Supplemental Material

Characterization of a novel eukaryal nick sealing RNA ligase from *Naegleria gruberi*

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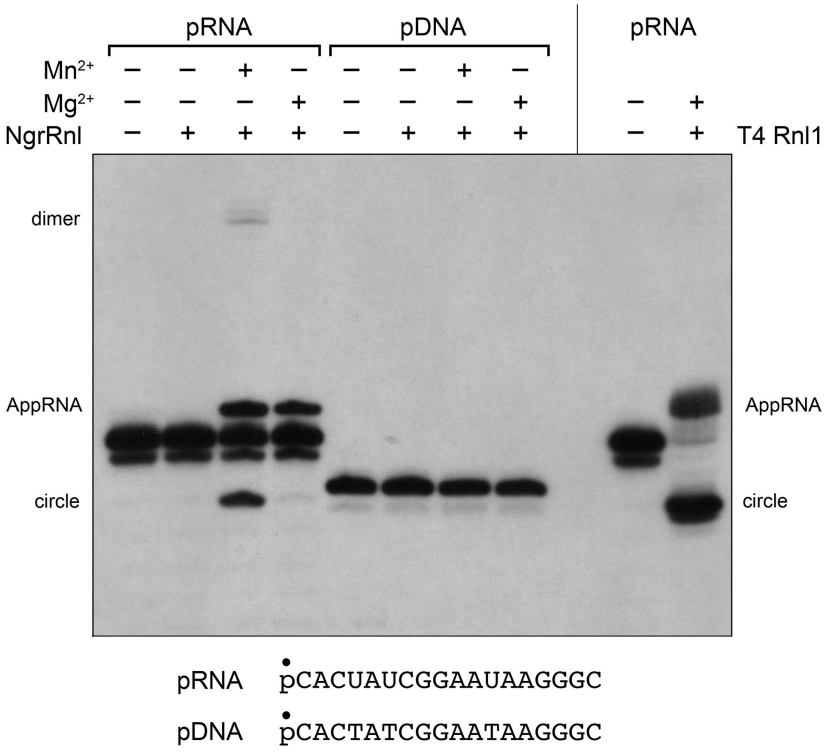

Figure S1. **Ligation of single-strand RNA**. NgrRnl ligation reaction mixtures (10 µl) containing 50 mM Tris-acetate (pH 6.0), 5 mM DTT, 0.2 mM ATP, 1 pmol (0.1 µM) ^{32}P-labeled 18-mer pRNA or pDNA as shown, 10 pmol (1 µM) NgrRnl (where indicated by +), and either no divalent cation (–) or 5 mM $MnCl_2$ or 5 mM MgCl2 (+) were incubated for 30 min at 37°C. T4 Rnl1 ligation reaction mixtures (10 µl) containing 50 mM Tris-HCl (pH 7.5), 1mM DTT, 10 mM $MgCl_2$, 0.2 mM ATP, 1 pmol (0.1 µM) ^{32}P-labeled 18-mer pRNA, and 10 units T4 Rnl1 (where indicased by +) were incubated for 30 min at 37°C. The products were analyzed by urea-PAGE (through a 40-cm gel) and visualized by autoradiography.

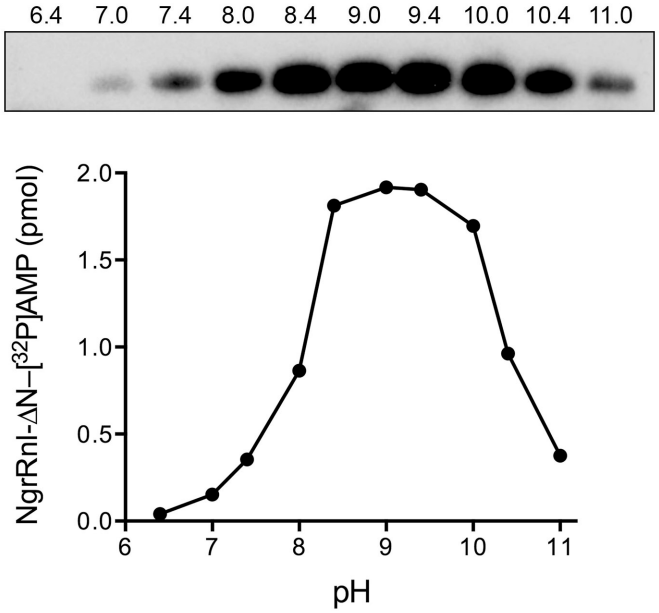

Figure S2. **pH profile for NgrRnl-ΔN adenylylation**. Reaction mixtures (10 µl) containing 50 mM buffer, either Tris-acetate (pH 6.4, 7.0), Tris-HCl (pH 7.4, 8.0, 8.4), 3-(cyclohexylamino)-2-hydroxypropanesulfonic acid (CAPSO; pH 9.0, 9.4), or 3-(cyclohexylamino)-1-propanesulfonic acid buffer (CAPS; pH 10, 10.4, 11.0), 5 mM $MgCl_2$, 50 µM [α^{32}P]ATP, and 80 pmol NgrRnl-ΔN were incubated for 30 min at 37°C.