

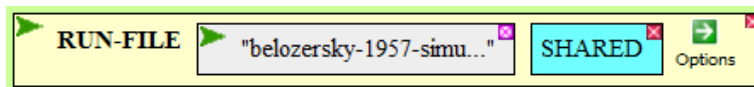
Molecular Biology Through Discovery

Problem Set 7: RNA

1. One of the most influential articles in the history of molecular biology was Belozersky and Spirin (1958)¹ even though the authors had no explanation for the remarkable observations they reported. Get the article and consider Tables 1 and 2 to see if you can do any better with the perspective of your modern understanding of molecular biology.
 - 1a. The two tables are show the results of two similar experiments repeated with a variety of different bacteria. First the authors isolated DNA from cultures of each bacterium, completely hydrolyzed it, and measured the relative amounts of each of the for deoxyribonucleotides (you'll recognize this type of experiment from the work of Erwin Chargaff). That produced the results shown in Table 1.
 - i. Do all bacteria have more or less the same deoxyribonucleotide base composition?
 - ii. Is there any general pattern that you can see in all DNA base compositions?
 - iii. Given your knowledge of DNA structure, what values would you expect for Pu/Py, (G+T)/(A+C), and (G+C)/(A+T)?
 - 1b. Table 2 was produced by a very similar experiment, isolating RNA and measuring relative amounts of ribonucleotides.
 - i. Would you expect the base compositions of RNA to be about the same as the base compositions of DNA for a given organism? Why or why not?
 - ii. In fact, within a given bacterium, are the two the same?
 - iii. Does Chargaff's rule hold for RNA? Why or why not?

What is the meaning of the discrepancy between Tables 1 and 2? To find out, do the experiment yourself:

- Go to the [BioBIKE Portal](#)
- Enter ViroBIKE (not CyanoBIKE).
- Log in as usual (or enter non-registered).
- Once into ViroBIKE, go to the INPUT/OUTPUT menu and click RUN-FILE.
- Fill in the *file-name* box with "belozersky-1957-simulation.bike" (in quotes, and yes, it's 1957 and not 1958).
- Mouse over the Options icon and click SHARED. You should get this:



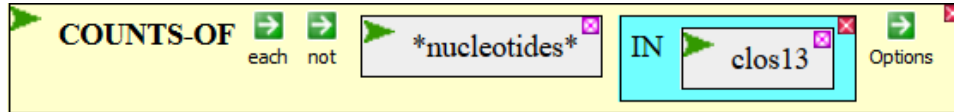
- Execute the now completed function by double-clicking RUN-FILE.

In a few seconds, you should get a message that loading completed successfully. As a result, you will have two new functions on your FUNCTIONS button.

- 1c. Reproduce a portion of Table 1 from Belozersky and Spirin (1958).¹ To do this, mouse over the FUNCTIONS button and click DNA-COMPOSITION-OF. In the *organism* box type in the nickname Clos13 (which stands for Clostridium perfringens str 13). Execute the function. Try it again with a different organism. How do your numbers relate to the numbers of Belozersky and Spirin (1958)?

1d. What do those numbers mean? Recall *What is a Gene?* You've done this problem before.

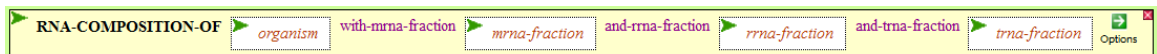
- From the green ALL menu, bring down the COUNTS-OF function.
- Click the *query* box to open it.
- Mouse over the blue DATA menu and click *nucleotides*.
- Mouse over the Options icon and click IN
- Open the *value* box of IN and enter a nickname of an organism. You should see:



- Execute the function. You should see four numbers in the Result pane at the bottom of the screen.
 - What do these numbers mean? Four nucleotides, four numbers... OK, but which number for which nucleotide? Make a guess, then check it by selecting the LABELED option and re-executing the function.
 - What do these numbers mean? How could you calculate them yourself? Recall from *What is a Gene* the LENGTH-OF function, which will tell you how many total nucleotides are in a given genome (or just add up the four numbers from the previous function). How could you use this number and the result of **1.d.i** to produce the values of **1.c**?
 - How do the fractions relate to the fractions you got in 1c? Why the difference? (Think about other options of COUNTS-OF)

1e. Now on to Table 2, a listing of RNA base composition. As before, you'll calculate this... wait a second! What does RNA composition mean? How could you calculate it from just the genome sequence? What would you need to know?

1d. Let's see what the function can do for you. Mouse over the FUNCTIONS button and click RNA-COMPOSITION-OF, getting:



The function demands four things from you. In the *organism* box, enter your favorite organism nickname. The other three boxes ask you for the fractions of RNA for mRNA, rRNA, and tRNA (which should add up to 1). How do you know what they are? You don't, but be thankful the function asked you for the fraction of *classes* of RNA and not the fraction of every gene in the organism! Where could you find rough estimates for these fractions? (Think back...)

What values for the three fractions enable you to reproduce Table 2 from Belozersky and Spirin? Use prior knowledge, if possible, to get in the general area, then trial and error to take you closer. How do you know your numbers are reasonable estimates?

2. Consider [two scenarios](#) intended to rationalize the paradox presented by Belozersky and Spirin (1958),¹ bearing in mind your experience with Question 1.

2a. Relate Scenario 1 (word length survey) to your answer to **1b**.

2b. Relate Scenario 2 (automobile structure) to your answer to **1b**.

3. Consider the results of Brenner et al (1961),² presuming alternate realities.
 - 3a. Redraw Fig. 2, presuming that ribosomes freely dissociate into their component proteins and then reassociate to form ribosomes again, prior to centrifugation.
 - 3b. Redraw Fig. 6, presuming that their Model I is correct.
 - 3c. Redraw Fig. 7, presuming that their Model II is correct.
 - 3d. Redraw Fig. 7, presuming that Model I is correct.

4. What were the two critical observations (i.e. experiment + evidence) of Astrachan and Volkin (1958)³? How did the experiments of Brenner *et al* show a way out of the dilemma posed by their work?

5. Below is the genome sequence of bacteriophage lambda in the region of the *cI* and *cro* genes.


```

5' -CTTTTTTGTGCTCATACGTTAAATCTATCACCGCAAGGGATAAATATCTAACACCGTGCGTGTTG-3'
3' -GAAAAAACACGAGTATGCAATTTAGATAGTGGCGTTCCTATTTATAGATTGTGGCAGCACAAC-5'

5' -ACTATTTTACCTCTGGCGGTGATAATGGTTGCATGTACTAAGGAGGTTGTATGGAACAACGCATA-3'
3' -TGATAAAATGGAGACCGCCACTATTACCAACGTACATGATTCCCTCCAACATACCTTGTTGCGTAT-5'
      
```

 - 5a. Copy the sequence shown above and paste it into your favorite word processor. Highlight or otherwise mark the beginnings of the two genes, the -35 and -10 regions of the promoters, and the Repressor/Cro binding sites.
 - 5b. Extract all instances of the promoters and align them (single stranded, 5'→3'). Highlight or otherwise mark deviations from the consensus -35 and -10 regions.
 - 5c. Extract all instances of the Repressor/Cro binding sites and align them (single stranded, 5'→3'). Use the alignment to guess at what might be a consensus binding site. Do any of the binding sites contain palindromic sequences?

6. See if you can identify regulatory elements without much prior knowledge. Is the repressor of Lactobacillus Phage A2 regulated in a manner similar to the lambda repressor? Go to ViroBIKE and bring up the SEQUENCE-OF *lact-a2* (*lact-a2* is the nickname of Lactobacillus phage A2). Now go to the *cI* repressor – *cro* region.... (Hmmm, that might be a problem).
 - 6a. In ViroBIKE look for the repressor gene by going to the alphabetized list of functions (the ALL button) and clicking GENE/S-DESCRIBED-BY. Then fill in the *query* box with "repressor", choose the IN option and give its *value* as *lact-a2*, then execute the function. A suggested gene should pop up. Is that the one you want? If not, then try the same thing with "cro" in the *query* box.
 - 6b. Copy the full name of the desired gene (beginning NC_004112.gene...) and paste it into the **Go to** box of the sequence viewer. Locate the *cro* gene. If Lact-A2 is like Lambda, then where should the *cI* repressor be? Find it. Note that you can use the **Go to** box to specify the first nucleotide you want to appear on the screen.
 - 6c. Make the display double-stranded by clicking the **Double-stranded?** check box and clicking **Go**. Then copy the double-stranded intergenic region between *cI* and *cro* and paste it into your favorite word processor.

6d. Look for sequences that might be -35 and -10 regions of promoters. Highlight them.

6e. Look for sequences that might be Repressor/Cro binding sites. This is not easy. Repressor binding sites are notorious for changing from organism to organism. Do you see any sequences that appear more than once on the two strands? Do you see any sequences that contain palindromes?

References

1. Belozersky, A. N. & Spirin, A. S. A correlation between the compositions of deoxyribonucleic and ribonucleic acids. *Nature* **182**, 111-112 (1958).
2. Brenner S, Jacob F, Meselson M (1961). An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* 190:576-581.
3. Astrachan L, Volkin E, (1958). Properties of ribonucleic acid turnover in T2-infected *Escherichia coli*. *Biochim Biophys Acta* 29:536-544.