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^YYYY

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

Reverse: 5'nnnnnYYYYYNNNNNNNNNNNNNNNNNNNNNNNNNNN

(Nucleotides added to the original primers are underlined.)

3. (3 points) Design left and right adaptors

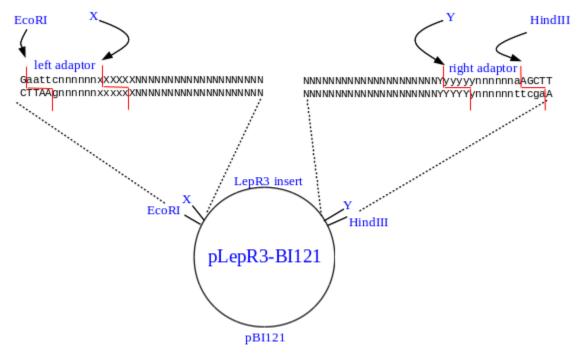
Left adaptors (EcoRI/X)

A. Oligonucleotides	B. How the oligonucleotides would pair in-vitro
L1: 5'AATTCnnnnnnX3' L2: 5'XXXXXnnnnnnG3'	5'AATTC <u>nnnnnX</u> 3' 3' GnnnnnXXXX5'

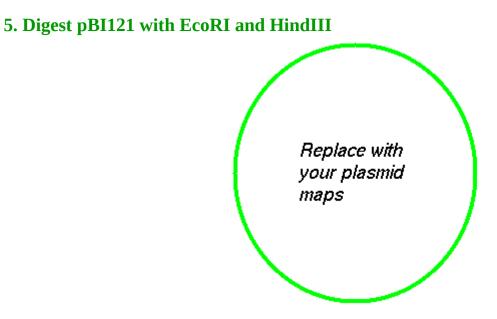
Right adaptors (Y/HindIII)

A. Oligonucleotides	B. How the oligonucleotides would pair in-vitro
R1: 5'AGCTTnnnnnnY3'	5'AGCTT <u>nnnnn</u> Y3'
R2: 5'YYYYYnnnnnA3'	3' AnnnnnYYYY5'

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.



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

Left

	ST EFLO PGGSTSSR/
	R L N S C S P G D P L V L E
	D *_ I P A _ A R G I H * F * S
	TCGACT <mark>GAATTCCTGCAG</mark> CCCGGGGGATCCACTAGTTCTAGAG(
	12 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
	AGCTGACTTAAGGACGTCGGGCCCCCTAGGTGATCAAGATCTC
	R S F E Q L G P S G S T R S F
_	V S N R C G P P D V L E L A
	SQIGAAR <mark>R</mark> IW*N*L

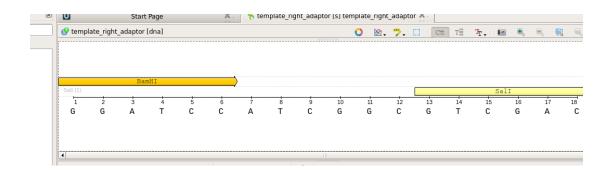
Right

=	R A R C H L C Y * I G C S S L A R G V I Y V T R S A A A
	R A V S S M L L D R <i>L</i> Q Q P GCGCGCGGTGTCATCTATGTTACTAGATCGG <mark>CTGCAG</mark> CAGCCT/
_	5 954 5960 5965 5970 5975 5980 5985 5990 5 997 CGCGCGCCACAGTAGATACAATGATCTAGCCGACGTCGTCGGA
•	A R P T M * T V L D A ^A A A A * A R H * R H * * I P O <i>L L</i> R

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

		adaptor [0 🖄		[]] Ca	<u> </u>	Tr 🚬 🛍			18
1																	
	HpaI Smal (1) 1 2 3 4 5 6 G T T A A C		<u> </u>														
Smal (1														Sm	aI		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
G	Т	Т	Α	Α	С	Т	Т	Т	Α	G	G	С	С	С	G	G	

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

8. (4 points) Assemble the completed construct

