

022-practicals

Insertion of gene for cry protein in a plasmid with the sequence of GFP protein, called 35S-eGFP-nosT, and used for transient expression

To complete this task follow the steps described in 2.1 section, except two important things:

- sequences for the restriction enzymes should be replaced. Which restriction enzymes can be used to open the plasmid between CaMV 35S promoter and eGFP gene? You have to select restriction enzymes which don't have recognition sites in the cry gene.*
 - when you will design reverse primer you should skip the last three nucleotides (represent stop codon) in order to keep ORF open*
-

1. Importam plasmid

Import Addgene Plasmids

35S-eGFP-nosT

☐ Addgene plasmid numbers: [Format Tips](#)

35S-eGFP-nosT

None selected

☐ Convert plasmid sequences to uppercase

☒ Include Addgene sequence verification results

☒ Preserve format settings from the original files

Imported sequences:

☒ Open

☐ Save

[Import](#) [Cancel](#)

Da vstavim cry gen moram izbrat restrikcijska mesta ki niso v njemu, torej.. če grem na **Enzymes** tab od cry gena in izberem print lahko dobim seznam:

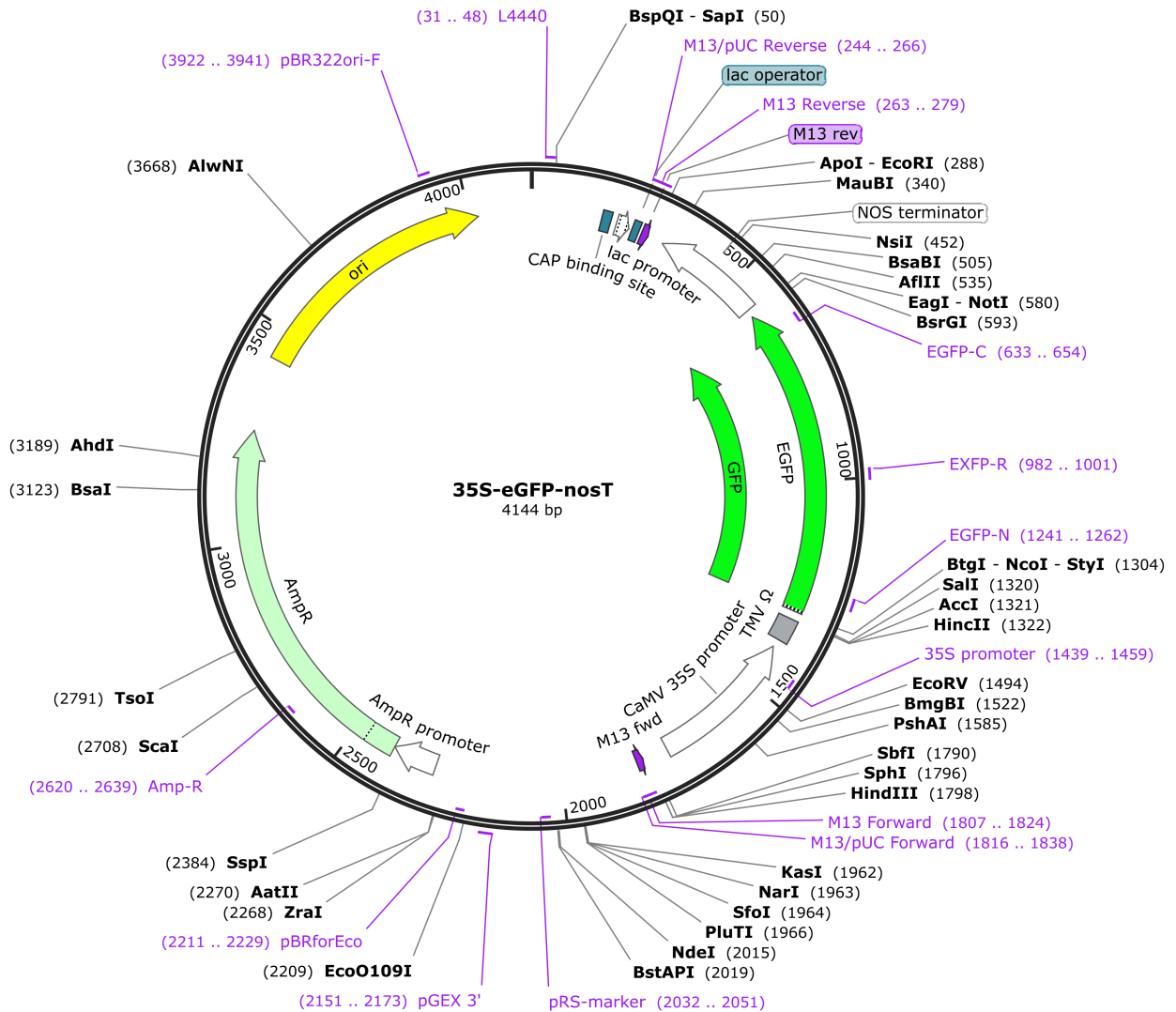
Sequence: Amplified_from021.dna (Linear / 1794 bp)

Enzymes: < Unsaved Enzyme Set > (36 of 686 total)

Enzyme	Sites	Location
AccI	1	1352
AflIII	1	889
ApaI	1	1605
ApaLI	1	1476
BbsI	1	751
BlpI	1	1702
BpuEI	1	1054
BsaI	1	1228
BseRI	1	520
BsmBI	1	327
BstXI	1	1437
BtgZI	1	516
DraIII	1	588
Esp3I	1	327
HincII	1	1353
MluI	1	889
MreI	1	1744
MscI	1*	1678*
NaeI	1	1746
NgoMIV	1	1744
NspI	1	402
PaeR7I	1	324
PaqCI	1	183
PpuMI	1	168
PspOMI	1	1601
PstI	1	1101
SalI	1	1351
Sau3AI	8	53 218 695 1367 1382 1574 1721 1769
SphI	1	402
TsoI	1	1057
XbaI	1	609
XhoI	1	324
XmnI	1	181

3 of 36 enzymes in the chosen set do not cut

Moram pa odpret med CaMV 35S promoter in eGFP gene ...



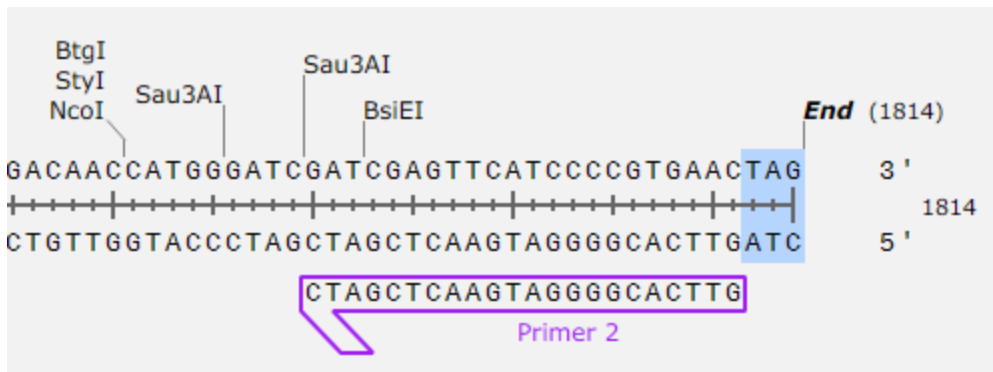
Okej, sem kopiral seznam encimov od obeh v svoje datoteke, jih odpr z R-jem in preveril kateri so unikatni za gen (glej `candidate_REs.html`):

Encimi, ki jih lahko izberem za vstavljanje (prisotni sami v cry genu):

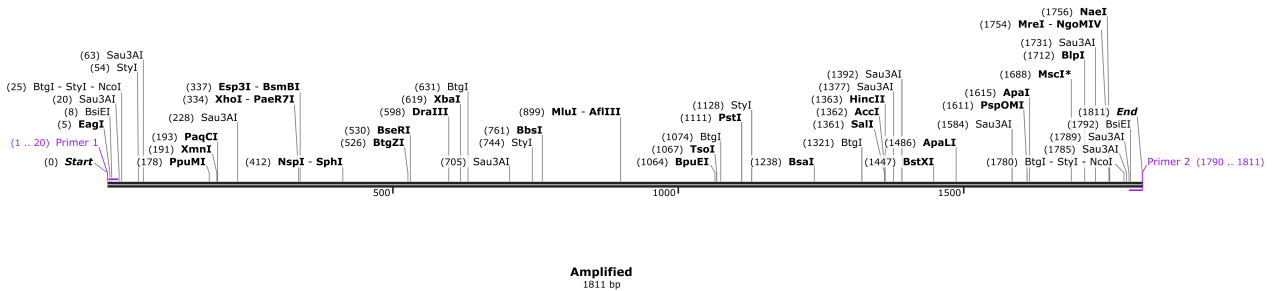
uncommon_f

```
## AflIII  ApaI  ApaLI  BbsI  BlpI  BpuEI  BseRI  BsmBI  BstXI  BtgZI  DraIII
##      2    3    4    5    6    7    9    10   11   12   13
##  Esp3I  MluI  MreI  MscI  NaeI  NgoMIV  NspI  Paer7I  PaqCI  PpuMI  PspOMI
##      14   16   17   18   19   20   21   22   23   24   25
##  PstI  Sau3AI  XbaI  XhoI  XmnI
##      26   28   31   32   33
```

In zdaj lahko bi spogramiral da najde še kateri so med promotorjem in GFPjem, ampak bom na oko pogledal ker jih ni precej. Mislim da moram te gledat:



In dobim tole



In zdaj to vstavim v plazmid...

Hold up...



Pač kaže mi da ne morem izbrat dva na istem baznem paru... ampak vsi te ki so na desni, so prisotni v fragmentu in jih ne smem izbrat...

Siva regija pa je enhancer in tega ne smem izrezat, kot smo imeli pri 2.1 MCS... I don't know what to do.

TMV

1327 .. 1382

56 bp

misc_feature

NOTE

translational enhancer from the tobacco mosaic virus 5'-leader sequence (Gallie et al., 1988)

Pusti mi če izberem samo eno regijo pri plazmidu in encimu... sem izbral **NcoI** . Anyways,

Restriction and Insertion Cloning: Insert Fragment

File Edit View Enzymes Features Primers Actions Tools Window Help

Vector Fragment Product

Map Sequence Enzymes

EGFP-N

10 20 30 40 50 60 70 80 90

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Display

↑ ↓

2970

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EGFP

Met

Unique 6+ Cutters | Nonredundant

Linearized Vector	4144 bp
-------------------	---------

☐ Make file:

Linearized Vector.dna

Fragment	1755 bp
----------	---------

Source: Amplified.dna
☐ Make file:

☐ Make file:
Fragment.dna

Alternative Product with Flipped Fragment

☐ Make file: Alternative

Ready to clone
Product: 5899 bp

1 warning

Create product: ☒ and close this window

Cloned.dna

Clone Cancel

Clone Cancel