



Advanced Cell Biology & Biotechnology

Biotechnology Project Lab

Giovanna Gambarotta
& Isabella Tarulli

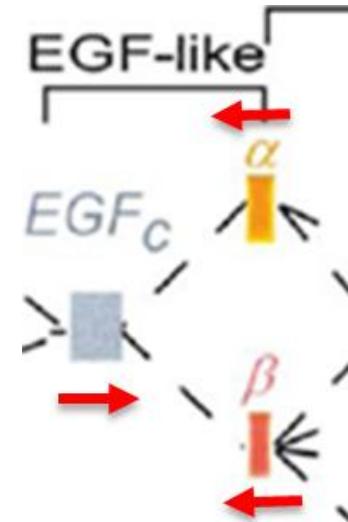
The lecture of November 8th 2021 is about to begin...

Summary of the previous lesson activity

1-find the length of the intron between domain EGF-like and domains alpha and beta

2-find coding exons (number and length) and introns (length) of TBP and of GAPDH to decide where to design primer.

3-project overview



PROJECT OVERVIEW

Genebank sequence analysis



primer design



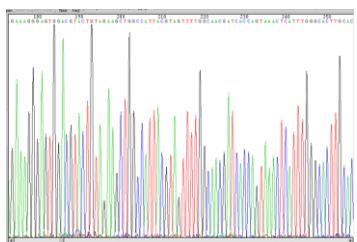
RT-PCR



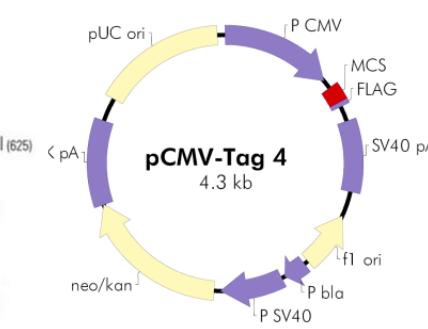
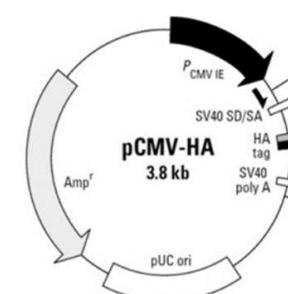
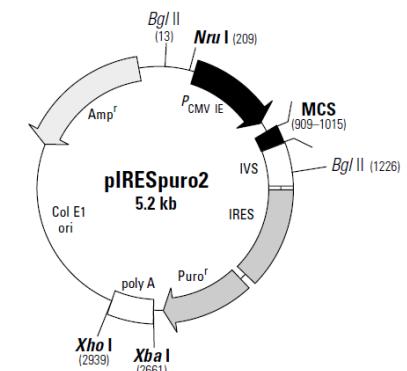
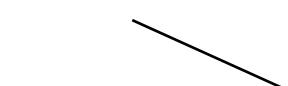
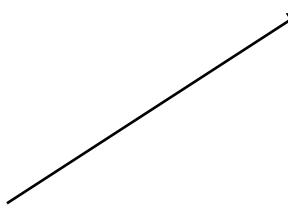
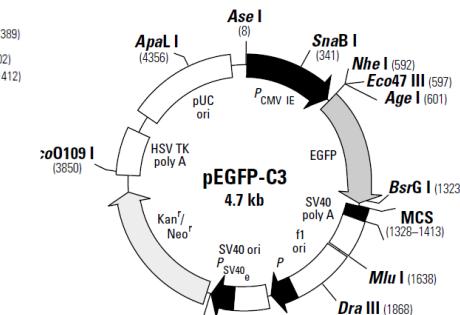
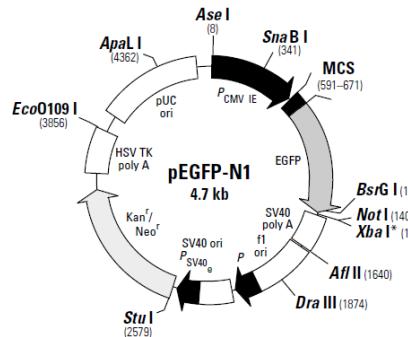
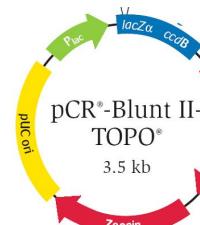
cloning in the vector pCRII-blunt



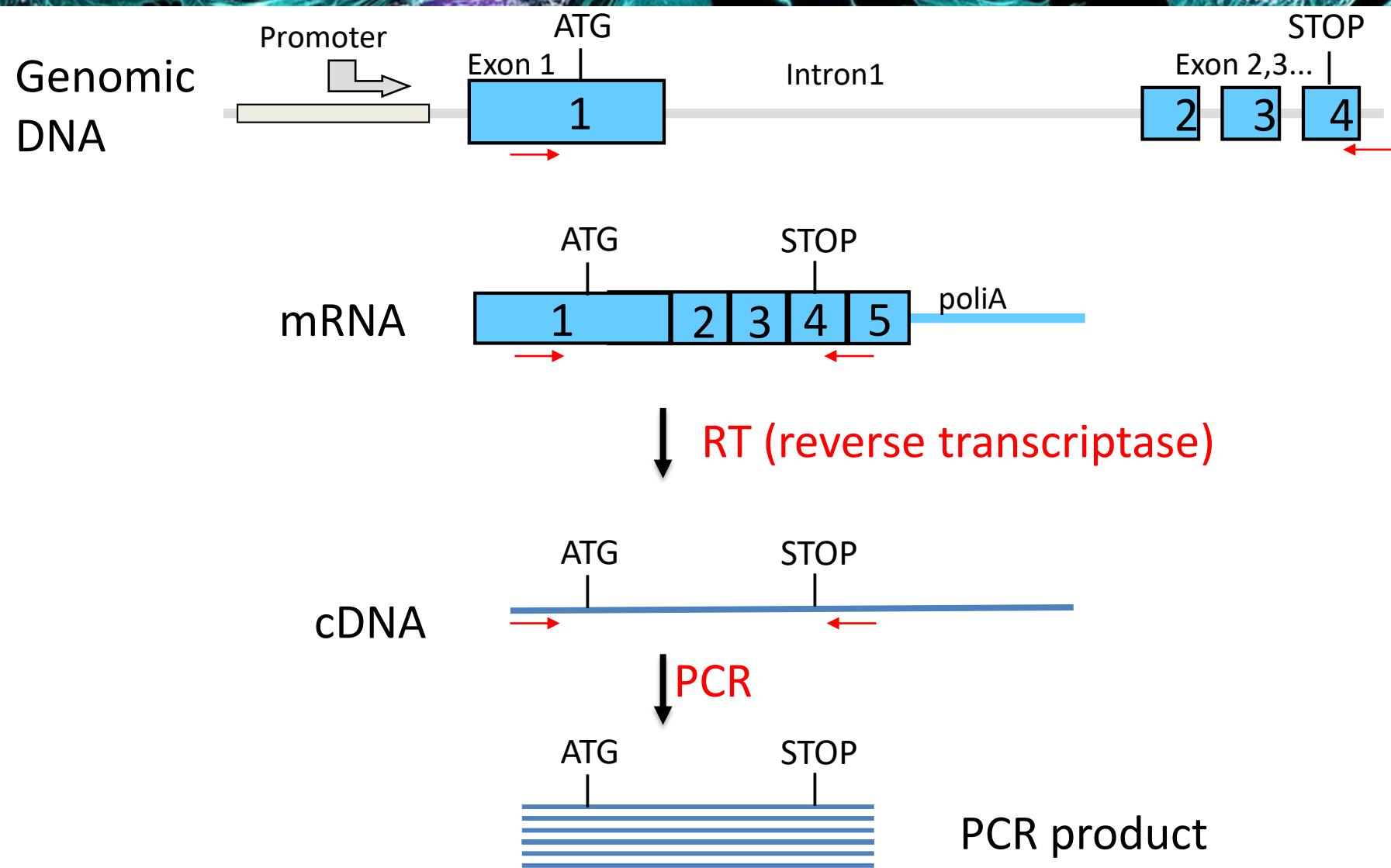
sequence analysis



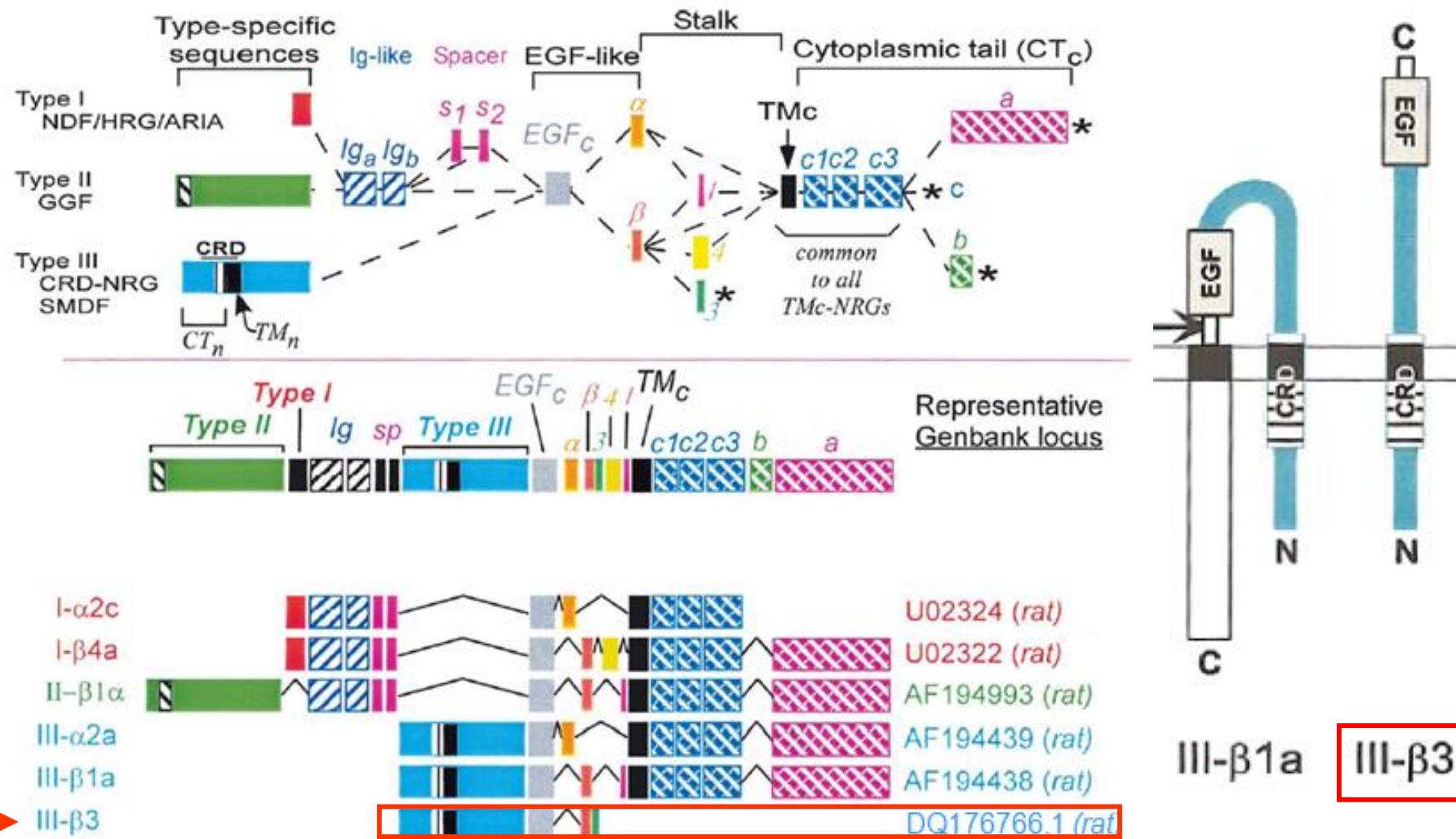
- real time PCR
- protein quantification

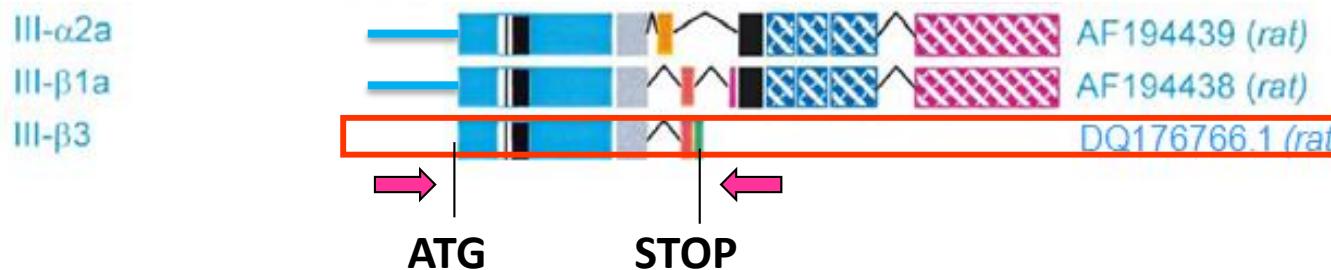
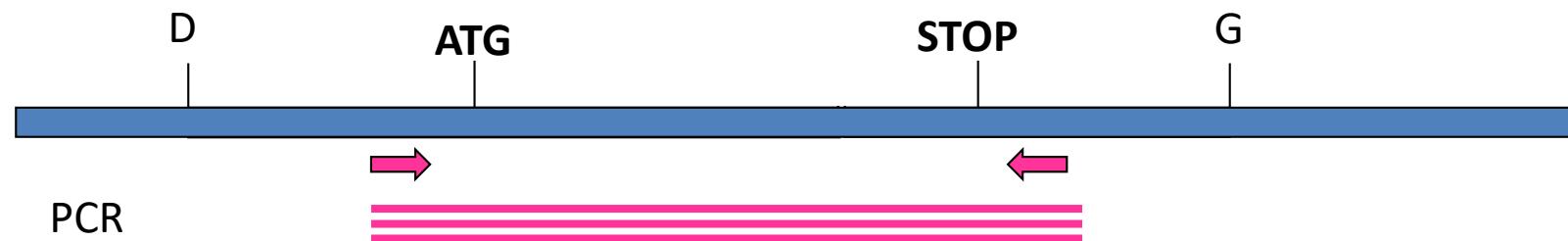


Cloning the full length cDNA to express NRG1 type III beta3



Cloning of rat NRG1-typeIII β 3





The sequence DQ176766 is starting from the ATG and is ending after the STOP. Therefore I suggest you to copy 30 bases upstream ATG from AF194438 or AF194439 to have 30 bp sequence before ATG, to be able to design a good primer before or near ATG.

You can copy 30 bases before ATG and copy them before the sequence DQ176766, as shown in the next slide

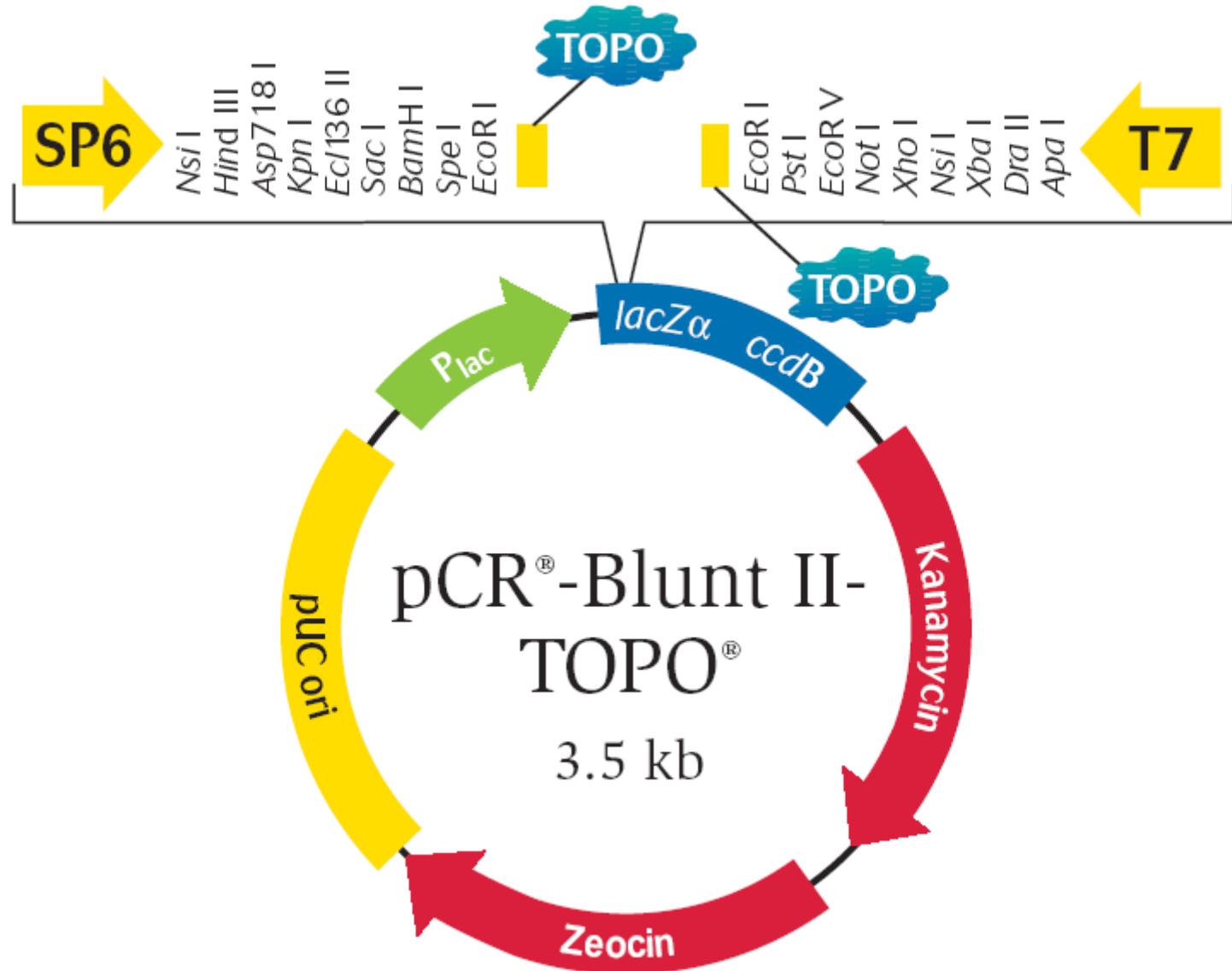
AGAGAGGGCCA GGCCTTCTGG AGGTGAGCCG

(Remember that ATG and STOP position will be shifted 30 bases!).

AGAGAGGCCA GGCCTTCTGG AGGTGAGCCG

1 atggagattt attccccaga catgtctgag gtagctggcg ggaggtcctc cagccctcc
61 actcagctga gtgcagcccc atctcttgat gggcttccgg cagcggagga acatatacca
121 gacaccaca cagaagatga gagaagccct ggactcctgg gcctggcggt gccctgctgt
181 gtgtgcctgg aagctgagcg cctgagaggg tgtctcaact ccgagaagat ctgcattgtt
241 cccattctgg cttgcctagt cagcctctgc ctctgcattg ctggcctgaa gtgggtattt
301 gtggacaaga tatttgaata cgacttcctt acccacctt accctgggg gttaggccag
361 gaccctgtga tttctctgga tccaaactgct gccccagcca ttttgtatc atctgaggca
421 tacacttcac ctgtctctaa ggctcagtct gaagctgggg ctcatgttac agtacaagg
481 gaccatgctg ctgtggcctc tgaaccttca gcagtaaccga cccggaagaa ccggctgtct
541 gctttcctc ccttcactc tactgcaccc cccttccctt ctccagctcg gacccttgag
601 gtgagaacac ccaagtcagg aactcagcca caaacaacag aaactaacct gcaaactgct
661 cctaaacttt ccacatcaac atccacgact gggaccagcc atctcataaaa gtgtgcggag
721 aaggagaaaa cttctgtgt gaatgggggc gagtgcttca cggtgaagga cctgtcaa
781 ccgtcaagat acttgtgcaa gtgcccataat gagttactg gtgatcggtt ccaaaaactac
841 gtaatggcca gcttctacag tacgtccact ccctttctgt ctctgcctga gtag **GAGCAT**

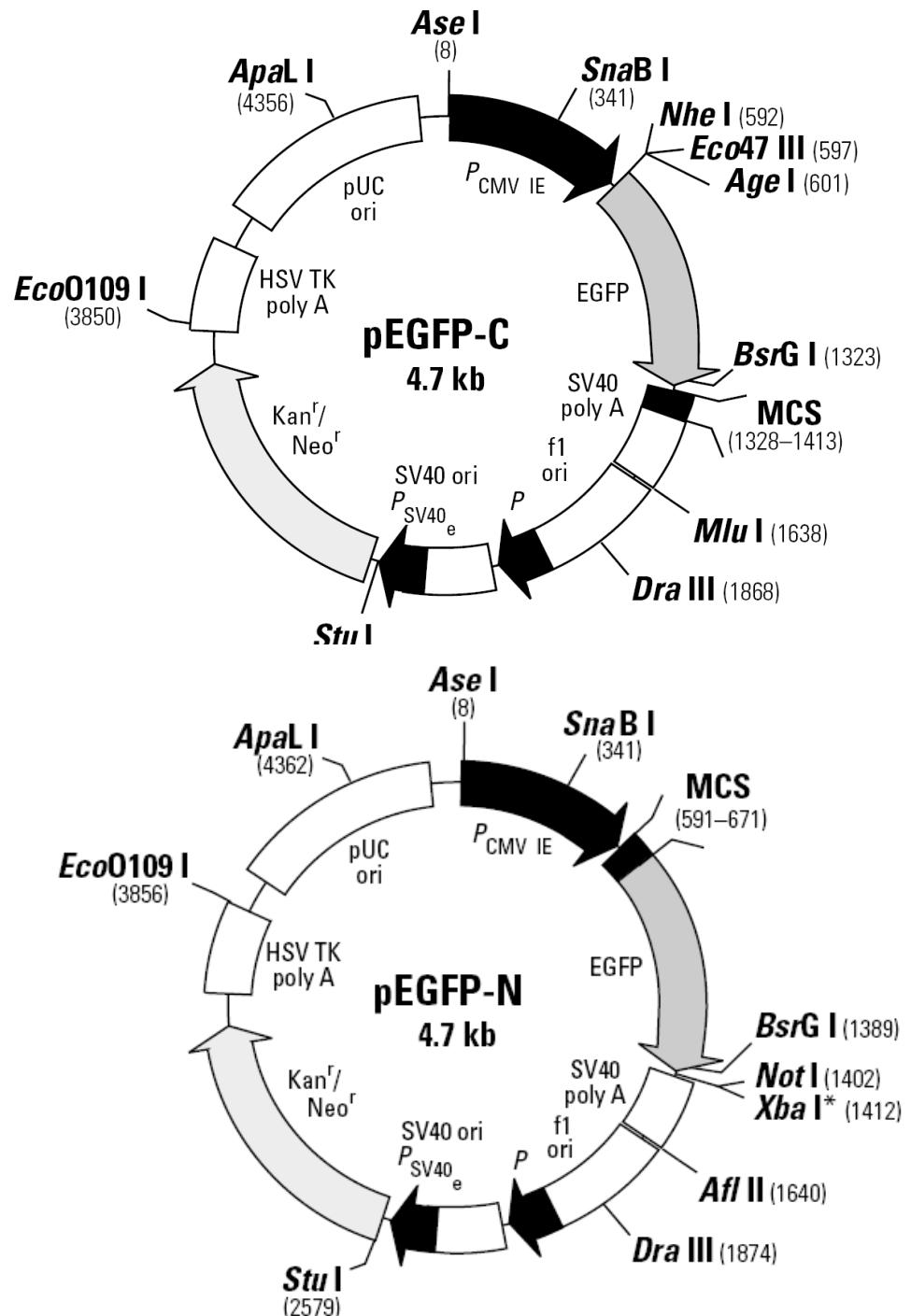
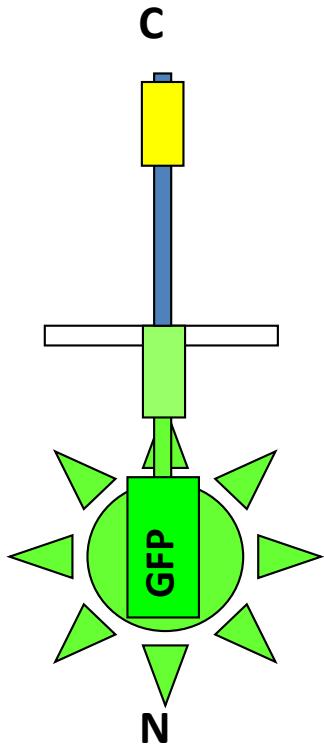
901 GCTCAGTCGA TGCT



<http://www.invitrogen.com/>

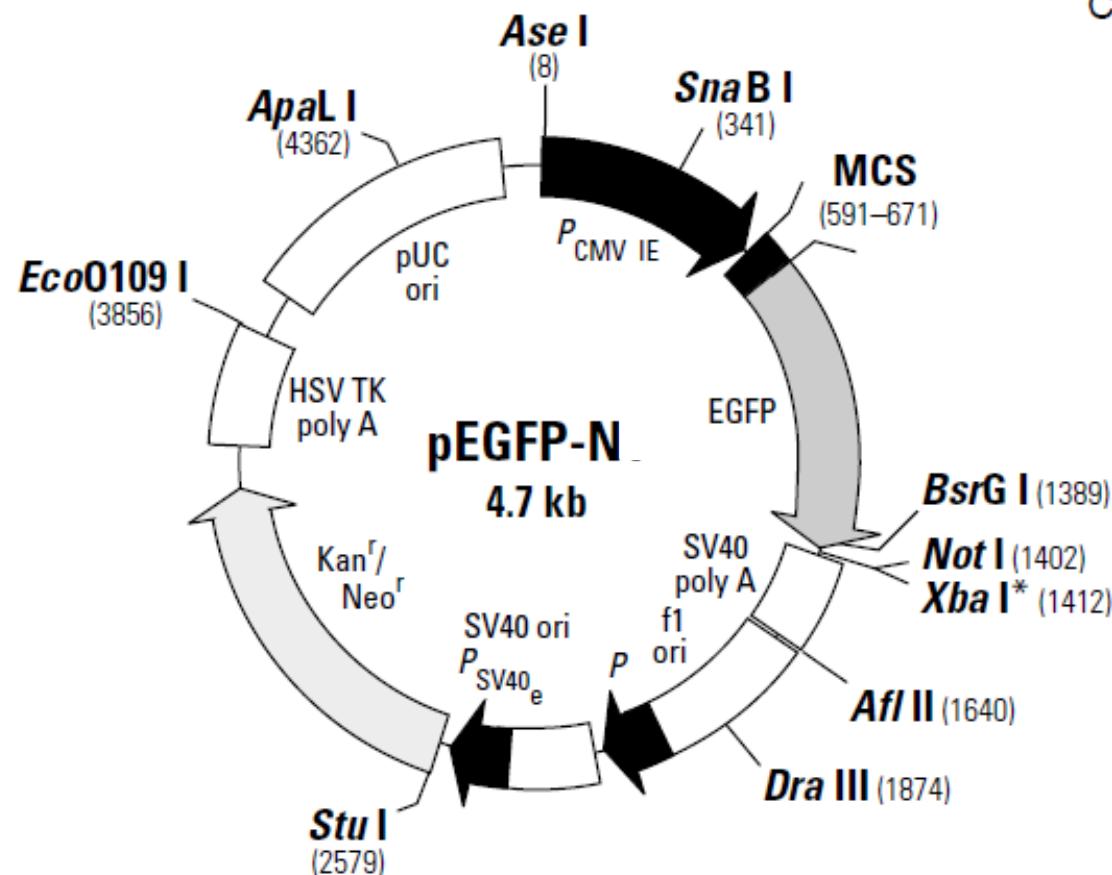
Cloning vector for the RT-PCR amplification product

Which expression vector will you use to have GFP in the cytoplasmic region, considering that N terminus is in the cytoplasm and C terminus is extracellular??



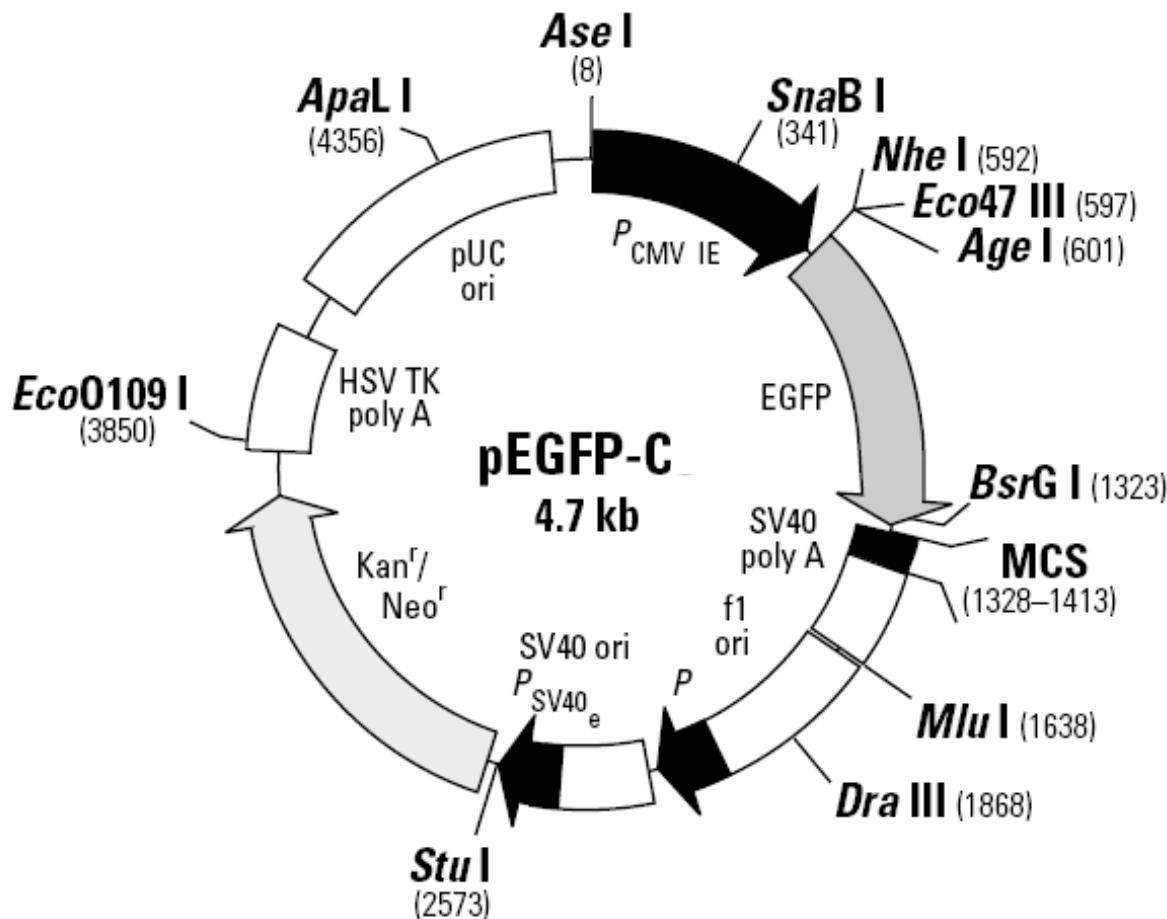
PT3027-5

Catalog #6085-1

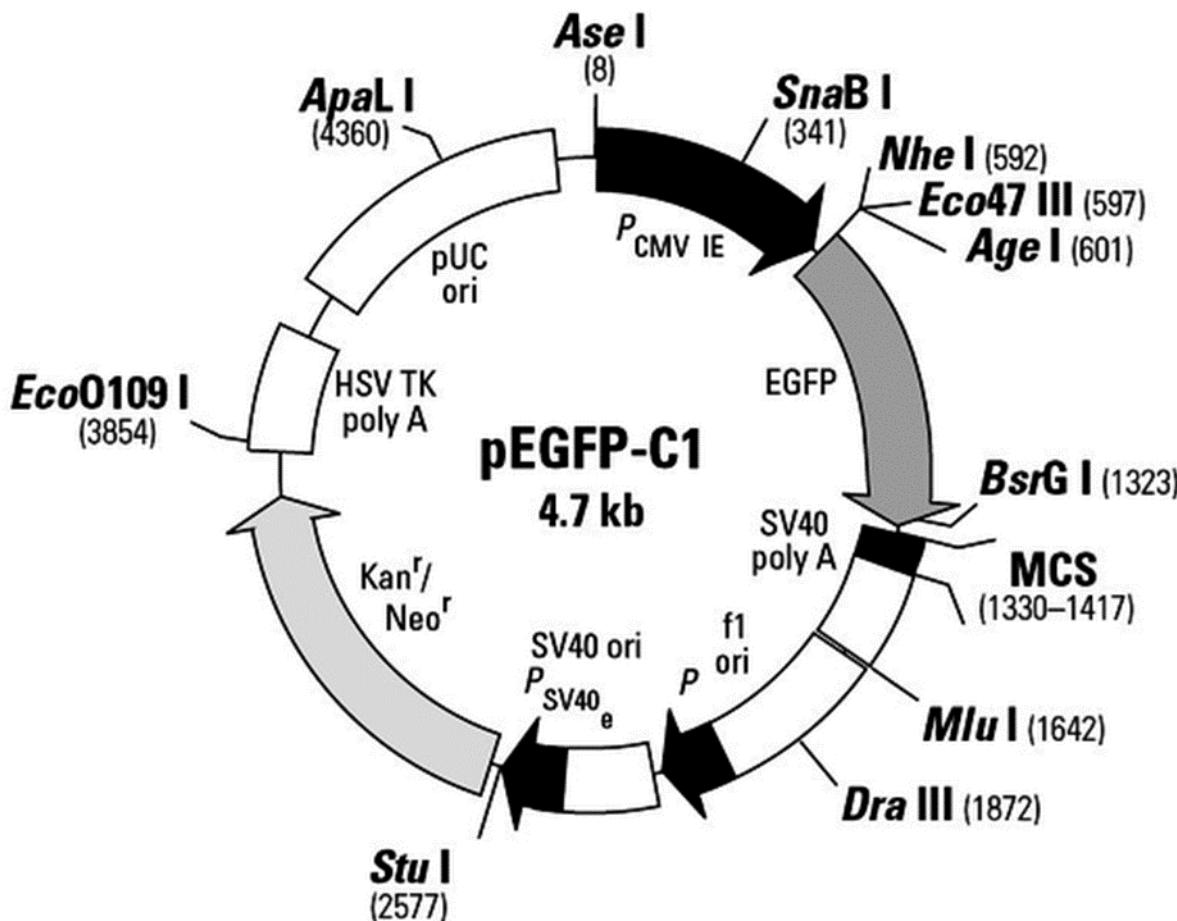


591 601 611 621 631 641 651 661 671
• G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC ATG GTG
Nhe I Eco47 III Bgl II Xho I Sac I Hind III EcoR I Pst I Sal I Acc I Asp718 I Kpn I Apa I Bsp120 I BamH I Xma I Sma I Age I EGFP →
Ecl136 II

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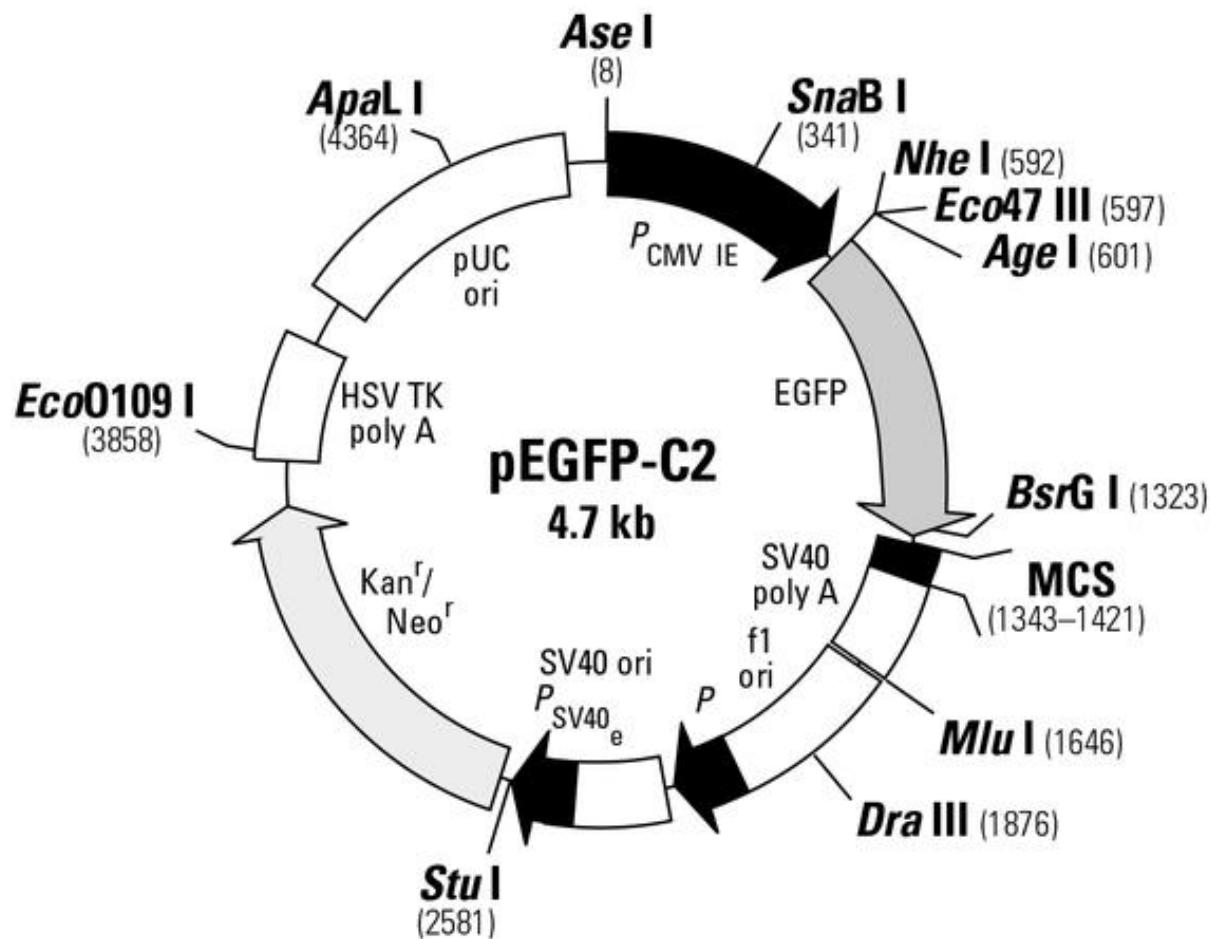


1330 1340 1350 1360 1370 1380 1390
 EGFP → TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GAT CTA GAT AAC TGA TCA
*Sca*I *Bgl*II *Xho*I *Sac*I *Hind*III *Eco*R I *Pst*I *Sal*I *Acc*I *Kpn*I *Asp*718 I *Apa*I *Bsp*120 I *Bam*H I *Xba*I* *Bcl*I*
*Ec*I/136 II *Sac*II *Xma*I *Sma*I STOPs



EGFP → 1330 . TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA CTG ATC A
 BspE I Bgl II Xho I Sac I Hind III Eco RI Pst I Sal I Acc I Kpn I Asp718 I Sac II Apa I Bsp120 I Bam H I Xma I Sma I Xba I* Bcl I*

Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. (Unique restriction sites are in bold). The **Xba I** and **Bcl I** sites (*) are methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.



Sequence:

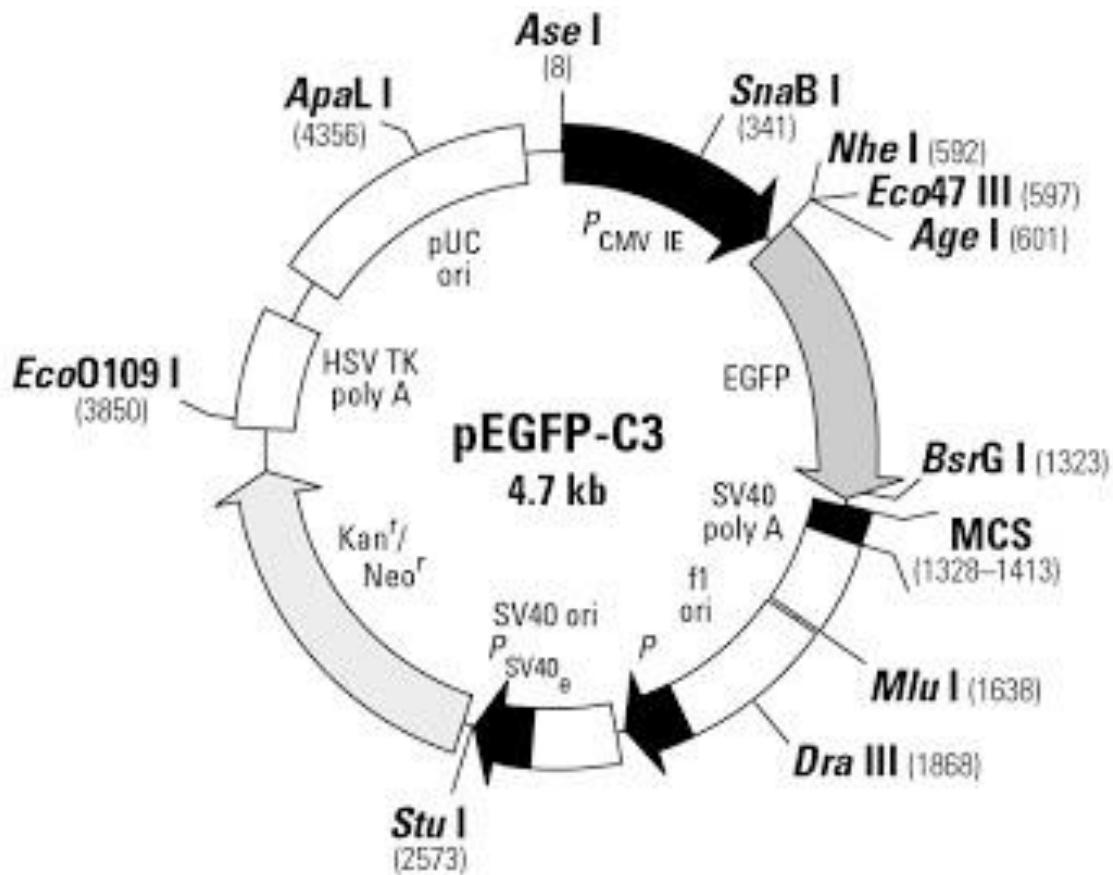
EGFP → TAC AAG TCC GGC CGG ACT CAG ATC TCG AGC TCA AGC TTC GAA TTC TGC AGT CGA CGG TAC CGC GGG CCC GGG ATC CAC CGG ATC TAG ATA ACT GAT CA

Enzyme Digestion:

Eag I Bgl II Xba I Sac I Hind III EcoRI Pst I Sal I Kpn I Apa I Bam HI Xba I* Bcl I*

Acc I Asp718 I Bsp120 I Sac II Xma I Sma I

STOPs:



EGFP	1330	1340	1350	1360	1370	1380	1390					
TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CCG GCC CGG GAT CCA CCG GAT CTA GAT AAC TGA TCA												
Scal	Bgl II	Xba I	Sac I	Hind III	EcoR I	Pst I	SaI	Kpn I	Apa I	BamH I	Xba I*	Bcl I*
			<i>Ecl36II</i>				<i>AccI</i>	<i>Asp718I</i>	<i>Bsp120I</i>	<i>Xma I</i>		
							<i>Sac II</i>			<i>Sma I</i>		
											STOPs	

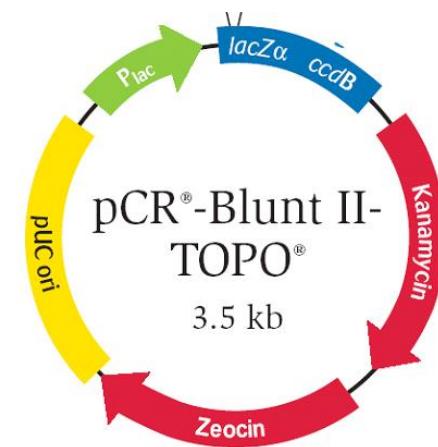
Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C3. (Unique restriction sites are in bold). The **Bcl I** site cannot be used for fusions since it contains an in-frame stop codon. The **Xba I** and **Bcl I** sites (*) are methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

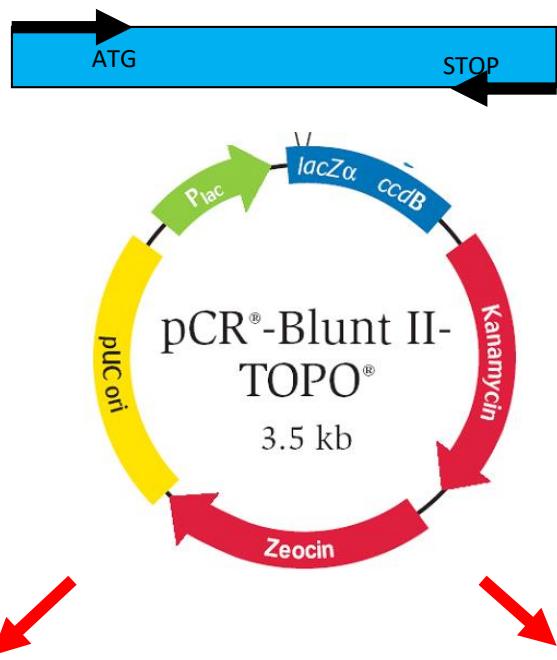
First AIM

* you have to prepare two primers, sense and antisense, to clone the full length NRG1 type III beta 3. One primer immediately before ATG, one after STOP.

* pairs of primers should meet the following criteria:

- 1- Tm similar (about 60° C calculated by the Allawi's method)
- 2- finish with G or C
- 3- have a content of G and C \geq 50% (if possible!)
- 4- not form secondary structures
- 5-18-28 base long

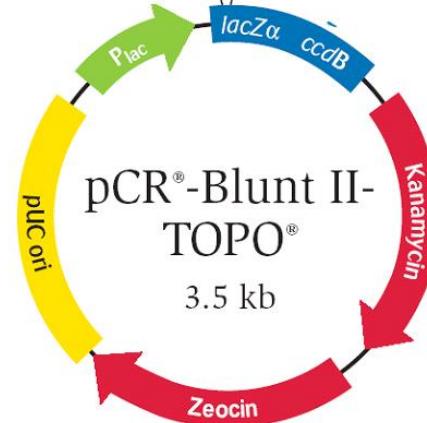
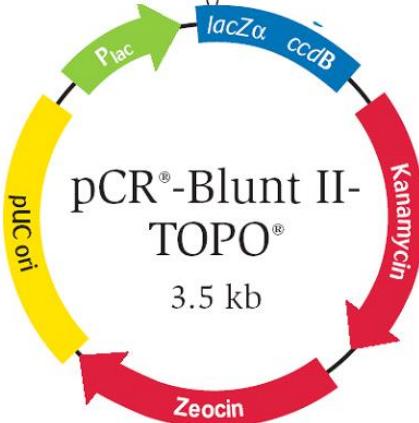
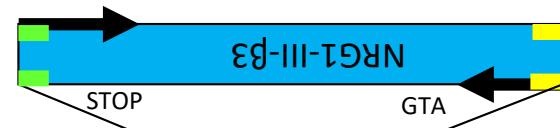


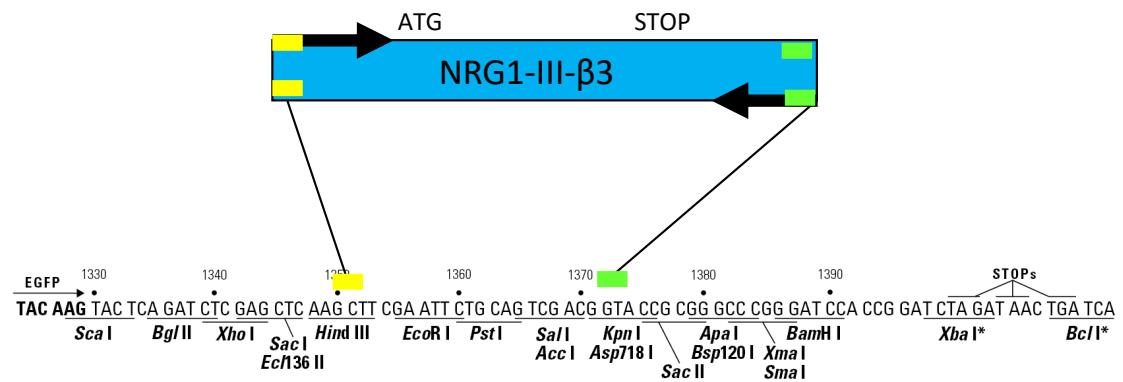
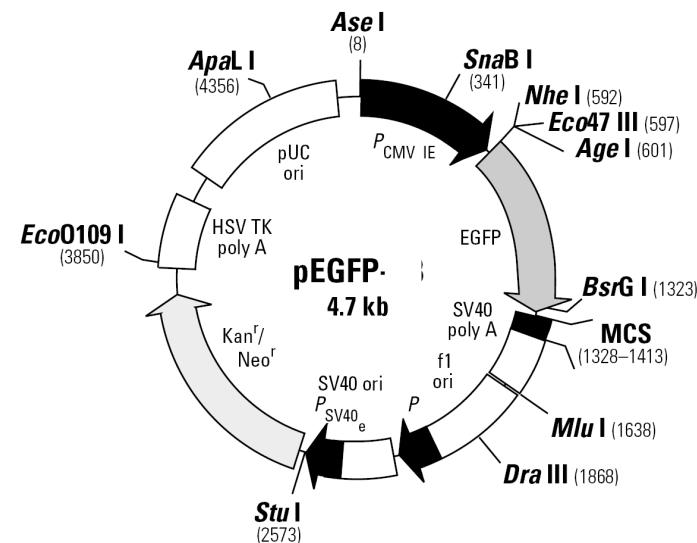
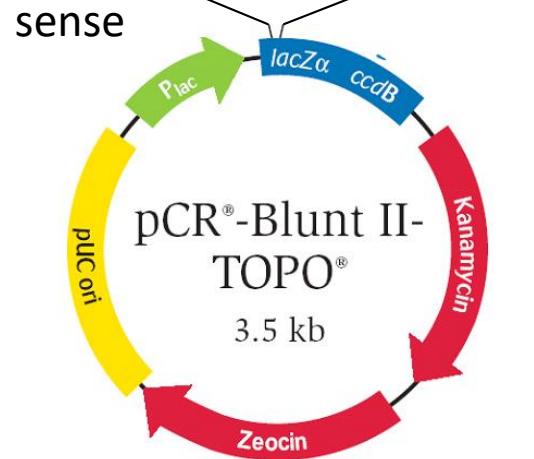
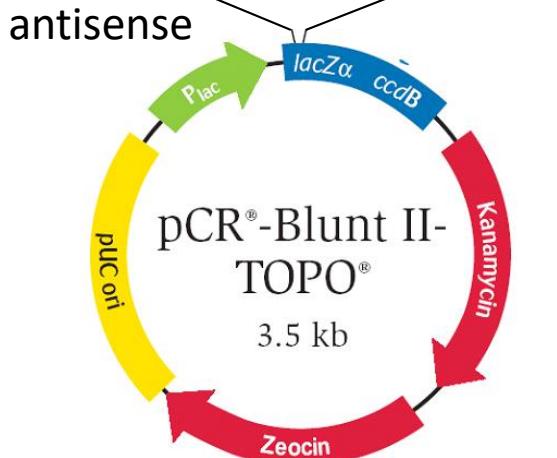


insert sense



insert antisense

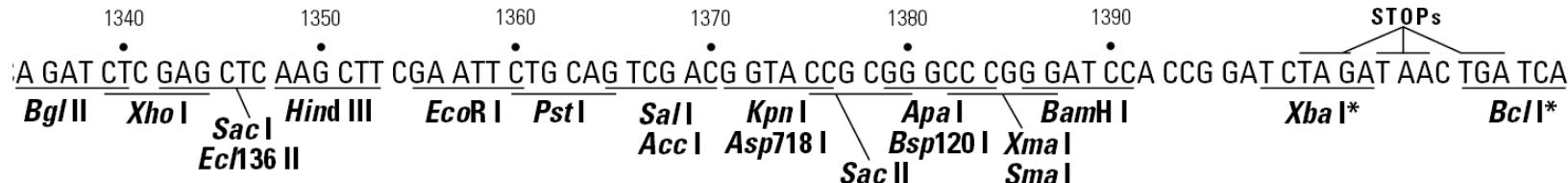
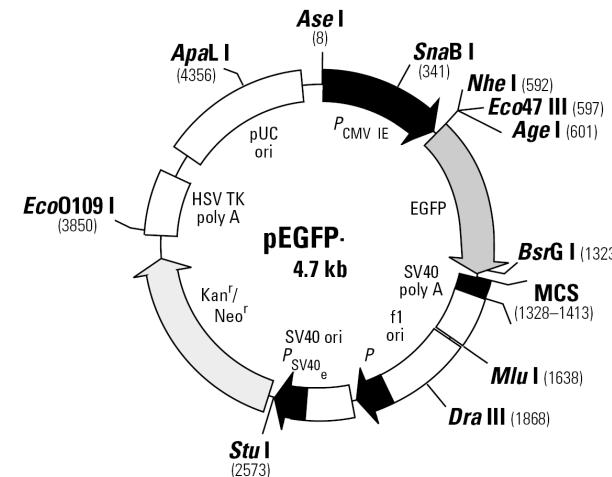




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Second AIM

- * because NRG1typeIII β 3 will be cloned in frame downstream EGFP in one vector pEGFP: insert restriction sites in the 5' of the primers to facilitate following subcloning from pCR-bluntII-TOPO
- * restriction sites must be compatible with the multiple cloning site of the vector pEGFP (from BgIII to BamHI)
- * restriction sites do not have to cut NRG1
 - > Make the list of enzymes in the MCS of the vector and investigate which of them do not cut NRG1



File Edit View History Bookmarks Tools Help

<http://tools.neb.com/NEBcutter2/index.php>

Google



Come iniziare Ultime notizie cocultura - co-cultura... Segreteria di Preside... UCSD-Nature Signalin... Università degli Studi... Sito Ufficiale della Re... La sessione di lavoro... AIAT Cogne Gran Par...

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G Search RS Bookmarks Check AutoLink AutoFill Send to neb cutter

Settings

NEBcutter V2.0



NEBcutter V2.0

Program Guide

Help

Comments

This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBytes.**

[What's new in V2.0](#)

Local sequence file: [Browse...](#)

GenBank number: [Browse GenBank](#)

or paste in your DNA sequence: (plain or FASTA format)

Standard sequences:

Plasmid vectors [▼](#)

Viral + phage [▼](#)

The sequence is: Linear Circular

Enzymes to use:

NEB enzymes
 All commercially available specificities
 All specificities
 All + defined oligonucleotide sequences
 Only defined oligonucleotide sequences
[\[define oligos\]](#)

Minimum ORF length to display: a.a.

Name of sequence: (optional)

Earlier projects:

Note: Your earlier projects will be deleted 2 days after they were last accessed.
You need to have cookies enabled in your browser for this feature to work.

Disable NEBcutter cookies

NEBcutter V2.0

ence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commer sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence **maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBases.**

EBcutter

Local sequence file: Nessun file selezionato.

GenBank number: [\[Browse GenBank\]](#)

or paste in your DNA sequence: (*plain or FASTA format*)

```
atttcaaagt
 2161 ctcactttta ttgataaaat aaaaatcatt ctactgaaca gtccatcttc
tttataacaat
 2221 gaccacatcc tgaaaagggt gttgctaagc tgtaaccgat atgcacttga
aatgatggta
 2281 agttaatttt gattcagaat gtgttatttg tcacaataaa acataataaa
aggagttcag
 2341 atgtttttct tcattaacca aaaaaaaaaa aaaa
//|
```

The sequence is: Linear Circular

Enzymes to use:

- NEB enzymes
- All commercially available specificities
- All specificities
- All + defined oligonucleotide sequences
- Only defined oligonucleotide sequences
[\[define oligos\]](#)

Minimum ORF length to display: a.a.

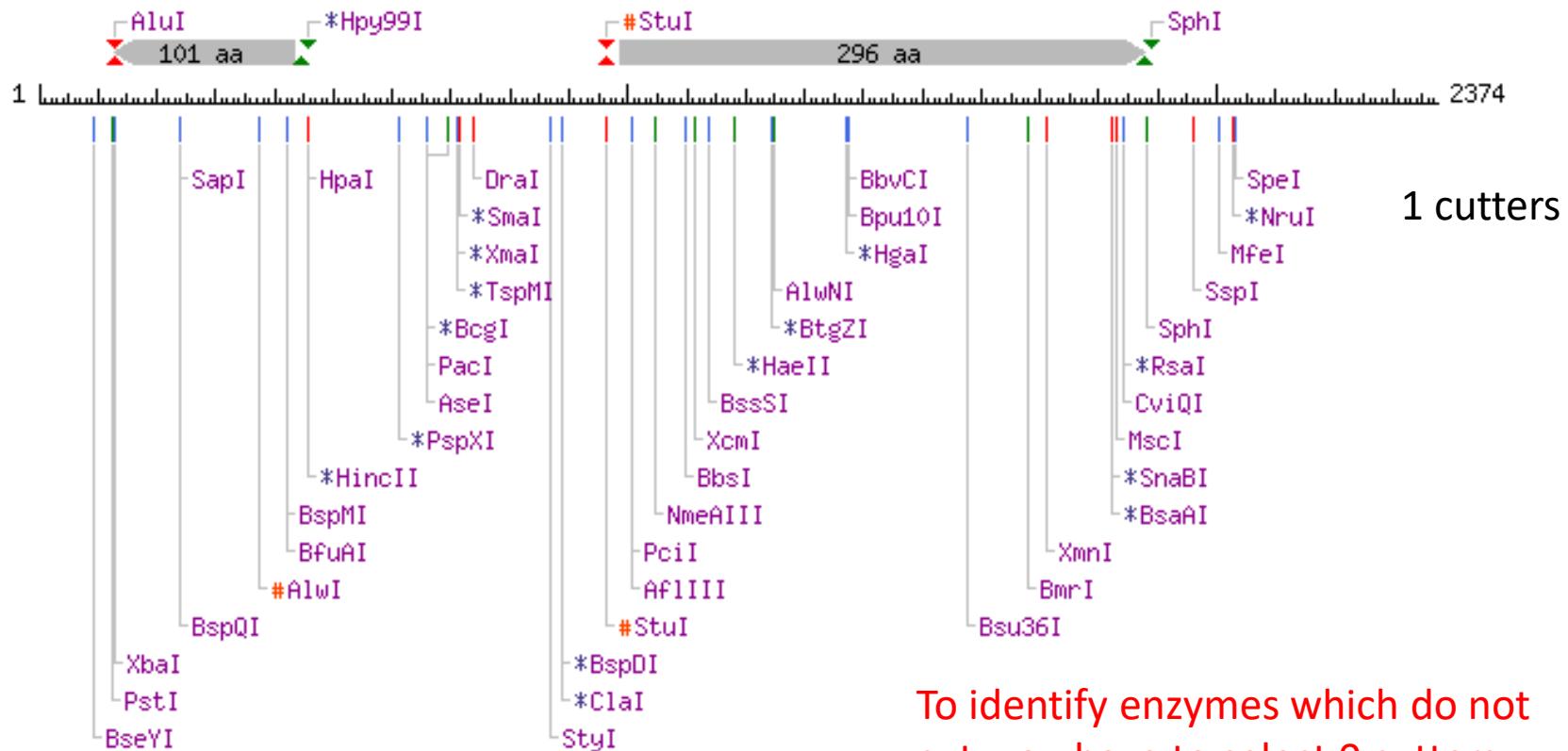
Name of sequence: (*optional*)

Standard sequences:
Plasmid vectors
Viral + phage



- The system automatically shows 1 cutter enzymes

I cuts 1 st



To identify enzymes which do not cut, you have to select 0 cutters

Only for teaching purposes - not for reproduction or sale

- Main options
- New DNA
 - Custom digest
 - View sequence
 - ORF summary
 - Save project
 - Print

Availability

- All commercial
- All

Display

- 2 cutters
- 3 cutters

Zoom

- Zoom in
- More...

List

- 0 cutters
- 1 cutters
- All sites
- Save all sites
- Flanking enzymes

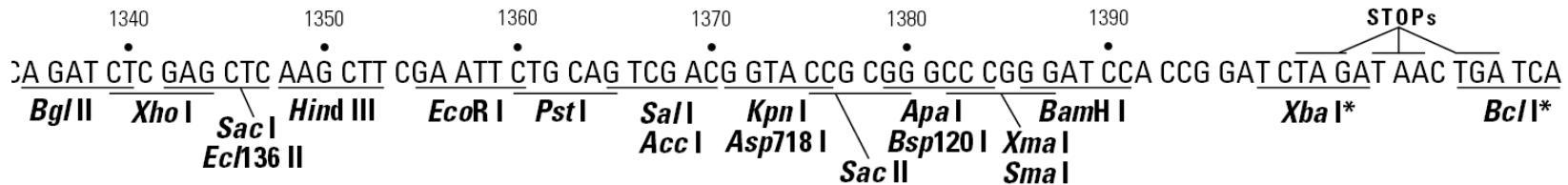
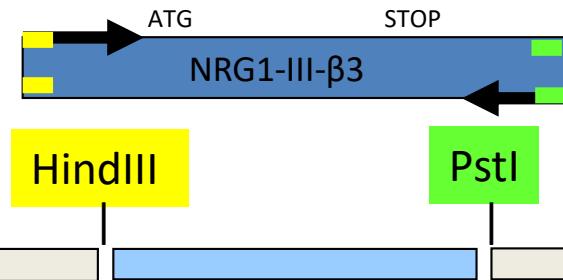
Minimum ORF length to display: 100 aa. OK

Enzymes that don't cut

[Help](#) [Comments](#)[\[Back to main display\]](#)*unnamed sequence*Number of cuts =

#	Enzyme	Specificity
1	Acc65I	G ^y GTAC _x C
2	AccI	GT ^y MK _x AC
3	AclI	AA ^y CG _x TT
4	AfeI	AGC _x GCT
5	AflII	C ^y TTAA _x G
6	AgeI	A ^y CCGG _x T
7	AleI	CACNN _x NNGTG
8	ApaLI	G ^y TGCA _x C
9	AscI	GG ^y CGCG _x CC
10	AseI	AT ^y TA _x AT
11	AsiSI	GCG _x AT ^y CGC
12	AvaI	C ^y YCGR _x G
13	AvrII	C ^y CTAG _x G
14	BaeI	(N) ₅ _x (N) ₁₀ ACNNNNNGTAYC(N) ₇ _x (N) ₅
15	BbvCI	CC ^y TCA _x GC
16	BceAI	ACGGC(N) ₁₂ _x NN _x
17	BciVI	GTATCC(N) ₅ _x N ^y
18	BmgBI	CAC _x GTC
19	BmtI	G _x CTAG ^y C
20	BsaBI	GATNN _x NNATC
21	DraI	GCTTCCTCTTATATATATAT

Example: in the list of enzymes that do not cut NRG1, I found HindIII and PstI



5' -CGTTAACTTGACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

3' -GCAATTGAACGGTACACGTAGATCGAG**GTACCGTACG**-5'

First AIM

Primer sense: 5' -**CGTTAACTTG**-3'

Primer antisense: 5' -**GCATGCCATG**-3'

5' -CGTTAACTTGACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

3' -GCAATTGAAC~~TGGTACACGTAGATCGAG~~**GTACCGTACG**-5'

First AIM

Primer sense: 5' -**CGTTAACTTG**-3'

Primer antisense: 5' -**GCATGCCATG**-3'

Add the **HindIII site**
to the 5' of the primer
sense

5' -AAGCTT-3'

3' -TTCGAA-5'

Add the **PstI site**
to the 5' of the primer
antisense

5' -CTGCAG-3'

3' -GACGTC-5'

Second AIM

Primer sense: 5' -AAGCTTCGTTAACTTG-3'

Primer antisense: 5' -CTGCAGGCATGCCATG-3'

5' -CGTTAACTTGACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

3' -GCAATTGAAC $\text{TGGTACACGTAGATCGAG}$ **GTACCGTACG**-5'

First AIM

Primer sense: 5' -**CGTTAACTTG**-3'
Primer antisense: 5' -**GCATGCCATG**-3'

Added the **HindIII site**
to the 5' of the primer
sense

5' -**AAGCTT**-3'
3' -**TTCGAA**-5'

Added the **PstI site**
to the 5' of the primer
antisense

5' -**CTGCAG**-3'
3' -**GACGTC**-5'

Second AIM

Primer sense: 5' -**AAGCTT****CGTTAACTTG**-3'
Primer antisense: 5' -**CTGCAG****GCATGCCATG**-3'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'

3' -GCAATTGAAC $\text{TGGTACACGTAGATCGAG}$ **GTACCGTACG**-5'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'
3' -GCAATTGAAC~~TGGTACACGTAGATCGAG~~**GTACCGTACG**-5'



denaturation + primer annealing

5' -**AAGCTTCGTTAACTTG**-3' ->
3' -GCAATTGAAC~~TGGTACACGTAGATCGAG~~**GTACCGTACG**-5'

5' -**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'
 <- 3' -**GTACCGTACGGACGTC**-5'



elongation

5' -**AAGCTTCGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3'
3' -GCAATTGAAC~~TGGTACACGTAGATCGAG~~**GTACCGTACGGACGTC**-5'

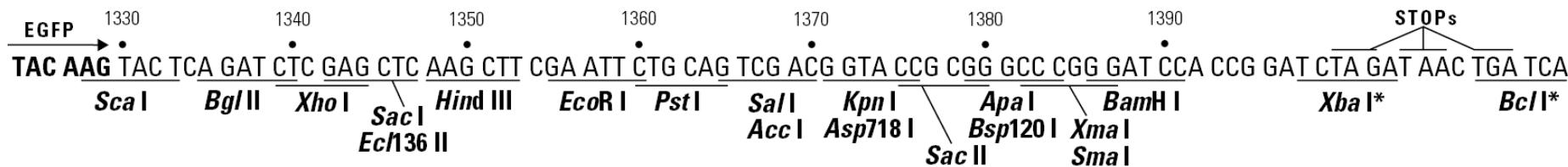


..... several amplification cycles



5' -**AAGCTTCGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC**CCTGCAG**-3'
3' -**TTCGAA**GCAATTGAAC~~TGGTACACGTAGATCGAG~~**GTACCGTACGGACGTC**-5'

Pay attention to the reading frame!

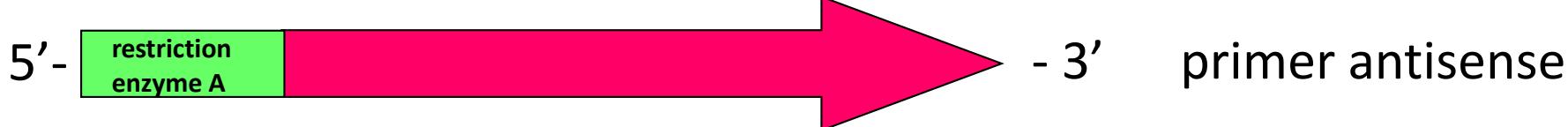
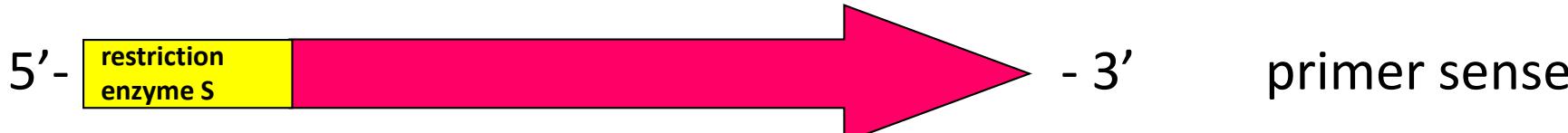


Example: you decide to include *Hind*III site upstream the primer:



EGFP

TAC AAG TAC TCA GAT CTC GAG CTC **AAG CTT** CGA ATT CTG CAG TCG



TAC AAG TAC TCA GAT CTC GAG CTC **AAG CTT** NNN NNN NNN NAT GNN N

EGFP

linker

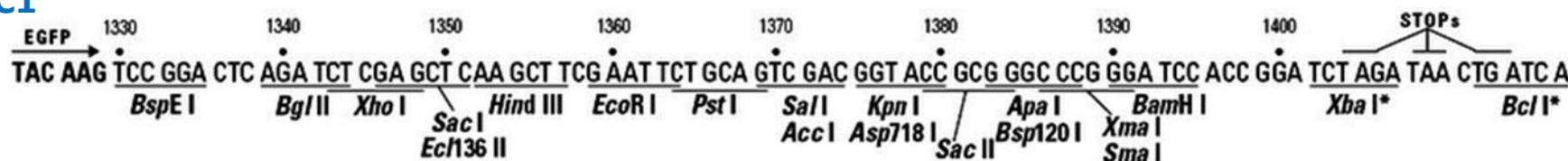
NRG1

TAC AAG TAC TCA GAT CTC GAG CTC **AAG CTT** NNN NNN NNN NNN NNN NNN **ATG** NNN
 EGFP linker NRG1

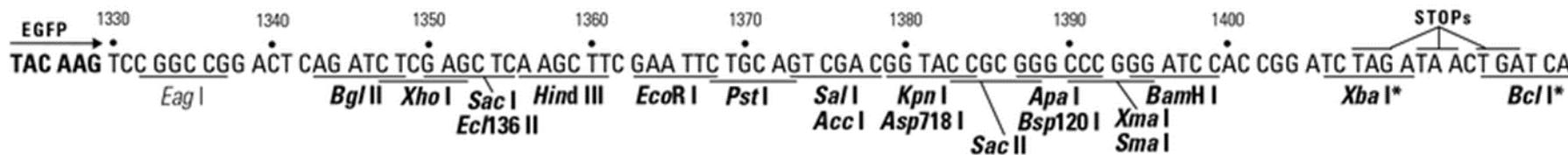
* translate the triplets between GFP and NRG1 into amino acids :
 there must be no STOP

* the triplets must be in frame: coding from EGFP to NRG1

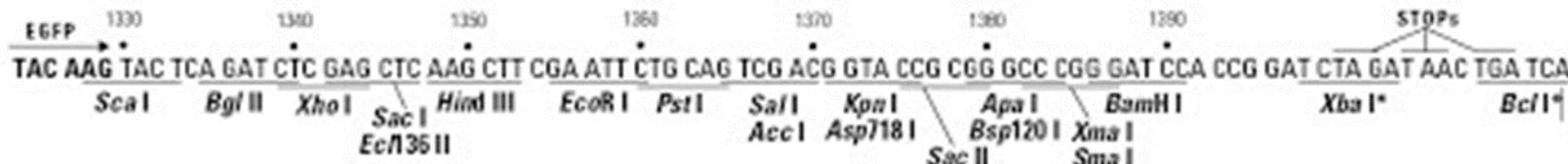
EGFP-C1



EGFP-C2



EGFP-C3



- * insert your sequence in vector pEGFP-C1
- * translate the triplets between the GFP and the NRG1 into amino acids : there must be no STOP & the triplets must be in frame: coding from EGFP to NRG1
- * if it does not work try with pEGFP-C2
- * if it does not work try with pEGFP-C3

Primer: 5'-**AAGCTTNNNNNNNNNNATGNNN-3'**

EGFP-C1

TAC AAG	TCC GGA CTC AGA TCT CGA GCT	CAA GCT T	NN NNN NNN NN	A TG	N NN
EGFP	linker	HindIII			NRG1

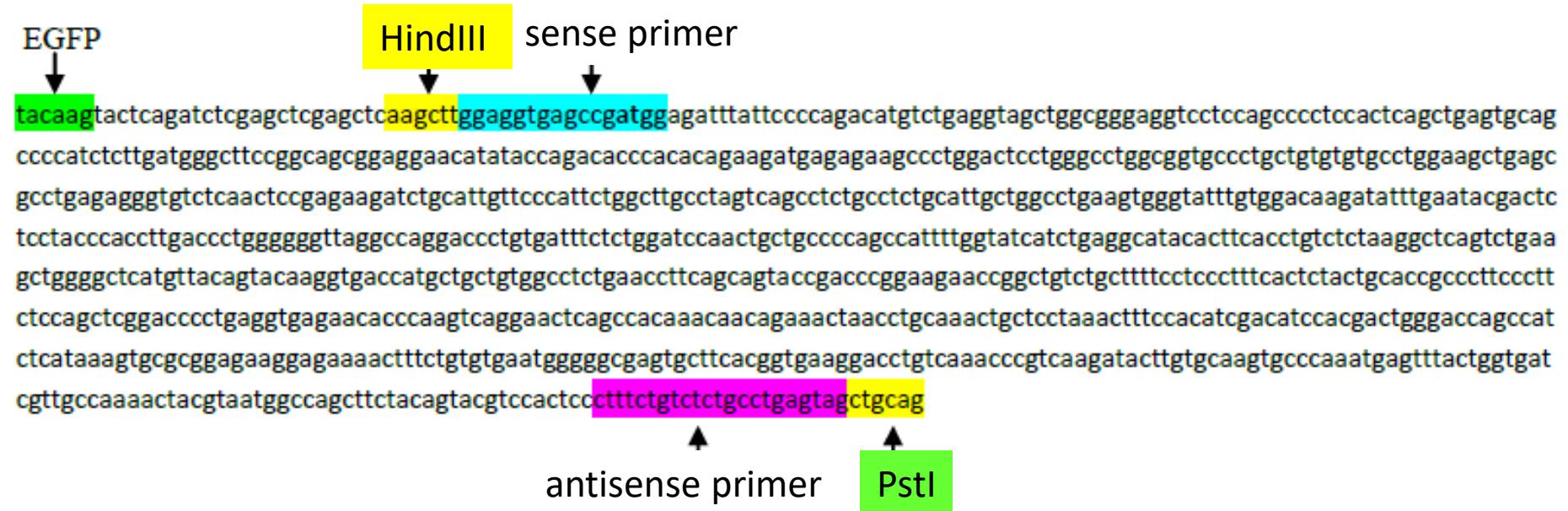
EGFP-C2

TAC AAG	TCC GGC CGG ACT CAG ATC TCG AGC	TCA AGC TT	N NNN NNN NNN	ATG NNN	
EGFP	linker	HindIII			NRG1

EGFP-C3

TAC AAG	TAC TCA GAT CTC GAG CTC	AAG CTT	NNN NNN NNN N	ATG NNN N	
EGFP	linker	HindIII			NRG1

- 1-prepare primers
- 2-add restriction sites (not cutting the NRG1 cDNA insert!)
- 3-verify the correct frame to choose the suitable expression vector
- 4-insert your construct into the expression vector, to obtain the final map and verify the correct frame.



pEGFP-C3

TAGTTATTAATAGTAATCAATTACGGGTCATTAGTTCATAGCCCATAATGGAGTCCCGTTACATAACTTACGGTAA
ATGGCCCGCCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA
GGGACTTTCCATTGACGTCAATGGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTATGGGACTTTC
CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGCGGTTTGGCAGTACATCAATGGCGTGG
TAGCGGTTGACTCACGGGATTCCAAGTCTCACCCATTGACGTCAATGGAGTTGTTGGCACCAAAATCAACG
GGACTTCCAAAATGTCGTAACAACACTCCGCCCCATTGACGCAAATGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA
GCAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTACGGTCGCCACCAGGTGAGCAAGGGCGAGGAGCTGTTCA
CCGGGGTGGTGCCCATCCTGGTCAGCTGGACGGGAGCTAAACGCCACAAGTCAGCGTGTCCGGCGAGGGCGAGGGC
GATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGACCAACCGGCAAGCTGCCGTGCCCTGGCCACCCCTGAC
CACCCGTACGGCGTGCAGTGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG
GGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGACAAGCTGGA
GTACAACATACAACAGCCACAACGTCTATATCATGGCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCC
ACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACTACCAGCAGAACACCCCCATGGCGACGGCCCGTGTGCTG
CCCGACAACCACCTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAAACGAGAACGCGGATCACATGGCCTGCTGGA
GTTCGTGACCGCCGCCGGGATCACTCTGGCATGGACGAGCTGTACAAGTACTCAGATCTCGAGCTC**AAGCTT**CGAATT**C**
TGCAGTCGACGGTACCGCGGGCCGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTGTAGAGG
TTTACTTGCTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTG
TTTATTGCAAGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTTCACAAATAAGCATTTCAGCTGCATT
TAGTTGTGGTTGTCCAAACTCATCAATGTATCTAACCGTAAATTGTAAGCGTTAATATTTGTTAAAATTCCGCTTA
AATTTTGTAAATCAGCTATTAAACCAATAGGCCAAATCGGAAAATCCCTATAAAATCAAAAGAACGACCGA
GATAGGGTTGAGTGTGTTCCAGTTGGAACAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAA
CCGTCTATCAGGGCGATGGCCACTACGTGAACCATCACCCTAATCAAGTTTTGGGTCGAGGTGCCGTAAAGCACTA ...

AAGCTT=HindIII
TGCAG=PstI

pEGFP-C3

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTCATAGCCCATAATGGAGTCCCGCTTACATAACTACGGTAA
ATGGCCCGCCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA
GGGACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCGCCCTGGCATTATGCCAGTACATGACCTTATGGGACTTC
CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGCGGTTTGGCAGTACATCAATGGCGTGG
TAGCGGTTTGAECTCACGGGATTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTGTTGGCACCAAAATCAACG
GGACTTCCAAAATGTCGTAACAACCTCCGCCATTGACGCAAATGGCGGTAGGCAGTACGGTGGGAGGTCTATATAA
GCAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTACCAGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCA
CCGGGGTGGTGCCTCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGC
GATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCGTGCCCTGGCCACCCCTCGTGA
CACCCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG
GGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAGCTGGA
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ACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACCTACCAAGCAGAACACCCCCATCGCGACGGCCCCGTGCTGCTG
CCCGACAACCAACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAACGGCGATCACATGGCCTGCTGGA
GTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTACTCAGATCTCGAGCTCAAGCTT

HindIII

PstI

CTGCAGTCGACGGTACCGCGGGCCGGATCCACCGGATCTAGATAACTGATCATATACTAGCCATACCACTTTGTAGAGG
TTTACTTGCTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAACTTG
TTTATTGCAGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTTCACAAATAAGCATTTCACGATT
TAGTTGTGGTTGTCCAAACTCATCAATGTATCTAACCGTAAATTGTAAGCGTTAATATTGTTAAAATTGCGTTA
AATTTTGTTAAATCAGCTCATTTTAACCAATAGGCCGAAATCGGAAAATCCCTATAAAATCAAAGAACGAGG
GATAGGGTTGAGTGTGTTCCAGTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAAACGTCAAAGGGCGAAAAA
CCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCATAATCAAGTTTGGGTCGAGGTGCCGTAAAGCACTA ...

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAATGGAGTTCCCGGTTACATAACTTACGGTAA
ATGGCCCGCCTGGCTGACCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA
GGGACTTTCCATTGACGTCAATGGGTGGAGTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAAGTACGCCCTTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTC
CTACTTGGCAGTACATCTACGTTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACATCAATGGCGTGG
TAGCGGTTGACTCACGGGATTCCAAAGTCTCCACCCATTGACGTCAATGGGAGTTGTTGGCACC AAAATCAACG
GGACTTTCAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGCGGTAGGCCTGTACGGTGGGAGGTCTATAAA
GCAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTCA
CCGGGGTGGTGCCTCCTGGTCGAGCTGGACGGGACGTAAACGGCCACAAGTTCAAGCTGTCGGCGAGGGCGAGGGC
GATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGCCACCCCTCGTGAC
CACCCGTACGCTACGGCGTGCAGTGTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCAAGGACGACGGCAACTACAAGACCCGCCAGGTGAAGTTGAG
GGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGCAAGCTGGA
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ACAACATCGAGGACGGCAGCGTGAGCTCGCCGACCAC TACCAGCAGAACACCCCATCGCGACGGCCCCGTGCTGCTG
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aagcttggaggtagccatggagatttattccccagacatgtctgaggtagctggcgggagggtctccagccccctccact
Cagctgagtgcagccccatctcttgatggctccggcagcggaggaacatataccagacacccacacagaagatgagaga
Agccctggactcctggccctggcggtgcctgtgtgcctggaaagctgagcgcctgagagggtgtctcaactccgag
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Acagtacaaggtagccatgtctgtggctctgaaccttcagcagtaccgaccggaaagaaccggctgtctgtttcc
Cccttcactctactgcaccggcccttcccttcagtcggaccctgaggtgagaacaccccaagtccaggaaactcc
Caaacaacagaaaactaacctgcaaactgctctaaactttccacatcgacatccacgactggaccgcattcataaag
Tgcgcggagaaggaaaaactttctgtgtaatggggcgagtgctcacggtaaggaccctgtcaaacccgtcaagatac
Ttgtgcaagtgcctaaatgagttactggtgatcggtgcctactacgtaatggccagttctacagtacgtccactccc
tttctgtctctgcctgagtagctgcag
CTGCAGTCGACGGTACCGCGGGCCGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACTTTGTAGAGG
TTTACTTGCTTAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAACITG
TTTATTGCACTTATAATGGTTACAATGAGCAATAGCATCACAAATTTCACAAATAAGCATTTCACGTGCAATT
TAGTTGTGGTTGTCACACTCAATGATCTTAACGCGTAAATTGTAAGCGTTAATATTGTTAAAATTGCGTTA
AAATTTGTTAAATCAGCTCAATTTTAACCAATAGGCAGAAATGGCAAAATCCCTTATAAAATCAAAAGAATAGACCGA
GATAGGGTTGAGTGTGTTCCAGTTGGAACAAGAGTC CACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAAA
CCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTTAATCAAGTTTGTGGGTCGAGGTGCCGTAAAGCACTA ...

HindIII

PstI

AAGCTT=HindIII

CTGCAG=PstI

For restriction analysis and identification of ORF and maps, you can use NEB cutter:

<http://tools.neb.com/NEBcutter2/index.php>

NEBcutter V2.0 - Mozilla Firefox

File Edit View History Bookmarks Tools Help

<http://tools.neb.com/NEBcutter2/index.php>

Come iniziare Ultime notizie coculture - co-cultura... Segreteria di Preside... UCSD-Nature Signalin... Università degli Studi... Sito Ufficiale della Re... La sessione di lavoro... AIAT Cogne Gran Par...

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NEBcutter V2.0

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[NEB homepage]

NEBcutter V2.0

Program Guide Help Comments

This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBytes.**

[What's new in V2.0](#)

Local sequence file: [Browse...]
GenBank number: [Browse GenBank]
or paste in your DNA sequence: (plain or FASTA format)

Standard sequences:
Plasmid vectors
Viral + phage

Submit More options Set colors

The sequence is: Linear Circular

Enzymes to use:

- NEB enzymes
- All commercially available specificities
- All specificities
- All + defined oligonucleotide sequences
- Only defined oligonucleotide sequences
[define oligos]

Minimum ORF length to display: a.a.

Name of sequence: (optional)

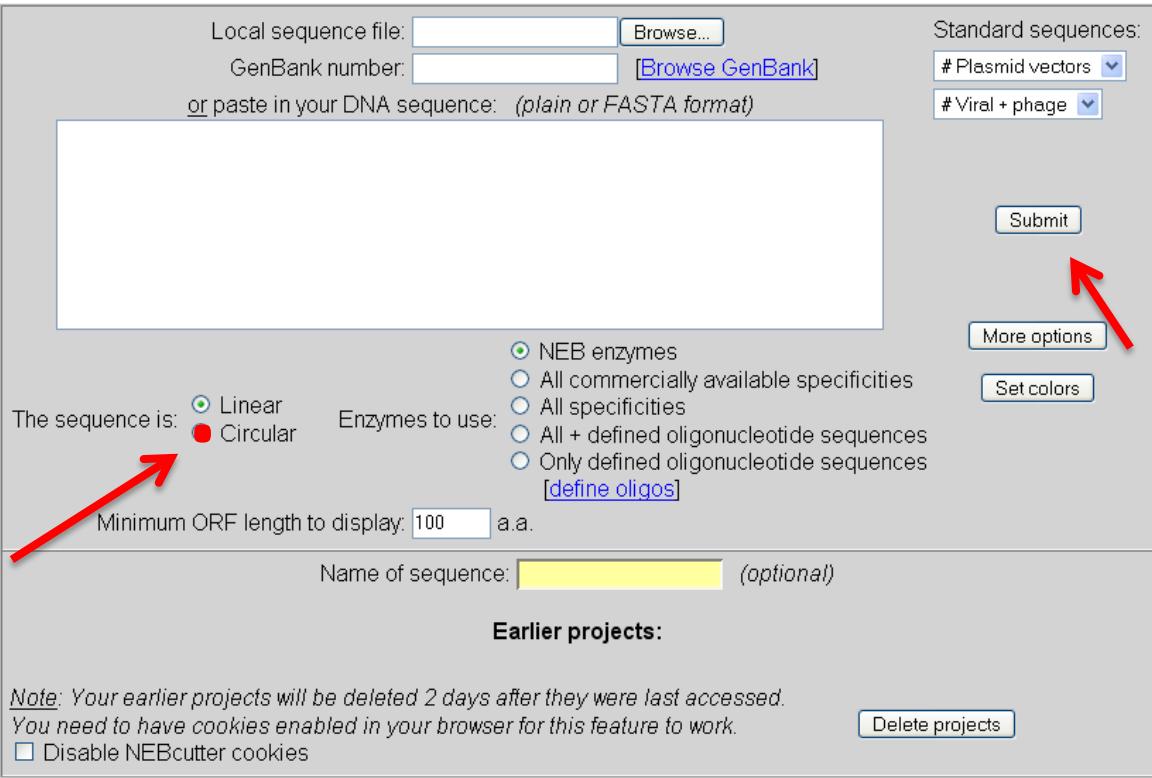
Earlier projects:

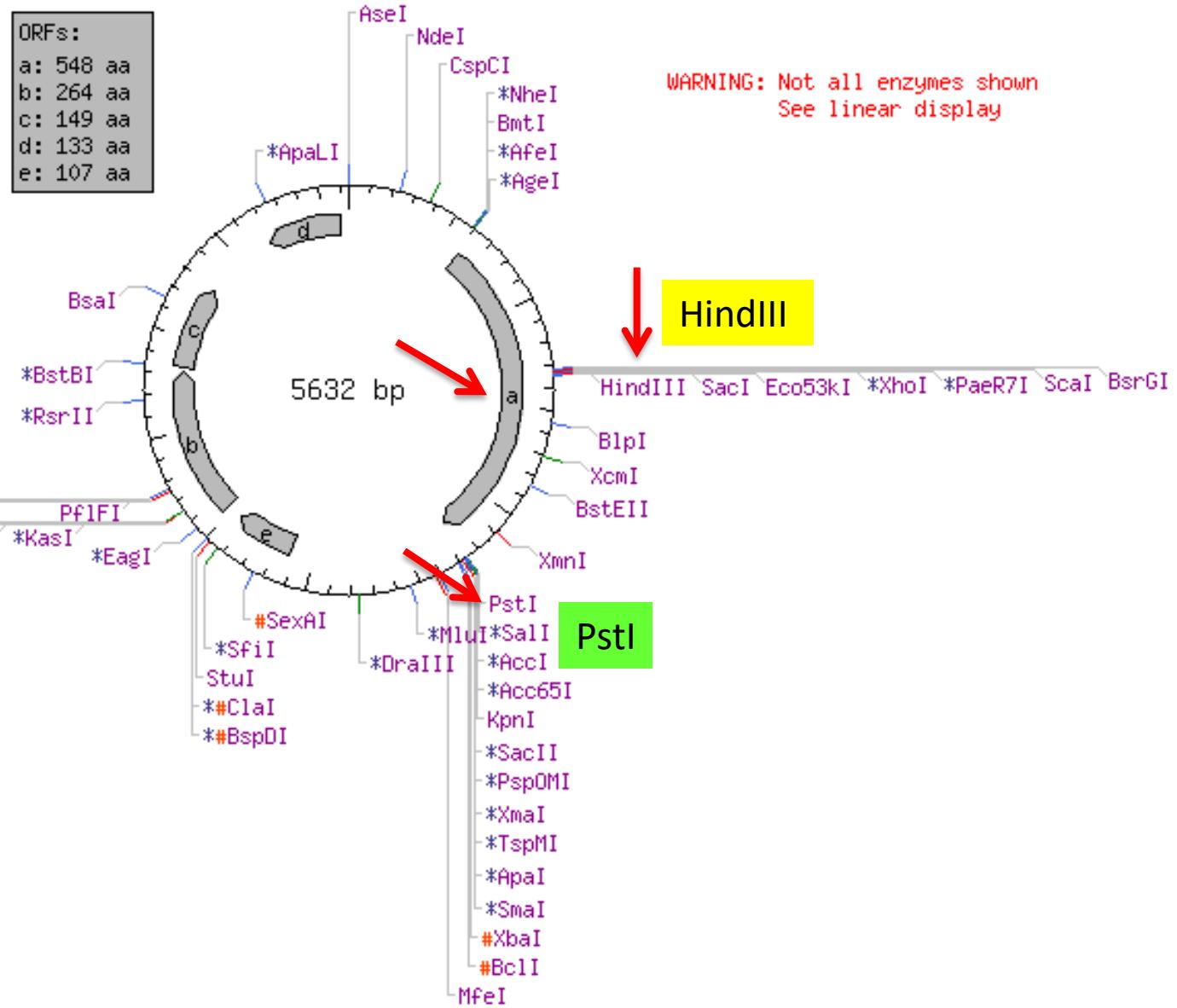
Note: Your earlier projects will be deleted 2 days after they were last accessed.
You need to have cookies enabled in your browser for this feature to work.

Disable NEBcutter cookies

Done

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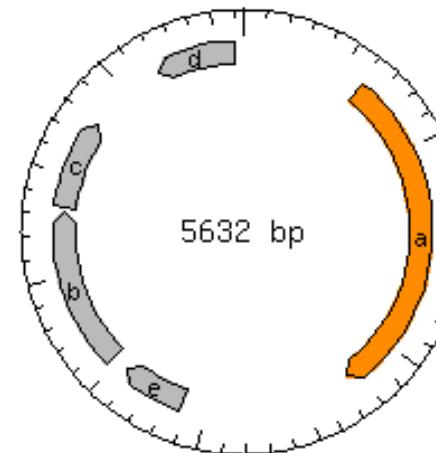
[\[click to main display\]](#)

ORF Sequence

unnamed sequence

Coding region: 613..2259

ORFs:
a: 548 aa
b: 264 aa
c: 149 aa
d: 133 aa
e: 107 aa



[\[Edit\]](#) - [\[Delete\]](#) - [\[Add new ORF\]](#) - [\[Locate multiple cutters that excise this ORF\]](#) - [\[Silent Mutagenesis\]](#)

Protein sequence:

> 548 aa
MVKGEELFT GVVPILVLED GDVNNGHKFSV SGEGEGGDATY GKLTLKFICT
TGKLPVPWPPT LVTTILTYGVQ CFSRYPDHKMK QHDFFKSAMP EGYVQERTIF
FKDDDGNYKTR AEVKFEGDTL VNRIELKGID FKEDGNILGH KLEYNLYNSHN
VYIMADKQKN GIKVNFKIRH NIEDGSVQLA DHYQQNTPIG DGPVLLPDNH
YLSTQSALSK DPNEKRDHMV LLEFVTAAAGI TIGMDELYKY SDLELKLGGE
PMEIYSPDMS EVAGGRSSSSP STQLSAAPSL DGLPAAEEHI PDTHTEDERS
PGILGLAVPC CVCLEAERLR GCLNSEKICI VPILACLVSL CLCIAGLK WV
FVDKIFEYDS PTHLDPGGLG QDPVISLDPT AAPAILVSSE AYTSPVSKAQ
SEAGAHVTVQ GDHAAVASEP SAVPTRKNRL SAFPPFHSTA PPPPSPARTP
EVRTPKSGTQ PQTTETNLQT APKLSTSTST TGTSHLIKCA EKEKTFCVNG
GECFTVKDLS NPSRYLCKCP NEFTGDRCQN YVMASFYSTS TPFLSLPE

EGFP

NRG1

Exercize: prepare a single slide like the following one, containing all necessary information:

- 1-the entire sequence from EGFP to the antisense primer, highlighting the primers, the restriction sites, the ATG, the STOP codon)
- 2- the vector used (1, 2 or 3)
- 3- restriction sites used (do not use HindIII!!)
- 4- the primers (written correctly, with restriction sites in **bold**)
- 5- the protein translation
- 6- the full map showing the fusion protein

EGFP

HindIII

sense primer

DO NOT use the enzyme HindIII!

tacaagtactcagatctcgagctcgagtcaggctggaggtagccgatggagattttcccccagacatgtctgaggtagctggccggaggtctccagccccctccactcagctgagtgcag
 ccccatcttgcgtggctccggcagcggaggaacatataccagacacccacacagaagatgagagaagccctggactcctggccctggccctgtgtgtgcctggaaagctgagc
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 gctggggctcatgttacagttacaagggtgaccatgctgtggctctgaaccttcagcgtaccgaccggagaaccggctgtctgtttcccttcactctactgcaccgc
 ctccagctggaccctgaggtgagaacacccaagtcaggaactcagccacaaacaacagaaactaacctgcaaacttccacatcgacatccacgactggacc
 ctcataaaagtgcgcggagaaggagaaaaacttctgtgtgaatggggcgagtgcttacggtaaggaccgtcaaacccgtcaagatacttgcaagtgc
 cgttccaaaactacgtaatggccagcttctacagtaacgtccactcccttctgtctgcctgagtagctgcag

antisense primer

PstI

PRIMERS

Sense: 5'-AAGCTTGGAGGTGAGCCGATGG-3'

Antisense: 5'-CTGCAGCTACTCAGGCAGAGACAGAAAG-3'

Vector: pEGFP-C3

TRANSLATION

> 548 aa

MVSKGEELFT GVVPILVLED GDVNNGHKFSV SGEGEGDATY GKLTLLKFI CT
 TGKLPLPVWPT LVTTLTGYGVQ CFSRYPDHMK QHDFFKSAMP EGYVQERTIF
 FKDDDNYKTR AEVKFEGDTL VNRIELKGID FKEDGNILGH KLEYNLYSHN
 VYIMADKQKN GIKVNFKIRH NIEDGSVQLA DHYQNTPIG DGPVLLPDNH
 YLSTTQSALSK DPNEKRDHMV LLEFVTAAIGI TLGMDELYKY SDLELKLGE
 PMEIIYSPDMS EVAGGRSSSP STQLSAAPSL DGLPAAEEHI PDTHTEDERS
 PGLLGLAVPC CVCLAEERLR GCLNSEKICI VPILACLVSL CLCIAGLKVV
 FVDKIFYEYDS PTHLDPGGLG QDPVISLDPT AAPAILVSSE AYTSPVSKAQ
 SEAGAHVTVQ GDHAHAVASEP SAVPTRKNRL SAFFPFHSTA PPFPSPARTP
 EVRTPKSGTQ PQTETNLQT APKLSTSTST TGTSHLIKCA EKEKTFCVNG
 GECFTVKDLS NPSRYLCKCP NEFTGDRCQN YVMASFYSTS TPFLSLE

ORFs:
a: 548 aa
b: 264 aa
c: 149 aa
d: 133 aa
e: 107 aa

