Moderní analytická instrumentace pro genetický výzkum, lékařskou diagnostiku a molekulární identifikaci organizmů

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Oddělení bioanalytické instrumentace
Ústav analytické chemie
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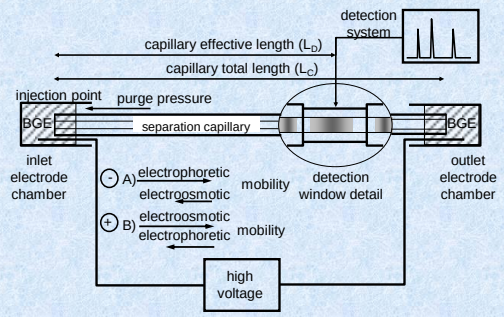
Brno

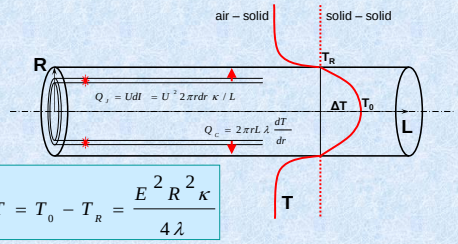
Capillary electrophoresis

CE

Capillary electrophoresis scheme



Why capillary electrophoresis?



$$\Delta T = T_0 - T_R = \frac{E^2 R^2 \kappa}{4\lambda}$$

Miniature capillary: low R => fast separation

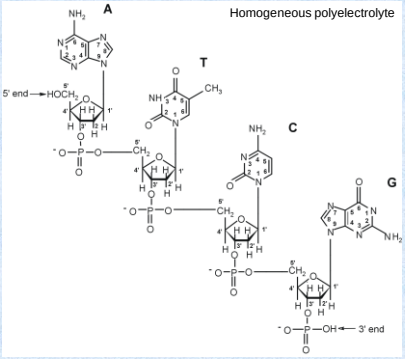
1) high resistivity ↻ low current at high voltage ↻ low heat production

2) efficient heat transport ↻ low temperature difference inside the capillary

DNA electromigration

K. Klepárník, P. Boček, DNA diagnostics by Capillary Electrophoresis Chemical Reviews 107, 5279 – 5317, 2007.

DNA primary structure



DNA electromigration regimes in sieving media

Size separations of homogeneous polyelectrolytes are impossible in free solutions

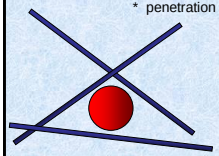
Short DNA fragments
Low concentration of media

Long DNA fragments
High concentration of media

Ogston regime

Ogston (1958): * distribution of spaces in a random network of rigid rods available to a spherical molecule

* penetration probability



$$P_{D \geq r} = \exp -(2\pi\nu L r^2 + \frac{4\pi\nu r^3}{3})$$

ν average density of number of fibers
$2L$ fiber length
D „pore" radius
r particle radius

Rodbard Chrambach (1970): $P_{D \geq r} = \frac{\mu}{\mu_0} = \frac{V_a}{V_0} = \exp -(K_r c)$

μ el. mobility
μ_0 free electrolyte el. mobility
V_a accessible volume
V_0 void volume
K_r retardation coef.
c gel concentration
d gel fibre radius

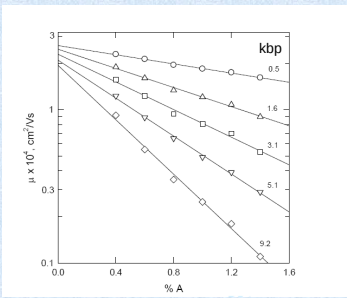
$$K_r \approx (r + d)^{n-1-3}$$

Ferguson plot (1964): $\log \mu = \log \mu_0 - K_r c$

Fergusson plot

$$\log \mu = \log \mu_0 - K_r c$$

$$\mu_0 = \frac{q}{6\pi r \eta}$$



Ferguson plots of DNA molecules in agarose gels. The logarithm of the mobility, extrapolated to zero electric field strength at each gel concentration, is plotted as a function of agarose concentration, %A.

Biased Reptation Model

$$\mu \propto \frac{1}{M}$$

$$\mu = \frac{q}{\xi} \times \frac{\langle h_x^2 \rangle}{L^2}$$

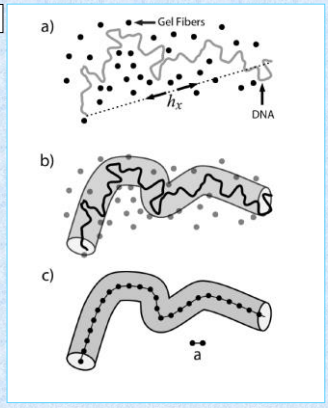
L tube legth
ξ friction inside
x field direction

$$\mu = \frac{q}{3\xi} \times \left[\frac{1}{N} + \frac{\varepsilon^2}{3}\right] \quad \varepsilon << 1$$

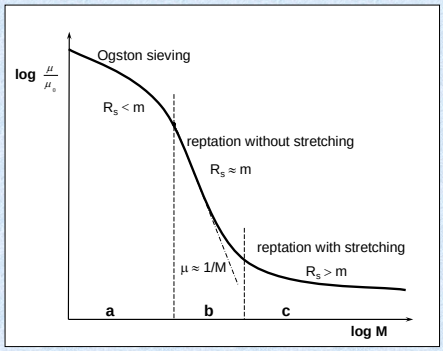
$$\varepsilon = \frac{qEa}{2 k_B T}$$

N reptation segments
ε scaled el. field
q segment charge
a segment length

$\mu/\mu_0 \sim$ 1/(3N) N<<ε^{-2}
ε^2/9 N>> ε^{-2}



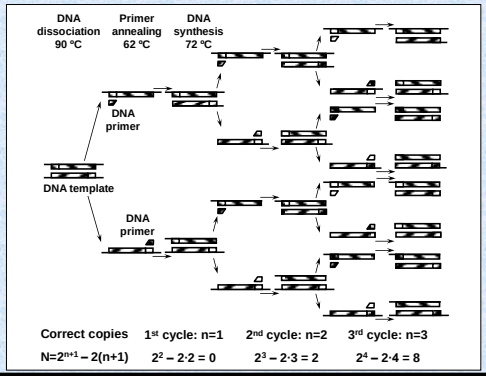
Dependence of DNA electrophoretic mobility on molecular mass



Polymerase chain reaction

PCR amplification

PCR amplification scheme



Human Genome Project

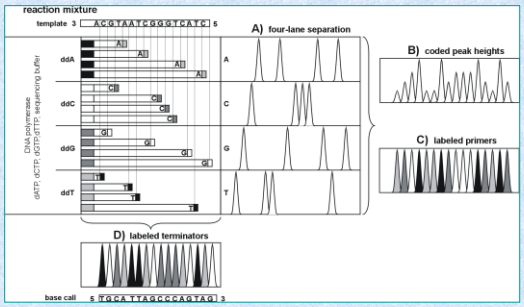
J. CRAIG VENTER, Ph.D., PRESIDENT, CELERA GENOMICS
REMARKS AT THE HUMAN GENOME ANNOUNCEMENT
THE WHITE HOUSE
MONDAY, JUNE 26, 2000

Mr. President, Honorable members of the Cabinet, Honorable members of Congress, distinguished guests.
Today, June 26, 2000 marks an historic point in the 100,000-year record of humanity. We are announcing today that for the first time our species can read the chemical letters of its genetic code. At 12:30 p.m. today, in a joint press conference with the public genome effort, Celera Genomics will describe the first assembly of the human genetic code from the whole genome shotgun sequencing method. Starting only nine months ago on September 8, 1999, eighteen miles from the White House, a small team of scientists headed by myself, Hamilton O. Smith, Mark Adams, Gene Myers and Granger Sutton began sequencing the DNA of the human genome using a novel method pioneered by essentially the same team five years earlier at The Institute for Genomic Research in Rockville, Maryland. The method used by Celera has determined the genetic code of five individuals....

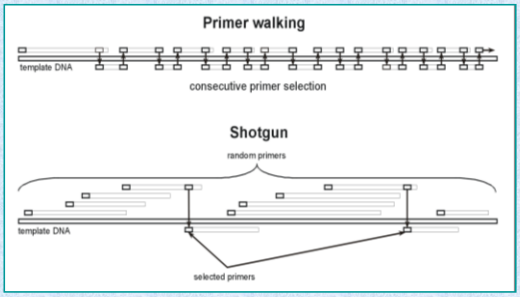
…There would be no announcement today if it were not for the more than **$1 billion that PE Biosystems invested in Celera and in the development of the automated DNA sequencer** that both Celera and the public effort used to sequence the genome…

DNA sequencing

Analysis of Sanger sequencing fragments

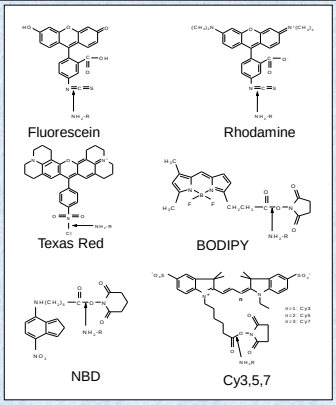


DNA sequencing strategy

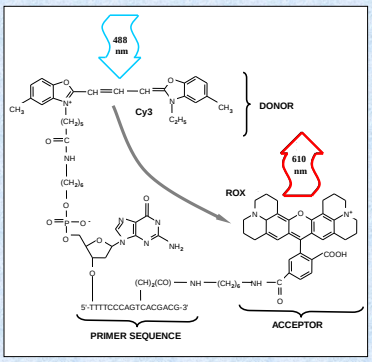


Fluorescence chemistry

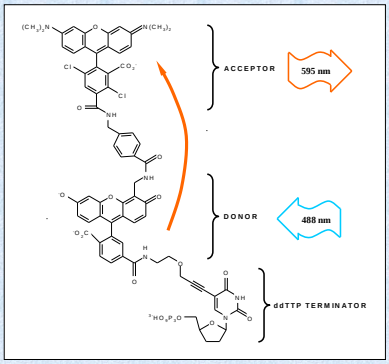
Fluorescent labels



Sequencing primer attached to Fluorescence Resonance Energy Transfer



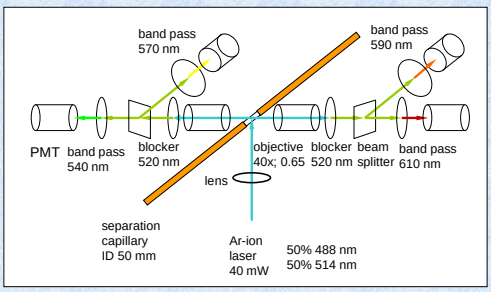
Dideoxy terminator attached to Fluorescence Resonance Energy Transfer



LIF detection

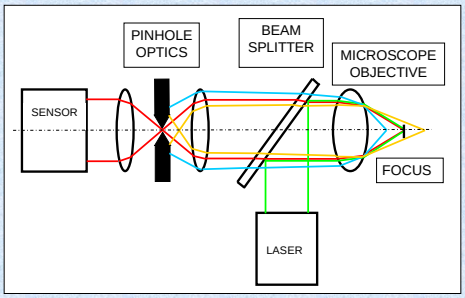
Spectral filtering

Four channel LIF detection arrangement

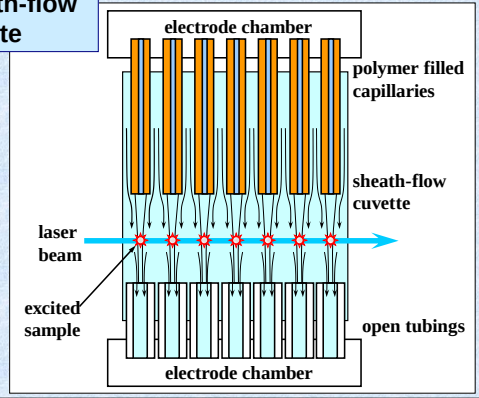


Space filtering

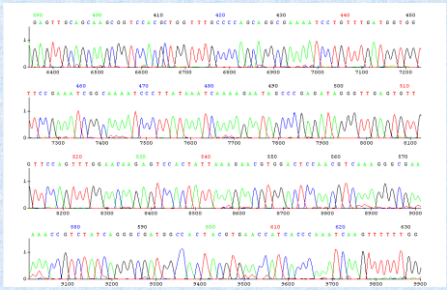
SCHEME OF CONFOCAL DETECTOR



Sheath-flow cuvette

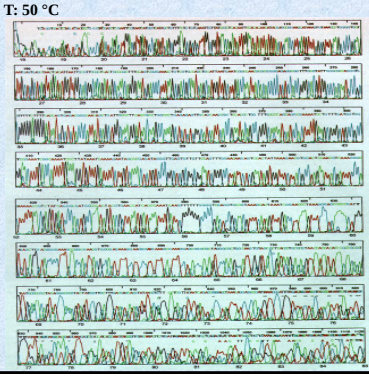


DNA sequencing record



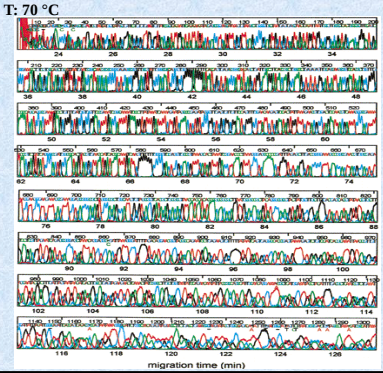
DNA sequencing over 1000 bases in 1.5 hour

Separation matrix: LPA 2.0% (w/v) 5.5 MDa
E: 150 V/cm, T: 50 °C



DNA sequencing up to 1300 bases in 2 hours

Separation matrix: LPA 2.0% (w/w) 17 MDa, 0.5% (w/w) 270 kDa
E: 125 V/cm, T: 70 °C



DNA mutation analysis

Restriction (amplification) fragment legth polymorphism

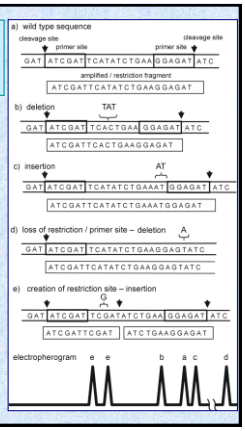
RFLP (AFLP)

Size based separation of ds or ss DNA fragments

Resolution:

ss > 1000

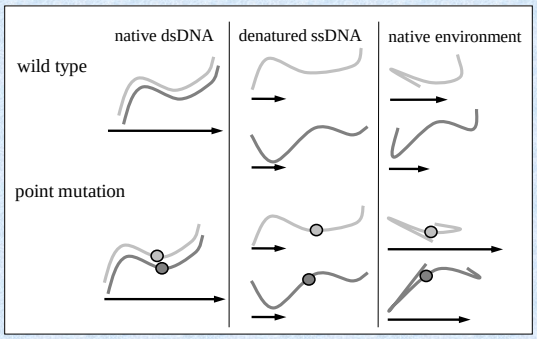
ds > 400



Single Strand Conformation Polymorphism

SSCP

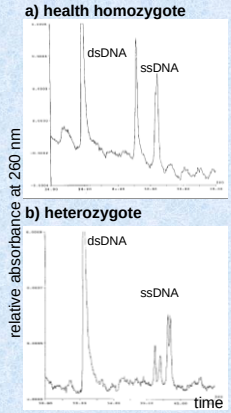
Principle of SSCP technique



Phenylketonuria

SSCP analysis
Detection of point mutation C > T in phenylalanine hydroxylase gene on chromosome 12

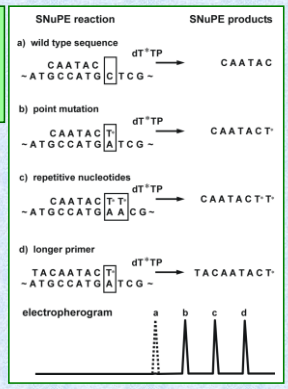
Separation conditions:
2% solution of agarose SeaPrep in 1xTBE with 10% formamide
T - 30 °C
LC - 55 cm
LD - 50 cm
E – a) 183 V/cm, b) 135 V/cm.



Single nucleotide primer extension

Minisequencing

SNuPE



Next generation sequencing

Parallel single molecule sequencing by synthesis

Helicos

The HeliScope™ Sequencer

2 . 10^9 b/day

10^9 reads/run
25 – 55 bp read lengths

454 LIFE SCIENCES

Genome Sequencer FLX System

3 . 10^8 b/day

100 Mb/7.5 hour run
400 000 reads/7.5 hour
200 – 300 bp read lengths

Solexa

Illumina Genome Analyzer

6 . 10^8 b / day

3 . 10^9 b / 5 days run
50 . 10^6 oligo clusters
36 – 50 bp read lengths

Photocleavable dideoxy nucleotides

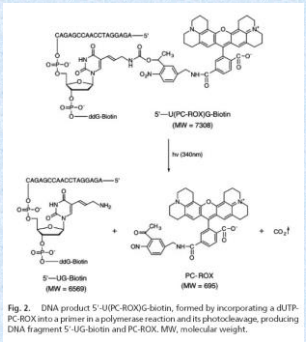


Fig. 2. DNA product 5'-U(PC-ROX)G-biotin, formed by incorporating a dUTP-PC-ROX into a primer in a polymerase reaction and its photocleavage, producing DNA fragment 5'-UG-biotin and PC-ROX. MW, molecular weight.

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DNA sequencing – DNA polymerase
RNA sequencing – reverse transcriptase
Codone-resolved translation elongation by single ribosomes
Tens of nucleotide peaks in 1 sec
Read length 1 – 15 kb
80 000 detection points
15 min/genome: 50 n/s * 80 000 points * 15 min * 60 s = 3.6 Gb
DNA polymerase 529 processivity 20 kB – 400 b/s
Some enzymes are not processive
$ 100/genome

Ion Torrent
The Ion Personal Genome Machine (PGM™) sequencer

http://www.iontorrent.com/

- Different templates in microwells
- Washing steps by individual nucleotides G, C, T, A
- The world's smallest solid-state pH meter
- Digital output