

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

Frederick Betz

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Smith, 2022-02-03 Prepare students with complete coverage of the revised Cambridge IGCSETM Biology syllabus 0610 0970 for examination from 2023 Collins Cambridge IGCSE Biology Teacher's Guide is full of lesson ideas practical instructions technicians notes planning support and more New Technical Books New York Public Library, 1992 BBC Worldwide

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