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As I leaned over the light, UV rays bathed my protective face mask, *"Please! Let it show products!"* For many months, I had been working on creating a chimera of my proteins. The lack of bands on the gel meant another failure, and another week of work. This project would be my first and it was not progressing well.

I am working with two proteins: Alpha and Beta. Each protein targets to separate areas of the cell. My goal is to discover what domains in Alpha target it to the membrane. The data I collected over the summer indicates that the N-terminal of Alpha is necessary for membrane targeting. To confirm this hypothesis, we proposed a "swapping" project: replace the N-terminal of another protein, Beta, with that of Alpha to build a chimera. If HeLa cells transfected with this chimera show colocalization of Beta at the membrane then the N-terminal of each is responsible for targeting. A simple polymerase chain reaction (PCR) protocol would do all the work.

I underestimated the whimsical nature of PCR. This simple undertaking proved more difficult than said. At first I tried to make blunt-end pieces of each protein to ligate together. While I received PCR products for each individual piece, the pieces did not ligate properly. "If at first you don't succeed, try, try again." I tried altering the PCR annealing temperature to make it less specific but still no product. Next I tried overlapping PCR to eliminate the need for ligation. In this method, each individual piece contains a portion of the other and so serves as a template for each the other. The same problem persisted. Again I changed the annealing temperature to no avail. I could make the individual Alpha and Beta pieces but the chimera continued to be elusive. After consulting with the post-doctoral fellow, who I work with, we decided to redesign the primers to make them longer. The new primers did not solve the problem. In fact, now I could not even produce the protein fragments.

After months of arduous work, I still possessed no results. The post-doc continued to insist I was doing something wrong. In my mind, this possibility did not exist. I added every necessary ingredient to the mix and in the correct amounts. I double, triple, and quadruple checked my primers and the mix before running the PCR. No reason existed for the experiment to fail and yet it did.

The post-doc wanted to observe me performing the experiment insisting that I must have left out a step or done something wrong. I prepared my lab bench setting out the necessary reagents. I pulled gloves onto my hands and began combining reagents. Because of the small volumes used in the experiment I made sure to observe the reagent in the pipette tips. Having performed the protocol as specified, I turned to my post-doc with a victorious look. He shattered that victory with a single question, "Did you mix it? Do you mix it every time?"

"What mixing?!" Having performed the protocol many times over, I could recite it from memory at any moment. Mixing was not one of the steps. He informed me, much to chagrin, that the mixture must be homogenized because the polymerase is in a glycerol solution, which causes it to sink to the bottom of the reaction tube. As such, the polymerase does not mix with the other elements of the reaction resulting in low concentrations or none of the desired products. Turning back to the PCR tubes, I reached for the p20 and homogenized the mixture. This time I got products.

The saying "if at first you don't succeed, try again" is lacking. If the first attempt does not succeed, find out why. Change something in the next attempt. Only then will knowledge be gained and success one step closer.