

Virginia State Science Olympiad Regional Tournament

2013 – Division C Designer Genes

- You may write your name on this sheet before the event examination has begun.
- The reverse of this exam cover contains the Genetic Code; you are welcome to use your own copy.
- Two (2) non-programmable non-graphing calculators, one (1) standard-size (8.5"x11") double-sided sheet (*not two one-side sheets*) of paper containing any information, and writing implements may be used.
- Any other electronic devices are ***not*** allowed for this event; please consult the proctor about a safe location to store these devices for the duration of the event if you happen to carry one; *any team caught with an electronic device during the event will be immediately disqualified.*
- Tiebreakers will be tallied according to specified questions and will be used in the order from the front to back; tiebreakers will *only* be used to break ties and do not count towards your overall score.

Student Names: _____

School: _____

Team #: _____

	T			C			A			G			
T	TTT	Phe	F	TCT	Ser	S	TAT	Tyr	Y	TGT	Cys	C	T
	TTC			TCC			TAC			TGC			C
	TTA	Leu	L	TCA			TAA	ochre	TGA	Stop	amber	A	
C	TTG	Leu	L	TCG	Pro	P	TAG	opal	TGG	Trp	W	G	
	CTT			CCT			CAT	CGT	T				
	CTC			CCC			CAC	CGC	C				
A	CTA	Leu	L	CCA	Gln	Q	CAA	His	H	CGA	Arg	R	A
	CTG			CCG			CAG			CGG			G
	ATT			ACT			AAT			AGT			T
G	ATC	Ile	I	ACC	Thr	T	AAC	Asn	N	AGC	Ser	S	C
	ATA			ACA			AAA			AGA			A
	ATG	Met	M	ACG			AAG	Lys	L	AGG	Arg	R	G
G	GTT	Val	V	GCT	Ala	A	GAT	Asp	D	GGT	Gly	G	T
	GTC			GCC			GAC			GGC			C
	GTA			GCA			GAA	GAG	Glu	E			GGA
	GTG			GCG			GAG		GGG				G

PART I - TATA

As far as we are aware, all genes require promoters. In many eukaryotic genes, these promoters contain a *cis*-regulatory element called a TATA box. The transcription factor binds to this protein is called a TATA-binding protein (TBP).

1. TBP forms part of a complex protein complex generally called *Transcription Factor II D* (TFIID). What is the general purpose of these transcription factors in the first place? [1pt]
2. While TATA boxes are found in many eukaryotic genes, you seldom find evidence of TATA box schemes in isolated mRNA extracts. Why is this so? [2pt]
3. There are relatively few known TBP mutations in nature. Why is this so? [2pt]
4. The TATA box is called a “*cis*-regulatory element”, which the TBP is called a “*trans*-regulatory element”. What’s the difference between the two? [3pt]
5. The following is a fragment of the cDNA sequence of the DNA-binding domain (DBD) of the TBP gene sequence. Fill in the sequence data for the mRNA transcript, the tRNA anti-codon sequence, and the polypeptide primary sequence. [6pt]

cDNA sequence (5' -> 3')
 AGAATTGTTCTCCTT ATTTTTGTTTCTGGA AAAGTTGTATTAACA

mRNA sequence (5' -> 3')

tRNA anti-codon sequence

polypeptide primary sequence

6. Another known mutation of the TBP gene produces the following sequence over the same domain:

cDNA sequence (5' -> 3')
 AGATTTGTTCTCCTT ATTTTGGTTTCTGGA AAGACTGTAGTAACA

polypeptide primary sequence

Annotate the five (5) sequence changes above and write in the new polypeptide sequence. [5pt]

7. Most of the changes in question 6 are probably an example of which kind of mutation? [1pt]
- | | |
|-----------------------------|--------------------------|
| a. frameshift (in/del) | d. inversion |
| b. point substitution | e. double-stranded break |
| c. polynucleotide expansion | |
8. One of the changes indicated in question 6 does not alter the primary sequence of the polypeptide. Of what phenomenon is this an example? [1pt]
9. The formation of the preinitiation complex requires the recruitment of multiple transcription activating factors (TAFs), which help bind RNA polymerase to the transcription origin. Explain briefly how this process helps speed up the rate of transcription. [3pt]
10. The mutation indicated in question 6 alters the TBP structure sufficiently to enhance DNA binding. What effects on transcription might result from this change? [2pt]
11. Part of the preinitiation complex includes multiple domains that influence helicase and ATPase activity. Briefly explain the purpose of both: [4pt]

helicase -

ATPase -

PART II - Smorgasbord!

The following statements compare/contrast replication and transcription. Determine whether each statement is true or false, and briefly explain your choice if the statement is false. [2pt each]

12. RNA polymerase is required to form primers for replication and transcription.
13. Topoisomerase is only used to unkink DNA strands during replication, not during transcription.
14. Okazaki fragments are produced by the lagging strand during transcription.
15. DNA ligase is required to bind together the leading and lagging strand after replication is complete.
16. In replication, only DNA molecules are synthesized.
17. In transcription, only RNA molecules are synthesized.
18. Deoxyribonucleoside-triphosphates are used for replication, while ribonucleoside-triphosphates are used for transcription.
19. Termination of replication usually involves the formation of a poly-adenylated tail on the lagging strand.
20. Termination of transcription usually involves the formation of a poly-adenylated tail on the mRNA transcript.
21. Transcription of DNA is required for cells undergoing mitosis.
22. Replication of DNA is required for the proper expression of genes.

23. Match the following scientists to their contributions to the field of genetics [1pt each]:

Person		Contributions	
	Oswald Avery	A	Helped discover that DNA replication occurs via semi-conservative methods
	Erwin Chargaff	B	Developed one of the first successful methods for in vitro sequencing of DNA
	Francis Crick	C	One of the first researchers to suggest that DNA was the “transforming principle” after confirming the results of prior experiments
	Frederick Griffith	D	One of the first researchers to propose the “one gene, one protein (enzyme)” hypothesis
	Alfred Hershey	E	First to associate genes to chromosomes, and identified some of the first few mutations associated to chromosomal defects
	Gregor Mendel	F	Discovered that the proportion of adenine/thymine and cytosine/guanine residues are equal to one another, and that $A+G=C+T$
	Matthew Meselson	G	First to extract and characterize DNA
	Friedrich Miescher	H	Coined “Father of Genetics” - discovered that genes assort separately and independently, leading to the famous 3:1 ratio of observable traits in a monohybrid cross
	Thomas Morgan	I	Helped discover the structure of DNA
	Kary Mullis	J	Helped discover the genetic code using synthetic ribonucleotide sequences
	Marshall W. Nirenberg	K	In addition to discovering the structure of DNA, helped discover that DNA is transcribed via a three-nucleotide set called “codons”
	Frederick Sanger	L	Helped determine that DNA - as opposed to proteins - were the molecules responsible for heredity
	Edward Tatum	M	Discovered that bacteria can absorb a “transforming factor” that gave it characteristics of recently killed bacteria
	James Watson	N	Helped develop the process of in vitro replication and amplification of DNA sequences

PART III – Forensics!

You are working at a forensics lab, and unlike all of those people masquerading as “forensics experts”, you’re going to do all of your work in the lab. Your first case is to determine the paternity of an infant child. A multi-billionaire heiress has suddenly died, leaving her infant daughter in the capable hands of the woman’s husband (A). However, five other men have come forward claiming paternity of the child: a famous celebrity (B); a random paparazzo photographer (C); a foreign prince (D); a former bodyguard of the late heiress (E); and the butler (F).

You first decide to test the paternity of the child using the ABO blood test. You obtain the following results:

	Child	Mother	Person A	Person B	Person C	Person D	Person E	Person F
Bloodtype	O	O	A	A	O	B	AB	O

24. Explain briefly why it is *not* necessary to include the heiress’s blood type as a standard for comparison or a reference. [2pt]

25. Based on the data above, calculate the probability that Person A cannot be excluded as the actual father of the child; be sure you include *all* possibilities in your calculations. [4pt]

26. Based on the data above, who is the only person who can be *absolutely* excluded as a potential father? [1pt]

27. Based on the data above, which individuals *cannot* be absolutely excluded as a potential father? [2pt]

28. Explain briefly why using ABO markers is a very *bad* system for distinguishing individuals apart in forensic cases. [2pt]

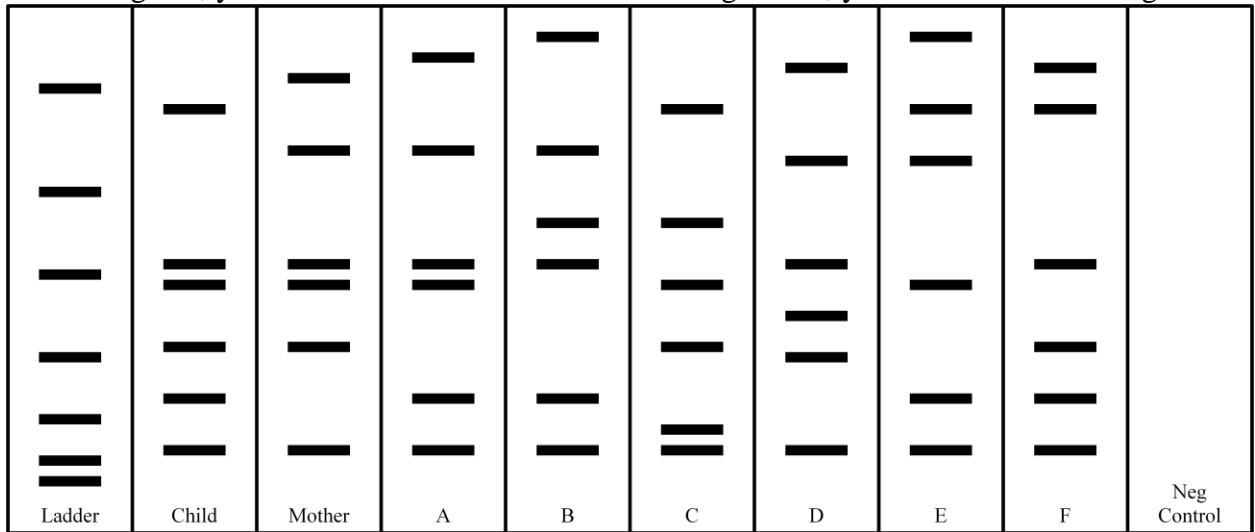
You decide replicate the previous experiment, including the Rh antigens this time. With this setup, you obtain the following data:

	Child	Mother	Person A	Person B	Person C	Person D	Person E	Person F
Bloodtype	O+	O-	A+	A-	O+	B+	AB+	O+

29. Assuming that Person D is heterozygous at the Rh locus (+/-), construct a Punnett square showing a cross between Person D and the heiress. What is the probability that the Person D could have fathered the child? [4pt]

30. Based on this information, which individuals can be *absolutely* excluded as possible fathers (including those you'd determined for question 26)? [2 pt]

31. At long last, you decide to run a RFLP screen. Using *EcoRI*, you obtain the following results:



On the results, circle the bands that are most informative for determining paternity [2pt].

TIE-BREAKERS

(1) What does RFLP mean?

(2) Determine the probable identity of the father.

PART IV – Super Mice!

In an attempt to create knock-out mice, you have accidentally created a strain of mice that actually knock-out researchers with super-mouse strength (which is sufficient to knock out people). After cordoning the area off, you start to use your knowledge of genetics to see if there is anything that you can do.

32. The genes that you have mutated appear to be responsible for a transcription factor. How can a mutation on this type of gene have such a drastic effect on the mouse phenotype and physiology? [3pt]
33. Fortunately, it only appears that a minority of your mutated mice carry the knock-out effect. Moreover, the knock-out phenotype is most prevalent in male mice, whereas female mice can *only* have the phenotype if the father exhibits the knock-out effect. Based on this limited information, which of the following best describes the inheritance pattern? [1pt]
- a. Autosomal dominant
 - b. Autosomal recessive
 - c. X-linked dominant
 - d. X-linked recessive
 - e. Y-linked
34. Assuming that one of the male knock-out mice escapes into the general mouse population and starts breeding, calculate the percentage of knock-out (phenotype) mice that would be created in the **first** generation. *Use a Punnett square to calculate your work.* [4pt]
35. Fortunately, all of the mice in your lab are labeled with a protein that causes the mice to glow in the dark. Unfortunately, the glowing and knock-out genes are *not* linked. Construct a Punnett Square in the space below to help calculate the probability a mouse with the knock-out allele (genotype) *also* carries the glow-in-the-dark allele, which is an autosomal dominant trait. [5pt]

36. In question 35, why is it important to distinguish whether the two genes are linked? [3pt]
37. Unfortunately, some of the mice have escaped – a total of 20 male and 20 female knock-out (phenotype) mice. Assuming that the normal mouse population contains 1000 male and 1000 female mice, calculate the *allele frequency* of the knock-out gene compared to the wildtype (non-mutated) gene. [4pt]
38. Fortunately, it appears that the knock-out mice selectively mate only with other knock-out mice. Why is this advantageous for you? [3pt]
39. It is discovered that the mutated gene in the knock-out mice no longer works, and by no longer working produces the phenotype. In an unusual “fix” to your genetics problem, you propose to “rescue” the mutant phenotype with a normal, functioning gene. Propose briefly how you intend to do this; include a brief discussion about the costs and benefits of the methodology that you specifically choose. [6pt]

TIE-BREAKERS

(1) In genetics parlance, explain what “knock-out” means.

(2) Explain briefly why “rescuing” the mutant phenotype in adult mice would be futile.

PART V – Create a gene!

You want to create a synthetic gene that produces a final pentapeptide product with the following sequence: Met-Ala-Pro-Lys-Glu. However, you want to be sure that the peptide product is produced only if enough lysine is present. Moreover, you want to ensure that the peptide product is synthesized only in specific cell lines. Using your knowledge of gene expression, your task is to design a simple template for creating your gene.

40. Using the space below, draw a simple sketch showing the relevant sequences of DNA (no actual DNA sequences – use boxes) for your gene. Be sure to include *cis*- and *trans*-regulatory elements and any necessary elements. Do *not* include any introns. [4pt]

41. Assuming that you've managed to sequence all of the relevant regulatory elements, your next task is to design the actual DNA sequence to code for the pentapeptide product. Be sure to include the untranslated regions (UTR), the coding sequence (CDS), and the complementary strand. [4pt]

42. Using the sequence above that you designed, transcribe the sequence into a polished mRNA transcript – do not forget the poly-A tail and the 5'-cap. Underline the coding sequence of your transcript as well. [4pt]

43. Assuming that you've managed to get everything all synthesized properly, you now have a functioning gene, but no way to verify that it works. Moreover, you don't have enough copies to work with, so you decide to clone your whole gene template into a plasmid. What is the main benefit of using plasmids for gene cloning? [2pt]

44. You choose a plasmid that was engineered to include the entire *lac* operon. What is the purpose of including the *lac* operon on this plasmid? [2pt]

45. The plasmid also contains a gene that confers to the host bacterium resistance to antibiotics. What is the purpose including this gene on the plasmid? [2pt]

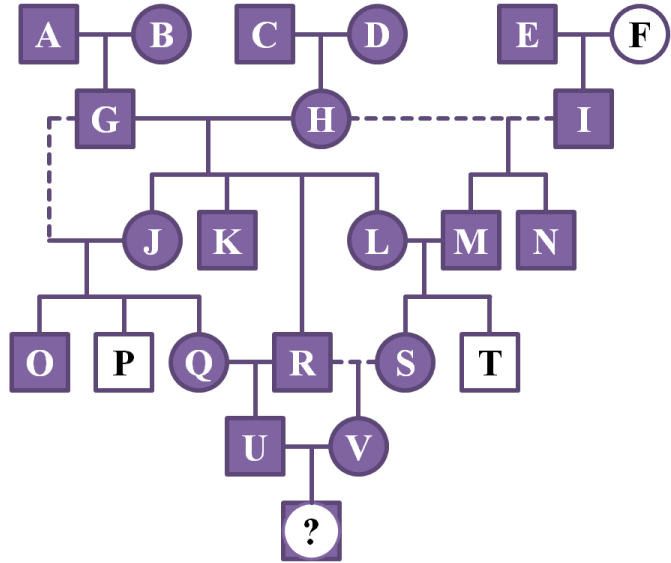
46. In the middle of the *lac-Z* gene is a restriction that is recognized by the *HinDIII* restriction endonuclease. When the restriction site is cleaved, *lac-Z* is broken. What is the net effect of this action? [2pt]
47. After digesting the plasmid lightly in *HinDIII*, you add in your own sequence and attempt to transform competent *E.coli* bacteria to clone the gene. It ends in failure. What was your mistake? [2pt]
48. Having fixed the mistake in the previous question, you successfully transform bacteria and try to streak plates coated in X-gal and ampicillin. What is the purpose of each substance? [4pt]
- X-gal:
- Ampicillin:
49. When you come back to select your colonies, you discover that most colonies are blue, while a few are white. What is the meaning of each colored colony and which one(s) do you want to select for further analysis? [3pt]
50. Having picked the right colony, you discover the gene that you have constructed was successfully cloned. The next big step is to transfect the synthetic gene into a new cell line and to integrate the gene into the genome. Without using a viral vector, identify three common methods of transfection to get your gene into the cells. [3pt]

TIE-BREAKER

You notice – belatedly – that your gene acquired a mutation, such that the 15th nucleotide in your coding sequence is now a “G”. How will this change affect your gene product?

PART VI – Smorgasbord Again!

Using the pedigree to the right, answer questions 51 to 55



51. Which of the following is not an inbred (intrafamilial) mating? [1pt]

- a. G+J
- b. I+H
- c. R+Q
- d. R+S
- e. U+V

52. Assuming that “shaded” boxes show a particular trait, while “non-shaded” boxes do not show the trait, which of the following explains the inheritance pattern?

- a. Autosomal dominant
- b. Autosomal recessive
- c. X-linked dominant
- d. X-linked recessive
- e. Y-linked

53. Given the information in the pedigree, which of the following best describes genotype of Individual I?

- a. homozygous dominant
- b. homozygous recessive
- c. codominant
- d. hemizygous recessive
- e. heterozygous

54. Which of the following statements are true?

- I.** The genotype of Individual G must be heterozygous.
- II.** The genotype of Individual H must be heterozygous.
- III.** The genotype of Individual M must be heterozygous.

- a. I only
- b. II only
- c. I & II
- d. I & III
- e. I, II, & III

55. Putative Individual “X” (I know I skipped “W”) is the resulting offspring of Individuals U and V. Calculate the probability that Individual X will be homozygous recessive for the trait indicated on the pedigree (*hint*: calculate each parent’s genotype probabilities first!) [6pt]

56. In a special culture of paramecia, “AA” individuals are red, “BB” individuals are blue, while “AB” individuals are purple. Which of the following best describes this system?
- a. complete dominance
 - b. incomplete dominance
 - c. codominance
 - d. epistasis
 - e. all of the above

57. In a strict cross between only red paramecia and only blue paramecia, what is the expected ratio of phenotypes?
- a. 50% red; 50% purple; 50% blue
 - b. 100% red; 0% purple; 0% blue
 - c. 0% red; 0% purple; 100% blue
 - d. 0% red; 100% purple; 0% blue
 - e. 25% red; 50% purple; 25% blue

58. You observe a population of paramecia and count the numbers of individuals as follows:

Red	Purple	Blue
2314	2246	7293

Calculate the proportion of “A” and “B” alleles in the population. [3pt]

59. Is this population currently in Hardy-Weinberg equilibrium? [2pt]

60. After counting all of the paramecia, you realize that you accidentally spilled a culture of another, more aggressive paramecia (which are not red, blue, or purple). You observe changes to your existing culture of paramecia after the first two generation of changes:

	Red	Purple	Blue
1 st	1157	1797	3647
2 nd	579	2396	1824

Calculate the proportion of “A” and “B” alleles in the population for each generation. [6pt]

61. It is evident after two generations that the purple paramecia have some sort of advantage that is not evident in either the red or blue paramecia. This is a very explicit example of what genetics phenomenon? [1pt]