- 1P266 SNOMによるBACクローン直接マッピング及びAFM切断染色体ナノ断片のDNA増幅
○杉山 滋¹、山内 武志¹、塚本 和巳¹、桑崎 誠剛²、高橋 宏和¹、山本 公子²、大谷 敏郎¹
¹食品総合研究所、²農業生物資源研究所
Direct BAC clone mapping by SNOM and DNA amplification from chromosome nano-fragment dissected by AFM
Shigeru, Sugiyama (1), Takeshi Yamauchi (1), Kazumi Tsukamoto (1), Seigo Kuwazaki (2), Hirokazu Takahashi (1), Kimiko Yamamoto (2) and Toshio Ohtani (1). (1: National Food Research Institute, 2: National Institute of Agrobiological Sciences)

We developed a novel application of scanning probe microscope (SPM) technology, such as scanning near-field optical microscope (SNOM) and atomic force microscopy (AFM), to genome analysis. Here we report the detection of BAC clone position on the chromosome using improved SNOM and the amplification of genomic DNA from nano-size chromosome fragments dissected by AFM. For the target, we adopted silkworm genome since its whole genome analysis project is just on going. Silkworm chromosome in meiotic pachytene phase was used as an experiment material. The high-resolution detection of BAC clone positions on the chromosomes became possible by a newly constructed SNOM system that can simultaneously obtain two color fluorescent images and topography. By the system, very adjacent BAC markers on a silkworm chromosome at the same position on a linkage map were found to be overlapped but had different distributions and center positions. A nanometer-sized chromosome fragments (ca. 300 nm wide) could be reproducibly dissected and recovered by AFM, and its partial nucleotide sequences were successfully amplified with improved DOP-PCR. The amplified DNAs were sequenced and identified as originating from silkworm genome and was used for genomic analysis (see abstract of K. Yamamoto et al.). This work was supported by the funds from the Bio-Oriented Technology Research Advancement Institution (BRAIN).

- 1P268 タイリングアレイを用いた大腸菌トランスクリプトームの定量的解析
○小野 直亮¹、鈴木 真吾²、縣 朋治²、古澤 力^{1,2}、清水 浩²、四方 哲也^{2,3}
¹JST・ERATO、²阪大院・情報科学、³阪大院・生命機能
Quantitative analysis of Escherichia coli Transcriptome using A Full tiling Microarray
Naoaki ONO (1), Shingo SUZUKI (2), Tomoharu AGATA (2), Chikara FURUSAWA (1,2), Hiroshi SHIMIZU (2), Tetsuya YOMO (1,2,3). (1: ERATO, JST, 2: Dept Bioinfo Eng, Osaka Univ, 3: Grad School of Frontier Biosciences, Osaka Univ)

DNA microarray have been used for a wide range of DNA/RNA analysis. Recently, high density oligonucleotide arrays allow us to measure the expression of all genes by a single experiment. In this study, we designed a tiling microarray that covers the whole genome of Escherichia coli, in order to investigate detail information of expression patterns. Furthermore, we developed a new data analysis method addressing more quantitative measurement. This model is based on thermodynamical representation of probe-target hybridization. By taking depletion of both probe and target molecules into account, the dynamic range of the measurement was widely expanded. We also introduced the effects of dissociation and other interactions between probe and target molecules in order to increase the accuracy. The results of spike-in control experiment show that our method measures gene expression level precisely from 0.01 ~ 10000 mRNA molecules per cell. Using this method, we investigate changes of expression patterns in E. coli in the adaptation to several different environments in detail. The method will provide us useful tools for analysis of expression dynamics.

- 1P267 走査型プローブ顕微鏡で回収したナノサイズ染色体断片を用いた局所的ゲノム解析法
○山本 公子¹、桑崎 誠剛¹、塚本 和巳²、末次 克行¹、生川 潤子¹、大谷 敏郎²、杉山 滋²
¹農業生物資源研究所、²食品総合研究所
Region focusing genome analysis using nano-size chromosome fragment dissected by scanning probe microscopy.
Kimiko Yamamoto (1), Seigo Kuwazaki (1), Kazumi Tsukamoto (2), Yoshitaka Suetsugu (1), Junko Narukawa (1), Toshio Ohtani (2) and Shigeru Sugiyama (2). (1: National Institute of Agrobiological Sciences, 2: National Food Research Institute)

Scanning probe microscopy (SPM), such as atomic force microscopy (AFM), can obtain topographic images of bio-materials with nanometer scale resolution without any staining nor coating. We have developed a technique that dissects and recovers the DNA at specific target regions of a silkworm chromosome with a width of approx. 300 nm by AFM. The dissected genomic DNA fragments were amplified by a modified DOP-PCR method (see abstract of S. Sugiyama et al).

We succeeded the sequencing of the amplification products. Using the obtained sequence information, we screened a BAC clone that includes the target genome region, and searched a single nucleotide polymorphism (SNP) in the region between two silkworm strains. Then we performed a linkage analysis and successfully created a new linkage marker on the linkage map. The neighboring BACs were screened from 3D-pool of BAC library by walking with end-sequence of the first selected BAC clone, and we could construct a partial BAC physical map of the target genome region. Our newly developed technology will be very useful tool for local or point analyses of desired regions on the genomes and for accelerating the present genome analysis. This research is supported by a fund from Bio-oriented Technology Research Advancement Institution (BRAIN).

- 1P269 酵素を使わない光ライゲーション法の開発と応用
○吉村 嘉永²、網 健裕¹、荻野 雅之²、藤本 健造^{1,2}
¹北陸先端大マテリアル、²JST
Development and application of non-enzymatic DNA photoligation
Yoshinaga Yoshimura (2), Takehiro Ami (1), Masayuki Ogino (2) and Kenzo Fujimoto (1, 2). (1: School of Materials Science, Japan Advanced Institute of Science and Technology; 2: JST)

While many methods for template-directed chemical ligation of oligonucleotides via a native phosphodiester bond or non-native linkages have been demonstrated, there are only a few methods for photoinduced non-enzymatic chemical ligation. The merit of the photochemical ligation avoiding the need for additional reagents is obvious. Their actions are controllable within space and time by the choice of proper irradiation methods. However, these methods have serious problems for practical applications, such as low yields of photoligation, the use of short wavelength that is injurious to other components, the occurrence of undesirable photocrosslinking reaction and the lack of photoreversibility. In our continuing efforts to develop a novel method for template-directed DNA photoligation, we demonstrated an efficient and reversible template-directed photoligation of ODNs using 5-substituted pyrimidine analogues. By the use of ODNs containing modified pyrimidine nucleosides at the 5 end or 3 end, we have succeeded in the synthesis of complex DNA structures, such as tandem ligated DNA, branched DNA or padlocked plasmid DNA at desired sites, and the reversible photocleavage of the ligated DNA by photoirradiation at a shorter wavelength. Therefore, our photoligation system using vinyl-substituted pyrimidine nucleosides can be a powerful tool for easy and accurate DNA handling, leading to the development of new DNA nanotechnology as represented by DNA chips and DNA computers.