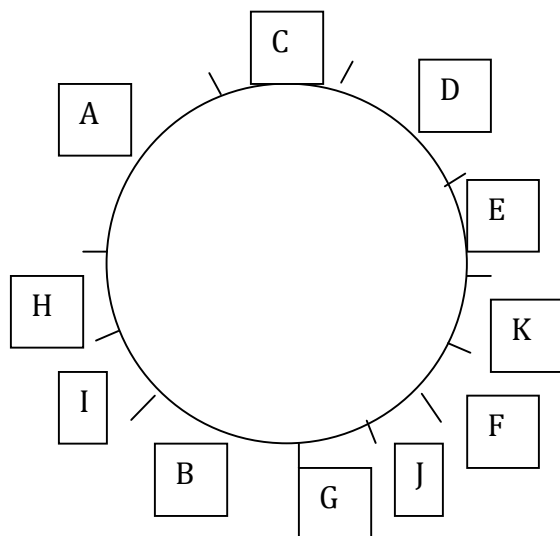


**Exercise 5: DNA properties**

1. Restriction endonucleases are bacterial enzymes that cleave duplex (double-stranded) DNA at specific nucleotide sequences. The mode of replication of the animal virus SV40 has been investigated by using restriction endonucleases that cleave SV40 DNA into a number of unique segments. The map positions of the 11 fragments produced by a pair of restriction endonucleases are shown below. Immediately following a 5 or 10 minute pulse of radioactively labeled thymidine, labeled SV40 molecules that have completed replication during the pulse are isolated. These newly replicated DNA molecules are digested by the restriction endonucleases and the resulting fragments are analyzed for the relative amounts of pulse label they contain. Assume that at the time the label was added there was a random population of replicating SV40 DNA molecules in all possible stages of synthesis. From the information given below, decide whether SV40 replication is unidirectional or bidirectional, and assign the origin and terminus to specific restriction fragments.

Relative amount of pulse label (corrected for fragment size and normalized to 1.0 for fragment A)		
Fragment	after 5 minutes	after 10 minutes
A	1.0	1.0
B	3.9	3.0
C	0	0.8
D	0.9	0.9
E	1.8	2.0
F	4.0	3.1
G	5.4	4.2
H	1.7	2.5
I	2.7	3.0
J	4.9	3.7
K	2.4	2.9



2. A bacterium-like microorganism is brought back from Mars and found to contain double-stranded DNA as its genetic material. To determine its mode of replication, the following test is run: cells are grown several generations in “heavy” medium containing  $^{15}\text{N}$ , then transferred to a “light”  $^{14}\text{N}$ -containing medium, and sampled for DNA extraction after 0, 1 and 2 generations. The three DNA samples are sheared to  $10^6$ -dalton fragments and centrifuged to equilibrium in a CsCl gradient. The results shown in the table below are obtained. In an attempt to understand this puzzling result, extracts of the organism are assayed for various enzyme activities related to nucleic acid synthesis, and only the following are found:

- a. some RNA polymerase activity
- b. a DNA polymerase that functions only with single-stranded DNA as a template
- c. a new enzyme that catalyzes the formation of a deoxyribonuclease-sensitive product when supplied with NADPH and a heat-stable deoxyribonuclease-insensitive, non-dialyzable factor present in the extract. How does the organism replicate its DNA?

Generations after transfer to “light” medium	amount of fully heavy DNA (1.0 = original amount)	amount of half-heavy, half-light DNA	amount of fully light DNA
0	1	0	0
1	1	0	1
2	1	0	2