

GENETICS

Supporting Information

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DMR1 (CCM1/YGR150C) of Saccharomyces cerevisiae
Encodes an RNA-Binding Protein From the Pentatricopeptide
Repeat Family Required for the Maintenance of the Mitochondrial
15S Ribosomal RNA

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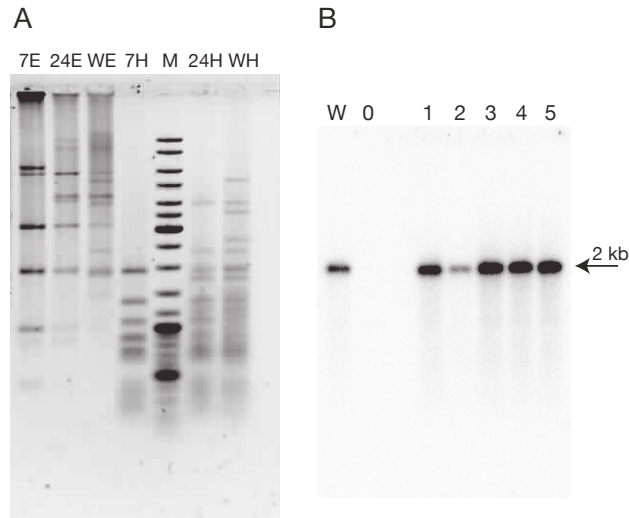


FIGURE S1.—Restriction and Southern analysis of different ρ^- clones expressing 15S rRNA. (A.) Mitochondrial DNA from two independent ρ^- clones expressing 15S rRNA, DPPR2/15S-7 (7) and DPPR2/15S-24 (24) and the wild-type ρ^+ strain CW04 (W) was purified by centrifugation in CsCl/bis-benzimide gradient, digested with *EcoRV* (E) or *HapII* (H) and separated in a 0.7% agarose gel. M is the GeneRuler™ DNA Ladder Mix (Fermentas). Different restriction patterns of the two independent ρ^- clones are apparent. (B.) Southern blot analysis of total DNA from five independent ρ^- clones expressing 15S rRNA (1 to 5), the wild-type ρ^+ strain CW04 (W) and a ρ^0 negative control (0). DNA was digested with *HapII*, separated in a 0.7% agarose gel, blotted on a Hybond N+ membrane and probed with the 15S_3ter oligonucleotide probe detecting the 3' fragment of the 15S rRNA gene. Presence of the 2kb fragment, known to contain the entire 15S rRNA gene (OSINGA *et al.* 1981) is detected in all the ρ^- clones.

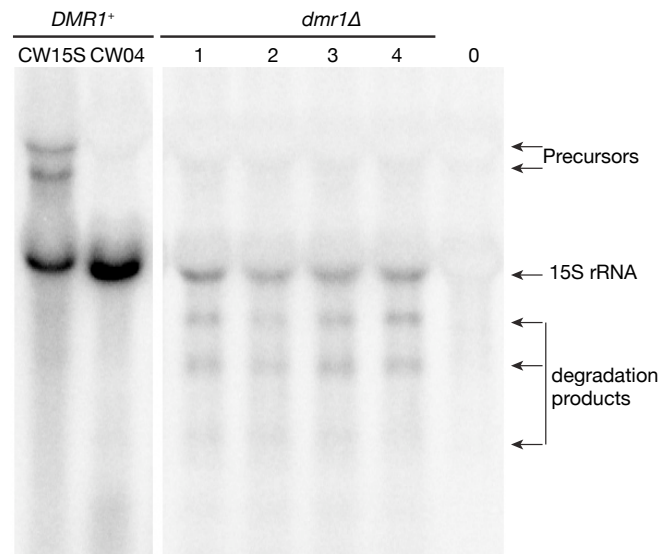


FIGURE S2.—Northern analysis of total RNA from different ρ^- clones expressing 15S rRNA. Total RNA from four independent *dmr1 Δ* ρ^- clones expressing 15S rRNA (1 to 4), the wild-type ρ^+ strain (CW04), a strain with the ρ^- genome expressing 15S rRNA introduced into wild-type nuclear background (CW15S), and a ρ^0 negative control (0) was hybridized with an oligonucleotide probe recognizing the 3' fragment of the 15S rRNA (15S_3ter). Neither the wild-type ρ^+ strain (CW252), nor a strain with the same ρ^- genome expressing 15S rRNA introduced into wild-type nuclear background (CW15S), show any signs of degradation, while all the *dmr1 Δ* ρ^- clones show a distinct degradation pattern, similar to the one observed in RNA preparations from purified mitochondria. The total amount of 15S rRNA fragments recognized by the probe in the *dmr1 Δ* ρ^- clones is decreased in comparison with the *DMR1⁺* controls. The amounts of RNA in each lane were normalized using methylene blue staining of cytoplasmic rRNA bands on the blot.