



**Figure S1: Effect of dRTP during PCR.** **A)** Ribavirin (RTP) is a nucleotide that displays antiviral activity. Due to its structure, we considered deoxyribavirin (dRTP) as an alternative to dKTP when performing DENT-seq for the ability to base pair with both C and T residues. Dashed black lines represent hydrogen bonds. **B)** dKTP and dRTP (Biolog Life Science Institute, special order) were compared for their mutagenic abilities by incorporation into the nick-containing biotinylated oligonucleotide (Fig. 2A). Initial nick translations were performed with either Taq DNA polymerase, Terminator DNA polymerase (New England Biolabs), or Sulfolobus DNA polymerase IV (New England Biolabs). Taq and dRTP lead to an unexpected result: a mutational signal 5' of the SSB that would interfere with precise resolution of SSB location. Pol IV led to a stronger signal one nucleotide 3' of the SSB with dKTP but much weaker signal at consecutive nucleotides and was unable to incorporate dRTP. While Terminator would incorporate dRTP, it does not appear to provide any significant benefit over using Taq with dKTP.