You are planning to study the activity of a protein which participates in proliferation of Vaccinia

virus. You need to clone the relevant DNA in *E. coli* cell line. The gene of interest of your project

is 1711 bp long, linear and the details can be found in NCBI GenBank (accession number E02780,

version E02780.1). You are planning to introduce the DNA sequence in a pET-43.1(+) vector.

Linear DNA sequence can be found here:

<https://www.ncbi.nlm.nih.gov/nuccore/E02780.1>

1) Find out suitable restriction enzymes for double digestion during your cloning

procedure.

2) Write down your cloning strategy mentioning important steps. Use schematics for

better explanation, if necessary.

3) For PCR amplification of your insert, design a forward and a reverse primer with

appropriate restriction enzyme and write down the primer sequences.

4) Check the reading frame and decide which pET-43.1(+) is suitable for you.

5) Write down the amino acid sequence that should be produced from your insert.

6) Attach relevant images you use for the report (Digestion results, pET 43.1 map etc.)

\*\*\* I am expecting that each student would work individually and therefore would not pick

exact same set of enzymes.

You may use the following sites for supporting information:

https://www.neb.com/

http://www.expasy.org/

https://molbiol-tools.ca/Restriction\_endonuclease.htm