Chapter 2

Theory

2.1 Plasma definition

First and foremost, a plasma is an ionized gas. When a solid is heated sufficiently that the thermal motions of the atoms break the crystal lattice structure apart, usually a liquid is formed. When a liquid is heated enough that atoms vaporize off the surface faster than they re-condense, a gas is formed. When a gas is heated enough that the atoms collide with each other and knock their electrons off in the process, a plasma is formed: the so-called 'fourth state of matter'. But any ionized gas cannot be called a plasma, so we define "a plasma is a quasineutral gas of charged and neutral particles which exhibits collective behavior [16]."

The important point is that an ionized gas has unique properties. In most materials the dynamics of motion are determined by forces between near-neighbor regions of the material. In a plasma, charge separation between ions and electrons gives rise to electric fields, and charged particle flows give rise to currents and magnetic fields. These fields result in 'action at a distance', and a range of phenomena of startling complexity, of considerable practical utility and sometimes of great beauty. Neutral is no net electromagnetic force on it and the force of gravity is negligible. The molecule moves undistributed until it makes a collision with another molecule, and these collisions control the particle's motion [15-17].

2.2 Plasma parameters

Three fundamental parameters characterize a plasma:

1. The particle density n (measured in particles per cubic meter),
2. The temperature T of each species (usually measured in eV, where 1 eV=11,605 K),
3. The steady state magnetic field B (measured in Tesla).

A host of subsidiary parameters (e.g., Debye length, Larmor radius, plasma frequency, cyclotron frequency, thermal velocity) can be derived from these three fundamental parameters. For partially-ionized plasmas, the fractional ionization and cross-sections of neutrals are also important [18].

"Debye shielding" is an important parameter of a plasma. The plasma has its ability to shield out electric potential that is applied to it. This property is related to sheath whose thickness depends on the potential applied. Consider a finite-temperature plasma in a statistically large number of electrons and ions and assume that the ion densities (n_i) and the electron densities (n_e) are initially equal and spatially uniform. Which $n_i \approx n_e$ is called "quasineutrality" and is probably the most important characteristic of a plasma.

The ions and electrons need not be in thermal equilibrium with each other, and so the ions and electrons will have separate Maxwell-Boltzmann distributions with different temperatures by the ion temperature T_i and the electron temperature T_e. That the electron thermal velocity is at least 100 times the ion thermal velocity because of mass so $T_e \gtrsim T_i$.

So, the electron density at position x is

$$n_e(x) = n_0 \exp\left(\frac{e\phi(x)}{KT_e}\right), \qquad (2.1)$$

and the ion density at position x is

$$n_i(x) = n_0 \exp\left(-\frac{e\phi(x)}{KT_e}\right), \qquad (2.2)$$

where e is magnitude of charge on electron (1.6022 x 10^{-19} C),

ϕ is potential,

K is Boltzmann Constant (1.3807 x 10^{-23} J/K).

If we define the Debye length to be the measure of the shielding distance or the thickness of the sheath so we can find the Debye length (λ_D) from

$$\lambda_D \equiv \left(\frac{\varepsilon_0 KT_e}{ne^2}\right)^{1/2}, \qquad (2.3)$$

So, when the density increases, λ_D will decrease and when KT_e increases, λ_D will increase. So we can define λ_D from the electron temperature T_e because of the electrons being more mobile.

2.3 Sheaths

Plasma, which is quasineutral $n_i \approx n_e$, is joined to floating or low-voltage wall surfaces across thin positively charged layers called "sheaths"[19]. We confine our attention to one dimension with no magnetic field as shown in Fig. 2.1. We can let the potential be zero there. When electrons and ions hit the wall, they recombine or are lost when $n_i >> n_e$. Since electrons have much higher thermal velocities than ions, they are lost faster and leave the plasma with the positive charge. The plasma must then have a potential positive with respect to the wall; i.e., the wall or sample holder is potentially negative.

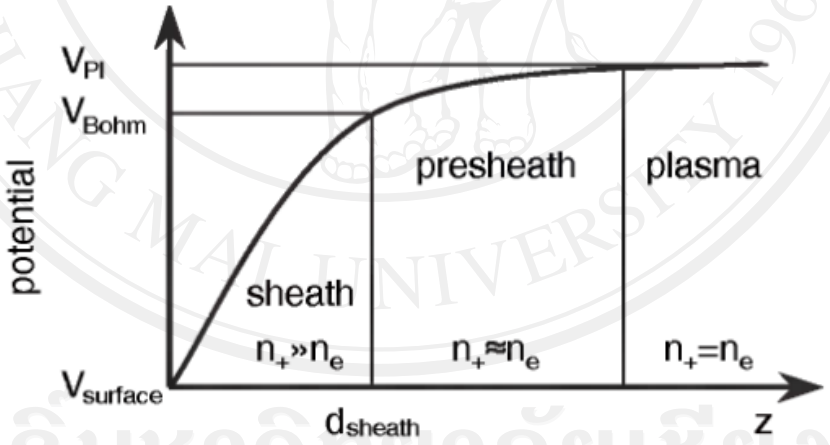

Figure 2.1 Plasma boundary sheath. Typical potential change in dependence on the distance from the surface [20].

2.4 Elements of an RF discharge plasma source

There are a number of ways to generate plasma, such as radio-frequency (RF), DC discharge, microwave, etc. In this study, an RF-driven plasma source is used. There are four main subsystems for the plasma source as shown in Fig. 2.2: the vacuum system, the cooling system, the gas holding system and the discharge power

source. For ion implantation, the plasma system needs another subsystem, the pulse high voltages DC supply.

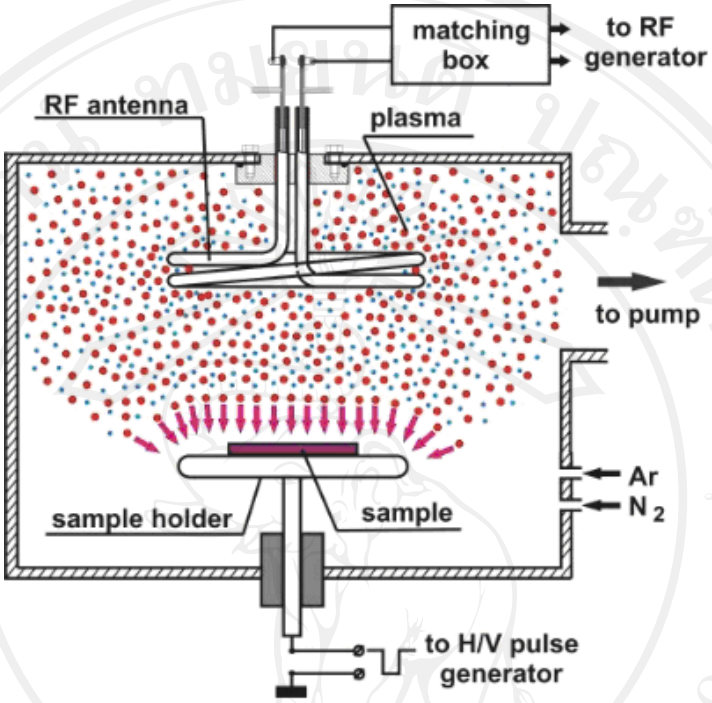

Figure 2.2 Elements of a RF discharge plasma source [21].

Vacuum system

To make plasma, creating a vacuum is very important. The base pressure, before filling gas, is at least 10^{-5} torr for experiment. Vacuum is obtained normally by using a turbomolecular pump or a diffusion pump supported by a rotary pump.

Gas holding system

Gas is the origin material to make a type of plasma. And we can use gases mixed to make the plasma too. The mixture of gases to be used in a process is formed in a gas manifold, into which gases from different tanks are fed through mass flow controller that the flow rate is measured in sccm (standard cubic centimeters per minute), which is the number of cubic centimeters of gas at STP flowing through per minute. The gas is then in put into and processing chamber and pumping will make the equilibrium pressure waiting for applying RF power generators.

Cooling system

The cooling system is only a technical support system. While the plasma processing is started a lot of heat is generated. Many electronic circuits require cooling by the water, air, liquid nitrogen, helium or other techniques.

Discharge power source

Electrical power is applied by radio-frequency (RF), DC discharge or microwave to ionize and heat a plasma. The vast majority of sources use the industrially assigned frequency of 13.56 MHz for RF power. Some work at a harmonic or subharmonic of this, and some experimental sources run at frequencies higher or lower than this range. Electron cyclotron resonance (ECR) sources are driven at 2.45 GHz, the same as used in microwave ovens.

RF discharges are widely used in plasma technologies for thin film deposition and surface treatment. RF sources are usually driven by a solid-state power with a matching network, or matching box, which performs the important function of transforming the impedance of the antenna-plasma system to the impedance of the rest of the circuit. Before passing through the matching network, the power goes through directional couplers which measure the power flowing into the antenna and back from it. This reflection has to be kept low (< 1%) to protect the amplifier and to make the best use of its power. In an inductive discharge, the power goes to an external antenna, which is wound around the chamber in various ways depending on the type of source. In the systems there may be sensors to measure the RF voltage and current applied to the antenna.

The induced electric field generates dense low temperature plasma so called inductively coupled plasmas (ICP). ICP may be operated by the internal antenna or the external antenna as shown in Fig. 2.3–2.4. ICP has many advantages. First no internal electrodes are needed as in capacitively coupled systems, and second no dc magnetic field is required as in ECR reactors and the plasma is in high density and clean. These benefits make ICPs probably the most common plasma tools. These devices come in many different configurations.

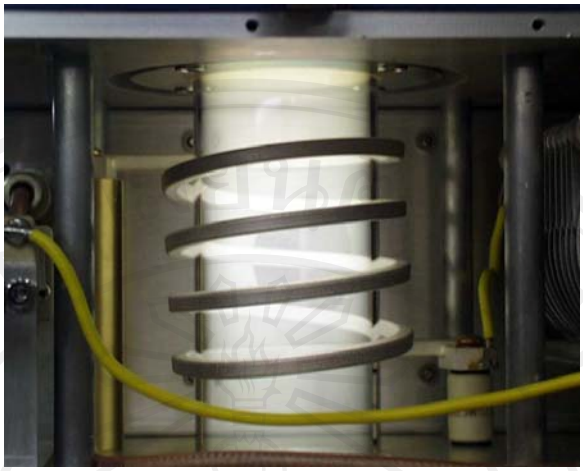

Figure 2.3 The radio frequency electric field is induced in the plasma by an external antenna [22].

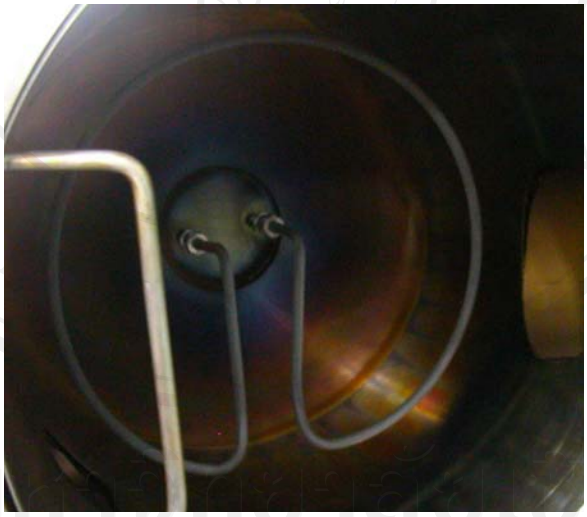

Figure 2.4 An internal antenna inside the chamber that the radio frequency electric field is induced the plasma.

Pulse high voltages DC supply

A plasma immersion ion implantation (PIII) system needs pulse high voltages DC supply for implantation. It is also used for thin film deposition and surface treatment. When a sample is immersed, ion energy is not enough for implantation, so we need DC bias for accelerating ions to implant in the sample.

2.5 Ion energy

Ion energy in a plasma without a bias is only an order of thermal energy, or eV. When a bias voltage U is applied to the sample holder in the plasma, the energy of a singly charged ion is then eU. Since the bias voltage is normally at least kV, the accelerated ion thus has energy of keV. In this case the original ion energy that is in the thermal energy level can be neglected. In our experiment, when nitrogen plasma is applied, the energy of N-ions is complicated. There are both molecular (N_2) and atomic (N) nitrogen ions in the plasma. For a singly charged N-ion, after acceleration with a voltage U, its energy is eU. However, for a singly charged N_2-ion, after acceleration with U, the energy of a molecular ion eU, but because when a N_2-ion impact with the target it immediately dissociate into two N atoms and thus each N-atom gets energy of ½(eU). In N-plasma, N_2-ions are normally dominant while N-ions are in minority. Therefore, after ion implantation using a bias U for N-plasma, the energy of the majority of N-ions is ½(eU) and the energy of the minority of N-ions is eU.

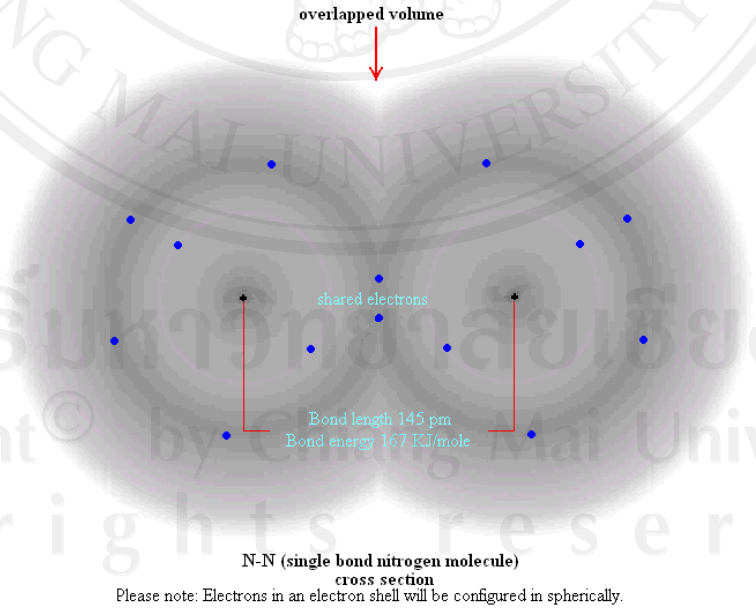

Figure 2.5 The bond energy of nitrogen atom is 167 KJ/mole (2 eV/mole)[23].

2.6 Fluence

The important parameter for ion implantation is fluence. When the ions implanted into the surface of a sample, density of ions per unit area affects property modifications. The thickness of modified surface layer is usually limited to the projected range that depends on ion species, ions energy, target materials and fluence. We define fluence by the ion density per cm^2. Normally the ion a real density can be represented by ion current, which is measured by Faraday cup. For this system, the current is not measured by Faraday cup but measured by the sample holder while bias voltage is applied. In this case, the secondary electron emission coefficient is taken into account. As the chamber and the sample holder are made from stainless steel, the secondary electron emission coefficient used should be of stainless steel. The ion fluence can then be estimated by

$$Fluence = \frac{It}{eA\gamma}, \qquad (2.4)$$

or

$$Fluence = \frac{If\eta}{eA\gamma} \qquad (2.5)$$

where I is the target current in ampere

e is magnitude of charge on electron (1.6022×10^{-19} C)

A is the implantation surface area in cm^2

t is the total implantation time in second

γ is the electron emission coefficient

η is pulse width time in second

f is frequency in hertz

2.7 Fundamentals of ion implantation

2.7.1 Interaction of energetic ions with solid surface

When a primary ion bombards a solid target surface and penetrate inside the solid, various physical processes occur, as shown in Fig. 2.6. At the top surface of solid, some electrons in the target may be emitted due to secondary electron emission. The elastic collisions between ions and the target atom may be back-scattered. When the ions impacts to the target atom the electrons may be excited and fall to the lower level, so it produces X-ray ,visible light, UV and photon emission. When the ions penetrate in solid, momentum and charge are transferred to target atoms. If the collision energy is larger than the critical energy for displacement of the solid atom, solid atom is displaced. A series of collisions and displacements occur in the solid, called a collision cascade. A consequence of the collision cascade is damage to the target material structure, creating vacancies, interstitial, etc. When an ion loses energy continuously until it finally stops below the surface, it is called an implanted ion.

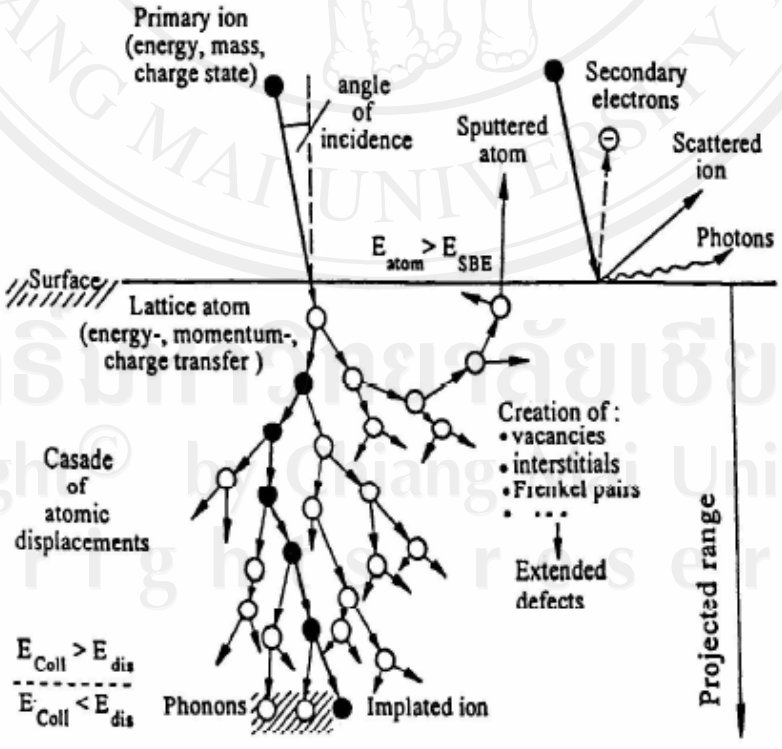

Figure 2.6 Schematic drawing of ions and solid interactions [24].

2.7.2 Stopping cross section

The energy loss during ion penetrating in a solid can be described by the stopping cross section *S(E)*, which is defined by

$$S(E) = -\frac{1}{N}\frac{dE}{dX} \tag{2.6}$$

In a unit of

$$\left(\frac{eV/cm}{atom/cm^3}\right) = \left(\frac{eVcm^2}{atom}\right),$$

where dE is the energy loss of the ion traveling a distance dX

N is the number density of target atoms.

For a two-element target with compositions of A_mB_n, the mean stoping cross section is

$$S^{A_mB_n} = mS^A + nS^B \tag{2.7}$$

(average S per molecule, for compounds)

$$S^{A_mB_n} = \frac{m}{m+n}S^A + \frac{n}{m+n}S^B \tag{2.8}$$

(average S per atom, for mixtures).

2.7.3 Ion implantation range and projected range

From the stopping cross section we can determine the penetration depth or projected range of ions. The total path length R of the implanted ion in solid is calculated by

$$R = \int_0^{E_0} \frac{1}{N}\frac{dE}{S(E)} \tag{2.9}$$

where E_0 is the initial ion energy. The shortest distance from the top surface of solid to the depth where ions stop is called projected range, R_p is defined by

$$R_P = R\cos\phi \tag{2.10}$$

where ϕ is the deviating angle of the incident ion from the normal. Normally, for the case of low energy, there is a relationship between the range and the projected range as shown by

$$\frac{\overline{R}}{\overline{R_P}} \approx 1 + \frac{1}{3}\frac{M_2}{M_1} \qquad (2.11)$$

where $\overline{R}$ is the average range, $\overline{R_P}$ is the average projected range, M_1 and M_2 are the masses of ion and target atom respectively. Fig.2.7 shows the relationship between range and projected range. This is a fairly good approximation for the case of low energies.

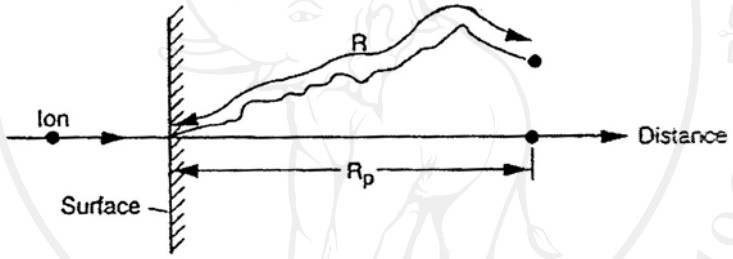

Figure 2.7 Schematic drawing of ion's total path length and its projected range [24].

2.7.4 Radiation damage

Radiation is energy propagation in space. Materials absorbing radiation will undergo structural damage. This kind of damage is called radiation damage. When the ion passes through a solid these two kinds of collision processes occur simultaneously. When the ion energy is higher, inelastic collision dominates, and when the ion energy is lower, elastic collision dominates [25].

When the ions penetrate in solid, momentum and charge are transferred to lattice atom due to collision and causes it to displace from lattice site. This energy is called the displacement energy, E_{dis} , which is at least equal to the displacement threshold of lattice atom. The displaced atom, as shown in Fig. 2.8, leaves a vacancy and forms an interstitial site in lattice. This vacancy interstitial defect is referred to as a Frenkel-pair or Frenkel-defect. A high density cascade occupies a limited volume,

which results in motion of temporary atoms and is defined as spike [26], as shown in Fig. 2.9.

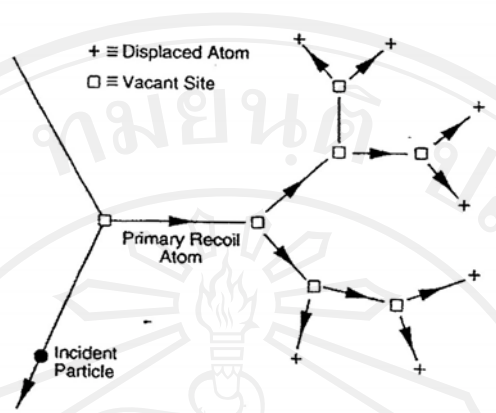

Figure 2.8 Schematic of collision cascade formation by a primary knock-on Atom [27].

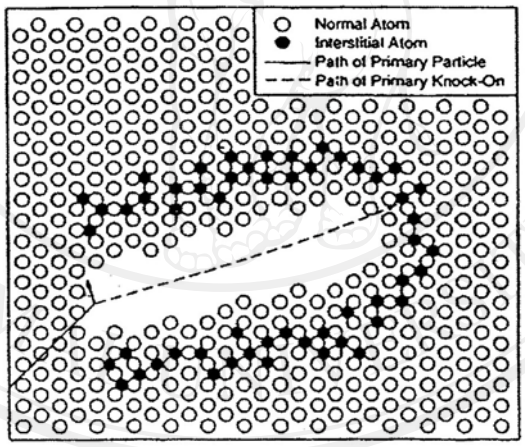

Figure 2.9 Schematic of highly damaged volume to form dense cascade of a displacement spike [27].

2.8 Interaction between energetic ions and biological organisms

The biological organism is a complex system. It is not crystalline, non-crystalline or amorphous but a disordered system. The biological organism is composed of solid, fluid and gas, not as stable as a solid, and it has multiple layers, multiple phases and multiple channel structures. The organism structure is heterogeneous and specific elements are concentrated in some places. There are holes,

tubes and voids which have no stopping powers to ions and the energy loss is discontinuous. Collisions of the energetic particle with the biological organism are random. This makes the ion range greater in biological cells than in solids. Ion and living call interactions are in some ways quite different from conventional ion-solid.

However, in the case of low energy ion implantation, an ion transfers some of its energy to a target atom and is deflected through a large angle, and the energy transferred causes the target atom to be displaced; this is the nuclear collision energy loss.

Ion implantation in biological organism is normally performed under dry vacuum conditions. Although ion implantation may produce molecular radicals, it is not possible, when water solutions participate, for the $OH^{\bullet}$ radicals to form from the radiation induced dissociation of water to bring about indirect damage. For solid-state biological small molecules, ion implantation plays a direct role in causing damage. The results of this action includes (1) breaking of bonds or strands by ionization, excitation or displacement of bio-molecules, (2) formation of new molecules or groups from the recombination of implanted ions, displaced atoms or substrate elemental atoms, and (3) production of free radicals [25].

2.8.1 Biological organism structures

i) Cell structures

Cell is a small bag of liquid, which is the cytoplasm. The cytoplasm is contained within a cell membrane. Cell membrane is a phospholypid bilayer. Inside the membrane, proteins are embedded into the bilipid layer and are more or less free to move around within the membrane. Outside of the membrane, some cells may have additional structures. For instance, many bacterial and plant cells have thick cell walls that confer more rigidity to the cell as well as better defense against mechanical, chemical or biological insults. Some cells also have hair-like cilia on the surface.

In the world cells have two basic types, prokaryotic cell and eukaryotic cell. Prokaryotic and eukaryotic come from Greek words. Prokaryotic means before a nucleus and eukaryotic means possessing a true nucleus [28]. The meanings of their

names are showing the differences between these two cell types. If we look at the comparison of these cells as showing in Fig. 2.10, we see the following differences:

- Eukaryotic cells have a true nucleus, bound by a double membrane. Prokaryotic cells have no nucleus.
- Eukaryotic DNA is linear; prokaryotic DNA is circular.
- Eukaryotic DNA is complexed with proteins called "histones," and is organized into chromosomes but prokaryotic is not. Eukaryotic cells have chromosomes but prokaryotic cell contains only one circular DNA molecule called "plasmids"
- The ribosomes of the eukaryotic cells are larger and more complex than the prokaryotic cell. Eukaryotic ribosome is composed of five kinds of rRNA and about eighty kinds of proteins. Prokaryotic ribosomes are composed of only three kinds of rRNA and about fifty kinds of protein.
- The cytoplasm of eukaryotic cells is filled with a large, complex collection of organelles, many of them enclosed in their own membranes; the prokaryotic cell contains no membrane-bound organelles which are independent of the plasma membrane.
- Membrane, as shown in Fig. 2.11, the head of the phospholipid is polar and hydrophilic (water-loving), and these heads make up the outside of the phospholipid bilayer. The tail of the phospholipid that is located inside the membrane is nonpolar and hydrophobic(water-fearing). Because one end of the phospholipid is hydrophobic and the other is hydrophilic, phospholipids naturally form bilayers in which the heads are facing outward (toward the water), and the tails are facing inward (away from the water). Therefore, the characteristics of phospholipids enable the phospholipids to form a stable structure.

Bacterial *Escherichia coli (E. coli)* used in this study is prokaryotic.

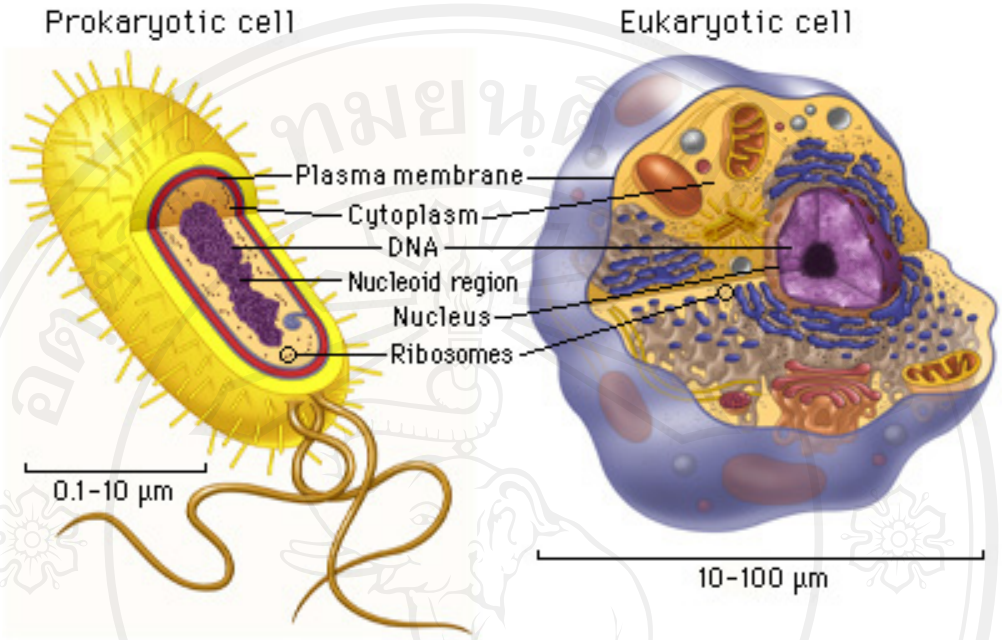

Figure 2.10 The comparison of prokaryotic and eukaryotic cell structure [29].

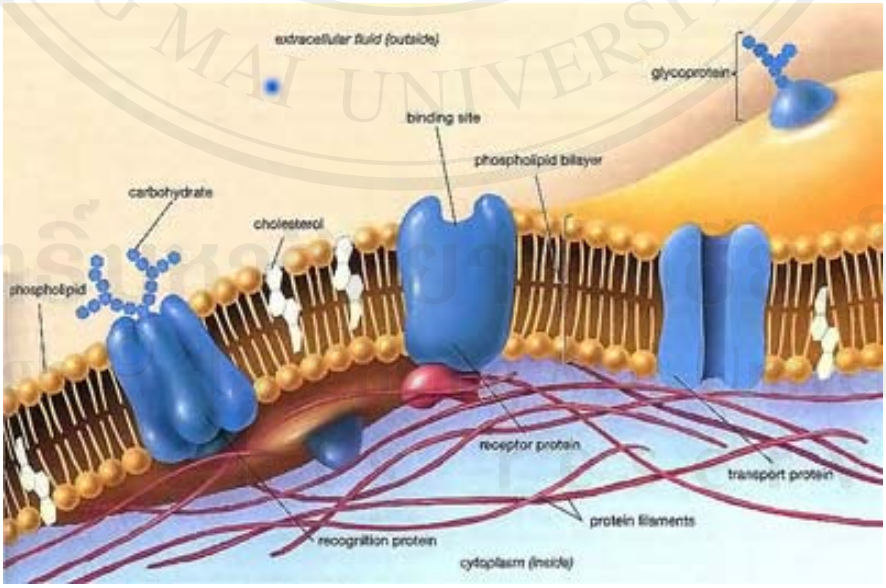

Figure 2.11 Diagram show the fluid mosaic model of a biological membrane [30].

ii) DNA structures

A DNA molecule is composed of two poly-deoxyribonucleotide strands. The backbone of each strand is made from a connection between phosphodiester and two nucleoside furan β-D-deoxyriboses via 3' and 5' bonds. Both strands are dextrogyrate. They wind in opposite direction around the same axis, forming a dextrogyrate double helical structure. The helix rise per base pair is 3.4 Å. Every ten nucleotides form a helical turn and the height of each turn is 34 Å. The bases are inside the helix with their planes perpendicular to the helical axis. Phosphates are outside. The average diameter of the helix is 20 Å. The two strands are linked by hydrogen bonds between base pairs.

The spatial arrangement of base pairs is remarkably specific. Each adenine residue is paired with a thymine residue (A=T) by two hydrogen bonds, and each guanine residue is paired with a cytosine residue (G≡C) by three hydrogen bonds. Since the bases from two strands are on the same plane, they must be adapted to the phospho-deoxyribose backbone. Thus a base pair must be composed of a purine and a pyrimidine. At the positions opposite to purine must be pyrimidine, and at positions opposite to pyrimidine must be purine as shown in Fig.2.12. However, note that adenine (A) and cytosine (C) cannot form a base pair, neither can guanine (G) and thymine (T) form a hydrogen-bonded pair. This means that on the position opposite to A must be T and on the position opposite to G must be C. The total number of the purine bases in a DNA molecule is the same as the number of pyrimidine bases. This is determined by the strict pairing rule between two single strands in the DNA molecule. But on each strand the base types in front and behind are not controlled by any rule. Hence in a DNA molecule containing a large number of bases, four bases are arbitrary and their arrangements are unlimited, and thus the genetic information that can be carried is extremely large. However, the versatile structure is maintained in the replication process only if the DNA molecule suffers no damage [25].

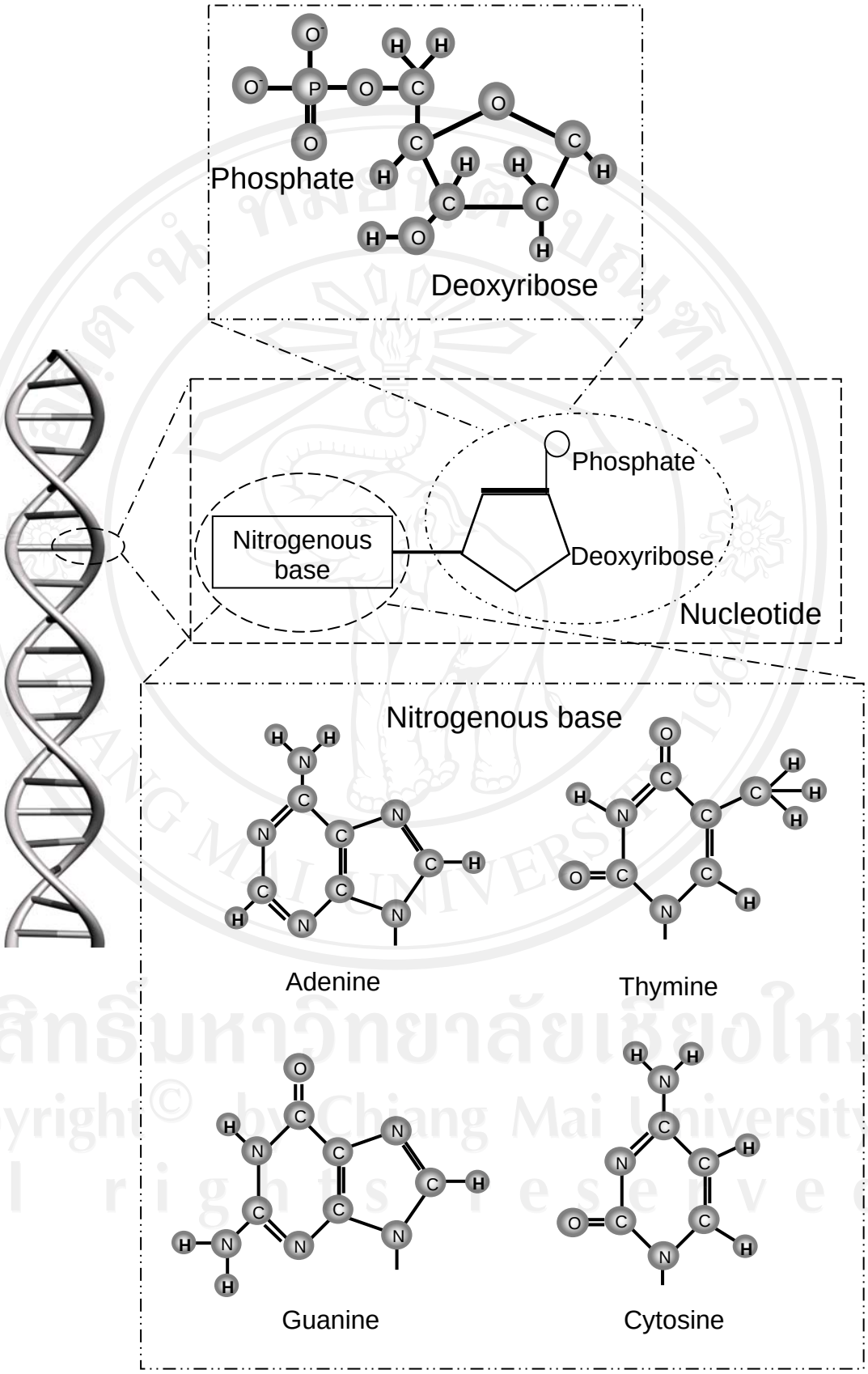

Figure 2.12 Molecular structure of DNA.

iii) Plasmid DNA used

Plasmid DNA is a kind of circular DNA molecule that can autonomously replicate in the host cells and is often used as a gene bearer. This naked DNA can suffer from radiation damage under ion implantation. The naked DNA in this thesis is pGFP(plasmid green fluorescent protein). Green fluorescent protein (GFP) is a naturally fluorescent protein first isolated from the jellyfish Aequorea victoria. Because GFP can be functionally expressed in bacteria and nematodes, it has opened exciting new avenues of investigation in cell, developmental and molecular biology. Now GFP can be developed in several types such as in particular blue fluorescent protein (EBFP, EBFP2, Azurite, mKalama1), cyan fluorescent protein (ECFP, Cerulean, CyPet) and yellow fluorescent protein derivatives (YFP, Citrine, Venus, YPet).

Structure of GFP, as shown in Fig. 2.13, is β-barrels with 11 strands on the outside of cylinders. These cylinders have a diameter of about 30 Å and a length of about 40 Å. Inspection of the density within the cylinders revealed the fluorescent center of the molecule, a modified tyrosine side chain and cyclized protein backbone as a part of an irregular a-helical segment. Small sections of a helices and loops also form caps on the ends of the cylinders. This motif, with a single a helix inside a very uniform cylinder of β -sheet structure, represents a new protein class, which we have named the β-can [31].

The plasmid DNA forms can be observed by electrophoresis. We found three forms of an uncut plasmid with all of the same size and molecular weight as shown in Fig 2.14. They do not behave the same way during electrophoresis. Because the SUPERCOILED molecules are more compact, they move more easily through the gel, and are found farther. The RELAXED CIRCULAR molecules and the FULL-LENGTH LINEAR molecules usually run very close together and are not always resolved from one another. When they do run as separate bands, either relaxed circles or linears may run faster in the gel, so the DNA fragments typically used as standards on agarose gels are linear molecules for compared to these experiment and the sizes of unknown fragments are determined.

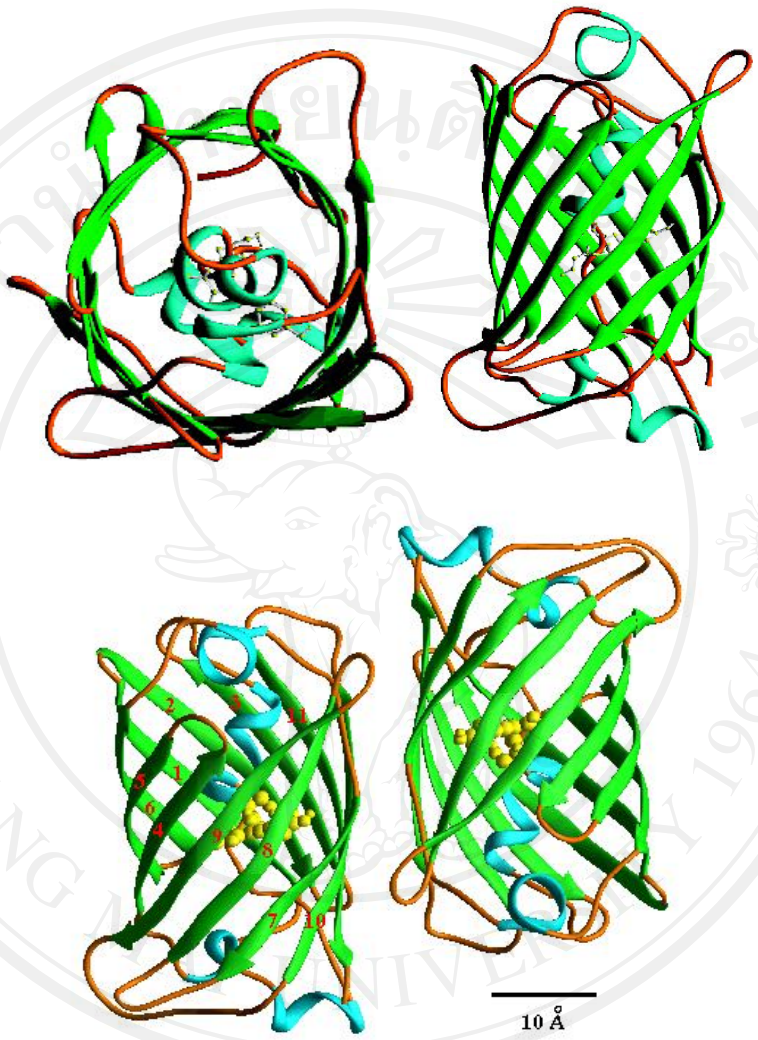

Figure 2.13 The overall shape of the protein and its association into dimers. Eleven strands of -sheet (green) form the walls of a cylinder. Short segments of helices (blue) cap the top and bottom of the ' β -can' and also provide a scaffold for the fluorophore which is near geometric center of the can. This folding motif, with -sheet outside and helix inside, represents a new class of proteins. Two monomers are associated into a dimer in the crystal and in solution at low ionic strengths. This view is directly down the two-fold axis of the non-crystallographic symmetry [31-32].

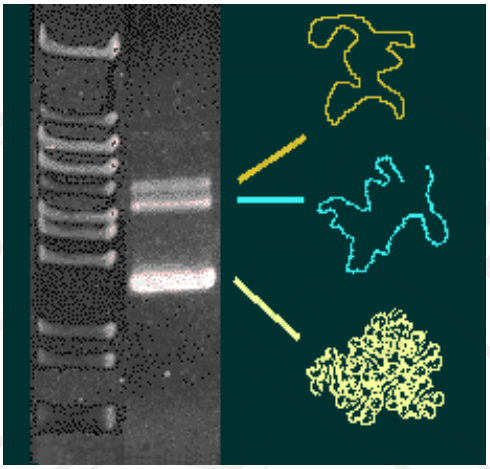

Figure 2.14 There are three forms of the plasmid DNA: supercoiled, relaxed circular, full-length linear supercoiled [33].

2.8.2 Charge exchange effect

The surface of a biological contains negative ion groups that can be observed to move to the anode during electrophoresis. In the case of interaction between energetic ions and biological organisms, energy deposition plays the role of ionizing radiation in damaging the ionized groups on the biological organism surface.

As the incident ion loses energy in the target material, every collision with target atoms has a certain probability for loss or capture of electrons from the target material. This phenomenon is called the charge exchange effect of the incident ion. If the ion has charge state *i* before the charge exchange collision and *f* after the collision, the reaction cross section for loss or capture of electrons is σ_{if}. The process of electron capture or loss in a collision is most important. The cross section is strongly dependent on the incident ion velocity and charge number, but almost independent of the charge number of the target material [25].

2.8.3 Production of free radicals

Free radicals are independently existing atoms or a group of atoms which are contain unpaired electrons. In radiobiology is important because in many radiation

processes the primary products are free radicals that can become an active factor in secondary reactions. These indirect interactions cause damage to biological molecules, particularly to genetic substances, and are one of the most important factors in genetic mutation.

Free radicals are produced in the collision volume when energetic ions are implanted into biological organisms, due to excitation and ionization of the target molecules. The ways in which free radicals are formed can be summarized as follows:

Dissociation of an excited molecule:	$AB^{*} \rightarrow A^{\circ} + B^{\circ}$,
Dissociation of an excited ion:	$(A^{+})^{*} \rightarrow R^{\circ +} + S^{\circ}$,
Excited molecule - molecule reaction:	$A^{*} + RH \rightarrow AH + R^{\circ}$,
Dissociation due to capture of a slow-electron:	$AB + e \rightarrow A^{\circ} + B^{-}$
Ion - molecule reaction:	$RH^{+} + RH \rightarrow RH_2 + R^{\circ}$,
Dissociation due to ion neutralization:	$A^{+} + A^{-} \rightarrow A^{*} + A$,
	$\rightarrow R^{\circ} + S^{\circ}$.

A, B: neutral atom or molecule

R, S: alkyl group is a group of carbon and hydrogen atoms derives from an alkane molecule by removing one hydrogen atom. When a functional group is joined with an alkyl group, replacing the hydrogen that was removed, a compound is formed whose characteristics depend largely on the functional group.

A^{*}: excited atom or molecule.

A°: free radical atom or molecule.

2.8.4 Mass and energy deposition

The radiation causes damage to the organism structure. If the damage cannot be repaired the DNA duplication will induce mutation. Actually, the radiation induced damage to the body has long been concerned and the cosmic ray particles from the universe are always deposited in biological organisms.

Based on the same principle of the ion implantation into biological organisms, when the ion energy is decreased down to less than 20 eV, ions are deposited [25]. Then the deposit concentration increases, and the deposits are

competed with the substrate for free radicals and competitive reactions. For the biological organisms and inorganic materials dispersed in the water, the acid-alkali balance should also be considered when ion deposited in to the biological organisms. The acid-alkali level of a solution is can describe by the pH value.

2.8.5 Structural damage

A nucleotide molecule, has a phosphate base, and is an ampholite, which is unstable in acid solution but very stable in alkaline and neutral solution. One of the important damage mechanisms to a nucleotide molecule under ion implantation is dephosphorylation. Various nucleotides have different sensitivities to ion implantation induced damage, namely, 5'-dTMP > 5'-CMP > 5'-GMP > 5'-AMP. Generally, pyrimidine nucleotides are more sensitive to ion implantation damage than purine nucleotides. For nucleosides, the sensitivities to ion beam irradiation are found in an order of dTR>GR>AR>CR>UR [25].

2.8.6 DNA damage and mutation

DNA damage in environment factor occurs everyday. Physical DNA damage can be recognized by enzyme, thus they can be correctly repaired if redundant information is available for copying. If the cell retain DNA damage replications may be blocked and/or the cell may die. There are four types of DNA damage.

1. All four base of DNA can be covalently modified at various positions.
2. Mismatches of normal base because of a failure of proofreading during DNA replication.
3. Crosslink covalent linkages can be formed between base on the same DNA strand or on the opposite stand.
4. Breaks in the backbone can be limited to one of two strands or on both strands and ionizing radiation is a frequent cause, but some chemicals produce break as well.

In the case of ion implantation, making DNA molecules to damage is normally breaking. Low energy ion implantation can damage the nucleotide by breaking the bonds between oxygen and phosphate groups then the damage is single strand break. It can be recognized by enzyme. If it cannot be recognized by enzymes once the base change is present in both DNA strands, DNA damage cannot be repaired and then mutation happens as shown in Fig. 2.15. Although the damage is double strand break, there are two mechanisms by which the cell attempts to repair a complete break in DNA molecule. Direct joining of the broken ends is recognized by proteins binding the exposed end together and homologous recombination is repaired using the information on the intrac sister chromatid or homologous chromosome or same chromosome. Thus in the repairing process there may be some mistakes to lead to this mutation happening.

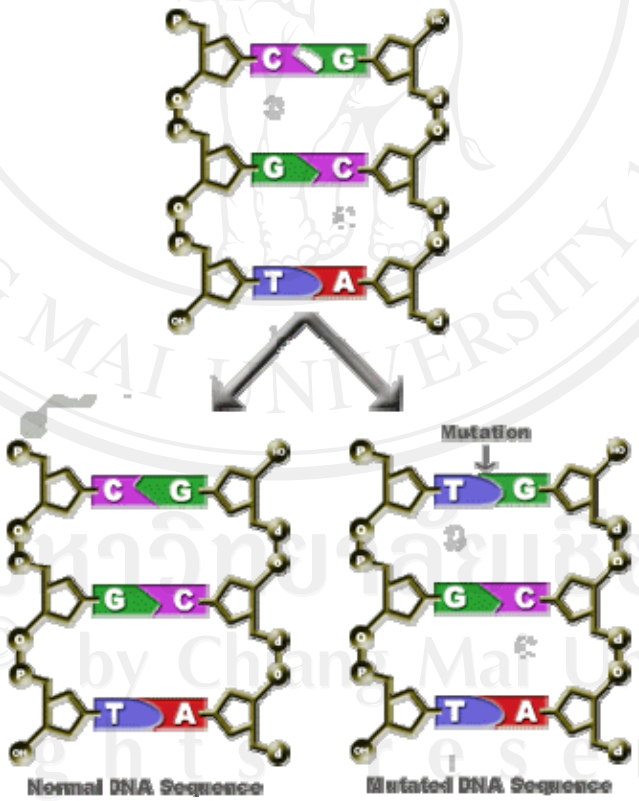

Figure 2.15 There are breaking the phosphate [34].

There are varieties of mutations and all of them affect the sequence of DNA. We can separate mutation types as follows.

Point mutation: This is a single change in one nucleotide (Fig. 2.16): an A might change to a C, for example, or a C to a G. Sometimes a single nucleotide change will not have any effect. Other times, it can change the type of amino acid that is added to the protein, or it can lead to a shortened (truncated) protein. Sickle cell anemia is an example of a disease caused by point mutations.

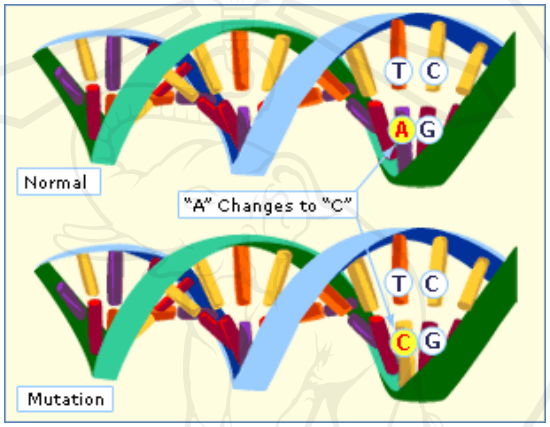

Figure 2.16 Point Mutation: this is a single change in one of the four nucleotide bases. An" A " might change to a" C " for example [35].

In this case we can separate point mutation into three types:

1) Missense mutation : point mutation that translation process changed the type of amino acid in polypeptide thus in this mutation we found one disorder in that location such as the original base is GCA (Ala) changed to GAA (Glu) which in second order of codon changed result is amino acid changed from Ala to Glu in polypeptide.

2) Silent mutation : point mutation is translation process cannot changed the type of amino acid in polypeptide thus in this mutation we found the same order in that location such as the original base is GCA (Ala) changed to GCG (Ala) which in third order of codon changed but the amino acid is not changed in polypeptide.

3) Nonsense mutation : point mutation that translation process changed the type of amino acid in to stop codon which translation process stopped so polypeptide shorter than the original such as the original base is TGG (Trp) changed to TGA that

translated to mRNA (UGA), that mean stop codon, result is translation process stopped.

Deletion: Part of the DNA sequence anywhere from one nucleotide to a large section of DNA is missing (Fig. 2.17). Deletions can cause the cell to use the wrong amino acids to build the protein. Deletions can also shorten the protein. In some cases, a chunk of the protein will be left out. Many cases of Duchenne muscular dystrophy are caused by deletions.

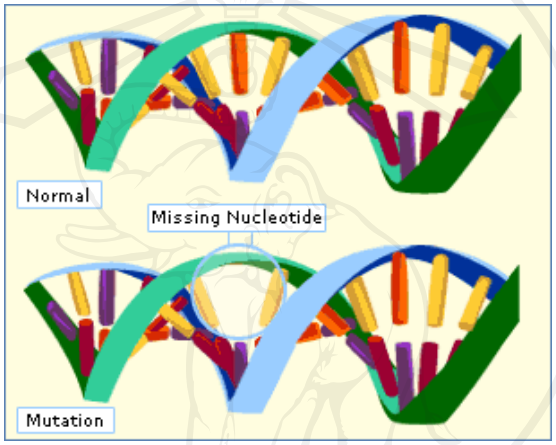

Figure 2.17 Deletion: part of the DNA sequence is missing [35].

Insertions: Extra DNA is added to the normal DNA sequence (Fig. 2.18). Similar to deletions, addition of DNA sequence can cause the cell to use the wrong amino acids to build the protein or shorten the protein. In some cases, the protein will have extra amino acids, which could affect protein function.

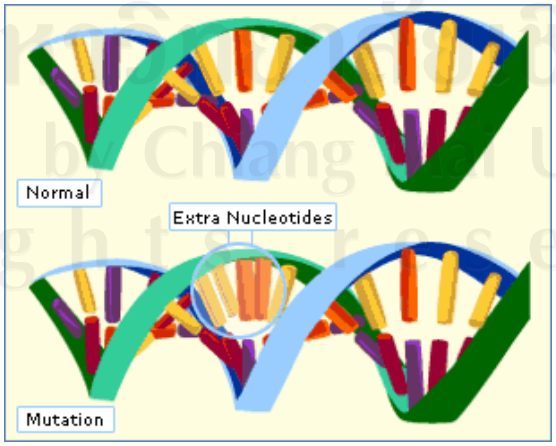

Figure 2.18 Insertion: extra nucleotides are added to the sequence [35].

Inversion: A portion of the DNA sequence is reversed. Inversions can be small or large, and can affect one or more genes. The bleeding disorder hemophilia A is caused by an inversion in the Factor VIII (F8) gene. The effects of an inversion can be similar to those of a deletion or insertion as shown in Fig. 2.19.

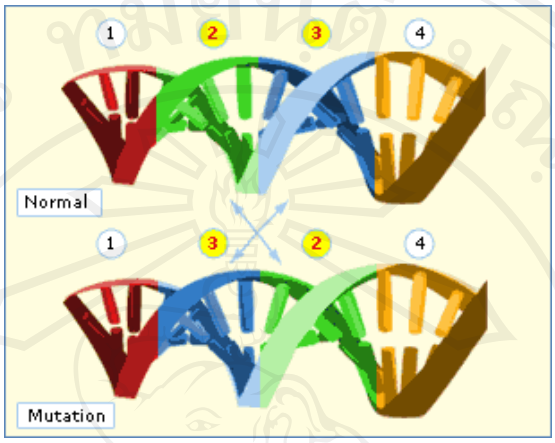

Figure 2.19 Inversion: a portion of the DNA sequence is reversed [35].

2.8.7 Technique for analyzing DNA damage

Electrophoresis

Electrophoresis is a simple technique for analyzing DNA damage because this technique is used for separation of deoxyribonucleic acid, ribonucleic acid, or protein molecules. Biological molecules have an electric surface charge. By placing the molecules in wells in the gel and applying an electric current in a non-conducting medium, the molecules will move through the matrix at different rates (Fig. 2.20), usually determined by mass, form, medium, gel and voltage, toward the positive anode if negatively charged or toward the negative cathode if positively charged.

Figure 2.20 The molecules moving in solution of an electric field [36].

The agarose gel electrophoresis is easy and suitable to analyze DNA damage. The various sized DNA fragments that are present in some but not all of the samples as shown in Fig. 2.21. After the electrophoresis is completed, the molecules in the gel can be stained to make them visible by using ethidium bromide. If the analyzed molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions.

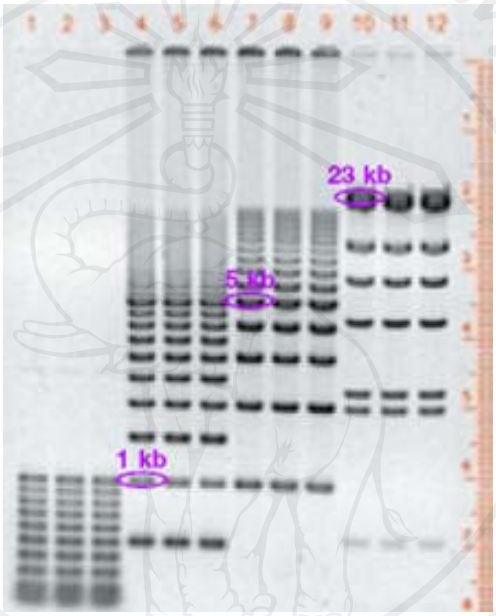

Figure 2.21 The molecules will move through the matrix at different rates [37].

DNA sequencing

The purpose of sequencing is to determine the order of the nucleotides of a gene. The most popular sequencing is called the dideoxy method or Sanger method (named after its inventor, Frederick Sanger, who was awarded the 1980 Nobel prize in chemistry [his second] for this achievement).

DNA sequencing is a complex nucleotide-sequencing technique including three identifiable steps (Fig 2.22):

- Polymerase Chain Reaction (PCR)
- Sequencing Reaction
- Gel Electrophoresis & Computer Processing

Chromosomes, which range in size from 50 million to 250 million bases, must first be broken into much shorter pieces (PCR step). Each short piece is used as

a template to generate a set of fragments that differ in length from each other by a single base that will be identified in a later step (template preparation and sequencing reaction steps). The fragments in a set are separated by gel electrophoresis (separation step). New fluorescent dyes allow separation of all four fragments in a single lane on the gel. The final base at the end of each fragment is identified (base-calling step). This process recreates the original sequence of As, Ts, Cs, and Gs for each short piece generated in the first step. Current electrophoresis limits are about 500 to 700 bases sequenced per read. Automated sequencers analyze the resulting electropherograms and the output is a four-color chromatogram showing peaks that represent each of the 4 DNA bases.

The fluorescently labeled fragments that migrate through the gel, are passed through a laser beam at the bottom of the gel. The laser exits the fluorescent molecule, which sends out light of a distinct color. That light is collected and focused by lenses into a spectrograph. Based on the wavelength, the spectrograph separates the light across a CCD camera (charge coupled device). Each base has its own color, so the sequencer can detect the order of the bases in the sequenced gene.

Fig. 2.23 shows that after the bases are "read," computers are used to assemble the short sequences (in blocks of about 500 bases each, called the read length) into long continuous stretches that are analyzed for errors, gene-coding regions, and other characteristics [38].

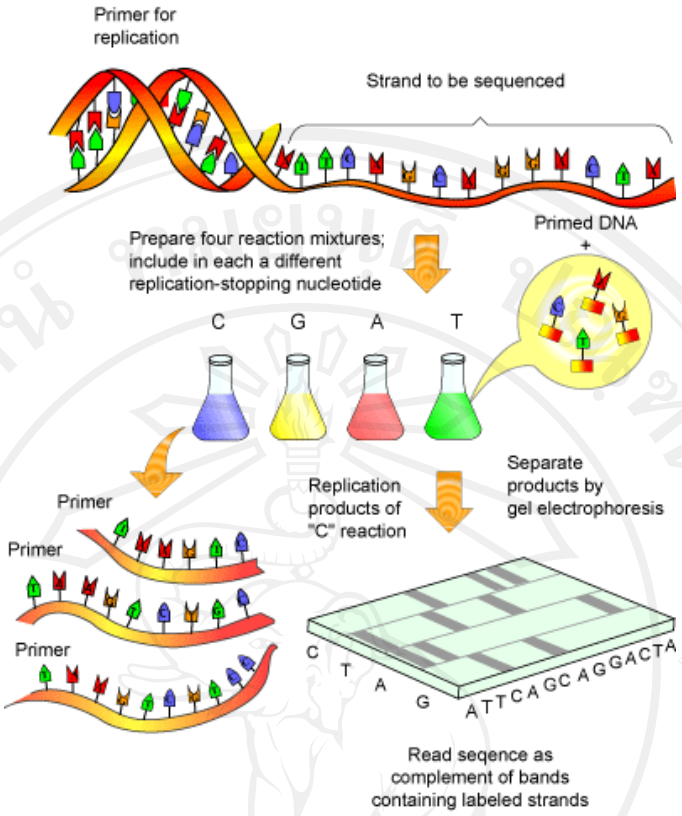

Figure 2.22 The DNA sequencing process [39].

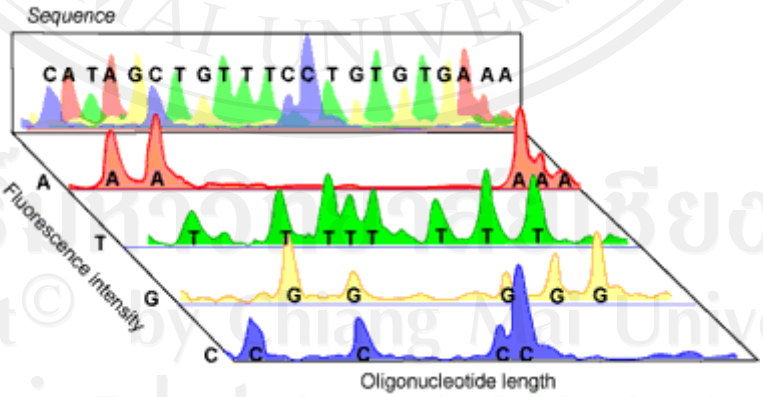

Figure 2.23 Computer programs are used to assemble the short sequences for analysis [39].