GENETICS 603 NAME_____

Exam II

Fall, 2003

I. A) Shown below are a series of *E. coli* strains that have 1 or 2 copies of the lac operon as shown. Defective components are indicated by a superscript minus (for example Y^-), except FS^{FS} is used for frameshift mutations and S for i^S mutations. For each strain tell whether β-galactosidase, permease and transacetylase would be constitutive (C) , regulated (R) or always absent (A).

E. coli Strain	β-gal'ase	permease	TA'ase
$P^- I \quad P \; O \; Z \; Y^- \; A$	C	A	C
$P \; I \; P \; O \; Z \; Y^{FS} \; A$	R	A	A
$P \; I^S \; P \; O \quad Z^- \; Y \; A$	A	A	A
$P \; I \; P \; O^- \; Z \; Y \; A^-$	C	C	A
$\frac{P \; I^- \; P \; O \; Z \; Y^- \; A}{P \; I \; P \; O^- \; Z \; Y \; A^-}$	C	C	R
$\frac{P \; I^S \; P \; O \quad Z^- \; Y \; A}{P \; I \; P \; O^- \; Z \; Y^{FS} \; A}$	C	A	A

B) Why do researchers use the synthetic compound IPTG rather than lactose to test regulation in the lac operon?

It can enter without the permease and can interact directly with the repressor; with lactose, Z^- (no allolactose) and Y^- mutations would interfere with normal regulation

C) What is an I^S mutation?

Super suppressor; the repressor cannot interact with lactose but still binds the operator

D) Predict the effect of a point mutation in the *TrpL* leader region of the Tryptophan operon of *E. coli* that changes the second of two trp codons (UGG) to CGG, a code word for arginine.

Very likely would alter attenuation; it may cause low arginine to increase tryptophane biosynthesis whereas low tryp would not stall the ribosomes long enough to continue transcription.

E) Give examples of mechanisms that can be used for regulation of gene expression that would be classified as:

post-transcriptional regulation

differential intron splicing, mRNA editing, stored mRNA, poor leader, mRNA stability via hormones, etc.

regulation during translation

codon preference (modulation) attenuation, etc.

genomic or pre-transcriptional regulation

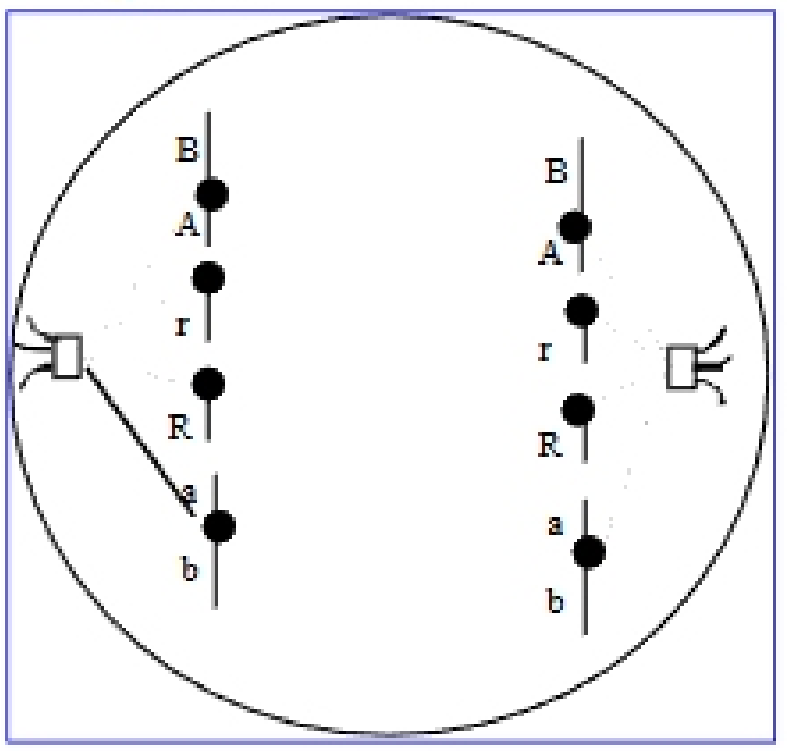
gene copy number, promoter efficiency, DNA methylation, acetylation of histones, etc.

post-translational regulation

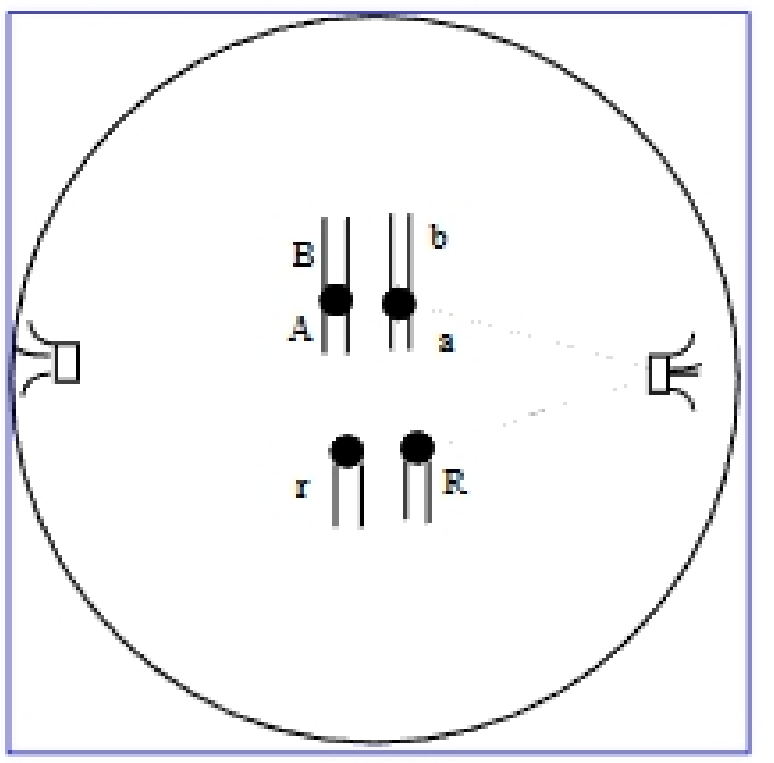
Enzyme stability, phosphorylation, glycosylation, pre-pro-forms of proteins

II. Genes A, and B are on the p and q arms respectively of a submetacentric chromosome and gene R is on a telocentric chromosome. Use labeled line drawings to show how the chromosomes of a triple heterozygote (AB/ab, R/r) would appear at the stages in the labeled "cells" below:

Anaphase of **Mitosis**



Metaphase of **Meiosis I**



How many chromosomes are present in each cell?

8

4

III. A procedure called "differential display" is often used to clone amplified fragments of messages present in "plus" versus "minus" samples (for example, induced versus un-induced cells, two different tissues, or cells at different developmental stages).

a) What enzyme and primer would be used to convert the mRNAs into cDNA?

enzyme = Reverse transcriptase, primer = oligo dT (or U)

b) Subsets of cDNAs are amplified using one "fixed" primer and one random primer. What enzyme, substrates and fixed primer will be used for the amplification process?

Enzyme = Taq polymerase (or equal) ; fixed primer, oligo dT,

substrates: dNTPS, cDNAs, MgCl2

c) The amplified products are separated on "sequencing" type gels. What is the name of this separation process and what is the basis for separation?

electrophoresis, size of the products (since DNA, charge is also OK)

d) Bands that are present in lanes from the plus or minus lanes and missing in the other are cut from the gel and cloned. Describe a set of enzymes, DNA oligomers, a vector and the steps you might use to clone the amplified cDNA fragments.

(Example answer)

open plasmid pUC vector with *Eco*RI restriction endonuclease

treat with phosphatase to eliminate religation

Use T4 DNA ligase to attach *Eco*RI linkers to the cDNA fragments (blunt end ligation); digest with *Fco* RI to create tails that complement those of vector

Mix adapter-converted cDNAs and the open vector, heat cool slowly and ligate with DNA ligase.

Transform into competent *E. coli* cells by electroporation, grow on ampicillin-containing medium with X-gal & IPTG

Select white colonies for testing