Consider the following circuit diagram for detecting ultra-low light levels (~pW).

When a photon strikes the photocathode of a photomultiplier tube (PMT), it ejects an electron and initiates a fast electron cascade that produces a significant flow of electrons out of the PMT anode (or equivalently, a flow of electrical current into the PMT). Piece together the following circuit to determine its function.

1. For I., II., III., and IV., describe the function of each segment and the qualitative relationships between the inputs and outputs.
   
   I. (15 pts) This circuit acts as a current to voltage converter with a low output impedance. The output voltage is inversely proportional to the current coming out of the PMT.
   
   II. (15 pts) The circuit in II. is a simple comparator, yielding an output voltage corresponding to "ON" when the output of I. is exceeds the threshold (i.e., for a voltage spike) and "OFF" when the output of I. is approx. zero.
III. (15 pts) The circuit in III. is a four bit digital counter. The output of the comparator acts as the clock for the counter.

IV. (15 pts) The final circuit is a digital to analog converter. The output voltage is proportional to the digital count from the four bit counter.

2. (25 pts) Sketch a plot of $V_{out}$ vs. time after the segment in III. is reset.

3. (15 pts) If $V_{out}$ is recorded just before the system is periodically reset, what is the function of the total circuit?

The first circuit converts the current generated from a single incident photon to an output voltage. If the output voltage exceeds a certain threshold, the single photon event can be isolated from the background noise and counted using the comparator circuit. The digital counter simply stores the total number of photons arriving at the PMT in 1 μs (for this circuit, that had better be less than 16). Finally, the digital to analog converter measured just before resetting generates an output voltage proportional to the number of photons arriving at the PMT in 1 μs. Consequently, the total circuit is a photon counter with analog output.
1. Describe the basic elements that should be contained in a plasmid vector for use in cloning and sequencing a novel human gene. Please use a sketch to assist your description. (35 points)

Answer:

Plasmids should:

a. contain a polylinker site for restriction enzyme digestion; these restriction enzymes will hopefully cut the plasmid only once to allow gene insertion (8 Points)

b. contain two separate selectable markers, one to assure the transfected vector contains a transgene insert and the second to insure all surviving cells contain a desired plasmid (10 Points)

c. be able to accommodate substantial foreign DNA (3 Points)

d. be transferable among bacterial cells so that transfection of all bacteria is not necessary (3 Points)

e. contain a strong origin of replication whose replication is cell independent (7 Points)

f. have promoters from T7, T3, or SP6 phages adjacent to polylinker site to allow transcription directly from the plasmid using the phage RNA polymerase (4 Points)

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A restriction map of the plasmid pUC18 indicating the positions of its ampr, lacZ', and lacI genes. The ampr gene confers resistance to the antibiotic ampicillin (a penicillin derivative); lacZ' is a modified form of the lacZ gene, which encodes the enzyme β-galactosidase (Section 10-1C); and lacI encodes the lac repressor, a protein that controls the transcription of lacZ as is discussed in Sections 29-1A and 3B. The polylinker, which encodes an 18-residue polypeptide segment inserted near the N-terminus of β-galactosidase, incorporates 13 different restriction sites that do not occur elsewhere in the plasmid.
2. Describe the composition of a cDNA library and explain how it might be screened to obtain the complete sequence of a protein for which you only know the sequence of 25 amino acids. (40 points)

Answer:

A cDNA library is comprised of DNA copies of all mRNA species in a particular tissue or cell sample. Because it is prepared by reverse transcription of the purified mRNA present in the same sample, its composition reflects the abundance of each mRNA species in the sample. Thus, some genes are represented by many more copies in the library than others. The library can be screened using one of the three methods, each of which begins with a transfer of individual bacterial clones from a solid growth medium such as agar to a paper or membrane such as nitrocellulose. Briefly, for use of a labeled oligonucleotide probe (screening method #1), cells from the transformation reaction are plated onto solid medium under conditions that permit transformed but not nontransformed cells to grow. (1) From each discrete colony formed on this master plate, a sample is transferred to a solid matrix such as a nitrocellulose or nylon membrane. The pattern of the colonies on the master plate is retained on the matrix. (2) The cells on the matrix are lysed, and the released DNA is denatured, deproteinized, and irreversibly bound to the matrix. (3) A labeled DNA probe is added to the matrix under hybridization conditions. After the nonhybridized probe molecules are washed away, the matrix is processed by autoradiography to determine which cells have bound labeled DNA. (4) A colony on the master plate that corresponds to the region of a positive response on the X-ray film is identified. Cells from the positive colony on the master plate are subcultured because they may carry the desired plasmid-cloned DNA construct.

For antibody screening (screening method #2), the nitrocellulose is reacted with an antibody that specifically recognizes the protein of interest. Subsequent staining with the appropriate enzyme-couple second antibody then allows visualization of bacterial colonies that express the desired protein.

For enzyme activity staining/screening (method #3), the protein of interest must have a unique enzyme activity that is completely absent in the nontransformed bacteria. The enzyme activity must also be adaptable to a visually distinct assay method. The nitrocellulose paper is then reacted with the colored substrates and clones containing the desired enzyme are thereby identified.
3. Describe how you can best evaluate the tissues in which your novel gene is expressed. Please include all steps necessary. (25 points)

Answer:

The best method to identify tissues that express a specific gene is via Northern blot analysis. In this procedure, mRNA from each tissue to be examined is isolated in the presence of an RNase inhibitor to prevent digestion. The mRNA is then separated electrophoretically on a polyacrylamide or agarose gel and transferred onto a nitrocellulose or nylon sheet. The blotted mRNA is finally probed with a radiolabelled oligonucleotide complementary to the gene of interest and hybridizing samples are visualized by autoradiography.

Alternatively, and less desirably, the various tissues can be homogenized in the presence of proteinase inhibitors and their component proteins separated by SDS polyacrylamide gel electrophoresis. As above, the material in the gel can be transferred to a nitrocellulose membrane and the membrane can be probed with an antibody specific for the gene product of interest. Staining with a secondary enzyme-coupled antibody will then reveal which tissues express the gene of interest. This method is less desirable, because nonspecific immunological cross-reactivity can lead to false positive results.
Organic Cume (9/21/02)

1. (30pts) Give the value of each of the following quantities:

(a) The diameter of a benzene molecule
\[ 2 \times (1.40 + 1.08 + 0.8) = 5.1\text{Å} \]
\[ \text{C-C} \quad \text{C-H} \quad \text{van de Waals} \]

(b) The pK_a of acetone
19

(c) The C-C bond dissociation energy in ethane
90 kCal/mol

(d) The energy of the π- π* absorption in benzene
\[ 257 \text{ nm} = 96 \text{ kCal/mol} \]

(e) The frequency in \text{sec}^{-1} of an H-C vibration in methanol
\[ 3000 \text{ cm}^{-1} = 9 \times 10^{13} \text{ s}^{-1} \]

(f) The rate of chair to chair flipping in cyclohexane at room temperature
\[ E_a = 10.6 \text{ kCal/mol} \quad \text{and rate} = 10^5 \text{ s}^{-1} \]

(g) The boiling point of methylene chloride
42

(h) The $^{13}$C chemical shift of CHCl_3
78 ppm

(i) Arrange the indicated C-H bonds in order of \text{DECREASING} bond dissociation energy.

![Chemical structure diagram]

BDE is energy of process \text{R-H} \rightarrow \text{R}^+ + \text{H}^-
\[ a < e < c < b < d \]

Explain why your selected lowest value has such a low bond dissociation energy.

The benzylic/allylic radical is very stable because of conjugation.
2. (15 pts) Knochel reported the following transformations. Identify compounds A and B and write a stepwise mechanism for the reaction of A to give B.

\[
\begin{align*}
\text{I} & \xrightarrow{\text{i-PrMgCl}} \text{A} + \text{B} \\
\text{Br} & \xrightarrow{-20 \degree C, 30 \text{ min}} \\
\text{FeCl}_2 \ 2 \text{ equiv} & \xrightarrow{\text{NaBH}_4 \ 1 \text{ equiv}} \\
\text{H}_2\text{O} & \\
\text{I} \xrightarrow{1) \text{i-PrMgCl}} \text{A} \\
\text{R} & \xrightarrow{-20 \degree C, 30 \text{ min}} \\
\text{MgCl} & \\
\end{align*}
\]

Note Grignard attack at O of N=O then elimination of phenoxide. Alternatively, Grignard attack at N of N=O (no pentacoordinate N's) then elimination of MgO! from anion, and addition of the Grignard to form phenoxy 4.

\[
\begin{align*}
\text{Ar}^2-\text{N}^+ \xrightarrow{3) \text{H}_2\text{O}} & \xrightarrow{1) \text{FeCl}_2 \ (2 \text{ equiv})} \text{Ar}^2-\text{N}^+ \xrightarrow{2) \text{NaBH}_4 \ (1 \text{ equiv})} \text{Ar}^2-\text{H}^+ \\
\text{Ar}^2-\text{N}^+ & \xrightarrow{4) \text{H}_2\text{O}} \text{Ar}^2-\text{H}^+ \\
\text{Ar}^2-\text{MgCl} & \xrightarrow{5) \text{Ar}^2-\text{H}^+} \\
\end{align*}
\]
3. (15pts) Grubbs recently reported a method for preparing macrocycles 4 and 5 using the following reaction. He found that the overall isolated yield of 4 and 5 dropped, but the ratio of 5:4 increased when the total concentration was increased. What is the role of 2 and why is this the preferred catalyst for the reaction? Identify the intermediates formed in the transformation of cyclopentene into 4 and 5. Account for the change in yield and product ratio with concentration.

Ref: J. AM. CHEM. SOC. 2002, 124, 3224–3225

This is the second generation Grubbs metathesis catalyst. It is stable to functional groups, highly active and sterically selective.

Note that the cyclopentene is opened first to give the new metathesis species which then couples with the vinyl ketone regenerating the methyldiene Ru catalyst and releasing the new diene. A second catalyst moiety brings about the macrocyclization to form 4. Formation of 5 requires the coupling of two equivalents of the intermediate diene. As formation of 4 is a “unimolecular” process, while formation of 5 is bimolecular, increase in the ratio of 5/4 is achieved at higher concentrations. However higher concentration also leads to increasing the rate of competitive side reactions such as polymerization and so lowers the overall yield.
4. (40 pts) Recently Billups et al (J. Org. Chem. 2002, 67, 4436-4440) reported the following reactions. The 500 MHz proton (see next page) and carbon NMR spectra showed that the dimers were symmetrical and fused via the 1,2 bonds of the monomer.
(a) (20 pts) Give a stepwise mechanism for each step in the above the reaction sequence – only one dimer need be shown.

(b) (20 pts) Only two dimers were formed. They were identified by the coupling patterns of the carbon-13 satellites in the proton spectra and from nOe data. The splitting patterns for the $H_A$ protons in isomer $X$ were found to be 9.4, 3.7, 2.5, and 1.9 Hz and for isomer $Y$ were found to be 4.4, 2.9, 2.9, and 1.2 Hz.

Construct splitting diagrams for the two isomers and hence assign $X$ and $Y$ to the spectra $E$ and $F$ below.

$X$ is split by 4 non-equivalent protons into a 16 line pattern of equal intensity. This appears as a large doublet (9.4 Hz) of overlapped multiplets with 8 lines each. Total splitting = 17.6 Hz = 0.035 ppm.

$Y$ is split by 4 non-equivalent protons into a “dti” pattern as two of the couplings are equal. Thus the outer lines are half as intense as the inner lines. Total splitting = 11.4 Hz = 0.022 ppm.

So $X$ is $E$ and $Y$ is $F$
(Also shown by full spectra and their chemical shifts)

One isomer was identified from this data. Which one? Justify your choice by assigning the couplings.
Only isomer 6 can have the large 3 bond 9Hz coupling. Note that these protons are eclipsed and the dihedral angle is zero and follows the Karplus relationship. The trans coupling (angle 120) is small in 3 and 4 membered rings.

9.4 Hz \( H_A - H_A' \); 3.7 Hz, \( H_A - H_M \) vicinal; 2.5 Hz, \( H_A - H_P \) allylic; and 1.9 Hz \( H_A - H_K \) homoallylic.

*Explain why the pattern for the carbon satellite spectra were used for this analysis rather than the standard proton spectrum.*

Because \( H_A \) and \( H_A' \) have the same chemical shift their spectra are highly second order with no resolved couplings. The original paper shows simulated spectra matching the observed and calculated spectra using the coupling constants from the satellite spectra as input.

*Both isomers showed similar nOe patterns. The absence of any new strong nOe interactions was used to identify the second isomer from the remaining three possibilities. Identify it and justify your choice.*

Isomer Y is 7. nOe patterns show protons which are within 4Å of each other. In 8 and 9, there are close methyl contact distances. The supplementary Information for the paper has a table showing the data.

![500-MHz \(^1\text{H} \) NMR spectrum](image)
a). (10 points)

\[ \frac{dN}{dt} = -\lambda N \]

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\[ \int_{N_0}^{N} \frac{dN}{N} = -\lambda \int_0^t dt \]

where \( N_0 \) is the no. of atoms at \( t = 0 \)

\[ \ln \frac{N}{N_0} = -\lambda t \]

\[ N = N_0 e^{-\lambda t} \]

b). (10 points)

When \( t = \frac{t_1}{2} \), \( N = N_0 / 2 \)

\[ \frac{N_0 / 2}{N_0} = e^{-\lambda t_1 / 2} \]

\[ \ln \frac{1}{2} = -\lambda t_1 / 2 \]

\[ t_1 / 2 = \frac{\ln 2}{\lambda} \]

1st order kinetics

\[ \frac{dN_2}{dt} = \lambda_1 N_1 - \lambda_2 N_2 \]

formation decay
Assuming \( N_2 = f e^{-\lambda_2 t} \) where \( f = f(t) \)
and differentiating:

\[
\frac{dN_2}{dt} = -f \lambda_2 e^{-\lambda_2 t} + e^{-\lambda_2 t} \frac{df}{dt}
\]

Setting the expressions for \( dN_2/dt \) equal to each other:

\[
\lambda_1 N_1 - \lambda_2 N_2 = -f \lambda_2 e^{-\lambda_2 t} + e^{-\lambda_2 t} \frac{df}{dt}
\]

Substituting \( N_1 = N_1^0 e^{-\lambda_1 t} \) (form) and \( N_2 = f e^{-\lambda_2 t} \):

\[
\lambda_1 N_1^0 e^{-\lambda_1 t} - \lambda_2 f e^{-\lambda_2 t} = -f \lambda_2 e^{-\lambda_2 t} + e^{-\lambda_2 t} \frac{df}{dt}
\]

So \( e^{-\lambda_2 t} \frac{df}{dt} = \lambda_1 N_1^0 e^{-\lambda_1 t} \)

and \( \frac{df}{dt} = \lambda_1 N_1^0 e^{-(\lambda_1 - \lambda_2) t} \)

Integrating:

\[
f = \int \lambda_1 N_1^0 e^{-(\lambda_1 - \lambda_2) t} \, dt = \frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0 e^{-(\lambda_1 - \lambda_2) t} + C
\]

Since \( N_2 = f e^{-\lambda_2 t} \),

\[
N_2 = \frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0 e^{-\lambda_1 t} + C e^{-\lambda_2 t}
\]

Since \( N_2 = 0 \) when \( t = 0 \),

\[
0 = \frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0 + C
\]

\[
C = -\frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0
\]

and

\[
N_2 = \frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0 (e^{-\lambda_1 t} - e^{-\lambda_2 t})
\]
(20 points)

1. When \( \lambda_1 \gg \lambda_2 \) or \( t_2 \gg t_1 \)

\[
\ln N = N_2 \quad \text{N}_2 \text{ decays with its own half life}
\]

When \( \lambda_1 \ll \lambda_2 \) or \( t_2 \gg t_1 \)

\[
\ln N = N_1 \quad \text{N}_2 \text{ decays with half life of 1}
\]

2. (20 points) \( N_2 = \frac{\lambda_1}{\lambda_2 - \lambda_1} N_1^0 (e^{-\lambda_1 t} - e^{-\lambda_2 t}) \)

Differentiating:
\[
dN_2/dt = \frac{-\lambda_1}{\lambda_2 - \lambda_1} N_1^0 (-\lambda_1 e^{-\lambda_1 t}) + \frac{\lambda_1 \lambda_2}{\lambda_2 - \lambda_1} N_1^0 e^{-\lambda_2 t}
\]

Setting derivative to zero when \( t = t_{\text{max}} \):
\[
\frac{\lambda_2}{\lambda_1} = e^{-(\lambda_2 - \lambda_1) t_{\text{max}}}
\]

\[
t_{\text{max}} = \frac{1}{\lambda_2 - \lambda_1} \ln \frac{\lambda_2}{\lambda_1}
\]
\[ f. \ (10 \text{ points}) \]

\[ N = N_0 e^{-\frac{t}{t_d}} \]

\[ N_0 = \frac{1.00 \text{ mg}}{10 \text{ g/mg}} \times 6.022 \times 10^{23} \text{ mol} = 2.87 \times 10^{15} \text{ atoms} \]

\[ N = (2.87 \times 10^{15}) \exp \left[ -\frac{t}{138 \text{ yr}} \right] = 1.74 \times 10^{15} \]

No. of atoms decayed = \( N_2 \) = 2.87 \times 10^{15} - 1.74 \times 10^{15} = 1.13 \times 10^{15}

\[ PV = nRT \quad \text{where} \quad n = \frac{1.13 \times 10^{15}}{6.022 \times 10^{23} \text{ mol}^{-1}} = 1.88 \times 10^{-9} \text{ mol} \]

\[ V = \left(1.88 \times 10^{-9} \text{ mol}\right) \left(0.08206 \text{ atm} \cdot \text{L} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}\right) \left(273 \text{ K}\right) \]

\[ = 4.21 \times 10^{-8} \text{ L} \]