

Name

Student ID number

Assignment 4

**1. Use BACHREST to generate a list of restriction enzymes that do NOT cut within the 5.3 kb PCR fragment for LepR3**

Enzyme X: **name** X^XXXXX

Enzyme Y: **name** Y^YYYYY

*Cutting sites in this example are arbitrary. Show the true cutting sites for your chosen enzymes.*

**2. (3 points) Modify your PCR primers to include unique restriction sites and at the 5' ends of both primers**

Forward: 5' nnnnnnXXXXXNNNNNNNNNNNNNNNNNNNNNNNN3'

Reverse: 5' nnnnnnYYYYYNNNNNNNNNNNNNNNNNNNNNNNN3'

*(Nucleotides added to the original primers are underlined.)*

**3. (3 points) Design left and right adaptors**

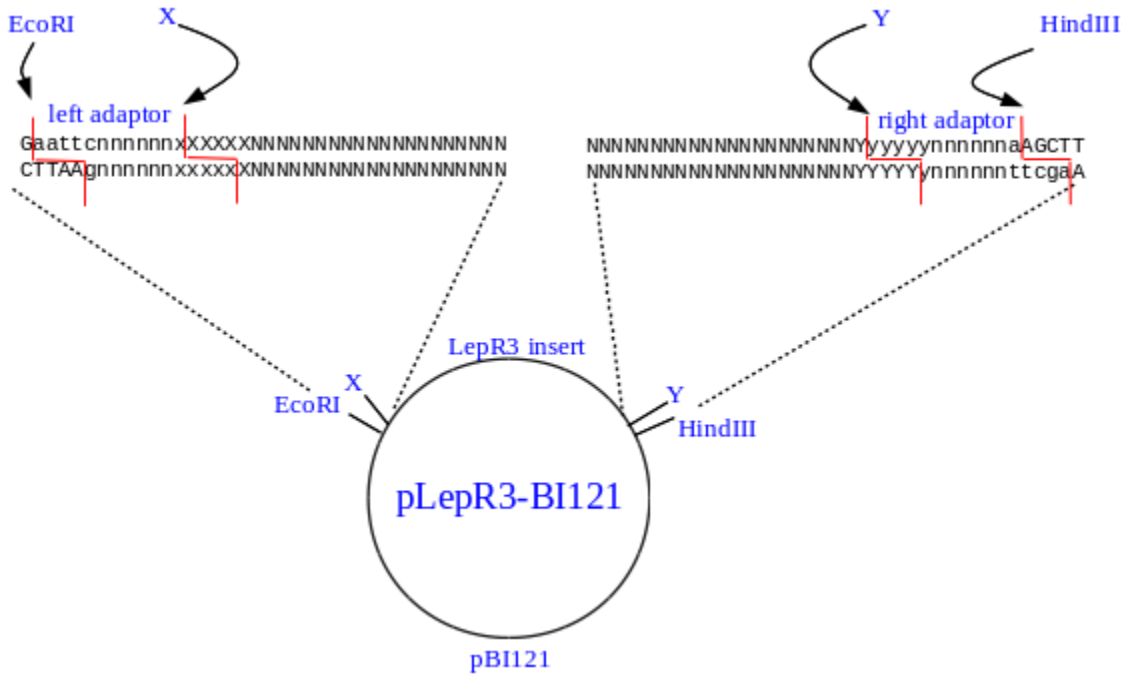
**Left adaptors (EcoRI/X)**

A. Oligonucleotides	B. How the oligonucleotides would pair in-vitro
L1: 5' AATTCnnnnnnX3' L2: 5' XXXXXnnnnnnG3'	5' AATTC <u>nnnnnn</u> X3' 3' GnnnnnnXXXXX5'

**Right adaptors (Y/HindIII)**

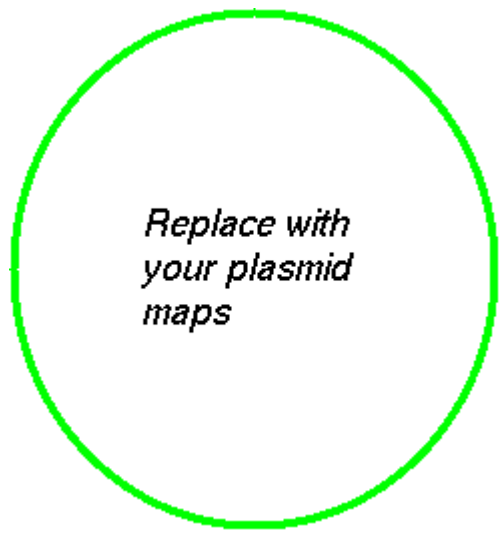
A. Oligonucleotides	B. How the oligonucleotides would pair in-vitro
R1: 5' AGCTTnnnnnnY3' R2: 5' YYYYYnnnnnnA3'	5' AGCTT <u>nnnnnn</u> Y3' 3' AnnnnnnYYYYY5'

#### 4. (3 points) Plan your construct



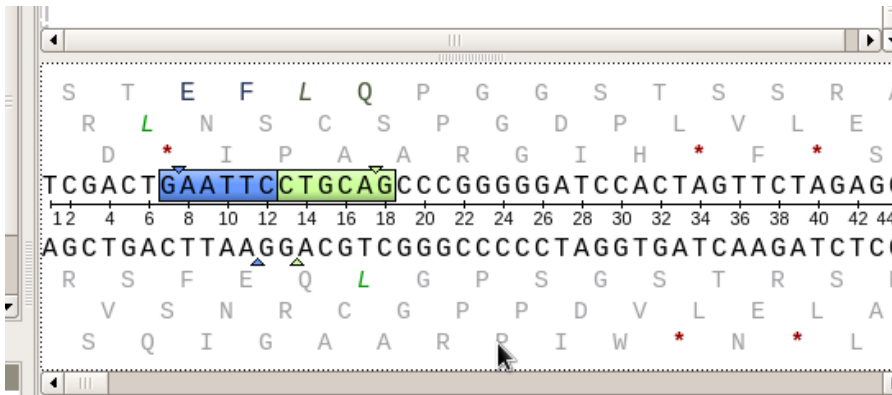
Replace this figure with your own image, created from construct\_template.odt. Replace ambiguous nucleotides (eg. n,N,x,X,y,Y) with the actual sequences as described in part 4.

#### 5. Digest pBI121 with EcoRI and HindIII

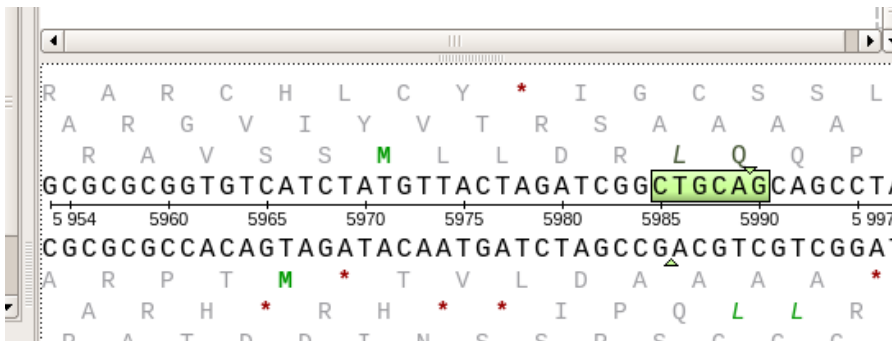


6. (3 points) In UGENE, create the X/Y restriction fragment for the PCR product.

Left



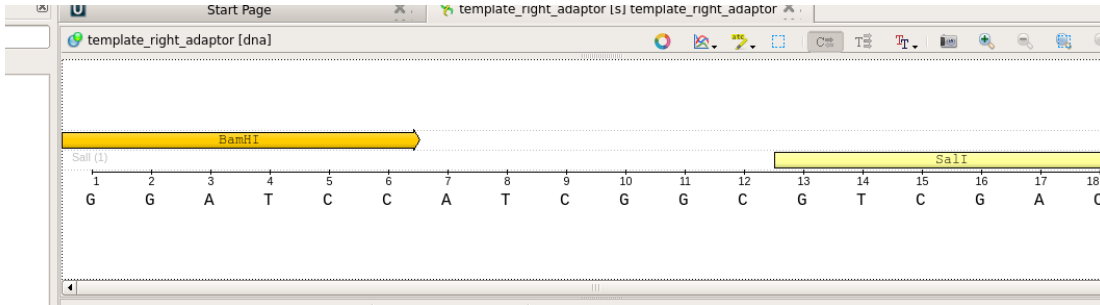
Right



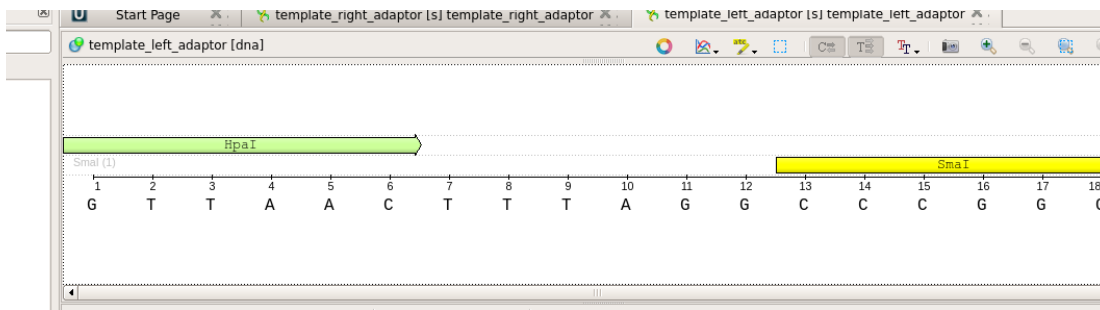
Replace these images own cropped screenshots as described for part 6.

## 7. (2 points) Create your two adaptor sequences (2 points)

### Left adaptor



### Right adaptor



*Replace these images own cropped screenshots as described for part 7.*

**8. (4 points) Assemble the completed construct**

