



**Figure 2 - Supplement 1.**

**Flanking DNA does not influence the orientation of the hexasome.**

(A) Schematic representation of the 0-601-80 nucleosome and hexasome. As shown for 80-601-0, limiting amounts of H2A/H2B favors a hexasome that lacks the dimer on the TA-poor side of the Widom 601 sequence.

(B) Exonuclease III digestions performed as in Figure 2 except using 0-601-80 nucleosomes and hexasomes, where flanking DNA was on the opposite (right) side of the 601. Digestion of hexasomes from the right of the 601 (lanes 17-20) indicated that hexasomes lacked H2A/H2B on the TA poor side of the 601 regardless of flanking DNA. Addition of two-fold molar excess H2A/H2B to hexasomes restored nucleosome digestion patterns (lanes 21-24). ExoIII digestions of 80-601-0 and 0-601-80 hexasomes and nucleosomes were performed in parallel, run on the same gel and are each representative of two independent experiments.