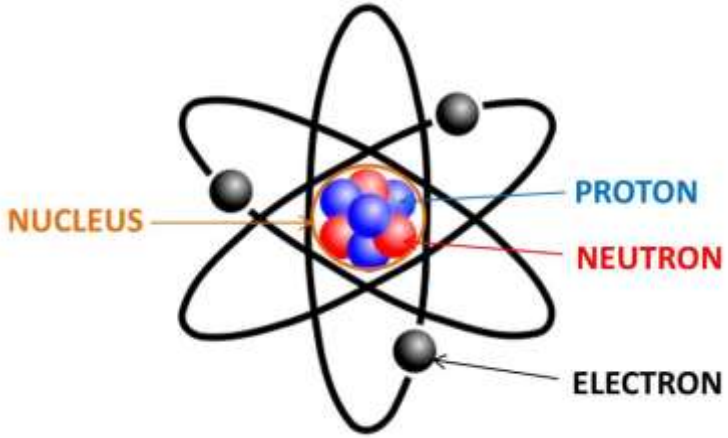
DNA DETECTION

- Radioactivity and stable isotopes
- Rate of Radioactive decay with units
- Geiger-Muller Counter

An atom is composed of positively charged nucleus that is surrounded by negatively charged electrons.

- **The number of electrons is equal to the number of protons = Atomic Number**
- **Sum of protons and neutrons present in the nucleus = Atomic mass**



Strong nuclear forces holds the positive protons and neutral neutrons together in the nucleus.

In stable nuclei, nuclear force is strong enough to hold the nucleus permanently.

Unstable nuclei (either have too many neutrons or too many protons): These unstable nuclei balance themselves by giving off the excess protons or neutrons. This is called radioactive decay. Unstable nuclei are radioactive and emits radiation.

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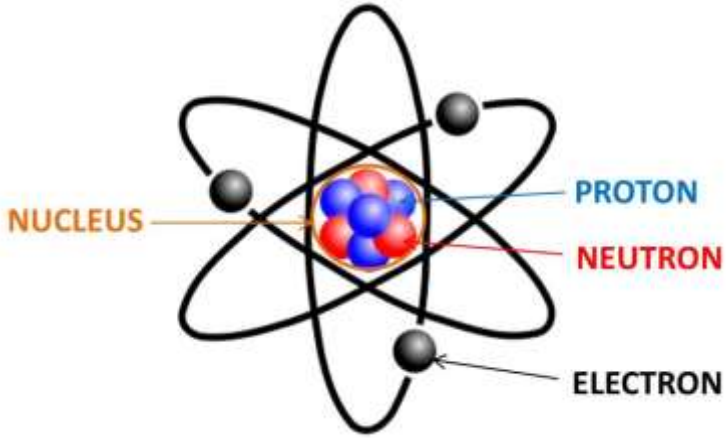

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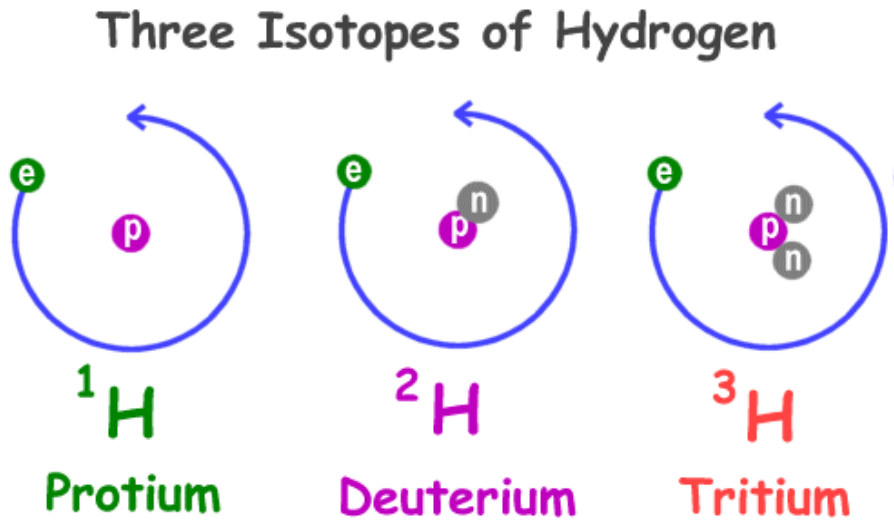
Atoms that have the same amount of protons but differs in neutrons amount are called isotopes.



For example, carbon-12, carbon-13, and carbon-14 are three isotopes of the element carbon with mass numbers 12, 13, and 14, respectively. The atomic number of carbon is 6, which means that every carbon atom has 6 protons so that the neutron numbers of these isotopes are 6, 7, and 8 respectively.

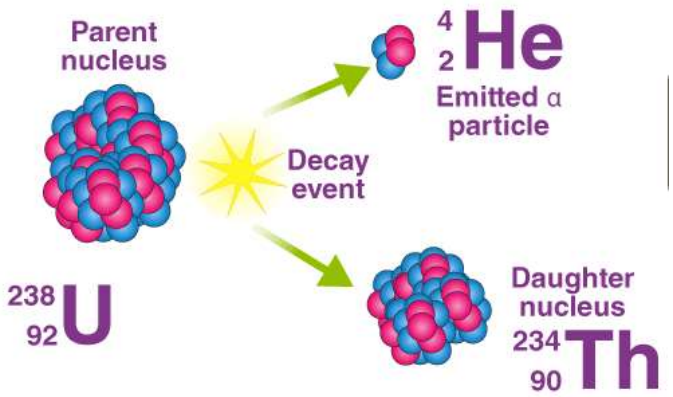
The spontaneous degradation of nucleus & transmission of one element to another with consequent emission of rays (or) particles is known as radioactivity.

Three types of Decay:

1. Alpha (α) Decay or alpha radiation : Release of α particle ($_2He^4$)

Parent nucleus ⟶ **Daughter nucleus + alpha particle**

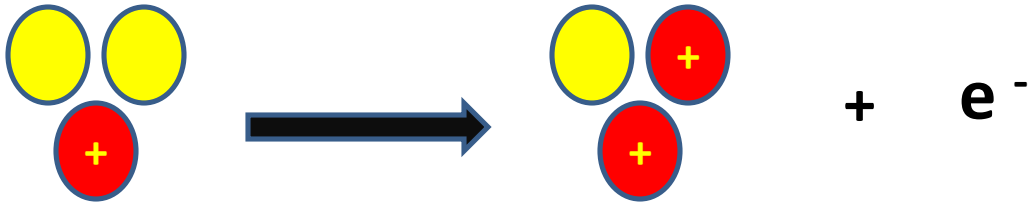
An alpha particle is identical to the nucleus of a normal Helium atom i.e., doubly ionized helium atom (He^{2+})



When an atom emits an alpha particle in alpha decay, the **atom's mass number decreases by four** .

The **atomic number of the atom goes down by two**, as a result of the loss of two protons – the atom becomes a new element.

2. Beta (β^-) Decay or beta radiation (too many neutrons)



Lost high energy electron is called β particle.
Atomic mass will be same, however, atomic number will be enhanced by one.
New element is formed; one place higher in the periodic table.

Example: The decay of Carbon 14

$$^{14}_{6}C \rightarrow ^{14}_{7}N + ^{0}_{-1}\beta^-$$

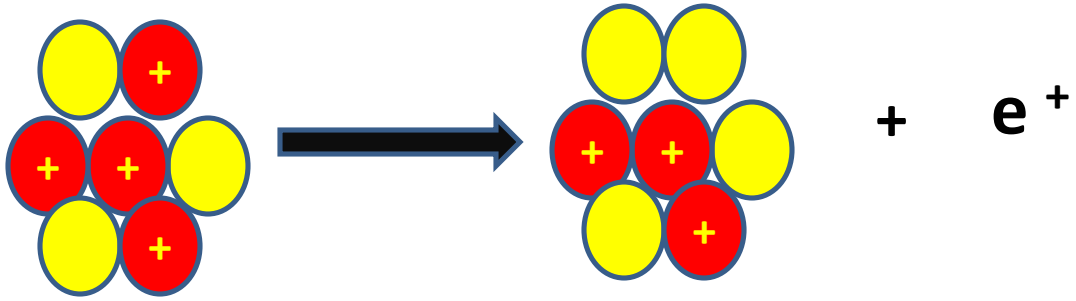
Carbon 14 decays to **Nitrogen 14** plus a **beta particle**.

Notes:

1. The beta particle, being negatively charged, has an effective atomic number of minus one.

2. The beta particle can also be notated as: $^{0}_{-1}e$

3. Beta (β^+) Decay or positron radiation (too many protons)



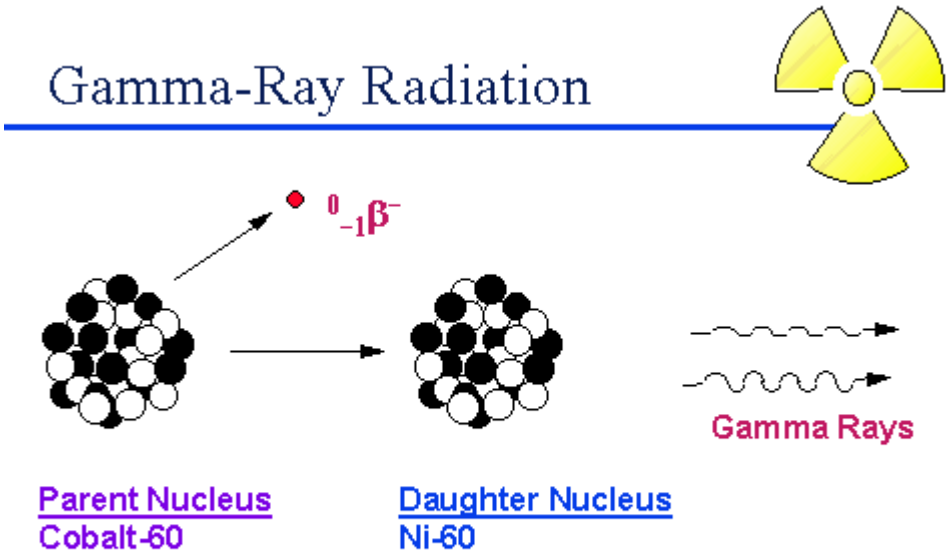
Beta-plus decay (also called positron emission) occurs when a proton is converted to a neutron, and in the process, emits a positively charged electron (a positron).
A positron is a particle identical to an electron except that it has a positive charge

Atomic number decreases by one and mass number remains the same . One place lower in the periodic table.

$$^{11}_{6}C \longrightarrow {}^{0}_{+1}e + {}^{11}_{5}B$$

Gamma Radiation

- A gamma ray is a high-energy photon emitted by a radioisotope.
- Often are emitted along with alpha and beta particles.
- Gamma radiation doesn't have a positive or negative charge.
- Gamma rays are similar to X-rays, but they have even greater energy.
- Gamma radiation can only be stopped by a thick layer of lead or concrete.



Other examples:

$$^{234}_{90}Th \rightarrow {}^{234}_{91}Pa + {}^{0}_{-1}\beta + {}^{0}_{0}\gamma$$

$$^{234}_{91}Pa \rightarrow {}^{234}_{92}U + {}^{0}_{-1}\beta + {}^{0}_{0}\gamma$$

$$^{60}_{27}Co \longrightarrow {}^{60}_{28}Ni + {}^{0}_{-1}e + \gamma$$

(gamma rays distroys cancer cells)

The SI unit of radioactive activity is the becquerel (Bq), in honor of the scientist Henri Becquerel. One Bq is defined as one transformation, decay, or disintegration per second. Since sensible sizes of radioactive material contain many atoms, a Bq is a tiny measure of activity; amounts giving activities on the order of GBq (gigabecquerel, 1×10^9 decays per second) or TBq (terabecquerel, 1×10^{12} decays per second) are commonly used.

Another unit of radioactivity is the curie, Ci, it is equal, by definition, to the activity of any radionuclide decaying with a disintegration rate of 3.7×10^{10} Bq, so that 1 curie (Ci) = 3.7×10^{10} Bq.

Counts per minute (cpm) is a measure of radioactivity. It is the number of atoms in a given quantity of radioactive material that are detected to have decayed in one minute. Disintegrations per minute (dpm) is also a measure of radioactivity.

Radioactivity measurement:

Geiger-Muller counter (G-M Counter)

- The Geiger Muller counter (Gas based counter) is an instrument used for measuring ionizing radiation.
- It detects ionizing radiation such as alpha particles, beta particles and gamma rays using the ionization effect produced in a Geiger–Müller tube.
- It is one of the best-known radiation detection instruments

Readout: Count per second
Absorbed dose

Radioisotopes which are commonly used in the biological research

^{32}P, ^{33}P, ^{131}I, ^{35}S, ^{14}C, ^{45}Ca, ^{3}H

Labeling and Detection of Nucleic acid

In molecular biology, hybridization (or hybridisation) is a phenomenon in which single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to complementary DNA or RNA.

Though a double-stranded DNA sequence is generally stable under physiological conditions, changing these conditions in the laboratory (generally by raising the surrounding temperature) will cause the molecules to separate into single strands. These strands are complementary to each other but may also be complementary to other sequences present in their surroundings. Lowering the surrounding temperature allows the single-stranded molecules to anneal or "hybridize" to each other.

Nucleic acid hybrids can be formed between two strands of DNA, two strands of RNA or one strand of DNA and one of RNA.

Nucleic acid hybridization with a labeled probe is the only way to detect a Complementary target sequence in a complex nucleic acid mixture.

Nucleic acid probes are oligonucleotides or polynucleotides that can bind with high specificity to complementary sequences.

Probes can be complementary to either DNA or RNA and can be from as few as 20 nt to hundreds of nt long.

- **DNA probes**
- **RNA probes**
- **Oligonucleotide probes**

In molecular biology, a nucleic acid probe is a fragment of DNA or RNA which can be radioactively or fluorescently labeled. HP can be used to detect the presence of nucleotide sequences in analyzed RNA or DNA that are complementary to the sequence in the probe.

The labeled probe is first denatured (by heating or under alkaline conditions such as exposure to sodium hydroxide) into single stranded DNA (ssDNA) and then hybridized to the target ssDNA (Southern blotting) or RNA (northern blotting) immobilized on a membrane or in situ.

❖DNA probes

DS OR SS

❖RNA probes

SS

❖Oligonucleotide probes

SS

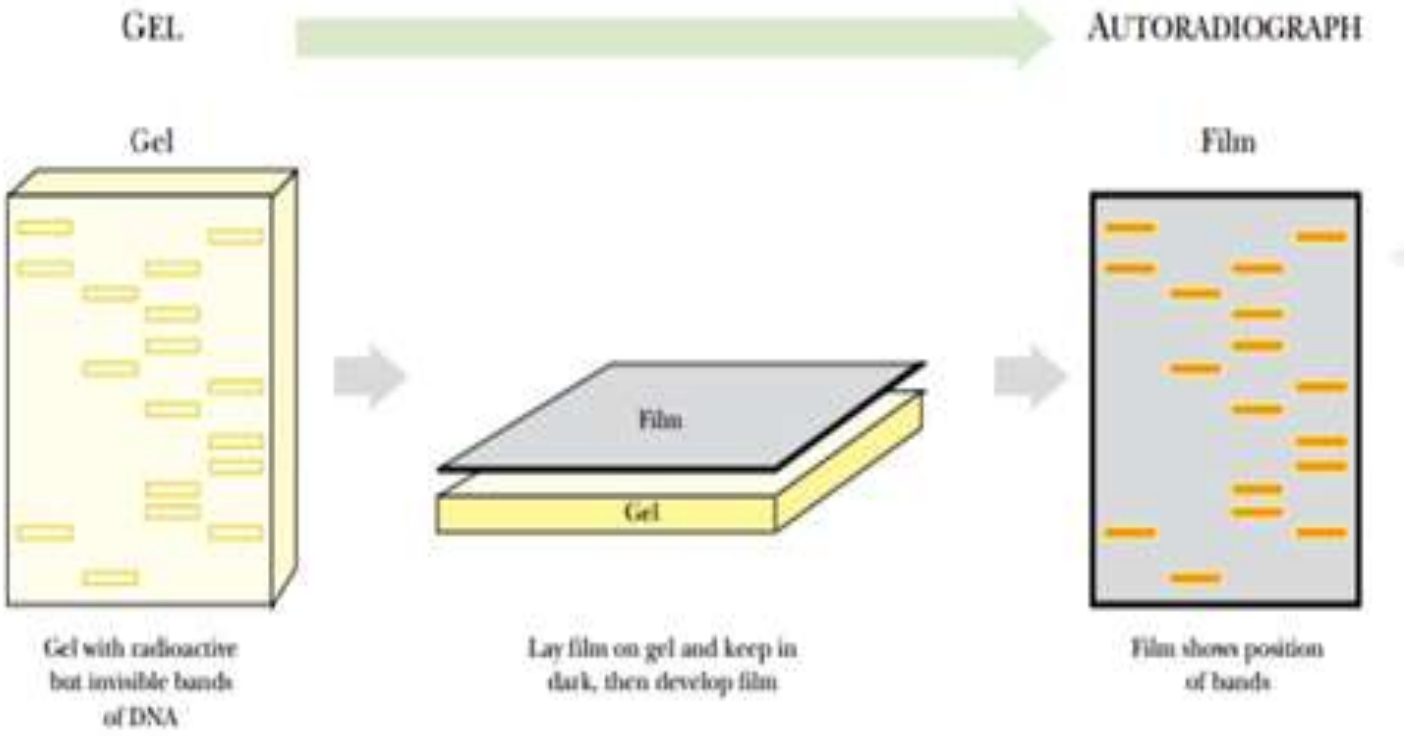
1. Heterologous Probe: Probe that is similar, but not exactly the same

Mouse probe can be used to search a human genomic library.

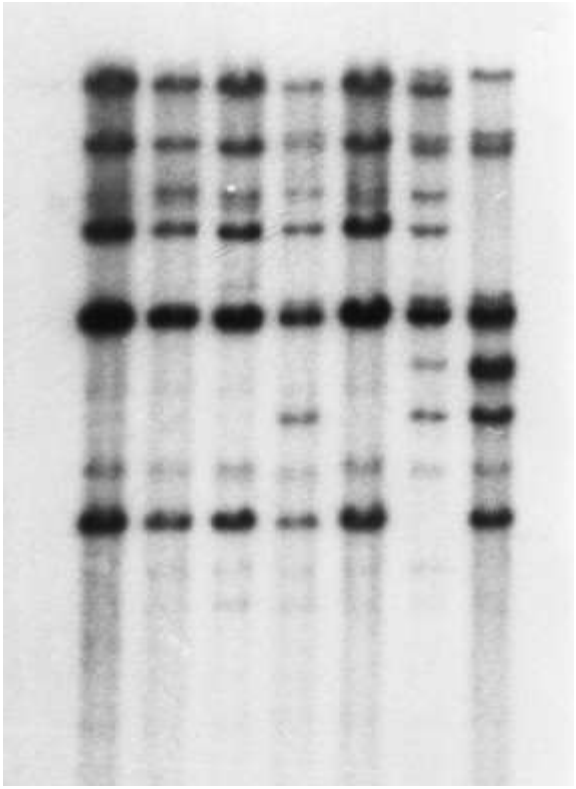
2. Homologous: Probe that is exactly complementary to the nucleic acid sequence of interest

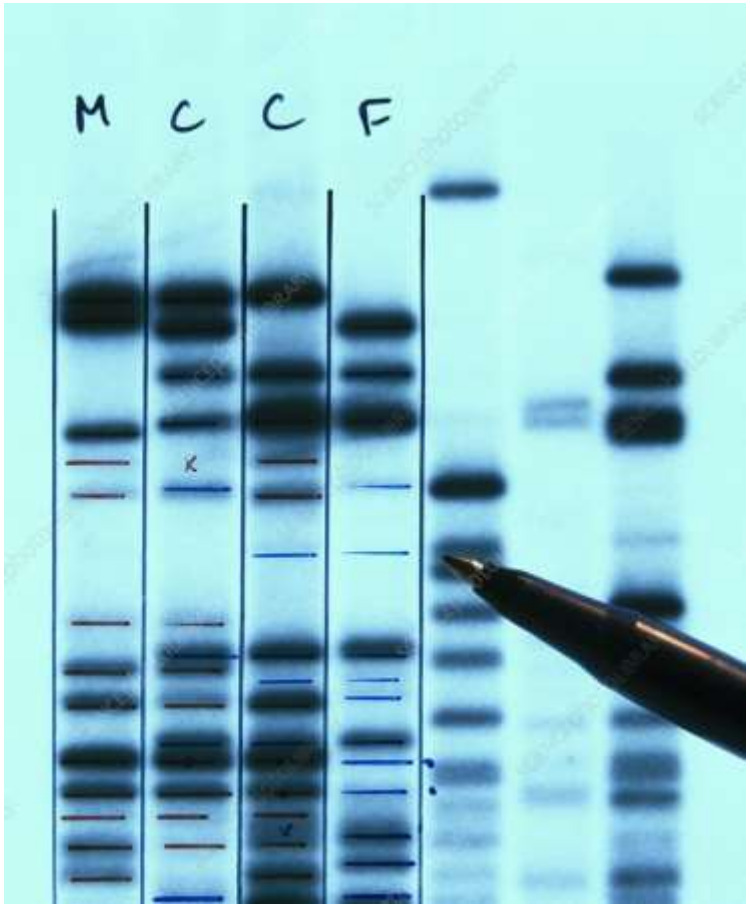
The labeling with radioisotopes or radioactive isotopes is called radiolabelling.

Autoradiography



Autoradiography





Label Location

There are two ways to label a DNA molecule –
by the ends (end labeling) OR all along the molecule (uniform labeling)

Uniform labeling:

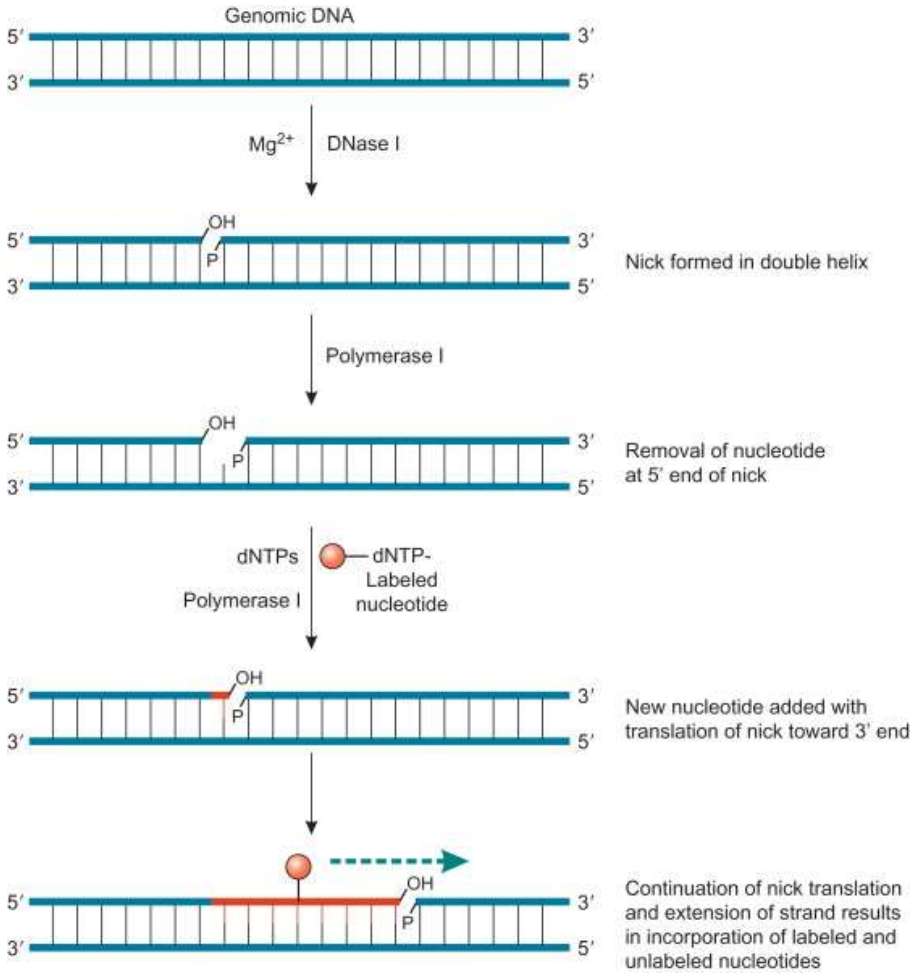
A. Nick translation

The endonuclease DNase I is used to create nicks at random sites in the strand of double stranded target DNA

DNA polymerase I is used to add nucleotide residues to the free 3'-hydroxyl ends created during the DNase I nicking process.

As the DNA polymerase I extends the 3'-end, the 5'- tp 3' exonulecase activity of the enzyme simultaneously removes bases from the 5'-end of the nick.

Sequential addition of bases on to 3'-end with the simultaneous removal of bases from the 5'-end results in translation of the nick Along the DNA molecule.



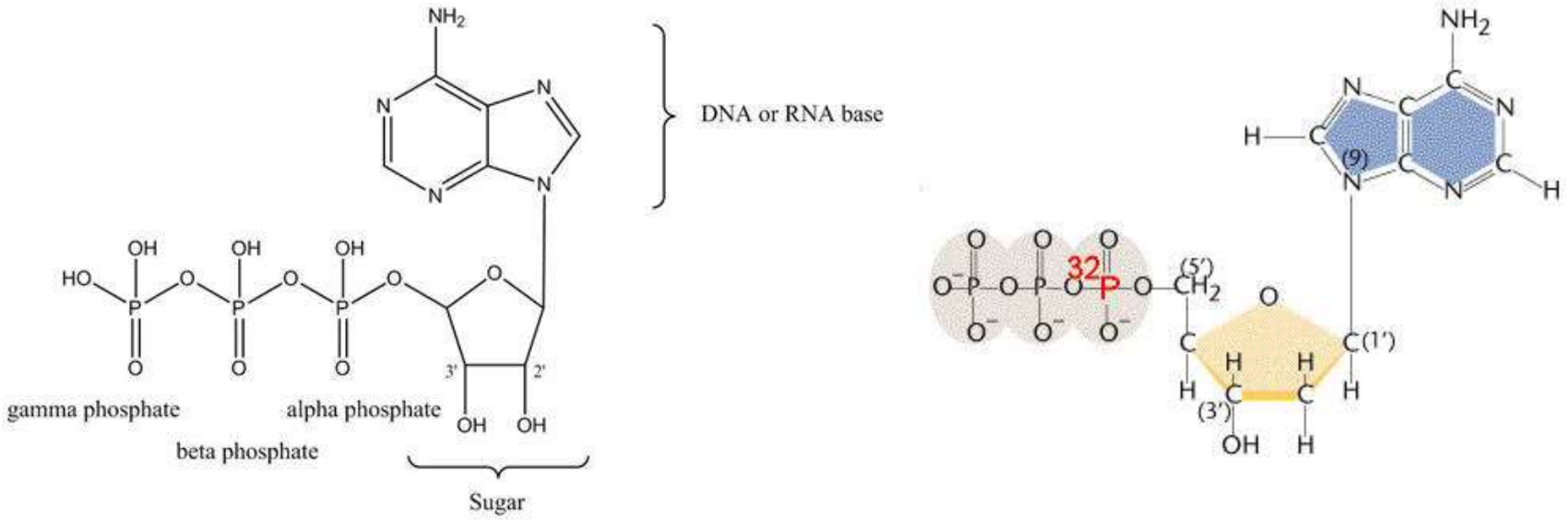
DNA ligase

Pancreatic DNase I

E. coli DNA polymerase I

DNA ligase

When performed in the presence of a radioactive ([α-^{32}P]dNTP the newly synthesized strand becomes radioactivity labeled.



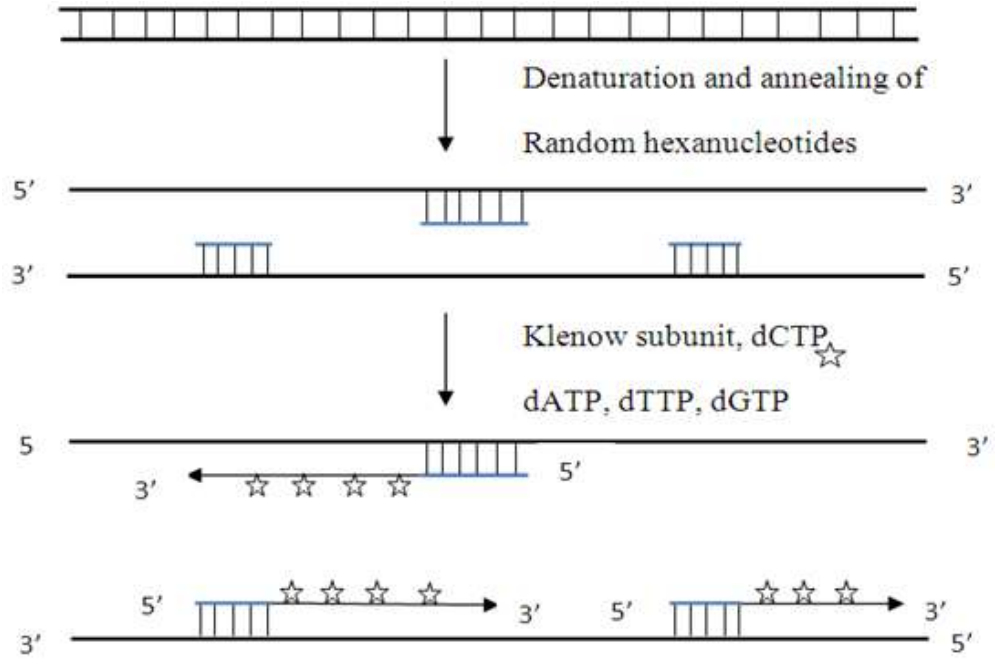
B. Random priming:

This is an alternative method for preparing uniformly labeled DNA is by oligo-nucleotide –primed DNA synthesis with hexanucleotide (or longer oligomers) of random sequences

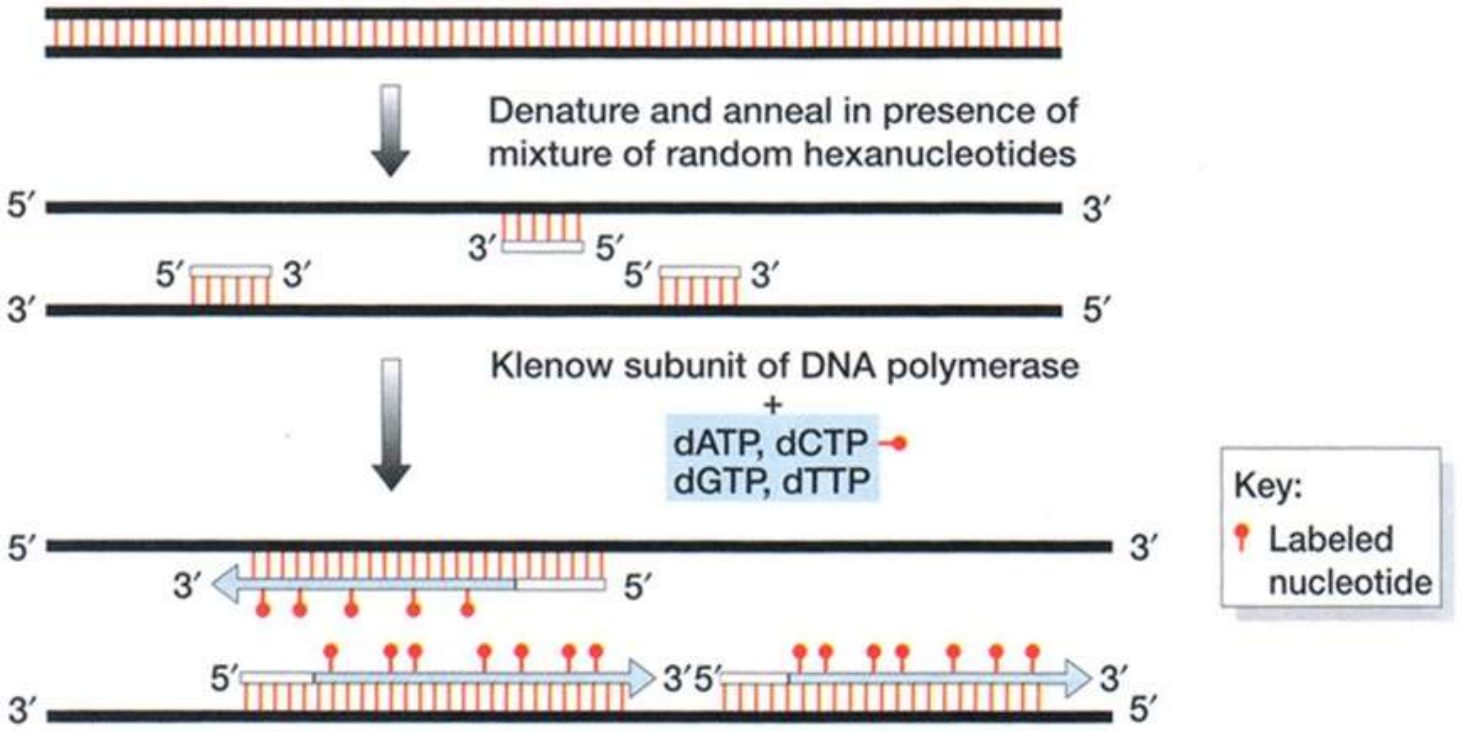
The klenow fragment is used as this enzyme lacks the 5'-3' exonuclease activity of DNA Polymerase I.

It fills gaps between adjacent primers.

Labeled nucleotides are incorporated Into new DNA that is synthesized.



Random primed labeling



End-Labeling of DNA

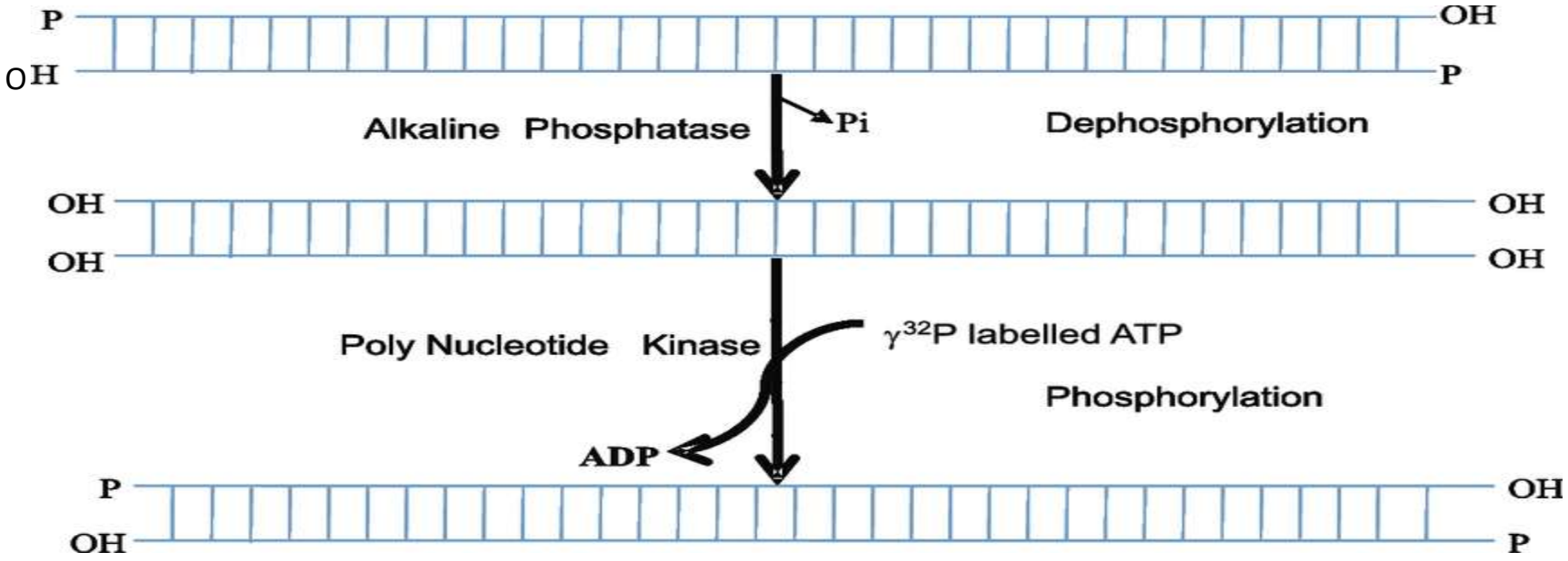
- End labeling can be performed at the 3' or 5'-end.

3'-End labeling-

- Template- dependent polymerization of [α-^{32}P] NTP to the 3' terminus of DNA is Catalyzed by calf thymus terminal deoxynucleotidyl **transferase.**
- Terminal deoxynucleotidyl transferase [TdT] is a template independent polymerase that incorporates dNTPs to the 3' hydroxyl end of the single or double stranded DNA and RNA in an irreversible manner.

B. 5’-end labeling

Alkaline Phosphatase
T4 polynucleotide Kinase



End labeling

Single stranded DNA/RNA

5’ -end labeling: polynucleotide kinase (PNK)

3’-end labeling: terminal transferase

5'-end labeling for DNA we use gamma P, but it is alpha that is used at the 3' end?