# Murder by HIV? Undergraduate Edition

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Naowarat (Ann) Cheeptham, Department of Biological Sciences, Thompson Rivers University Laura B. Regassa, Department of Biology, Georgia Southern University Michèle I. Shuster, Department of Biology, New Mexico State University

## Part I – An III-Fated Argument

In July of 1994, a nurse broke off her relationship with her married boyfriend (a doctor). On August 4, 1994, her ex-boyfriend showed up at her residence and administered a shot that he claimed was a vitamin B-12 injection. He had given her vitamin B-12 injections in the past, but this one was very painful. Prior to that time, the nurse had had several HIV tests (each time she gave blood, and one after having the saliva of an infected patient splash on her skin) and she had always tested negative. Her most recent blood donation was in April of 1994, and her blood tested negative for HIV at that time. In January of 1995, however, she tested positive for HIV. At that time, she accused her ex-boyfriend of deliberately infecting her during the argument back in August. He was brought to trial on charges of attempted second-degree murder.

#### Questions

1. You can imagine that the defense team posed alternative means by which the woman could have become infected. What are some other possibilities? List them below.

2. What kinds of tests or information could be used to rule out these alternative hypotheses for her infection with HIV?

## Part II – Circumstantial Evidence

Other possible sources of the infection included the woman's prior sexual contacts and occupational exposure, given that she was a nurse.

All seven of the men that she had been in sexual contact with (including her former boyfriend) were tested and found to be HIV-negative.

Her employment records were examined, and there were no reports of any accidental or occupational exposures other than the saliva that was splashed on her skin sometime in the mid-1980s. Her file did not have any documentation of any needle sticks at work.

As the investigation proceeded, it was found that an HIV-positive patient under the care of the ex-boyfriend/doctor had blood drawn at the physician's offices on August 4, 1994. The paperwork for this procedure was deliberately hidden (the log book was found in a box of "1982 records" in a storage room with other records from the 1980s) and was not filled out in a manner that was consistent with standard office practices.

Based on the circumstantial case against the physician, the reverse transcriptase (RT) sequences from the victim (the nurse/ex-girlfriend) and from the physician's HIV-positive patient (the putative source of the nurse's infection via the injection administered during the ill-fated argument) were analyzed. The reverse transcriptase (pol) gene was used in the actual criminal trial. Reverse transcriptase has a unique set of selective pressures exerted on it and is therefore a useful gene/protein to explore in order to establish relationships between different strains of HIV.

#### Question

1. HIV-1 mutates rapidly. Based on this, would you expect to find a single, identical sequence in the victim and the patient, or would you expect to find a set of related sequences that share a common ancestor? Explain your answer.

### Part III – Comparing Sequences

You will be examining the relationship between patient and victim sequences from the actual criminal trial. Here's how:

1. Go to the NCBI homepage (http://www.ncbi.nlm.nih.gov/). On the right, you will see the link "Nucleotide" for searching for nucleotide sequences. Click on this link (see red arrow in screen capture below).

SNCBI Resources 🗹 How 1	ro 🗵			Sign in to NCBI
SNCBI National Center for Biotechnology Information	atabases 🔻			Search
NCBI Home	Welcome to NCBI			Popular Resources
Resource List (A-Z)	The National Center for Biotechnolo	ogy Information advances science a	nd health by providing access to	PubMed
All Resources	biomedical and genomic informatio	n.		Bookshelf
Chemicals & Bioassays	About the NCBI   Mission   Organ	ization   NCBI News & Blog		PubMed Central
Data & Software				BLAST
DNA & RNA	Submit	Download	Learn	Nucleotide
Domains & Structures	Deposit data or manuscripts	Transfer NCBI data to your	Find help documents, attend a	Genome
Genes & Expression	into NCBI databases	computer	class or watch a tutorial	SNP
Genetics & Medicine		-	1.	Gene
Genomes & Maps				Protein
Homology				PubChem
Literature				
Proteins				NCBI News & Blog

2. You are now prepared to search for a sequence from the actual trial. In order to find sequences from the trial, enter "AY156803" into the search box at the top of the page. This is the ID number for a patient ("P") sequence from the trial. When you click on the "Search" button, you will find this particular sequence from the trial.

S NCBI Resources 🗹 How To 🗹		Sign in to NCBI
Nucleotide Nucleotide		Search
Advanced		Help
ACCCACACACACATT	Nucleotide	
TGTAGCTTACCTCCTC	The Nucleotide database is a collection of sequences from s PDB. Genome, gene and transcript sequence data provide t	everal sources, including GenBank, RefSeq, TPA and he foundation for biomedical research and discovery.
Using Nucleotide	Nucleotide Tools	Other Resources
Quick Start Guide	Submit to GenBank	GenBank Home
FAQ	LinkOut	RefSeq Home
Help	<u>E-Utilities</u>	Gene Home
GenBank FTP	BLAST	SRA Home
RefSeq FTP	Batch Entrez	INSDC

3. Once the search result loads, click on FASTA towards the top (see below). This will open up a new window with that Patient HIV sequence in a format (FASTA) suitable for alignment and tree building in Bioinformatics programs. Copy and paste that sequence (starting with the first nucleotide) into a text file.

	Nucleotide V Advanced	Search	
GenBank <del>-</del>	Send to: ◄	Change region shown	
HIV-1 cl	one V2.MIC.RT from USA reverse transcriptase (pol) gene, partial cds	Customize view	
GenBank: A	156807.1	Customize view	
FASTA Gra	phics PopSet	Analyze this sequence	
<u>Go to:</u> ♥		Run BLAST	
LOCUS	AY156807 805 bp DNA linear VRL 22-OCT-2002	Pick Primers	
D D D D D D D D D D D D D D D D D D D	HIV-I CLONE V2.MIC.KT from USA reverse transcriptase (pol) gene, partial cds.	Highlight Sequence Features	
DEFINITION			
DEFINITION ACCESSION VERSION	AY156807 AY156807.1	Find in this Sequence	

- 4. Now that you have the nucleotide sequence from one HIV strain from this trial, you are going to use that sequence to find related sequences in the database. You will use a program called "BLAST" to do this (BLAST makes sequence comparisons, and finds closely related sequences in the database).
- 5. Navigate to BLAST (back on the NCBI homepage, on the right column, above the first link you clicked—see figure for step 1 above). You will then come to a page that looks like the figure below. Click on Nucleotide BLAST (as you want to compare nucleotide sequences).

BLAST <sup>®</sup>	Home Recent Results Saved Strategies Help
Basic Local Alignment Search Tool BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.	Search Betacoronavirus Database         We have created a new BLAST database focused on the SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) Sequences. For further detail please visit         NCBI GenBank.         Mon, 03 Feb 2020 10:00:00 EST
Web BLAST	stx totide ▶ protein astn slated nucleotide

6. When you get to the nucleotide blast page, paste your FASTA sequence (from your text file) into the top box (Enter Query Sequence).

BLAST <sup>®</sup> Home Rece	Basic Local Alignment Search Tool nt Results Saved Strategies Help	
NCBI/ BLAST/ blastn	suite	Standard Nucleotide BLAST
blastn <u>blastp</u> b	alastx tblastn tblastx	
Enter Query S	Sequence	BLASTN programs search nucleotide databases using a nucleot
Enter accession n	umber(s), gi(s), or FASTA sequence(s) 😡 <u>Clear</u> Query s	ubrange 😡
Paste	Your Sequence Here	
Or, upload file Job Title	Browse	
	Enter a descriptive title for your BLAST search 😡	
Align two or m	ore sequences 🥹	
Choose Searc	ch Set	
Database	Human genomic + transcript OMouse genomic + transcript Others (nr Nucleotide collection (nr/nt)	etc.):

7. On that same screen, under "Choose Search Set," select "Standard databases (nr etc.)" for the database and "nucleotide collection (nr/nt)" from the database dropdown menu.

Choose Sear	ab Sat
Choose Searc	IT SEL
Database	Standard databases (nr etc.):      RNA/ITS databases      Genomic + transcript databases      Betacoronavirus     Nucleotide collection (nr/nt)
Organism Optional	Enter organism name or idcompletions will be suggested
	Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown 🥹
Exclude Optional	□ Models (XM/XP) □ Uncultured/environmental sample sequences
Limit to	Sequences from type material
Entrez Query Optional	You Tube Create custom database
	Enter an Entrez query to limit search 😡

8. Under Program Selection (towards the bottom of the same screen), select the button to optimize for somewhat similar sequences (blastn):



9. A little further below, click on the BLAST button.



- 10. After a few moments, you will get a list of "hits" that have nucleotide similarities to your Query sequence (from the patient). Not surprisingly, the top hits are patient and victim sequences from this case. You now need to select several sequences to compare and build a phylogenetic tree. Scroll down to the "Alignments" section of this page. This is where individual sequences have been aligned with the patient sequence that you entered. Select several victim and patient sequences by clicking in their check boxes. You should include between five and eight sequences, including both patient and victim sequences. Also select some sequences that are from other sources (e.g., USA sequences or sequences from other countries).
- 11. Once you have checked the sequences you want to compare, click on "Distance tree of results":



This will build a phylogenetic tree of the sequences that you have selected.

12. The phylogenetic tree will open in a new window. The sequences will have the same labels as they did when you checked them from the Alignments listing in step 10. Remember that sequences from our patient and victim are labeled with a P and V, respectively.

#### Questions

1. Describe the tree (in general terms). On the next page, draw a quick sketch of the tree.

2. Does there appear to be a relationship between the patient and victim sequences? Do they appear to diverge from a common ancestor?

3. What conclusion can you draw from this tree?

4. Given the circumstantial evidence and the phylogenetic evidence, what do you think the verdict was in this case?

#### Reference

Metzker, M.L., et al. 2002. Molecular evidence of HIV-1 transmission in a criminal case. PNAS 99 (22): 14292–14297.

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