Integrative Molecular and Cellular Bioengineering, SPRING '06
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Exam 1: March 21, 2006, 80 minutes

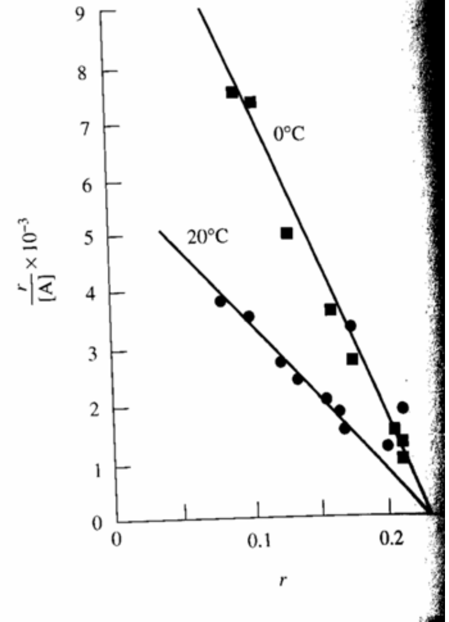
1. (8 points) List at least two relative advantages each for viral vs. non-viral gene therapy

a) *Viral*

    1.
    2.

b) *Non-viral*

    1.
    2.



2. (10 points) The accompanying Scatchard plot shows the binding of ethidium to DNA at two temperatures. In this figure, *r* is the number of bound ethidium molecules per base pair of DNA, and [*A*] is the concentration of DNA base pairs in units of μM.

a) What is the affinity of ethidium for DNA at 0ºC? At 20ºC?

b) What is the number of binding sites for ethidium per base pair of DNA at 0ºC? At 20ºC?

c) What are the main assumptions of the Scatchard equation? Are they likely to be satisfied here?

Scatchard equation $\frac{r}{[A]} = K(n-r)$

(Note: Feel free to leave your answer in the form of an arithmetic expression if you don't have a calculator)

3. (12 points) In the accompanying figure, HL-60 cells were treated with PMA (an inflammatory inducer) for 0, 0.5, 4 or 24 hours, after which the expression levels of 6000 genes were probed by Affymetrix microarray. After filtering, 567 genes remained for analysis and were clustered using self-organizing maps (SOM).

a) What types of processes were most likely used to filter the genes from 6000 to 567?

b) How is the number of clusters determined? Do you think it is appropriate?

c) The genes in cluster 11 are shown in the following table. Many of these are "unexpected". If we're skeptical about these genes, describe one way to find out if they are truly differentially expressed after PMA treatment.

d) What types of associations might we look for among the genes that are coexpressed (e.g., in cluster 11)?

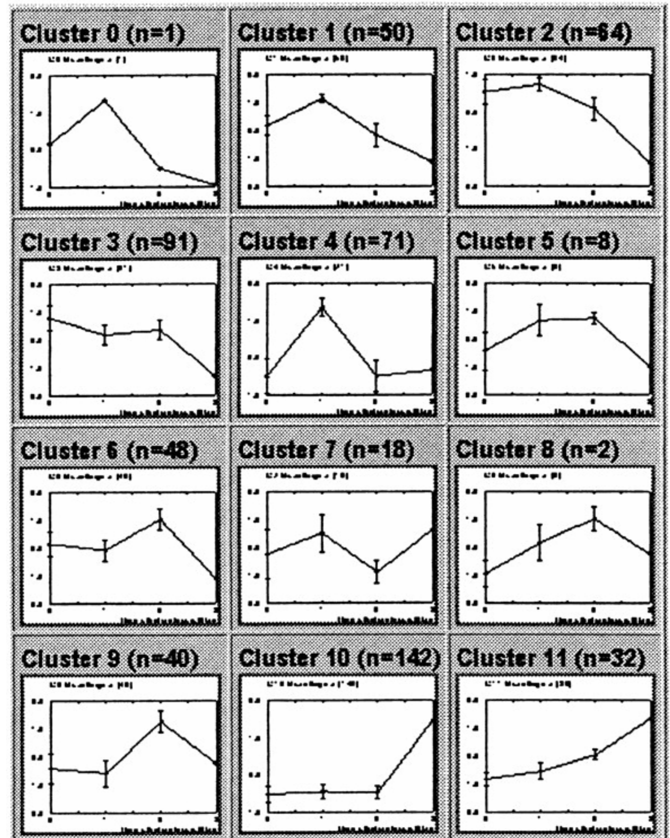


Table 1. Genes in cluster 11 (PMA-induced genes in HL-60 cells)

Gene	Description
Expected	
MIP1α	Macrophage Inflammatory Protein 1 α
BFL-1 (Bcl-2 related)	
PEA-15	Major astrocytic phosphoprotein
CD83 antigen	
DTR	Diphteria toxin receptor (heparin-binding EGF-like growth factor)
JUNB	Protooncogene
P4HA	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), polypeptide
DAF	Decay accelerating factor for complement (CD55)
EGR2	Early growth response 2
SLP-76	76-kDa tyrosine phosphoprotein
TNFAIP1	Tumor necrosis factor α-inducible protein A20
KNG	Kininogen
Fc-receptor γ-chain	
Tryptophanyl-tRNA synthetase	
BTG1	B cell translocation gene 1
RASA1	GTPase-activating protein ras p21 (RASA)
CRFB4	Cytokine receptor family II, member 4
Homeobox c1 protein	
Unexpected	
GLVR1	Leukemia virus receptor 1
PTPN12	Protein tyrosine phosphatase, non-receptor type 12
FKBP25	FK506-binding protein
CSNK1A1	Casein kinase 1, alpha 1
CSNK2A2	Casein kinase 2, alpha prime polypeptide
RPL3	Ribosomal protein L3
RPL4	Ribosomal protein L4
HIP	Putative tumor suppressor (HNC6)
EST	GenBank accession no. H80240
EST	GenBank accession no. T53118

4. (10 points) I am studying blood clotting in a porcine (pig) model and would like to develop an ELISA assay to measure the concentration of fibrinogen in pig sera. I cannot find an antibody for porcine fibrinogen but did find the following from Sigma: Mouse Anti-Fibrinogen Monoclonal Antibody, Unconjugated. Pig is listed under Reactivity, along with several other species. Assume that I can also purify or obtain pig fibrinogen.

a) What other reagents do I need to make the ELISA work?

b) What format of ELISA would be used (e.g., sandwich, competitive)? Describe the arrangement – i.e., what would be coated, what would be added in what order.

c) Sketch the expected shape of the resulting standard curve. Label your axes.