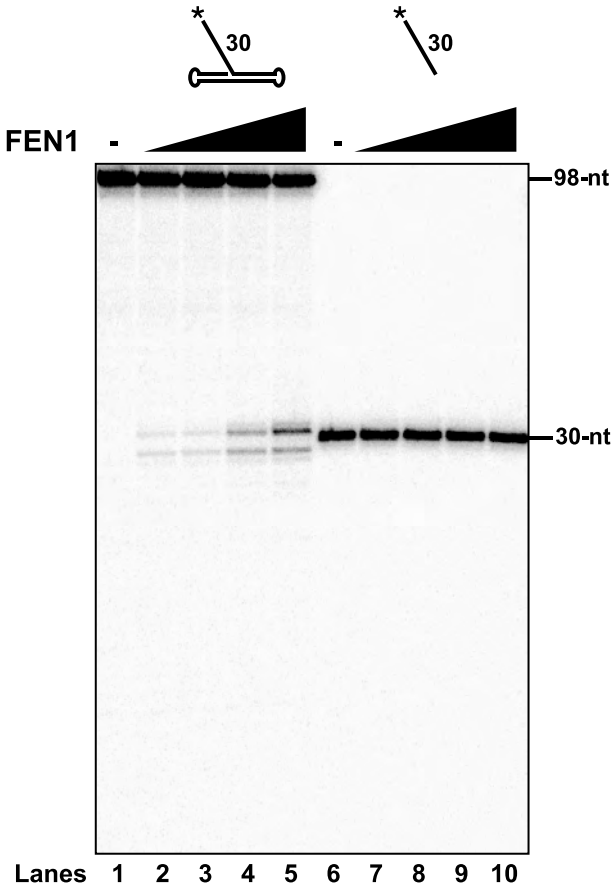
Supplementary Data

Dna2 is a structure-specific nuclease, with affinity for the base of 5'-flap intermediates

Jason A. Stewart[1], Judith L. Campbell[2] & Robert A. Bambara[1,*]

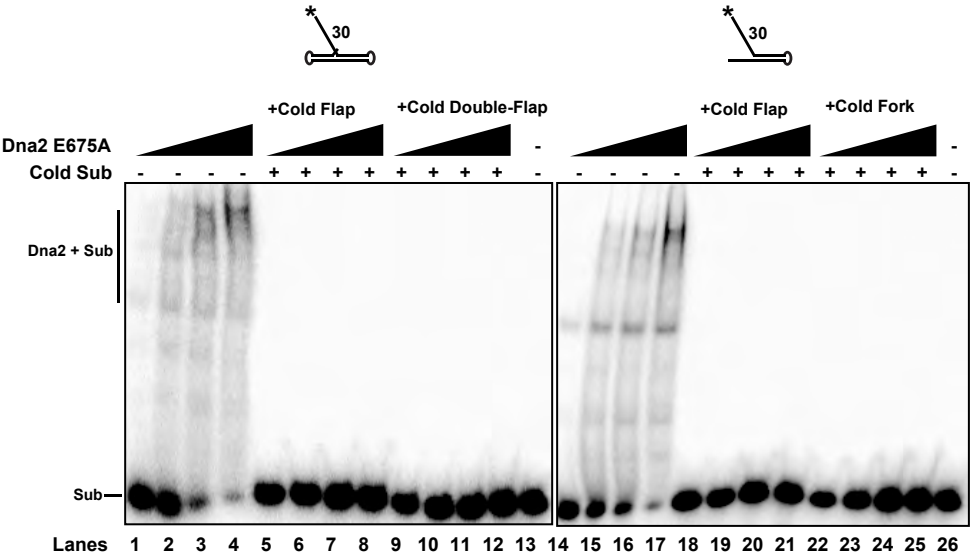
[1]Department of Biochemistry and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York, 14642, [2]Braun Laboratories, California Institute of Technology, Pasadena, California, 91125.
[*]To whom correspondence should be addressed. Tel: +1 585 275 3269; Email: Robert_Bambara@urmc.rochester.edu

Supplementary Figure 1



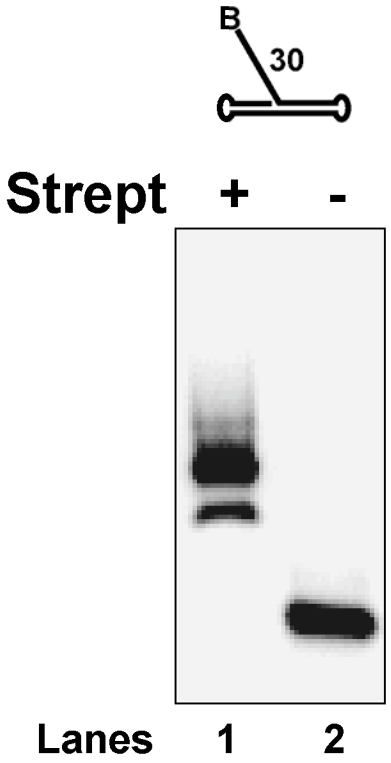
Supplementary Figure 1. FEN1 cleavage of the 30 nt flap and ssDNA substrates. FEN1 (0.5, 1, 2, 5 fmol) was incubated with 5 fmol of either the labeled flap (lanes 1-5) or ssDNA (lanes 5-10). Reactions were incubated at 37C for 10 min followed by denaturing PAGE analysis. Lanes 1 and 5 are the flap alone and ssDNA alone, respectively. Substrates are depicted above the gel with the asterisk indicating the site of the 32P label.

Supplementary Figure 2



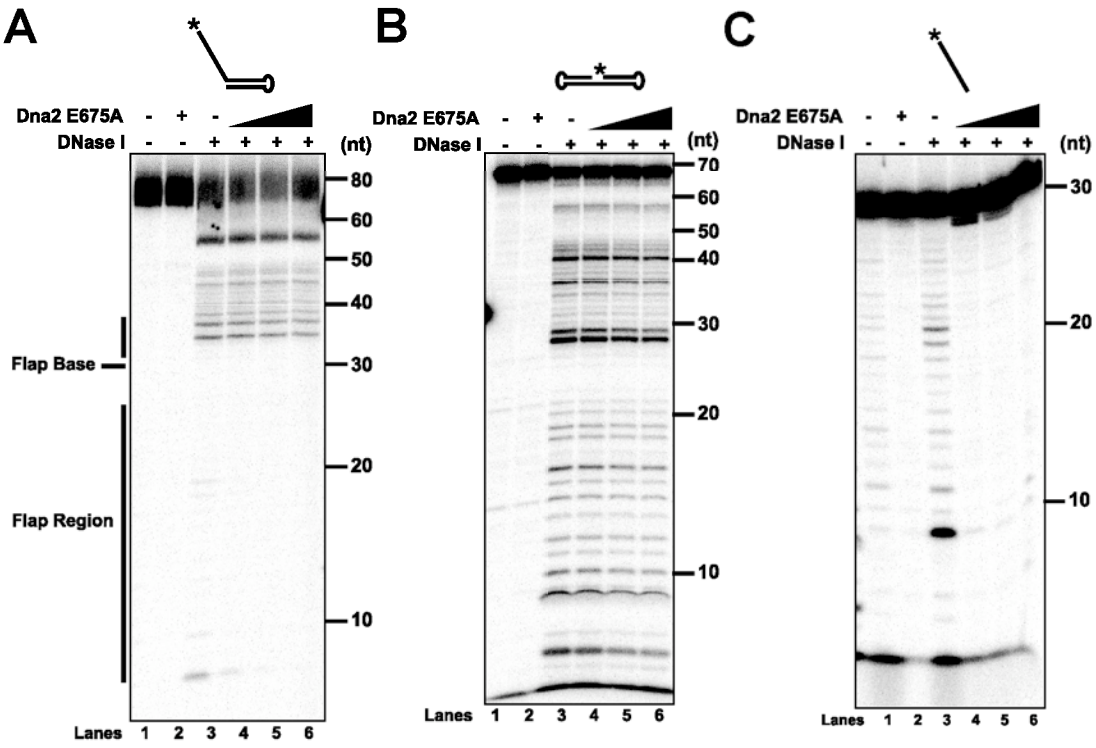
Supplementary Figure 2. Binding competition assays between the labeled double-flap or lableledfork substrate and an excess of unlabeled flap substrate. The labeled and unlabeled substrates were mixed prior to the addition of Dna2. Reactions contained 5 fmol of the labeled flap substrate and 1 pmol of the unlabeled substrate. Dna2 E675A (50, 100, 200, 400 fmol) binding of the labeled double-flap substrate was measured in either the absence of unlabeled substrate (lanes 1-4) or the presence of the unlabeled flap (lanes 5-8) or unlabeled double-flap (lanes 9-12) substrate. Lane 13 is the double-flap substrate alone. Dna2 E675A (50, 100, 200, 400 fmol) binding of the labeled fork substrate was measured in either the absence of unlabeled substrate (lanes 14-17) or the presence of the unlabeled flap (lanes 18-21) or unlabeled fork (lanes 22-25) substrate. Lane 26 is the fork substrate alone.

Supplementary Figure 3



Supplementary Figure 3 Streptavidin conjugation of the biotin-flap substrate. The biotin-flap (10 pmol) was incubated with (lane 1) or without (lane 2) streptavidin (50 fmol). Reactions were then analyzed on a 1% agarose (0.5 x TBE) gel. Substrate is depicted above gel.

Supplementary Figure 4



Supplemental Figure 4. Footprinting analysis of Dna2 on the various substrates substrate. (**A**) Dna2 E675A (200, 400, 800 fmol) was incubated with the 5'-tail substrate followed by DNase I footprinting analysis. (**B** and **C**) Dna2 E675A (1, 2, 3 pmol) was incubated with either the nick substrate (B) or ssDNA (**C**) followed by DNase I footprinting analysis. Reactions were then analyzed by denaturing PAGE. Lanes 1 and 2 are the substrate alone and substrate with Dna2 E675A, respectively. Lane 3 is the flap substrate alone treated with DNase I. FEN1 cleavage was used to identify the flap base.