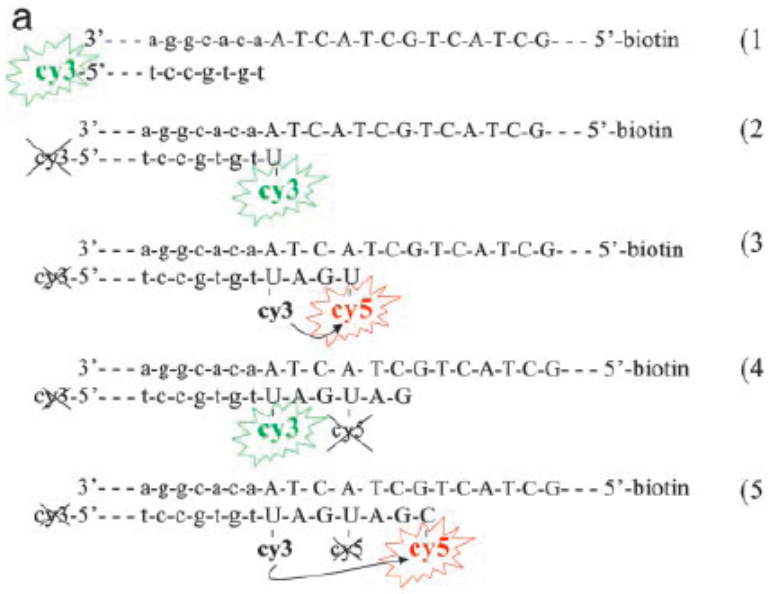
Sequencing by Synthesis: Why FRET about it?

Braslavsky, Ida, et al. (2003)."Sequence information can be obtained from single DNA molecules." *Proc. Natl. Acad. Sci.* **100**(7):3969-3964

MODEL 1 Basics of SBS

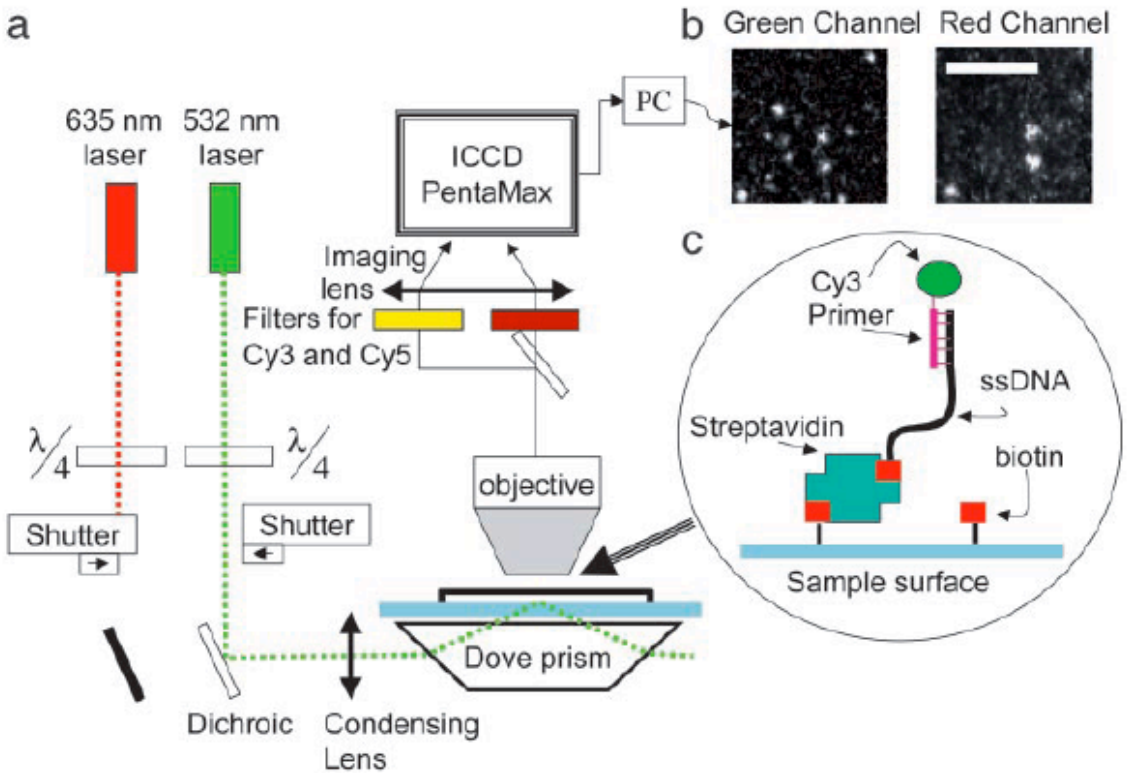


1. Sequencing by synthesis involves attaching a nucleotide-dye complex to a template DNA strand. The dye-complex blocks any further elongation, so in order to sequence the entire strand, the dye molecules must be cleaved by irradiation. This means that each base pair is determined one step at a time: first with one base (A, U, G, or C) and the other three following after rinsing. In one method, only base pairs U and C were dyed. How many different colors would one expect to see with these two? If all four bases are dyed, how many colors does one expect to see?

2. Add only **one** kind of dyed base pair to be incorporated in the DNA template (either dATP, dUTP, dGTP. or dCTP). Cleave the dye molecule from the template strand, rinse, incorporate the next base. Using figure 4 as a reference (and the listed molecules) diagram the steps it would take to sequence the 4 bp strand GATC. I'll get you started:

3'-GATC-5' ⟶ ⟶ ⟶

MODEL 2 Instrumentation



1. Why do the authors use biotin and streptavidin? How do they attach these to the surface? Why doesn't the template bind directly to the surface?

2. What is meant by total internal reflection? Why is it useful in this experiment?

3. Explain the function of each component in the system: λ/4 plates, dichroic, filters, ICCD camera, condensing lens, shutters. What is the advantage of a CCD over a PMT?

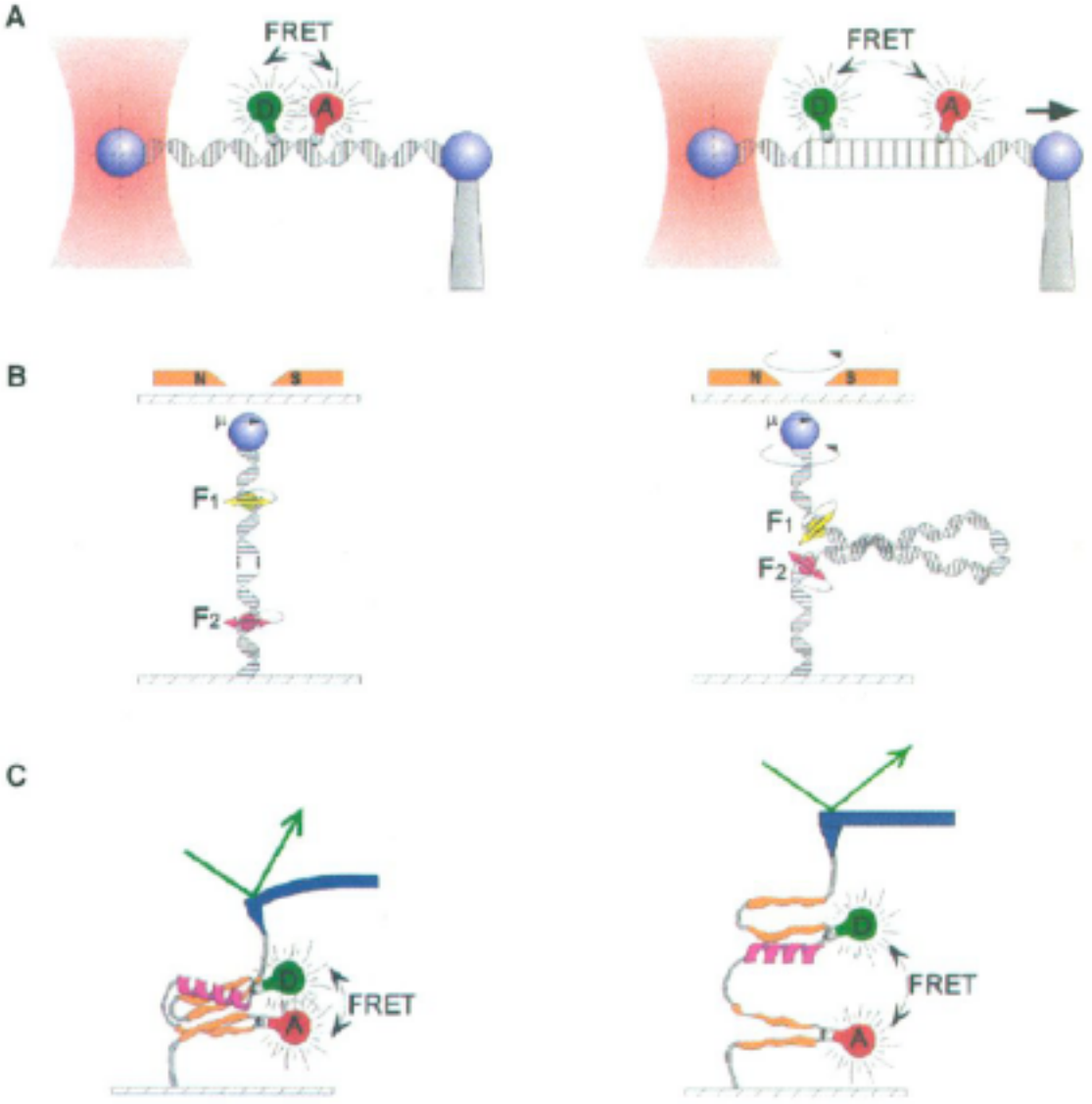
4. What is photobleaching? How is this used in the experiment?

MODEL 3 Why FRET about it?

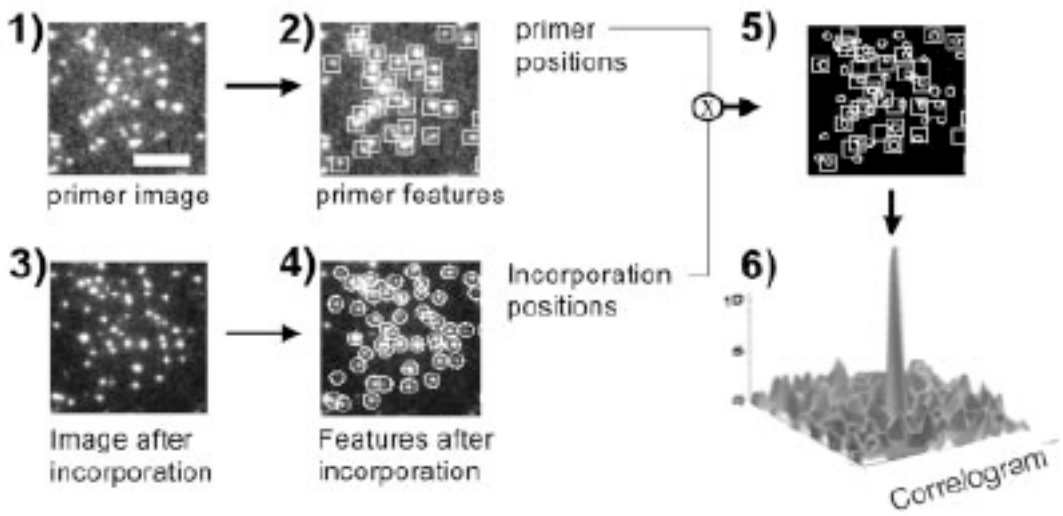
1. What does the acronym FRET represent?

2. Fluorescence indicators are very sensitive to changes in charge, potential, pH, and ion concentration. Using figure 1 as a guide, what are a few uses for fluorescence indicators that take advantage of its chemical and physical properties?

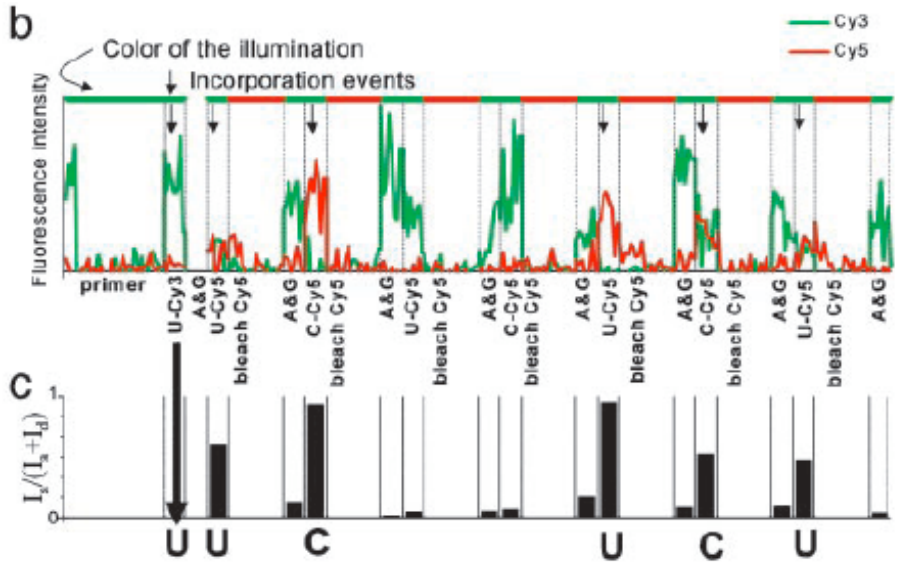
3. Below are illustrations of how FRET can be used to make measurements in different biological processes. Can you infer what the uses of FRET in A and B are illustrating?



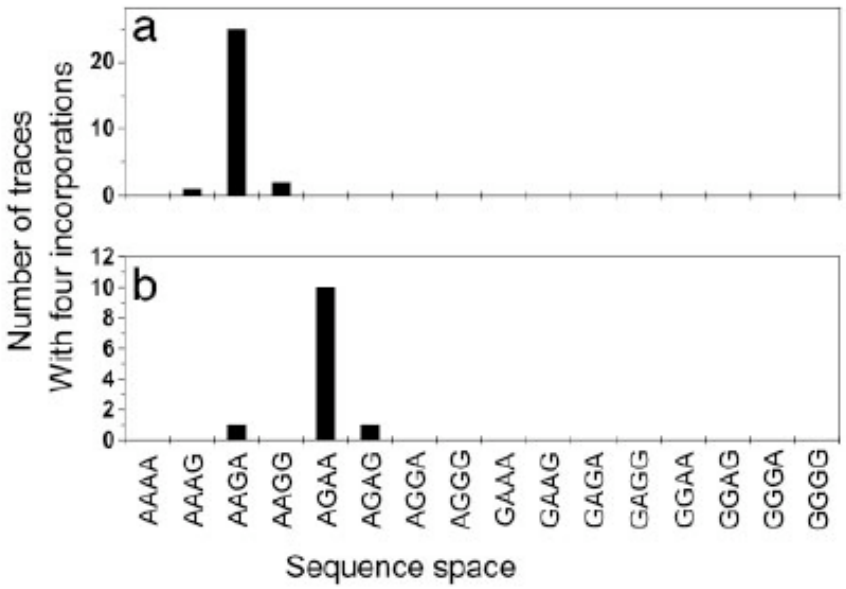
MODEL 4 Data analysis



1. Interpret the correlogram above.



2. Describe the meaning of the axes and the relation of diagram b and c.



3. How did the authors get the data in a and b?

Now you see how many steps it would take to complete an *entire* strand? The advantage however, is that you can place several template strands on a DNA chip and perform this procedure, sequencing the genes *in parallel*. Can you think of any advantages or disadvantages to this process (time, cost, resources, error)?

Think about this: One hundred DNA templates can be placed on a chip that is 100 um in diameter. The use of reagents for this ranges in microliters. With automated scanning, the time it takes is rather short. How many DNA templates can one sequence on a $25mm^2$ chip?

Certain cells in the body express only certain genes because of their function. An eye cell does not express the same combination of genes as a liver cell, even though both cells have the *entire* genome in their nuclei. What could sequencing by synthesis measure in terms of gene expression in cells?