

### LABORATORY EXERCISE: CLADISTICS II

In December 1987, a young Florida woman named Kimberly Bergalis had her two upper wisdom teeth extracted by a dentist named Dr. David J. Acer. A few months later, she began showing signs of infection, and in December 1989 was diagnosed with AIDS. Dr. Acer was openly gay, and had been diagnosed with AIDS three months before he performed the tooth extraction on Bergalis.

Bergalis alleged that she had been infected by Acer during her dental procedure; she had no other risk factors for HIV infection, such as IV drug use, blood transfusions, tattoos, or sexual activity. She died in December 1991. Acer himself closed his practice in July 1989 and died in September 1990. The case of the “Florida AIDS dentist” drew national attention; as she was dying, Bergalis spoke to Congress in support of a law (which didn’t pass) enforcing stricter controls on HIV+ health care workers. Eight other patients of Dr. Acer also became infected with HIV, and claimed that they had been infected by Acer. Suspicion still lingers that Dr. Acer *might* have infected his patients deliberately, possibly as a way of drawing attention to what at the time was marginalized as a “gay disease”.

But the case turned out not to be nearly that simple:

- Bergalis’s case is still the only incidence ever reported of a health care worker allegedly transmitting HIV to patients through routine procedures. Other dentists and doctors have practiced long after being diagnosed HIV+, without infecting their patients.

- A devout Catholic, Bergalis (Patient A) publically claimed to be a virgin, but a physical examination revealed that she was not—she had human papilloma virus, the causative agent of genital warts and a common sexually transmitted disease. She had admitted earlier to having had sex with two different men.

- Some of the dentist’s patients had other risk factors for HIV infection. In some cases the patients didn’t admit these to investigators. Patient G, for example, admitted to only two female sex partners since 1986, but other witnesses claimed that he frequented crack houses and had sex with multiple prostitutes. Patient C, a man, claimed to have never had homosexual contact, but investigators later tracked down both multiple female sex partners and a male sex partner. Patient B had had major surgery; it’s not certain whether she had received blood transfusions or not.

- All of Acer’s patients, except for Bergalis herself, knew about the accusations against Acer. They may have chosen to hide information that would suggest other means of HIV transmission.

## MATERIALS:

1. A very short snippet of the V3 coding region of the HIV virus (positions 226-242 in our datafile) looks like this:

Dentist	TACTTGGATGGCTAAT
PatientA	TATTTGGATAGCTAAT
PatientC	TACTTGGGTGGCTAAT
PatientF	TACTTGGGAATGATACT
LocalControl3	TACCTGGAATGATACT
LocalControl4	TACCTGGAAGTTTGAT
LocalControl5	TACTTGGGGGTTTAAT

Just for fun, take a moment to try to draw up a cladogram based on these sixteen nucleotide positions. This is going to be a bit of a pain in the sit-upon; you just look for nucleotide matches between sequences and try to group those with the most unique matches (synapomorphies). Some of the nucleotides won't be helpful; for example, the TA at the beginning of all seven, and the T at the end of all seven, won't be useful in figuring out which sequence is related to which. It may help to use a highlighter marker to highlight nucleotide matches.

2. There are two full data sets that you'll need to download; one is **HIV.nexus** (based on the V3 region of the HIV genome) and the other is **HIV2.nexus** (based on the V4CV5 region of the genome). Each one of these contains sequences from Dr. Acer, his patients, and "controls"—i.e., HIV-positive people in the area who had no known link to Dr. Acer or any of his patients. HIV.nexus has sequences from seven patients, lettered A through G; HIV2.nexus contains sequences from these seven plus one more (A through H), as well as many more controls.

3. There are many computer programs that can do various kinds of analyses of sequence data to try to reconstruct the evolutionary tree. We don't need to get into all of them. Fortunately, free Web-based programs are freely available. So go to the website <http://www.phylogeny.fr/> and select the option "One Click". Upload the file **HIV.nexus** and click **Submit**. You may opt to have the results e-mailed to you, in which case you'll enter your e-mail address in the appropriate window.

The programs will align the sequences, calculate the tree, and draw it. (Technically, the tree is drawn by means of an algorithm called *maximum likelihood*, which is a little different from standard cladistics. Maximum likelihood methods can only be used with molecular sequence data; we don't need to get into them further here.) Record the tree—scroll down and you should see where you can click to save the tree as a PNG graphics file or a PDF document, among others; these are easy to print. Include it in your writeup.

4. Repeat step 3 with the file **HIV2.nexus**.

5. In your report, include a brief introduction, a description of your methods, the trees you got, and—most importantly—a full discussion of what they mean. Are they both “telling the same story”, or do they radically differ? Which patients, if any, are likely to have contracted HIV from Dr. Acer? Which, if any, probably did not get HIV from Dr. Acer?

