Assignment: Illumina sequencing

1. How do the nucleotides in Illumina sequencing differ from those in PCR?

    - ❏ they have colored tags
    - ❏ they have a blocker preventing another nucleotide from being added
    - ❏ they have 3 phosphate groups on the 5' carbon
    - ❏ they have a hydroxyl group (-OH) on the 2' carbon

2. What is the purpose of the colored tags on the nucleotides used in sequencing?
    - ❏ to make sure that only one base is added at a time
    - ❏ to indicate what base is being added
    - ❏ to block the 5' carbon
    - ❏ to make the reaction more colorful
3. Why is only one nucleotide added to the growing DNA strand in each cycle?

    - ❏ 3' end has a blocker
    - ❏ only nucleotides with a single base are added to the flow cell at a time
    - ❏ only one DNA polymerase molecule is used

4. Why can another nucleotide be added in the next cycle?

    - ❏ the strand is ended after a modified nucleotide is added
    - ❏ a nucleotide with a different base is added in the next cycle
    - ❏ 3' blocker is removed

5. Do the DNA strands that are produced in a PCR reaction serve as a template in the next cycle of the same PCR reaction?

- ❏ yes
- ❏ no

6. Do the DNA strands produced in Illumina sequencing serve as a template in the next cycle of the same sequencing reaction?
   - ❏ yes
   - ❏ no
7. How do the components of the Illumina sequencing reaction ensure this?
8. In Illumina sequencing, the different steps are initiated by washing different chemicals over flow cell (just like in bridge PCR). Put the steps in Illumina sequencing in the right order
   - color tells what base has been added to the growing DNA strand
   - add DNA polymerase and nucleotides
   - add more polymerase and more nucleotides
   - bridge PCR to produce clusters
   - remove blocker on 3' carbon
   - one nucleotide is added to the growing DNA strand
   - add primer
9. Suppose that there was a mistake and the nucleotides you ordered for sequencing were all labeled with blue tags. How would this affect the results? Explain your reasoning.
10. Challenge: When template is added to the flow cell for bridge PCR, the concentration of template is critical. What would happen if too many template molecules were added to the flow cell?