Technical University of Denmark

Biobrick Workshop Agenda

This will be a general assembly tutorial that will cover skills independent of organism and project. Lab coats will be provided.

Project

In this lab tutorial, you will integrate a green fluorescent protein (GFP) expression cassette into a target location on the chromosomal DNA of E. coli. Half of the groups will do this using the USER-cloning technique while the other half will use traditional restriction enzyme/ligase cloning technique.

Timeline

Preparation (Done at DTU, by Kristian Davidsen earlier in the week)

Prepare and order primers, check PCR with template DNA, start overnight cultures for minipreps, obtain competent cells

Friday, April 24th, 2015

Location: Meeting Center (Building 101) Room S04, See attached Map

16:00 -- Welcome, Introduction to iGEM Workshop and the Competition (Chris Workman)

16:30 -- Lecture: Introduction to Biobrick Design (Pernille Myers)

17:00 -- Lecture: Introduction to USER cloning and Primer Design Exercise (Pernille Myers)

18:30 -- Tour of the lab & Introduction to the equipment

Saturday, April 25th, 2015

Location: Building 301, See attached Map

8:30 – Welcome, Safety Considerations and Pipetting Technique in the Lab (Scott Myers) – See attached safety documents.

9:00 -- Introduction to lab work (Kristian Davidsen)

Lab Work Outline:

Time	USER group	Restriction cloning
10:00 - 11:00	PCR (45 min, 3 hour incubation)	Miniprep
11:00 - 12:00	Make LB agar plates	PCR
13:00 - 14:00	Run agarose gel and purify PCR product	Run agarose gel and purify PCR product
14:00 - 15:30	USER-treatment	Restriction digestion, heat-inactivation and ligation
15:30 - 17:30	Transformation and plate	Transformation and plate

Details:
10:00 -- Begin work on the project

1. Restriction/ligation teams spin down cultures for miniprep of plasmid (8,000g for 10 min)
2. Both teams setup PCR reactions following SOP1 (USER teams need 3 PCR reactions (2 for GFP and 1 for backbone), restriction/ligation groups need one (GFP))
3. Restriction/ligation groups continue with the miniprep (Qiagen protocol). Nanodrop when done. SOP2 step 2.
4. USER teams make LB agar plates with kanamycin (each team needs 2 plates) and supervisors prepare agarose gels for gel electrophoresis after lunch.

Step 2 and 3 can both be stored several hours at 4°C.

12:00 -- Lunch

13:00 -- Analysis of PCR (SOP2 Step 1)

1. Load sample of PCR reaction on agarose gel
2. While the gel is running, purify your PCR reactions using Qiagen PCR Purification kit (Qiagen protocol)
3. Observe DNA on gel

14:00 -- Transformation

1. USER teams set up USER reaction and incubate it first at 37 degrees to make USER-overhangs and then at room temperature to allow parts to assemble. SOP3 Step 1.
2. Restriction/ligation teams set up restriction digest with two different enzymes according to the vector backbone (miniprep from morning) and the PCR. After digestion for 15 minutes, restriction digestion is stopped by heat inactivation for 20 minutes. Ligase enzyme and ATP is then added to ligate GFP into the vector backbone. SOP3 Step 2.

3. Both teams transform chemically competent E. coli cells by mixing either USER reaction or ligation reaction with E. coli cells and heat-shocking the cells to allow entry of DNA (30 seconds at 42 degrees). SOP3 Step 3.
4. In order to improve transformation efficiencies (= number of transformants) we will add media (SOC or LB) to the cells and incubate them for half an hour at 37 degrees before plating them on LB + kanamycin.

18:00 - Dinner and party
Social Dinner at Mythos
Adress:
Egegårdsvej 1
2800 Lyngby

Sunday, April 26th, 2015

Location: Building 301 (lab) and Meeting Center (Building 101) S09, See attached Map

10:00 -- Continue work on project in the lab observe plates for expression of GFP.

1. Observe plates for colonies that fluoresce under UV light
2. Prepare colony PCR reaction (important for when a positive result is not easily observed through visual methods)
3. Prepare analytical agarose gel for verifying the colony PCR reaction was a success in the afternoon

12:00 -- Lunch (Sandwiches) Meeting Room Building 101 S09

12:30 -- Lecture: How to get gold in iGEM (Chris Workman)
Discuss some of the frequently overlooked aspects of iGEM such as human practices and characterization of Biobricks that are necessary to achieve a gold medal.

13:00 -- Lecture: Wiki design from Thøger J Krogh from SDU

14:00 -- Lecture: Wiki design from Mads Anderson from DTU

14:30 -- Complete lab work with verification of Colony PCR results with Gel electrophoresis of PCR fragments. Confirm that proper band patterns appear on gel. Clean up lab.

16:00 -- Wrap up, Thanks (Chris Workman)