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An improved thermodynamical model of hybridization on high-densit y oligonucleotide microarrays.
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Recently, physico-chemical models of probe-target hybridization on short oligonucleotide microarrays have been studied to estimate expression levels quantitatively from observed probe intensities. However, the complex mixture of the targets provides high level of chemical background, so that researchers have been puzzled by the effect of cross-hybridization. In order to understand the true behavior of probe-target interaction, it is required to observe hybridization under a controlled condition where the effect of cross-hybridization is negligible. To address this issue, we designed a custom microarray for a broad survey of the effect of probe length and types of mismatch, and applied artificially synthesized oligonucleotide as targets instead of genome/transcriptome. The results showed that the intensities saturate at lower level than the Langmuir model expects. In this study, we present an improved thermodynamical model of hybridization that considers the effect of target depletion. We extended the nearest neighbor model that estimate free energy of hybridization from probe sequences and introduced other detail effects, e.g. dependence on a secondary structure of the probe. The model explains the observed data very accurately through a wide range of target concentration from 1 fM to 1 nM. The improved model will help to design useful tools for both genotyping and expression analysis.

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Strategy to evaluate the effect of individual E. coli protein on the protein translation machinery
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Protein translation machinery is one of the most well studied mechanisms in the cell. Major components involved in this machinery have been identified, however, it is yet possible that there are number of unidentified proteins that play a role during the translation reaction. While E. coli is predicted to have more than 4000 different open reading frames, approximately half of these do not have identified functions. In order to fully understand the molecules involved in the E. coli derived translation reaction, we aimed to establish a strategy to evaluate the effect of each individual protein derived from E. coli. This was done by expressing the green fluorescence protein (GFP) as a reporter protein using the in vitro translation system in the presence of individual E. coli derived proteins. It is often the case that some proteins can not be generated in vitro. Indeed, it has been reported that only half of the E. coli derived proteins could be overexpression in E. coli. We overcame this problem by using in vitro translation system, and found that more than 95% of the E. coli proteins could be expressed. In this presentation, we show that the assay can be carried out in 96-well format with highly reproducibility. Moreover, we tested the effect of several well-known E. coli proteins on the translation reaction and found that these proteins function as expected, and thus the assay is applicable for the identification of the components involved in the translation reaction.

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Proteomic analysis of yeast ribosomal protein S10 mutant
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Ribosomes are large ribonucleoprotein particles, consisting of two unequal subunits (40S and 60S), that carry out chemical synthesis. Eukaryotic ribosomes contain one copy of each of four ribosomal RNAs (5S, 5.8S, 18S and 25-28S) and about 75 different ribosomal proteins. Recently, it has been suggested that many ribosomal protein genes might act as haploinsufficient tumor suppressors in zebrafish (Danio rerio). To obtain an insight into the roles of ribosomal proteins related to tumorigenesis, we sought to analyze changes in proteome expression in ribosomal protein mutants using Saccharomyces cerevisiae as a model system. We generated a haploid rps10Δ or rps10Δ mutant, and performed comparative proteome analysis between the wild-type and the mutant cells, using two-dimensional gel electrophoresis and mass spectrometry. We identified several underexpressed proteins in the ribosomal protein S10 mutant cells. Surprisingly, some proteins were found to be overexpressed in the mutant cells. Our results show that ribosomal protein mutation causes perturbation in expression of several proteins, which might form the basis for tumorigenesis in higher eukaryotes.

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Identification of the proteins involved in production of magnetosome in Magnetospirillum magnetotacticum MS-1
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Magnetic bacteria synthesize magnetic particles of 50 - 70 nm diameter, covered with plasma membrane and proteins. They are called as magnetosomes (MG) and form a chain in the cell. Since magnetic particles in the same size are hard to produce artificially, it is of great value to understand MG synthetic system in magnetic bacteria, which is remained unclear. To identify the protein involved in MG synthesis, we compared MS-1 proteins under different culture conditions. The bacteria can produce MG in the medium containing iron, which is named Fe(+). On the other hand, in the medium without iron, the bacteria cannot produce MG, called as Fe(-). Total proteins of both Fe(+) and Fe(-) were separately separated with two-dimensional gel electrophoresis (2D-gel) and compared with each other. The eight and seven of spots detected specifically in Fe(+) and Fe(-), respectively. The membrane, cytoplasm and MG fraction of Fe(+) were prepared and then loaded on 2D-gel. Two of eight spots were found in only cytoplasm fraction, and one, in only MG fraction. The other five spots were found in two or all fractions. It was supposed that these proteins might be involved in MG synthesis. Identification of these proteins are now in progress and we will discuss the relation between these proteins and MG synthetic system.