Hands on Simulation of Mutation

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ABSTRACT

This exercise is a hands-on simulation of mutations and their effects on protein encoding genes. Students are provided with different protein encoding genes to randomly “mutate”. Each time, students note the results of the "mutation" on the amino acid sequence. This is repeated and frequency of synonymous to non-synonymous substitutions with random mutation is noted for the whole class. Hands-on nature of this simple simulation makes the concepts clear. This exercise leads to a discussion of the effects of the random mutations to individuals with that mutation, and thus the actual frequency of synonymous to non-synonymous substitutions observed in genes.

Introduction

The purpose of this hands-on exercise is for students to see for themselves the effects of random mutations on protein encoding genes. I designed this exercise because students often don't seem to "get" the effects of mutation on genes when just described in lecture. Even students in upper division courses don't comprehend it, or have just memorized that extant genes typically have higher synonymous vs non-synonymous substitutions without realizing that that is the result of selection. Sophisticated computer simulations are not as helpful because students consider the computer as a "black box". This simple simulation illustrates the random nature of the mutation and their effects. If given a large enough sample size, this exercise results in the expected synonymous to non-synonymous substitution for random mutations found by using sophisticated computer simulations.

Student Outline

Directions given to students

This game allows you to see what happens when random mutations occur. Each team of 2 students will get a DNA sequence of the first 30 nucleotides of different human genes.

1) First transcribe the DNA sequence into the mRNA sequence.
2) Use the genetic code provided below to write down the amino acid sequence that these 30 nucleotides encode beginning with the first nucleotide.
3) Now randomly choose the DNA nucleotide to "mutate" by using the birth day of a student in the class as the position along the gene to mutate.
4) Then "roll" the tetrahedron "mutator" dice. Note the letter on the side that is flat on the table. That is the nucleotide that will replace the nucleotide in the DNA at the position decided in the previous step. Write the nucleotide in the row for Mutation 1
    a. If it is the same nucleotide, write **same nucleotide** under the Results column
    b. If it is a different nucleotide, look up what is encoded by the new triplet code.
    c. If it is the same amino acid, write **same amino acid** under the Results column
    d. If it is a different amino acid write the new amino acid in the row for amino acid and write **different amino acid** under the Results column.
    e. If the new triplet code encodes a stop codon, write **STOP** in the row for amino acid and write **stop** under the Results column.
    f. If the new triplet code changes the start codon at the beginning of the sequence into any other amino acid or STOP codon, write the amino acid or STOP in the row for amino acid and write **stop** under the Results column, since no protein will be made.
5) Now, repeat from step 3, only this time use the birth day of another student. Be sure to mutate the original DNA sequence since mutations occur in the DNA.
6) Depending upon how long it takes, we will do 10-20 "mutations" and tally the results for the whole class.

UUU - Phe, **F**	UCU - Ser, **S**	UAU - Tyr, **Y**	UGU - Cys, **C**
UUC - Phe, **F**	UCC - Ser, **S**	UAC - Tyr, **Y**	UGC - Cys, **C**

UUA - Leu, **L** UUG - Leu, **L**	UCA - Ser, **S** UCG - Ser, **S**	UAA - **Stop** UAG - **Stop**	UGA - **Stop** UGG - Trp, **W**
CUU - Leu, **L** CUC - Leu, **L** CUA - Leu, **L** CUG - Leu, **L**	CCU - Pro, **P** CCC - Pro, **P** CCA - Pro, **P** CCG - Pro, **P**	CAU - His, **H** CAC - His, **H** CAA - Gln, **Q** CAG - Gln, **Q**	CGU - Arg, **R** CGC - Arg, **R** CGA - Arg, **R** CGG - Arg, **R**
AUU - Ile, **I** AUC - Ile, **I** AUA - Ile, **I** AUG - Met, **M**, **Start**	ACU - Thr, **T** ACC - Thr, **T** ACA - Thr, **T** ACG - Thr, **T**	AAU - Asn, **N** AAC - Asn, **N** AAA - Lys, **K** AAG - Lys, **K**	AGU - Ser, **S** AGC - Ser, **S** AGA - Arg, **R** AGG - Arg, **R**
GUU - Val, **V** GUC - Val, **V** GUA - Val, **V** GUG - Val, **V**	GCU - Ala, **A** GCC - Ala, **A** GCA - Ala, **A** GCG - Ala, **A**	GAU - Asp, **D** GAC - Asp, **D** GAA - Glu, **E** GAG - Glu, **E**	GGU - Gly, **G** GGC - Gly, **G** GGA - Gly, **G** GGG - Gly, **G**

Sample worksheet

Human Phenylalanine Hydroxylate (gene defective in PKU)

Gene	tac agg tga cgc cag gac ctt ttg ggt ccg	
mRNA		
Translation		result
Mutation 1 Amino acid		
Mutation 2 Amino acid		
Mutation 3 Amino acid		
Mutation 4 Amino acid		
Mutation 5 Amino acid		

Mutation 6 Amino acid		
Mutation 7 Amino acid		
Mutation 8 Amino acid		
Mutation 9 Amino acid		
Mutation 10 Amino acid		
Mutation 11 Amino acid		
Mutation 12 Amino acid		
Mutation 13 Amino acid		
Mutation 14 Amino acid		
Mutation 15 Amino acid		
Mutation 16 Amino acid		
Mutation 17 Amino acid		

Total: no nucleotide change, that is no mutation = _____

No amino acid change or synonymous substitution= _____

Change in amino acid or non-synonymous substitution= _____

Stop = .

Instructor's Notes

General notes

Prior to this exercise, students should have learned about transcription and translation so that they can do the first two steps. Alternatively instruction on how genes code for proteins can be introduced at the beginning of the exercise if it is part of a 3 hour lab session.

This simulation provides a good foundation to discuss mutations as truly random events. The choice of birth days and rolling the tetrahedron dice clearly demonstrates that this simulates random mutations at random locations. Given a reasonable number of trials/student pair (10-20) and a reasonable number of students in the class, at least 20, this simulation will show a significantly higher number of non-synonymous substitutions over synonymous substitutions.

Students of wide ranging backgrounds can learn from this simulation. For beginning students or non-majors, one can discuss the consequences of different type of mutations. What would happen if an amino acid turned into a STOP? What might happen if a crucial amino acid in a gene is altered? The genes chosen include those involved in human genetic diseases so that can be incorporated into this discussion. The beginning students can be guided to recognize that mutations that result in STOP codons and some non-synonymous substitutions will be deleterious and be selected against. For more advanced students this exercise may be followed by comparison of homologous genes, or alleles of a gene in a population using using available programs such as SNAP (http://www.hiv.lanl.gov/content/hiv-db/SNAP/WEBSNAP/SNAP.html). Such comparisons typically show a higher synonymous to non-synonymous substitution ratio, due to selection, despite the fact that random mutations (as seen in the game) produces lower synonymous to non-synonymous substitution ratio. It can be pointed out that one evidence of recent and strong selection for a favorable mutation, known as selective sweep, is an unexpectedly lower synonymous to non-synonymous substitution ratio.

Additional sample genes

Following are some additional human genes that can be used in the exercise. Of course, any protein-encoding genes can be used. More information on the genetic diseases may be found on Online Mendelian Inheritance in Man at http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM

Human β-hemoglobin gene – mutated in sickle cell anemia

tac cac gtg gac tga gga cac ctc ttc aga..

Human fibrillin gene – mutated in Marfan Syndrome

tac gca gct ccc gca gac gac ctc tag cgg..

Human lamin gene – mutated in progeria

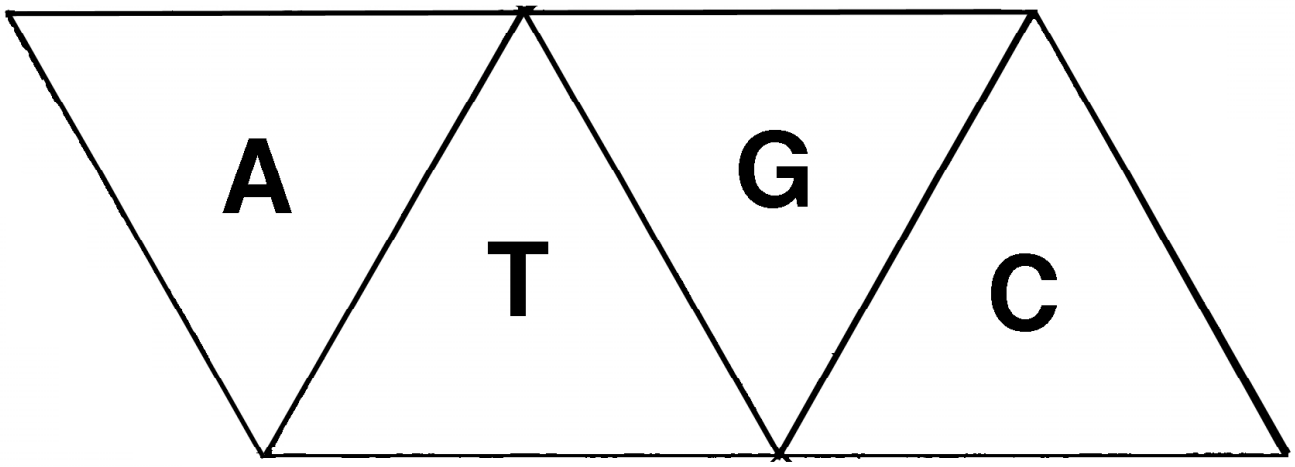
tac ctc tgg ggc agg gtc gcc gcg cgg tgg..

Human galactose metabolizing gene – mutated in galastosemia

tac agc gcg tca cct tgg cta gga gtc gtt..

Tetrahedron mutator

Here is a sample sheet for making the "mutator" tetrahedron. It is best made with a heavy paper. Cut around the outer edge, score on the inner 3 lines and fold. Tape together,



About the Author

Charlotte Omoto is a Professor and Associate Director of Undergraduate Studies in the School of Biological Sciences at Washington State University where she has been since 1984. She received her B.S. in Biology from the University of Washington, Seattle and her Ph.D. from University of Wisconsin, Madison. She has done post-doctoral work at Princeton, Caltech and Penn. State University. Her research interest focuses on gregarines, protozoan parasite of invertebrates. She teaches introductory biology course for majors and a genetics course for non-science majors.