Optimization of prion transformation conditions in different strains. Indicated strains were transformed with 500 ng pH125 and 500 nM Ure2p^{1-90}-AAT filaments. After incubation with
buffer PTC, the transformation mixtures were not pelleted, but instead 1/10 aliquots were directly diluted into 10 ml HC-Ade,L top agar containing 1 M sorbitol and the indicated concentrations of adenine, then plated on top of 20 ml HC-Ade,L bottom agar containing 1 M sorbitol. For comparison, the same transformations were performed using the standard procedure (see Materials and methods). Similar experiments with Ure2p filaments gave comparable results (data not shown). (A) Transformation plates after incubation at 30°C for 6 days. Bar, 2 mm. Note the appearance of red Ade⁻ [ure-o] clones among the white Ade⁺ [URE3] clones with increasing adenine concentrations. At a concentration of 20 mg/l adenine in the top agar, even Ade⁻ clones appear white, particularly those embedded in the top agar. Photos were taken using a Zeiss M²Bio microscope with an Axiophot camera and OpenLab software. (B) Number of [URE3] clones as a function of adenine concentration in transformation plates. Black bars indicate the infectivity of parallel transformations performed using the standard transformation procedure. Mean values of two independent experiments are presented. (C) Spectrum of [URE3] variants obtained from transformations in (B). Randomly chosen clones were streaked on 1/2 YPD plates and incubated at 30°C for 5 days. Note that the [URE3] variant spectrum is independent of the adenine concentration in the selection plates but differs between strains.