

Solution key

Question 1

During a summer hike you suddenly spy a grizzly bear. This triggers a fight or flight response activating the signaling pathway that is shown on last Page.

a) **Circle** the best option. This is an example of *autocrine/ paracrine/ juxtacrine/ endocrine* signaling. **Explain** why you selected this option.

This is an example of endocrine signaling since adrenaline hormone travels through the blood stream to reach the target cells that are expressing the β -adrenergic receptors thus triggering this signaling pathway.

b) Which component functions as the **second messenger**? *cAMP*

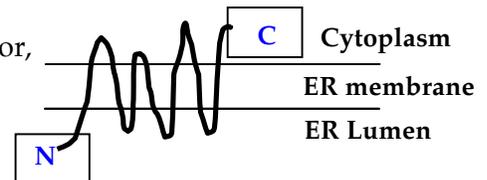
c) Consider the following **perturbations** in different components of this signaling pathway in cells.

- #1: The promoter region of the gene encoding β -adrenergic receptor is constitutively methylated.
- #2: BARK lacks its kinase-binding domain.
- #3: Cells are treated with an allosteric inhibitor of adenylate cyclase enzyme.
- #4: Protein kinase A has a constitutively active kinase domain.

Complete the table for each of the following **perturbations relative to the wild-type cells** in the **presence** of adrenaline. **Please note:** Consider each perturbation independently.

Perturbations	G protein activated (Yes/No?)	cAMP produced (Yes/No?)	Protein kinase A activated (Yes/No?)	Effect on heart rate and glycogen breakdown relative to the wild-type in the presence of adrenaline? (increased/ decreased /unchanged)
1	No	No	No	Decreased
2	Yes	Yes	Yes	Increased
3	Yes	No	No	Decreased
4	Yes	Yes	Yes	Increased

d) On the schematic label the N and C ends of β -adrenergic receptor, as it is translated in endoplasmic reticulum, by filling in the boxes.



e) You identify a mutant cell line, which produces β -adrenergic receptor as a secretory protein. **Circle** the domain that is absent in this receptor that prevents its localization in the plasma membrane?

Signal sequence Kinase domain Phosphatase domain Transmembrane domain

f) Name the **feedback inhibitory component** of this signaling pathway. *β -arrestin*

Question 2

The following is the DNA sequence for the transcription initiation region of the **PKA gene**. *Please note: Part of the promoter region is shown and boxed. Transcription begins at and includes the bold and underlined T/A base pair.*

```
5' -TGGACTGCTATAATAGCAGTTCTGCTGAGATGATGGCCATACGGCCATGGTTCTTAAAGT-3' TOP
3' -ACCTGACGATATTATCGTCAAGACGACTCTACTACCGGTATGCCGGTACCAAGAATTTC-5' BOTTOM
```

- a) **Box** the template strand (*Top or Bottom*) for transcription.
- b) Write the first 6 nucleotides of the nascent PKA mRNA. *5' UGCUGA 3'*
- c) Write the first 3 amino acids of the newly synthesized PKA protein. *N- Met¹- Met²- Ala³-C*
- d) You identify a mutant cell line that shows a single mutation in the PKA gene. You find that although PKA gene has the same **protein coding sequence** in the wild-type and mutant cells, it is **NOT transcribed** in mutant cells.
- iii. Based on this information, **circle** the sequence from the choices below that has a mutation and **explain** why you selected this sequence.

Promoter Introns Exons 3' Untranslated region 5'Cap Ribosome binding site
A mutation in the promoter region may prevent the binding of transcription factor(s) and RNA polymerase thus preventing the formation of the transcription initiation complex, which is required to trigger the transcription of a gene.

- iv. Is the above sequence present in PKA gene/ nascent PKA mRNA transcript/ spliced PKA transcript?
- e) The last (C-terminal) **5 amino acids (301-305)** of the **wild-type** form of the **PKA protein** are shown below.

N -Val³⁰¹-Ser³⁰²-Asn³⁰³-Ser³⁰⁴-Met³⁰⁵- C

The DNA sequence encoding the C- terminus of the wild- type and mutant forms of the PKA protein is included within the sequence below. The **point mutations** in the mutant alleles are **bold & shaded**.

Wild-type allele: 5' -TCGTATCGAATTCCATC TAGC-3'
3' -AGCATAGCTTAAGGTACATCG-5'

Mutant allele 1: 5' -TCGTATAGAAATTCATGTAGC-3'
3' -AGCATATCTTAAGGTACATCG-5'

Mutant allele 2: 5' -TCGTATCGAACCTCCATGTAGC-3'
3' -AGCATAGCTTGAGGTACATCG-5'

- ii. Underline the codon corresponding to the **Met³⁰⁵** in the wild- type allele of the PKA gene.

- iii. Compared to the wild- type, which mutant allele will encode a **truncated PKA protein**? **Explain** why you selected this option.

Mutant 1 will encode a truncated protein. The codon corresponding to the amino acid 302 in the PKA transcript of this mutant is a stop codon. Thus the PKA protein produced by mutant 1 will only be 301 amino acids long instead of 305. In comparison, the mutation in mutant 2 is a silent mutation and hence it will produce the wild-type protein.

- f) You observe that PKA gene is expressed in the muscle cells (as a 200 amino acids long membrane protein) and also in the heart cells (as a 64 amino acids long cytosolic protein). Briefly **explain** how the same gene can encode two different proteins in two different cell types.
Same gene can produce different proteins in different cell types due to the alternative splicing of the introns in the mRNA transcript. Alternative promoters is also an acceptable answer.

Question 2 continued

g) You are comparing the activities of DNA polymerase, RNA polymerase and telomerase enzymes. Which of these enzymes...

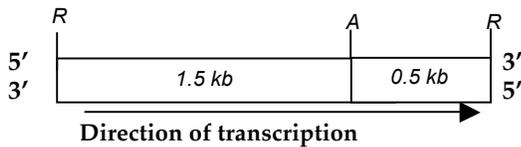
- v. Is primase dependent? *DNA polymerase*
- vi. Is active in wild- type **non- dividing** somatic cells in an adult? *RNA polymerase*
- vii. Is functional **only** in embryonic and tumor cells? *Telomerase*
- viii. Has lowest overall error rate? *DNA polymerase*

Question 3

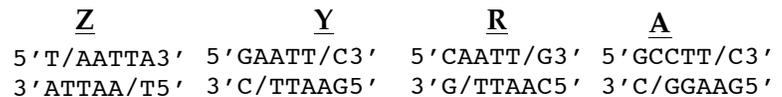
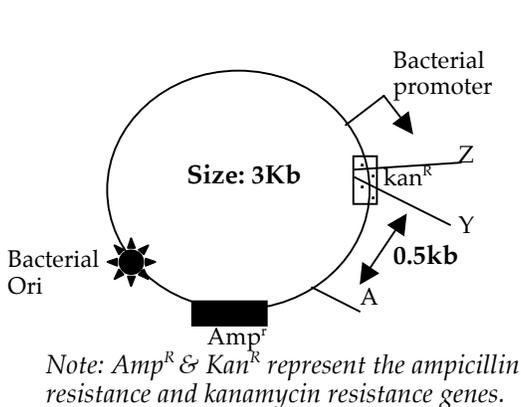
a) You make the PKA cDNA using the spliced mRNA derived from the wild- type allele of the PKA gene. You use a Poly- T primer to make the **first strand of cDNA** using the PKA mRNA template. What feature does the spliced mRNA have that allows you to use the Poly T primer?

The spliced mRNA has a 3' Poly A tail with which the PolyT primer can base pair in an anti- parallel fashion. The reverse transcriptase can then add the complementary bases to the 3'OH end of this poly T primer to make the first cDNA trans that is complementary to the processed mRNA transcript.

b) The PKA cDNA has the recognition site for restriction enzymes R and A as shown below.



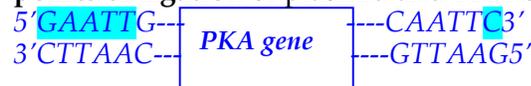
You want to clone PKA cDNA into the following plasmid that has recognition sites for restriction enzyme Z, Y and A as shown. **Please note:** A slash (/) represents the cutting site for each restriction enzyme.



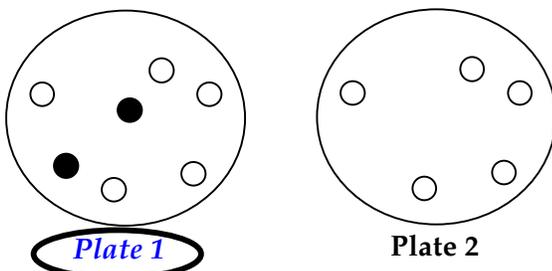
iii. Which enzyme (Z/ Y/ A) would you use to cut the plasmid so that it has ends that are compatible to the ends of the PKA cDNA cut with restriction enzyme R?

Enzyme Y

iv. Write the resulting 6- base pair sequences at the two points of ligation of plasmid and PKA cDNA.



c) You then plan to amplify the recombinant plasmid in *E. coli* bacterial cells. You transform the *E. coli* with the ligation mix and plate them on a master plate (growth medium with no antibiotics). You then replica- plate the colonies on **plate 1** (growth medium + ampicillin) and **plate 2** (growth medium containing both ampicillin and kanamycin). You obtain the following colonies.



- iii. **Circle** the plate (1/2) in the schematic that contains bacterial colonies that have the **recombinant plasmid**.
- iv. In the plate that you circled, **fill in/ color** the colonies that contain the **recombinant plasmid**. **Explain** why you selected these colonies.

*The recombinant plasmid will have an intact and functional Amp^R gene but a disrupted Kan^R gene where Gene D is inserted. So any *E. coli* cell that will receive the recombinant plasmid will be Amp^R Kan^S and will therefore grow on plate 1 but not on plate 2.*

Question 3 continued

d) You purify the recombinant plasmids from two *E. coli* colonies (Colony A containing plasmid 1 and Colony B containing plasmid 2). You digest both plasmids with Restriction enzyme A and resolve the digested fragments by DNA gel electrophoresis. You observe the following DNA fragments on the gel.

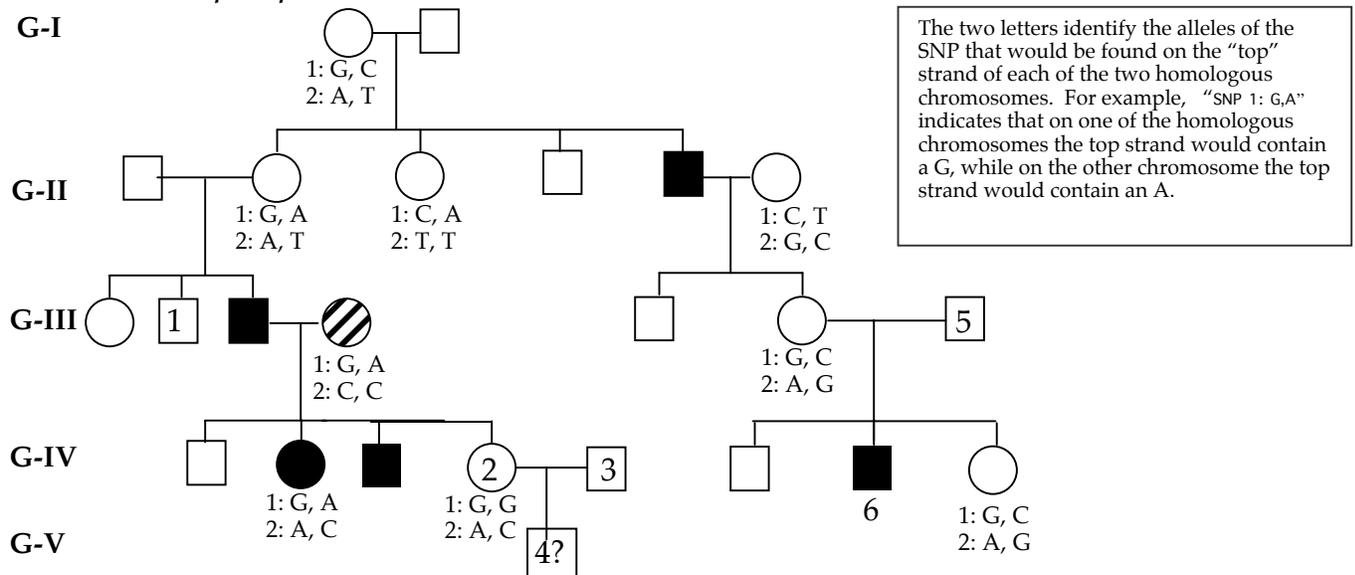
- Recombinant plasmid 1 from Colony A gives fragments of 3kb and 2 kb.
- Recombinant plasmid 2 from Colony B gives fragments of 4kb and 1kb.

Which bacterial colony (A or B) has plasmid that can express PKA cDNA? **Explain** your choice in words or by a drawing.

Plasmid 2 that is derived from colony 2 has the plasmid in the correct orientation, based on the size of Restriction enzyme A cut DNA fragments. In this orientation the direction of transcription of the plasmid is compatible to the orientation of bacterial promoter located on the plasmid. Plasmid 1 derived from colony 1 has the plasmid in the opposite orientation to the promoter. Hence the PKA cDNA insert in this recombinant plasmid will not be expressed.

Question 4

You are studying the following pedigree that shows a specific mode of inheritance of a disease, which is associated with mutations in the PKA gene. You identify two rare SNPs (SNP1 & SNP2), which are a **part of the PKA gene**. **Please note:** All the individuals with the disease phenotype are shaded. People marrying into the family only have the wild-type alleles of the PKA genes unless **striped**. Also listed are the alleles of SNP1 and SNP2 for some individuals. Assume **complete penetrance**.



a) Give the **mode of inheritance** of this disease. *X-linked recessive*

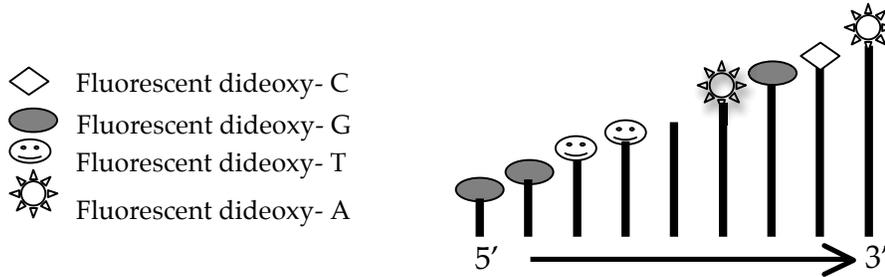
b) Give the **SNP1 and SNP2 genotype** for Individual 1 & Individual 6 in the pedigree.

Individual	SNP1 Genotype	SNP2 Genotype
1	A	T
6	G	A

c) Individual 2 and 3 have a **son** (shown as #4). What is the probability of him **being affected**? *It is 50% since his mother is a carrier.*

Question 4 continued

d) You sequence the allele of PKA gene from Individuals 5 & 6. You identify a third SNP; SNP3 that is located within the coding region that corresponds to amino acids N-gly⁹⁵-trp⁹⁶-ala⁹⁷-C in Individual 5. The following is the sequencing profile of the same region for Individual 6. Based on this profile...



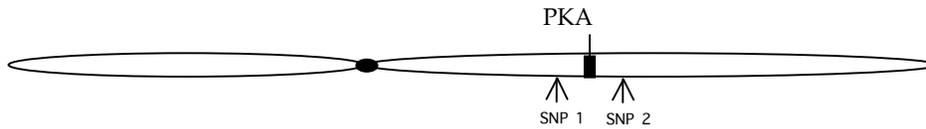
Give the alleles of SNP3 for Individuals 5 & 6.

SNP3 for Individual 5: **G**

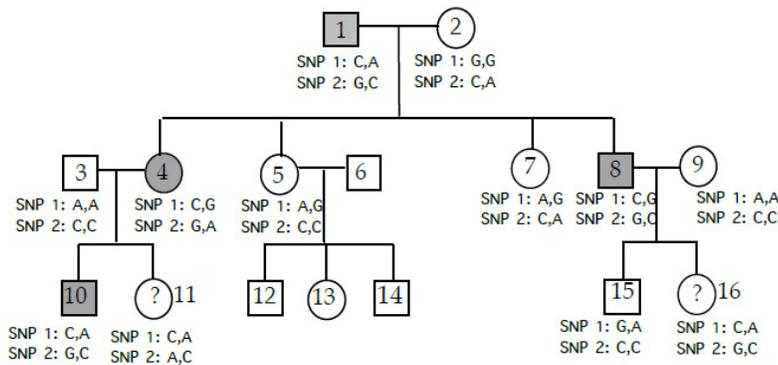
SNP3 for Individual 6: **A**

Question 5

The chromosomal position of the PKA is diagrammed below, as is the location of two single nucleotide polymorphisms (SNPs). Mutations in the PKA cause the rare inherited form of cancer.



The following pedigree represents the inheritance of PKA. **Note:** All affected individuals are shaded. Also listed are the alleles of SNP 1 and 2 for some individuals. People marrying into the family only have the wild-type copy of the PKA gene.



Note: The two letters identify the alleles of the SNP that would be found on the “top” strand of each of the two homologous chromosomes. For example, “SNP 1: C,A” indicates that on one of the homologous chromosomes the top strand would contain a C (that is, the chromosome would have a C/G base pair in this position), while on the other chromosome the top strand would contain an A (that is, the chromosome would have an A/T base pair in this position).

a) Circle the most likely mode of inheritance of predisposition to retinoblastoma.

- Autosomal Dominant
 Autosomal recessive
 X-linked dominant
 X-linked recessive

b) Give the most likely **genotype** at the PKA locus for each of the following individuals. **Note:** Use + for the allele regulating the wild-type phenotype and - for the allele regulating the cancer phenotype.

Individual 2: **++** Individual 8: **+-**

c) Individuals 5 and 6 have no affected children. Can you predict the genotype for individual 6 at SNP 1 and SNP2 loci (Yes/ No)? **Explain** why you selected this option.

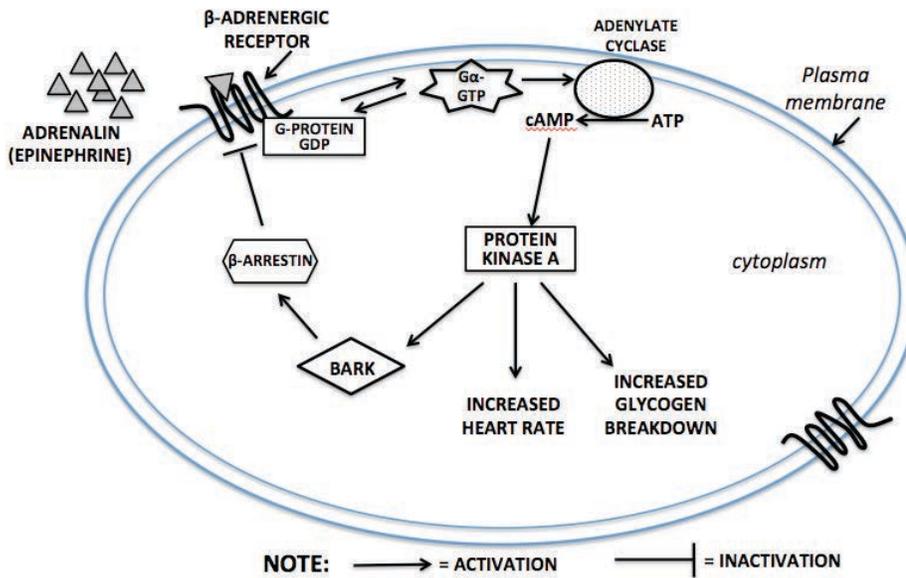
No, you cannot predict the SNP genotype of Individual #6 since he is marrying into the family and can have different SNPs flanking the RB gene.

d) Assuming no recombination between SNPs and the PKA locus, which alleles of SNP1 and SNP2 are linked to the mutated allele of the PKA gene in this pedigree?

SNP1: C

SNP2: G

Signal transduction pathway for Question 1. Please note: You can detach this page.



- The adrenaline hormone, circulating in the blood stream, binds to and activates the **β 1-adrenergic membrane receptor**.
- Following the binding to adrenaline, this receptor acts as a kinase and converts the inactive G proteins (GDP bound form) to active **$G\alpha$ -GTP complex**.
- The $G\alpha$ -GTP complex **activates adenylate cyclase** enzyme.
- The $G\alpha$ -GTP is **inactivated** by conversion back to $G\alpha$ -GDP.
- Activated adenylate cyclase converts ATP to cyclic AMP (**cAMP**).
- The cAMP binds and activates **Protein Kinase A**.
- This results in an increased heart rate and breakdown of glycogen to glucose to trigger the “**Fight and Flight**” response.
- Protein kinase A also activates **BARK** kinase.
- Activated BARK activates **β -arrestin** that inhibits **β 1-adrenergic membrane receptor**.

		2nd base in codon					
		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

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