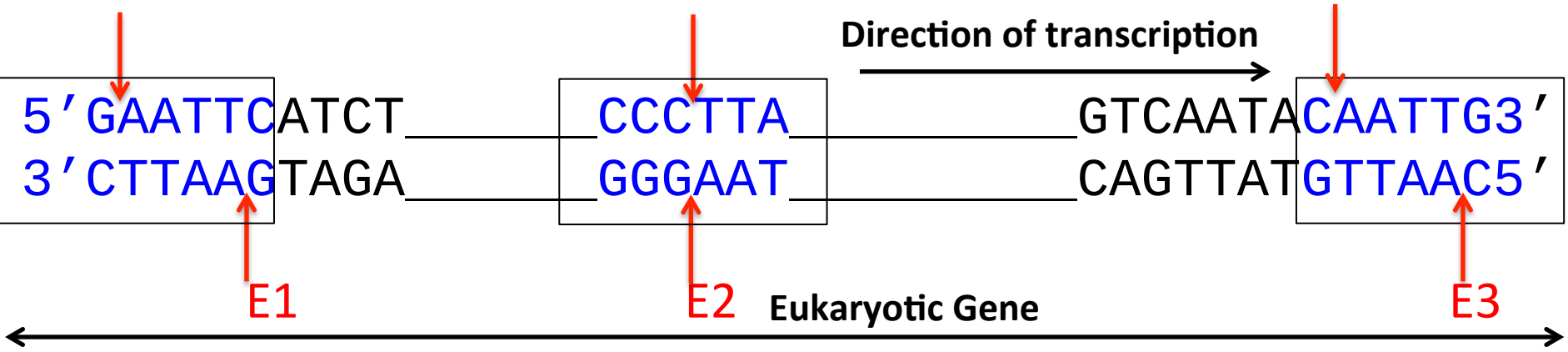
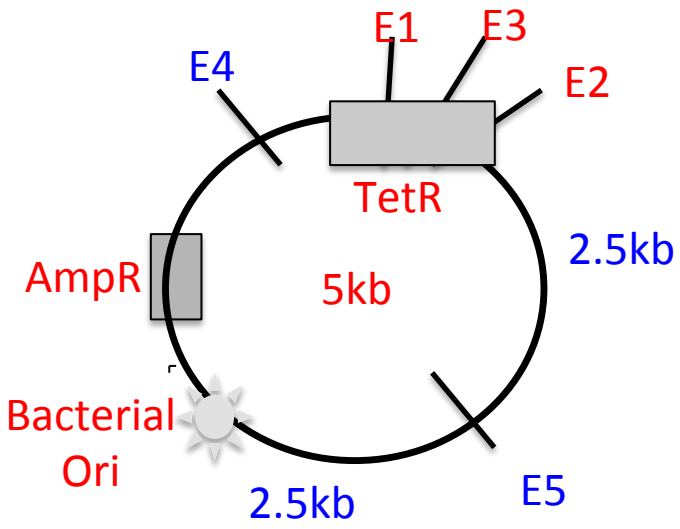
Eukaryotic gene with restriction enzyme sites



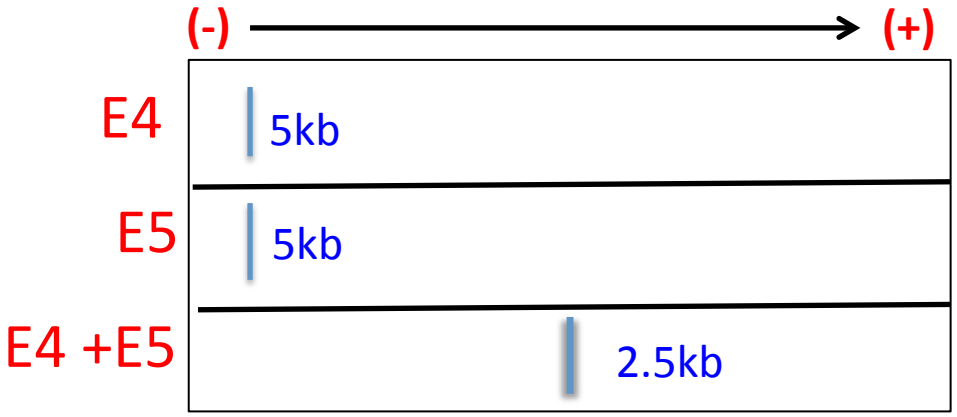
- Blunt cutter(s): E1/ E2/ E3? Circle all correct options **E2**
- Staggered cutter(s): E1/ E2/ E3? Circle all correct options **E1 and E3**
- Enzyme generating 3'overhang: E1/ E2/ E3/none? None
- Enzyme generating 5'overhang: E1/ E2/ E3/none? E1 and E3
- Enzyme recognizing a palindromic sequence: E1/ E2/ E3? E1 and E3
- Enzymes you will use to cut this gene: E1/ E2/ E3?

Vector / Plasmid



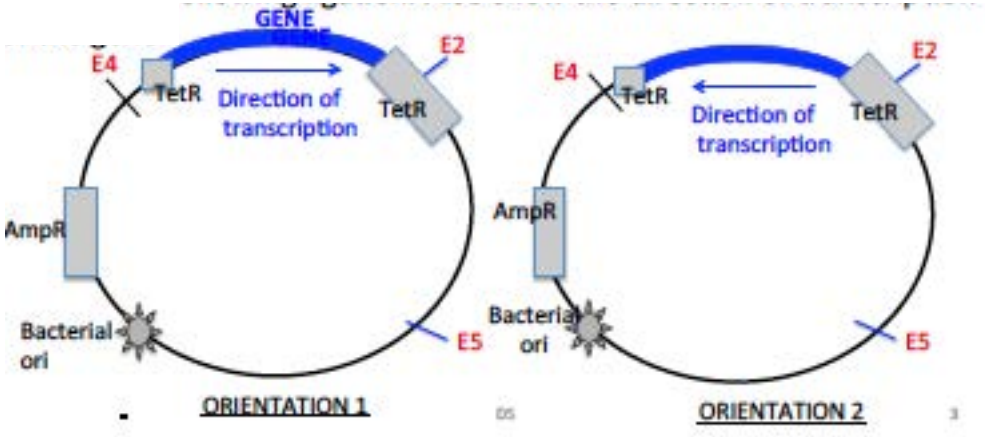
AmpR: ampicillin resistance gene
TetR: tetracyclin resisitance gene

- 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.



-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

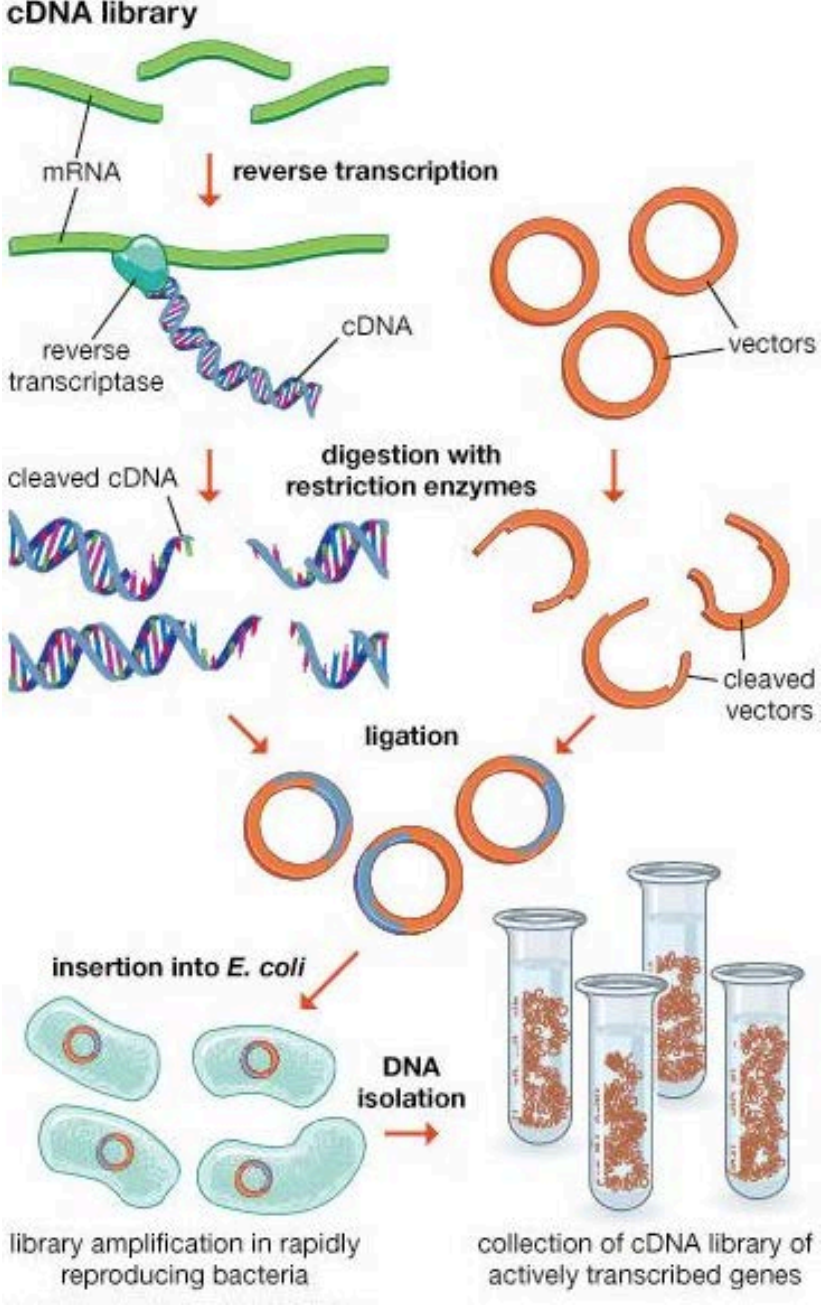
-You transform the bacteria with the ligation mix. Give the phenotype of bacteria PRIOR TO transformation: *Amp and Tet sensitive*

-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* $Amp^R Tet^S$

Image removed due to copyright restrictions. Please see:
Synthesis of complementary DNA (cDNA) from mRNA by reverse transcription.
Griffiths, et al., 2002. https://www.mun.ca/biology/scarr/MGA2-08-04.html



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