1. What is the significance and advantages of temperature-sensitive mutants?

Temperature-sensitive (ts) mutants only exhibit a mutant phenotype at a high temperature. Hence, it is particularly useful when one studies mutations in genes encoding essential functions. This is because such mutants of essential genes are generally lethal making it impossible to propagate and grow the mutants. The ts mutants will allow us to grow and maintain the mutants at a low temperature but study the mutant phenotype at a high temperature.

2. What does “complementation test” mean in classical genetics?
What does “complementation” mean in modern plant molecular biology?

Complementation test in classical genetics is used to test if two mutations of similar phenotypes reside in the same gene or in different genes. By crossing these two mutants with each other, the phenotype of the F1 progeny will tell if the two mutations complement (thus reside in two different genes) or fail to complement (thus reside in the same gene).

Modern complementation test is used to test if a piece of DNA containing the wild type gene can rescue and restore the phenotype of a mutant individual. If it can rescue the mutant phenotype, then one can conclude that the wild type DNA encodes the gene which is mutated in the mutant individual.

3. What is the advantage of “genetic selection” vs. “genetic screen”?

Genetic selection is a technique that allows one to select for a specific type of mutants which survive the specific growth condition while the wild type plants all die. This allows one to find the desirable mutants without much effort.

Genetic screen is to allow all plants (mutant or wild type) grow and the mutant plant with a specific phenotype will be screened for (looked for). During the screen, we are required to examine all plants individually to identify the desirable ones that exhibit the specific phenotype. Thus it is more time consuming.
4. Give an example of a phenotype that is visually visible and an example of a phenotype that is not visually visible but testable?

Visible: flower morphology, pigment color
Not visible: biosynthetic pathway mutants. One can identify them by feeding them with specific compounds.

5. What is the advantage of EMS mutagenesis vs. transposon insertion mutagenesis?

EMS chemical is easy to use, can generate all kinds of mutations such as missense, nonsense and its effect is largely random (ie. Every gene has an equal opportunity of getting mutated by EMS).

Transposon: insertion is not random (there are hot spot and cold spot). Insertion generally disrupt the function of the gene in a dramatic way (such as frameship, truncation). However, transposon tagging allows one to isolate the mutant gene easily and quickly (transposon tagging).

6. What is the difference between Ds and AC elements?

Ac (activator) is autonomous and encodes the transposase.
Ds (Dissociation) is nonautonomous, it does not encode its own transposase and its mobility depends on the external supply of transposase (such as by a Ac element).

7. Describe what each of the following mutations are. Indicate what types of mutagens are used in generating each.

a. double-stranded breaks
Sugar-phosphate bonds in both strands of DNA are broken. Often result in deletions. Mutagen: X-ray.

b. missense mutation
A mutation that results in a single amino acid substitution in the protein. Often caused by chemical mutagens such as alkylating agent (for example, EMS) and hydroxylating agents.

c. frame shift mutation
Insertions or deletions of base pairs within the coding region causing the shifting of the reading frames (codes) resulting in the translation of an entirely different protein after the point of insertion/deletion. Mutagen: intercalating agents such as Acridine Orange and Proflavins. Transposon/T-DNA insertions can also cause frame shift.
d. thymidine dimmer
The formation of covalent bonds between two adjacent thymidines in the DNA strand.
Mutagen: UV irradiation

8. Briefly explain what each of the following markers are and methods of their detection.

a. RFLP
Restriction fragment length polymorphism. It reflects differences in the DNA sequences between two different individuals or between two different strains/ecotypes. This DNA sequence polymorphism can be detected because the DNA sequence difference creates a new restriction enzyme site (or destroys a restriction enzyme site). Thus by subjecting to restriction enzyme digestion followed by Southern blot, one can detect the RFLP.

b. CAPS
Cleaved Amplified polymorphic sequences. It is based on the similar principal of RFLP except that the method of detection is through PCR. Hence, the sequence information flanking the polymorphism should be known. Based on the sequence, one can design specific PCR primers which allow for the PCR amplification of relevant sequences. The amplified DNA fragments will be digested with a specific restriction enzyme and then run on an agarose gel and stained with Ethidium Bromide. Differences in the banding pattern between two individuals will be revealed.

c. SNP
Single nucleotide polymorphism. Changes of single nucleotides between two different individual (or ecotype). This change (in the case it does not affect restriction pattern) can be detected by direct sequencing or detected by DNA chip hybridized to 20 nucleotide long probes. Such short probes can only hybridize to the DNA chip when there is 100% homology. A single base change (mismatch) can eliminate the hybridization altogether.

d. SSLP
Simple sequence length polymorphism. Differences between two individual (or ecotypes) in the number of dinucleotide repeats. This can be easily visualized by PCR amplification and then run on a gel which is then stained with ethidium bromide. A change in the size of the band reflects a difference in the number of the dinucleotide repeats. This is also called microsatellite marker.
9. A mutant plant with small flower (s/s) and curly leaf (c/c) was crossed into wild type to generate F1 (s c /+ +), which is wild type in phenotype. The F1 was used to pollenate the mutant parent (s c /s c) to generate F2 plants with following phenotypes. Specifically, 43 plants showed curly leaves but developed normal flower; 2146 plants showed both small flowers and curly leaves; 2302 plants are wild type; and 22 plants developed small flowers but normal leaves. Calculate the recombination% between the locus controlling the flower size (s) and the locus controlling the leaf shape (c).

F1 backcross: sc/++ X sc/sc
F2 progeny:

Parental type: sc/sc 2146
++/sc 2302
Recombinant type: s+/sc 22
+c/sc 43

Recombinant frequency: 22+43/2146+2302+22+43 = 1.44%

Note: since in the backcross, one of the F1 parent is a sc/sc homozygote and not possible to observe recombination in this parent. Thus the total chromosomes should not be multiplied by 2. I still give full credit if you did multiply by 2 as I feel it is my mistake not bringing this up in class.

10. uns1 is a mutant that is hypersensitive to UV irradiation. In an effort to map and then clone the UVS1 gene in Arabidopsis, Dr. Franks discovered that UVS is linked to a molecular marker AAS1. Specifically, uvs1 mutation was isolated in the Ler ecotype and was crossed into a wild-type plant (Col ecotype). The F1 progeny of this cross is wild-type in phenotype but is heterozygous for uvs1 in genotype. He let the F1 plants self-cross and then isolated DNA from 19 F2 uvs1 mutant plants. The AAS1 primers were used to PCR-amplify the AAS1 locus from these 19 F2 individual uvs1 mutants. The PCR reactions were run on a 3% agarose gel, an image of which is shown below:

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Calculate the distance (in % recombination) between AAS1 and UVS1

Recombinant: individual 4, 5, and 15. In individual 15, both chromosomes are recombinants. Recombination% = (2 +2X2)/19X2 = 10.53%