

Telephone DNA mutation activity description (Worksheet below)

Remind students of the structure of virus: nucleic acid, protein coat, envelope. This article and activity focus on the nucleic acid portion of the virus.

Read: Gire et al, 2014. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science, 345:1369-1372. DOI: 10.1126/science.1259657.

<http://www.sciencemag.org/content/345/6202/1369.full>

Abstract:

In its largest outbreak, Ebola virus disease is spreading through Guinea, Liberia, Sierra Leone, and Nigeria. We sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone to ~2000x coverage. We observed a rapid accumulation of interhost and intrahost genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African lineages around 2004, crossed from Guinea to Sierra Leone in May 2014, and has exhibited sustained human-to-human transmission subsequently, with no evidence of additional zoonotic sources. Because many of the mutations alter protein sequences and other biologically meaningful targets, they should be monitored for impact on diagnostics, vaccines, and therapies critical to outbreak response.

Discussion points

1. Central Dogma for Obligate Intracellular Parasites: DNA->RNA->Protein. Where does viral replication/transcription/translation take place?
2. Viral evolution
3. Point mutations: Nonsense/missense/silent mutations
4. Co-evolution of reservoir vs. humans: Why is it advantageous for a virus and reservoir to co-evolve? What is the

Learning objectives:

- Spillover
- Reservoir coevolution.
- Understand random mutations, mutation rate

The Activity: A random sequence of letters and numbers is transmitted from host to host, first through a non-human reservoir species and then, as a result of a spillover event, through a human host.

Mode of transmission: whispering of the sequence into the ear of the next host in the chain.

R₀ for this disease: 1

Events:

Transmission: Whispering of sequence into the ear of the next organism to be infected

Replication: writing the sequence down on a piece of paper

Gene sequencing: each student will have the sequence written down

The starting sequence: 7 P K 7 V 3 T G H 5 6

Rationale for letter/number choices: Chose numbers and letters that sound the same to increase potential for mutations.

Divide the class into 2 groups about evenly:

Group 1: Reservoir species

Group 2: Human spillover

The reservoir species and agent have co-evolved over millennia so that the agent does not cause major disease, and the agent is very efficiently transmitted from reservoir to reservoir. The transmission of the agent among the reservoir species is associated with a relatively low level of genetic mutation.

The agent-human relationship has not experienced any co-evolution, so the agent causes significant disease to the human. In addition, the human body environment is not as well-suited to the maintenance of the agent, and more genetic mutations are noted in human-to-human transmission.

Reservoir transmission:

1. Student 1 (who is transmitting the agent) whispers the sequence into the ear of the next student in line, Student 2. Just as in the game telephone, the sequence can only be whispered once.
 2. Student 2 listens to the entire sequence and then writes it down on a piece of paper.
 3. Student 2 then reads the sequence into the ear of the next student in line to transmit the agent..
- All reservoir transmissions will proceed by the same pattern, where the transmitting student whispers the whole sequence to the next student in line, and the recipient of the agent waits to hear the entire sequence before writing it down to prepare for transmission to the next student in line.

Human transmission:

Because the human and agent have not coevolved, transmission is not as efficient and mutations are much more likely to occur. To simulate the fact that the relationship between the agent and the human host isn't 100% optimal, the students will sing a nursery rhyme in between the transmission event (hearing the sequence) and the replication event (writing the sequence down).

All human transmissions will proceed by the same pattern, where the transmitting student whispers the whole sequence to the next student in line. The recipient then sings "Mary had a little lamb" or "Twinkle twinkle little star" (or some familiar short song), then writes down the sequence, and prepares to transmit it to the next student in line. I also tell loud, involved stories about historical disease outbreaks or the rabies vaccine over the human transmission chain in humans as "environmental noise."

Spillover:

The spillover event is a normal human-type transmission event, only the first student transmitting the agent is from the reservoir group and the recipient of the agent is in the human group.

Quantification:

After all transmission is completed, students compare their sequence to neighbors and count the number of mutations for each transmission event. For instance, if the transmitting student had the sequence 7 P K 7 V 3 **T G H** 5 6 and the receiving student had 7 P K 7 V 3 **3 G 8** 5 6, that is 2 mutations for that transmission event. Find average mutation rate for reservoir transmissions versus human transmission. The expected result is that the mutation rate in the reservoir species will be lower than that in the human species due to the lower "co-evolution" (i.e. less efficient/more distraction) for human transmission.

Discussion:

1. What are the differences between transmission in reservoir species and human species in this activity?
2. How might these differences reflect co-evolution?
3. What are some of the potential "molecular distractions" that enhance mutation rate?
4. What are the potential outcomes of a mutation event?

Viral Mutation Telephone Analysis - Lab 02

Analysis questions (in pairs) due Wednesday 04 Feb (20 points)

Transmission Instructions:

All hosts: DO NOT WRITE YOUR SEQUENCE DOWN AS YOU HEAR IT.

Reservoir hosts: Listen to the whole sequence and then write your sequence below.

Human hosts: Listen to the whole sequence, then sing ALL OF "Mary Had a Little Lamb", THEN write your sequence below.

Individual transmission data

Are you a reservoir or human host?

Your order in the transmission cycle:

Your viral sequence:

Data to collect after transmission is completed through all hosts

Input your viral sequence (in order) to the google spreadsheet linked on Moodle.

mutations in your sequence compared to the previous host:

mutations in the next host's sequence compared to yours:

Determine the average mutation rate (# of mutations/transmission) for all reservoir-to-reservoir transmissions.

Determine the average mutation rate for all human-to-human transmissions.

Analysis questions - To be completed in pairs and submitted via Moodle - Due 04 Feb.

1. Which part of viral replication, nucleic acid synthesis or protein synthesis, is more sensitive to mutation, and why?
2. How are non-synonymous mutations different than synonymous mutations in terms of virulence?
3. What are the contributing factors to any similarities or differences in the mutation rate in the reservoirs versus the humans? Comment on the factors specific to our simulation and how these factors are similar to or different from factors that might impact mutation rates in nature.
4. Looking over all the sequences, identify the major types of sequence mutations. Create a data chart to quantify each type of mutation. Include your chart in your results section. **Include this data chart with your file submission.**
5. Based on the types of mutations that you identified in question 4, which types of mutations do you predict would be non-synonymous?