SUPPLEMENTARY INFORMATION

Spontaneous advent of genetic diversity in RNA populations through multiple recombination mechanisms

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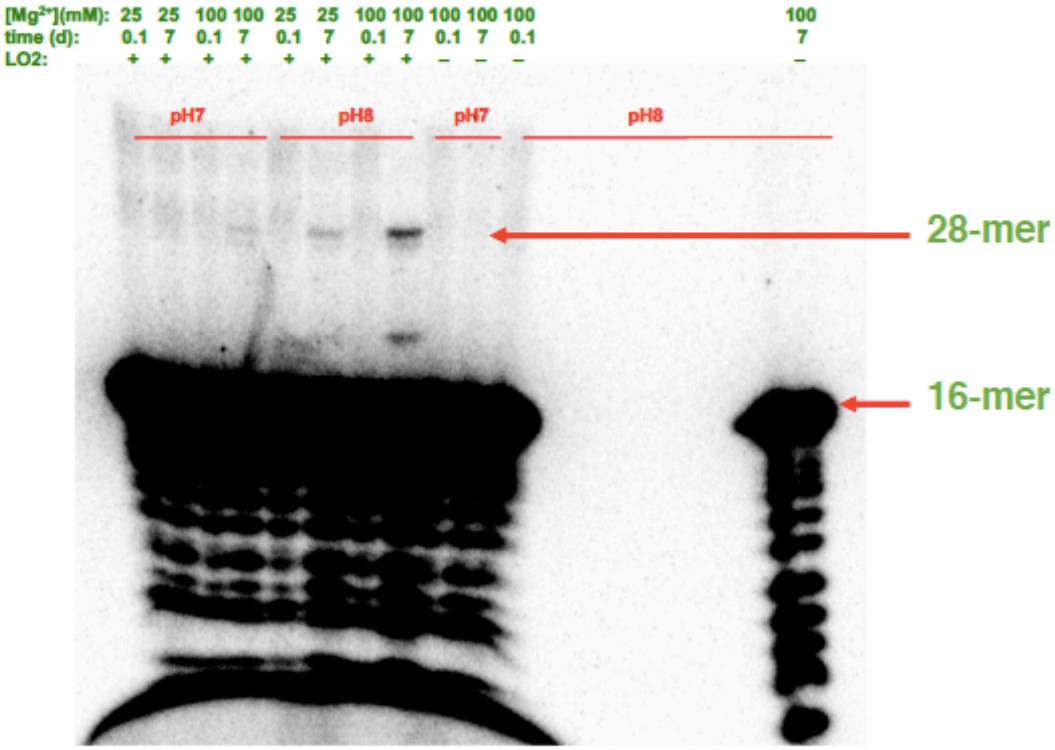
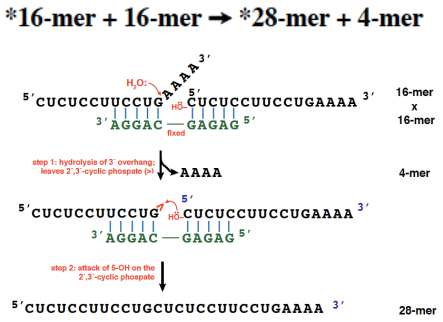
Table of Contents:

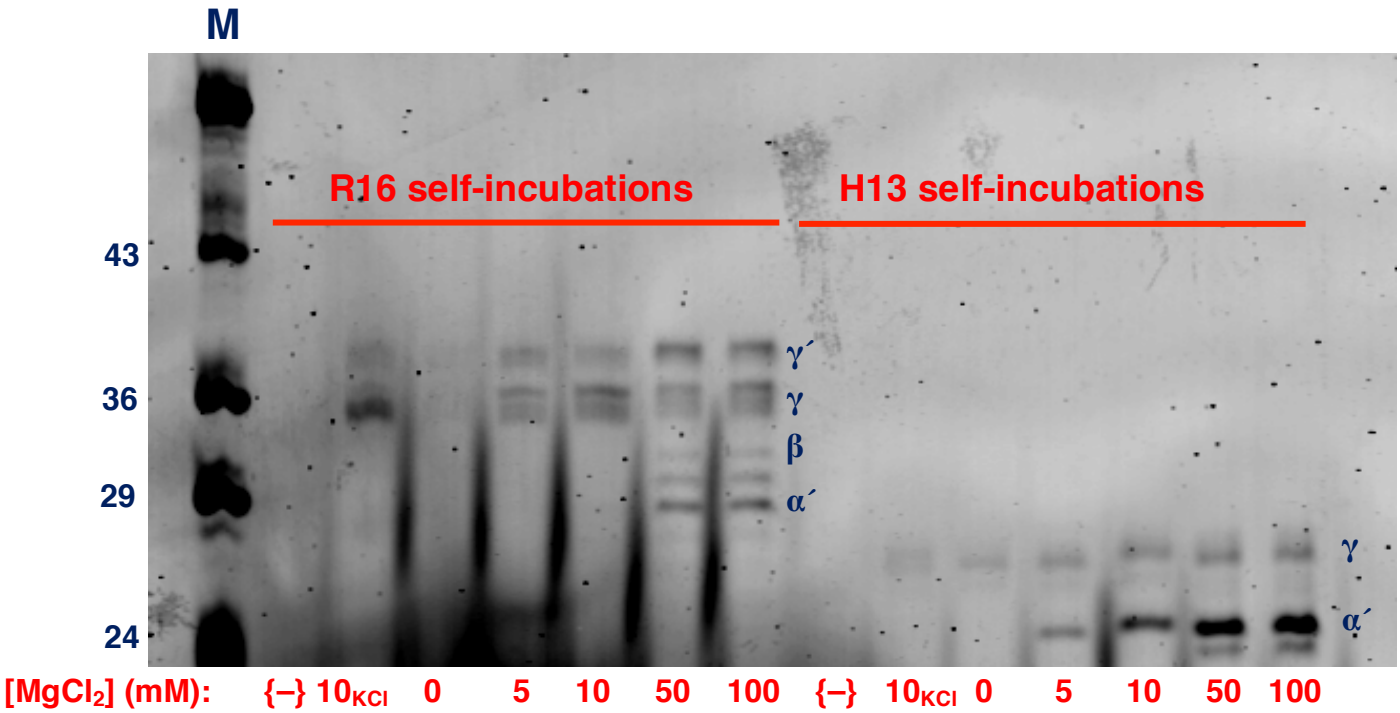




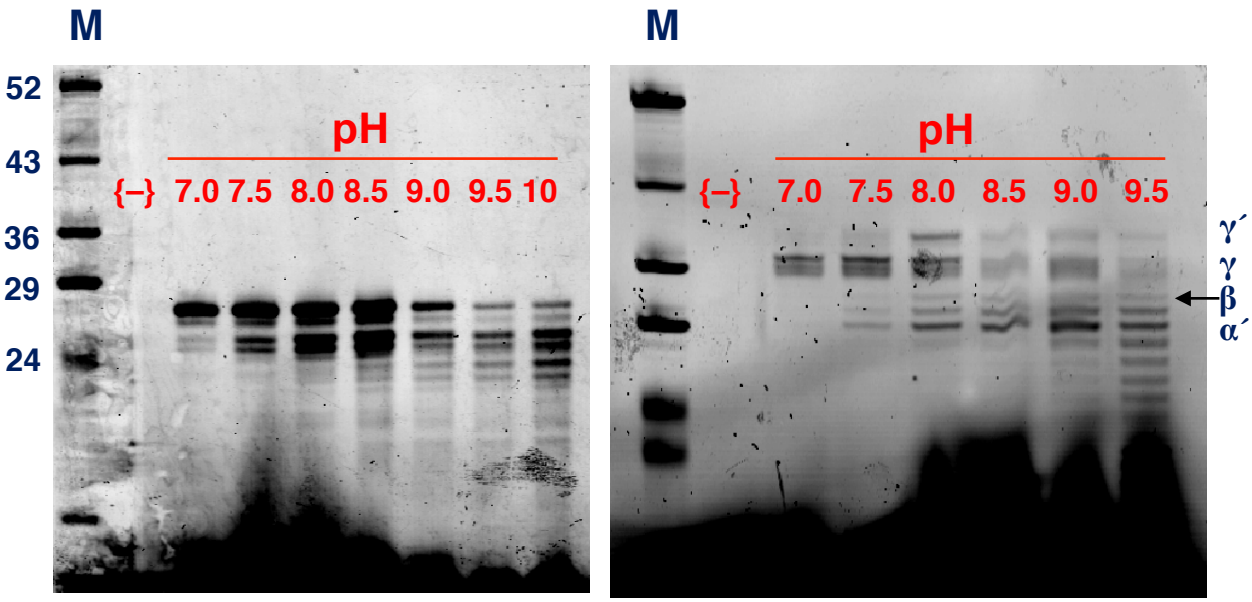
***Supplementary Figure 1* – Optimization of the α mechanism.** Prior to this electrophoretogram on a 15% denaturing polyacrylamide gel, equimolar ^{32}P-radiolabeled **LO1** (5′-CUCUCCUUCCUGAAA-3′) and cold **LO2** (5′-GAGAGCAGGA-3′) were incubated, at 20 μM each, for 1 hour or seven days at various pH values and $MgCl_2$ concentrations. These are the exact sequences used previously in the discovery of the **α** mechanism (Lutay et al. 2007). The maximum extent of the 28-mer product in this experiment is 2%. From this, and similar subsequent experiments, we have determined that the optimum **α** reaction conditions are:

- 10 μM each (if >1) or 100 μM (if only 1) RNA oligomer
- 100 mM $MgCl_2$
- pH 9.9 (*e.g.*, 30 mM CHES, pH 9.9)
- 7 days at 22˚C

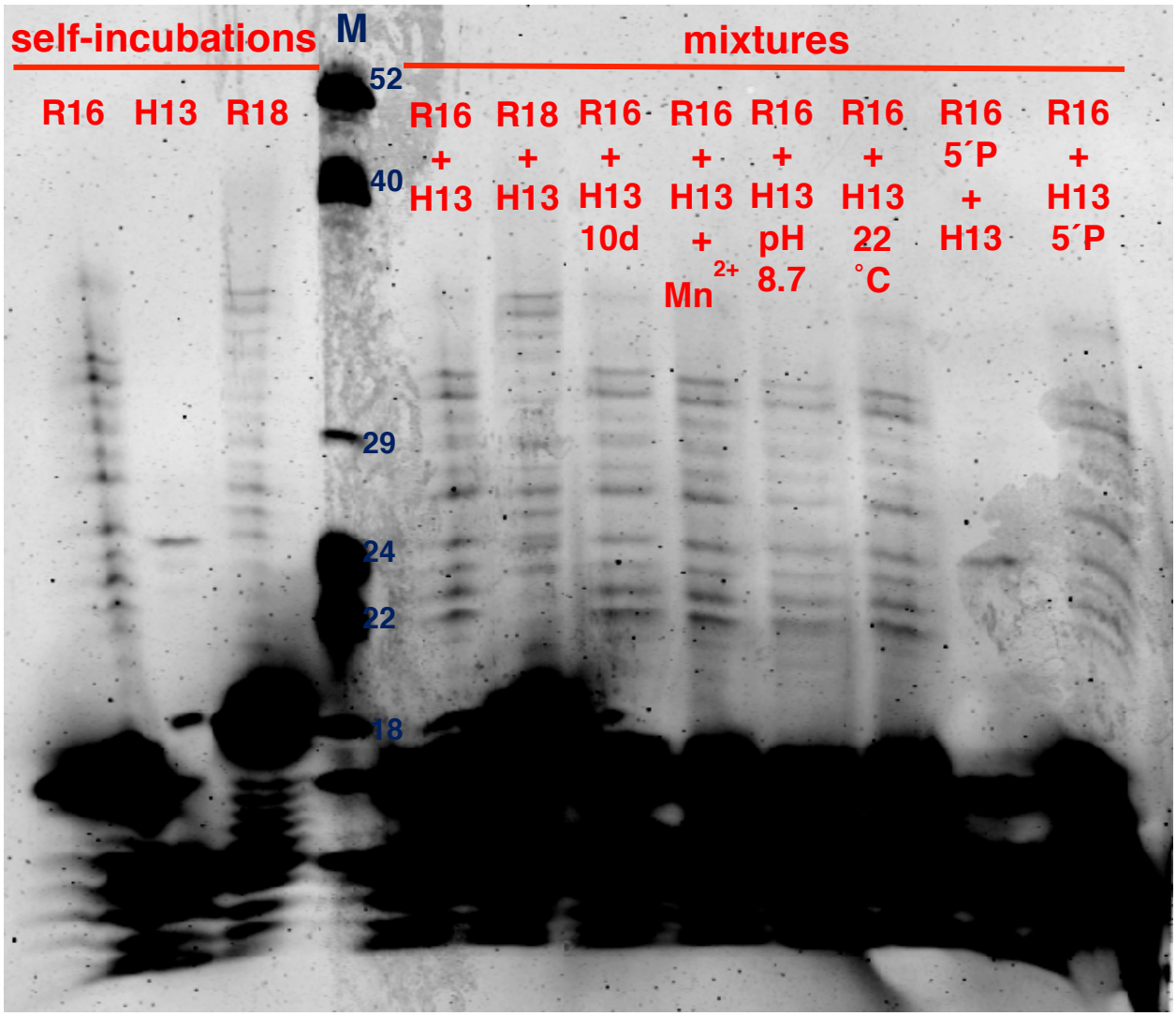
These conditions hold true for the optimum **α′** reaction conditions as well, although a 3-day reaction produces 80–90% of the yield seen after 7 days, and 3-h 0˚C/22˚C cycles improve the yield somewhat.



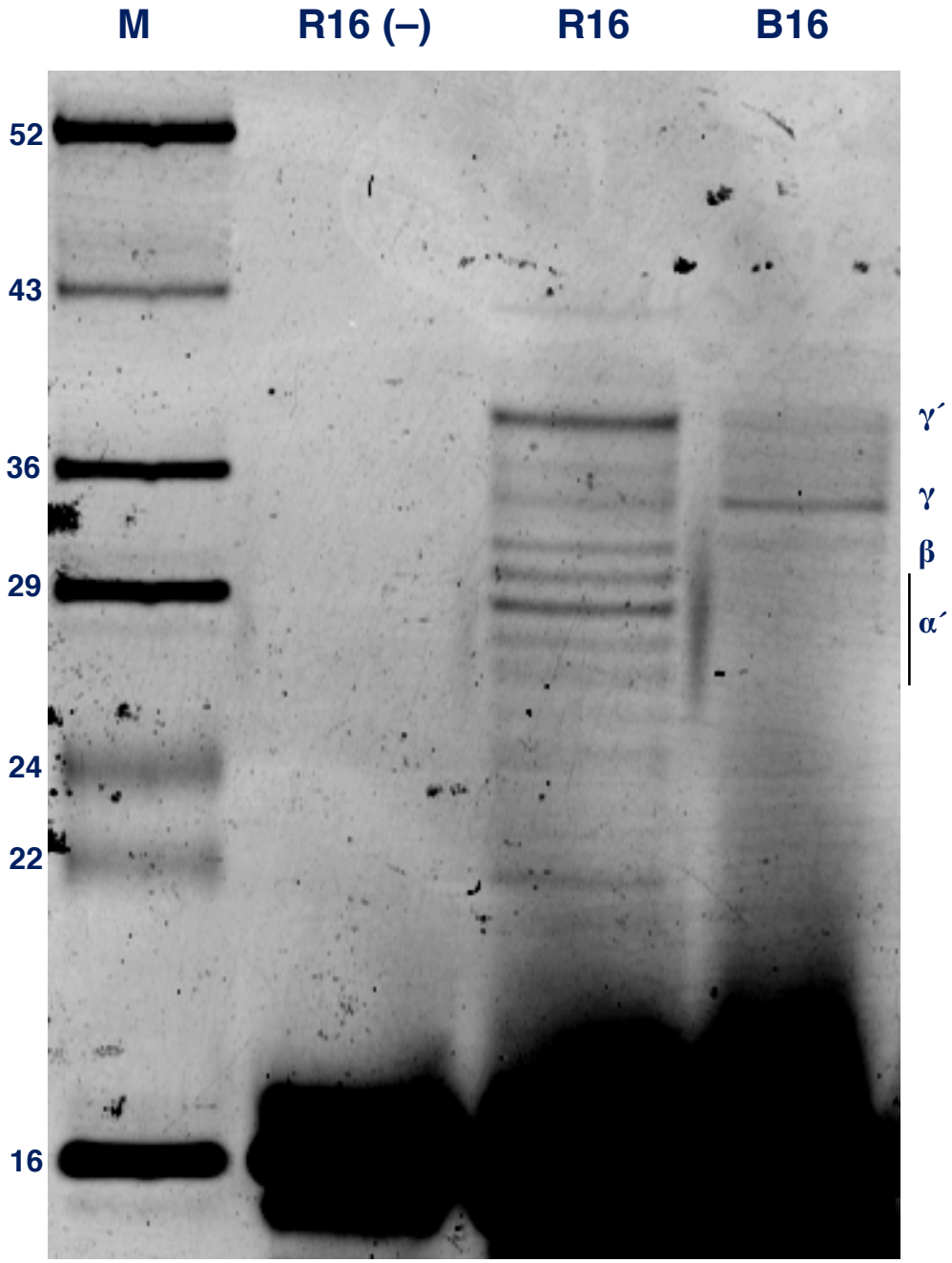
Supplementary Figure 2 **– Effect of $MgCl_2$ on the α´, β, and γ reactions.** Prior to this electrophoretogram on a 15% denaturing polyacrylamide gel, the reactions contained either cold **R16** or cold **H13** were incubated, at 100 μM each (or both), for 5 days at pH 8.0 with 3-hour cycles of 22˚C/0˚C at pH 8.0 in varying $MgCl_2$ concentrations. The designation 10_{KCl} refers to 10 mM KCl, which seems to permit the **γ** reaction. The **α´** reaction seems to require at least 50 mM $MgCl_2$ for **R16**, but only 5 mM for **H13**. In the marker (M) lane, size designations are in nucleotides.



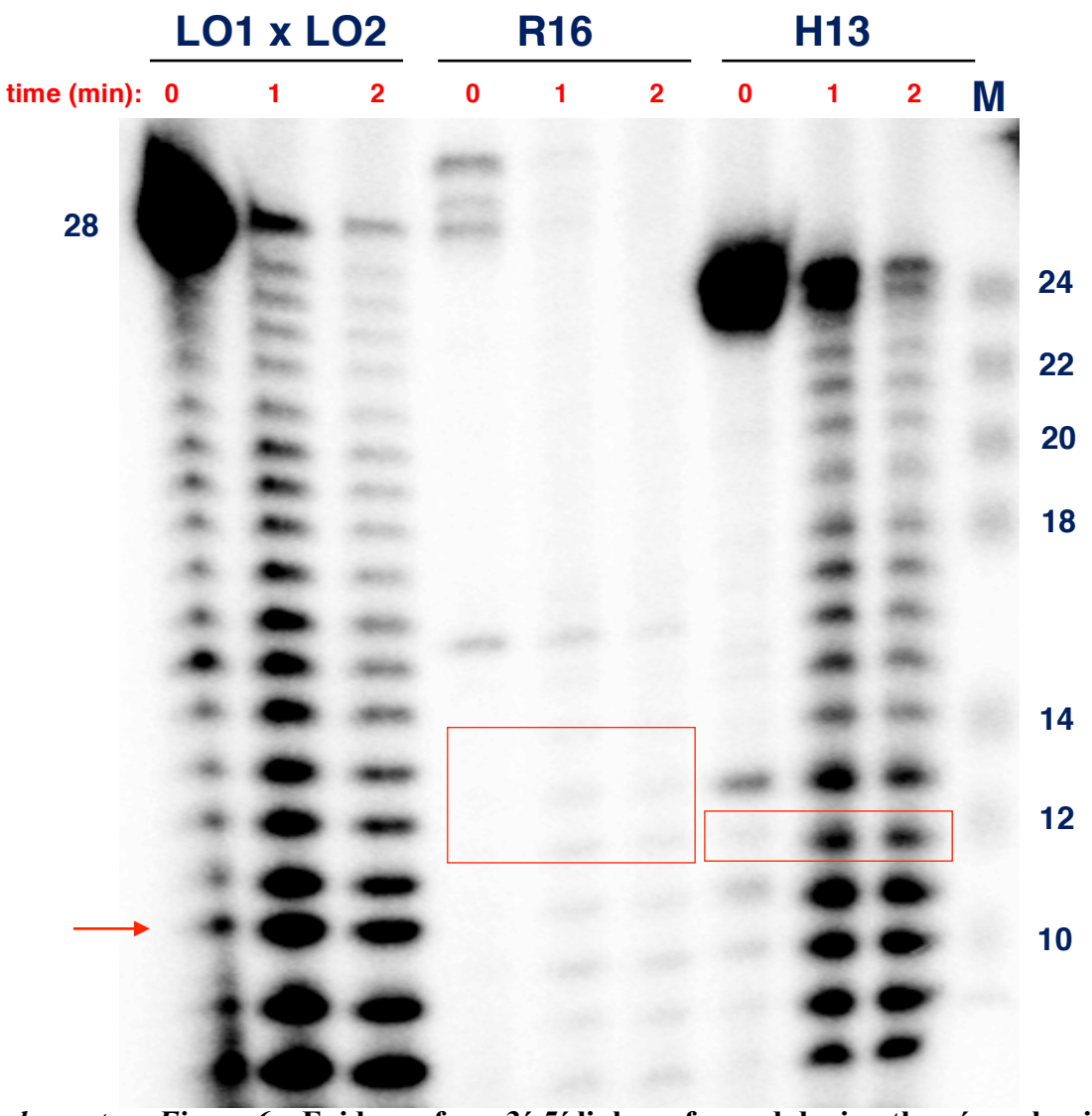
***Supplementary Figure 3* – pH profiles of H13 and R16 self-incubations.** Prior to these electrophoretograms on 15% denaturing polyacrylamide gels, the reactions contained either cold **H13** (left) or cold **R16** (right) were incubated, at 100 µM each, for 5 days at various pH values as indicated, with 3-hour cycles of 22˚C/0˚C in 100 mM $MgCl_2$. Reactions at pH 7.0, 7.5, and 8.0 occurred in 30 mM Tris, reactions at pH 8.5 occurred in 30 mM EPPS, and reactions at pH 9.0, 9.5, and 10 occurred in 30 mM CHES. The uppermost bands (**γ** and **γ´**) are dominant at lower pH values, while the lower bands (**α´** and **β**) are dominant at higher pH values. In the marker (M) lane, size designations are in nucleotides.



Supplementary Figure 4 **– Reaction of equimolar R16 and H13.** Prior to this electrophoretogram on a 15% denaturing polyacrylamide gel, the reactions to the right of the marker contained equimolar cold **R16** and cold **H13** were incubated, at 40 μM each, for (typically) 7 days with 3-hour cycles of 22˚C/0˚C in 100 mM $MgCl_2$ at pH 8.0. The reactions to the left of the ladder contained only **R16** (CGUACCGUUGCAUUUG) or **H13 (**CUGCAACGGUACG**)** or **R16** with two extra nucleotides (**R18** = CGUACCGUUGCAUUUUUG). The suite of products on the left match those on the right, for equivalent conditions, indicating that there is no appreciable reaction between **R16** and **H13**, as one would expect from the cross-strand attack model (Pino et al. 2013). In the marker (M) lane, size designations are in nucleotides.



***Supplementary Figure 5* – R16 and B16 self-incubations.** Prior to this electrophoretogram on a 15% denaturing polyacrylamide gel, 94 μM of **R16** or 64 μM of **B16** (5´-CAG CUU AGU CCG GUU C-3´; a 16-mer that, when self-complementary, would give a 4-nt rather than a 3-nt bulge in an α´ configuration) were incubated for 10 days in 100 mM $MgCl_2$ and 50 mM Tris (pH 8.0) with 3-hour cycling between 0 ˚C and 22 ˚C. The image was produced after staining with SYBR Gold. The lane adjacent to the marker (M) lane (sizes in nucleotides) contains unreacted (unincubated) **R16**. No α´ products are visible in the **B16** self-incubation.



***Supplementary Figure 6* – Evidence for a 3′-5′ linkage formed during the α′ mechanism.** Prior to this electrophoretogram on a 15% denaturing polyacrylamide gel, the reaction products from 5-day incubations of 85 μM RNAs in 100 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.0) using 3-h temperature cycles (22 ˚C / 0˚ C) were excised from a 15% denaturing polyacrylamide gel, concentrated by precipitation in ethanol and then rehydrated in RNase-free water. They were then 5′-end-labeled with OptiKinase (USB) and γ^{32}P•ATP and subsequently subjected to a 2-minute incubation in 50 mM NaOH at 90 ˚C. Aliquots from 0, 1, and 2 minute time points were electrophoresed as shown here. In the case of the LO1 x LO2, the relative proportions of band intensities (at 0 min) for the 17-, 16-, 15-, 14-, 13-, and 12-mer hydrolysis bands are 0.245, 0.156, 0.104, 0.140, 0.142, and 0.214, respectively. This indicates an over-representation of the 12-mer product in the LO1 x LO2 reaction (see red arrow; migration rates differ from marker (M) lane as a consequence of high pyrimidine content), a result of the increased lability of the 2′-5′ linkage seen in the **α** mechanism (Lutay et al. 2007). The lack of corresponding over-represented products in the **R16** reaction (at 12–14 nt) and in the **H13** reaction (at 12 nt) suggests that the reaction products contain 3′-5′ linkages at the recombination junction (see red boxes).

In the case of **R16** reaction at 0 min 12.3% of the total product manifests as a 16-mer, while in the case of H13, 1.96% of the total product has manifest as a 13-mer. These bands are likely a result of carry-over starting material trapped in the self-recombination products. This phenomenon is consistent with the 32-mer band seen in Figure 1A, and also helps to explain partially the unreacted RNA sequences seen in the HTS analysis (Tables 1–2).

References

Lutay AV, Zenkova MA, Vlassov VV. 2007. Nonenzymatic recombination of RNA: possible mechanism for the formation of novel sequences. *Chemistry & Biodiversity* **4**:762–767.

Pino S, Costanzo G, Giorgi A, Šponer J, Šponer JE, Di Mauro E. 2013. Ribozyme activity of RNA nonenzymatically polymerized from 3′,5′-cyclic GMP. *Entropy* **15:**5362–5383.