



**Figure S4** *In vivo* codon-optimized luciferase (*luc*) constructs integrated at *his-3* or *csr-1* for assessing uORF and 3'UTR intron effects on mRNA stability.

Schematic representation of the reporters integrated at (A) *his-3* locus or (B) *csr-1* locus. For both recipient loci, boxes indicate mRNA exons; green indicates the 5'UTR, blue indicates coding sequences, and red indicates the 3'UTR. All reporters contained the *cox-5* promoter. Constructs contained the wild-type arginine attenuator peptide (AAP) or the mutated or D12N AAP (AAP<sub>wt</sub>-*luc* or AAP<sub>D12N</sub>-*luc*) for analyzing the role of the *arg-2*-encoded uORF. To examine the roles of 3'UTR introns, the *cox-5* 3'UTR (*luc-cox-5*) served as a negative control. The shaded boxes surrounding the constructs indicate the reporter mRNAs contain specific features: blue, uORFs that trigger NMD; yellow, spliced 3'UTRs that trigger NMD; gray, no NMD-triggering features. The functions of 3'UTR introns were examined by comparing intron-containing or intronless *eif4a3* 3'UTRs

(*luc-eIF4A3+I* or *luc-eIF4A-I*) or *erf1* 3'UTRs (*luc-eRF1+I* or *luc-eRF1-I*). (C) 3'RACE analyses of *luc* and *eif4a3* mRNA 3'UTRs in wt and mutant strains. 3'RACE analysis was performed with specific oligos as described in Materials and Methods. The major and minor bands were excised and sequenced. The major 3'RACE fragment from *luc-cox-5* with oligos oYZ287~oYZ294 was 282 bp (top panel, lanes 1-6, 11,12). The minor larger band was 437 bp and represents an mRNA whose 3'UTR was 155-nt longer. The major fragment from *luc-eif4a3* (spliced-intron or intronless reporters) using oligos oYZ287~oYZ294 was 328 bp (top panel, lanes 7-10, 13-16, position indicated by "\*" ; the position for unspliced mRNA is 390 bp and indicated by "-"). No fragments were amplified with these *luc*-specific oligo sets from the wt strain lacking a *luc* reporter or from a no-cDNA control (top panel, lanes 17-18). The same cDNAs were used to obtain the endogenous *eif4a3* 3'RACE fragment of 300 bp using oYZ330~oYZ294 (bottom panel, lanes 1~17, position indicated by "\*" ; the position for unspliced mRNA is 362 bp and indicated by "-"). The larger fragment indicated by "#" observed for *eif4a3* in the  $\Delta y14$  strain (387 bp) represents a 3' extension of the spliced mRNA. This 3' extension (415 bp fragment, indicated by "#") is also observed for the spliced-intron *luc-eif4a3* reporter mRNA in the  $\Delta upf1$  and  $\Delta y14$  strains as determined by sequencing these fragments.