



Week 3 Manipulating DNA

Status Done

DNA Ligase

- Most commonly — T4 DNA Ligase (T4 phage)
- Cofactor — ATP
 - forms enzyme-AMP intermediate bound to the 5' phosphate
 - absolute requirement for **5' terminal phosphate**
 - In vivo function (37°) — seals single strand nicks at optimum efficiency

But....

- Blunt Ligations
 - sticky ends stick better at 12~16c, so we compromise.
 - At room temperature — takes several hours (less efficient)

Alkaline Phosphatase

- Removes 5' (terminal) phosphate groups from DNA/ RNA
 - Prevents self-ligation (5' phosphate required for ligation)
 - Why? — bacteria has self-replicating plasmid, and has the tendency to ligate back to it's own strand.

T4 Polynucleotide kinase

- Adds a phosphate to the 5' end of DNA/ RNA
 - Reverses effects of Alkaline Phosphatase

Manipulating Dna Answer Key

**D. Peter Snustad, Harold James
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