

核酸定序的基礎原理與應用



ABI DNA analyzer 3730xl

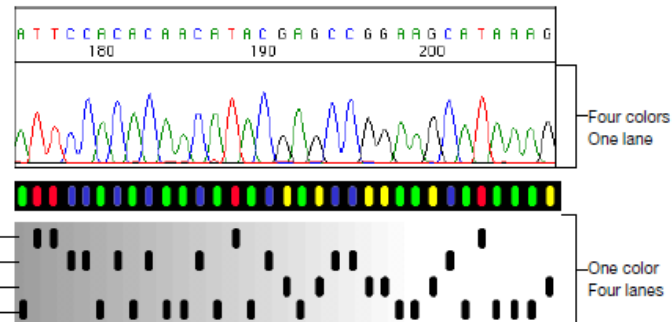
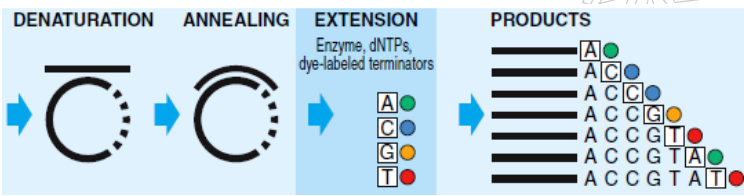
核酸定序反應是採用
Sanger sequencing
原理

- 螢光標定 DNA 片段的尾端 則合成中止，產生各種長短不一的 DNA 片段。
- 最終的合成產物以毛細管電泳方式，分離 DNA 片段。
- 透過雷射光激發，偵測每一片段不同發光的組合來判讀 DNA 序列。

Fluorescent sequencing compared with radioactive sequencing

Dye terminator chemistry

With dye terminator chemistry, each of the four dideoxynucleotide terminators is tagged with a different fluorescent dye. One reaction is performed, containing the enzyme, nucleotides, and all dye-labeled dideoxynucleotides. The products from this reaction are injected into one capillary.



Capillary Electrophoresis

Historically, DNA sequencing products were separated using polyacrylamide Gels that were manually poured between two glass plates. Capillary electrophoresis using a denaturing flowable polymer has largely replaced the use of gel separation techniques due to significant gains in workflow, throughput, and ease of use. Fluorescently labeled DNA fragments are separated according to molecular weight. Because you do not need to pour gels with capillary electrophoresis, you can automate DNA sequence analysis more easily and process more samples at once.

Shortly before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size, move across the path of a laser beam. The laser beam causes the dyes on the fragments to fluoresce. An optical detection device on Applied Biosystems genetic analyzers detects the fluorescence. The Data Collection Software converts the fluorescence signal to digital data, then records the data in a *.ab1 file. Because each dye emits light at a different wavelength when excited by the laser, all four colors, and therefore all four bases, can be detected and distinguished in one capillary injection.

「Applications overview
De novo sequencing of genomes」

- **Detection of variants (SNPs) and mutations**
- **Confirmation of clone constructs**
- **Detection of methylation events**
- **Gene expression studies**
- **Detection of copy number variation**