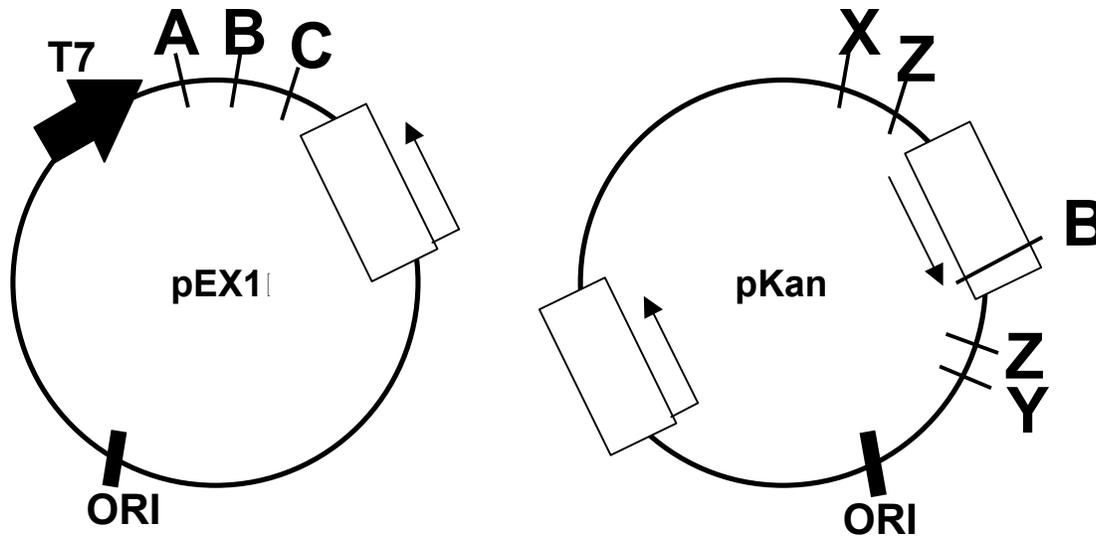


Question 3

As part of a cloning project in your laboratory, you want to create a plasmid that expresses two antibiotic resistance genes: AmpR and KanR.

As starting material, you have two plasmids: pEX1 and pKan. A diagram of each plasmid and a description of each plasmid's features are provided below:



pEX1 contains:

- AmpR gene (with its own promoter);
- origin of replication (ori)
- T7 promoter (T7)
- restriction sites for enzymes A, B, C

pKan contains:

- AmpR gene (with its own promoter)
- origin of replication (ori)
- **promoterless** KanR gene
- restriction sites for enzymes B, X, Y, and Z

**For each plasmid, an arrow indicates the direction of transcription of AmpR and KanR.

Here are the restriction enzyme recognition sequences for enzymes A, B, C, X, Y, and Z and where each enzyme cuts within its recognition sequence:

Enzyme A:

5'—G ACGTC—3'
3'—CTGCA G—5'

Enzyme B:

5'—G GTACC—3'
3'—CCATG G—5'

Enzyme C:

5'—AGC GCT—3'
3'—TCG CGA—5'

Enzyme X:

5'—T ACGTA—3'
3'—ATGCA T—5'

Enzyme Y:

5'—A GTACT—3'
3'—TCATG A—5'

Enzyme Z:

5'—ATC GAT—3'
3'—TAG CTA—5'