01-practicals

- Bacterial plasmids are cyclic, but can be cut by restriction enzymes (exonucleases) into a linear shape

> E.g. we want to develop plant resistant to something, we find an appropriate bacterial gene, cut it out and splice it with a plant promotor (it won't work with a bacterial one) and insert it into back into a plant, so it can start expression when neeeded... **or?**

- Combination of restriction enzymes (cut DNA) and ligases (splice DNA back together)
- Gibson assembly

SnapGene

Data

- GenBank accession numbers for DCA15 (FJ492763.1) and EMO3 (AJ416321.1) loci
- Primers for amplification of DCA15:

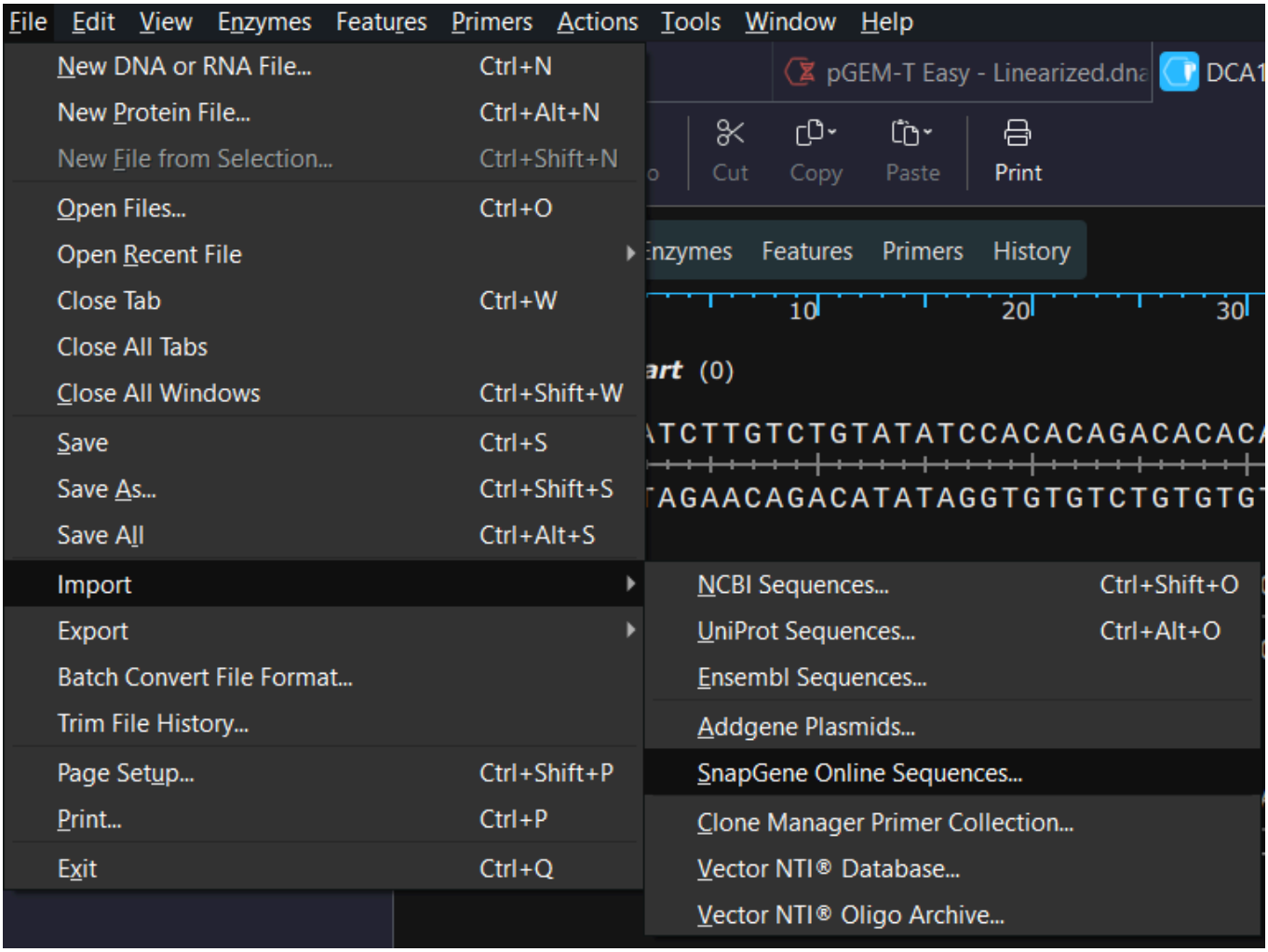
```
DCA-15-forward
GATCTTGTCTGTATATCCACAC

DCA-15-reverse
TATACCTTTTCCATCTTGACGC
```

- Primers for amplification of EMO3:

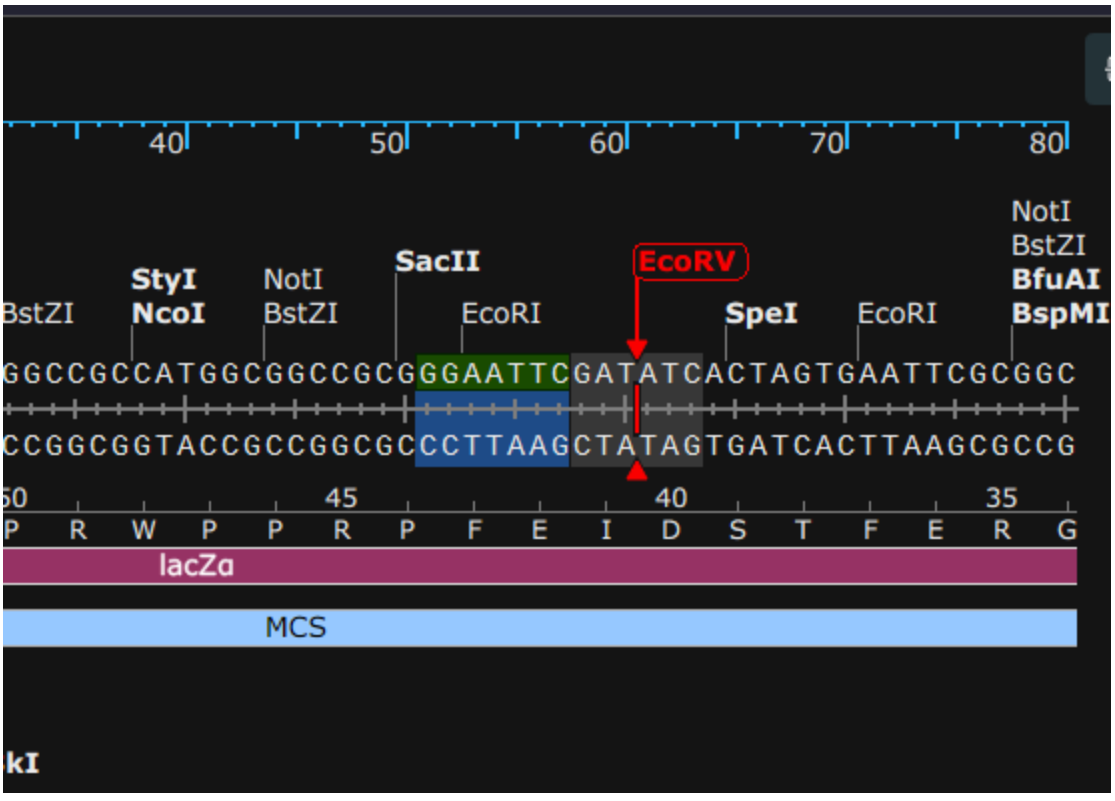
```
EMO-03-forward
GGTGTAGCCCAAGCCCTTAT

EMO-03-reverse
GCATGACCGTGGTGTAAGT
```

Workflow

1. *Import pGEM-T Easy Vector sequence from "SnapGene Online Sequences..."*

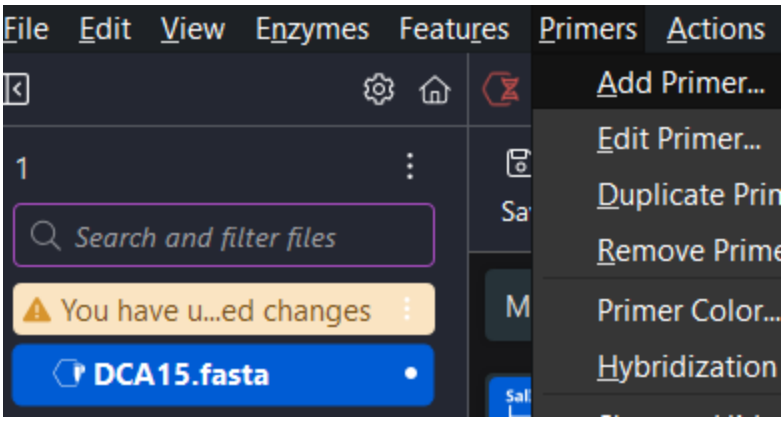


2. *Linearize the vector (Actions/Linearize...) and add T to the 3' ends (two AA should be on each 5' end with blunt ends)*



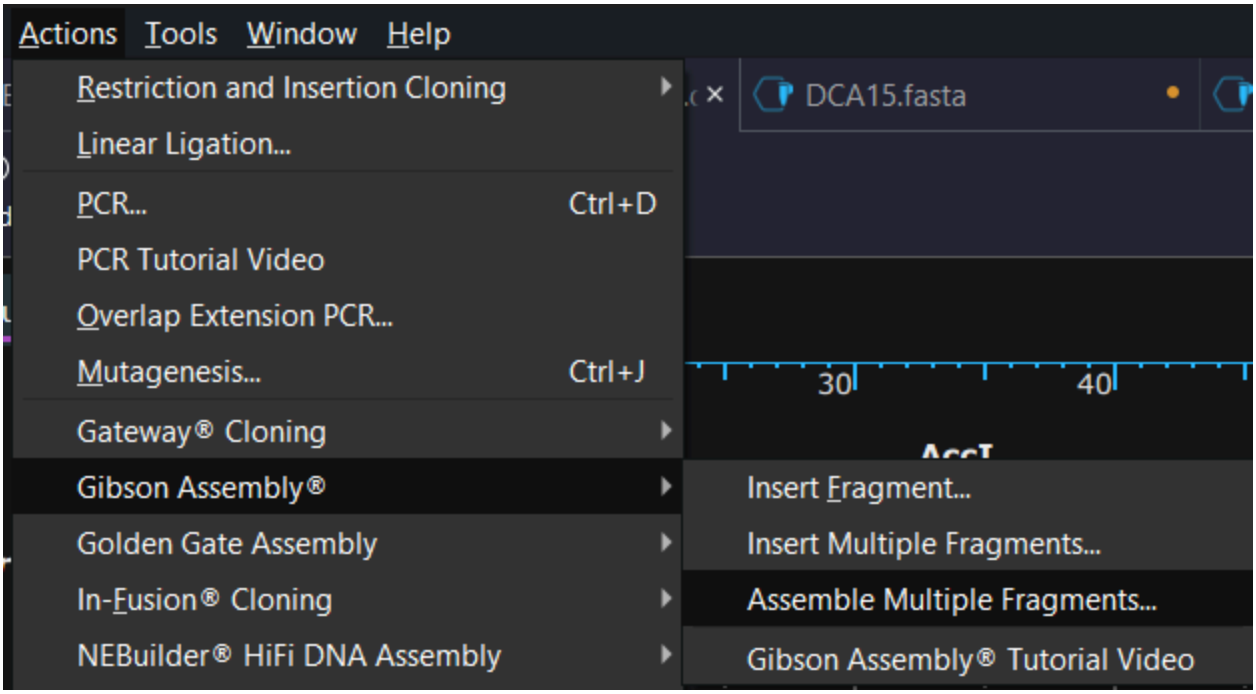
> EcoRV is the restriction enzyme that cuts at position 60

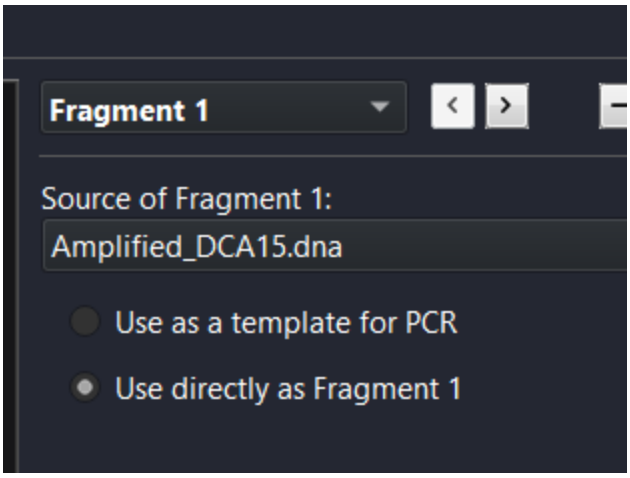
3. *Open fasta files with DCA15 and EMO3 sequences and add/annotate (Primers/Add primer...) the primers*
    - Click one fasta file
    - copy forward and reverse primers from above



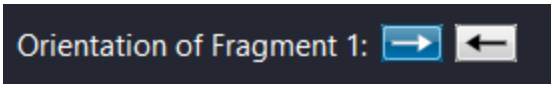
4. *Simulate PCR to get final sequences for both fragments (Actions/PCR...)*
5. *Design Gibson Assembly cloning experiment (Actions/Gibson Assembly) and save file Assembled.dna*

Select linearized sequence before doing this

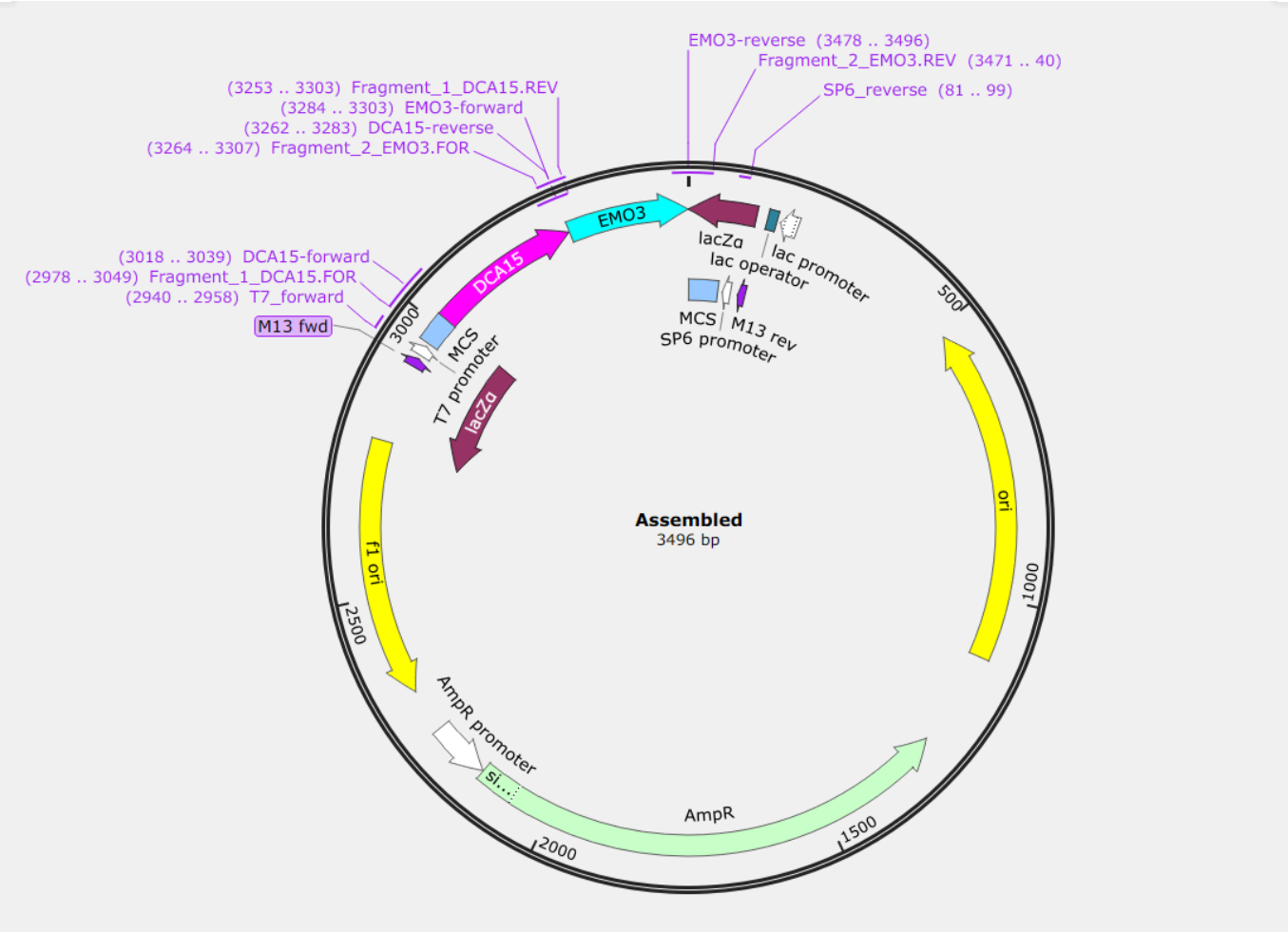




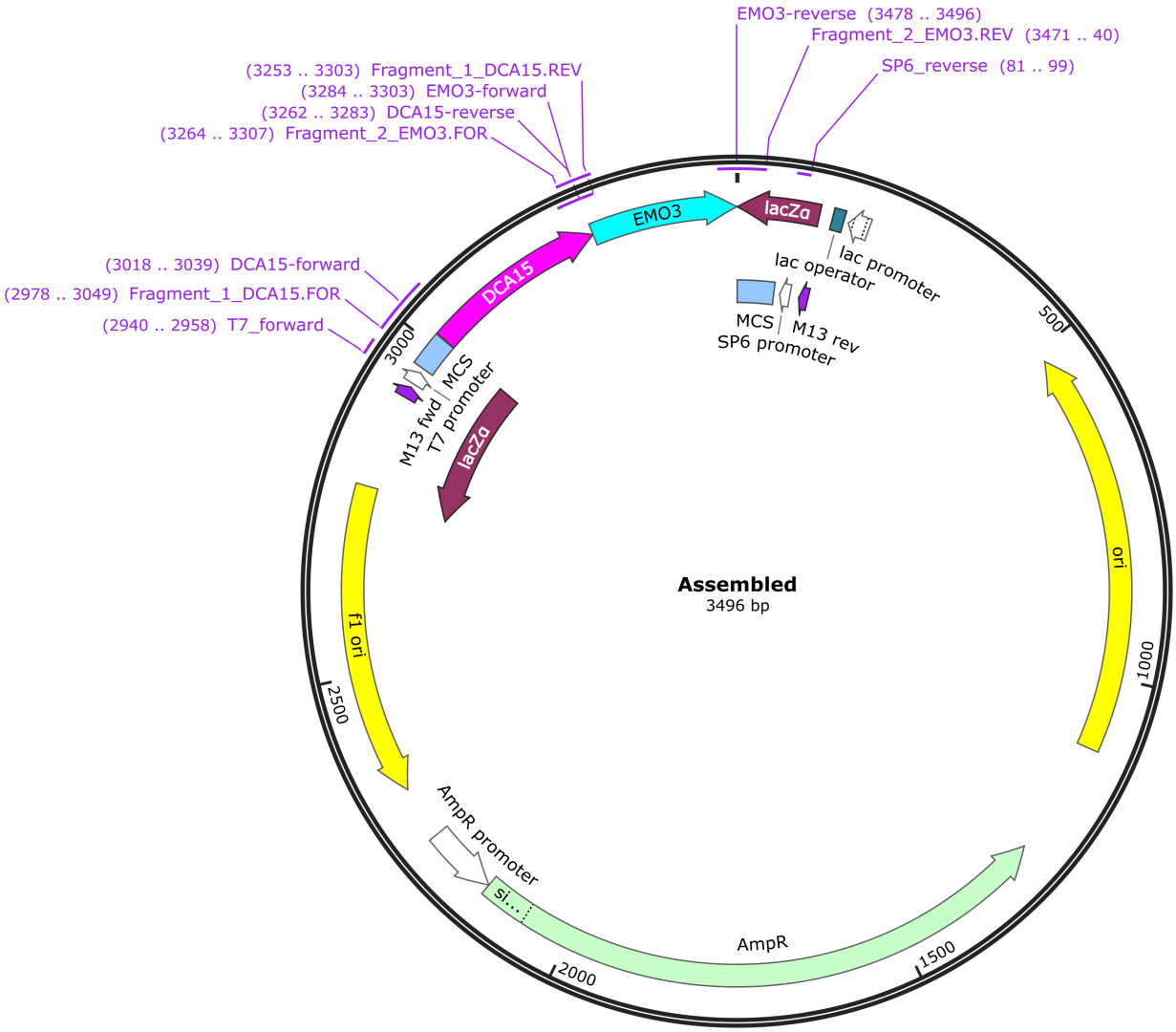
NOTE: choose "Use as a template for PCR"!!



6. *Annotate the sequences of DCA15 and EMO3 with function (Features/Add Feature…)*
    1. Copy first amplified primer (select all, ctrl-c :3)
    2. goto amplified DNA, ctrl-f search for region
    3. Features → Add Feature
7. *Select "Map" tab, take a screenshot and include it in the notebook. Go to "Primers" tab and near the Save button click the arrow and select Export Primer Data…. Paste designed primers in the notebook.*

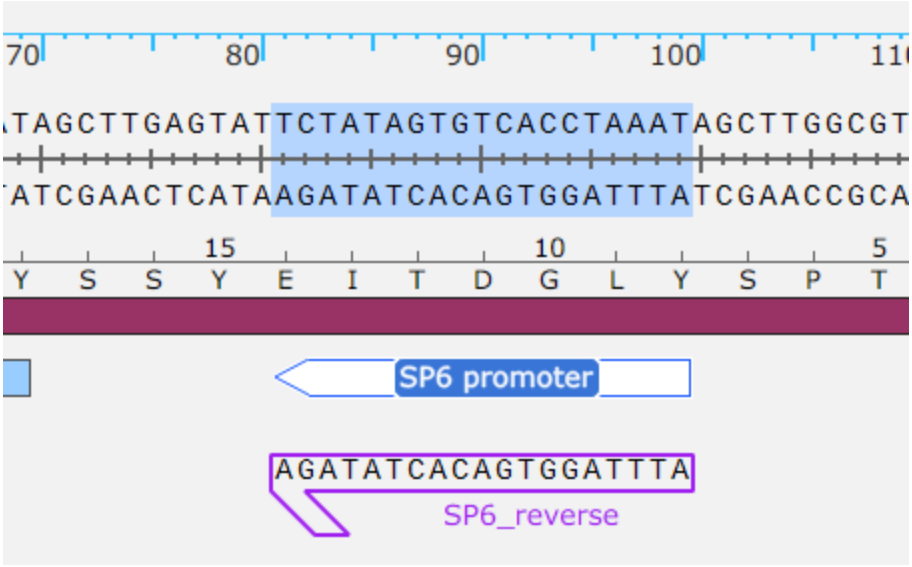


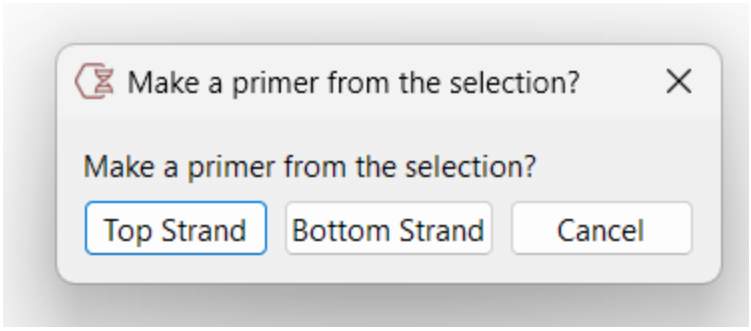
Lahko tudi izbereš `File > Export > Map` :



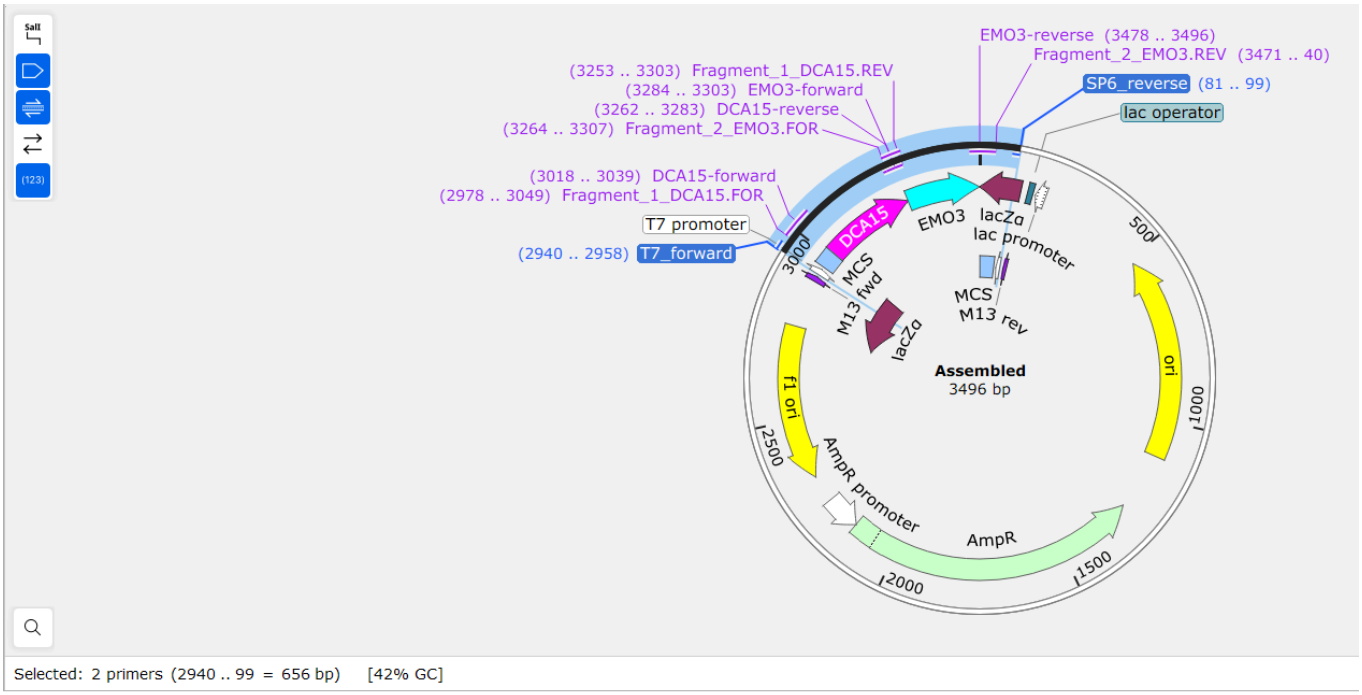
8. *Add primers on the site of T7 and SP6 promoters and simulate the PCR.*

Notice that **T7 is left of SP6**. T7 primer will be the **forward** (top) strand, while SP6 primer will be the **bottom** one. Click on the promotor region in the assembled sequence, `Primers → Add primer → Make a primer from selection [top|bottom]` and name them correctly.

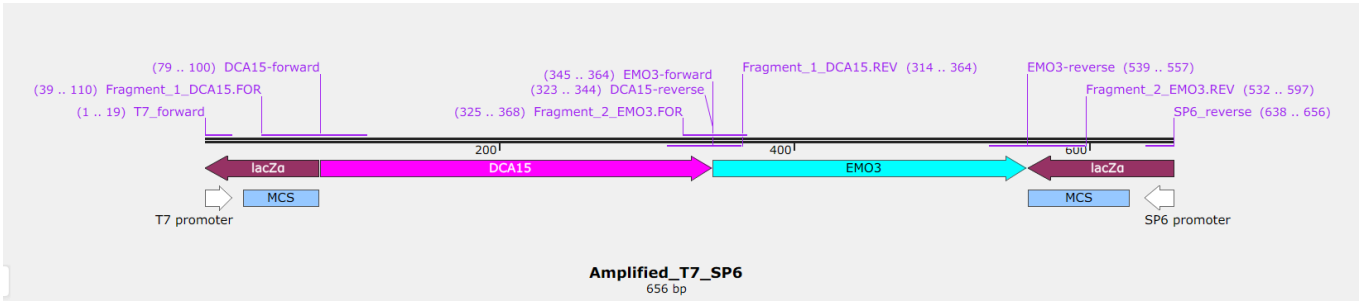




Goto `Actions → PCR` and select new primers.



Don't choose anything else, just run the PCR. Amplified region:



Questions

1. What will the length of the PCR product be using T7 and SP6 primers?

Has length of 656 bp.

2. Which part of the assembled vector will be amplified with SP6 and T7 primers?

DCA15 and EMO3 regions.

3. Export the amplified region as a fasta file and paste the sequence to your notebook.

```
>Amplified_T7_SP6  (656 bp)
TAATACGACTCACTATAGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCGGGAATTCGATTGA
TCTTGTCTGTATATCCACACAGACACACACACACACACACACACACACACACAAAGAATAGAAAAACAGACAGAAAAAGAAT
CAGAGACAACACTATACTGAGAAATTATACGTCTGCTAACTACACAGGTGGGAAAAAATTACCAGTTCTGGGGTCAACTT
CTCAGCTGACTTTGCAGCTGTCAAAACATAATGCATATTAAATTAAAAAACTAAAAGAGAAAGGCTAATTAGAGCGATAA
ATGCGTCAAGATGGAAAAGGTATAGGTGTAGCCCAAGCCCTTATAACTGACATGGGATTTTACCCCTAACAAACGAAAGA
GAAGGCACTGAAAAAATGAAAAAGAAAAAAAAAAAAAAACACACACACACACATACGTTATGGAAGAAATTTGTAAGCAG
CAAAATCTAAGTAATGCTAGACCAGAACCAGAGAAGATCATCAGAGGTAAACAAGATAACTTACACCACGGTCATGCAAT
CACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCT
ATAGTGTCACCTAAAT
```