Polonies

Shendure, J., G. J. Porreca, et al. (2005). "Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome." *Science* **309**: 1728-1732.

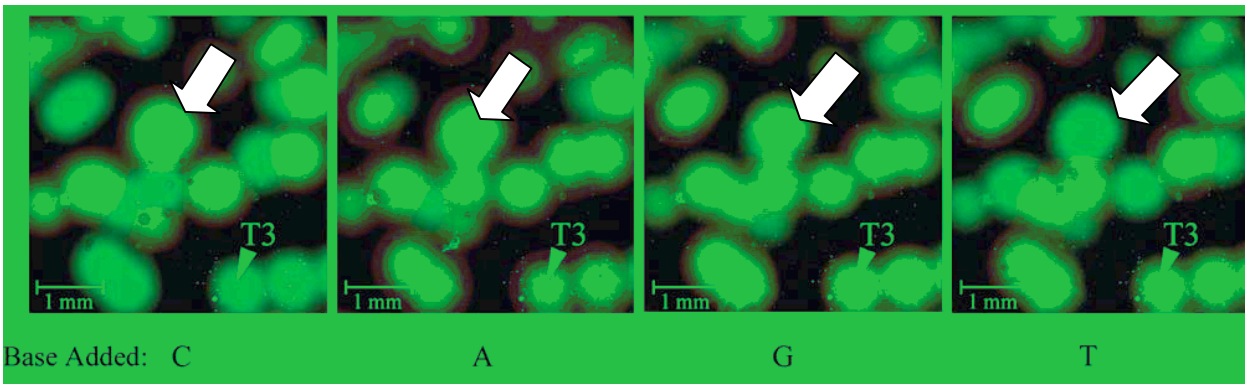
Church, G. M. (2006). "Genomes for all." *Scientific American* **294**(1): 46-54.

Model 1 What is a polony?

1) What is PCR and how does it work?

2) A bead in a polony contains thousands of copies of the same PCR product. Taking that into consideration, what is the advantage of having so many copies of the same strand?

3) When detecting fluorescence, the green channel and the red channel are merged. All the polonies are visible in the green channel, and only the ones fluorescing are visible in the red channel as yellow dots. Taking this into consideration, look at the figure below and determine the sequence of the two polonies marked by the arrows.



Model 2 Two Types of Sequencing

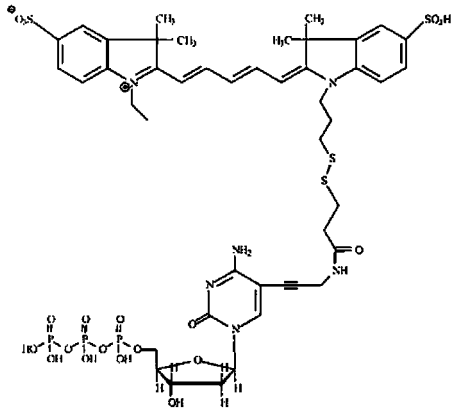
1. What is the function of ligase in DNA replication?

2. Looking at the figures from Church's article in Scientific American, give a brief description of how sequencing by ligation works.

3. Refer to Figure 7 of supplemental material from Shendure's article. After sequencing the fifth base into the tag, we want to sequence the sixth base into the tag. What would we need to remove? Draw the new degenerate nonamers needed.

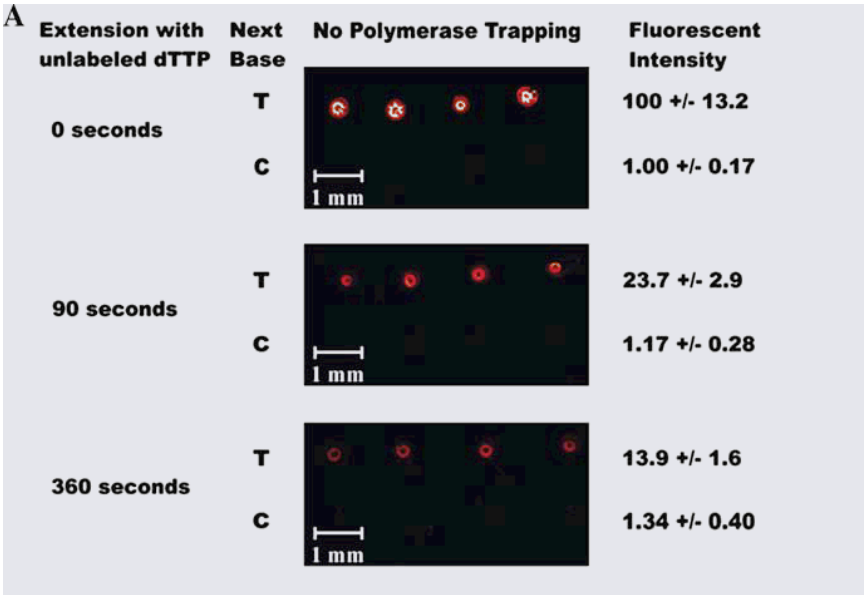
4. Looking at the figures in Church's article, explain how sequencing by base extension works.

5. The structure of Cy5-SS-dCTP (dCTP + fluorophore) is shown below. The structure of Cy5-SS-dNTP would be the same but with its corresponding nucleotide. After each cycle of sequencing by base extension, all fluorescence must be washed away. What might be the easiest way to break up Cy5-SS-dNTP into dNTP and the fluorophore?

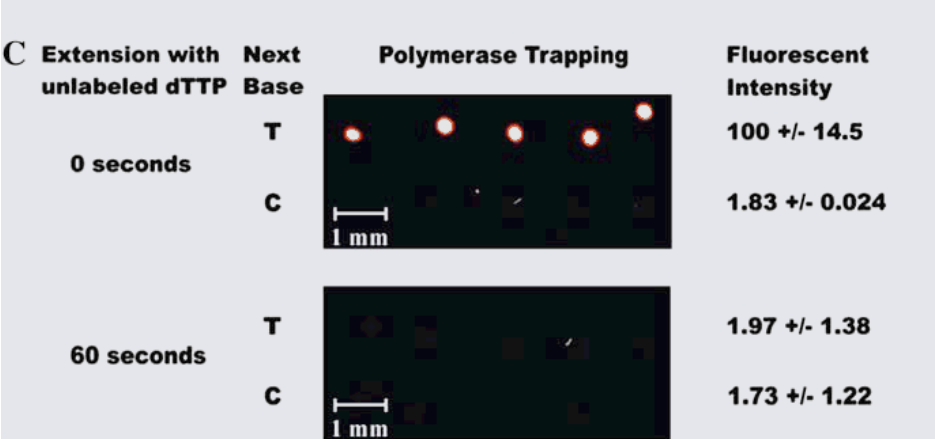


Model 3 Optimizing the sequencing procedure.

1) To quantify the efficiency of polymerase, the authors added unlabeled dNTPs to be incorporated into the complementary strand, and then added labeled dNTPs after a certain amount of time. The results are provided below. Explain the results and determine qualitatively the efficiency of this polymerase.



2) The authors test the efficiency of a technique they called "polymerase trapping" by adding a polyacrylamide matrix to the polymerase. Explain why the polyacrylamide prevents the polymerase from diffusing away from the template/primer. They followed the same procedure to find the efficiency and obtain the results below. Compare these results to those obtained in Q1.



3) Think of how DNA is sequenced using polonies and labeled nucleotides. Consider the sequence ACCGTA to be determined by this method. What result would polony sequencing give? What is the inherent flaw of polony sequencing?

4) Can you think of any solutions to this problem?

5) What are the advantages and disadvantages of polony sequencing compared with other approaches. Is it best suited to *de novo* genome sequencing or re-sequencing?