

In silico Restriction Digestion (activity)

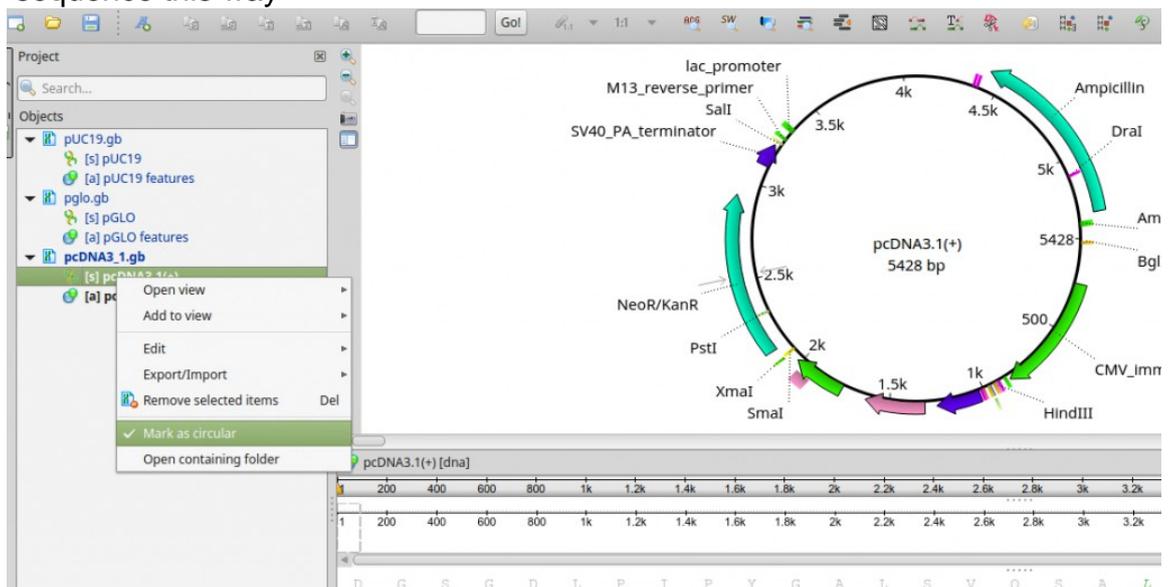
Concept

Restriction enzymes act as molecular scissors. The ones we use in Molecular Biology are those that cut within known sequences that occur often enough, yet rare enough to cut our DNA into analyzable fragments. Molecular Biologists often use 6-cutters. This means that the site of digestion is “restricted” to a recognition sequence of 6 nucleotides. These nucleotides are usually palindromic as discussed [before](#).

Imagine a linear piece of DNA as a piece of string. When cutting the string once, you result in 2 pieces. Now consider a plasmid. This was already discussed to be a circular piece of DNA. With a circle, there are no ends. cutting the plasmid once results in 1 piece of DNA as opposed to 2. Keep this in mind when digesting circular plasmids. In this case, nucleotide 1 is adjacent to and contiguous with the last nucleotide of the sequence.

In silico digestion

1. This activity is meant to supplement [Identification of DNA \(activity\)](#)
2. Launch [UGENE](#) and open the following files:
 - o [pGlo.gb](#)
 - o [pUC19.gb](#)
 - o [pcDNA3.1.gb](#)
 - o [pUC with Insert](#)
3. In the **Objects** menu, right-click on the sequences and select **“Mark as circular”**
 - o the sequences will now be treated as circular DNA
 - o the first nucleotide and the last nucleotide become adjacent as a continuous sequence this way



4. From top menu, select **Actions ? Analyze ? Find restriction sites**

- this will load a set of default restriction enzymes
- if none are loaded, select them individually (found in alphabetical order)
 - choose: BamHI, BglII, ClaI, DraI, EcoRI, EcoRV, HindIII, KpnI, PstI, Sall, SmaI, XbaI, XmaI, NotI

5. Actions ? Cloning ? Digest into fragments

- Choose your enzyme
- Try *HindIII* for each plasmid
- Fragments will be added to the circular view and annotations for these fragments will be added
- You can use this information to calculate how many fragments come from enzymes based on how many times they cut and by the nucleotide coordinates found in the annotation of the fragment
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