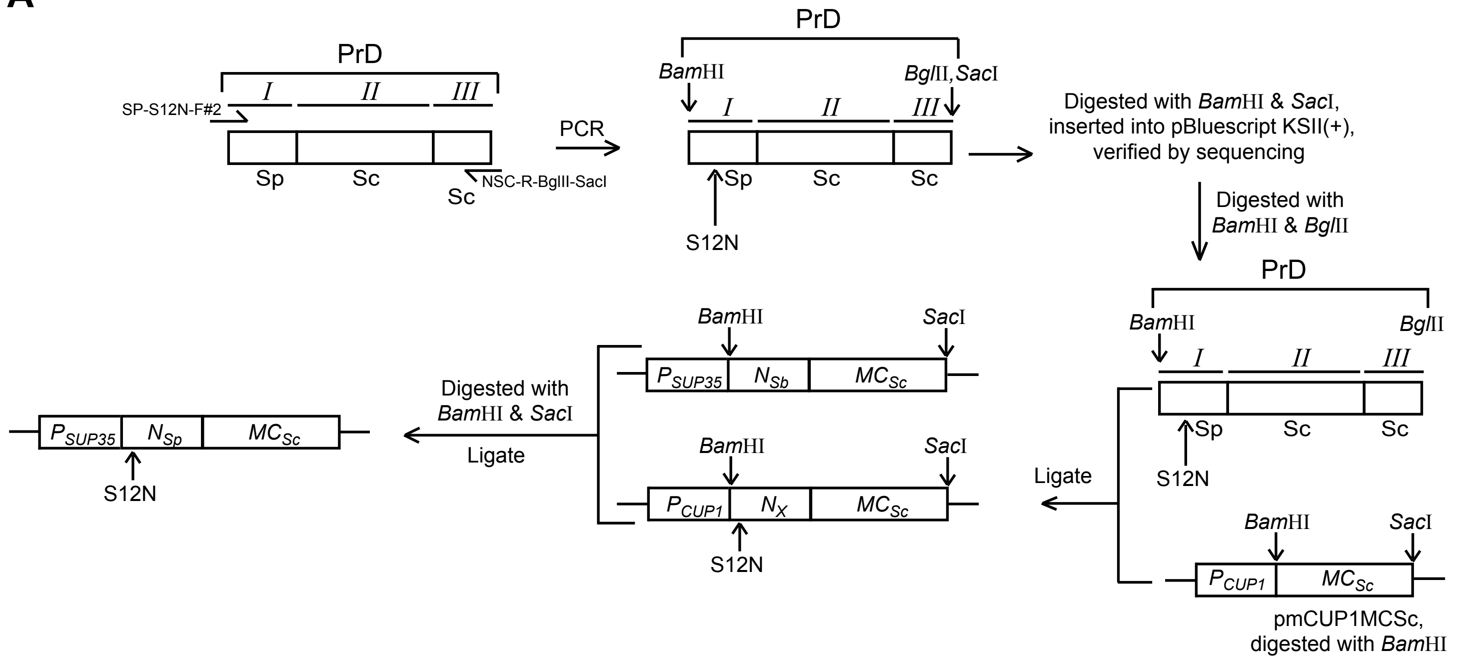


Figure S4. Levels of the Sup35 protein produced by the chimeric or mutant constructs. Proteins were isolated from the derivatives of the strain GT256-23C (see Materials and methods) bearing *URA3* plasmids with either chimeric or mutant *SUP35* PrDs fused to the *MC* regions of *S. cerevisiae and expressed from the* P_{SUP35} promoter, (see Table S1). Protein isolation and Western analysis were as described in Supplemental materials and methods. (**A**) and (**B**) refer to two different gels, each containing Sc control (Sc-Sc-Sc). Each Sup35 chimeric construct was produced at the same level as control.

A



B

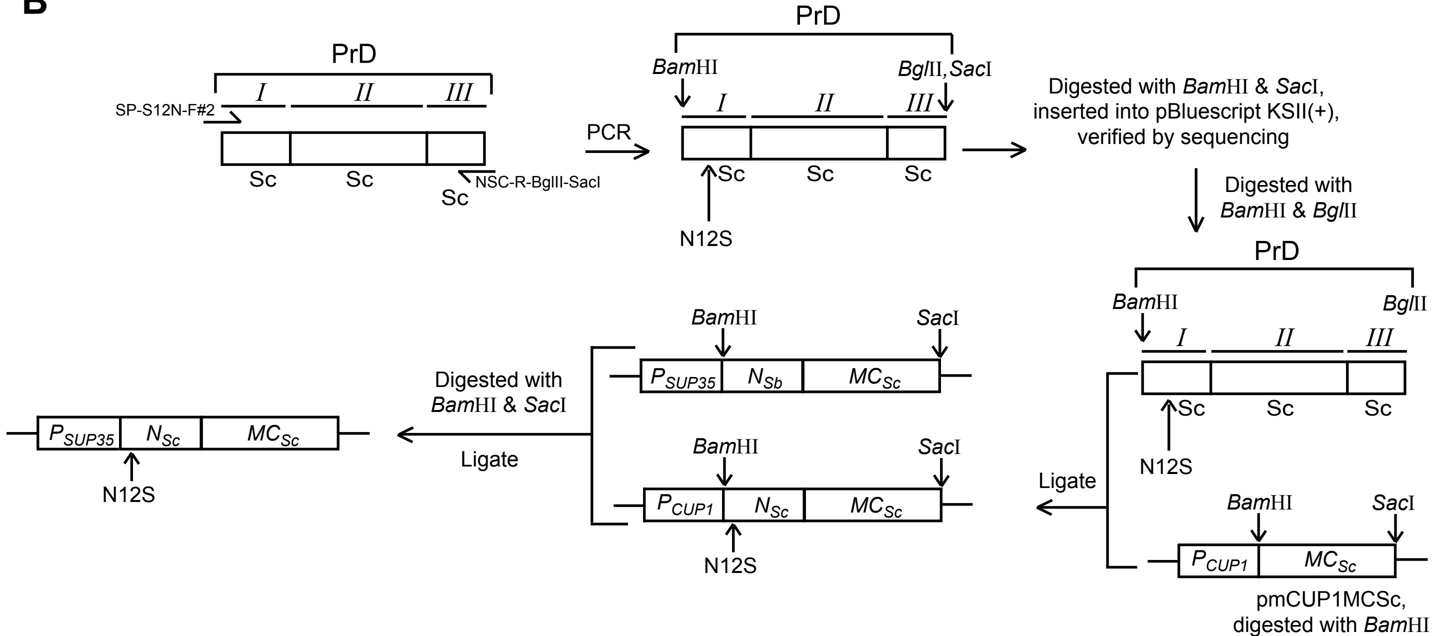
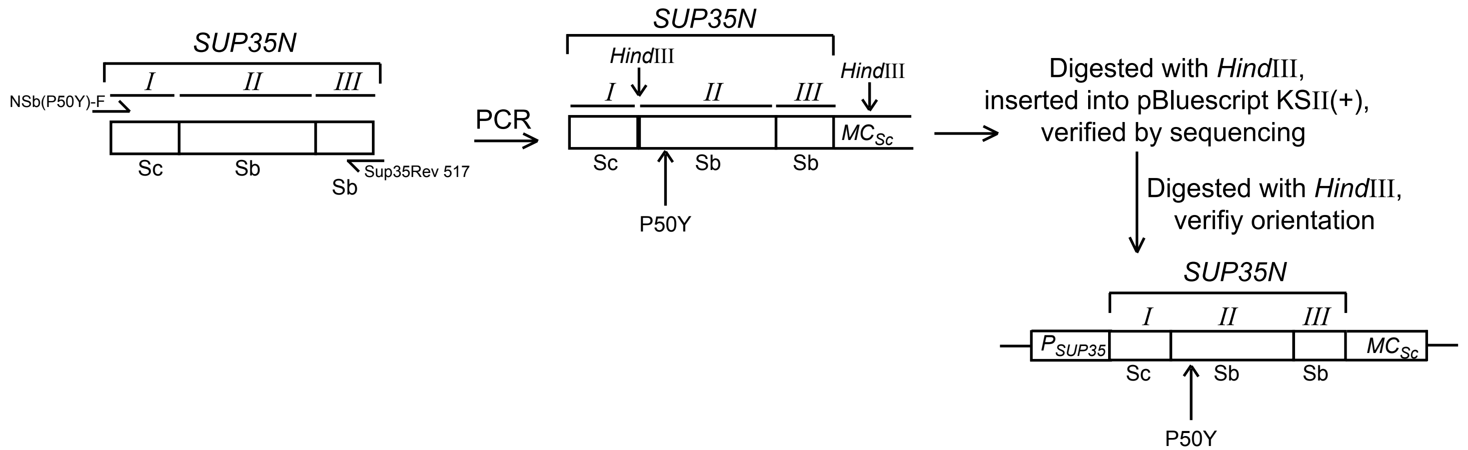


Figure S5. Construction of the *SUP35* derivatives with a mutation at position 12. For designations of the *SUP35* domain and modules, see Fig. S1 legend. (**A**) Plasmid pBC109 (Table S1) was PCR-amplified using primers SP-S12N-F#2 and NSC-R-BglII-SacI (Table S2). The former primer introduces a G to A substitution at nucleotide position 35, changing coding capacity of codon 12 from serine (S) to asparagine (N). Resulting fragment was digested by *Bam*HI and *Sac*I, inserted into plasmid pBluescript KSII (+) from Stratagene (cut with the same enzymes), verified by sequencing, digested with *Bam*HI and *Bgl*II, fused to the *SUP35MC* region of *S. cerevisiae* by inserting into pmCUP1MCSC (Table S1) cut with *Bam*HI, and the BamHI-SacI fragment encompassing full-size gene of chimeric SUP35 bearing the S12N was inserted into plasmid pBC104 (Table S1) bearing P_{SUP35} promoter cut with *Bam*HI and SacI. A similar strategy was used for construction of pKB101 (Table S1). (**B**) Plasmid pBC102 (Table S1) was PCR-amplified using primers NSC-MCSC(N12S) and NSC-R-BglII-SacI (Table S2). The former primer introduces an A to G substitution at nucleotide position 35, changing coding capacity of codon 12 from asparagine (N) to serine (S). Resulting fragment was digested with *Bam*HI and *Sac*I, inserted into plasmid pBluescript KSII (+) from Stratagene (cut with the same enzymes), verified by sequencing, digested with *Bam*HI and *Bgl*II, fused to the *SUP35MC* region of *S. cerevisiae* by inserting into pmCUP1MCSC (Table S1) cut with *Bam*HI, and the *Bam*HI-*Sac*I fragment encompassing the full-size gene of *SUP35* of *S. cerevisiae* bearing the N12S was inserted into plasmid pBC104 (Table S1) bearing P_{SUP35} promoter cut with *Bam*HI and *Sac*I.



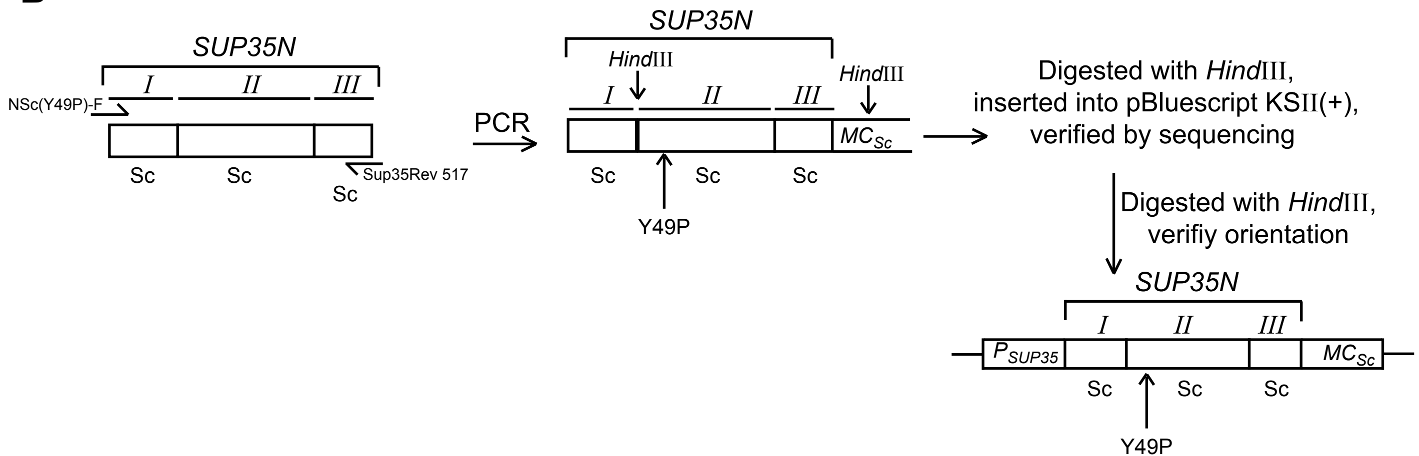


Figure S6. Construction of the *SUP35* derivatives with a mutation at position 49/50. For designations of the *SUP35N* domains and modules, see Fig. S2 legend.(**A**) Plasmid pBC110 (Table S1) was PCR-amplified using primers NSB(P50Y)-F and SUP35REV-517 (Table S2). The former primer also introduces a CC to TA substitution at nucleotide position 148 and 149, changing coding capacity of codon 50 from proline to tyrosine. Resulting fragment was digested with *Hind*III, inserted into plasmid pBluescript KSII (+) from Stratagene (cut with the same enzyme), verified by sequencing, digested with *Hind*III, inserted into plasmid pBC110 (Table S1) cut with *Hind*III and verified orientation of the insert. A similar strategy was performed to construct the plasmids pGN101 and pGN103 (Table S1). (**B**) Plasmid pBC102 (Table S1) was PCR-amplified using primers NSC(Y49P)-F and SUP35REV-517 (Table S2). The former primer also introduces a TA to CC substitution at nucleotide position 145 and 146, changing coding capacity of codon 49 from tyrosine to proline. Resulting fragment was digested with *Hind*III, inserted into plasmid pBluescript KSII (+) from Stratagene (cut with the same enzyme), verified by sequencing, digested with *Hind*III, inserted into plasmid pBC102 (Table S1) cut with *Hind*III and verified orientation of the insert.

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