N-66

Development of new premix-type DNA ligases: advantages of premix-type DNA ligase over solution-type DNA ligase

Jin-Su Park, Seong-Youl Kim, Han-Oh Park and Hae-Joon Park
Molecular diagnostic research center, Bioneer corporation, Daejeon, 306-220, Korea

DNA ligases have been used extensively in molecular biology laboratories for DNA recombination, repair and replication experiments. DNA ligases catalyze the formation of phosphodiester bonds that can link together DNA strands with double-strand breaks. Most of commercially available DNA Ligases are solution-type products. However, these products have several disadvantages in terms of long-term stability, the possibility of cross-contamination, inconvenience of use. In this study, we developed new premix-type DNA ligases (AccuPower Ligation PreMix) and compared biochemical properties of premix-type DNA ligase with other commercially available solution-type DNA ligases. Materials required for ligation reaction, such as T4 DNA ligase and optimal ligation buffer, were added in one tube and then dried. The compositions of optimal stabilizers were tested to get improved stability. Over 95 percentage of DNA ligase activity retained over 24 months at -20°C. Activitiy of premix-type DNA ligase showed the equal or more extent, compared to those of solution-type DNA ligases. Our data suggests that AccuPower Ligation PreMix can provide more convenient and reliable ligation reactions without cross-contamination of samples for long-term usage, compared to solution-type DNA ligases.

N-67

Development of industrial culture medium to give high yield spore number and sporulation efficiency in Bacillus subtilis

Uyangaa Temuujin, Bayarbat Ishvaanjil, Joong-Kook Park, Chang-Hyun Kim and Soon-Youl Lee
Department of Genetic Information, Graduate School of Bio and Information Technology, Faculty of animal life and environment science and College of Agriculture & Life Science, School of Applied Life Science, Hankyong National University

The genus Bacillus subtilis is considered as a model endospore-forming Gram-positive bacterium for prokayotic developmental studies and also used widely for industrial purpose. We investigated optimal condition of culture medium for industrial purpose to give high yield spore number and sporulation efficiency in Bacillus subtilis. A study has been made on small and large-scale production of this investigating medium. We found that the highest total cell number and spore number was obtained at the glucose concentration of 1 g/l with the Bacillus subtilis strain we investigated. Also, three kinds of organic nitrogen sources (yeast extract, peptone, casein acid hydrolysate) were investigated at the various concentrations on Bacillus subtilis with the carbon source fixed. The best result regarding nitrogen source was obtained with yeast extract of 16 g/l. Studies of the cultivation conditions led to the maximum concentration of total cell from 1.00*108 to 3.36*108cells/ml and the spore concentration from 0.14*108 to 0.22*108cells/ml. And the sporulation efficiency was 68%- 80% in the Bacillus subtilis strain we investigated. The best result was obtained, when the culture temperature was 300C rather than 370C temperature. When we determine the enzyme activities of supernatant of Bacillus subtilis in different medium, we found that protease activities and xylanase activities are not changed much. However, the activity of amylase increased with the concentration of glucose increased.

N-68

Development of cell-based protease assay using HCV NS3 protease as a model system

Jeong Hee Kim[1,2], Min Jeon Lee[2], and Hyun Jin Hwang[3]
[1]Dept. of Biochemistry, College of Dentistry, [2]Dept. of Nano-Pharmaceutical and Life Sciences, Kyung Hee University and [3]R&D center, Ahram Biosystems Inc.

Nonstructural protein 3 (NS3) from hepatitis C virus (HCV) is a serine protease which plays a critical role in maturation of virus by cleaving the nonstructural regions of the viral proteins. Therefore, the NS3 protease of HCV has been a major target for the development of drugs against HCV infection. In the study, a sensitive subcellular translocation protease assay system has been developed that enables direct fluorescence detection of the NS3 protease and its inhibition inside living cells. This system provides a fluorescent molecular beacon protein that is designed to change its intracellular location upon cleavage by the NS3 protease, e.g., from cytosol to a subcellular organelle, or from a subcellular organelle to cytosol or another subcellular organelle. Details of the intracellular translocation mechanism and level of the protease action can be monitored at a single cell level. Clear change in the fluorescent image of the cell was observed with this assay system. The dose-response curve acquired with a known compound confirms that this novel in-cell assay system can be an ideal tool for a broad range of life science and drug discovery research

N-69

Development and characterization of monoclonal antibody for glycated antibody

Ju-Ram Jang, So-Hyeon Kim and Gun-Sik Tae
Dept. of Biology, Dankook University, Cheonan, 330-714

The glycated hemoglobin (HbA1c) can be used as an indicator of the diabetes state. The monoclonal antibody was, therefore, generated against HbA1c in this study, which can be used as the raw material to manufacture the immunodiagnostic strip or biosensor to detect the excreted HbA1c in blood from diabetes. The glycated N-terminal decapeptide of the HbA1c β-subunit was synthesized as antigen. First, the valine, the amino terminal residue of decapeptide (V-H-L-T-P-E-E-K-Y-Y), was incubated with D(+)-Glucose at room temperature for 4 days and its adduct was recrystallized with methanol. The glucose moiety has experienced the molecular rearrangement and converted to fructose. Then, the fructosyl-valine adduct was linked via peptide bond to the rest part of the decapeptide (H-L-T-P-E-E-K-Y-Y). The molecular weights of the fructosyl-valine adduct and the final glycated decapeptide were measured by mass spectroscopy after HPLC separations. The glycated decapeptide was conjugated to bovine serum albumin (BSA) or ovalbumin (OVA) with DCC and injected into BALB/c. The hybridoma cell lines, producing monoclonal antibody for HbA1c, were generated by the cell fusion of a myeloma cell (SP2/0) and a lymphocyte from the BALB/c immunized with the glycated decapeptide. The titers of monoclonal antibodies were high enough to go through the purification and characterizations. The results will be posted.

N-70

Cosmeceutical activity of Isatis tinctoria L.

Yeon-Kyoung Hwang, Su-Hyen Jung, Woo-A Jo[1], Young-Hun Kim, Jun-Sook Lee, Soon-Ju Cheon, Min-Jung Jang, Ji-Yeun Sung, Whan-Sin Oh, Won-Dae Ji[2], Hyang-Ja Choi[3], Dae-Ik Kim[4], Jung-Ok Kim[4], Jin-Tae Lee
Department of Cosmeceutical Science, Daegu Haany University, Gyeongbuk, Republic of Korea, [1]Department of Cosmetology Science, Nambu University, Gwangju, Republic of Korea, [2]Department of Innovation Supporting, Daegu Regional Innovation Agency, Republic of Korea, [3]Soriso Cosmetic Ltd., [4]Daegu Bio Industry Center

The purpose of this experiment was to determine cosmeceutical properties of Isatis tinctoria L. through 1,1-diphenyl-2-picrylhydrazyl (DPPH), Xanthine oxidase inhibition, Tyrosinase inhibition, and Nitrite scavenging ability. For preparing the samples, Isatis tinctoria L. was extracted three times in different solutions and temperatures, such as 70% Ethanol (E) at room temperature, Hot Water (HW) at 850C, and Supercritical Fluid (SF). In the test of DPPH, the scavenging ability of Isatis tinctoria L extracts with E, HW, and SF was showed to 66.8, 72.4, and 92.6 % at 5,000 ppm concentration, respectively. In the test of xanthine oxidase, the inhibition ability of Isatis tinctoria L. extracts with E, HW, and SF was determined 73.8, 61.6, and 98.6 % at 5,000 ppm concentration, respectively. Tyrosinase inhibition of Isatis tinctoria L. at 5,000 ppm was indicated 79.7, 71.7, and 91.2 % at E, HW, and SF, respectively. In the test of Nitrite scavenging ability, the scavenging ability of Isatis tinctoria L extracts with E, HW, and SF was showed 70.8, 92.8, and 68.6 % at 5,000 ppm concentration, respectively. Based on the results, it demonstrated that Isatis tinctoria L. has a sufficient potentiality to apply in the industry and can be utilized as natural materials in cosmetics.

N-71

Comparison of three different methods to prepare avidin-containing QCM sensor chip

Saem Mun, Borah Kim, So Young Sim, Kyunghwan Hyun and Suk-Jung Choi
Department of Chemistry, Kangnung National University, Gangneung 210-702, Korea

The highly specific interaction of avidin with biotin can be a useful tool in designing detection systems. Especially, avidin-containing biosensor chips have been widely used to capture biotinylated receptors. Here, we prepared and compared three types of quartz crystal microbalance (QCM) sensor chip with avidin immobilized on their surfaces. First, avidin was chemically immobilized through N-hydroxysuccinimide group presented on the self-assembled monolayer (SAM) surface. Second, avidin was attached to the biotin immobilized on the SAM surface. Third, lipid monolayer was prepared on the hydrophobic SAM and avidin was attached to the biotin incorporated in the lipid monolayer.