PLNT 2530 - Plant Biotechnology

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'nnnnnnXXXXXXNNNNNNNNNNNNNNNNNNNN3'  
Reverse:  5'nnnnnnYYYYYYNNNNNNNNNNNNNNNNNNNNNN3'

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

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

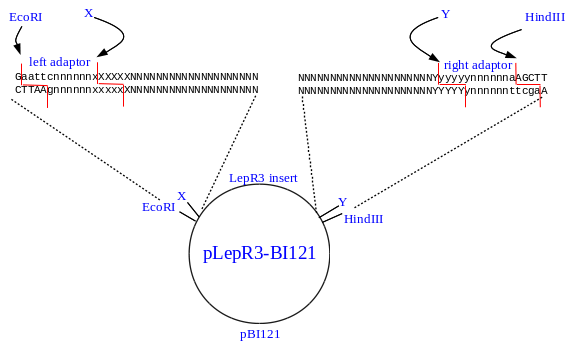
Left adaptors (EcoRI/X)

|  |  |
| --- | --- |
| A. Oligonucleotides   L1: 5'AATTCnnnnnnX3' L2: 5'XXXXXnnnnnnG3' | B. How the oligonucleotides would pair in-vitro  5'AATTCnnnnnnX3'  3' GnnnnnnXXXXX5' |

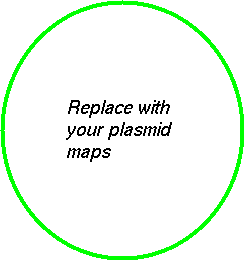
Right adaptors (Y/HindIII)

|  |  |
| --- | --- |
| A. Oligonucleotides   R1: 5'AGCTTnnnnnnY3' R2: 5'YYYYYnnnnnnA3' | B. How the oligonucleotides would pair in-vitro  5'AGCTTnnnnnnY3'  3' AnnnnnnYYYYY5' |

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

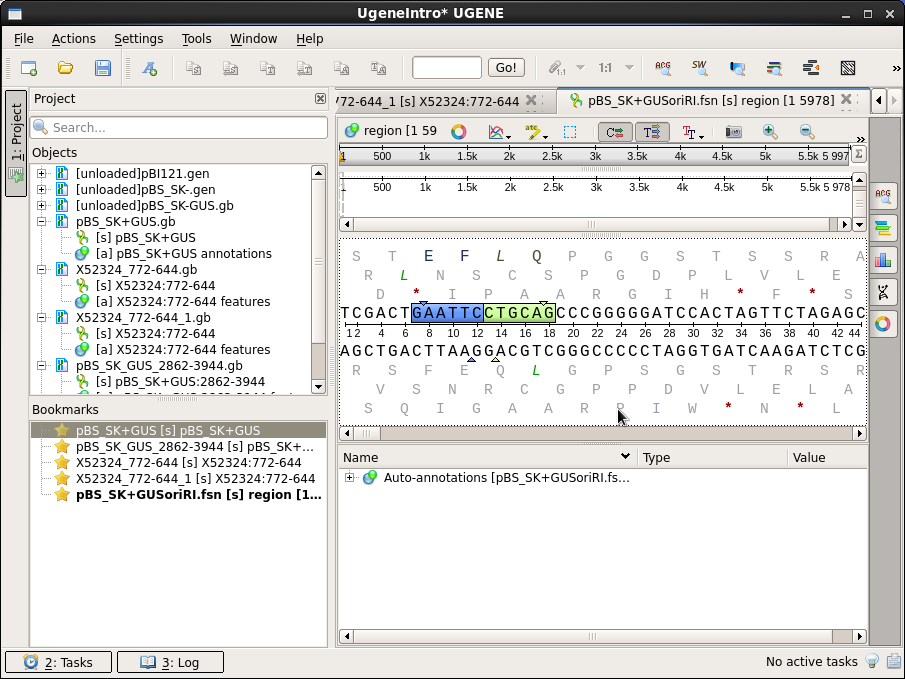
*Replace this figure with your own image, created from construct\_template.odt. Replace ambiguous nucleotides (eg. n,N,x,X,y,Y) with the actual sequences as described in part 4.*

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

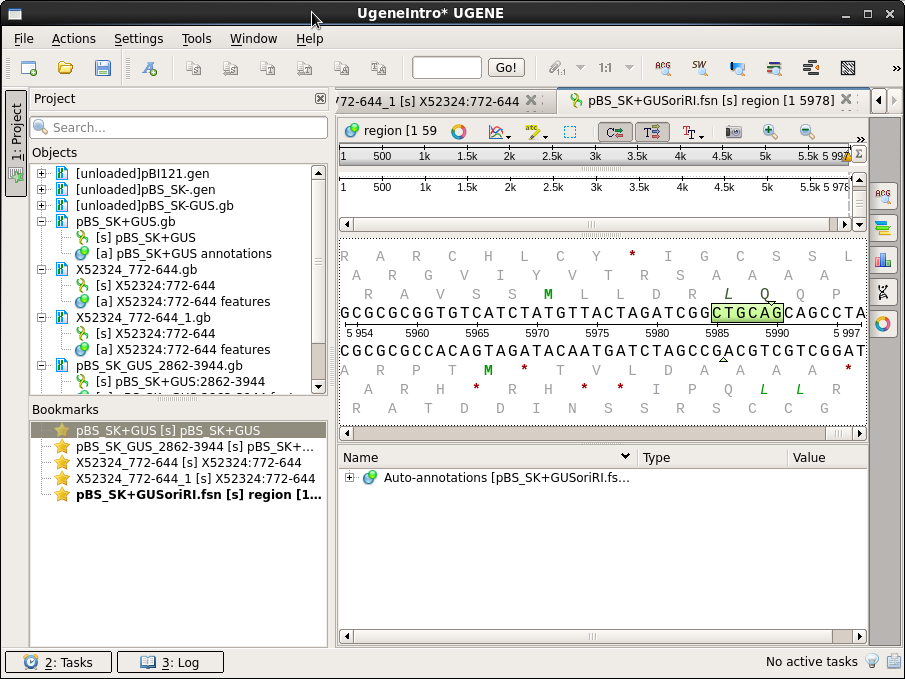


### 6. (3 points) In UGENE, create the X/Y restriction fragment for the PCR product.

Left



Right

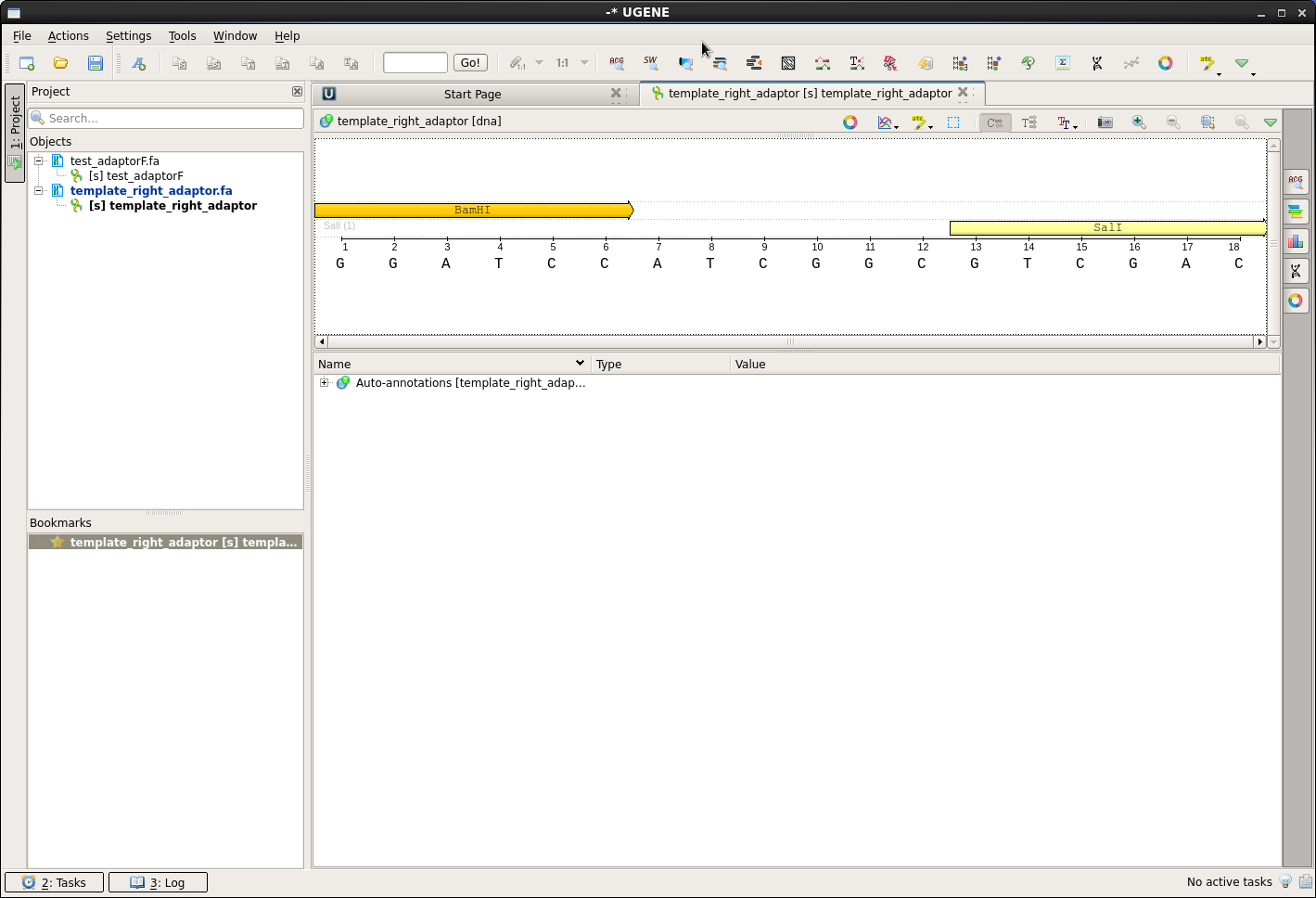


*Replace these images own cropped screenshots as described for part 6.*

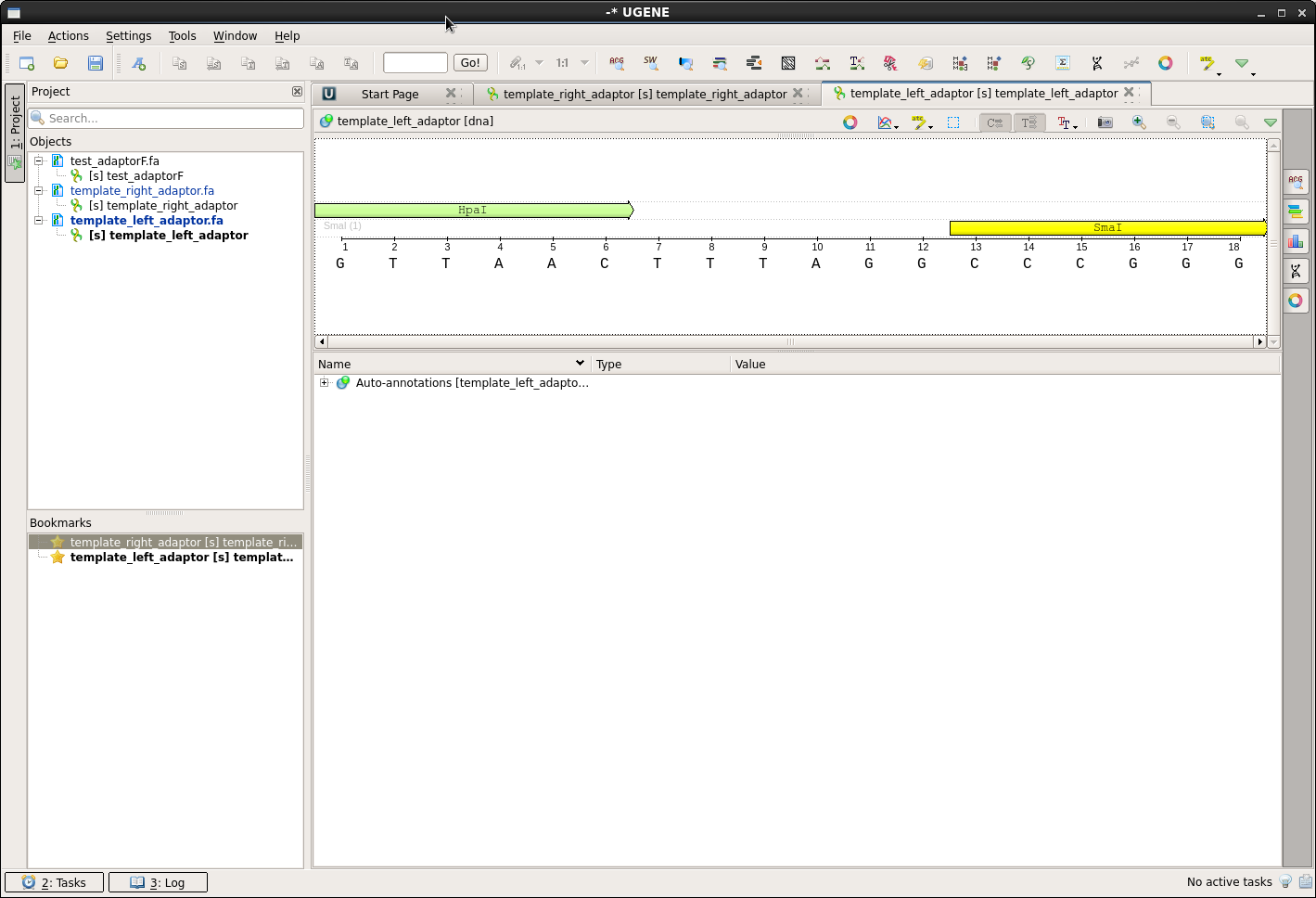
### **7. (2 points) Create your two adaptor sequences (2 points)**

**Left adaptor**

### 



**Right adaptor**



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

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

