GENETICS 603
Exam 1
Oct 26, 2008
Name

I. What is "attenuation"? Would it work in eukaryotes? Justify your answer.

It is a mechanism for transcriptional regulation of some amino acid biosynthetic pathways in bacteria where several codons in the leader of the mRNA call for that amino acid. If it (and its activated tRNA) is plentiful transcription ceases, but if too little is present, a different folding pattern allows transcription (and translation) to proceed.

It can only work in prokaryotes where transcription and translation are coupled.

II. A. List stepwise the reagents (other than buffers) and enzymes you would use to create a cDNA library from a specific tissue like mouse liver or plant leaf cells.

(many alternatives are possible!)

oligo-dT beads for separation of mRNA from cell extracts
oligo-dt primers and reverse transcriptase for first strand cDNA synthesis
RNAaseH to nick the mRNA strand
DNA polymerase to extend and replace the RNA primer fragments
DNA ligase to connect second strand fragments
ligate *Eco* or other linkers or primer binding sites to create useful ends
(can just blunt end ligate)
digest with *Eco* to create *Eco* ends
mix with *Eco* digested puc plasmid, re-anneal and ligate with ligase
transform into competent *E. coli*.

b. Suggest 3 different ways to try to determine if your library has a copy of a cDNA for anthranilate synthase, the next to the last step in tryptophan biosynthesis. (Tell what assumptions you are making in order to use each method).

1. Add to an anthranilate minus *E. coli* strian and look for growth on medium without tryptophan; assumes the native amino acid sequence produced in *E. coli* will function without modification.

2. Hybridize to an anthranilate clone from another species: assumes such a clone is available

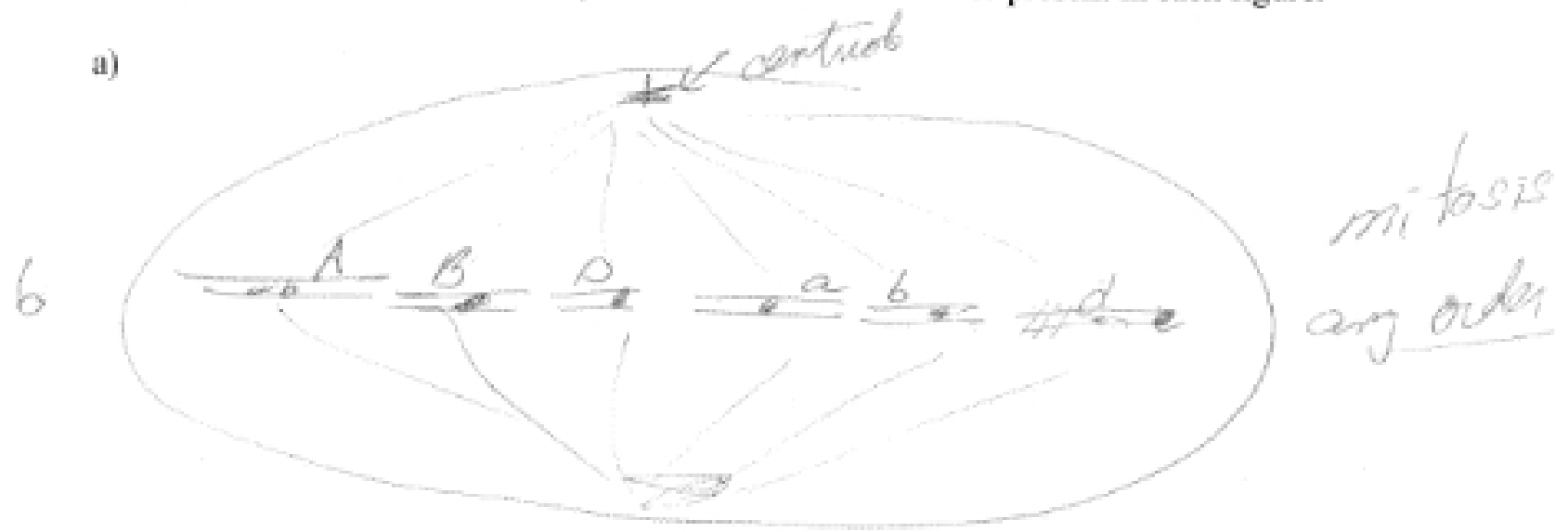
3. PCR using highly conserved sequences from anthranilate synthase from related species to obtain amplicon of predicted size; assumes sufficient sequence information available

4. Sequence large numbers of clones and compare the sequence to those in GenBank; assumes correct annotation of genes identified

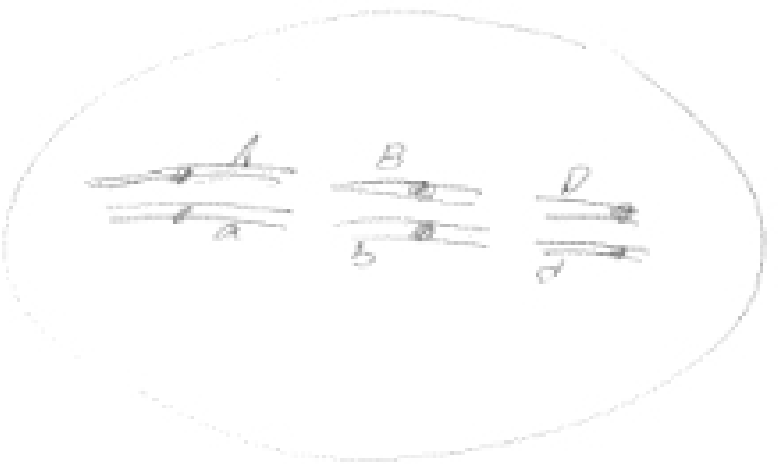
etc.

III. *Crepis capillaris* is a plant species where 2N=6. Assuming that genes A, B and D are heterozygous on chromosomes I (metacentric), II (submetacentric) and III (acrocentric) respectively, show how chromosomes would appear in metaphase of a) mitosis, b) meiosis I, c) meiosis II assuming only one crossover occurred and that was between the centromere and the A locus. Be sure to show the alleles, centromeres and chromatids present in each figure.

a)



b)



c)

IV. Assume that resistance to a specific disease in peas is inherited as a single gene defect and that you have purebreeding resistant and susceptible parents to work with. However, due to "escapes" in field inoculation tests, only 70% of the inoculated susceptible parent plants develop disease.

A. Predict the F1 and F2 phenotypic ratios that would be observed in field tests from a cross of the purebreeding resistant and susceptible parents under the assumption that:
(where res. means no disease detected, susc. is disease detected)

1) resistance is dominant F1 all resistant F2 82.5 % Res : 17.5% sus

2) resistance is recessive F1 30 no disease: 70 disease F2 52.5% sus : 47.5% res

B. If 6 F2 plants are observed in each case, what is the probability of seeing at least one of them will show the susceptible phenotype? (Filled in formulas are ok as an answer)

1) $1-(0.825)^6$ 2) $1-(0.475)^6$

C. Suppose you observe in the field that 60 F2 plants were disease free and 40 diseased. Would these data support either a single gene dominant or recessive model of resistance? Show your work.

For dominant resistance

	R	S
observed	60	40
expected	82.5	17.5
(O-E)	22.5	-22.5
$(O-E)^2/E$	6.25	28.9

Chi Square, 1 df: 35

Prob of a deviation this large or larger due to chance approx 0

resistance is 1 gene recessive

R	S
60	40
47.5	52.5
-12.5	12.5
3.3	2.98

6.2

Probability of a deviation this large or larger by chance is between 1 and 2%
Reject H0 unless using "highly sig"

D. Assume that the R gene is dominant and that the mutation rate to the recessive susceptible allele is 1 in 100,000 gametes. If 500,000 hybrid plants are grown from seeds from the RR by rr cross what is the probability 1) none will be susceptible or 2) at least one will be susceptible (show answer in terms of a formula only).
(using the poison where expect 5 to occur:)

1) e^{-5} 2) $1-e^{-5}$