STEPS Students Report

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First, I learned about basic dialysis method and Fluorescence resonance energy transfer (FRET) spectrum. The solution including DNA and Histones were dialyzed in NaCl salt solution step by step, from 2 M to 10 mM, and then the nucleosome reconstitution was obtained. All of the simples were checked by native gel electrophoresis whether they finally recombinant or not. The used DNA has 200~300 bp and is labeled by Cy3 and Cy5 on different position. The FRET data were below.

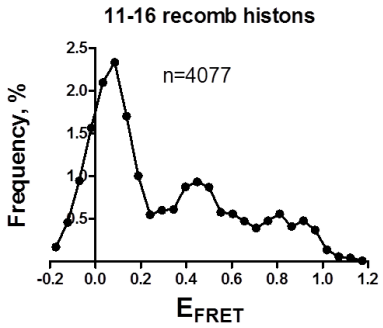

Figure 1: Analyzed spectra of 11-16 template with nucleosome assembled from purified histones.

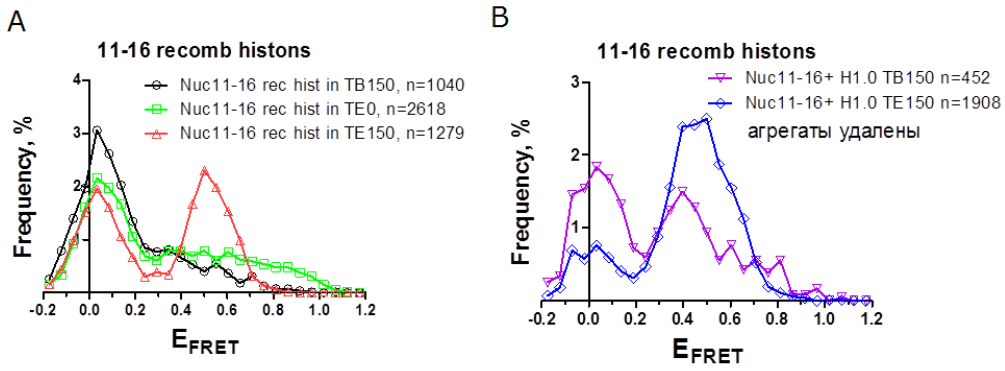

Figure 2: Analyzed spectra of 11-16 template with nucleosome assembled from purified histones in the presence of linker H1.5 histone (1:5 excess).

In Figure 1, the peak of the FRET efficiency is about 0%, hence without Histone, the probe does not work FRET system. This shows the characteristic of the probe. On the other hand, under the presence of the histones, one condition of the probe showed FRET.

In Figure 2A, the probe in TE150 (150 mM of KCl without Mg^{2+}) shows the peak at 0.5, in contrary, the probes without KCl or with Mg^{2+} showed similar peak to the original property. In Figure 2B, the aggregates are removed to obtain single particle. These results suggest that removal of Mg^{2+} contributes the probe structure to compact because the position of DNA linker changes.

What I learned in these experiments or this laboratory was followings. First, I obtained different procedures or viewpoints from our laboratory in Japan. I could confirm again what is important in the procedure and what can be removed or just be simplified. In addition, I can get the experimental tips in the bio-molecule field. There are a lot of differences between our laboratories for instance funds, background or interests but we and they also manage to carry out the experiments and discussion by ourselves. These kinds of difference gave me other viewpoints. The Second gift is, of course, the knowledge especially about the nucleosome and transcription. The professor gave me some essential and fresh papers related to their fields, and the lab doctors taught me procedures and the short lessons about nucleosome. Although the main topic of our laboratory is analysis and detection in the bio-molecule such as G-protein or RNA, the knowledge about the nucleosome must help me with understanding about the intercellular mechanism.