



**Figure S1.** Fluorescence readouts of separated cDNA products generated during reverse transcription at varied conditions. Shown is the region of the fluorescent signal starting from approximately 20 nt of cDNA (designated 3' on the top) and the full-length cDNA (designated 5' on the top). Reverse transcription reactions were set up with MMLV (**A, B, E**) or AMV (**C, D**) reverse transcriptase, pre- (**B**) or *in situ* (**A, C-E**) annealing of the primer and with different  $Mg^{2+}$  concentrations (shown on the right side). Reverse transcription reactions included 30 nM  $A_{25}$ -Luc mRNA (**A-C**) or a mixture of 15 nM  $A_{25}$ -Luc mRNA and 15 nM  $\beta$ -globin mRNA (**D, E**).