Eukaryotic gene with restriction enzyme sites

Direction of transcription

5' GAATTC ATCT________ CCCTTA_________ GTCAATA AATACAATTG3'  
3' CTTAAG TAGA________ GGGAAAT________ CAGTTATT GTTAAC5'  

E1 E2 E3

Eukaryotic Gene

- Blunt cutter(s): E1/ E2/ E3? Circle all correct options E2
- Staggered cutter(s): E1/ E2/ E3? Circle all correct options E1 and E3
- Enzyme generating 3’overhang: E1/ E2/ E3/none? None
- Enzyme generating 5’overhang: E1/ E2/ E3/none? E1 and E3
- Enzyme recognizing a palindromic sequence: E1/ E2/ E3? E1 and E3
- Enzymes you will use to cut this gene: E1/ E2/ E3?
- Bacterial cell receiving Vector will **grow/die** in the presence of ampicillin?

- For Vector to replicate in yeast, what additional feature should it have?
  **Yeast ori**

- Draw the DNA gel that you will obtain if you digest Vector A with E4 and E5.

**AmpR**: ampicillin resistance gene  
**TetR**: tetracyclin resistance gene
-You digest the Vector (shown in slide 2) and the gene (shown in slide 1) with appropriate restriction enzymes and then join them with the help of **Ligase** which forms a covalent **phosphodiester** bond in a **3’->5’/5’->3’** direction.

-Draw the two possible orientation of the recombinant Vector that you will get following ligation. Also show the direction of transcription of the gene.
-There are four possible types of sequences that your ligation mix can have. Either draw or state each.

- Gene A
- Plasmid alone
- Recombinant plasmid with Gene A insert in correct orientation
- Recombinant plasmid with Gene A insert in the incorrect orientation

- You transform the bacteria with the ligation mix. Give the phenotype of bacteria PRIOR TO transformation: Amp and Tet sensitive

- You replica plate the bacteria to identify those transformed with recombinant plasmid.

  **Plate 1:** Master plate with no antibiotics
  **Plate 2:** Plate containing ampicillin
  **Plate 3:** Plate containing tetracyclin.

- Which plate(s) will have bacterial colonies with recombinant vector (1/2/3)? **Explain.** *Plate 2 since these colonies will be Amp\textsuperscript{R}Tet\textsuperscript{S}*. 
**cDNA library**

cDNA library is different from different cell types

- It contains only the actively transcribed genes.

- The cDNA lacks the promoter and other regulatory regions.

**Genomic library**

- Has the information of entire genome

- Each gene has its own inherent promoter