

**Project-based Training Course (280995)**  
International Program of Frontier Biotechnology  
Autumn semester 2009

Dr. Clement Angkawidjaja ([clement@bio.mls.eng.osaka-u.ac.jp](mailto:clement@bio.mls.eng.osaka-u.ac.jp))

List of laboratories:

1. Fujiyama Laboratory (2 students)

Supervisor: Assist. Prof. Ryo Misaki ([misaki@icb.osaka-u.ac.jp](mailto:misaki@icb.osaka-u.ac.jp))

Students: Gulseren Gulcihan (Gul), Roslan Farah Wahidah (Farah)

Topic 1: Analysis of localization of glycosyltransferase from plant

Schedule:

Day 1: Transformation of tobacco suspension-cultured cells to express green fluorescent protein (GFP)-fused glycosyltransferase transmembrane domain

Day 2: Observation by fluorescence microscopy

Day 3: Fractionation and preparation of cytosol and microsomes

Day 4: SDS-PAGE

Day 5: Western blotting

Topic 2: Characterization of chimeric plant-glycosyltransferase

Day 1: Transformation of tobacco plant to express chimeric glycosyltransferase

Day 2: Preparation of fluorescence labeled sugar chain

Day 3: Fractionation and preparation of cytosol and microsomes

Day 4: Assay of glycosyltransferase activity by HPLC

Day 5: Determination of glycan structures

2. Fukui Laboratory (2 students)

Supervisor: Assoc. Prof. Sachihiro Matsunaga ([sachi@bio.eng.osaka-u.ac.jp](mailto:sachi@bio.eng.osaka-u.ac.jp))

Students: Lee Kyong Ho (Lee), Chanthaphan Atthawut (Vivi)

Topic1: Bioimaging of the chromosomal protein

Day 1. Transformation of E. coli

Day 2. Purification of vectors

Day 3. Transfection into HeLa cells

Day 4. Fluorescent microscopy

Day 5. Image analysis

Topic2: Bioimaging of cell cycle

Day 1. Transfection of Fucci

Day 2. Addition of chemicals to cells

Day 3. Live cell imaging

Day 4. Live cell imaging

Day 5. Image analysis

3. Fukusaki Laboratory (2 students)

Supervisor: Assist. Prof. Hisayo Ono ([ono@bio.eng.osaka-u.ac.jp](mailto:ono@bio.eng.osaka-u.ac.jp))

Students: Rachmawati Rina (Rina), Uratani Joao Marcus (John)

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: Assist. Prof. Minetaka Sugiyama ([sugi2@bio.eng.osaka-u.ac.jp](mailto:sugi2@bio.eng.osaka-u.ac.jp))

Students: Fitriainingsih (Fitri), Hu Jing (Jing)

Topic 1: Disruption of YAK1 gene that encodes a serine-threonine protein kinase in *Saccharomyces cerevisiae*

Schedule:

Day 1: Preparation of a disruption cassette for YAK1 by PCR

Day 2: Yeast transformation

Day 3: Pick up of transformants

Day 4: Isolation of genomic DNA from transformants

Day 5: PCR genotyping and identification of the correct disruptant

Topic 2: Overexpression of PDE1 gene that encodes a cyclic AMP phosphodiesterase in *Saccharomyces cerevisiae*

Schedule:

Day 1: Preparation of a overexpression cassette for PDE1 by PCR

Day 2: Yeast transformation

Day 3: Pick up of transformants

Day 4: Isolation of genomic DNA from transformants

Day 5: PCR genotyping and identification of the overexpression strain

5. Kanaya Laboratory (2 students)

Supervisor: S.A Assist. Prof. Clement Angkawidjaja ([clement@bio.mls.eng.osaka-u.ac.jp](mailto:clement@bio.mls.eng.osaka-u.ac.jp))

Students: Suroto Dian Anggraini (Dian), Sulaiman Sintawee (Aoi)

Topic 1: Production and crystallization of *Pseudomonas* sp. MIS38 lipase (PML)

Schedule:

Day 1: Secretory overproduction of PML by *E. coli*

Day 2: Purification 1: ammonium sulfate precipitation, dialysis

Day 3: Purification 2: anion exchange chromatography, gel filtration wash

Day 4: Purification 3: Gel filtration, concentration + Crystallization setting

Day 5: Crystal observation

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

Schedule:

Day 1: Overproduction SIB1 FKBP22

Day 2: Purification 1: Affinity chromatography ( $\text{Ni}^{2+}$  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. Kikuchi Laboratory (2 students).

Supervisor: Assist. Prof. Yuichiro Hori ([horiy@mls.eng.osaka-u.ac.jp](mailto:horiy@mls.eng.osaka-u.ac.jp))

Students: Srichaisupakit Akkaraphol (Toto), Dwiranti Astari (Astari)

Topic 1: Production and purification of histone demethylase, JMJD2A

Schedule:

Day 1: Transformation

Day 2: Culture of *E. coli* cells

Day 3: Overexpression

Day 4: Extraction and Purification: Affinity chromatography (glutathione column)

Day 5: Confirmation of purity and activity (Fluorescent spectroscopy)

Topic 2: Production and purification of histone deacetylase, HDAC8

Schedule:

Day 1: Transformation

Day 2: Culture of *E. coli* cells

Day 3: Overexpression

Day 4: Extraction and Purification: Affinity chromatography (glutathione column)

Day 5: Confirmation of purity and activity (Fluorescent spectroscopy)

7. Nihira Laboratory (2 students)

Supervisor: Assist. Prof. Shigeru Kitani ([kitani@icb.osaka-u.ac.jp](mailto:kitani@icb.osaka-u.ac.jp))

Students: Sharmin Dilruba (Sharmin), Sinsereekul Nitat (Nitat)

Topic: Measurement and detection of microbial secondary metabolites (for 2 students)

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

8. Noji Laboratory (1 student)

Supervisor: Assist. Prof. Ryota Iino ([iino@sanken.osaka-u.ac.jp](mailto:iino@sanken.osaka-u.ac.jp))

Student: Tannous Elias Antonios (Elias)

Topic: Purification and measurement of a rotary motor protein (F1-ATPase)

Schedule: Negotiable

Day 1: Purification of F1-ATPase expressed in *E. coli*

Day 2: Purification of F1-ATPase expressed in *E. coli*

Day 3: Biochemical (Ensemble-molecule) measurement of ATP hydrolysis activity

Day 4: Single-molecule observation of rotation

Day 5: Data analysis of biochemical and single-molecule measurements

9. Ohtake Laboratory (1 students)

Supervisor: Assist. Prof. Kohsuke Honda ([honda@bio.eng.osaka-u.ac.jp](mailto:honda@bio.eng.osaka-u.ac.jp))

Student: Hashim Zanariah Binti (Zanariah)

Topic: Bioconversion of water-immiscible chemical in aqueous/organic two phase cultivation system (for 2 students)

Schedule:

Day 1: Precultivation of recombinant microorganism in an Erlenmeyer flask

Day 2: Cultivation of the microorganism in a 5-L fermenter

Day 3: Conversion of an alkylbenzene to the corresponding phenol by the microorganism in a fermenter

Day 4: Quantification of the product with GC

Day 5: Data analysis

10. Watanabe Hajime Laboratory (0 student)

Supervisor: Assist. Prof. Koji Tsukada ([tsukada\\_koji@bio.eng.osaka-u.ac.jp](mailto:tsukada_koji@bio.eng.osaka-u.ac.jp))

Student: --

Topic: Evaluation of anti-microbial and anti-virus effects of silver-zeolyte.

Schedule:

Day 1: Discussion (what we can do? AND what we want to do?) and preparation: *E. coli* culture and Q-beta phage

Day 2: Basic procedures: How to count the number of bacterial cells and RNA phage

Day 3: Test 1: Silver-zeolyte effects on both bacterial growth and infectivity of RNA phage.

Day 4: Prepare specimens for electron microscopic observation of *E. coli* cells and Q-beta phage.

Day 5: Test 2: Silver-zeolyte effects on bacterial cells and infectivity of RNA phage by using electron microscopy.

11. Watanabe Heiji Laboratory (1 student)

Supervisor: Assist. Prof. Takuji Hosoi ([hosoi@mls.eng.osaka-u.ac.jp](mailto:hosoi@mls.eng.osaka-u.ac.jp))

Student: Ochieng Allan (Allan)

Topic: Fabrication and characterization of Germanium metal-oxide-semiconductor devices

Schedule:

Day 1: Introduction of semiconductor devices and gate insulator formation (oxidation and nitridation)

Day 2: Gate stack formation (gate electrode deposition)

Day 3: Gate patterning (lithography and etching)

Day 4: Electrical characterization (1)

Day 5: Electrical characterization (2)