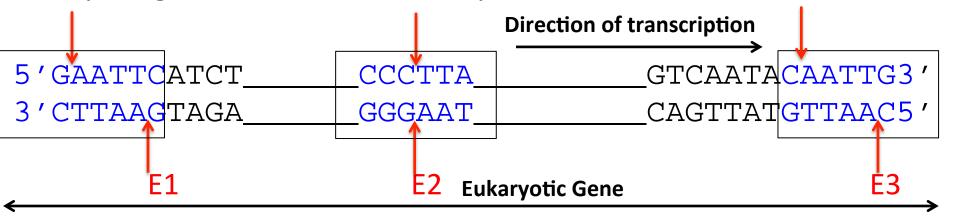
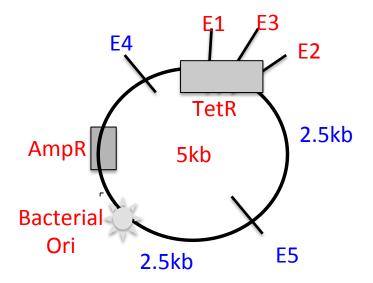
Eukaryotic gene with restriction enzyme sites



- 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?

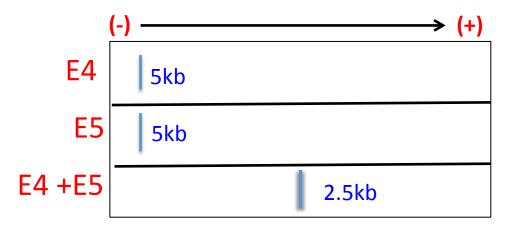
Vector / Plasmid



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?

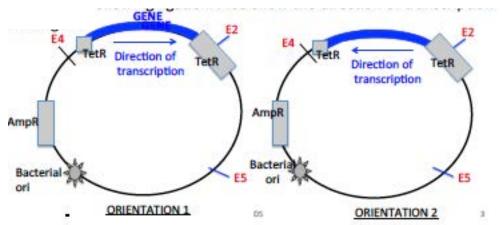
 Yeast ori
- Draw the DNA gel that you will obtain if you digest Vector A with E4 and E5.



2

-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 <u>Ligase</u> which forms a covalent <u>phosphodiester</u> bond in a <u>3'->5'</u>/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.



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- -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^RTet^S

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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 reverse transcription mRNA **cDNA** vectors transcriptase digestion with cleaved cDNA restriction enzymes vectors ligation insertion into E. coli DNA isolation library amplification in rapidly collection of cDNA library of reproducing bacteria actively transcribed genes

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

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