Types of Mutations

Mutations can be either **spontaneous** (occur without the known intervention of mutation-causing agents or occur due to spontaneous hydrolysis, errors in DNA replication, repair and recombination.) or **induced** mutations

The most common mutation is a ***point mutation*** or ***base substitution*****,** in which a single base in DNA is replaced with a different one. Such a substitution can to result in the incorporation of an incorrect amino acid in the synthesized protein, a result known as a ***missense mutation*** (Figure 1)**.**

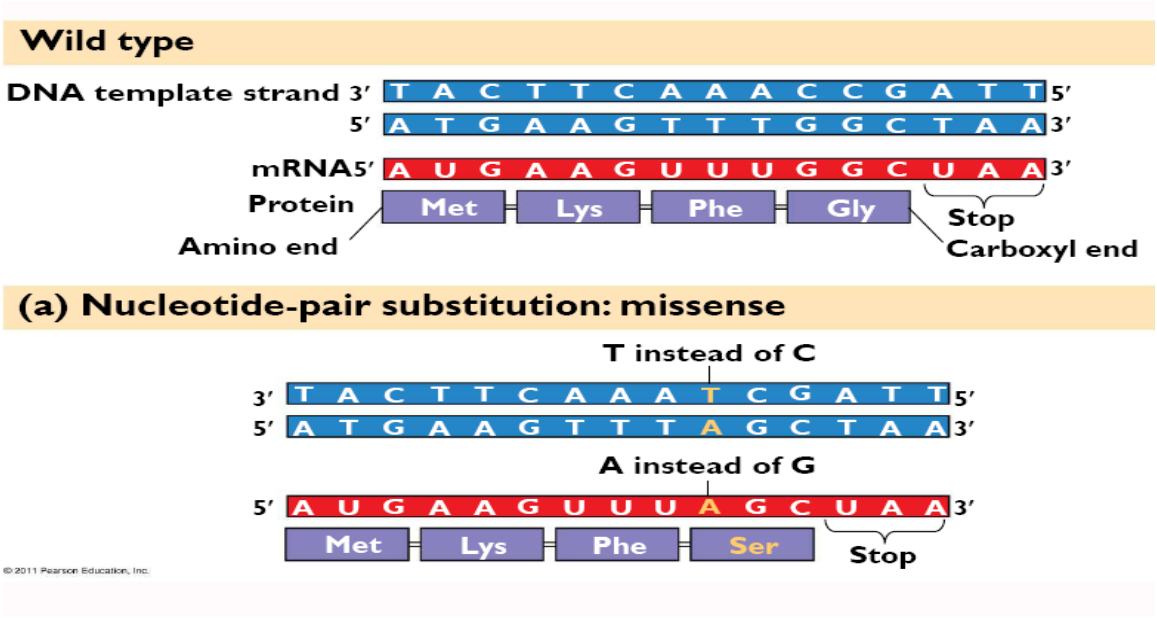

Figure 1: Nucleotide –pair substitution : missense (still code for an amino acid, but not the correct amino acid) [Pearson Education inc, 2011].

Some errors may create a stop codon, which stops protein synthesis before completion, resulting in type of mutation called a ***nonsense mutation*** (Figure 2)**.**
The other type of point mutation is **Silent mutations** (Figure 3) have no effect on the amino acid produced by a codon because of redundancy in the genetic code.

Deletion or addition of base pairs results in other type of mutation which called a ***frameshift mutation*****.** In this mutation, there is a shift in the "translational reading frame" (the three-by-three grouping of nucleotides), and a long stretch of missense and an inactive protein product result (Figure 4). All chemicals and radiation bring about mutations are called ***mutagens*****.**

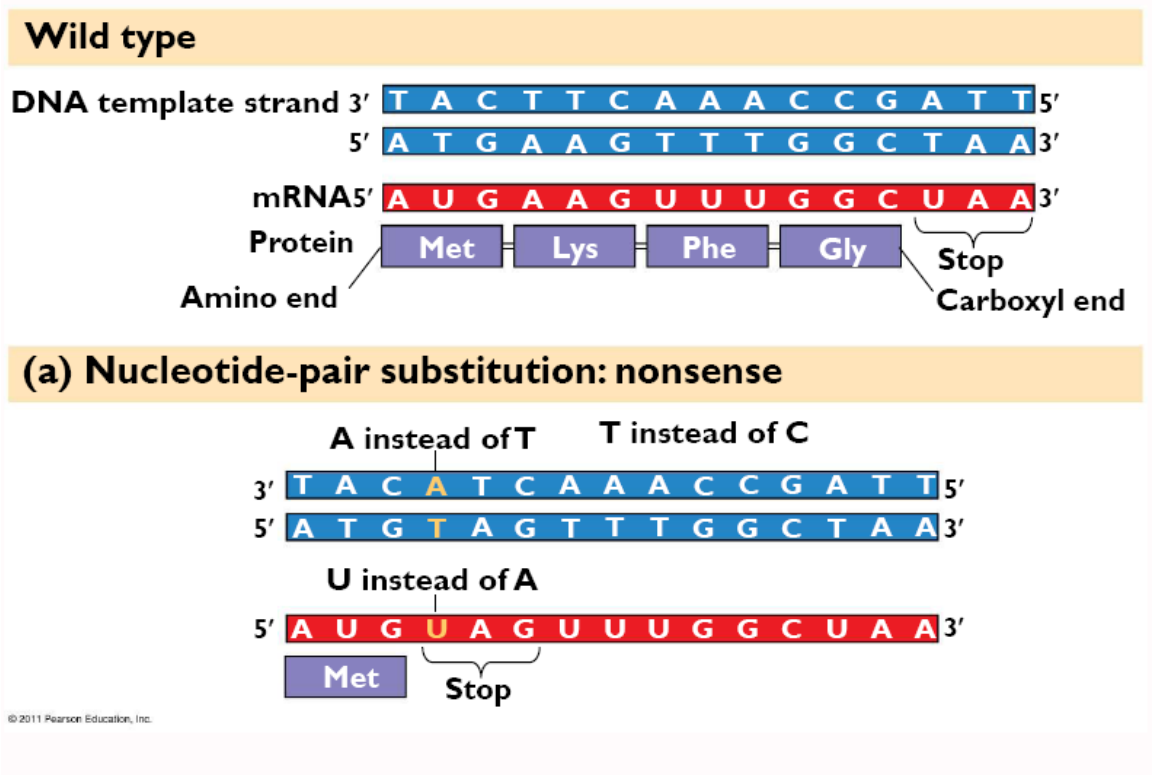

Figure 2: Nucleotide –pair substitution : Nucleotide –pair substitution : nonsense (change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein) [Pearson Education inc, 2011].

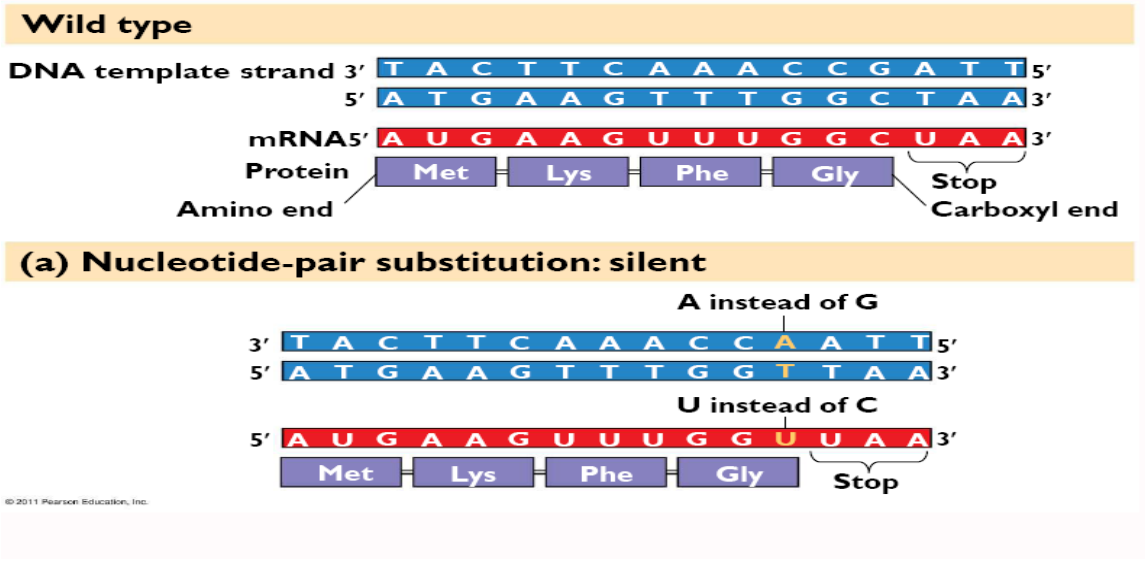

Figure 3: **Nucleotide –pair substitution : silent** [Pearson Education inc,2011].

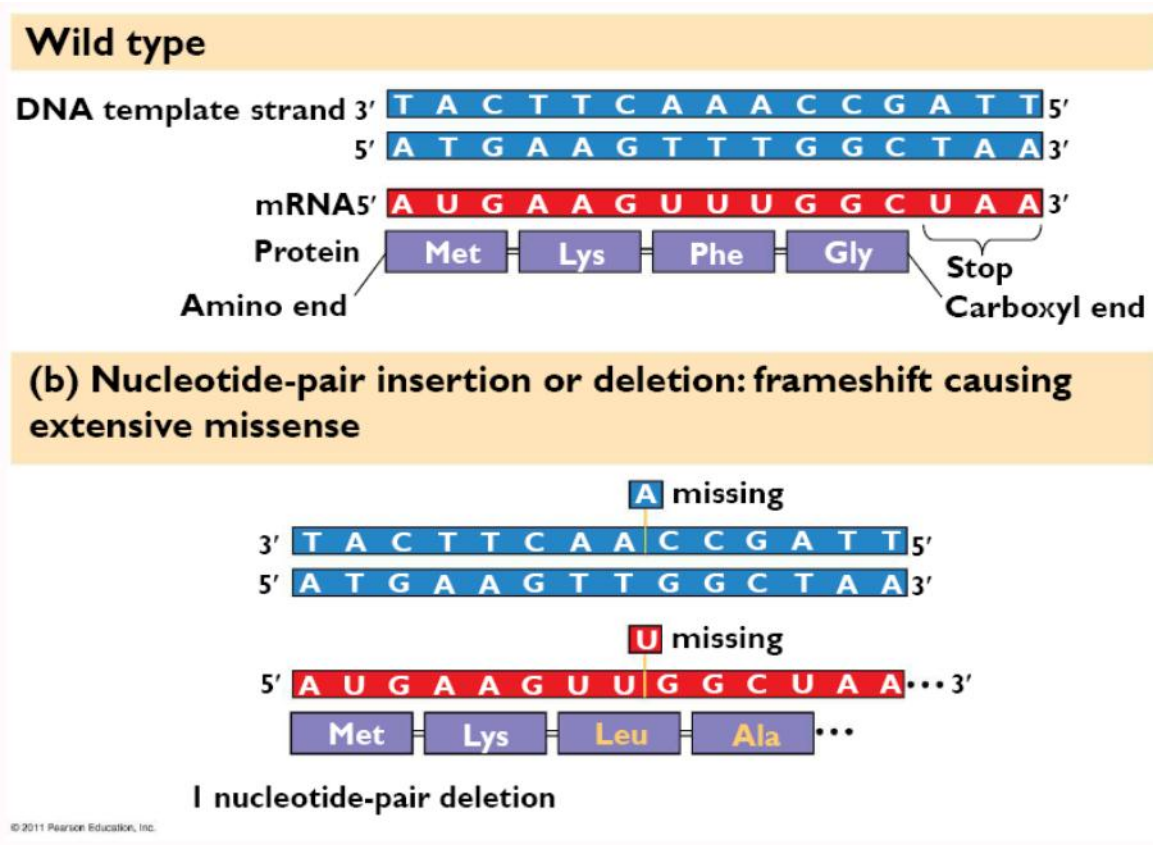

Figure 4: Nucleotide –pair insertion or deletion: extensive missense [Pearson Education inc,2011].

DNA modification repair pathways

Repair pathways that remove DNA modifications have Three basic mechanisms: direct repair, base-excision repair and nucleotide-excision repair.

A. Direct chemical reversal.

1. Photolyase, AKA photo-reactivation: UV light induces the formation of pyrimidine dimers between adjacent C or T bases in DNA. The photolyase enzyme break the cyclobutane dipyrimidine bond. To do so, the enzyme must absorb visible light, hence the name photo-reactivation. *E. coli* and the yeast *Saccharomyces cerevisiae* have such an enzyme.

2. Methyltransferase: The methyl groups from mutagenic O6-methylguanine (O6-MeG is particularly mutagenic) and O4-methylthymine can be removed directly by this enzyme.

B Glycosylase + AP endonuclease: Many modified bases are recognized by specific N-glycosylases that cleave the modified base. The resulting AP site is

repaired by AP endonucleases. Examples in *E. coli*: hypoxanthine-DNA glycosylase, 3-methyladenine glycosylase . formamidopyrimidine glycosylase, hydroxymethyl glycosylase.

C. Nucleotide excision repair: In this form of DNA repair, the damaged bases are removed from DNA as an oligonucleotide and the resulting gap is repaired by resynthesis. This pathway is used to remove many bulky adducts in DNA, including cross-links and UV-induced pyrimidine dimers.

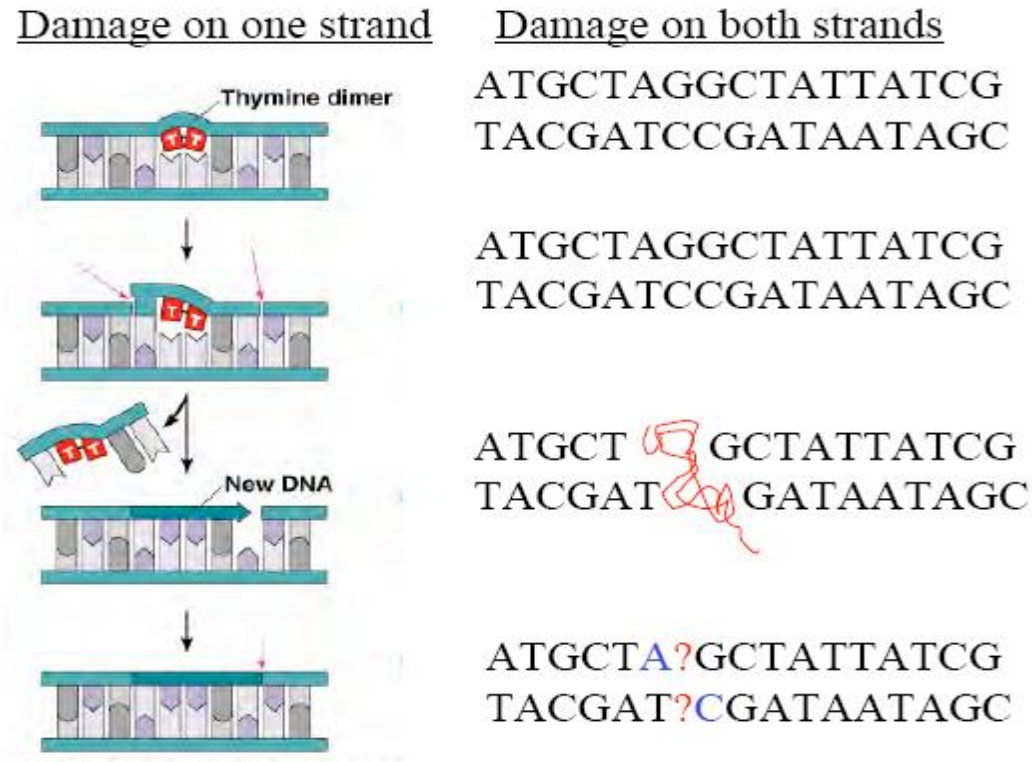

Figure 5: Nucleotide excision repair = enzymes that function to cut out and replace DNA damage [Pearson Education inc,2004]

Rate of Mutation

The ***mutation*** is a probability that gene will mutate when cell divides. DNA replication is very faithful, and only about once in 1 billion base pair replications does an error occur by Spontaneous mutation (If it was harmful, organism dies. If beneficial, organism thrives and passes mutation to offspring (This allows organisms to balance the need for genetic stability with that for evolutionary improvement). Mutagen play a role for increasing the rate of such errors from 10 to 1000 times.

The fact that organisms as phylogenetically disparate as hyper thermophilic Archaea and *Escherichia coli* have about the same mutation rate might make one believe that evolutionary pressure has selected for organisms with the lowest possible mutation rates. However, this is not so. The mutation rate in an organismis subject to change.

For example, mutants of some organisms have been selected in the laboratory that are hyperaccurate in DNA replication and repair.
However, in these strains, the improved proofreading repair mechanisms has a significant metabolic cost; thus, a hyperaccurate mutant might actually be at a disadvantage in its natural environment. On the other hand, some organisms seem to benefit from a hyperaccurate phenotype that enables them to occupy particular niches in nature. A good example is the bacterium
Deinococcus radiodurans . This organism is 20 times more resistant to UV radiation and 200 times more resistant to ionizing radiation than *E. coli.*

Identifying Mutants

With bacteria mutants can be identified more easily, because bacteria produce very large populations very quickly. ***Positive* (*direct*) *selection*** involves the selection of mutant cells and rejection of non mutant cells. For example: plating out bacteria on a medium containing penicillin. Survivors, which are penicillin - resistant mutants, can be isolated. ***replica plating***(Figure 6) is used for ***negative* (*indirect*) *selection***to detect, for example, ***auxotrophs*** that have nutritional requirements not possessed by the parent (non mutant) cell. To isolate auxotrophs, colonies growing on a master plate containing a complete medium can be transferred by a sterile velvet pad is pressed onto the master plate, and the colonies are transferred simultaneously to a ***minimal medium***, which lacks essential nutrients such as the required amino acid. An auxotrophic mutant will fail to appear on the minimal medium.

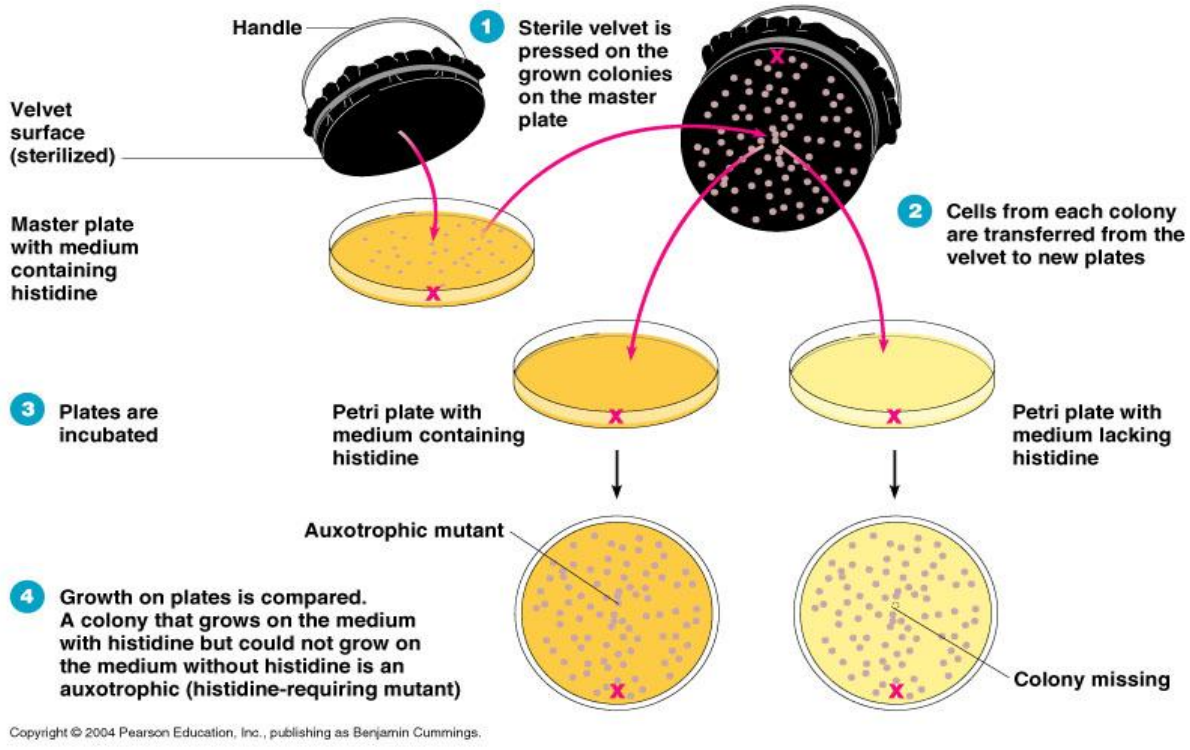

Figure 6: Replica plating process for detection the Auxotrophic mutation

Identifying Chemical Carcinogens

For identifying possible ***chemical carcinogens***, the ***Ames*** test is used as a relatively inexpensive and rapid test. is based on the ability of a mutated cell to mutate again and to revert to its original form. An auxotroph of *Salmonella,* which has lost the ability to synthesize the amino acid histidine, is plated out on a minimal medium without histidine. together with a rich source of activation enzymes found in rat liver extract called the test chemical (is placed on this plate also). Rat Mutations of the *Salmonella* to the normal histidine-synthesizing form are indicated by colonies growth near the chemical test. High mutation rates are indicate the effects of carcinogens (Figure 7).

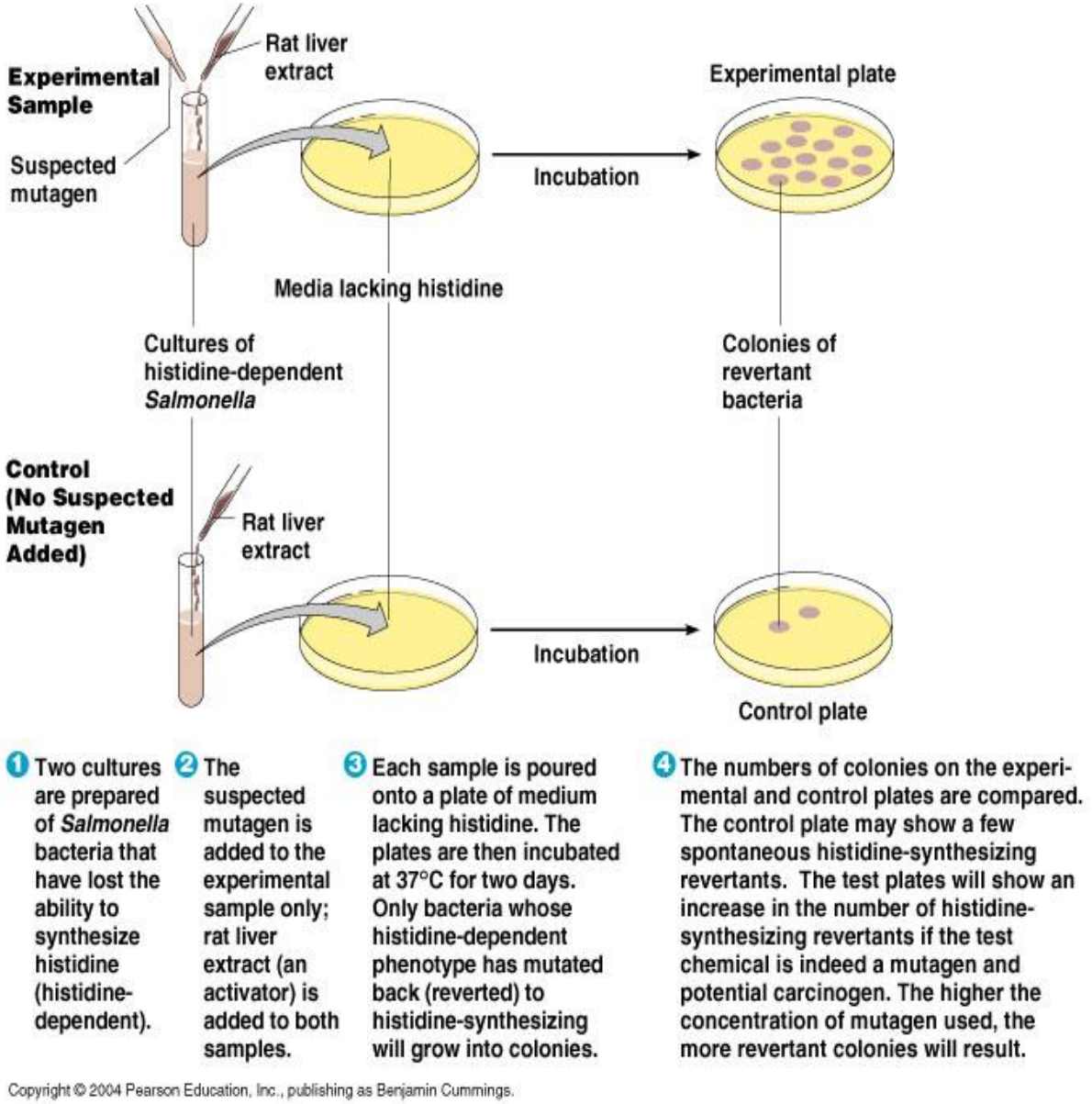

Figure 7: Ames test to identify chemical carcinogens.