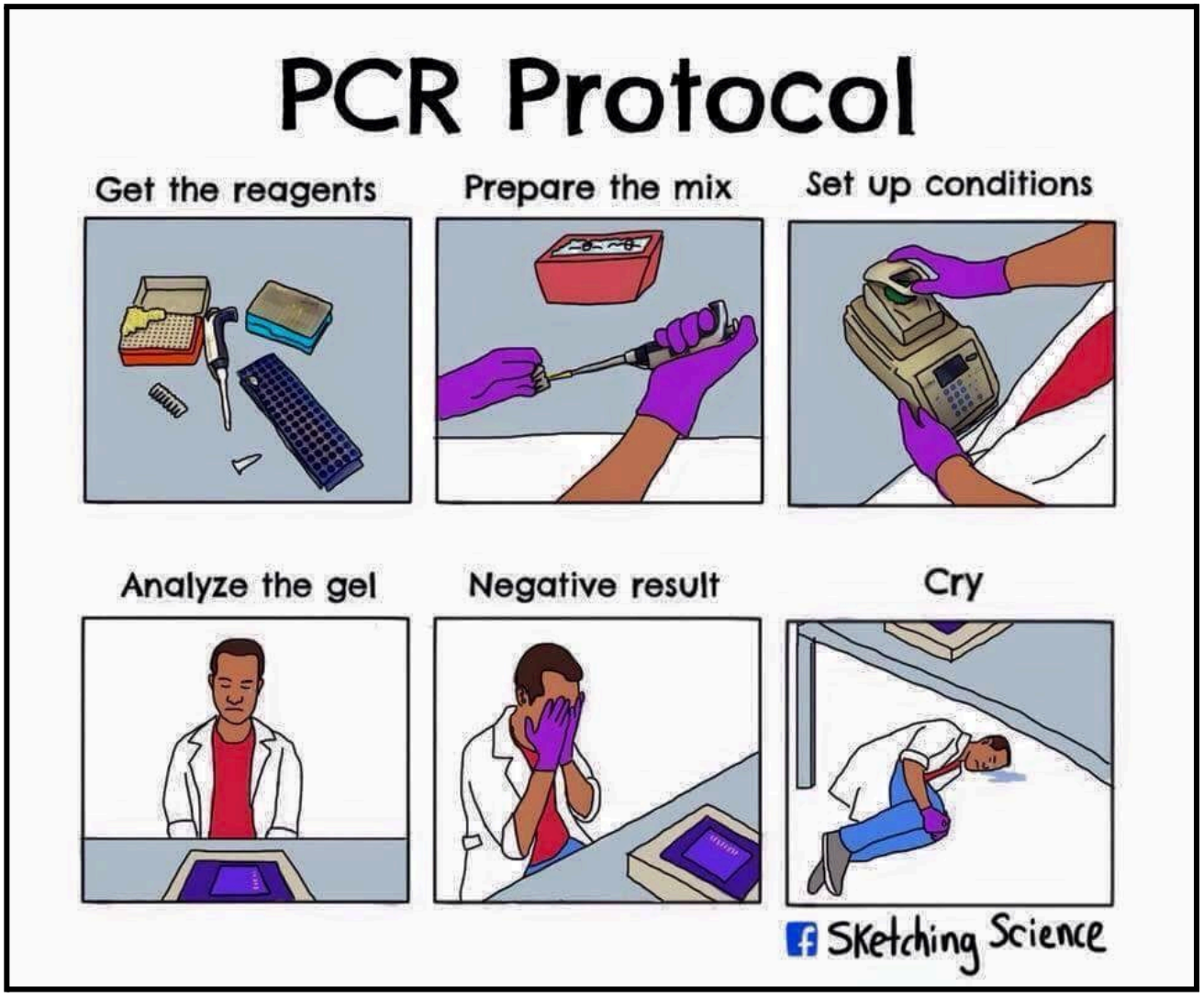
# A Level Biology.

# PCR, Sequencing and Profiling - Answers.

# Name:



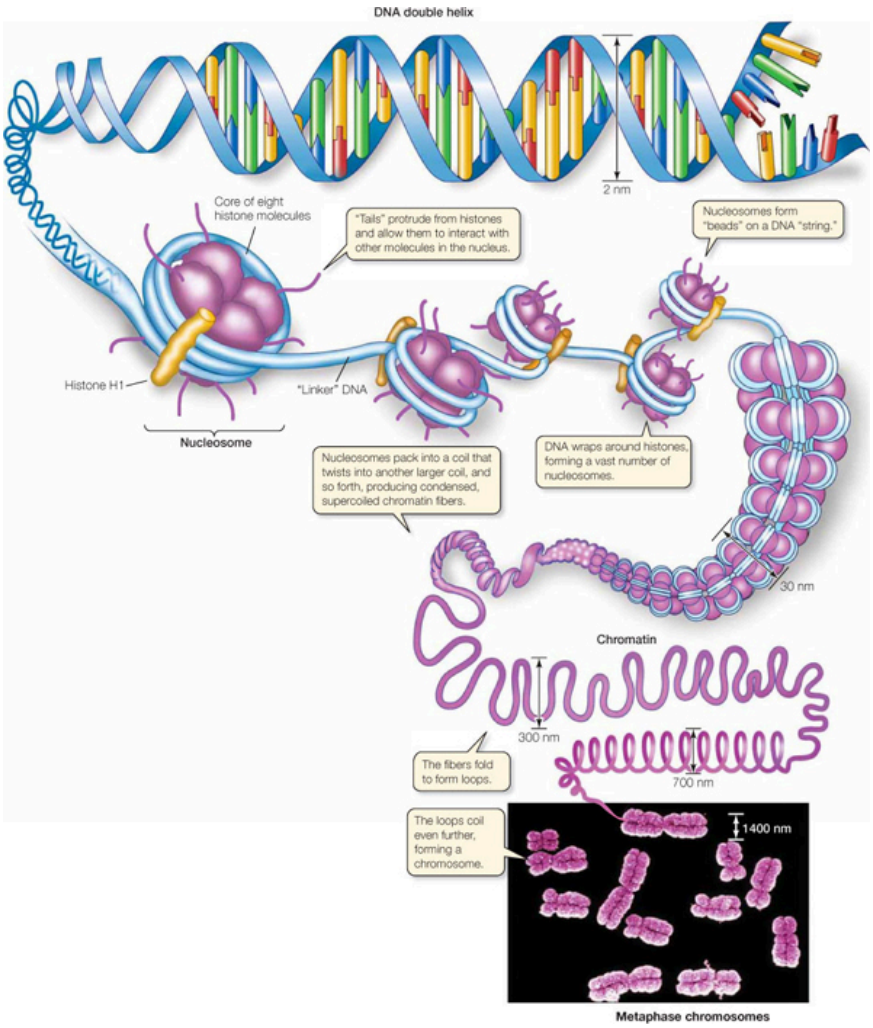
**Knowledge Organiser.**

| Term | Definition |
|---|---|
| Allele | Different form of a gene. |
| DNA or gene sequencing | Analysis of an individual base sequence along a DNA strand or individual gene. |
| DNA Profiling | Identification of repeating patterns in the non-coding regions of DNA. |
| Exons | Coding regions of DNA within genes |
| Gene variants | Different versions of a gene - alternative term for allele |
| Gene probe | Short DNA sequences (oligonucleotides) labeled with a fluorescent molecule that are complementary to specific DNA sequences which are being sought. |
| Genome | A genome is an organism's complete set of genetic instructions. Each genome contains all of the information needed to build that organism and allow it to grow and develop. This is considered to be the inherited genes in a haploid set of chromosomes. |
| Hybridisation | The binding of complementary gene probes to the complementary DNA strands. |
| Introns | Large non coding regions of DNA that are removed before messenger RNA is translated into proteins. |
| Massively parallel sequencing | Very rapid method of sequencing millions of DNA fragments at the same time. |
| Micro satellite | Section of DNA with a 2-6 base sequence repeated between 5 and 100 times. |
| Mini Satellite | Section of DNAwith a 10-100 base sequence repeated 50 to several hundred times. |
| Polymerase chain reaction | Reaction used to amplify a sample of DNA to make more copies of it very rapidly. When DNA is amplified, it is replicated repeatedly using the polymerase chain reaction to produce a much bigger sample. |
| Recognition sites | Specific base sequence (usually palindromic) where restriction endonuclease enzymes cleave the DNA molecule(breaking phosphodiester bonds) |
| Restriction endonuclease | Enzymes that cleave DNA at specific intron sequences |
| Southern blotting | A process that draws DNA fragments from an electrophoresis gel to a filter leaving the DNA as blots on the filter. The process also denatures the DNA fragments (dsDNA to ssDNA) so the separate bases and strands are exposed to probes. |
| Short tandem repeats (STR) | Micro satellite regions that are used in DNA identification (DNA fingerprinting) |
| Terminator bases | Modified versions of the four nucleotide bases that halt the production of a DNA molecule as soon as they are incorporated as no more bases can be added. |

**Specification guidance.**

**Gene sequencing.**

1. **Understand what is meant by the term genome.**
2. **Understand how PCR can be used to amplify DNA samples, and how these samples can be used:**
   a. **to predict the amino acid sequence of proteins and possible links to genetically determined conditions, using gene sequencing.**
   b. **in forensic science, to identify criminals and to test paternity, using DNA profiling.**

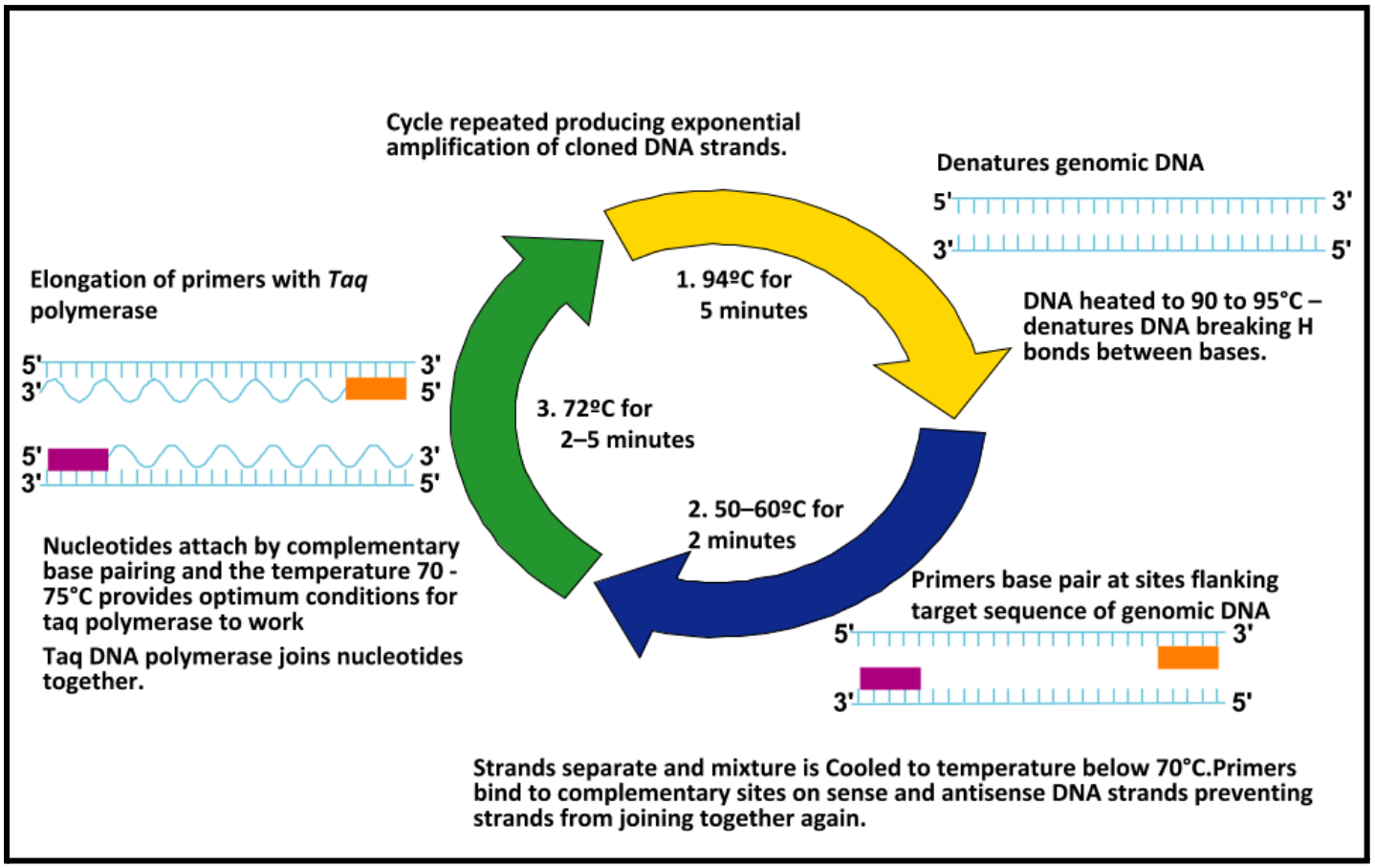


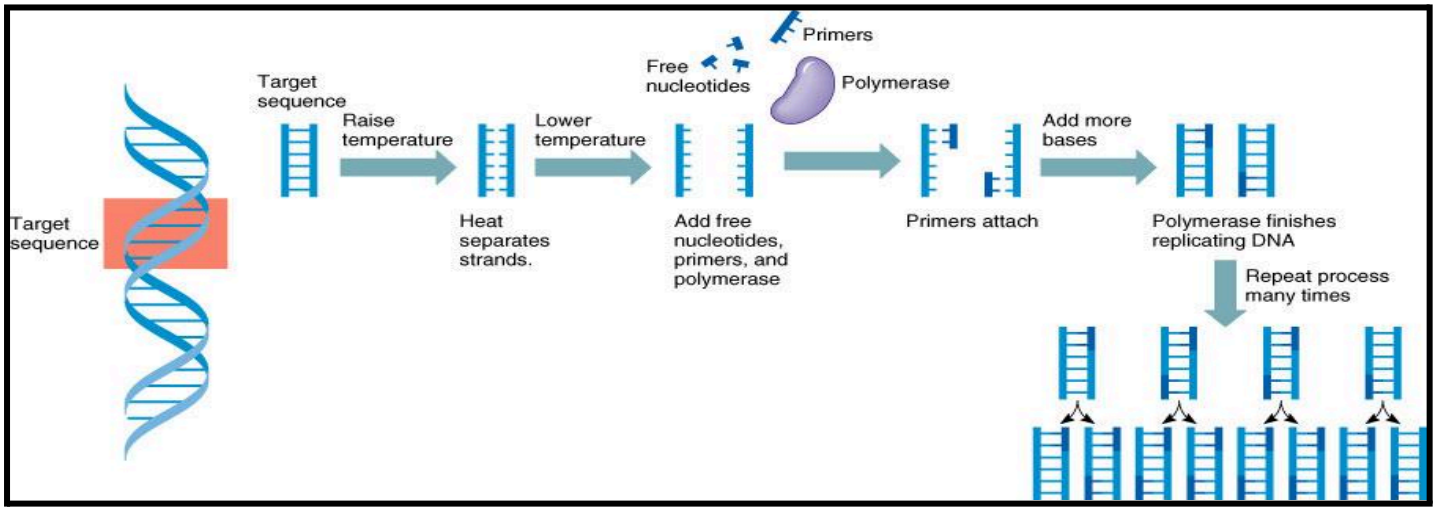

1. **What is the genome?**

**A genome is an organism's complete set of genetic instructions. Each genome contains all of the information needed to build that organism and allow it to grow and develop. This is considered to be the inherited genes in a haploid set of chromosomes.**

2. **Why might DNA need to be analysed?**

| |
|---|
| Paternity testing |
| Investigating evolutionary relationships between species |
| Forensic investigations |
| Diagnosis of genetic illness by identifying mutant alleles |
| Diagnosis of infectious disease eg PCR viral DNA |
| |

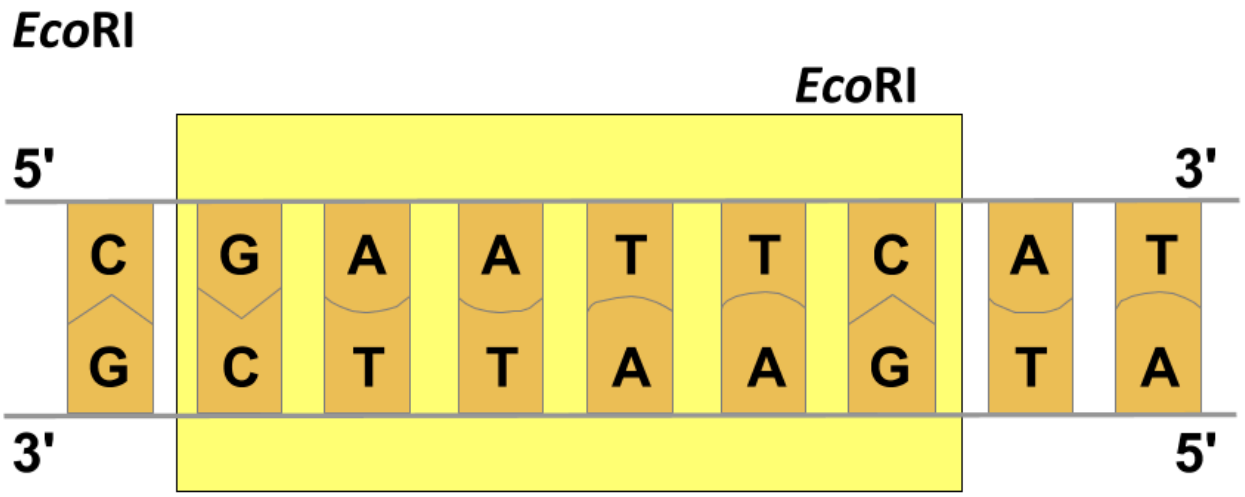


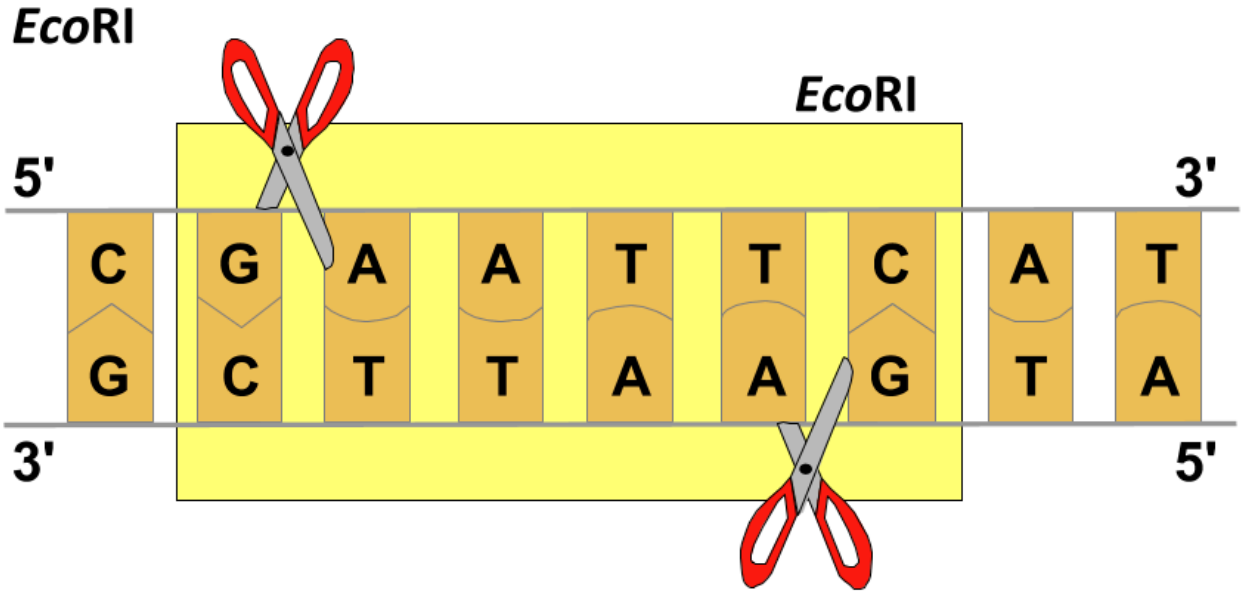
3. **Starting with reagents used, outline the process of PCR. Include the significance of using Taq polymerase in the process.**

- → PCR is a technique used to amplify a small amount of DNA to make many, many copies of a gene or sequence of DNA that a researcher is interested in.
- → PCR is used in paternity testing, forensic investigations, genetic diagnosis, diagnosis of infectious disease.
- → PCR is used to make enough of a target sequence to for it to be identified using electrophoresis and DNA Probes.
- → A thermocycler heats DNA to 90 to 95°C to break the Hydrogen bonds between nucleotides
- → Strands separate (dsDNA becomes ssDNA
- → The thermocycler is cooled to below 70°Cto allow the binding of primers
- → Primers are short sequences of ssDNA (oligonucleotides) around 20 nucleotides long, that provide a starting point for DNA synthesis.
- → Two primers must be used for section of DNA to be amplified. Each primer flanks the target sequences by binding to the complementary sequences and the ends of the sequence to be copied
- → The primers prevent the ssDNA reforming H bonds forming dsDNA again.
- → Thermocycler raises temperature to 72°C - optimum temperature for Taq Polymerase.
- → Free nucleotides bind to complementary bases on target sequences.
- → Taq polymerase joins the nucleotides together forming phosphodiester bonds.
- → The cycle then repeats to exponentially increase the amount of DNA

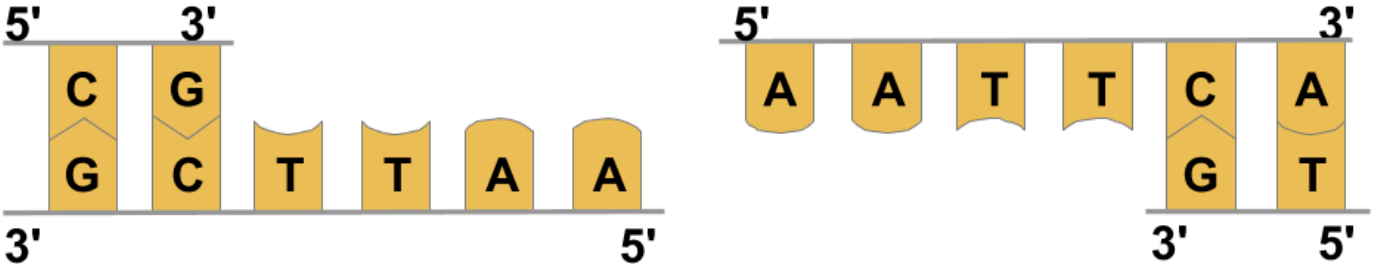
## Restriction Endonuclease - Palindromic Recognition sequences




## Restriction Endonuclease - Palindromic Recognition sequences



## Restriction Endonuclease - Palindromic Recognition sequences

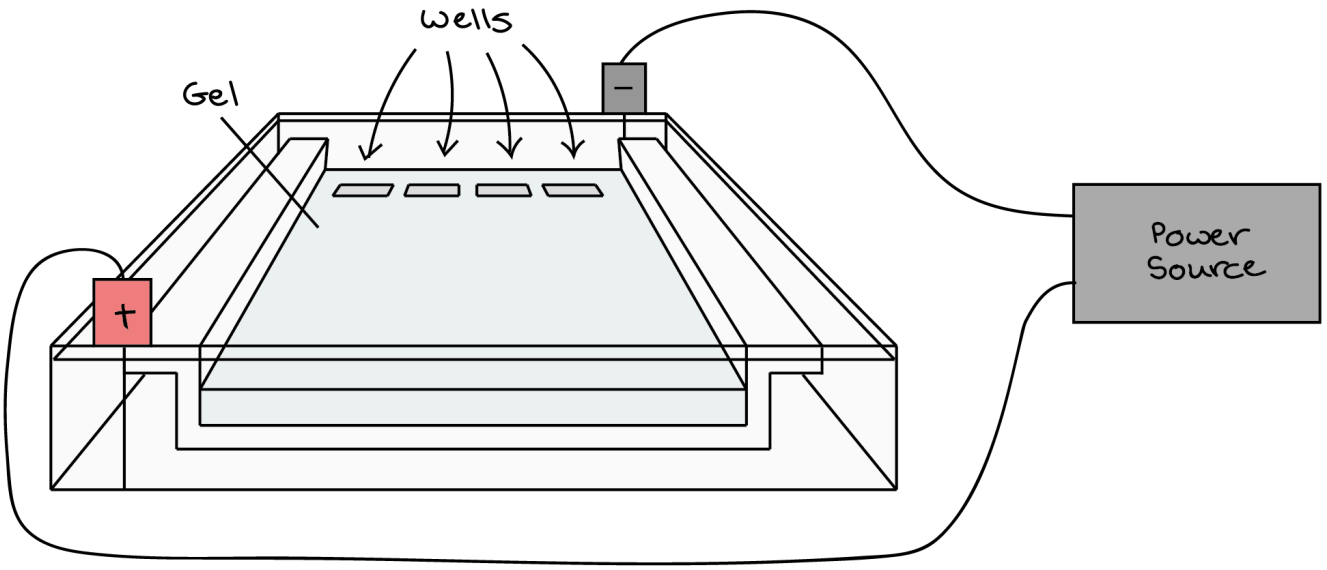



**Sticky Ends**

4. **Why are restriction endonucleases used prior to electrophoresis being carried out?**
5. **What are Palindromic Recognition sequences?**
6. **What are sticky ends?**

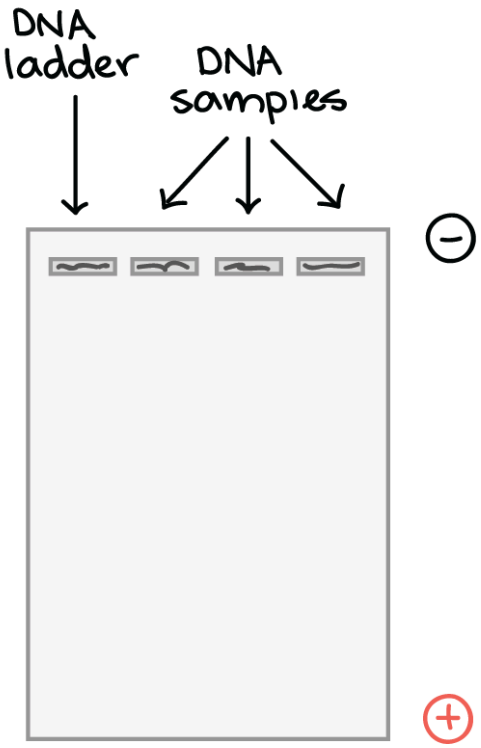
| |
|---|
| 4. Restriction endonucleases are enzymes that cleave DNA by breaking phosphodiester bonds at specific intron sequences. |
| 5.<br>→ Palindromic recognition sequences are DNA sequences that read the same sequence backward and forward eg GAATTC.<br>→ They are complementary to the active site of a restriction endonuclease allowing the formation of an enzyme substrate. |
| 6.<br>→ A sticky end results in a staggered cut in a DNA molecule, resulting in overhangs.<br>→ These are useful for producing recombinant DNA.<br>→ If two separate pieces of DNA have been cleaved using the same restriction endonuclease, the same stick ends / overgangs are produced.<br>→ This allows complementary base pairing to allow these separate strands to anneal - join together to make recombinant DNA (DNA sequence composed of DNA from two different species) |

**Electrophoresis**



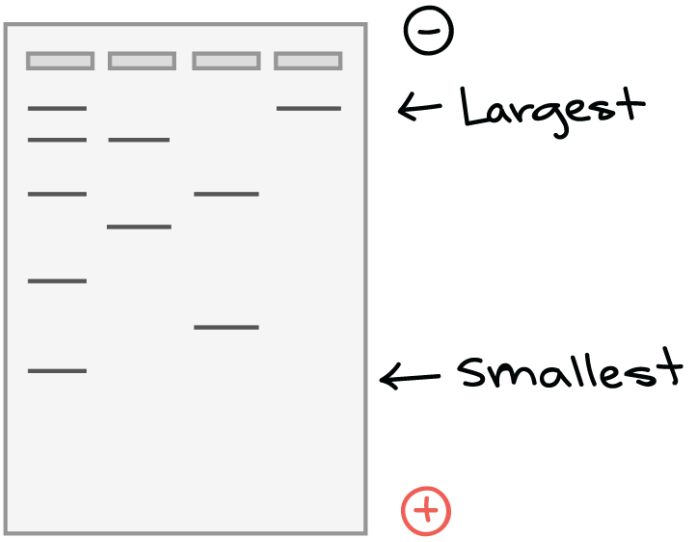

7. **Explain how electrophoresis works.**

| |
|---|
| → Gel electrophoresis is a technique that allows fragments of DNA to be separated according to their size. |
| → The DNA molecule is charged due to the presence of the phosphate on the sugar phosphate backbone. |
| → The DNA molecules are loaded into wells in an agarose gel matrix |
| → A potential difference is applied across the plate. This causes the DNA to migrate through the agarose gel matrix towards the positive end of the gel. |
| → The DNA fragments are made visible by combining the DNA loaded into the wells with a DNA binding dye. |
| → The size of the DNA fragments are measured against a DNA Ladder. |
| → The DNA Ladder is a mixture of fragments of known size which are also run in the agarose gel. |
| |
| |
| |
| |
| |
| |
| |
| |
| |

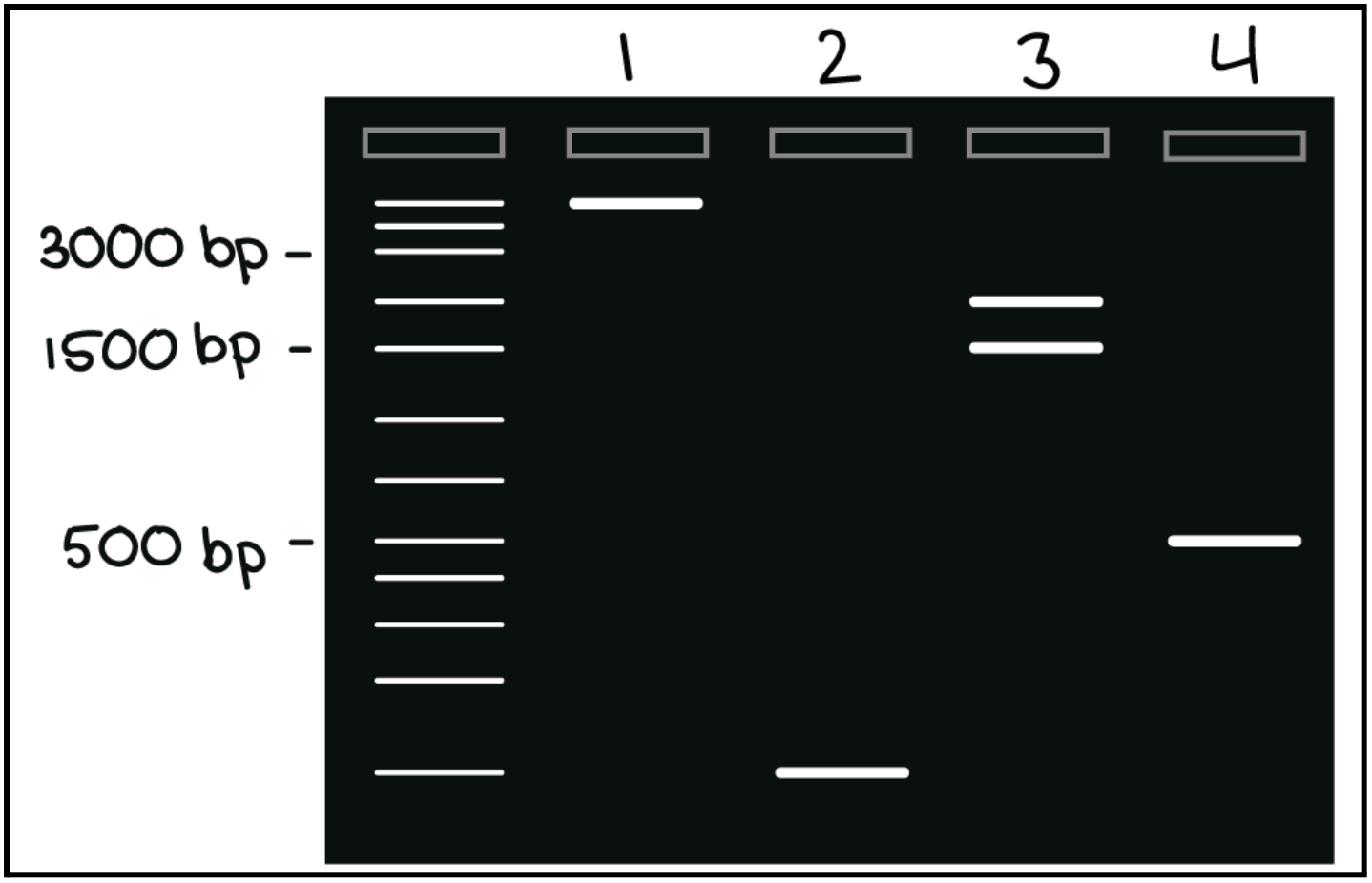



DNA samples are loaded into wells

Power is turned on and DNA fragments migrate through gel



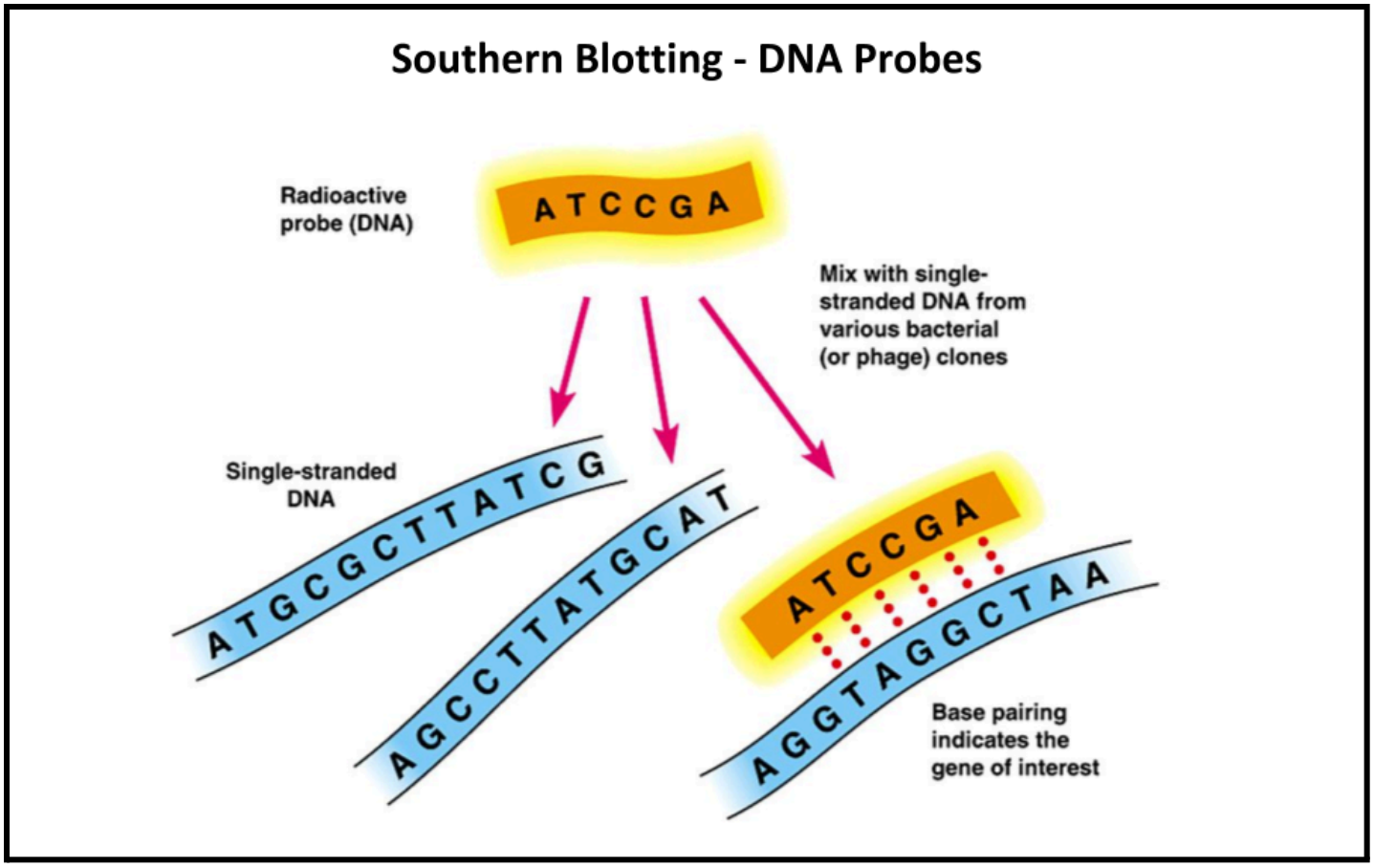

The fragments are now separated by size.




8. **Which lanes have the shortest and longest DNA fragments. Explain your answer.**

| |
|---|
| ➔ Lane 1 has the longest fragments as it the DNA fragment visible has moved the least in comparison to other fragments |
| ➔ It can also be measured using the DNA ladder as longer than 3000 base pairs |
| ➔ Lane 2 is the shortest fragment as it has travelled the furthest. |
| ➔ Using the ladder it can be measured as much shorter than 500 base pairs. |
| |
| |
| |
| |
| |
| |
| |
| |

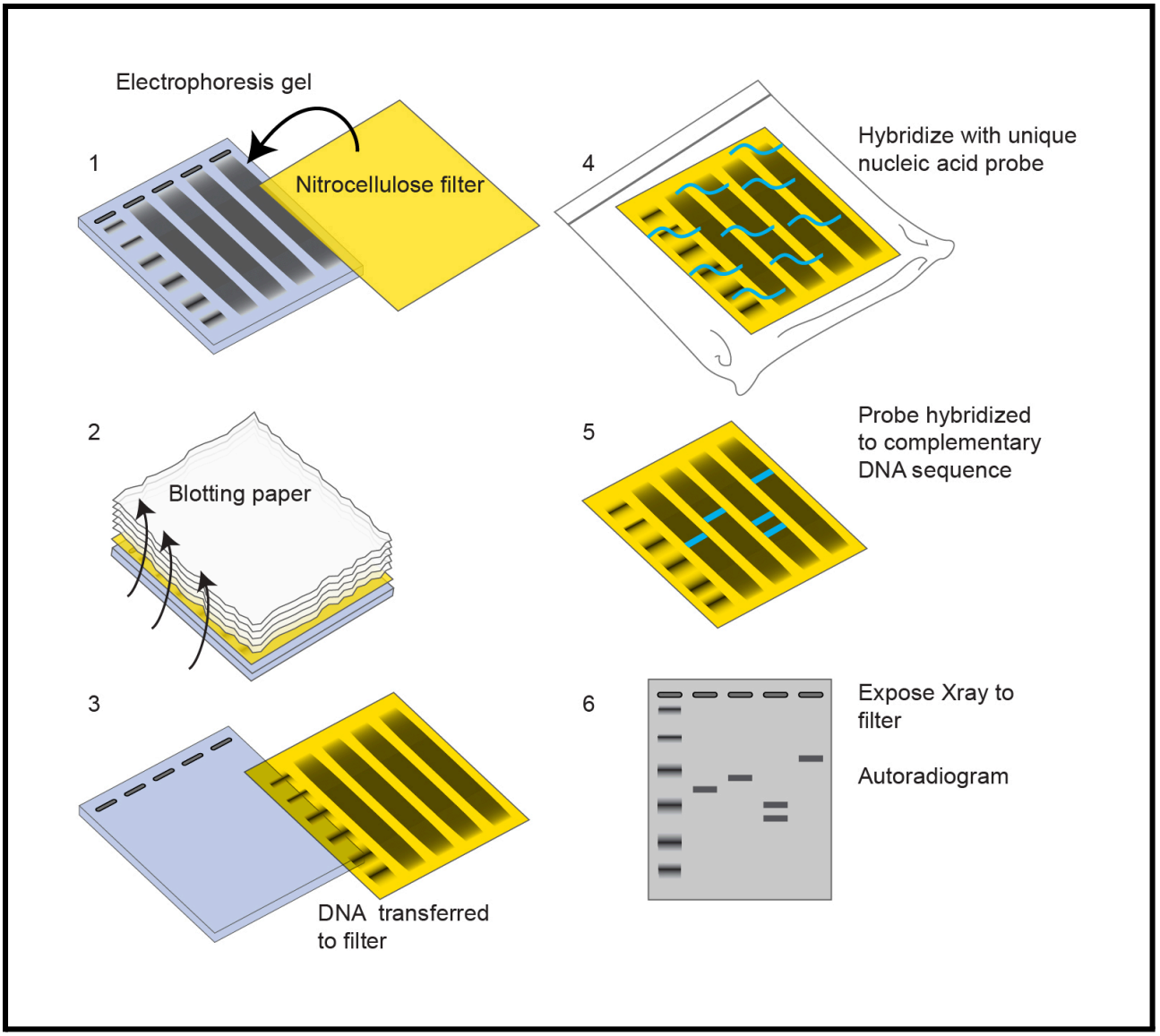
**DNA Probes.**




9. **What is DNA Hybridisation?**
10. **Explain what a DNA probe is and how hybridisation allows identification of target sequences.**

| |
|---|
| 9. **DNA hybridisation is the binding of two ssDNA strands due to complementary base pairing between strands.** |
| 10.<br>➔ **DNA probes are oligonucleotides - short sequences of DNA composed of between 100 - 10000 bases long.**<br>➔ **They are either radioactive (labelled with radioactive phosphorus) or fluorescently labeled.**<br>➔ **Probes are designed to hybridise to a target ssDNA or RNA sequence due to complementary base pairing.**<br>➔ **If a probe hybridises it indicates the presence of the target gene / DNA sequence.** |
| |
| |
| |
| |
| |
| |

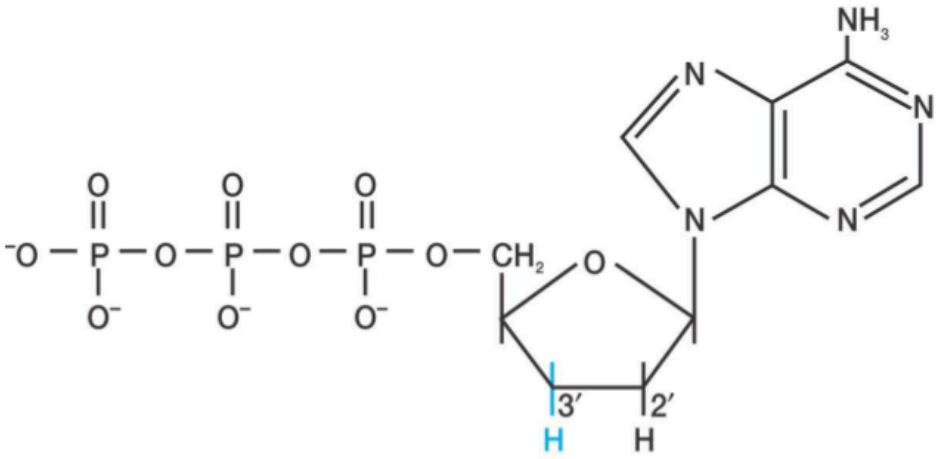
**Southern Blotting**




**11. Describe the procedure involved in carrying out a southern blot.**

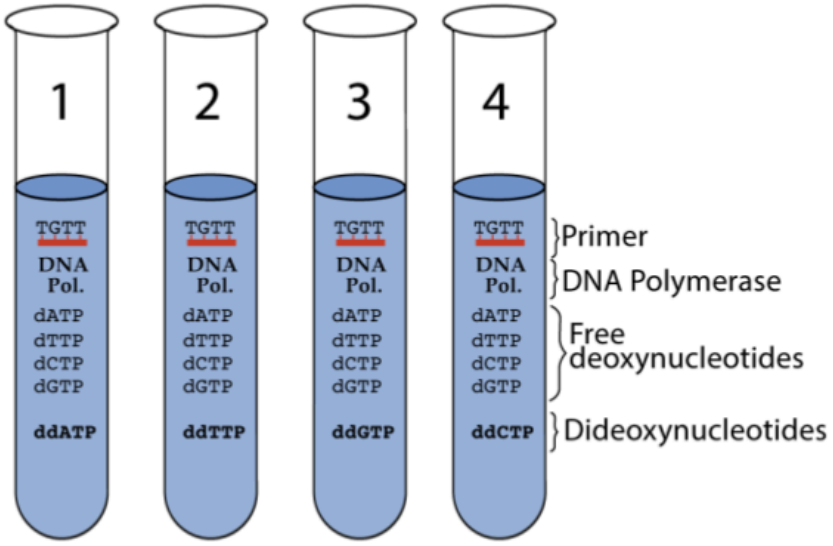
| |
|---|
| ➔ **Following a restriction digest and electrophoresis the restriction fragments separate out based on their size.** |
| ➔ **The agarose gel is then placed in an alkaline solution to allow the dsDNA to denmature - breaks the hydrogen bonds to form ssDNA** |
| ➔ **The Gel is then neutralised and placed on a nitrocellulose paper with blotting paper to allow the ssDNA to adhere to the nitrocellulose.** |
| ➔ **The nitrocellulose paper is then dried and exposed to the DNA probe (Radioactive or Fluorescent) to allow hybridisation with complementary target sequence.** |
| ➔ **The nitrocellulose is then rinsed to wash off the unbound probe.** |
| ➔ **If the probe has hybridised it's fluorescence is then detected or in the case of radioactive probes, left on photographic paper where the radioactivity will fog the paper indicating the presence of the probe.** |
| |

## Terminator Nucleotides - ddNTP dideoxy nucleotide triphosphate




- ➔ Modified versions of the four nucleotide bases that halt the production of a DNA molecule as soon as they are incorporated as no more bases can be added.
- ➔ This is due to the absence of -OH group on the 3' carbon on the ddNTP.
- ➔ This example is ddATP - dideoxy adenosine triphosphate - Terminator A

## Sequencing Reagents




**Outline the process of DNA sequencing and explain why ddNTPs are used in DNA sequencing.**

- → Sanger sequencing is similar to PCR.
- → Multiple copies of the target sequence need to be synthesised.
- → The difference is that four separate cycles completed with a terminator nucleotide are included in each.
- → Each tube contains complementary primers, DNA polymerase, deoxynucleotides and a terminator nucleotide eg
- → Tube 1 complementary primers, DNA polymerase, deoxynucleotides & terminator A
- → Tube 2 complementary primers, DNA polymerase, deoxynucleotides & terminator T
- → Tube 3 complementary primers, DNA polymerase, deoxynucleotides & terminator C
- → Tube 4 complementary primers, DNA polymerase, deoxynucleotides & terminator G
- → Terminator nucleotides (dideoxynucleotides) are nucleotides that are missing an -OH group on Carbon 3.
- → When these nucleotides are incorporated into the elongating DNA sequence this terminates the strand as a phosphodiester bond cannot form at the 3' carbon.
- → This produces fragments of many different lengths guaranteeing that a terminator nucleotide will have been incorporated at each position of the target DNA.
- → Each of the dideoxynucleotides used are labeled (radioactive or fluorescent) to allow detection by electrophoresis.

**Determining the Sequence.**