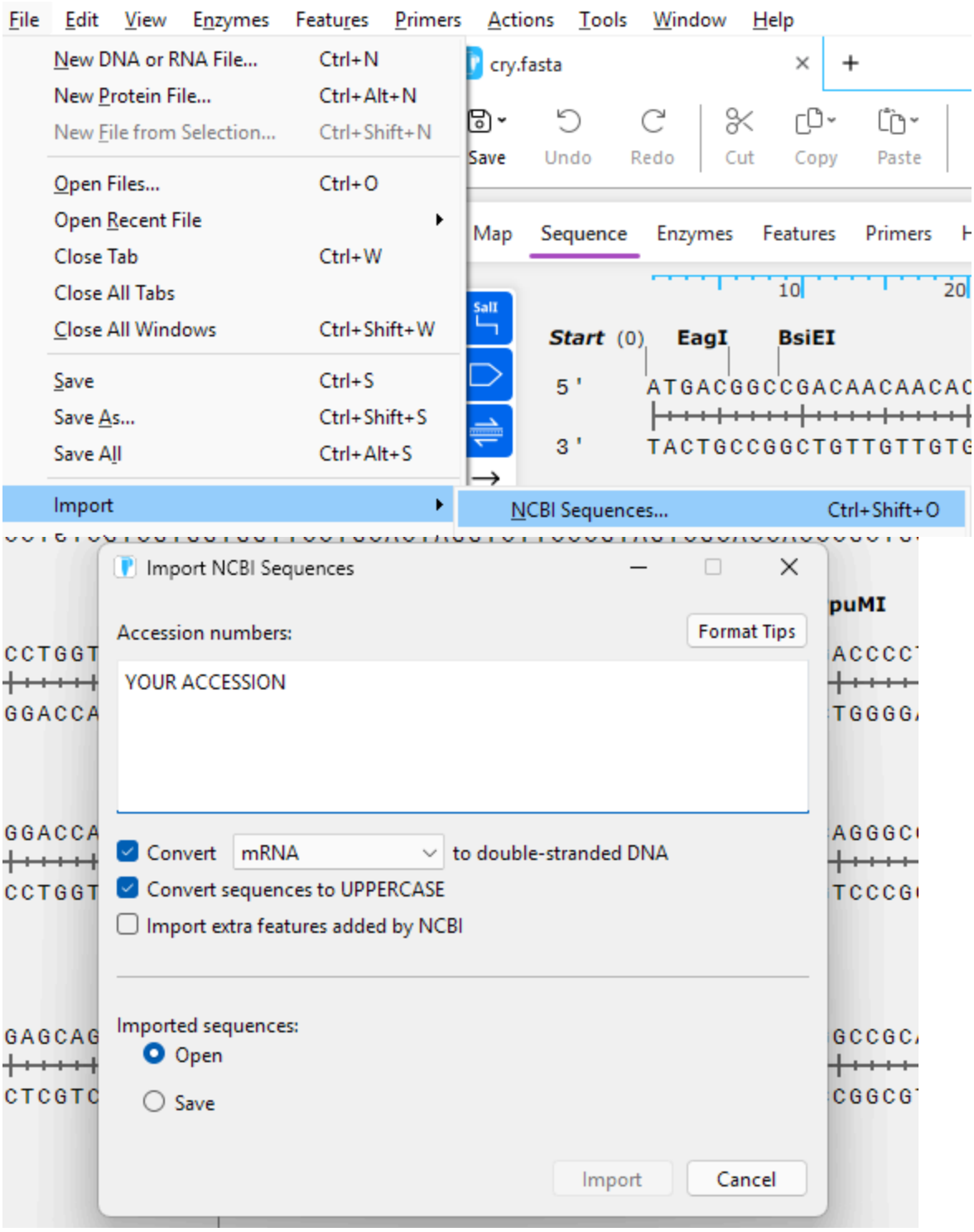
021-practicals

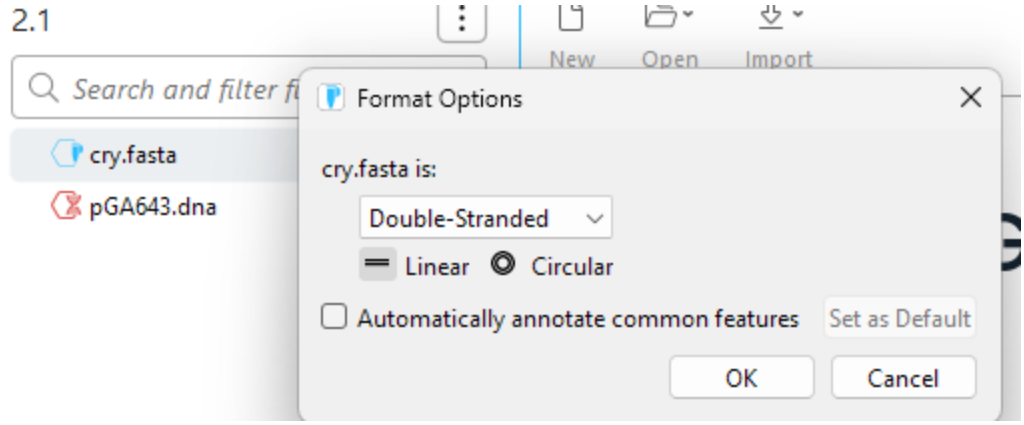
Insertion of gene for cry protein into a pGA643 plasmid.

1. *Import fasta sequence of cry protein in SnapGene.*

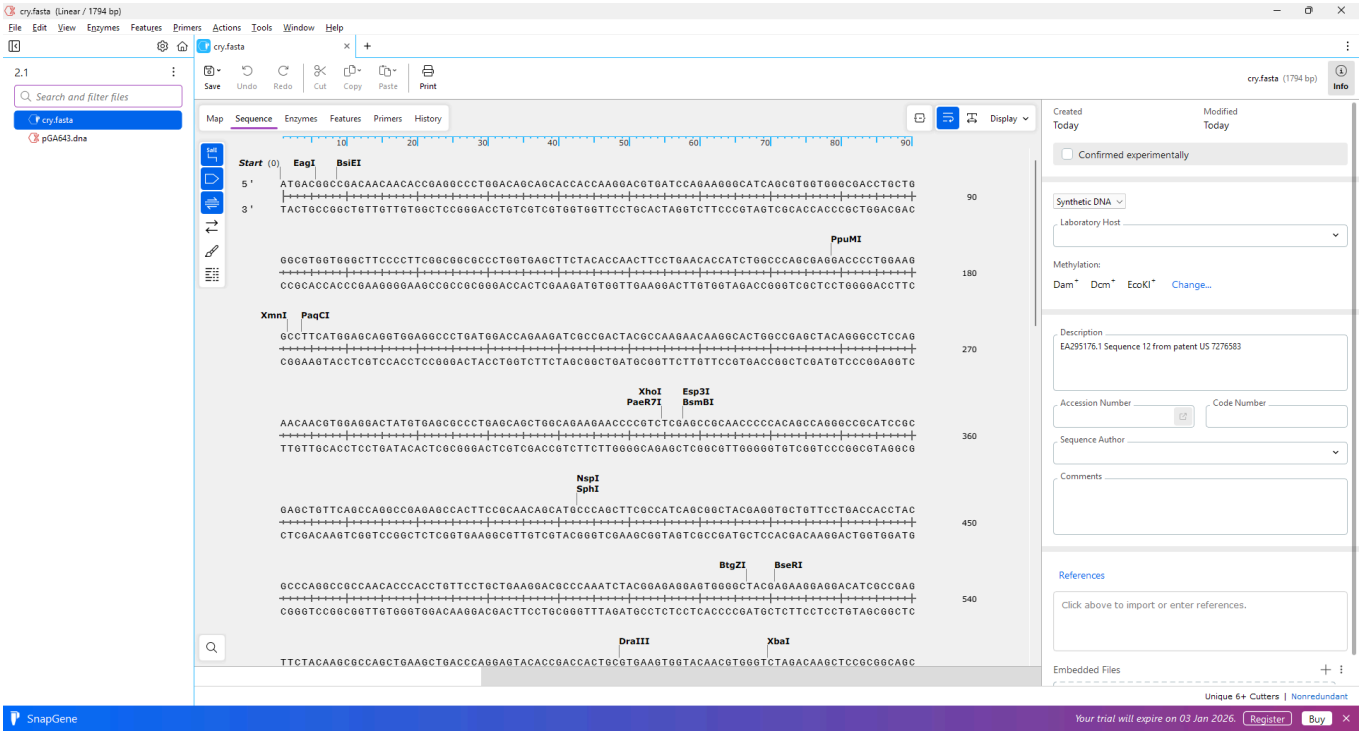
Either like this:



Or like this:



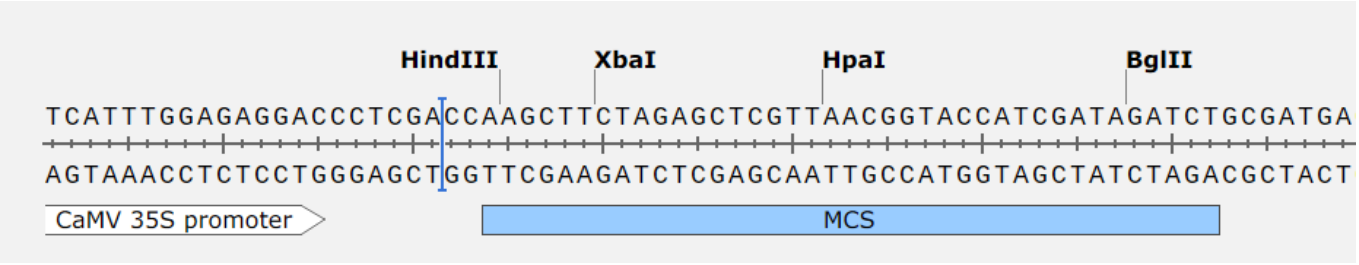
Imported sequence:



2. ***Select the first few nucleotides (until melting temperature 60 °C is reached) and click "Add primer".***

***Click the tab "Insertions" and select HindIII sequence to be attached to the 5' end to the forward primer and since restriction will be more efficient if recognition site is not at the beginning of the DNA, add additional restriction site of any other restriction enzyme.**

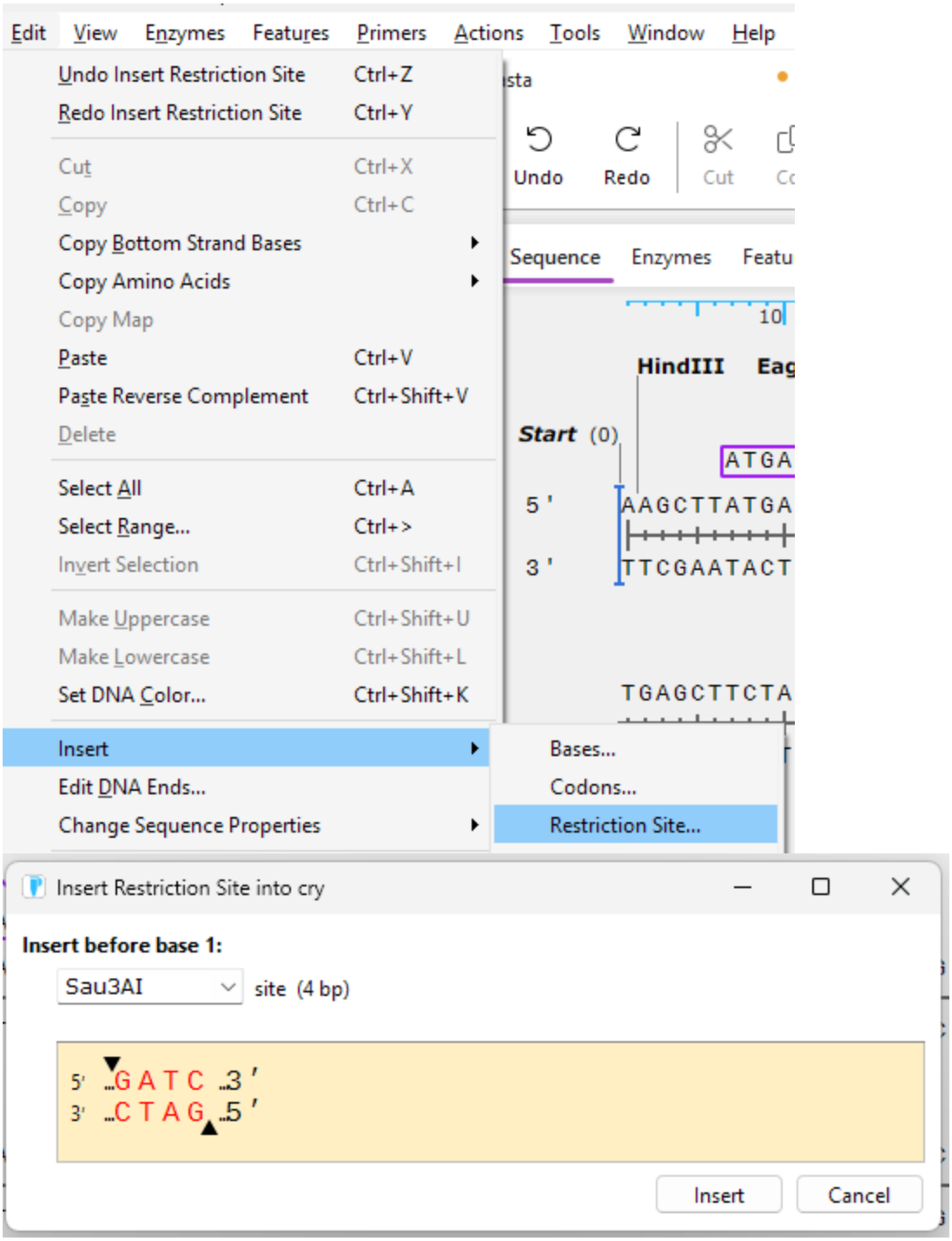
Primer zakaj izbiramo te encime:



Izločila se bo `MCS` regija (multiple cloning site)...

Klikneš na začetek zaporedja, najdeš `HindIII` in potem še za `Sau3AI` (ker je kratek in naredi sticky ends idk, to sem dal na začetek).

PAZI, DA DAŠ ZA PRIMERJEM, KER MI HOČEMO FRAGMENT NAMNOŽITI S TEMI REGIJAMI (na sliki je napačno).



Repeat the process for the reverse primer, except that the restriction site at the other site of DNA should be BglII (Bg”L”2).

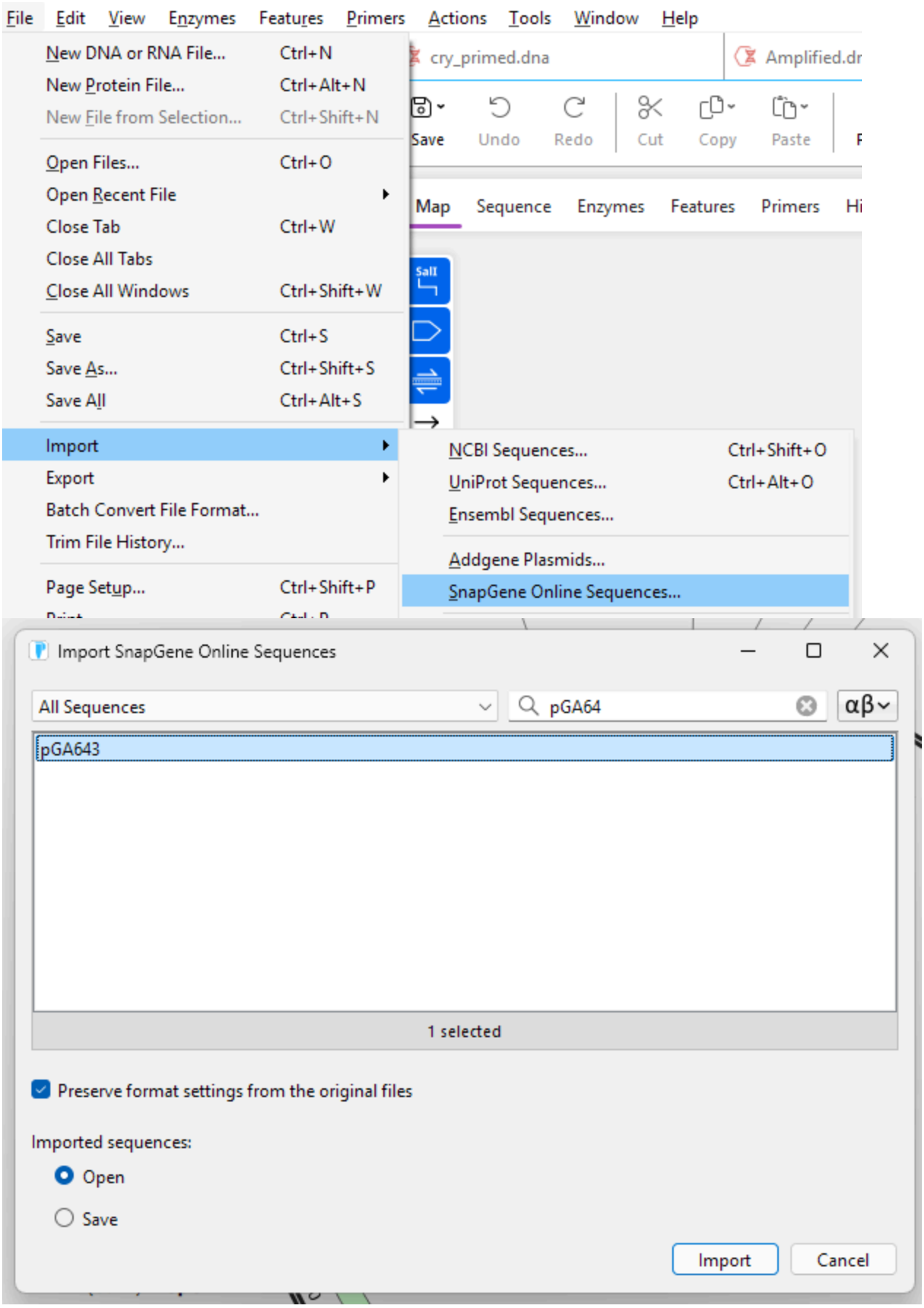
Enako, tudi tu sem dal `Sau3AI` na konec.

3. *Simulate PCR to get DNA with elongated primers.*

`Actions → PCR` in izbereš primerja, datoteka `Amplified.dna`.

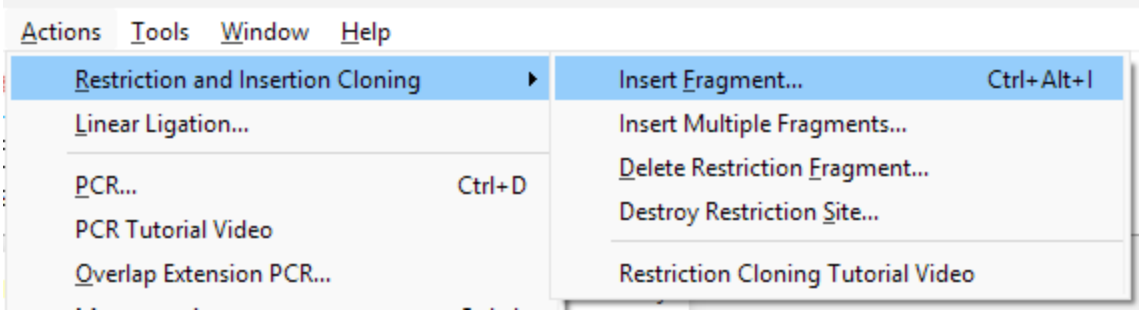
4. ***Import pGA643 plasmid.***

Odpreš prenešeno datoteko ali pa...

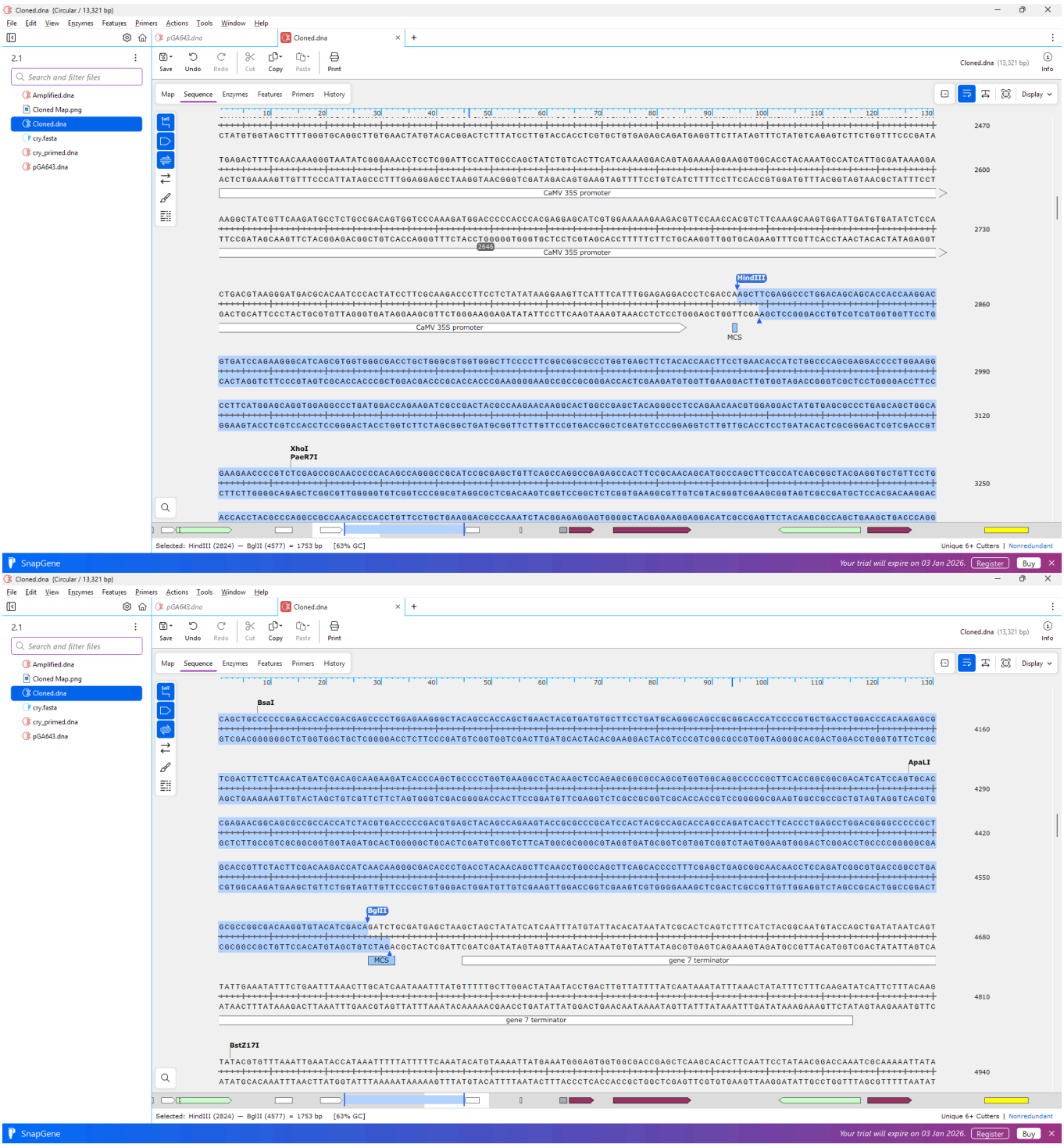


5. ***Click "Actions", then "Restriction and Ligation cloning" and "Insert fragment". Select vector in the first tab, amplified DNA in the second***

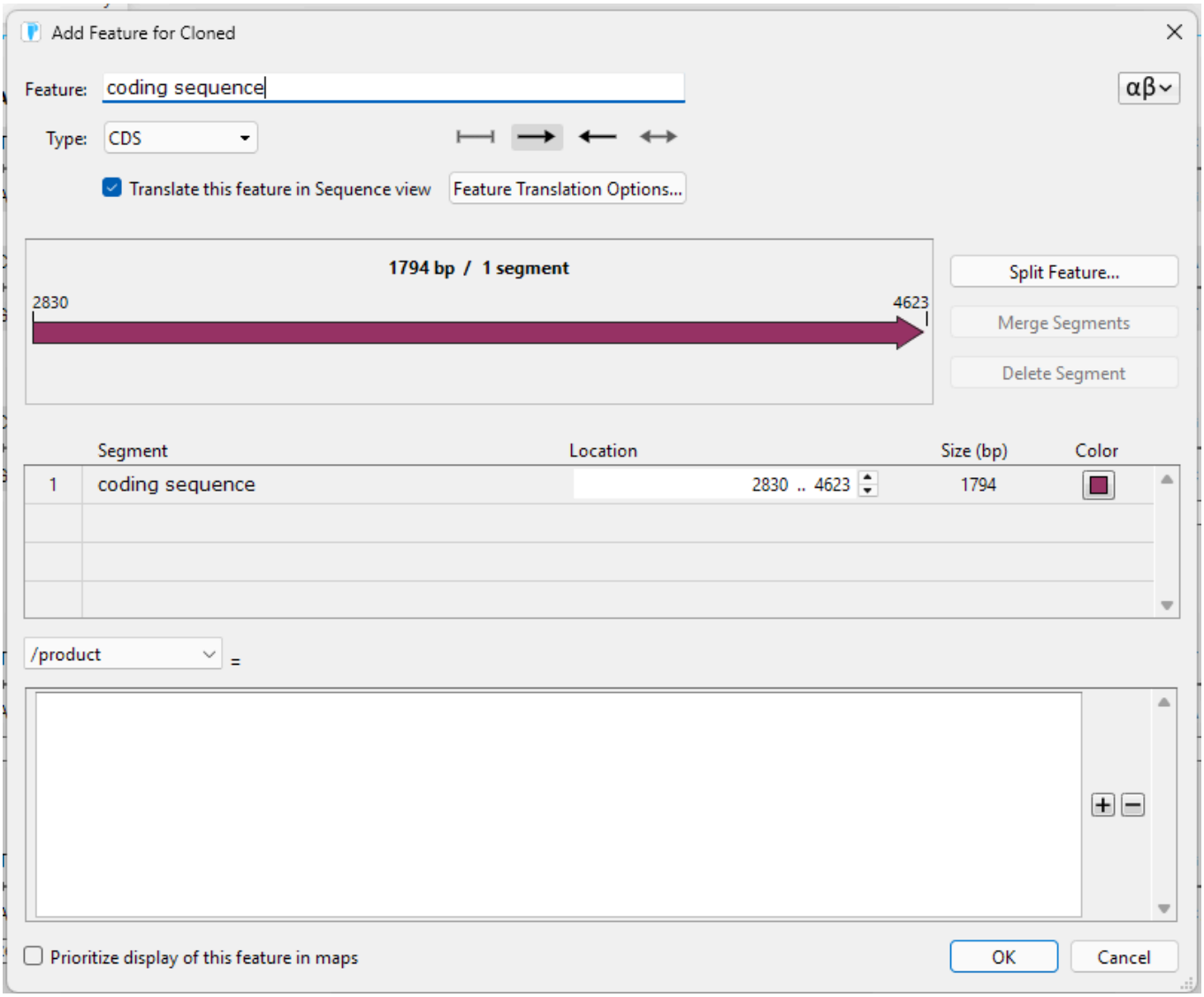
tab and press “Clone”

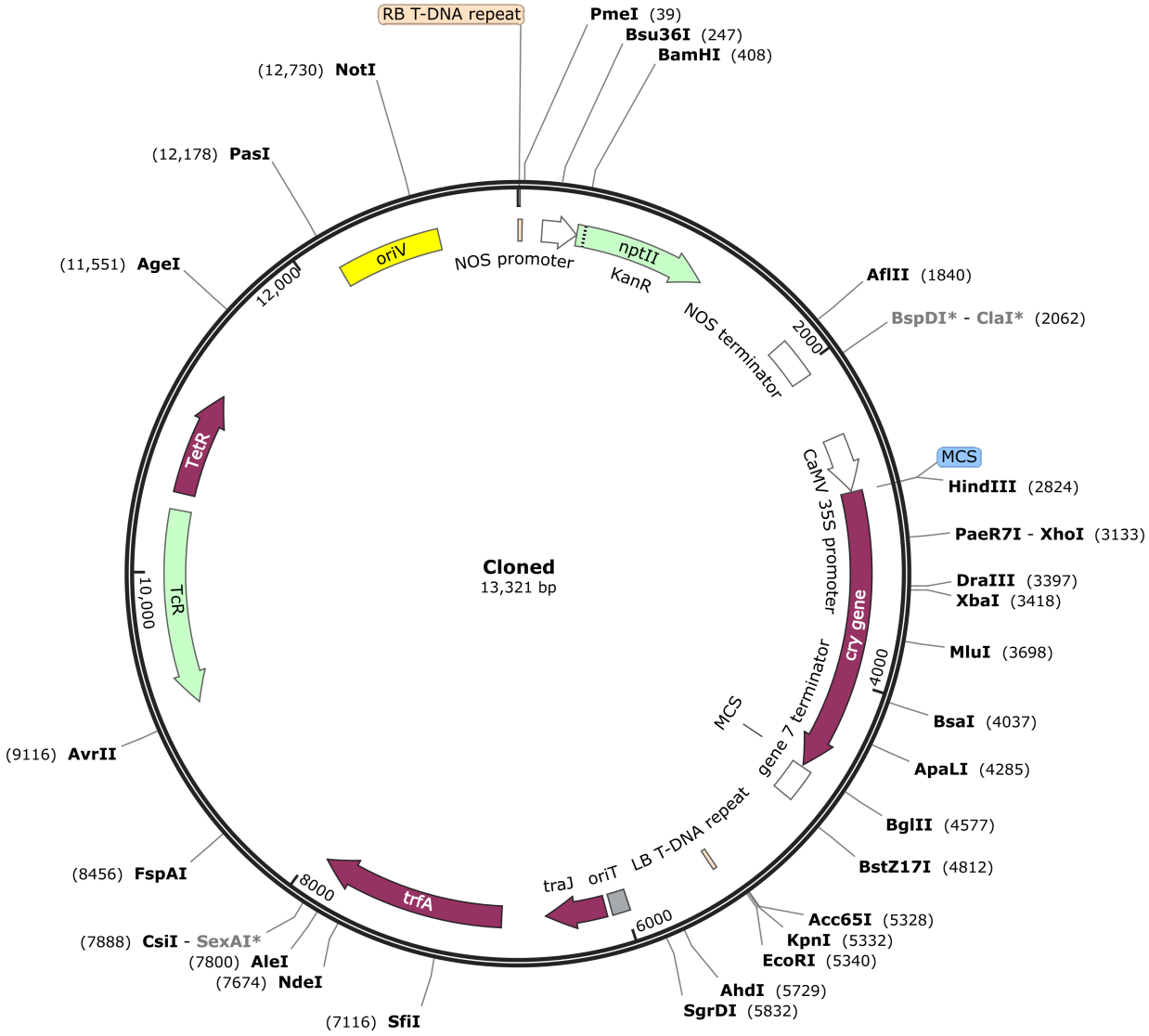


6. ***Select window with pGA643 and inserted DNA. Select inserted sequence from ATG to the TAG codon***



Click “Features”, “Add feature”, rename the inserted sequence and select CDS for the “type”


Add Feature for Cloned
Feature: coding sequence
αβ
Type: CDS
Translate this feature in Sequence view
Feature Translation Options...
1794 bp / 1 segment
2830
4623
Split Feature...
Merge Segments
Delete Segment
Segment
Location
Size (bp)
Color
1
coding sequence
2830 .. 4623
1794
/product
=
Prioritize display of this feature in maps
OK
Cancel


RB T-DNA repeat
PmeI (39)
Bsu36I (247)
BamHI (408)
(12,730) NotI
(12,178) PasI
(11,551) AgeI
oriV
NOS promoter
nptII
KanR
NOS terminator
AflII (1840)
BspDI* - ClaI* (2062)
2000
MCS
HindIII (2824)
CaMV 35S promoter
cry gene
PaeR7I - XhoI (3133)
DraIII (3397)
XbaI (3418)
MluI (3698)
4000
BsaI (4037)
ApaLI (4285)
BglII (4577)
BstZ17I (4812)
Acc65I (5328)
KpnI (5332)
EcoRI (5340)
AhdI (5729)
SgrDI (5832)
6000
LB T-DNA repeat
oriT
traJ
trfA
8000
(7116) SfiI
(7674) NdeI
(7800) AleI
(7888) CsiI - SexAI*
(8456) FspAI
(9116) AvrII
10,000
TcR
TetR
12,000
gene 7 terminator
MCS
Cloned
13,321 bp