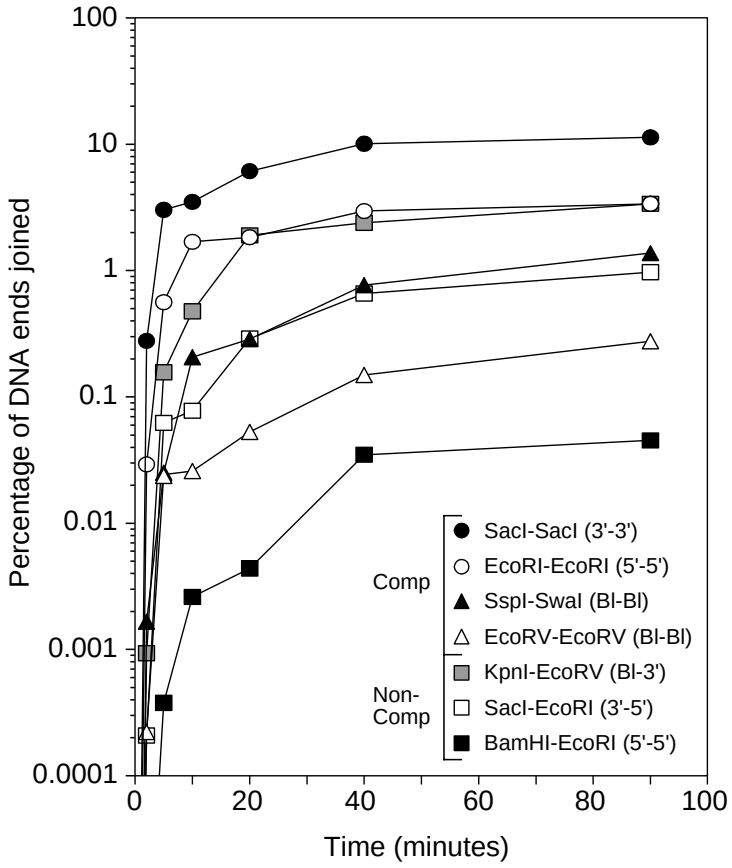
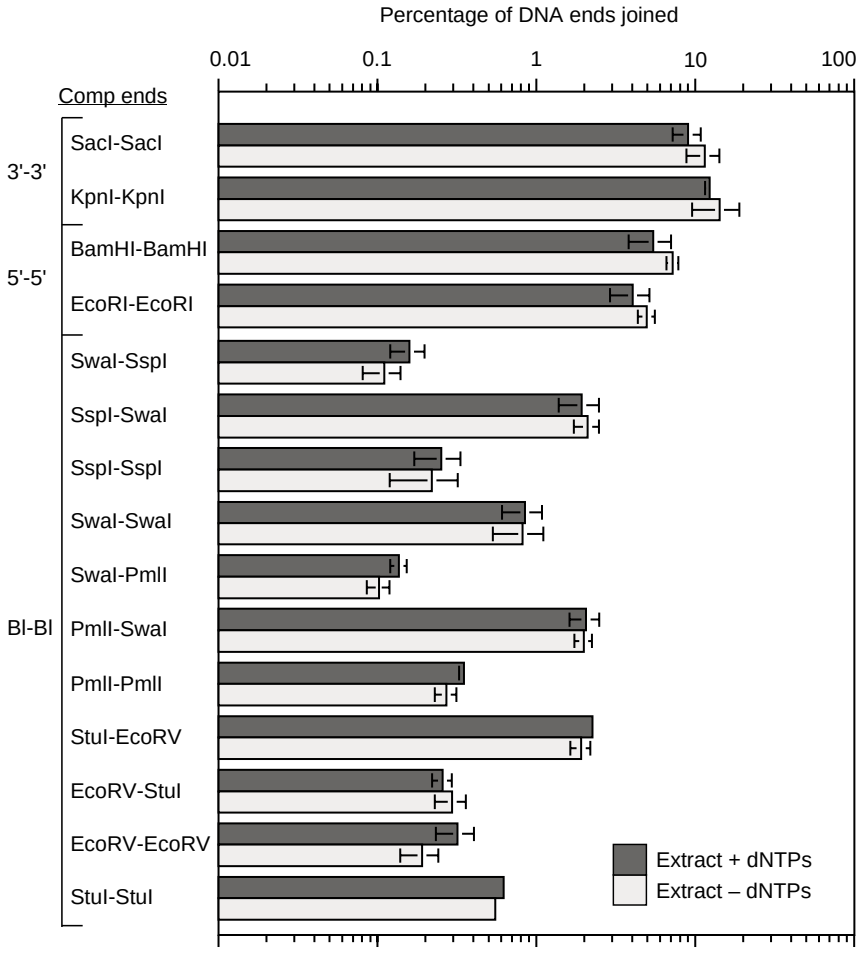
Supplementary Figure 1



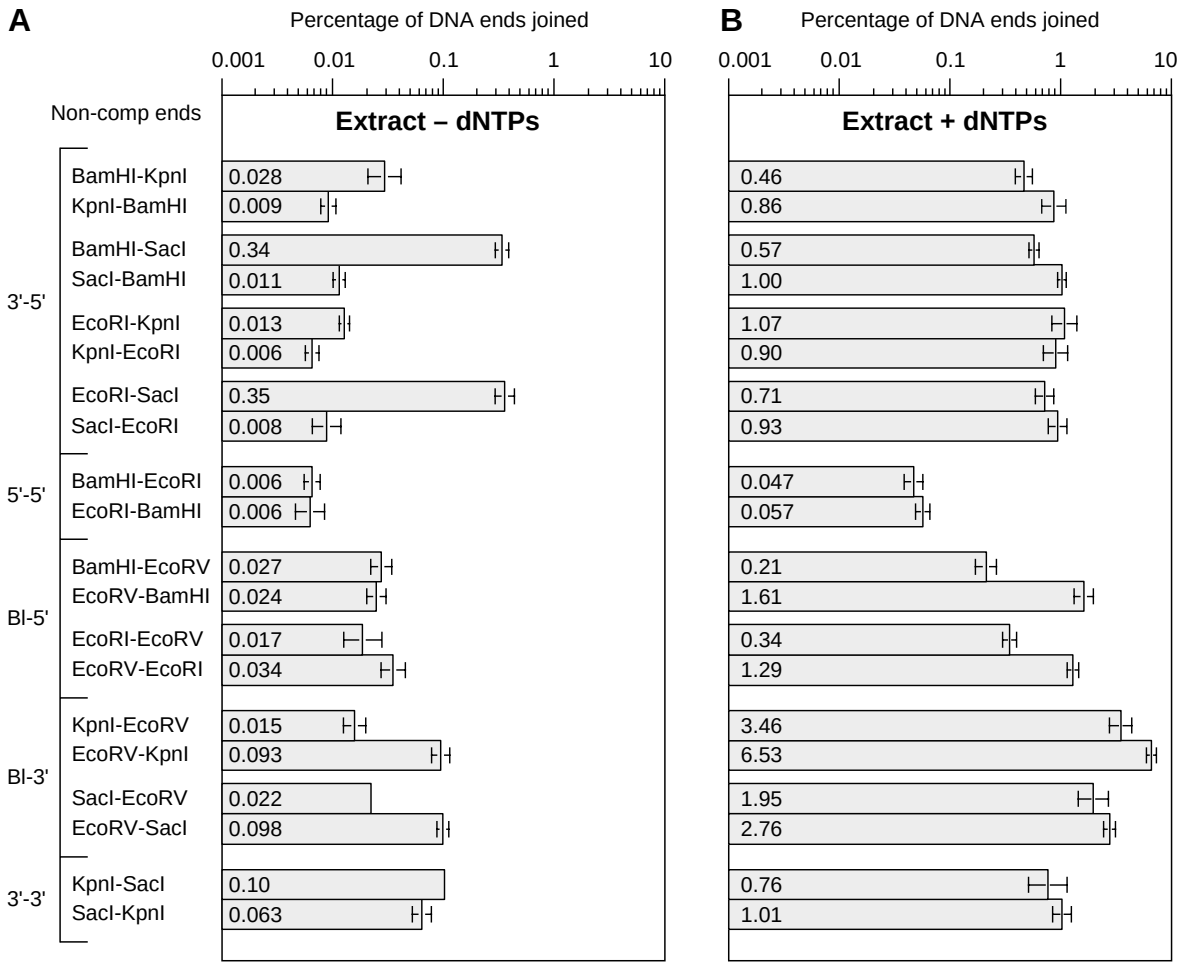
Supplementary Figure 1. Kinetics of NHEJ in vitro.
DNA substrates with compatible (comp) or non-compatible (non-comp) ends were incubated with extract in the presence of added dNTPs. We quenched the reaction at different times, purified the DNA products, and measured joining efficiency by quantitative PCR.

Supplementary Figure 2



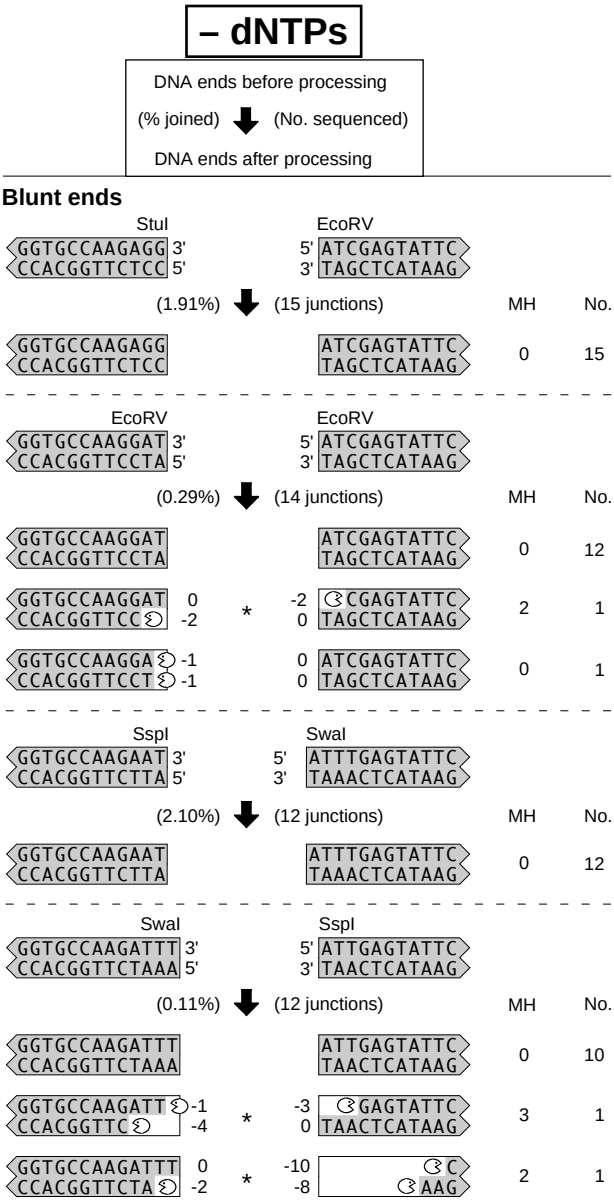
Supplementary Figure 2. Joining efficiency of compatible ends is not affected by dNTPs. DNA substrates with compatible ends were incubated with extract in the presence or absence of added dNTPs. We purified the DNA products and measured joining efficiency by quantitative PCR.

Supplementary Figure 3



Supplementary Figure 3. Joining efficiency of non-compatible ends is influenced by internal DNA sequence. (A) Extract in the absence of added dNTPs, or (B) extract with added dNTPs was incubated with DNA fragments containing non-compatible ends. Joining efficiency was measured by quantitative PCR. The NHEJ reactions are shown in matched sets. In each set of matched reactions, the ends were exchanged relative to the internal DNA sequences.

Supplementary Figure 4



Supplementary Figure 4. Blunt ends are joined without processing. We incubated blunt-ended DNA substrates with extract in the absence of added dNTPs and sequenced the junctions.

Supplemental Material for Materials and Methods

To amplify DNA1 with ends containing cleavage sites for BamHI, EcoRI, KpnI, EcoRV, SacI, StuI, SwaI, SspI, and PmlI, we used the following primers: 5'BamHIDNA1 (CGGGATCCGCCACCATGACTTCGAAAG) and 3'BamHIDNA1 (CGGGATCCTTGGCACCTTCAACAATAG); 5'EcoRIDNA1 (CGGAATTCGCCACCATGACTTCGAAAG) and 3'EcoRIDNA1 (CGGAATTCTTGGCACCTTCAACAATAG); 5'KpnIDNA1 (GGGGTACCGCCACCATGACTTCGAAAG) and 3'KpnIDNA1 (GGGGTACCTTGGCACCTTCAACAATAG); 5'EcoRVDNA1 (GAGATATCGCCACCATGACTTCGAAAG) and 3'EcoRVDNA1 (GAGATATCCTTGGCACCTTCAACAATAG); 5'SacIDNA1 (GGGAGCTCGCCACCATGACTTCGAAAG) and 3'SacIDNA1 (GGGAGCTCTTGGCACCTTCAACAATAG); 5'StuIDNA1 (GAAGGCCTGCCACCATGACTTCGAAAG) and 3'StuIDNA1 (GAAGGCCTCTTGGCACCTTCAACAATAG); 5'SwaIDNA1 (CGTAATTTAAATGCCACCATGACTTCGAAAG) and 3'SwaIDNA1 (CGTAATTTAAATCTTGGCACCTTCAACAATAG); 5'SspIDNA1 (CGTAAATATTGCCACCATGACTTCGAAAG) and 3'SspIDNA1 (CGTAAATATTCTTGGCACCTTCAACAATAG); 5'PmlIDNA1 (CGTACACGTGGCCACCATGACTTCGAAAG) and 3'PmlIDNA1 (CGTACACGTGCTTGGCACCTTCAACAATAG).

To amplify DNA2 with these same six cleavage sites, we used the following primers: 5'BamHIDNA2 (CGGGATCCGAGTATTCAACATTTCCGTGTC) and 3'BamHIDNA2 (CGGGATCCGGTCTGACAGTTACCAATG); 5'EcoRIDNA2 (CGGAATTCGAGTATTCAACATTTCCGTGTC) and 3'EcoRIDNA2 (CGGAATTCGGTCTGACAGTTACCAATG); 5'KpnIDNA2 (GGGGTACCGAGTATTCAACATTTCCGTGTC) and 3'KpnIDNA2 (GGGGTACCGGTCTGACAGTTACCAATG); 5'EcoRVDNA2 (GAGATATCGAGTATTCAACATTTCCGTGTC) and 3'EcoRVDNA2 (GAGATATCGGTCTGACAGTTACCAATG); 5'SacIDNA2

(GGGAGCTCGAGTATTCAACATTTCCGTGTC) and 3'SacIDNA2 (GGGAGCTCGGTCTGACAGTTACCAATG); 5'StuIDNA2 (GAAGGCCTGAGTATTCAACATTTCCGTGTC) and 3'StuIDNA2 (GAAGGCCTTGGTCTGACAGTTACCAATG); 5’SwaIDNA2 (CGTAATTTAAATGAGTATTCAACATTTCCGTGTC) and 3’SwaIDNA2 (CGTAATTTAAATGGTCTGACAGTTACCAATG); 5’SspIDNA2 (CGTAAATATTGAGTATTCAACATTTCCGTGTC) and 3’SspIDNA2 (CGTAAATATTGGTCTGACAGTTACCAATG); 5’PmlIDNA2 (CGTACACGTGGAGTATTCAACATTTCCGTGTC) and 3’PmlIDNA2 (CGTACACGTGGGTCTGACAGTTACCAATG).