Supplementary Figure 6

Spectrum of [URE3] variants from seeding experiments. (A) Solutions of purified Ure2p were incubated at 4°C for 12 hours without agitation with whole cell extracts from BY241 strains with
[ure-o] or one of the three [URE3] variants at a (w/w) protein ratio of about 1/150, 1/750, or 1/1500 (this corresponds to a Ure2p ratio of $1/3 \times 10^8$, $1/1.5 \times 10^9$, and $1/3 \times 10^9$, respectively). 2 μM of each mixture was used to transform strain BY241. Rates of conversion to [URE3] were for 1/150 solutions 0.7%, 2.6%, 0.9%, and 0.9%, for 1/750 solutions 1.9%, 1.2%, 0.6%, and 0.5%, and for 1/1500 solutions 5.4%, 0.6%, 1.4%, and 0.4% for seeding with [ure-o] and the three [URE3] variants, respectively. Randomly chosen clones were streaked on 1/2 YPD plates and incubated at 30°C for 5 days. (B) Reseeding experiments. Solutions of Ure2p and Ure2p$^{1-90}$-AAT were incubated at 4°C for 12 hours with agitation in the presence of whole cell extracts from BY241 [URE3] variant 1 at a (w/w) protein ratio of about 1/100 and 1/150, respectively. Mixture aliquots were diluted 1/100 in fresh Ure2p or Ure2p$^{1-90}$-AAT protein solutions and incubated in the same way. Each mixture was used to transform strain BY241 at a concentration of 400 nM (Ure2p) or 100 nM (Ure2p$^{1-90}$-AAT). Randomly chosen clones were streaked on 1/2 YPD plates and incubated at 30°C for 5 days.