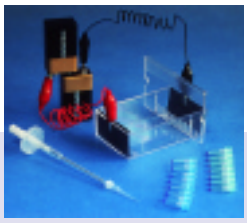
running gels



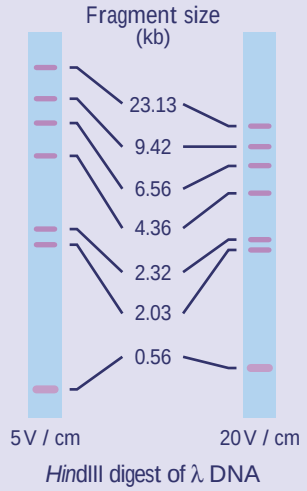
Effect of voltage on gel electrophoresis

What's the best voltage to use?
At low voltages, migration of linear DNA is proportional to the voltage applied. As the voltage is increased, the mobility of the higher molecular mass fragments is increased differentially (the larger fragments tend to 'catch up' with the smaller ones). Hence the effective range of separation is *decreased* as the voltage is increased. For the best resolution, 0.8% agarose gels should be run at no more than 5 V per cm (as determined by the distance between the electrodes).

Calculating the resolution of a gel
For λ DNA digested by *Hin*dIII (shown on the right), the resolution can be calculated by dividing the distance between the 23 and 2 kb fragments by the total distance travelled by the 2 kb fragment.

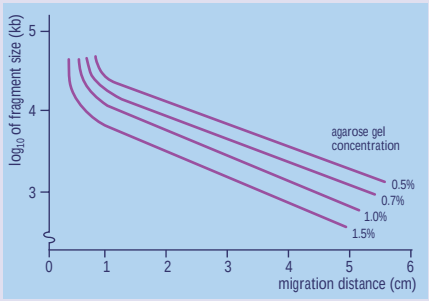
NCBE electrophoresis equipment
This particular equipment MUST NOT be connected directly to a mains electricity supply. It was designed to be used with batteries at low voltages and currents. You can use up to five 9 volt batteries (MN1604, 6LR61, 6LF22 or PP3).

Number of 9 V batteries	Time taken to run gel
2	4 hours
3	3.5 hours
4	2 hours
5	1 hour



Fragment size

Within limits, there is a linear relationship between the *logarithm* of the molecular mass of linear DNA molecules and their movement through a gel. The molecular mass of DNA fragments is roughly proportional to their size.



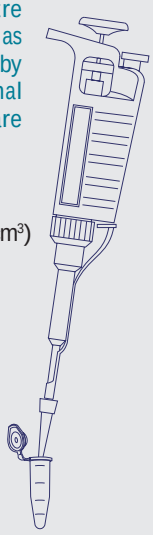
Gel concentration

There is a linear relationship between the *logarithm* of the mobility of the DNA and the gel concentration. By altering the agarose concentration it is possible to control the range of sizes of fragments that can be separated by electrophoresis.

Agarose (% w/v)	Separation range (kb)	Gel strength
0.3	60 – 5	very weak
0.6	20 – 1	weak
0.7	10 – 0.8	moderate
0.9	7 – 0.5	moderate
1.2	6 – 0.4	strong
1.5	4 – 0.2	strong
2.0	3 – 0.1	strong

UNITS OF MEASUREMENT

Small volumes and masses, which may be unfamiliar to school-based biologists, are referred to in this booklet. The units dm^3 and cm^3 have been used in preference to litre and millilitre (to which they are almost equivalent) as both cm^3 and dm^3 are often preferred in schools, by some examination boards and in the National Curriculum in England and Wales (although none are true SI units).



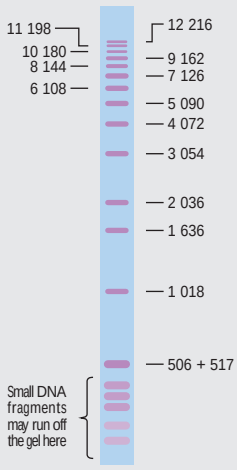
Volume
1 cubic decimetre (dm^3) = 1 000 cubic centimetres (cm^3)
1 cubic centimetre (cm^3) = 1 000 microlitres (µL)

Mass
1 gram (g) = 1 000 milligrams (mg)
1 milligram = 1 000 micrograms (µg)
1 microgram (µg) = 1 000 nanograms (ng)
1 nanogram (ng) = 1 000 picograms (pg)

Note: Various devices for pipetting microlitre volumes are available; the NCBE can advise on suppliers, suitability for school use and approximate costs.

1 kb DNA LADDER

This 'ladder' or 'ruler' provides DNA fragments of known sizes, for comparison with those whose size is not known. The sizes of the fragments are in base pairs, and they are spaced at roughly 1 kb intervals.



How much ladder should I use?
With the NCBE equipment, 1 µg of ladder is needed per well with the 6-toothed comb; with the 4-tooth comb, use 2 µg per well.

Note: Not all 1 kb DNA ladders have this selection of fragment sizes; details will be given by the supplier.

resources

The technical guides that accompany the NCBE's gel electrophoresis equipment are available from the Centre's Web site: www.ncbe.reading.ac.uk

DNA Science. A first course in recombinant DNA technology by David Micklos and Greg Freyer (1990) Carolina Biological Supply Company / Cold Spring Harbor Laboratory Press. ISBN: 0 89278 411 3.

This book includes a useful 'trouble-shooting' guide for diagnosing poor gels.

Biological nomenclature. Standard terms and expressions used in the teaching of biology by Alan Cadogan [Ed.] (2000) Institute of Biology. ISBN: 0 900 49036 5.

This book has details of the recommended units of measurement, terminology etc.