MCDB 3500
Exam #1
Fall, 2004

75 minutes, closed everything; be concise!

Name KEY

ID_____

Pseudonym _____ (We will post grades under this title rather than using name or ID#; for examples - kingkong, clamploader, Jo123, wombat#1.)

Q 1 (18 points)_____

Q 2 (15)_____

Q 3 (15)_____

Q 4 (15)_____

Q 5 (20)_____

Q 6 (17)_____

Total (100)_____

Grade_____

1. Here is a DNA sequencing gel done using the Sanger (enzymatic) method and radioactive primer, with the dideoxynucleotide indicated above each lane.

Write the sequence of the 20 template nucleotides nearest the primer, 5′ → 3′.

PRIMED STRAND → 5′ C AAAAAACGGACCGGGTGTAC 3′
3′ GTTTTTTGCCTGGCCCACAT 5′ ← TEMPLATE STRAND

Courtesy Life Technologies, Inc. Gaithersburg, MD.

Draw a circle around the unextended primer.

✓ see below left

Draw a box around the DNA molecule: 5′{primer}CA_6CG 3′.

✓ see below left

What nucleotides were in the reaction shown in the lane headed "G"?

dATP, dCTP, dTTP, dGTP plus dideoxyGTP

Draw an arrow beside the gel lanes showing the direction the polymerase moved.

How would the gel show you had sequenced to the end of the molecule?

A band at the top across all four lanes.

down here somewhere

2. There are only about 100 Northern hairy-nosed wombats left in the world; I propose that their DNA be put away to study / save them in the future. They have 7 chromosomes containing 3.6×10^9 nucleotide pairs.

How long (in cm), on the average, are the DNA molecules in each of a hairy-nosed wombat's chromosomes?

$3.6 \times 10^9 \frac{\text{NP}}{\text{genome}} / 7 = 5 \times 10^8$ NP/chromosome

$= 5 \times 10^7$ turns $\times$ 3.4 nM/turn

$= 20 \times 10^{-2}$ Meter $= 20$ cm

How many W/C turns (twists) are there in a wombat genome?

$\frac{3.6 \times 10^9 \text{ NP}}{10.5 \text{ NP/twist}} = 3.2 \times 10^8$ twists

320 Million!

How many 150 kb clones would be required in a genomic library that recovers any wombat sequence with probability = 0.999 ? [note that N = ln (1-P)/ln (1-1/n)]

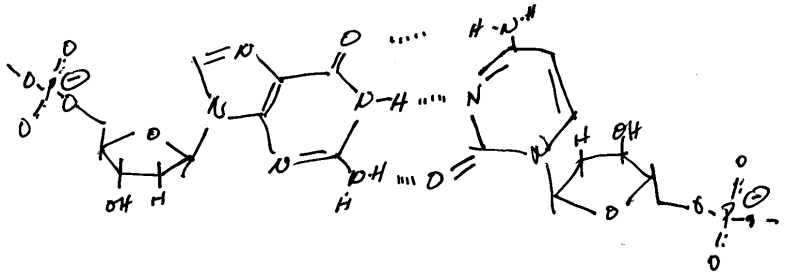
$$N = \frac{\ln(1-0.999)}{\ln\left(1 - \left(\frac{3.6\times10^9}{1.5\times10^5/\text{kb}}\right)^{-1}\right)} = 166{,}000$$

On the average, how many copies of each gene would such a library contain?

$\frac{166{,}000 \times 150{,}000 \text{ bp}}{3.6 \times 10^9} = \frac{2.5 \times 10^{10}}{3.6 \times 10^9} \cong 7$ copies of each gene

↖ 1 genome

Below, draw a wombat G-C pair, including all parts of the nucleotides.



3. Define the terms:

Haplotype

Sequences in a single chromosome.

Type 1 topoisomerase

Breaks single DNA strand and passes the other through to change Writhe.

VNTR

Variable Number Tandem Repeat - short tandemly repeated regs used for DNA fingerprinting AGGCAGGCAGGC..etc

β-clamp

Dimer donut (in bacteria) that holds DNA polymerase on template (leading) or holds & releases (on lagging)

telomerase

Special reverse transcriptase for replication telomeres

Base excision repair

Removes bases or repairs nucs in glycosidic bond: ex cise → repair → lig

Nick translation

5' exonuclease + polymerase, used for repair

Semidiscontinuous

DNA synthesis: continuous on one template (leading) and discontinuous on the other

SNP

Single Nucleotide Polymorphism - single nucleotides that differ when two genomes are compared.

Southern blot

Buffer flow through a DNA gel carries DNA onto paper to make a gel image that can be probed to identify different sequences.

4. For each phrase, describe briefly an experiment and its results, that:

Demonstrates Chargaff's rules.

Hydrolyze DNA into nucleotides; separate them & measure molar ratios.

result → A=T G=C

Makes it likely that DNA carries genetic information.

INJECT A MOUSE WITH DNA FROM VIRULEN STREPTOCOCCUS + NON-VIRULENT CELLS.

result → mouse croaks (AVERY-MCCARTY-MCL

Shows that DNA replication is semi-conservative.

GROW BACTERIA IN "HEAVY" MEDIUM ($^{15}NH_4Cl$)
TRANSFER TO "LIGHT" MEDIUM ($^{14}NH_4Cl$)

MEASURE DENSITY OF DNA OVER SEVERAL GENERATION

result → for example, all DNA is hybrid (half-heavy after 1 generation (MESELSON-ST

Separates proteins of varied sizes.

USE DENATURING GEL ELECTROPHORESIS (WITH STANDA

result - ⫶ ← big proteins
⫶
⫶ ← small

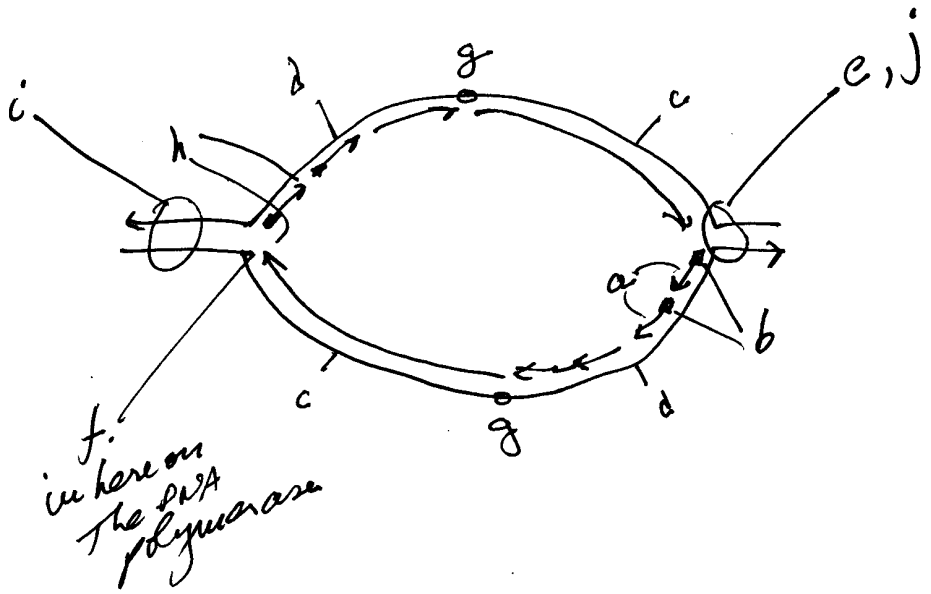
Identifies the sequences that comprise an origin of DNA replication.

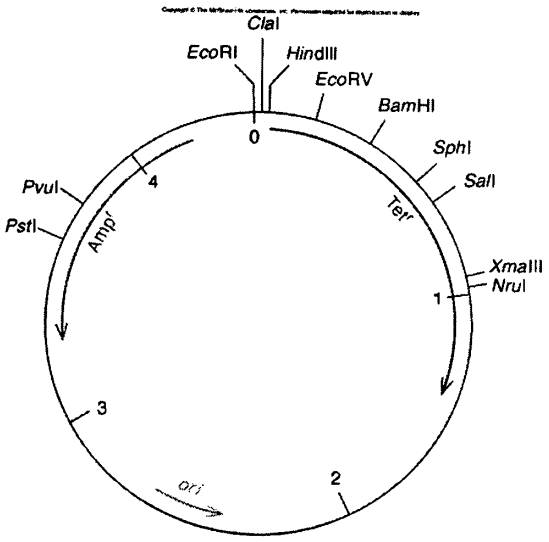
SEQUENCE AND COMPARE THE ~~SEQ~~ DNA'S THAT MADE IT POSSIBLE FOR AN ESSENTIAL GENE (LIKE ANTIBIOTIC RESISTANCE) TO PERSIST IN A CELL.

result → when closely related organisms are compared, find completely conserve repeats.

5. Draw a line diagram (using arrows to show polarity) that shows all DNA and RNA near an origin of replication from which a **bi**directional round of replication (as in a bacterium, for example) has recently been launched. In your diagram, label:

a. An Okazaki fragment
b. A primer
c. A leading strand template
d. A lagging strand template
e. A site where helicase might act
f. Proofreading exonuclease
g. Origin(s) of replication
h. Sites where dATP might have been incorporated during the last second
i. Site of possible topoisomerase II action
j. Site of SSB action





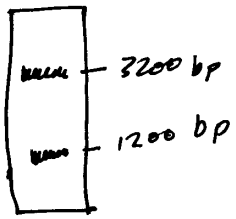
A restriction map of plasmid pBR322.

a. In which restriction fragment would you look for the terminus sequences for DNA replication (if any; you may use any of the enzymes implied above.)? Why?

the lower PstI – NruI fragment
(origin is unidirectional)

b. If the internal numbers are size in kbp, draw a Southern blot for a BamHI + PvuI digest probed with the radioactive messenger RNA from the ampicillin resistance (Amp^r) gene.

≈



c. Suppose you want to clone one and only one copy of a gene using the restriction enzyme Pst1. How could you know (without electrophoresis) you had cloned into the desired site?

Amp^r no longer exists
(use petri plate + amp)

d. How could you know you had only one copy of the target DNA?

For example, digest w/ flanking restriction enz:
w/ EcoRI + NruI → get 3.4 kb + size of cloned DNA

e. How could you clone in the same gene but force your clone to have only one orientation of its sequence?

Digest & clone with PstI plus PvuI
(or PstI + EcoRI, etc if your DNA does not have a PvuI site).