We changed the protocol for many times, but it still did not work well. (Fig4.1-Fig.4.6)
We then tried LCR assembly However, the result was not good. When we realized that we should try direct DNA synthesis, the time was not enough. Because of the unsuccessful DNA construction of p185*her2/neu*-ECD, we did not finish the FRET experiments before the jamboree. Looking back on all the problems we met, we find out that the original experiment plan was too idealized. We also have made some unwise decision due to lack of experience. We realized that to continue the experiment after the competition, **we have to make the experiments more practical for a high school team.** We cannot waste too much time on the DNA construction and protein expression experiments (We have wasted too much on them during these months and have paid the price for our mistakes), since the most important part of our experiment is the FRET part.
We chose SOE PCR as our way of antigen engineering, because we thought it would be a quick and simple method. We had to build 8 mutants of which the DNA sequences were almost the same, and if we chose to use the IDT synthesis we would have to synthesis the 8 similar sequences separately. We thought that we only had to order some primers and do several PCR to obtain the DNA sequence we needed if we use the SOE PCR. However, the p185*her2/neu*-ECD is a human-derived protein whose DNA sequence is very complex and contains high amount of continuous CG, and thus **the SOE PCR did not work.** Though the LCR assembly seems to be a more promising method, **we will not keep wasting time on DNA construction.** To continue, **we will directly synthesize all the sequences we need.**
The epitope of chA21 on p185*her2/neu*-ECD is discontinuous, which means **the affinity will be influenced significantly by protein folding.** We chose to express in pichia yeast because we have to make the folding mechanism as similar to the folding mechanism of human tumor cell as possible. However, the yeast expression system also has its disadvantages: long growing time, complex transformation, selection and inductive expression method.
Yeast expression is not so practical for us, but **we still think it is necessary for our project.** We are considering collaboration with a university which can finish the yeast expression for us. If we have to finish the yeast expression by ourselves, we will change the selection method. We used Zeocin selection but it was not effective enough even when, so we are planning to use auxotrophic selection instead. The transformation method will still be electrotransformation since it is more convenient and effective than chemical method. We still need to study the different methods and find those most suitable ones for us. Briefly speaking, we have to choose **cheap and simple methods which have been proved very effective** in previous studies.
We are not considering changing the FRET experiment. We still think that the FRET experiment will make sense and **we just need to perfect the group arrangement and the data analysis.** We will try our best to get to the FRET experiments and if the effect of the first 8 mutants is not satisfying, we will design more mutants based on the previous study and our structure prediction (both discussed in the Protein Engineering part).

In the last few days we tried to express the SCFV-eGFP fused protein in dh5alfa E.coli. We used 5 different IPTG concentrations (0.05\0.1\0.5\1.0\2.0 μM), but the expression was not detected. **We will use the BL21 strai**n, which is better for expression.