PCR
(polymerase chain reaction)

Objective

This experiment uses PCR as a means to detect the integration of CRIM (conditional-replication, integration, and modular) plasmid (pAH125lacIOPO) into *E. coli* (BW25113).

Day 1: PCR sample preparation, time 1 hour. The actual PCR reaction takes 3 hours, but samples can be left overnight in PCR machine at 4 degrees C.
Day2: Gel electrophoresis, total procedure 2 hours

Introduction

The principle of the polymerase chain reaction (PCR) is to amplify a specific DNA sequence from a genome or DNA sample. Oligonucleotide primers are annealed to the separate strands of denatured double stranded DNA by using hybridization conditions ensuring that only primers complementary the desired sequence will anneal. The primers are then extended using DNA polymerase and the four deoxynucleotide triphoshates (dNTPs), generating a new strand of DNA complementary to the template DNA. After primer extension, another round of denaturation separates the newly formed double strands. These three steps (annealing, elongation, and thermal denaturation) constitute one cycle of PCR DNA replication. If two primers are each extended to provide a template for annealing of the other primer a replicative chain reaction will result. Each round of synthesis, will double the number of templates in the previous round.

This experiment uses PCR as a means to detect the rearrangement of the DNA sequence of the CRIM (conditional-replication, integration, and modular) plasmid (pAH125) upon integration into the *E. coli* (BW25113) chromosome, at the *att*B site (fig.1). The primers used anneal the integrated chromosome in different places. The diagram illustrates the orientation of the primers.

Material (See drawer, freezer-box labeled: CRIM/Att, See TA for other materials)
Day 1
Materials
Sterile pipet tips,
3 - 1.5 ml micro centrifuge tubes
6 - PCR tubes (0.2 mL thinwall tubes)
PCR machine, ABI 7200

Reagents
Sterile water
Green GoTaq Master Mix, contains:
- Buffer, Glycerol and mobility Dyes for Electrophoresis
- Go-Taq DNA polymerase (freezer- keep it in the cryobox)
- dNTPs (2 mM of dATP, dCTP, dTTP, dGTP,

Primer mix (P1,P2,P3 and P4 each primer at 3 μM) sequences below

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ccaGGCATCACGGCAATATAC P1,
ggcTCTGGTCTGGTAGCAATG P4,
aacACTTAACGGCTGACATGG P2,
aACGAGTATCGAGATGGCAca P3,
TTttTtatActAA AttCore
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Plates of bacterial colonies (pick two)

Day 1: PCR

Procedures

A. Look up the concentration of the DNA that you purified in experiment 5. Calculate the volume you need to add to have at least 10 ng of template but not more than 250 ng in your PCR reaction. Record the volume and the mass of DNA.
B. Next, prepare the 6 PCR tubes. Label each tube and place 15 microliters of the mastermix in each tube. Follow by placing 5 μl of primer mix in each corresponding tube and 5 μl of DNA template* to each corresponding tube for a total volume of 25μL. Cap each tube and place them into the PCR machine and run the setting "att/crim2" (25 cycles). Leave overnight. The program will finish by keeping the tubes at 4ºC indefinitely.

* For the *E. coli* DNA template: Isolate one colony using sterile plastic tip and suspend in 20 μL water, vortex, extract 5 μL for template sample.

Final components for each tube

Sample	Mastermix Dyes, Buffer, Taq polym and dNTPs	Primers in μL	Template in μL	H_2O
Control (no Template DNA)	15 μL	5 μL	0 μL	10 μL
pAH125	15 μL	5 μL	x μL	10-x μL
pAH125lacIOPO	15 μL	5 μL	x μL	10-x μL
X90 (cells)	15 μL	5 μL	5 μL	5 μL
BW25113::pAH125lacIOPO (cells)	15 μL	5 μL	5 μL	5 μL
BW25113::pAH125lacIOPO (cells)	15 μL	5 μL	5 μL	5 μL

Day 2: Analysis

Use sequence extractor as directed from the page:
http://www.olemiss.edu/depts/chemistry/courses/chem472/PCR/exp6a_PCR.html

To determine expected fragment sizes

Gel Electrophoresis

Use the NEB cutter program to decide which gel percentage you should use to separate the expected fragments. Run gel and visualize as in Experiments 5 and 6.

Sources

Promega Protocol PDF (http://www.promega.com/tbs/Tb254/tb254.pdf)
Haldimann, Andreas and Barry L. Wanner. 2001. *Conditional-Replication, Integration, Excision, and Retrieval Plasmid-Host Systems for Gene Structure-Function Studies of Bacteria.* J. Bacteriology. p. 6384-6393.
Primer3 Output (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3)