ABSTRACT

TITLE:	Does Pseudoknot Formation Play a Role in HTLV-1 Pro-Pol Frameshift Efficiency?
AUTHOR(S):	GreyEyes, Shawn; Davis, Summer; Stelmaszek, Jordan; Knewitz, Allison; Mouzakis, Kathryn
PRESENTED AT:	2014 Undergraduate Research and Creative Activities Symposium, Fort Lewis College

ABSTRACT: The translation of the Human T-cell Lymphoma Virus Type-I (HTLV-1) pol gene is dependent on a programmed -1 ribosomal frameshift. The HTLV-1 frameshift site consists of a heptanucleotide slippery sequence (UUUAAAC) followed by a spacer region and a downstream pseudoknot. During translation, the pseudoknot is thought to pause the ribosome over the slippery sequence. At that time, the tRNAs can repair in the -1 reading frame. This study focuses on investigating whether or not pseudoknot formation affects the rate of HTLV-1 pro-pol frameshifting. We hypothesize that the pseudoknot structure will affect the frameshift efficiency. To test this hypothesis, three variant pro-pol frameshift sites were designed so their frameshift efficiencies could be compared to that of the wild-type frameshift site. Pseudoknot mutant 1 (PKM1) has sequence changes in the loop that eliminate base-pairing between the loop region and downstream RNA sequence. Pseudoknot mutant 2 (PKM2) has sequence changes in the downstream sequence that eliminate base-pairing between the loop region and downstream RNA sequence. Finally, the downstream sequence involved in pseudoknot base-pairing was eliminated in the third frameshift site, creating the stem-loop (SL) structure. Using molecular cloning techniques, these variant frameshift sites were inserted into a dual-luciferase reporter plasmid between the rluc and fluc genes. Two plasmids (experimental and control) were created for each frameshift site (WT, PKM1, PKM2, and SL). In the experimental plasmid, the fluc open reading frame is in the -1 reading frame relative to rluc. In the control plasmid, the slippery sequence is mutated so frameshifting cannot occur and rluc and fluc are in the same reading frame. At present, seven of the eight plasmids have been successfully cloned, each verified by DNA sequencing. Once all of the plasmids have been successfully cloned, in vitro transcription of the DNA will be used for RNA synthesis. This RNA will be purified and utilized in an in vitro frameshift assay to determine the frameshift efficiency for each frameshift site.