Exp. #1. Examining reversion of bacterial *His*- genetic mutants by direct and bioactivated chemical mutagens using the Ames Test

1-1. Purpose:

This laboratory has several goals:

1. To demonstrate the use of the Ames Test. This is a widely used screening tool for determining the mutagenicity of chemical compounds. It is used as an initial screen to test many types of potential mutagens, including environmental, industrial, and pharmaceutical samples that might be suspected as having an impact on human health

2. To demonstrate the concept and use of reverse mutations for genetic analysis. This laboratory will introduce different types of mutations, and show how different bacterial strains can be used to test for mutagenicity.

3. To introduce the concept of metabolic activation, also known as bio-activation, to show how a bacterial mutagen might be related to carcinogenesis in humans.

4. To demonstrate a unique color-based screening method to quantitate mutagenesis.

5. To properly design a genetic experiment that incorporates the use of independent variables, dependent variables, a negative and positive control, and how to determine the significance of test-sample results

6. To incorporate what you have learned about sterile technique, pipetting, and lab safety into a genetics laboratory exercise.

1-2. Overview:

The Ames test is used for determining if a chemical is a mutagen. It was developed by the laboratory of Bruce Ames at the University at California Berkeley in the early 1970s. This test uses strains of *S. typhimurium* or *E. coli* that contain different types of mutations in a gene that the bacteria use to make the amino acid Histidine. These *His*$^-$ strains cannot grow on minimal growth medium unless Histidine is added. This test is based on the assumption that mutagenic agents can cause changes in the mutant gene that encodes the defective Histidine-producing enzyme, restoring it back to the normal form that encodes the active enzyme. Note that the mutagen will also cause many other, undetected mutations, but this test is based on the recovery of revertant mutations that restore function to a mutant gene. These are also called reverse or back mutations. The Ames test measures the ability of *His- bacteria* to grow on media that does not contain histidine. Growth indicates that a reverse mutation has reverted the *His*$^-$ gene back to the active His+ form.

The Ames test is often used as an initial determination of oncogenesis (i.e. cancer causing activity) for various chemicals found in the environment. It is used to test chemicals and samples from pharmaceutical, industrial, waste management, and other sources of potential carcinogens. The assumption is that any substance that is mutagenic for the bacteria used in this test may also turn out to be carcinogenic in humans, meaning it might cause a mutation that would lead to cancer.

While this is not true for all chemicals, the ease and low cost of this test makes it a very valuable tool for screening substances in our environment for potential carcinogenicity.

Bacterial strains: The bacterium that is typically used for this test is an attenuated (non-pathogenic) laboratory strain of *Salmonella typhimurium* that contains different types of mutations in a gene necessary for Histidine production (*His-*). *E. coli His-* mutants are also sometimes used. This mutation makes the cells unable to synthesize the amino acid Histidine (His) on its own. For growth, these ***His-* strains** must have the growth medium supplemented with Histidine. However, some types of mutations can be reversed by a **revertant** or **back mutation**, restoring function to the gene. These **revertants** are able to grow on medium lacking Histidine.

There are three types of mutations that are relevant to this experiment, as described in the following figures:

Fig. 1-1. Point mutation. These are also called a base change or substitution mutation. This is a mutation that exchanges one base for another within the coding region of a gene on a DNA strand. For this laboratory, a Point mutation in the *His* gene will be reverted to wild type using the mutagenic chemical sodium azide (**NaN_3**).

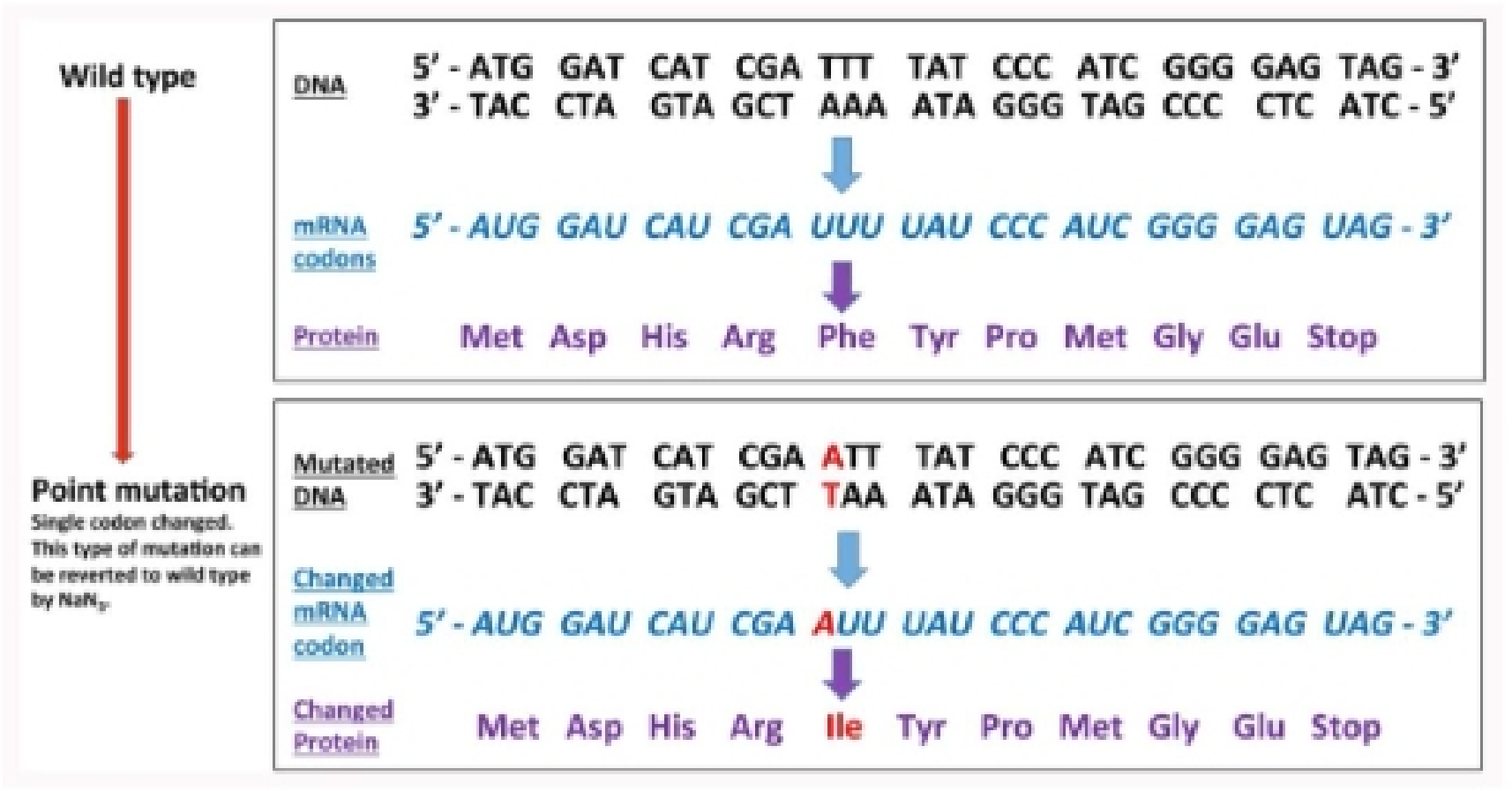


Fig. 1-2. Frame shift mutation. These are mutations in which an extra base or bases are inserted within the coding region of a gene on a DNA strand. This leads to a shifting of the genes reading frame downstream of the insertion site, causing new codons, loss of stop codons, and generation of new stop codons, depending on the sequence of the new reading frame. For this laboratory, a Frame shift mutation in the *His* gene will be reverted to wild type by using the mutagenic chemical 2-Nitrofluorene (**2NF**).

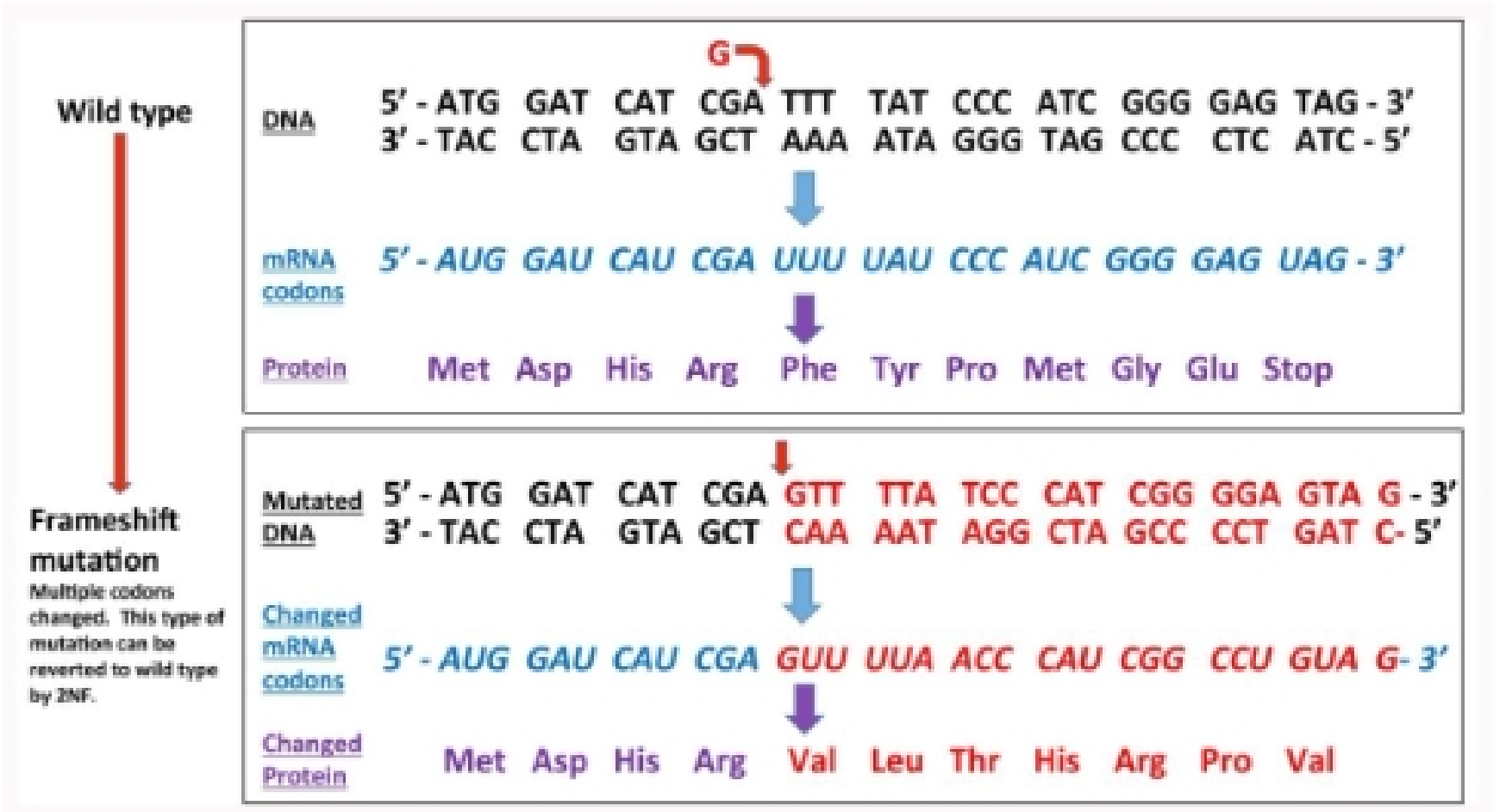
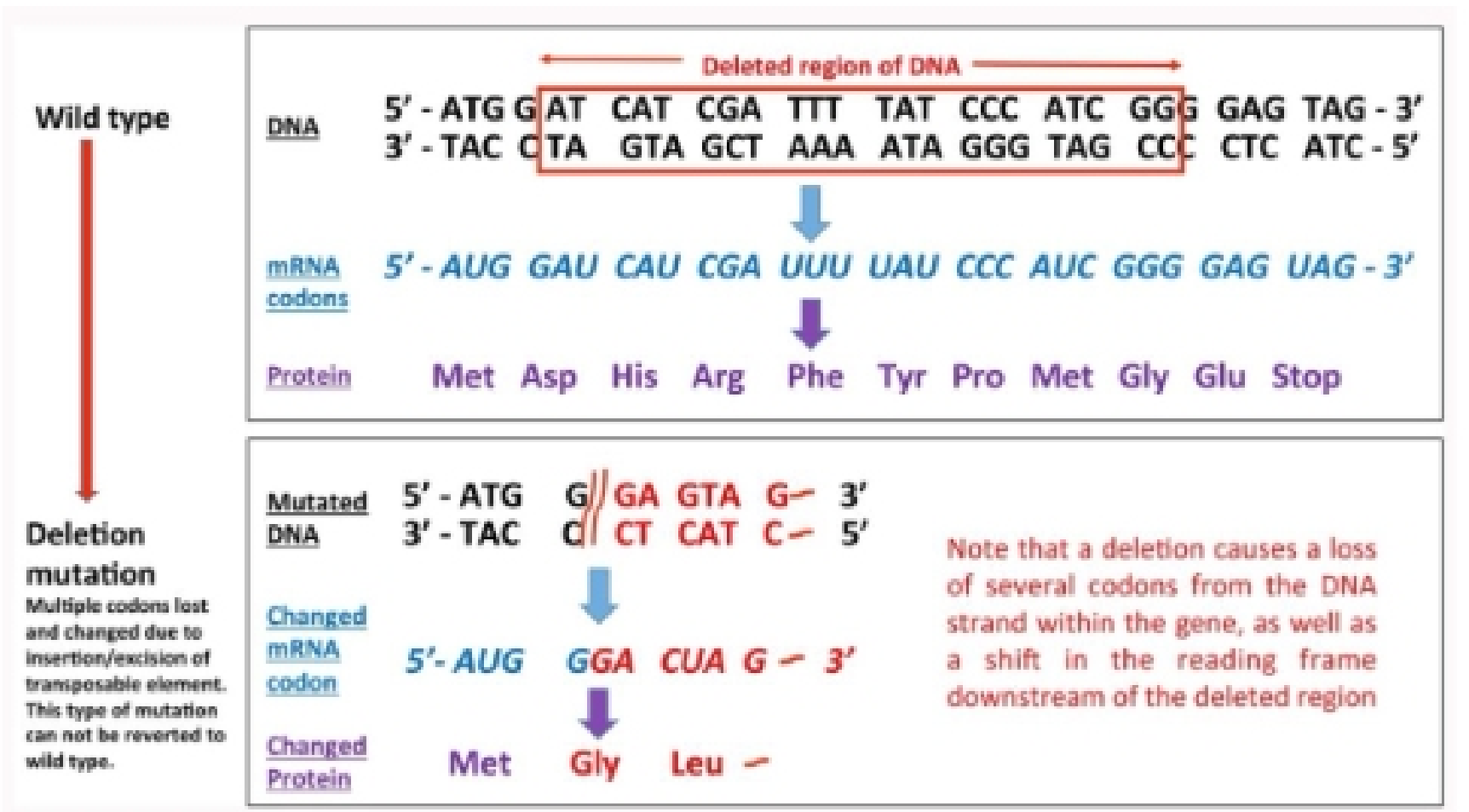


Fig. 1-3. Deletion mutation. These are mutations in which substantial regions of DNA have been lost or deleted within the coding region of a gene on a DNA strand. This leads to a loss of all of the codons within the deleted region, and usually a Frame shift downstream of the deleted region. This type of mutation cannot be reverted with any chemical reagent.



Note that for this lab, the *His*- Point and Frame shift mutations are in *Salmonella typhimurium*. The *His*- deletion mutation is in *E. coli*.