Project-based Training Course (280995)
International Program of Frontier Biotechnology
Autumn semester 2010

List of laboratories :

1. Fujiyama Laboratory (2 students)
   Supervisor : Assist. Prof. Takao Ohashi (ohashi@icb.osaka-u.ac.jp)
   Students : Topic 1 – Poonperm Rawin (Rawin), Topic 2 – Permanasari Etin Diah (Etin)

   Topic 1 : Analysis of localization of glycosyltransferase from the fission yeast
   Schedule :
   Day 1 : Transformation of green fluorescent protein (GFP)-fused glycosyltransferase into the fission yeast
   Day 2 : Picking up the transformants
   Day 3 : Cultivation of the transformants
   Day 4 : SDS-PAGE and observation by fluorescence microscopy
   Day 5 : Western blotting

   Topic 2 : Quantification of lactic acid produced by Lactobacillus using lactose dehydrogenase
   Schedule :
   Day 1 : Preparation of the buffers for enzymatic reaction
   Day 2 : Examine the effects for reaction time and inoculation of the Lactobacillus into the milk
   Day 3 : Examine the effects for the amounts of the enzyme and lactic acid concentrations
   Day 4 : Examine the effects for pH
   Day 5 : Quantification of lactic acid produced by Lactobacillus

2. Fukui Laboratory (2 students)
   Supervisor : Assoc. Prof. Sachihiro Matsunaga (sachi@bio.eng.osaka-u.ac.jp)
   Student : Ariyasrijit Chananya (Belle)

   Topic : Functional analyses of ASURA
   Schedule :
   Day 1 : Preparation of HeLa cells
   Day 2 : Preparation of cell lysates
   Day 3 : Western Blotting
   Day 4 : Immunostaining
   Day 5 : Data analyses

3. Fukusaki Laboratory (2 students)
   Supervisor : Assist. Prof. Hisayo Ono (ono@bio.eng.osaka-u.ac.jp)
   Students : Topic 1 - Peerakietkhajorn Saranya (Tip), Topic 2 – Jung Jieun (Jieun)

   Topic 1 : Metabolic profiling of different tea leave products, green tea, black tea, and oolong tea, based on gas chromatography/mass spectrometry (GC/MS)
   Schedule :
   Day 1 : Extraction of metabolites of tea leaves
   Day 2 : Derivatization and GC/MS analysis
   Day 3 : Data preprocessing for multivariate analysis
   Day 4 : Data analysis (multivariate analysis): Principle component analysis
   Day 5 : Data analysis (metabolite identification)

Topic 2： Metabolic profiling of ranked Japanese green tea based on gas chromatography/ mass spectrometry (GC/MS)
Schedule：
Day 1： Extraction of metabolites of tea leaves
Day 2： Derivatization and GC/MS analysis
Day 3： Data preprocessing for multivariate analysis
Day 4： Data analysis (multivariate analysis): Principle component analysis
Day 5： Data analysis (metabolite identification)

4. Harashima Laboratory (2 students)
Supervisor： Assoc. Prof. Yoshinobu Kaneko (kaneko@bio.eng.osaka-u.ac.jp)
Students： Topic 1 – Suandi Pratama Sultan (Andi), Topic 2 – Ly Nhung Hong (Nhung)

Topic 1： Isolation of yeast auxotrophic mutant by UV mutangenesis

Schedule：
Day 1： Prepreation of media and inculation of wild-type strain
Day 2： UV irradiation
Day 3： Incubation
Day 4： Replica plating
Day 5： Judgement of auxotrophy

Topic 2： Knockout yeast gene

Schedule：
Day 1： Preparation of media and inoculation of host strain
Day 2： Preparation of disruption cassette by PCR
Day 3： Transformation
Day 4： Incubation
Day 5： Picking up candidate and check by colony PCR

5. Kanaya Laboratory (2 students)
Supervisor： S. A. Assist. Prof. Eiko Kanaya (ekanaya@mls.eng.osaka-u.ac.jp)
Student： Topic 1 – Salayev Ahmed (Ahmed), Topic 2 – no one

Topic 1： Production and thermal denaturation of Tma-RNase H1

Schedule：
Day 1： Media preparation and pre-culture of Tma-RNase H1
Day 2： Overproduction of Tma-RNase H1
Day 3： Cell harvest, cell disruption, cell supernatant separation, dialysis, SDS-PAGE preparation
Day 4： Purification by cation and affinity column chromatography and SDS-PAGE analysis
Day 5： CD spectra and thermal denaturation of Tma-RNase H1

Topic 2： Chaperone function of SIB1 FKBP22, a peptidyl prolyl cis-trans isomerase from psychrotophic bacterium

Schedule：
Day 1： Overproduction SIB1 FKBP22
Day 2： Purification 1: Affinity chromatopgarhy (Ni2+ column); dialysis; gel filtration wash
Day 3： Purification 2: Gel filtration, dialysis

Day 4： Chaperone function 1： binding affinity to a folding intermediate protein
Day 5： Chaperone function 2： aggregation prevention

6. Kino-oka Laboratory (1 student)
Supervisor： Assist. Prof. Mee-Hae Kim (mh-kim@bio.eng.osaka-u.ac.jp)
Student： no one

Topic： Understanding the animal cell culture and characterization of cell properties
Schedule：
Day1： Culture of human fibroblasts
Day2： Taking cell pictures and counting number of adherent cells
Day3： Changing medium
Day4： Staining of F-actin and observation by fluorescence microscopy
Day5： Image and data analysis: cell attachment, morphology, and proliferation

7. Muranaka Laboratory (1 student)
Supervisor： Assist. Prof. Atsushi Okazawa (okazawa@bio.eng.osaka-u.ac.jp)
Student： Limkul Juthamard (Jah)

Topic： Cloning of cellulase from parasitic plant, Orobanche minor
Schedule：
Day 1： cDNA preparation from haustoria of O. minor
Day 2： PCR of partial sequences of cellulase genes
Day 3： RACE of the genes
Day 4： Cloning of full length genes
Day 5： Transformation (E. coli)

8. Nihira Laboratory (2 students)
Supervisor： Assist. Prof. Shigeru Kitani (kitani@icb.osaka-u.ac.jp)
Students： Maria Odaise Silva dos Santos (Daise), Jumhawan Udi (Udi)

Topic： Measurement and detection of microbial secondary metabolites
Schedule：
Day 1： Preparation of medium and inoculation
Day 2： Bioassay
Day 3： Extraction with organic solvent and evaporation
Day 4： HPLC detection
Day 5： TLC detection

9. Watanabe Hajime Laboratory (2 students)
Supervisor： Assist. Prof. Koji Tsukada (tsukada_koji@bio.eng.osaka-u.ac.jp)
Students： Hong Xun(Hong), Natesuntorn Waranya (Sine)

Topic： Study of the anti-virus effect of silver (Ag-Zeolyte) which was known as a bacteriostatic substance and electron microscopic study of natural coliphage species around us.
Schedule：
Day 1： Preparation of culture medium, agar plate, *E. coli* cells, and RNA phage. Sampling and partial purification of some phage samples (natural water) in our university.
Day 2： Training of basic procedures of virus research： Plaque assay, one-step growth experiment and single burst experiment.

Day 3 : Evaluation of Ag-zeolyte effect on the bacterial growth, the infectivity of RNA coliphage Qβ, and some natural phages.
Day 4 : Preparation of specimens for electron microscopic observation of phages and phage-infected cells.
Day 5 : Transmission electron microscopy observation of the shape of phage particles and infected bacterial cells by using a negative staining.