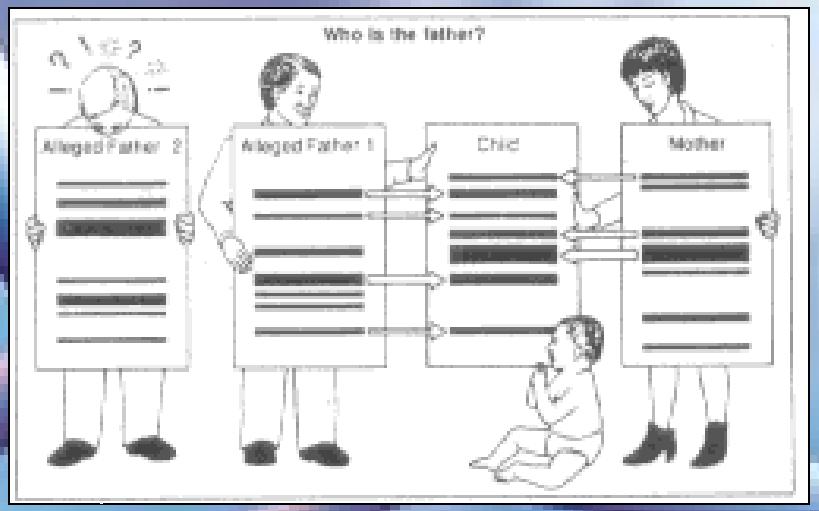
DNA sequencing and genetic fingerprinting

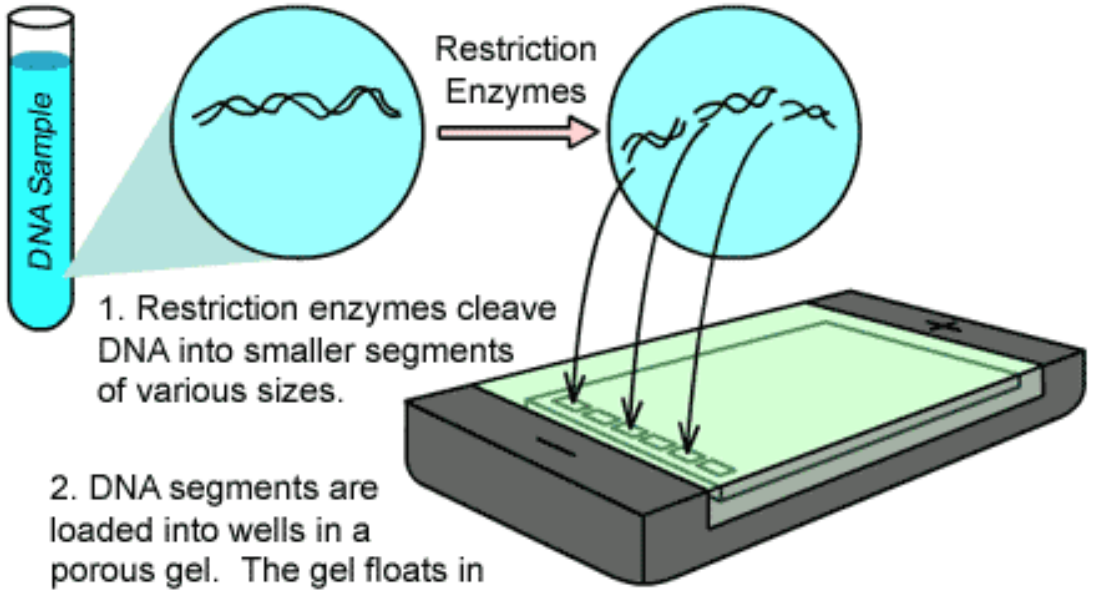


The use of electrophoresis in genetic fingerprinting and DNA sequencing

- **Electrophoresis**
  - A method of separating substances and analyzing molecular structure based on rate of movement of each component inn a liquid medium while under the influence of an electric field
  - Gel electrophoresis: gel is a colloid in which there are spaces between the molecules through which other molecules can move
  - Electrodes fragments of DNA of varied lengths
  - Direction of movement depends on the fact that DNA molecules and fragments of DNA are negatively charged – move towards the positive electrode (anode)
  - Distance moved in a given time will depend on mass of molecule of fragment
  - Human example
  - Fragments must be treated to make them visible:
    - Staining of all DNA fragments (EtBr, methylene blue and nile blue A)
    - Complementary gene probe

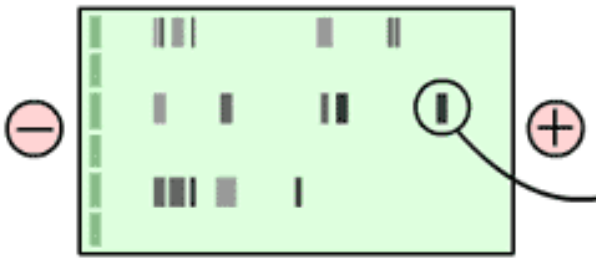
- Fragments must be treated to make them visible:
  - Staining of all DNA fragments (EtBr, methylene blue and nile blue A)
  - Complementary gene probe
    - To commonly repeated bit of DNA
    - To a base sequence that is specific to a particular gene or allele of a gene
- Gene probe: single stranded piece of DNA with a base sequence complementary to the DNA that you wish to identify and it must be labelled
- Common forms of labelling:
  - Making the probe radioactive and detect by its ability to expose the photographic film used to make X-ray photographs
  - To stain the probe with a fluorescent stain (vital red) that will fluoresce with bright visible light when placed in UV light

Figure S-2: Gel Electrophoresis



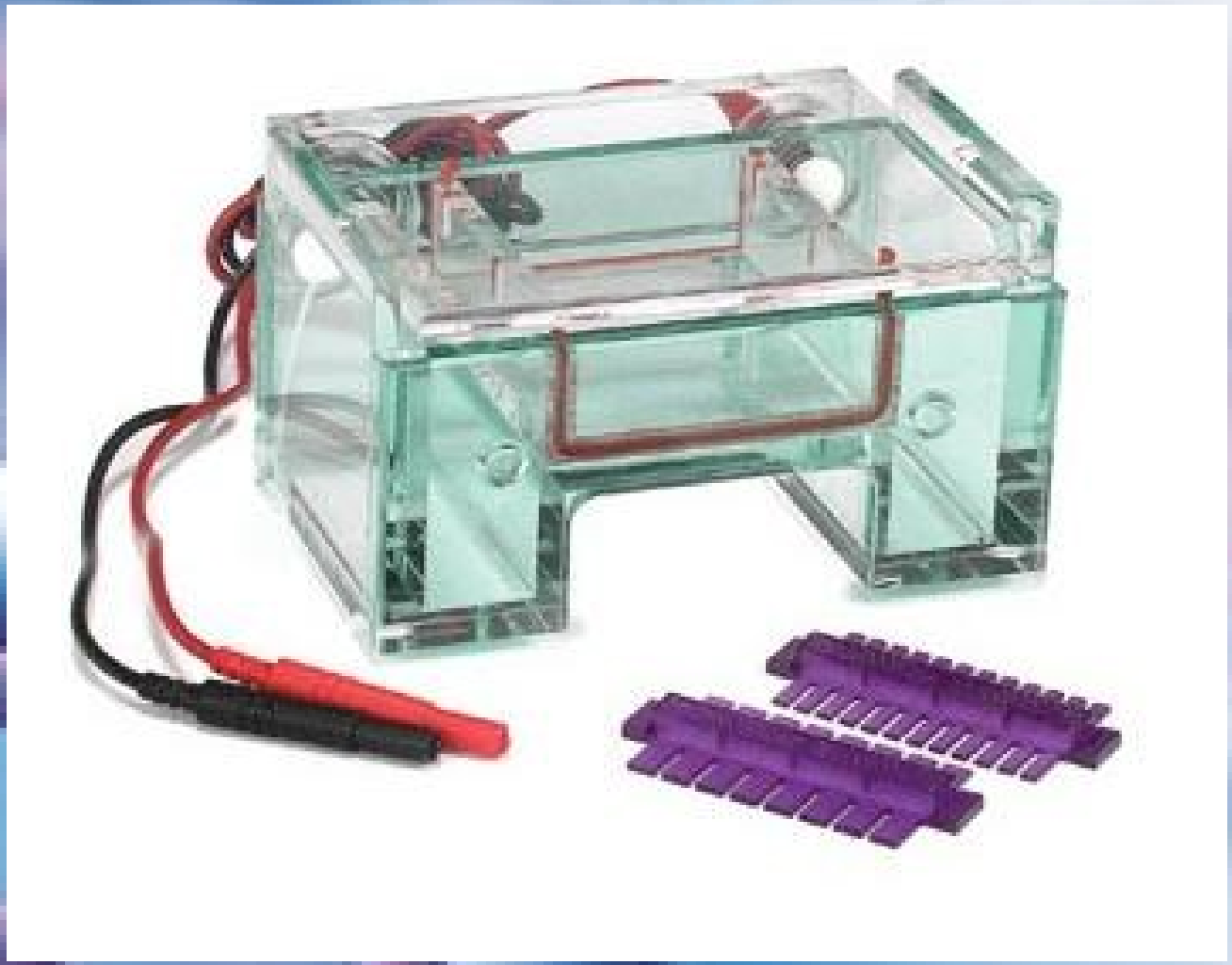
1. Restriction enzymes cleave DNA into smaller segments of various sizes.

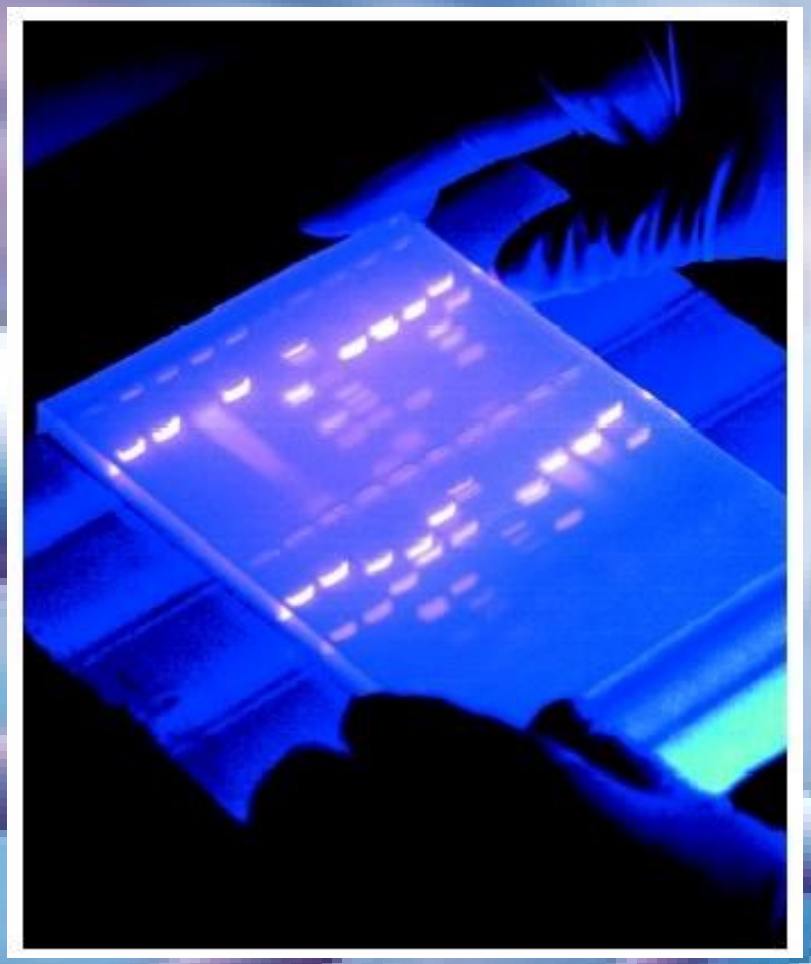
2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.

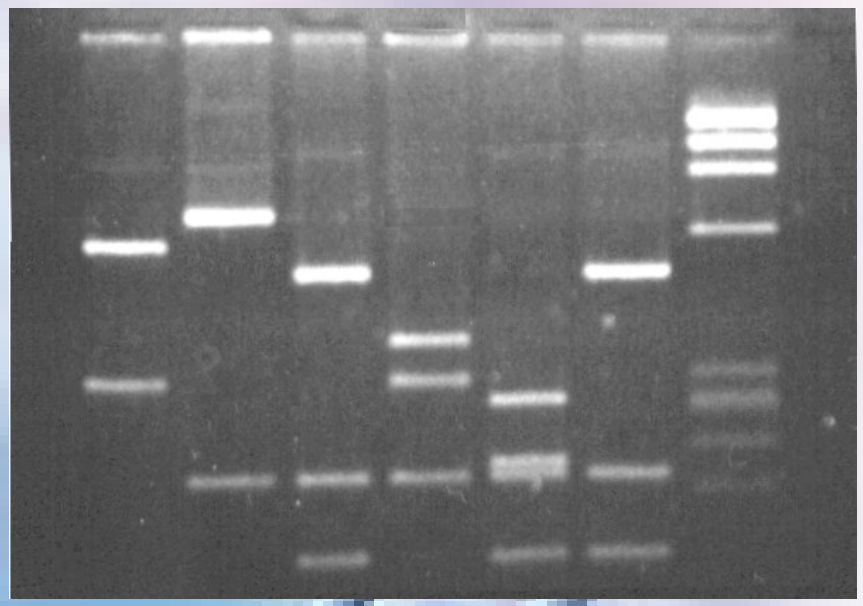


3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.

4. Smaller DNA segments move faster and farther than larger DNA segments.

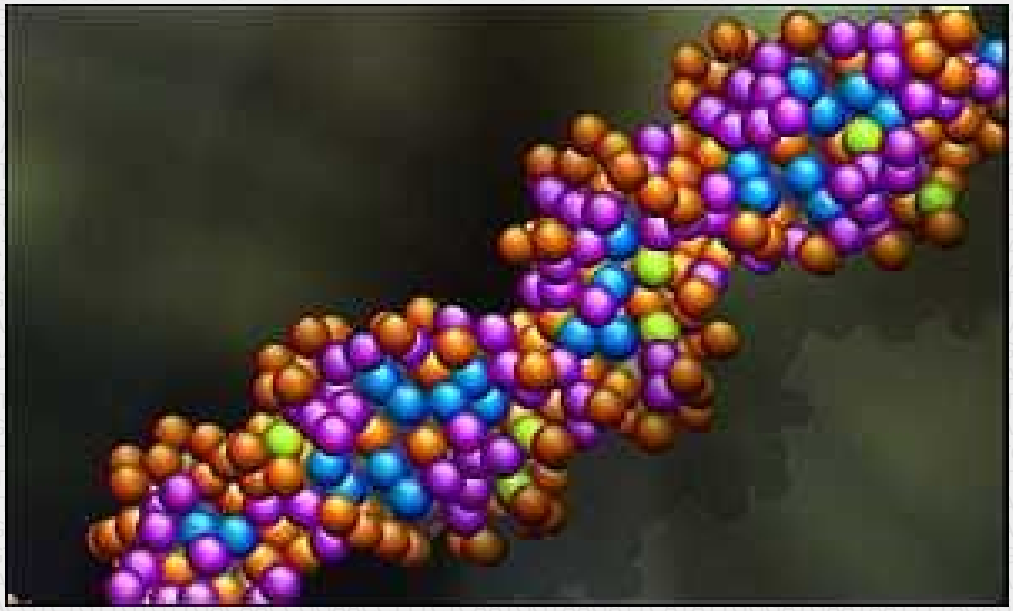


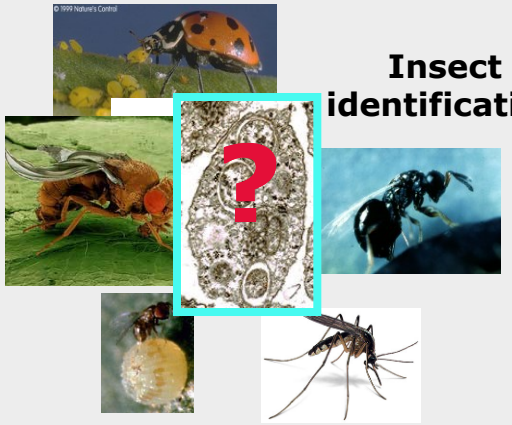




DNA sequencing

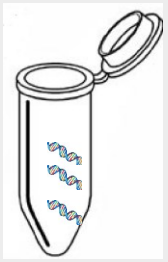
What, When, Why?
How?
Who and Where?



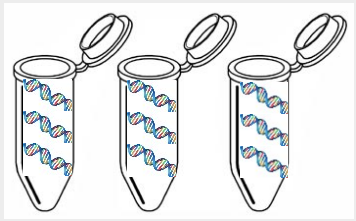


Insect identification

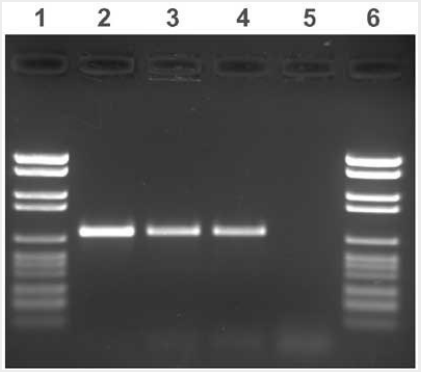
DNA extraction



PCR



Gel electrophoresis



DNA sequencing

ACAGATGTCTTGTAATCCGGC
CGTTGGTGGCATAGGGAAAG
GACATTTAGTGAAAGAAATTG
ATGCGATGGGTGGATCGATG
GCTTATGCTATCGATCAATCA
GGAATTCAATTTAGAGTACTT
AATAGTAGCAAAGGAGCTGC
TGTTAGAGCAACACGTGCTCA
GGCAGATAAAATATTATATCG
TCAAGCAATACGTAGTATTCT
TGAATATCAAAAATTTTTGTTG
GTTATTCA

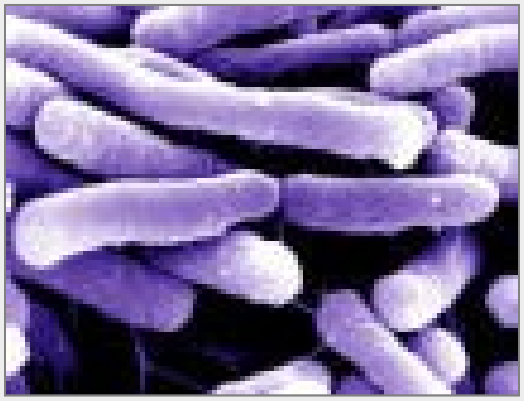
Bioinformatics

❖ WHAT types of biological insights can DNA sequence data provide?

❖WHEN and WHY would we want to obtain this detailed information?

❖ HOW is DNA sequencing actually performed?

❖ WHO conducts DNA sequencing, and WHERE?



DNA sequencing

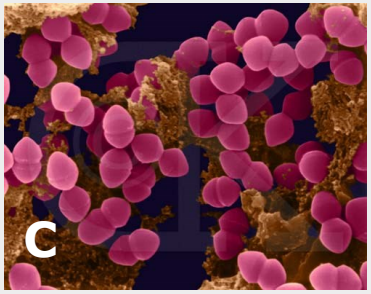
DNA sequence of a particular gene

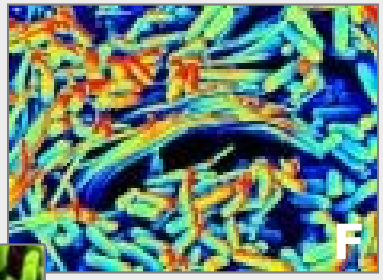
ACAGATGTCTTGTAATCCGGCCGTTGGTGGCATAGGGAAAG
GACATTTAGTGAAAGAAATTGATGCGATGGGTGGATCGATG
GCTTATGCTATCGATCAATCAGGAATTCAATTTAGAGTACTT
AATAGTAGCAAAGGAGCTGCTGTTAGAGCAACACGTGCTCA
GGCAGATAAAATATTATATCGTCAAGCAATACGTAGTATTCT
TGAATATCAAAAATTTTTGTTGGTTATTCAAAGTTCAGTAGA
AGATTTAATAGTTAGTGGGAACAAGAATTTTGGAGTAATTAC
TCCAAAATTAGGAATGAAATTTAGAGGTACGTCTGTTGTGTT
GACAACCGGAACCTTTCTCAAAGGTAAGATTCATATTGGGAT
GAATAATTTTAGAGGAATTCGATCTGGAGATTCGGAGTCAT
CGTCATTGTTATCATAGGGATTGAAAGAATTGTCTTTTCAGA
TTAGTCGGTTCAAACGGGTACTTCTCCTCGTGTGCATACCA
AA

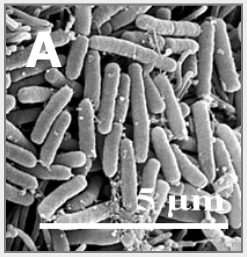
What good is it?

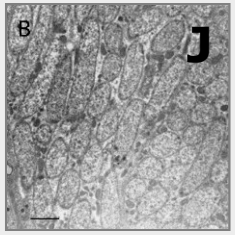
What questions about this organism can DNA data help us to address?

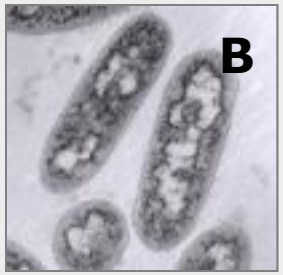
How is this organism related to other species?

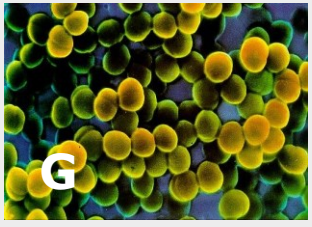






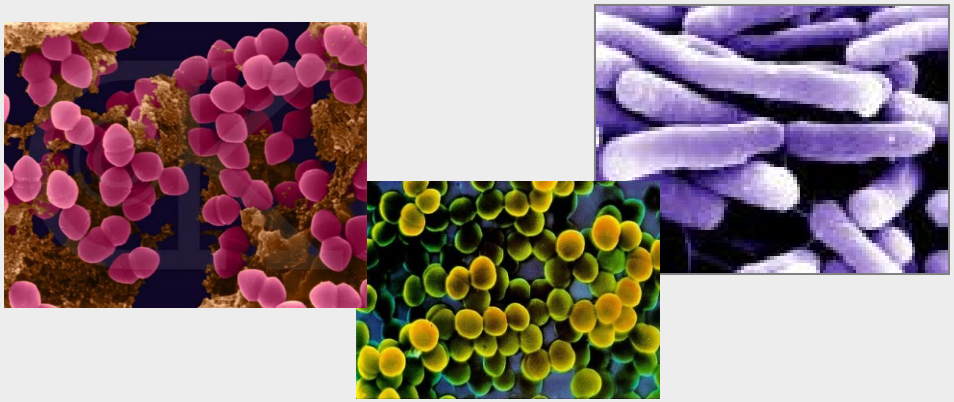






DNA sequences provide characters that are:

- Numerous, discrete characters (A, T, C, G)
- Directly comparable across species
- Unlikely to change due to culture conditions
- Can be sampled without ever culturing *(important for endosymbionts!)*



Molecular phylogenetics:

Inference of evolutionary relationships based on molecular data

ACAGATGTCTTGTAATCCGGCCGTTGGTGGCATAGGGAAAGGACATTTAGTGAAAGAAATTGATGCGATGGGTGGATCGATGGCTTATGCTATCGATCAATCAG
GAATTCAATTTAGAGTACTTAATAGTAGCAAAGGAGCTGCTGTTAGAGCAACACGTGCTCAGGCAGATAAAATATTATATCGTCAAGACAGATGTCTTGTAATCC
GGCCGTTGGTGGCATAGGGAAAGGACATTTAGTGAAAGAAATTGATGCGATGACAGATGTCTTGTAATCCGGCCGTTGGTGGCATAGGGAAAGGACATTTAGT
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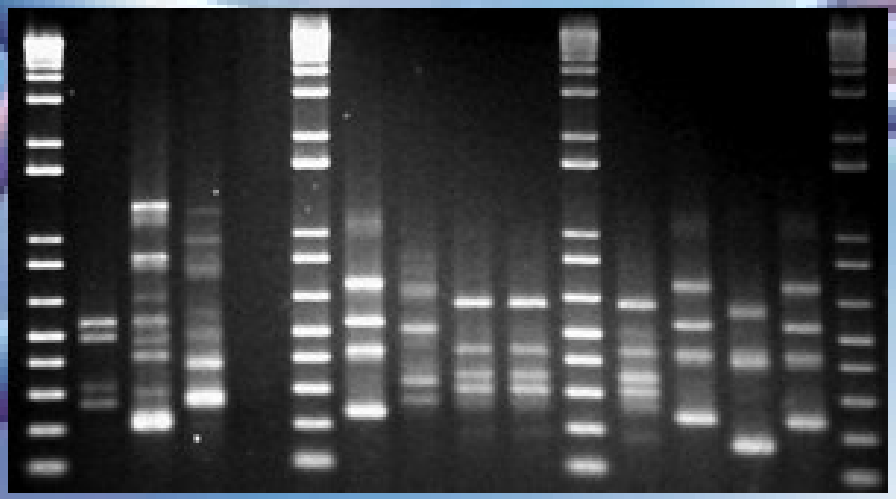
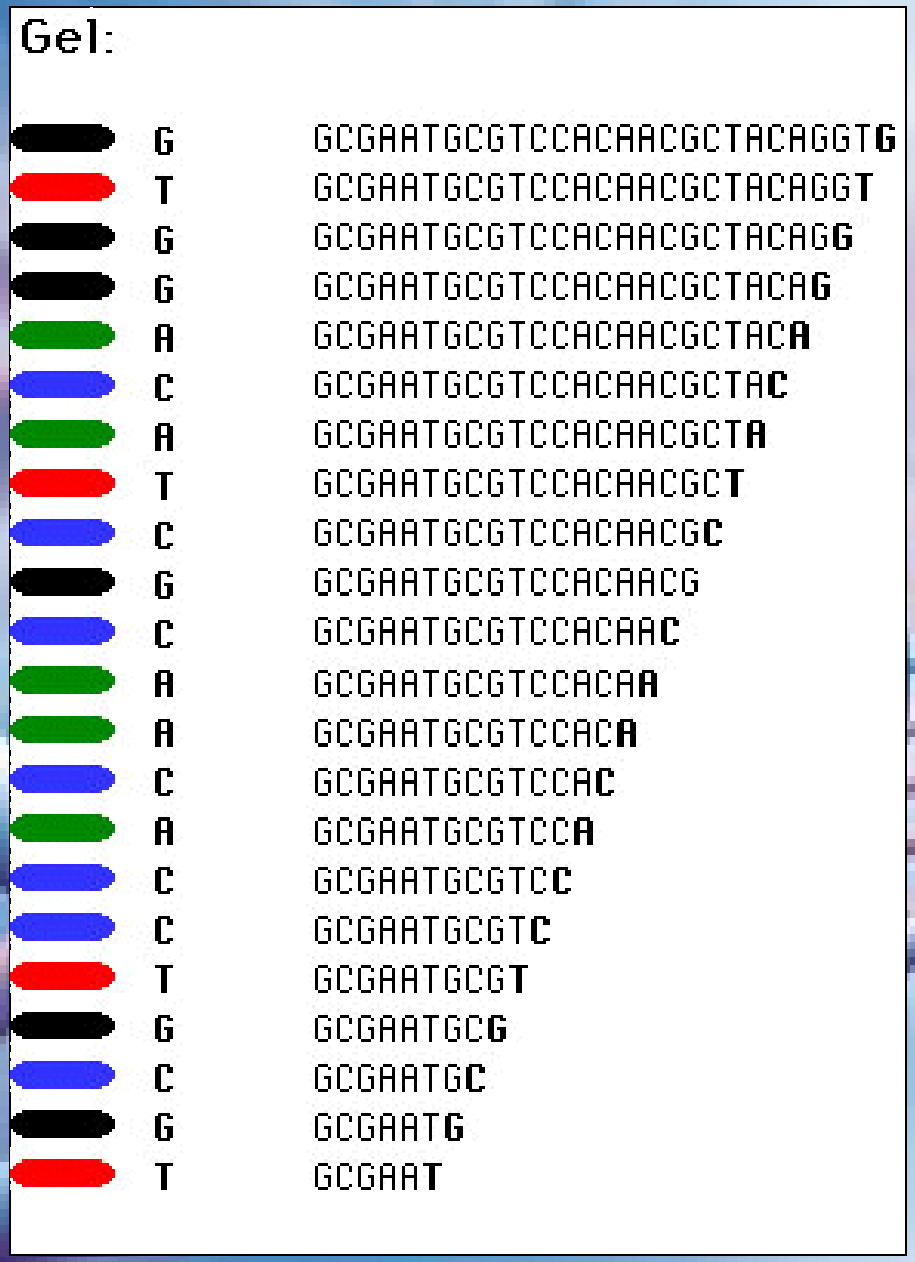
CTGCTGTTAGAGCAACACGTGCTCAGGCAGATAAAATATTATATCGTCAAGCAATACGTCAATACGCGTGCTCAGGCAGATAAAATATTA

- **Genetic fingerprinting**
  - DNA fragments separated by gel electrophoresis can be compared with other samples of DNA
  - Allowing determination of the source of the DNA (forensic investigations) or whether the samples are derived from related individuals



- **DNA sequencing**
  - Human Genome Project
  - Electrophoresis is used to separate fragments of DNA to enable determination of the order of bases within genes and chromosomes
  - The fragments vary in length by one base at a time and the last base on each can be identified
  - Sanger method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T GCGAATGCGTCCACAACGCTACAGGT
G GCGAATGCGTCCACAACGCTACAGG
G GCGAATGCGTCCACAACGCTACAG
A GCGAATGCGTCCACAACGCTAC
C GCGAATGCGTCCACAACGCTA
A GCGAATGCGTCCACAACGCT
T GCGAATGCGTCCACAACGC
C GCGAATGCGTCCACAACG
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C GCGAATGCGTCCACAA
A GCGAATGCGTCCACA
A GCGAATGCGTCCAC
C GCGAATGCGTCCA
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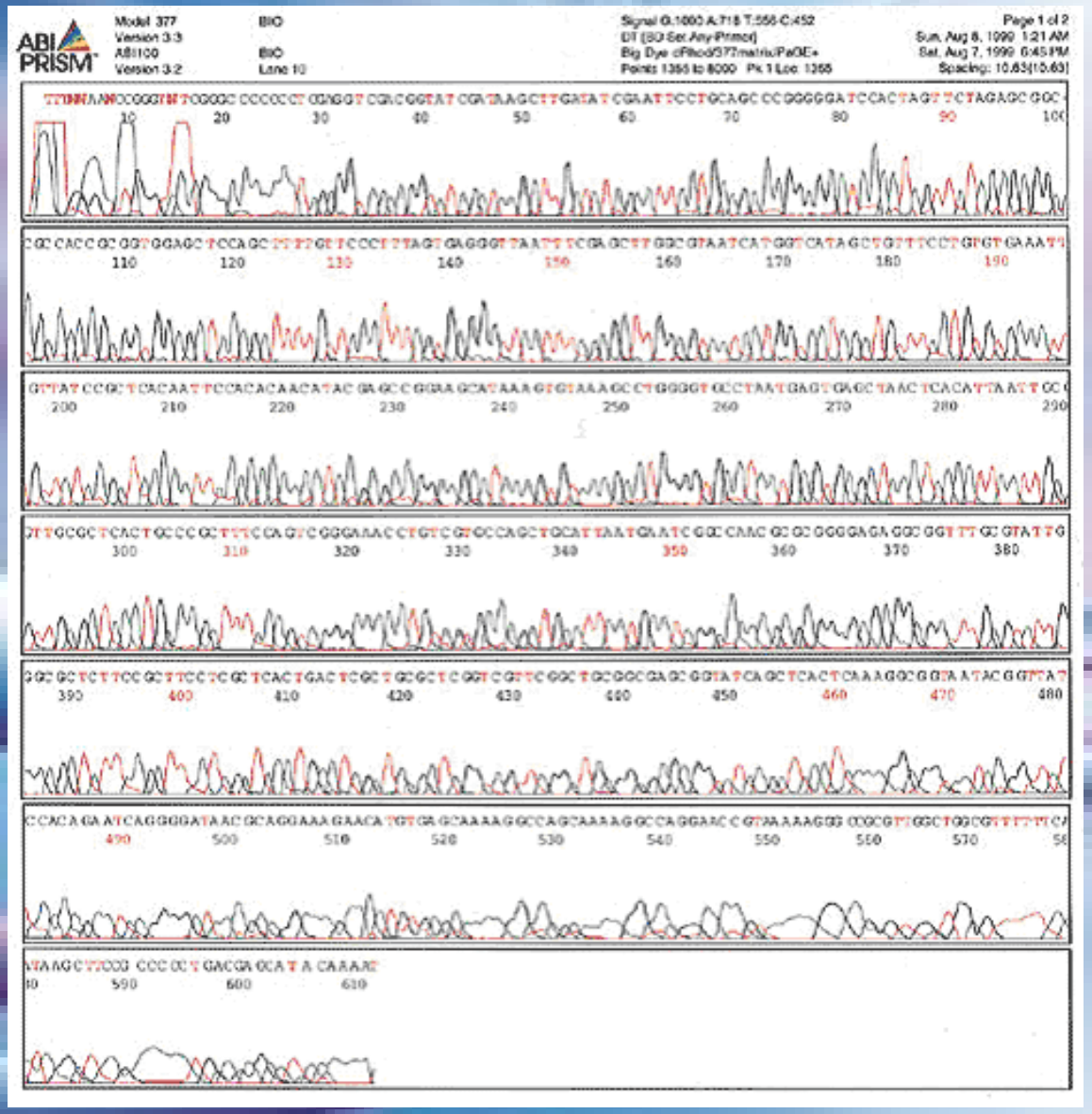
T GCGAA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ABI100
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