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Automated Genotyping System Using Bio-Nanomagnetic Beads

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Molecular genotyping has been important tool for molecular epidemiological approaches for analysis of disease and detection/identification of organisms in the fields of health and safety, environmental monitoring, food material evaluation and so on. Furthermore, a number of samples for the analysis with high throughput systems and simple and accurate methods have been required. We present our fully automated throughput system using bio-nanomagnetic beads for analyzing SNP in transforming growth factor- β1 (TGF- β 1) gene.

Bio-nanomagnetic beads

Bio-nanomagnetic beads (bacterial magnetic particles; BMPs, Fig.1) isolated from the magnetic bacterium *Magnetospirillum magneticum* AMB-1 (1) are small in size (50-100 nm) and are surrounded by a phospholipids bilayer membrane. Analyses in genomics, proteomics and mutagenesis led several hypotheses for biomagnetite formation processes which include vesicle formation, iron transporter across the vesicle membrane, oxidation/reduction of iron and crystallization (2).

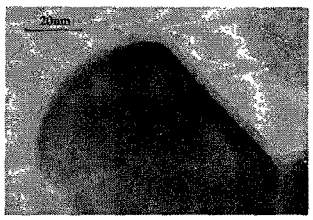

Fig.1 Photograph of BMP

BMPs offer high technological potential since these can be conveniently manipulated with magnetic force. The thin organic membrane enveloping the individual BMPs confers high and even dispersion in aqueous solutions compared to artificial magnetites making them ideal biotechnological materials. Furthermore, the findings in the molecular biological research for elucidation of BMP formation developed a superior strategy for novel new nanomaterials where functional proteins such as enzyme, antibody, and receptor were assembled on BMPs by using proteins isolated from BMP membrane as anchor. These BMPs have already been successfully used in immunoassay (3-5) and for DNA detection (6-11). Furthermore, it was reported that DNA extraction from whole blood was successfully performed efficiently using dendrimer-modified BMPs (12). Furthermore, seven-transmembrane proteins, G protein-coupled receptors (GPCRs) were also successfully assembled onto BMPs. GPCRs

play central roles in a wide range of biological processes and therefore may provide tremendous pharmaceutical potential (13). We have also immobilized various functional biomolecule using appropriate chemical cross-linking reagents targeting membrane amino-groups on BMPs.

Automated high-throughput SNP detection system using BMPs

Significant result of human genome project is decoding of human genome sequences completed in April 2003 and huge genetic data was built up. Single nucleotide polymorphisms (SNPs) are estimated to occur at 1 out of every 1,000 bases and some SNP databases containing several million of SNPs were constructed for analysis of cause of disease. Furthermore, analyzing SNP alleles in population-based studies to identify loci that are associated with a particular disease or phenotype using genetic statistics is the trend in the clinical investigator. The number of samples for the analysis in disease-associated study is important for reliability of the data. High-throughput SNP discrimination based on hybridization methods using BMPs was performed using automated detection robot (Fig.2) (14). This system has been developed into full automated including DNA extraction step from whole blood using dendrimer-BMPs, DNA hybridization and detection. In this study, SNP (869T→C: Leu10Pro) in transforming growth factor- β1 (TGF- β 1) gene were targeted, which is the cytokine concerned with bone remodeling and a candidate of genetic maker for hereditary risk of decrease in the bone mass. Although this gene has high GC content and short tandem repeat sequences at SNP site, accurate SNP detection with enough S/N ratio in 800 samples has been obtained (Fig. 3).

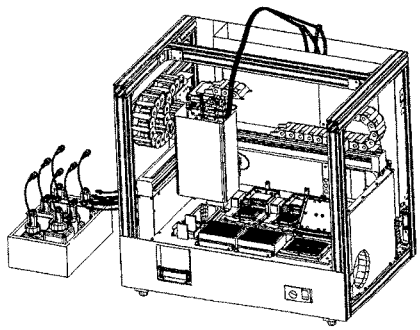

Fig.2 Fully Automated SNP detection robot

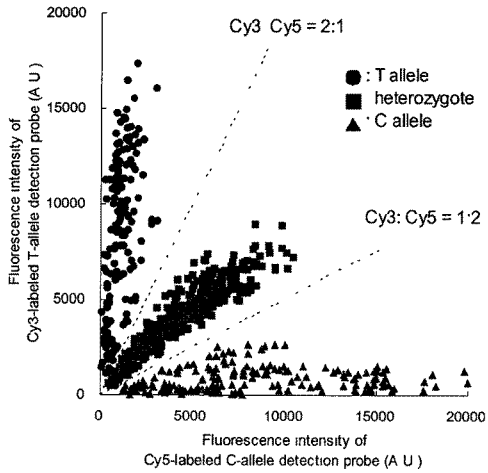

Fig.3 SNP detection in TGF-β1 using automated SNP detection system with BMPs.

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