PCR

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What is PCR?

- Polymerase chain reaction
- Invented in 1983 by Kary Mullis
- Used to amplify the amount of a certain DNA, starting from a small amount
 Most used DNA cloning technology today
- Applications:
 - Forensic science (DNA fingerprinting)
 - Molecular genetics (Phylogenetic analysis)
- Possible because of DNA polymerase

A reminder on DNA replication



https://www.nature.com/articles/439542a?draft=collection 2019/6/26

Materials

- DNA polymerases (esp. heat-stable polymerases: *Taq* polymerase etc.)
 Enzymes for synthesis of DNA
- Deoxynucleosides triphosphates (dNTPs)
 - Subunits (building blocks) of the DNA
- DNA primers
 - Short DNA strands to initiate the synthesis process
 - Designed based on sequence of interest
- DNA sample
 - DNA that is to be amplified

Process

- Sample DNA is heated until the strands separate (Denaturation)
- 2) DNA primers are added and the solution is cooled (Annealing)

- Primers will pair with complementary sequences on the sample DNA when cooled

- Taq DNA polymerase synthesises new complementary strands for both strands in 5' to 3' direction (Elongation)
 using dNTPs
 - Taq polymerase does not denature because of its thermal stability
- 4) Steps 1 to 3 are repeated to produce more copies of sample DNA



Dvid L. Nelson and Micfael M.Cox (2017) Lehninger principles of biochemistry W H Freeman & Co pp.301 FIGURE 8-33 https://www.amazon.co.jp/Lehninger-Principles-Biochemistry-David-Nelson/dp/1464126119 2019/6/26

After 20 cycles, the target sequence has been amplified about 10⁶-fold.



Garland Science, Essential Cell Biology 4th edition, Chapter 10: Modern Recombinant DNA Technology, DNA cloning by PCR (page 338)

Why is PCR good?

1. It's fast

2. It's easy

THANK YOU

Resources

1. Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., . . . Walter,

P. (2014). Essential cell biology. New York, NY: Garland Science.

2. Lehninger, A. L., Nelson, D. L., & Cox, M. M. (2013). Principles of

biochemistry. New York: W.H. Freeman.