



Figure 1. Activities of enzymes commonly used in cloning applications. Straight lines indicate DNA molecules, wavy lines indicate RNA molecules and arrowheads indicate the 3'-end of a nucleic acid molecule. **Panel A:** T4 DNA Ligase catalyzes the joining of two DNA strands between the 5'-phosphate and 3'-hydroxyl groups. T4 DNA Ligase can catalyze the joining of RNA to either a DNA or RNA strand in a duplex molecule but will not join single-stranded nucleic acids. **Panels B and C:** T4 RNA Ligase catalyzes the joining of single-stranded nucleic acids, including RNA/DNA hybrids. T4 RNA Ligase catalyzes the joining of the 5'-phosphate of single-stranded RNA (donor) to the 3'-hydroxyl of single-stranded RNA (acceptor; Panel B). Single-stranded DNA may also serve as a donor but is a poor acceptor (Panel C). **Panel D:** Calf Intestinal Alkaline Phosphatase catalyzes the hydrolysis of 5'-phosphate groups from DNA, RNA and ribo- and deoxyribonucleoside triphosphates. **Panels E-H:** T4 Polynucleotide Kinase has several activities including catalysis of the transfer of a γ -phosphate from a nucleotide triphosphate to the 5'-hydroxyl terminus of single- and double-stranded mono- and polynucleotides (DNA and RNA) by the *forward reaction* (Panels E and F). If excess ADP is present in the reaction the enzyme can catalyze the exchange of phosphates between the γ -phosphate of a NTP and the 5'-phosphate terminus of a DNA molecule by the *exchange reaction* (Panel G). T4 Polynucleotide Kinase also possesses 3'-phosphatase activity (Panel H). **Panel I:** RecA Protein, isolated from *E. coli*, facilitates the pairing of homologous sequences. In the presence of a nonhydrolyzable ATP analog, RecA Protein binds to ssDNA to form a RecA:ssDNA filament. This RecA-coated oligonucleotide can anneal with homologous duplex DNA to form a stable DNA-protein triplex.