**Microbial Genetics**

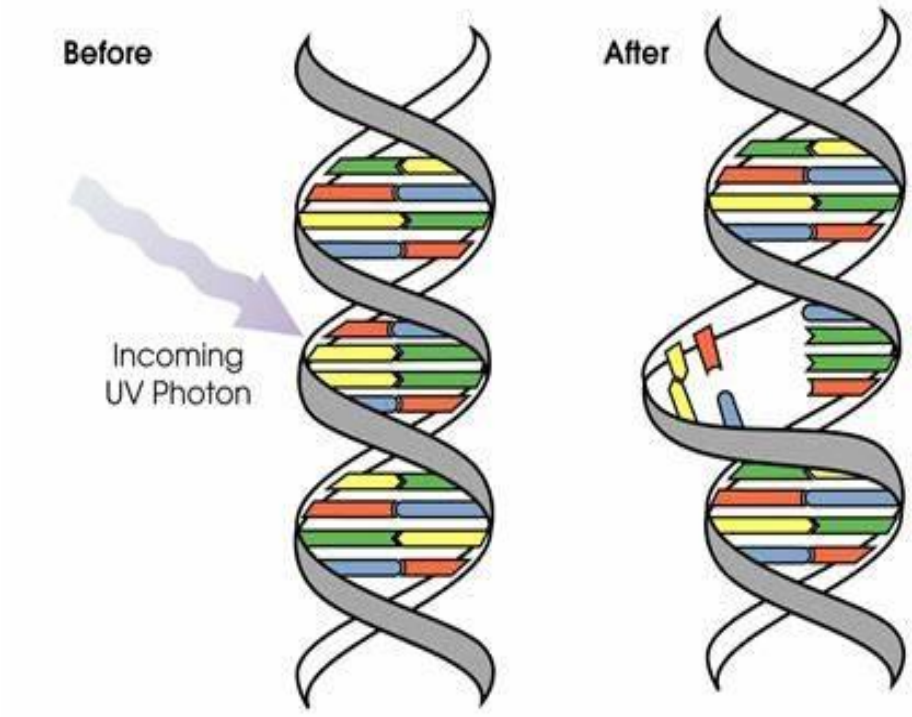
**Third Step** ***Assit. Prof. Alaa Naseer***

**Lab.4**

## Lethal Effect of U.V. Light and Photo reactivation

Ultraviolet (UV) radiation is a carcinogen because it damages DNA, resulting in mutations in proto-oncogenes and tumor suppressor genes . When UV light is absorbed by DNA, it can cause the formation of covalent bonds between adjacent pyrimidine bases, leading to the formation of pyrimidine dimers . These dimers can cause errors in DNA replication and transcription, leading to mutations and cell death .

Bacteria have been affected by the wavelength (10-250)nm which is called sub lethal effect , while the lethal effect is occurred in the wavelength (250-300)nm which causes the killing rate or LD50 that causes killing of 50% of the cells . The wavelength 260 nm is strongly affects mutation rates because of highest DNA optical absorbency in this wavelength which causes mutations through dimmers formation between pyrimidines (Thymine &Cytosine) on the same chain affects DNA replication or transcription , so the bacterial cell that exposes to the ray cannot grow and will die .



It can also reduce the lethal effect of U.V. rays on the culture that was exposed to the U.V. by exposure to the visible light with wavelength (365-450) nm that effect is called reversible effect of the lethal effect by photo reactivation where the visible light with activate cellular enzymes which are called catalyze enzymes or photolysis enzymes that break the double bonds between pyrimidine dimmers that caused by U.V. , so the DNA will returns to its natural state which calls Damage reversal , and on the basis the total number cells after exposure to the visible light immediately after treatment will be output times the number after treatment , with single note which is some of the cells that expose to lethal damage cannot be repaired .

**Materials and Methods**

E.coli , test tubes , nutrient agar , nutrient broth , plates .

1- Attend serial dilutions from bacterial broth (24) hrs.to $10^{-5}$ .

2. Take 0.1 ml of $10^{-5}$ and culture it on nutrient agar by spreading to get viable cell count .

3. Prepare 3 tubes each one of them contains 2ml of $10^{-5}$ and label them by writing the exposure period to U.V. (10,20,30) in wavelength 260nm.

4. The tubes are wrapped by isolator paper to prevent the exposure to the visible light .

5. Take 0.1 ml from each tube and culture them on nutrient agar separately, and warp these plates to prevent photo reactivation.

6. Leave these tubes under visible light without isolator paper for 30min for photo reactivation .

7. Take 0.1 ml from each tube and culture each one of them on nutrient agar and incubated them for 24hrs.

8. After incubation period count the viable colonies as following : Colonies exposed to the radiation before photo reactivation. Colonies exposed to the photo reactivation .

Killing rate of the U.V. exposed cell= (A-B/A)*100

(A): Viable cell rate before radiation treatment (v.c).

(B): Viable exposed cell or resistant cells .