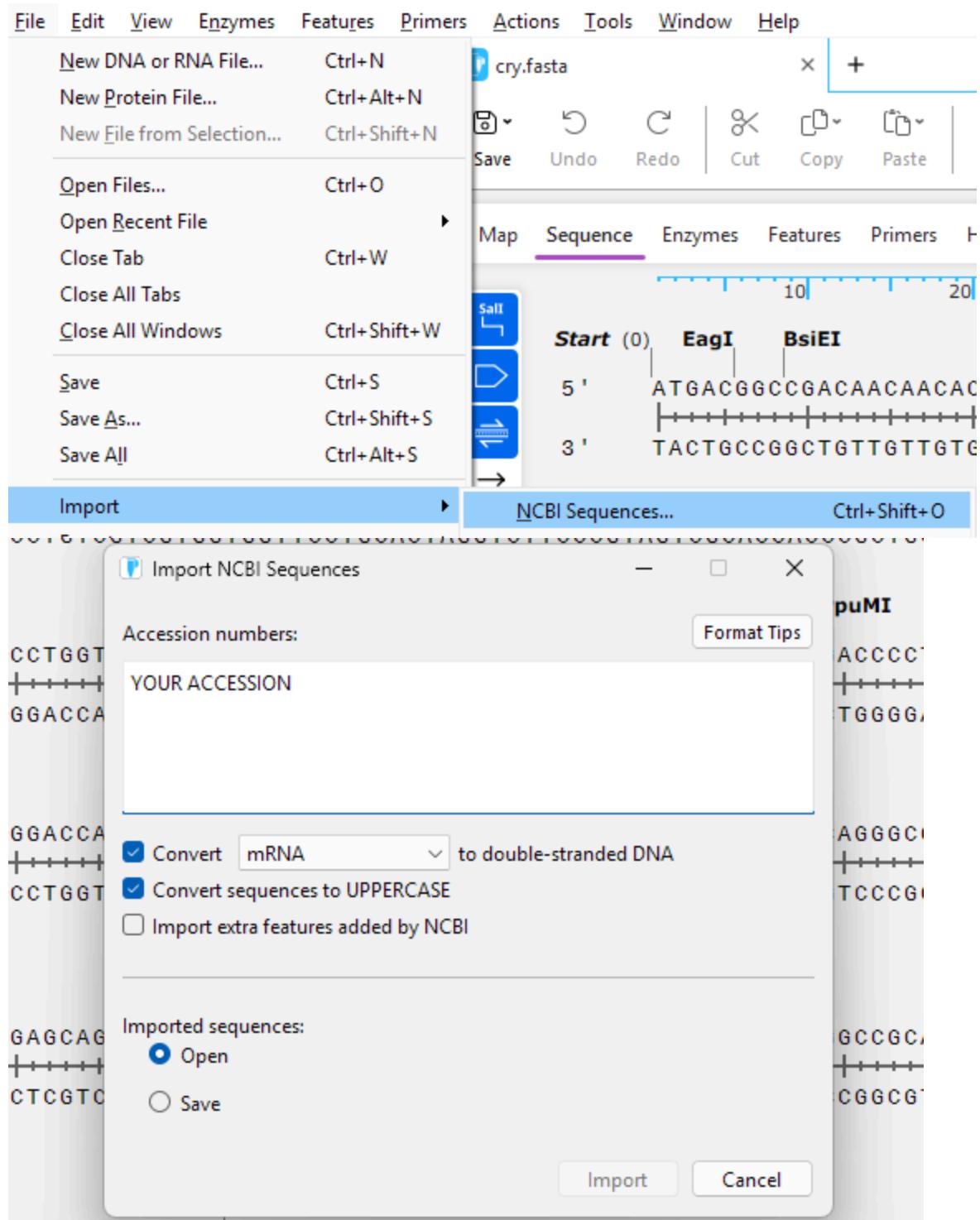


021-practicals

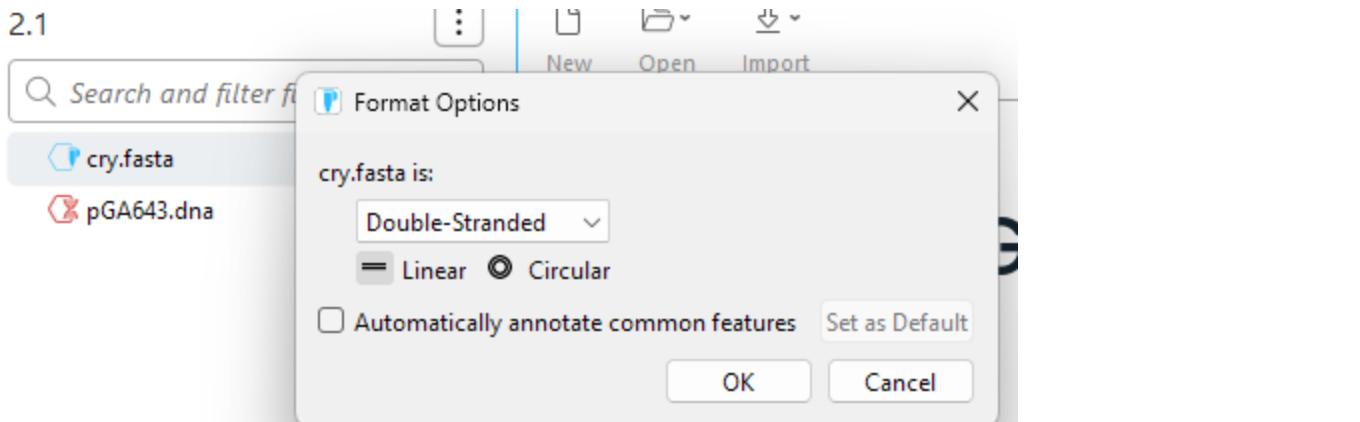
Insertion of gene for cry protein into a pGA643 plasmid.

1. Import fasta sequence of cry protein in SnapGene.

Either like this:



Or like this:



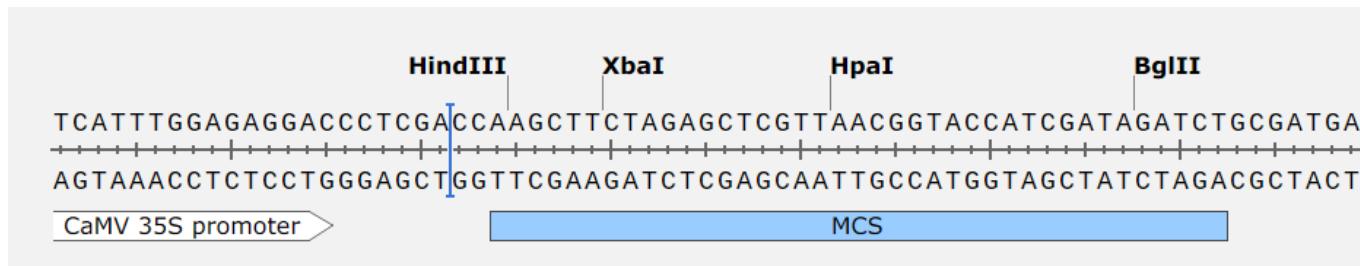
Imported sequence:

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).

The screenshot shows the BioEdit software interface. The main window displays a DNA sequence with restriction sites HindIII and EagI. A context menu is open, with the 'Insert' option selected. Under the 'Insert' option, 'Restriction Site...' is highlighted. A dialog box titled 'Insert Restriction Site into cry' is open, showing 'Sau3AI' selected for insertion before base 1. The sequence is shown with 5' and 3' ends, and the Sau3AI site (GATC) is indicated with red letters.

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

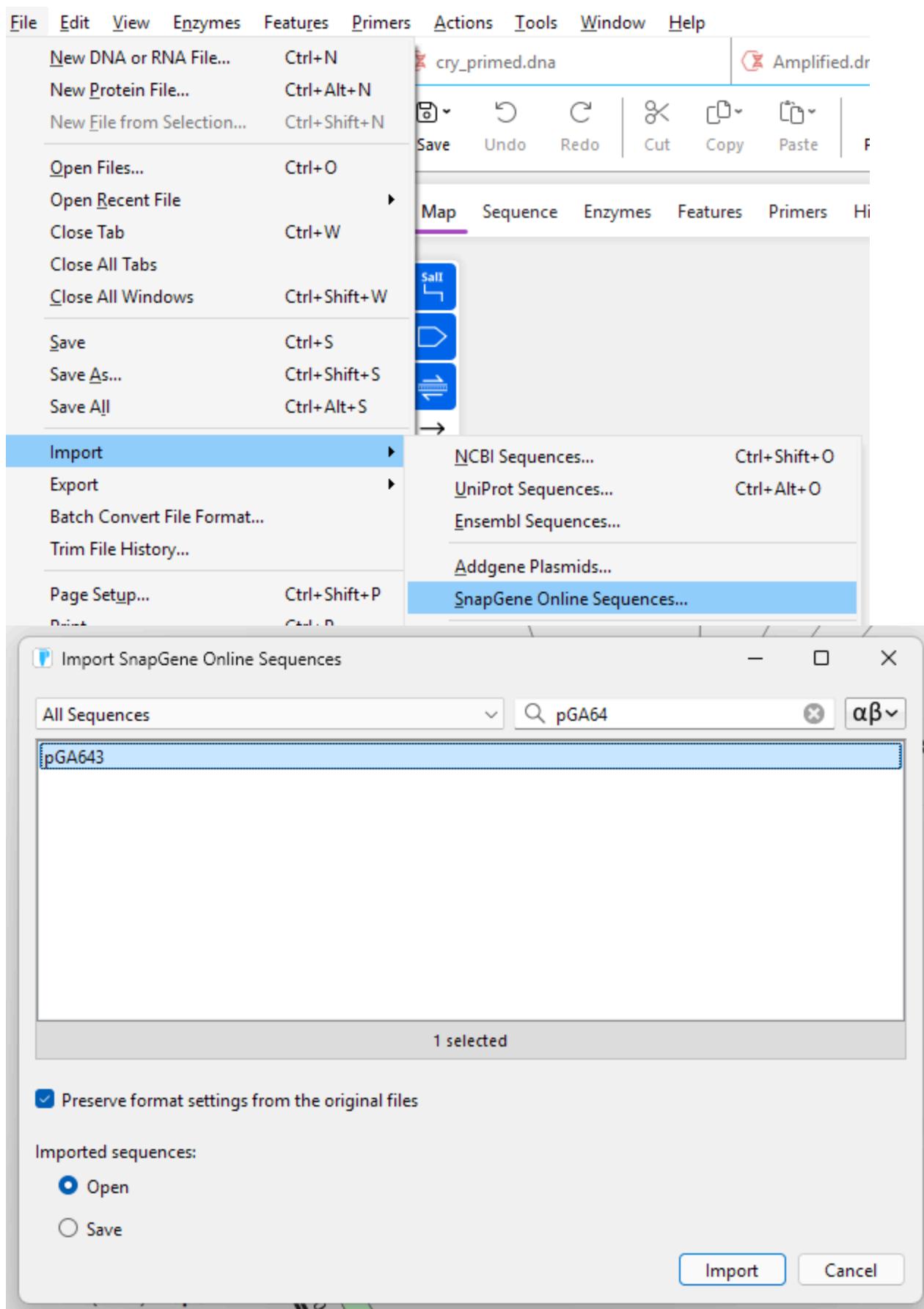
Enako, tudi tu sem dal **Sau3AI** na konec.

3. Simulate PCR to get DNA with elongated primers.

Actions → PCR in izberes primerja, datoteka **Amplified.dna**.

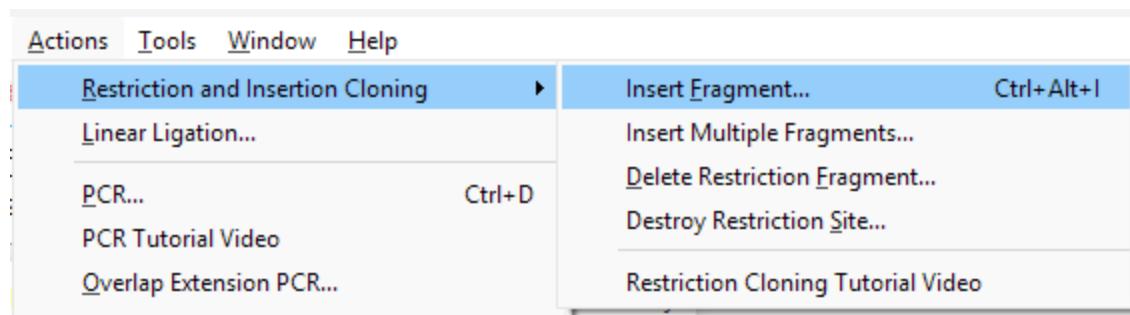
4. Import pGA643 plasmid.

Odpres preneseno 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

The figure consists of two vertically stacked screenshots of the SnapGene software interface, showing the analysis of a circular DNA molecule (13,321 bp) named "Cloned.dna".

Top Screenshot (Restriction Digestion with HindIII and BglII):

- Enzyme Selection:** HindIII (2824) and BglII (4577) are selected.
- Result:** A 1753 bp fragment is highlighted with a green bracket, representing 63% GC content.
- Promoter Regions:** CaMV 35S promoter is identified at positions 2470, 2600, and 2730.
- Other Enzymes:** XbaI, PstI, and KpnI are listed below.

Bottom Screenshot (Restriction Digestion with BsaI and ApaLI):

- Enzyme Selection:** BsaI (4160), ApaLI (4290), and BglII (4577) are listed.
- Result:** A 1753 bp fragment is highlighted with a green bracket, representing 63% GC content.
- Promoter Regions:** BglII 7' terminator and gene 7 terminator are identified.
- Other Enzymes:** BstZ1I (4680) and BstZ1I (4940) are listed.

Common Interface Elements:

- File Menu:** File, Edit, View, Enzymes, Features, Primers, Actions, Tools, Window, Help.
- Search Bar:** Search and filter files.
- Toolbars:** Save, Undo, Redo, Cut, Copy, Paste, Print.
- Sequence View:** Map, Sequence, Enzymes, Features, Primers, History tabs.
- Coordinates:** Ruler from 10 to 130.
- Annotations:** Various restriction sites (HindIII, BglII, BsaI, ApaLI, BstZ1I) and promoters (CaMV 35S, BglII 7' terminator, gene 7 terminator).
- Information:** Cloned.dna (13,321 bp), Info, Unique 6+ Cutters, Nonredundant.
- Trial Message:** Your trial will expire on 03 Jan 2026.

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

Add Feature for Cloned

Feature: **coding sequence** $\alpha\beta$

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](#)

