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*

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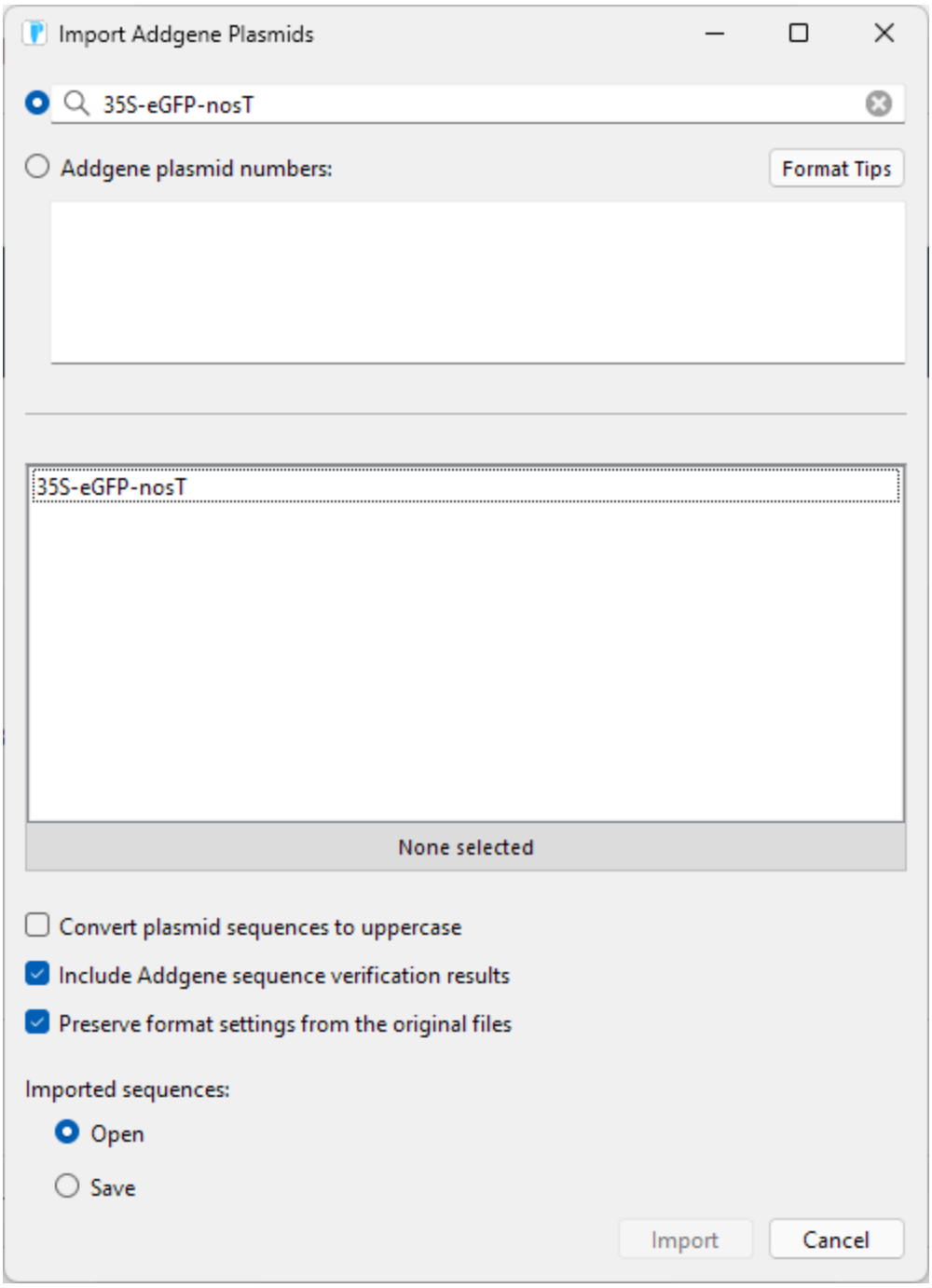
1. Importam plasmid



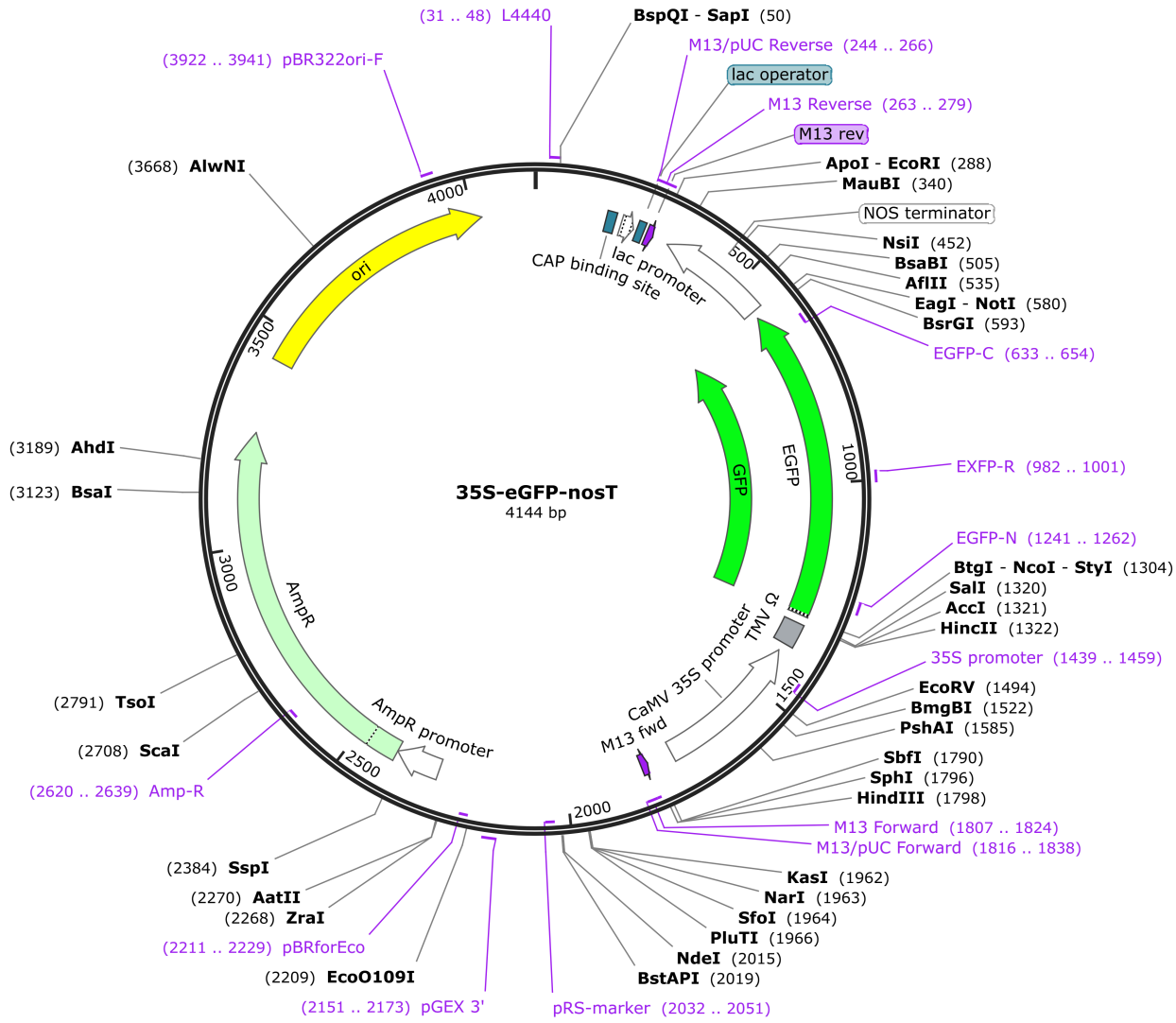
Da vstavim cry gen moram izbrat restrikcijska mesta ki niso v njemu, torej.. če grem na `Enzymes` tab od cry gena in izberem print lahko dobim seznam:

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`...



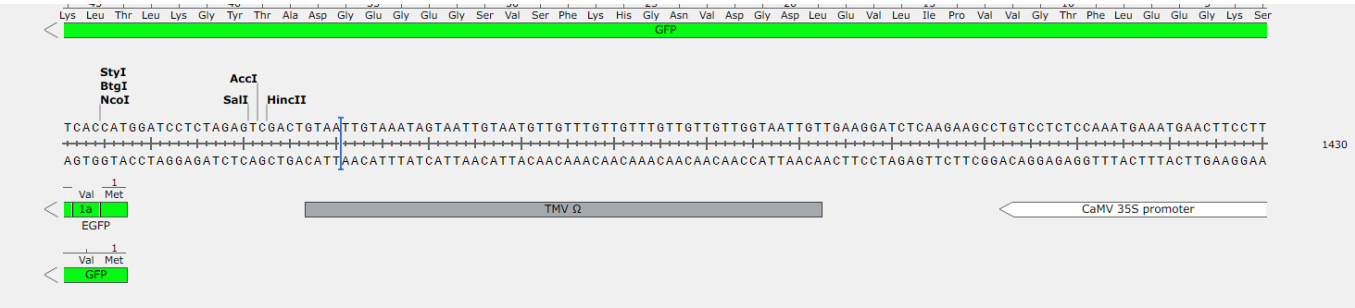
Okej, sem kopiral seznam encimov od obeh v svoje datoteke, jih odpr z R-jem in preveril kateri so unikatni za gen (glej `candidate_REs.html`):

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 sporgramiral 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:

Kateri od njih ni prisoten v fragmentu?

```
plasmid_candidates[which(!(plasmid_candidates %in% fvec2))]
```

```
## [1] "BtgI" "NcoI"
```

Te lahko zdaj uporabim za PCR in vstavljanje... to pomeni, da moram prilagoditi svoj gen - mu spremeniti primerje, narediti PCR in vstaviti. Hmm... a naj dam vsakega na en konec ali uporabim samo enega...? Glede na to, da smo prej tudi izbrali različna za isto regijo, se mi zdi prav da tudi zdaj naredim tako.

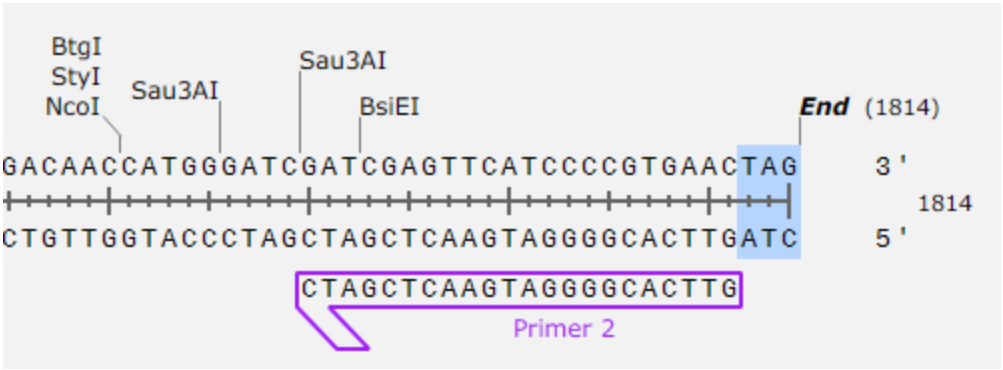
`BtgI` na začetek in `NcoI` na konec. Pazi, da dodaš encima za primerji (primerji so samo za PCR). Rekel je, da naj dodamo še en random site pred tem, da ni takoj za promotorjem.. ampak verjetno ne sme biti site prisoten v fragmentu..? Idk, prej sem dal za `SauAI3` pa je bilo okej... idk. Bom zdaj tudi.

No in tudi `BtgI` je med encimi ampak ga ni označilo v export... I dont fucking know.

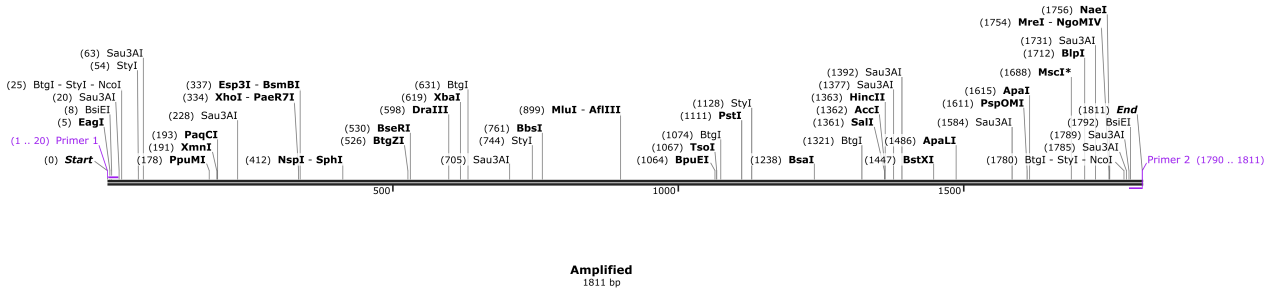
`NcoI` se osveti odebeljeno, `BtgI` pa ne.... bom `NcoI` uporabu...

No itak mi kaže da je isti site...

Pazil, da sem preskočil stop kodon.



In dobim tole

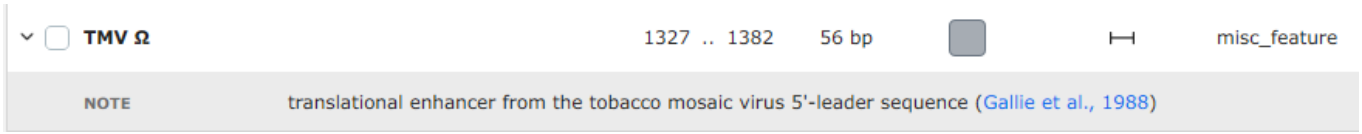


In zdaj to vstavim v plazmid...

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.



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
Display
BipI
MreI
NgoMIV
NaeI
EGFP
GFP
TMV Ω
Unique 6+ Cutters | Nonredundant
Bacterial Transformation Strain
Unspecified
Linearized Vector 4144 bp
Source: 35S-eGFP-nosT.dna
Make file:
Linearized Vector.dna
Fragment 1755 bp
Source: Amplified.dna
Make file:
Fragment.dna
Alternative Product with Flipped Fragment
Make file:
Alternative Product.dna

NcoI (1304)
NcoI (1304)
Vector 4144 bp
NcoI (25)
NcoI (1780)
Fragment 1755 bp
NcoI
NcoI
Product 5899 bp

Ready to clone
Product: 5899 bp
1 warning
Create product:
and close this window
cloned.dna
Clone
Cancel