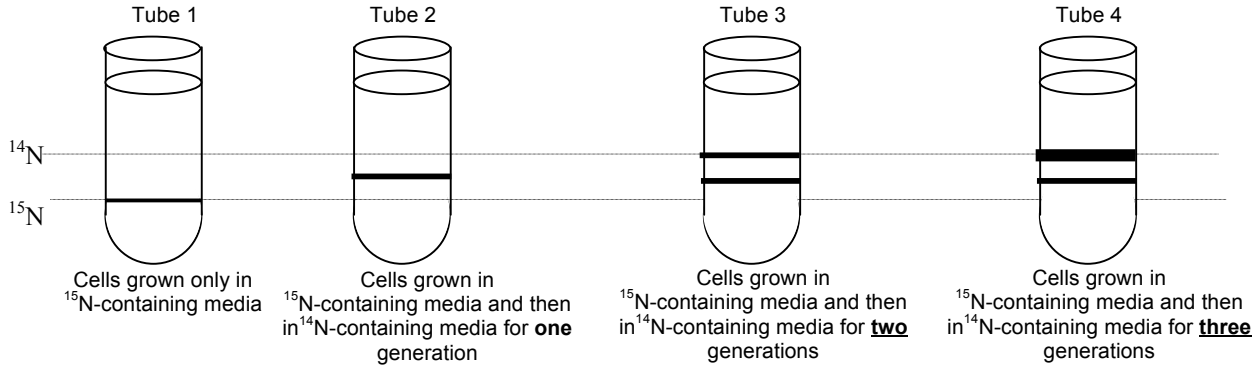
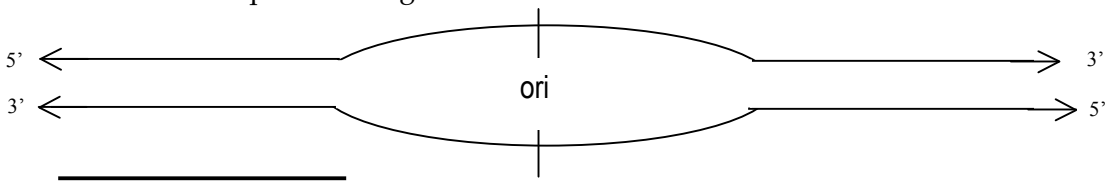
Solution key - 7.012 Recitation 6 - 2010

Questions:

1. You radiolabel the bacterial cells with N^{15}. You then grow them for three generations in N^{14} containing medium and separate the bands based on the difference in their density. Draw the band profile after the 1st, 2nd and 3rd generations.



2. Consider the following segment of the DNA that is a part of a much larger molecule constituting a chromosome. The sequence of region 1 is shown below.



Region 1: `5'…ATTCGTACGATCGACTGACTGACAGTC…3'`
`3'…TAAGCATGCTAGCTGACTGACTGTCAG…5'`

i. If we assume that a fragment of the lagging strand is made from region 1, what will be its sequence? Label the 5′ and the 3′ ends.

`5'…ATTCGTACGATCGACTGACTGACAGTC…3'`

ii. Why is DNA synthesis continuous at one strand and discontinuous at the other strand?

This is because the two strands of DNA duplex are anti-parallel but the replication always occurs in a 5′→3′direction. Accordingly, the synthesis is continuous at one strand and discontinuous at the other strand (this strand is made in the form of small okazaki fragments that are later joined by the action of ligase enzyme).

iii. DNA polymerase can add many thousands of nucleotides before it falls off. How does the DNA polymerase achieve this processive quality?

The newly replicated strand is stabilized by a sliding DNA clamp. This protein has multiple identical subunits assembled into doughnut shape. The doughnut's hole is just large to encircle the DNA double helix along with a single layer of water molecule for lubrication. The clamp binds to DNA just behind the DNA polymerase, keeping it associated tightly with the replicated DNA. If the clamp is absent, DNA polymerase dissociates from DNA after 20-100 polymerizations. With the clamp it can add up to 50,000 bases before it detaches.