Challenge 2 – Retron design automation

For gene editing in bacteria

Background: In lecture, we talked about how gene editing in bacteria can be performed using lambda recombineering. In this recombineering strategy, lambda phage proteins are expressed in a host that is then electroporated with ssDNA oligos. This method is great, but faces one limitation in that editing is not 100% efficient. Seth Shipman's group (UCSF) has been developing a newer strategy that utilizes retrons to continuously express a ssDNA inside a host. By placing retrons on a plasmid, they become heritable and therefore you can guarantee 100% genome editing after propagating a strain enough times.

Goal: I want to use retrons, but their design seems complicated. Make me a code that will automate the retron design for me.

- **Inputs:**
  - Host organism genome sequence
  - Sequence you want to end up with in a certain gene:
    - Easiest case - Can be a nucleotide sequence that contains the desired change (insertion, deletion, substitution)
- **Output:** Design of the msr/msd sequence that should be in my plasmid in order to do retrongineering.
  - This sequence will necessarily contain constant regions AND the region that will be variable.
  - Output can be in a FASTA format, or other DNA-sequence format.

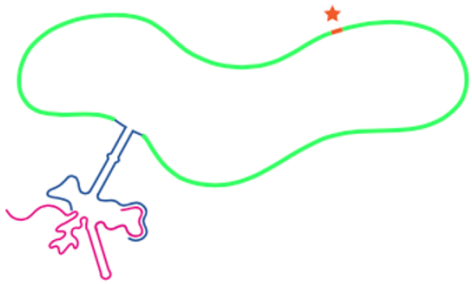

Figure 1: *Desired outcome of the automated retron design. Blue/pink regions will be constant. Green region is the homology regions, while red region is the desired edit. Users will be inputing a genome and a sequence that can be longer than the necessary green.*

Hint*: See lecture Module 2-1 slides 64-69*

Hint: *Input sequence length should be arbitrary and therefore not necessarily the correct length for putting into a retron. You will want to think about automatically trimming input sequences after you determine the homology regions.*

Hint: *You can download genome sequence from NCBI. Various formats to handle these genomes. (https://www.ncbi.nlm.nih.gov/nuccore/NC_000913.3)*

Hint: *You will need to read SI material of the retron paper in order to determine what regions are constant, and what regions are variable.*