Review for the "ga5-1 complementation" section.

A. Choose all the options that you think are correct. You may want to choose more than one option for some questions.

1. Gibberellins are:

___ Encoded in the genome of plants.
___Synthesized via the terpenoid pathway.
___Produced by certain bacteria.
___Produced by certain fungi.
___ Produced by plants.

2. What do you need to generate cDNA from Arabidopsis?

____ Total DNA
____ Total RNA
____ Messenger RNA
____ Genomic DNA

3. What template would you use to amplify the CDS of GA20OX1 gene of Arabidopsis by PCR?

____ RNA
____ Genomic DNA
____ cDNA
____The CDS of GA20OX1

4. You amplified the GA20OX1 gene from Arabidopsis using primers that introduced AscI and PacI restriction sites to the PCR product. After digesting the PCR product using AscI and PacI you run an agarose gel.

____ You run this restriction gel to see whether the PCR product was properly cut.
____ You run the gel to see whether you had a PCR product.
____ You run the gel to see whether the PCR product was the correct size.
____ You run the PCR product on the gel in order to remove the restriction enzymes.

5. What is the best way of making more copies of a plasmid?

____ Use PCR to make more copies of the DNA.
____ Transform it into E. coli.
____ Transform it into plants.

6. What do you use agarose gel electrophoresis for?

____ Visualize the DNA your are working on.
____ Analyze the length of the DNA you are working with by running it beside a standard of known size.
____ Cut your DNA using restriction enzymes.
____ Separate fragments of DNA of different sizes.
____ Amplify a fragment of DNA by PCR.

7. To sequence a fragment of DNA from a plasmid you need to:

____ Know some of the sequence of the plasmid in order to design primers flanking the region that needs to be sequenced.
____ Cut the DNA using restriction enzymes.
____ Purify your plasmid.
____ Know what is the concentration of your plasmid preparation.
____ Previously run your plasmid on an agarose gel.

8. In class you transformed your ligation reaction into E. coli cells. The transformed cells were able to grow on medium with the antibiotic kanamycin. Which of the following sentences explains why this was the case?

____ The transformation corrected a mutation in the E. coli genome that made the bacteria susceptible to the antibiotic.
____ The pMDC32 vector encodes for an enzyme that degrades kanamycin.
____A gene that encodes for an enzyme that degrades kanamycin was inserted into the bacterial genome.

9. We transformed the *ga5-1 Arabidopsis* mutant using a binary vector containing a wild type GA20OX1 gene to

___ Replace the mutated GA20OX gene found in the *ga5-1* mutant with the wild type GA20OX1 using Agrobacterium.
___ Introduce the wild type GA20OX1 in the genome of *ga5-1*.
___ Test whether this wild type GA20OX1 could complement the *ga5-1* semidwarf phenotype.

10. What substrates does the DNA polymerase use?

____ Nucleotide triphosphates
____ Deoxy nucleotide triphosphates
____ Nucleosides
____ Deoxynucleotide monophosphates

11. During the "Green Revolution" high yield varieties of wheat and rice where developed. The researchers

____ Realized that varieties with shorter stems were less prone to lodging.
____ Were trying to understand the role of gibberellins in plant growth.
____ Were looking to optimize the flowering time of these plants.
____ Were looking for varieties that produced more seeds.

12. You used the vector pMDC32 in class. pMDC32 is a binary vector. This vector

____ Can replicate in both E. coli and Agrobacterium.
____ Encodes for the proteins needed for plant transformation.
____ Has two origins of replication.
____ Is inserted twice in the Agrobacterium genome.

____ Is part of the binary vector system in Agrobacterium that is formed of two plasmids, one that contains the T-DNA, and one, the helper plasmid that encodes for the VIR genes needed to transfer the T-DNA to the plant.

B. You want to clone the genomic fragment of your favorite gene (YFG) from Arabidopsis into the binary vector pMDC32. Below is a list of the steps you need to do. Organize them in the right order. Some of the procedures described here might have to occur at the same time, you can label those 2a and 2b for example. Remember that you have done most of the steps yourself in class.

a-Sequence the insert in your newly generated plasmid to test whether its sequence matches that of YFG.
b-Check whether the E. coli cells transformed with the ligation reaction containing both the cut pMDC32 vector and your cut PCR product generated more colonies than the cells transformed with the ligation reaction that contained only your cut vector.
c-Set up a restriction digestion for both the pMDC32 vector and your purified PCR product.
d-Purify the PCR product to remove the DNA polymerase and the nucleotides.
e-Set up a ligation reaction using only your cut pMDC32 vector.
f-Plan your cloning strategy by looking for appropriate restriction sites in the pMDC32 vector
g-Design primers to amplify the genomic section of YFG and insert restriction sites to allow cloning into the pMDC32 vector.
h-Transform your ligation reaction into competent E. coli cells.
i-Run both your cut pMDC32 vector and your cut PCR product on an agarose gel.
j-Use the E. coli colonies on the LB medium containing kanamycin to inoculate a 3 ml overnight culture.
k-Set up a restriction digestion reaction of your new plasmids to test whether any of them contains your cut PCR product.
l-If you identify a new plasmid containing an insert of the correct size you will prepare this plasmid for sequencing.
m-See whether any of the new plasmids contain an insert of the size of your PCR product.
n-PCR YFG using your total DNA preparation and the primers you designed.
o-See whether your PCR product has the correct size.
p-Cut both the cut vector backbone and your cut PCR product from the gel and purify it using the gel purification kit.
q- Set up a ligation reaction using your cut pMDC32 vector and your cut PCR product.
r-Plate the transformed E. coli cells on LB medium containing kanamycin.
s-Run your restriction digestion on an agarose gel.
t-Prepare total DNA from a wild type Arabidopsis plant.
u-See whether your pMDC32 vector has been completely cut.
v-Use the overnight 3 ml E. coli culture to extract the plasmid product of your ligation reaction using a plasmid miniprep.