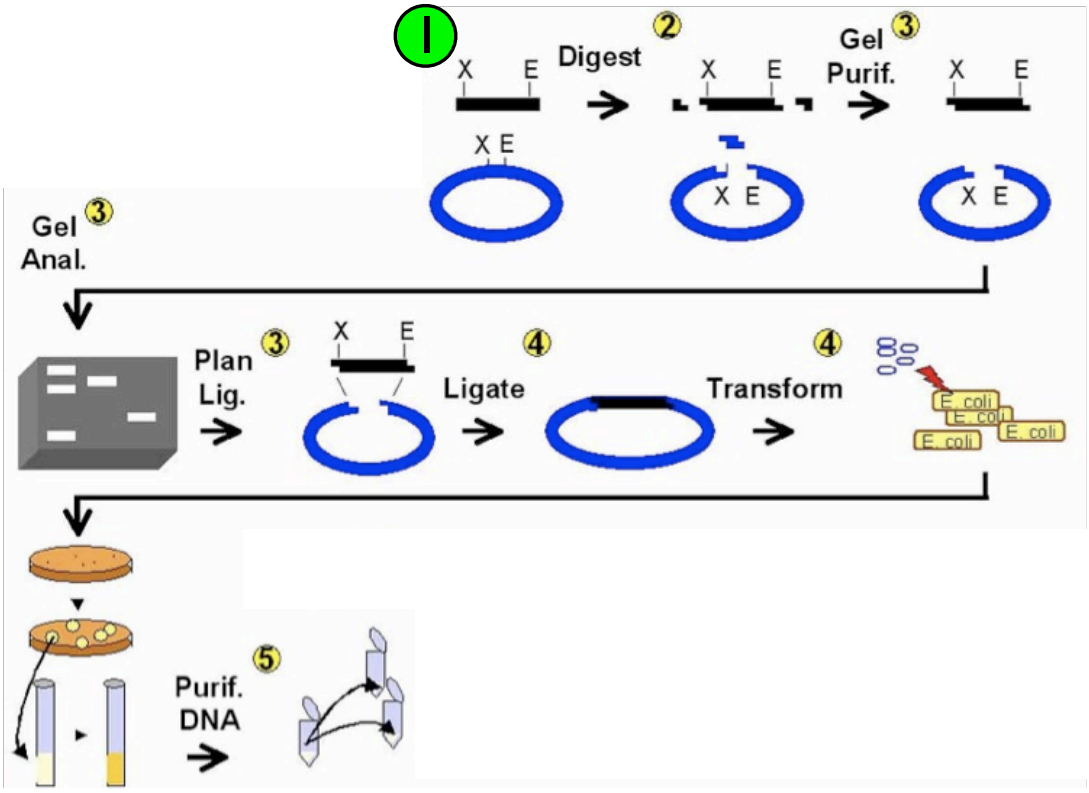
SEED Academy, Spring 2009
Synthetic Biology Module

Homework #4
Due March 14, 2009

1) **Laboratory Project Overview**
Here, again, is our lab schematic (adapted from MIT's 20.109 DNA Engineering Module: http://openwetware.org/wiki/20.109(F08):Module_1). Please answer the questions that follow.



a) Which steps in the process did you perform on Day 4? Refer to the step name(s) that appear(s) above the arrow (e.g. "Digest", "Ligate", etc.) rather than the number(s). Give a brief summary of the step(s).

b) One of the major things that you did on Day 4 is *not* on the schematic. What is it? Give a two sentence description of this step.

2) Assume that you gel purified 200 µL of vector at 75 ng/µL, and you purified 50 µL of PCR product at 1 µg/µL. After purification, you eluted into 30 µL and obtained 10 ng/µL for the vector and 50 ng/µL for the PCR product. Calculate the % yield or recovery from you gel purification for the PCR product and vector. (Hint: % Yield = 100% x $mass_{out}/mass_{in}$). **Show <u>ALL</u> work**.

3) Use the information at the end of the homework (NEB pages) to answer the following questions about the sequence below:

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5′ – ATTAGTCTAGAAATTCGCGACTAGTCAGCA - 3′
3′ – TAATCAGATCTTTAAGCGCTGATCAGTCGT - 5′
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a) Design the forward and reverse primers (20 base pairs long) you would use to PCR amplify this DNA sequence from your favorite organism.

b) Draw the products of XbaI digestion of this sequence.

c) Draw the products of SpeI digestion of this sequence.

d) Can any of these products (from the XbaI and SpeI digestions) be ligated to form a ***new*** product? Do not include potential products from blunt-end ligations.

e) Can this resulting fragment be cut by XbaI or SpeI? Why?

4) Read "Idempotent Vector Design for Standard Assembly of BioBricks" (http://hdl.handle.net/1721.1/21168 first 6 pages) and answer the following questions.
   a) What is the motivation for a standard assembly scheme?

   b) What does it mean for assembly to be idempotent?

   c) What are the 4 standard enzymes used in BioBricks assembly?

   d) What enzymes should be used to cut a BioBrick part that we wish to add to the back of another part?

e) Indicate the recipe for a ligation of a plasmid and an insert. Your plasmid is 3000 bp and the insert is 1000 bp. Assume that all nucleotides are 1000 Da. Assume that you want 1 nM concentrations of each of the DNA components in your ligation.

Plasmid (10 ng/μL)	_____ μL
Insert (50 ng/μL)	_____ μL
Ligation Buffer (10X)	_____ μL
T4 DNA Ligase (Enzyme)	_0.5_ μL
Water	___ μL
Total	_10_ μL

PstI

Nomenclature Update

Recognition Site:

Source:
A *E. coli* strain that carries the PstI gene from *Providencia stuartii* 164 (ATCC 49762).

Reagents Supplied:
NEBuffer 3 (10X)
BSA (100X)

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 75%
NEBuffer 2: 75%
NEBuffer 3: 100%
NEBuffer 4: 50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Methylation Sensitivity:

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Not sensitive

Heat Inactivation:
80°C for 20 minutes

Survival in a Reaction:
Minimum units to digest 1 µg of substrate DNA in 16 hours: 0.50 unit(s)

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer 3
Supplemented with 100 µg/ml Bovine Serum Albumin
Incubate at 37°C.

1X NEBuffer 3:
50 mM Tris-HCl
100 mM NaCl
10 mM $MgCl_2$
1 mM Dithiothreitol
pH 7.9 @ 25°C

Unit Definition:
One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Concentration:
20,000 units/ml and 100,000 units/ml

Unit Assay Substrate:
λ DNA

Storage Conditions:
10 mM Tris-HCl
200 mM NaCl
1 mM Dithiothreitol
0.1 mM EDTA
200 µg/ml BSA
50% Glycerol
0.15% Triton X-100
pH 7.4 @ 25°C

Storage Temperature:
-20°C

Diluent Compatibility:
Diluent C

Notes

General notes:

1. Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 1 unit, pBR322 = 1 unit, LITMUS = 1 unit.

XbaI

Nomenclature Update

Recognition Site:

Source:
A *E. coli* strain that carries the XbaI gene from *Xanthomonas badrii* (ATCC 11672).

Reagents Supplied:
NEBuffer 2
BSA

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 0%
NEBuffer 2: 100%
NEBuffer 3: 75%
NEBuffer 4: 75%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Methylation Sensitivity:

dam methylation: Blocked by overlapping
dcm methylation: Not sensitive
CpG methylation: Not sensitive

Heat Inactivation:
65°C for 20 minutes

Survival in a Reaction:
Minimum units to digest 1 µg of substrate DNA in 16 hours: 0.13 unit(s)

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer 2
Supplemented with 100 µg/ml Bovine Serum Albumin
Incubate at 37°C.

1X NEBuffer 2:
10 mM Tris-HCl
50 mM NaCl
10 mM $MgCl_2$
1 mM Dithiothreitol
pH 7.9 @ 25°C

Unit Definition:
One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA (dam^-/HindIII digest) in 1

hour at 37°C in a total reaction volume of 50 µl.

Concentration:
20,000 units/ml and 100,000 units/ml

Unit Assay Substrate:
λ DNA (*dam*$^-$/Hind III digest)

Storage Conditions:
10 mM Tris-HCl
50 mM NaCl
1 mM Dithiothreitol
0.1 mM EDTA
200 µg/ml BSA
50% Glycerol
pH 7.4 @ 25°C

Storage Temperature:
-20°C

Diluent Compatibility:
Diluent A

SpeI

Nomenclature Update

Recognition Site:

Source:
A *E. coli* strain that carries the SpeI gene from *Sphaerotilus* species (ATCC 13923).

Reagents Supplied:
NEBuffer 2
BSA

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 75%
NEBuffer 2: 100%
NEBuffer 3: 25%
NEBuffer 4: 75%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Methylation Sensitivity:

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Not sensitive

Heat Inactivation:
65°C for 20 minutes

Survival in a Reaction:
Minimum units to digest 1 µg of substrate DNA in 16 hours: 0.50 unit(s)

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer 2
Supplemented with 100 µg/ml Bovine Serum Albumin
Incubate at 37°C.

1X NEBuffer 2:
10 mM Tris-HCl
50 mM NaCl
10 mM $MgCl_2$
1 mM Dithiothreitol
pH 7.9 @ 25°C

Unit Definition:
One unit is defined as the amount of enzyme required to digest 1 µg of Adenovirus-2 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Concentration:
10,000 units/ml and 50,000 units/ml

Unit Assay Substrate:
Adenovirus-2 DNA

Storage Conditions:
10 mM Tris-HCl
50 mM KCl
1 mM Dithiothreitol
0.1 mM EDTA
200 µg/ml BSA
50% Glycerol
pH 7.4 @ 25°C

Storage Temperature:
-20°C

Diluent Compatibility:
Diluent A

Notes

General notes:

1. Cleaves to leave a 5´ CTAG extension which can be efficiently ligated to DNA fragments generated by AvrII, NheI, or XbaI.

EcoRI

Nomenclature Update

Recognition Site:

Source:
An *E. coli* strain that carries the cloned EcoRI gene from *E. coli* RY13 (R.N. Yoshimori).

Reagents Supplied:
NEBuffer EcoRI (10X)

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 100%
NEBuffer 2: 100%
NEBuffer 3: 100%
NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Methylation Sensitivity:

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Impaired by overlapping

Heat Inactivation:
65°C for 20 minutes

Survival in a Reaction:
Minimum units to digest 1 µg of substrate DNA in 16 hours: 0.13 unit(s)

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer EcoRI
Incubate at 37°C.

1X NEBuffer EcoRI:
100 mM Tris-HCl
50 mM NaCl
10 mM $MgCl_2$
0.025 % Triton X-100
pH 7.5 @ 25°C

Unit Definition:

One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Concentration:
20,000 units/ml and 100,000 units/ml

Unit Assay Substrate:
λ DNA

Storage Conditions:
300 mM NaCl
10 mM 2-Mercaptoethanol
0.1 mM EDTA
200 µg/ml BSA
50% Glycerol
0.15% Triton X-100
pH 7.5 @ 25°C

Storage Temperature:
-20°C

Diluent Compatibility:
Diluent C

Notes

General notes:

1. Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.