Supplementary Data for:

Kinetic Analysis of the Genome Packaging Reaction in Bacteriophage λ.

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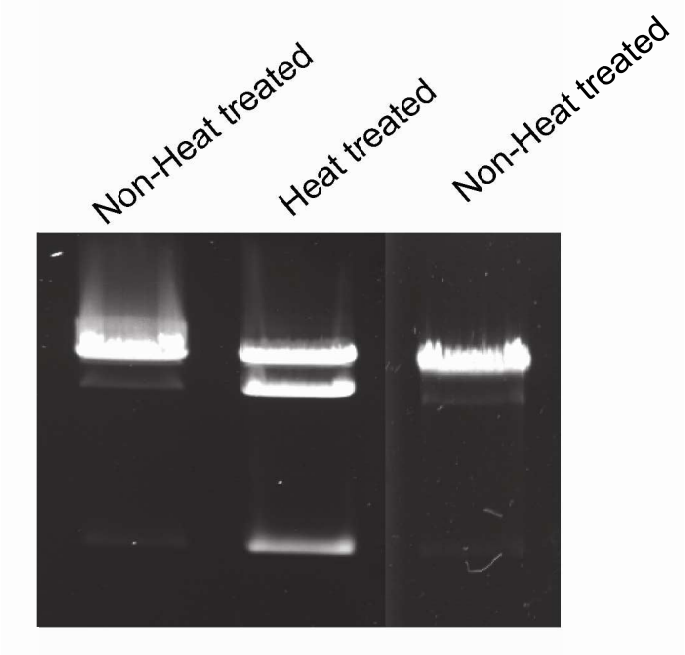

Figure S1. A DNA fragment, annealed at *cos*, which therefore contained the two symmetric nicks, was generated by complete digestion of purified λ genomic DNA with the Acc I restriction enzyme, followed by gel-purification of the 7.77 kbp DNA product that possessed the annealed *cos* site. This DNA was then taken through a mock DNA packaging reaction to assess its stability when subjected to native agarose gel electrophoresis. As expected, heating the DNA resulted in melting the 12 bp of complementary DNA, yielding two DNA fragments. This experiment shows that an annealed, and symmetrically nicked *cos* site, where the two nicks are separated by 12 bp of DNA, will remain annealed during native gel electrophoresis.

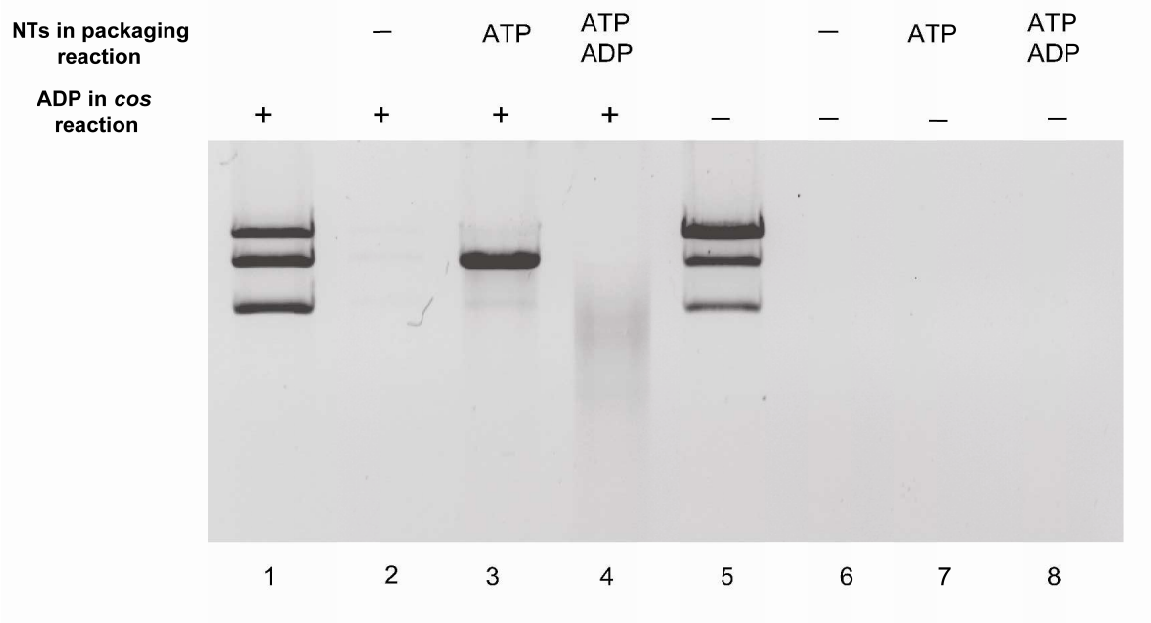

Figure S2. Demonstration that λ terminase cutting at *cos* in the absence of ADP will not support the DNA packaging reaction. The DNA packaging reaction was performed as described in Experimental Procedures, and 20 min time points are shown on this gel. When 1 mM ADP was included in the *cos* cleavage reaction, the matured *cos*-containing DNA (the 7.6 kbp DNA substrate) was successfully packaged. However, when the *cos* cleavage reaction was carried out in the absence of ADP, but still in the presence of Mg^{2+}, even though the *cos* site is cleaved, no DNA packaging is observed. Lanes 1 and 5 correspond to the products of the *cos* cleavage phase of the reaction, while Lanes 2-3 and 6-8 correspond to the quantity of DNA that was packaged during the DNA packaging phase of the reaction. Lane 1, *cos* cleavage reaction in the presence of 1 mM ADP; Lane 2, no ATP added during the DNA packaging reaction; Lane 3, 1 mM ATP was added during the DNA packaging reaction; Lane 4, 1 mM ATP and 5 mM ADP were added during the DNA packaging reaction; Lane 5, *cos* cleavage reaction in the absence of ADP; Lane 6, no ATP added during the DNA packaging reaction; Lane 7, 1 mM ATP was added during the DNA packaging reaction; Lane 8, 1 mM ATP and 5 mM ADP were added during the DNA packaging reaction.

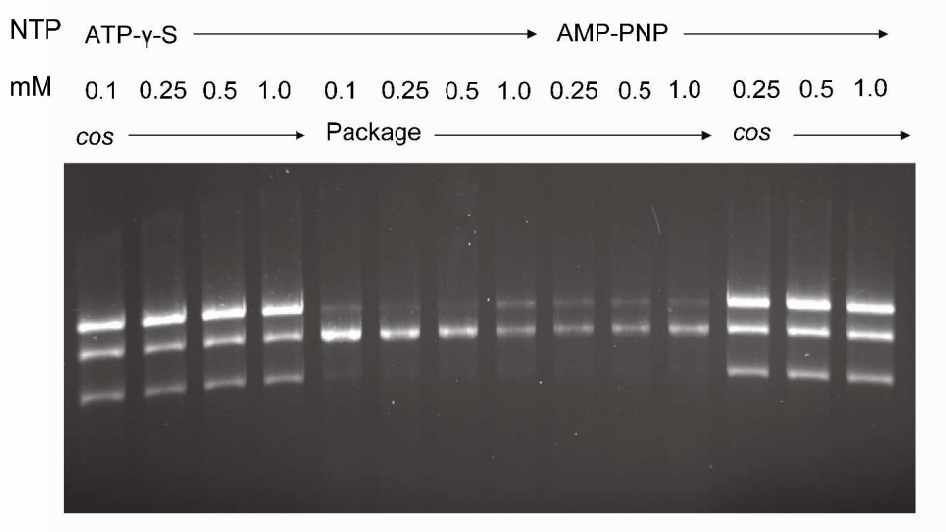

Figure S3. Demonstration that addition of either ATPγS or AMP-PNP to the *cos* cleavage step will support DNA packaging. In contrast to this, if the *cos* cleavage reaction is carried out in the absence of any nucleotide cofactor, no DNA packaging is observed. The reaction conditions are identical to those reported in the text, and the concentration of the added nucleotide cofactor is indicated in the figure. The lanes labeled *cos* refer to the *cos* cleavage reaction carried out in the presence of the indicated nucleotide cofactor, while the lanes labeled Package refer to the subsequent DNA packaging reaction carried out after either ATPγS or AMP-PNP were used in the *cos* cleavage reaction. The *cos* cleavage reaction was carried out as described in Experimental Procedures, and the DNA packaging reaction was allowed to proceed for 20 min before quenching.