MBCB course - tutorial problems week One

Problem 1 – How do enhancers work?

Enhancers work 'at a distance' from the start site of transcription, and may be upstream or down-stream of it. This posed a puzzle to molecular biologists – how did the action at a distance work? Three theories were proposed;

1) Scanning model: A transcription factor, having bound to the enhancer, would then trundle off along the DNA until it found a promoter
2) Twisting model: The binding of a transcription factor to the enhancer altered the twist (superhelicity) of the DNA, and this alteration facilitated transcription down at the start site.
3) Looping model, in which the intervening DNA loops out of the way and proteins at the enhancer are able to bind those at the promoter to form a complex.

Two key experiments were done to settle this issue.

1) Two pieces of DNA were made (by standard cloning techniques). One (X) contained a promoter and a reporter gene. The other (Y) contained an enhancer. One end of X, and one end of Y, were each linked covalently to biotin (vitamin H). Three *in vitro* translation reactions were set up. In one, X and Y were put in together, while in the other, X and Y were put in together and a protein called streptavidin was added to the mix. Streptavidin has the property that it binds biotin (even when the biotin is also bound to DNA), and up to four biotin molecules can bind to each molecule of streptavidin. In the third reaction, streptavidin and X were put together, without any Y. The reaction without the streptavidin, and the reaction with the streptavidin but without Y, failed to produce significant translation. The one with X,Y and streptavidin produced significant translation.

2) Two plasmids were made (by standard cloning techniques). Plasmid A had a promoter coupled to a reporter gene. Plasmid B was the same as plasmid A, except that it also had a region of DNA of a few hundred bases (call it 'Fred') that included an enhancer sequence, and which was followed, after a short spacer, by the complementary sequence of Fred in reverse. {So if Fred were ATGCC then the whole thing would read ATGCC….GGCAT}. The plasmids were cut in one place, denatured into single stranded DNA and were then mixed and allowed to anneal and ligate. Thus the experiment produced A type plasmids, B type plasmids, and plasmids with one A strand and one B strand. When the plasmids were assessed separately in *in vitro* transcription reactions, plasmid A produced little reporter gene, plasmid B a lot, and plasmid A/B also produced quite a lot of transcription.

Between them, these two experiments saw off two of the above hypotheses, leaving the other victorious. Explain how the experiments worked and lead to the conclusions that were drawn from them.

HINT: draw cartoons of the experiments to help you figure this out.