

PYROSEQUENCING

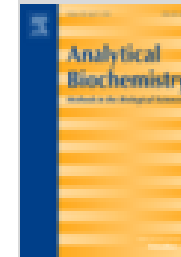
Polymerase has the Power

PYROSEQUENCING: THE ORIGINS



Analytical Biochemistry

Volume 167, Issue 2, December 1987, Pages 235-238



Enzymatic method for continuous monitoring of DNA
polymerase activity ☆

Pål Nyrén

“... One late afternoon in the beginning of January 1986, the idea for an alternative DNA sequencing technique came to my mind. The basic concept was to **follow the activity of DNA polymerase during nucleotide incorporation into a DNA strand by analyzing the pyrophosphate released during the process.**”

SNPs vs POINT MUTATIONS

Analytical Biochemistry **280**, 103–110 (2000)

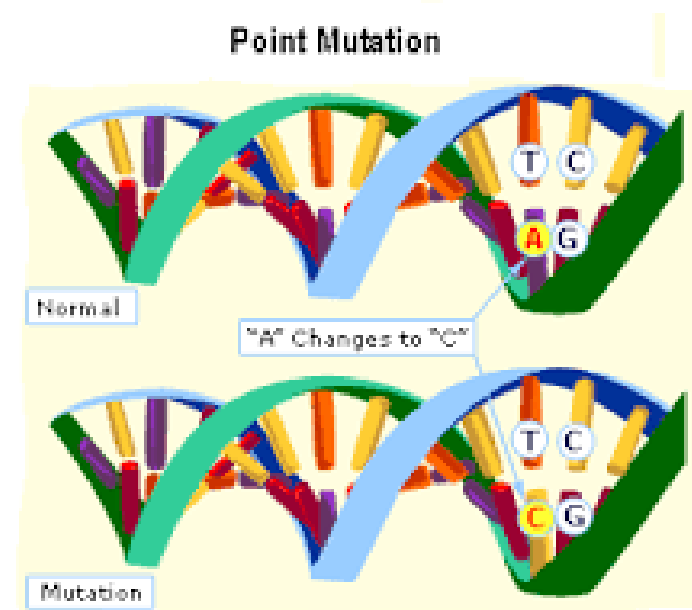
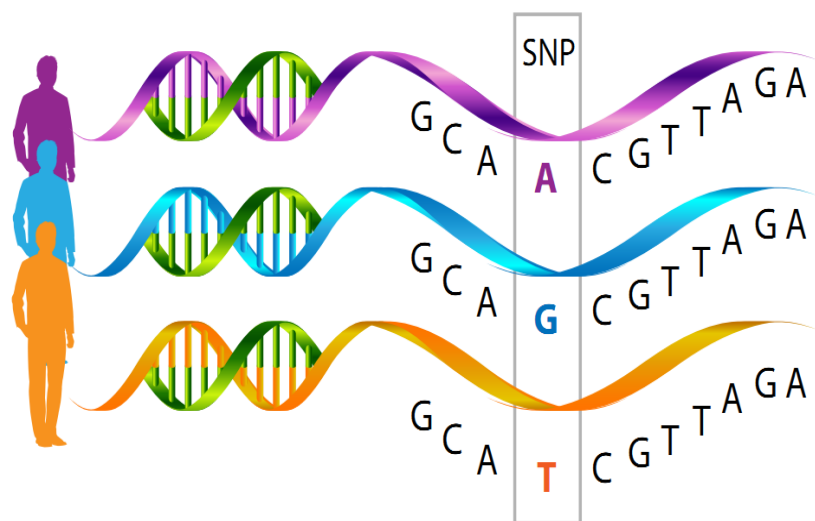
doi:10.1006/abio.2000.4493, available online at <http://www.idealibrary.com> on **IDEAL**®

Single-Nucleotide Polymorphism Analysis by Pyrosequencing

Afshin Ahmadian, Baback Gharizadeh, Anna C. Gustafsson, Fredrik Sterky, Pål Nyrén,
Mathias Uhlén, and Joakim Lundeberg¹

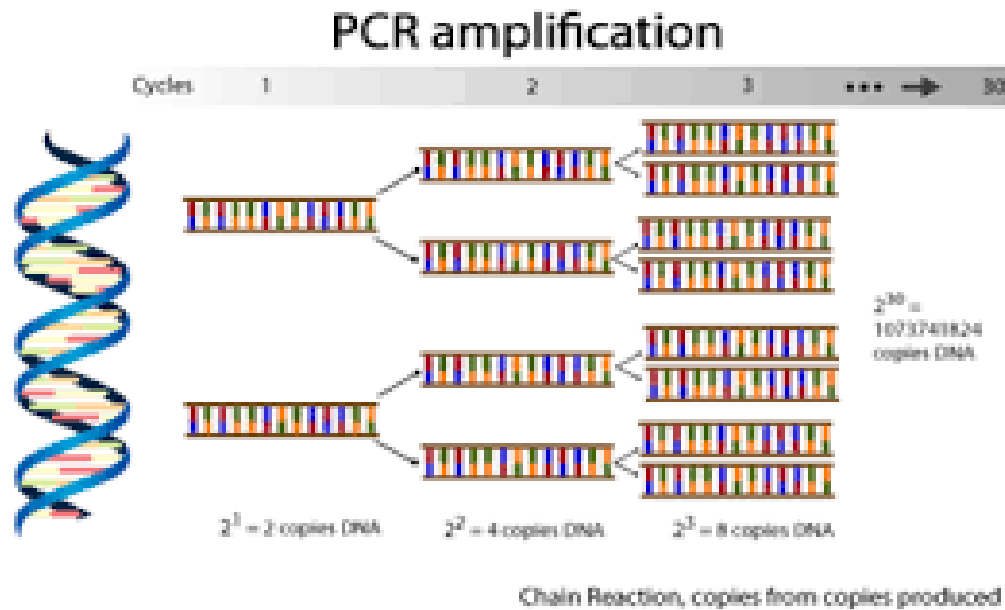
Department of Biotechnology, The Royal Institute of Technology (KTH), SE-100 44 Stockholm, Sweden

Received October 6, 1999

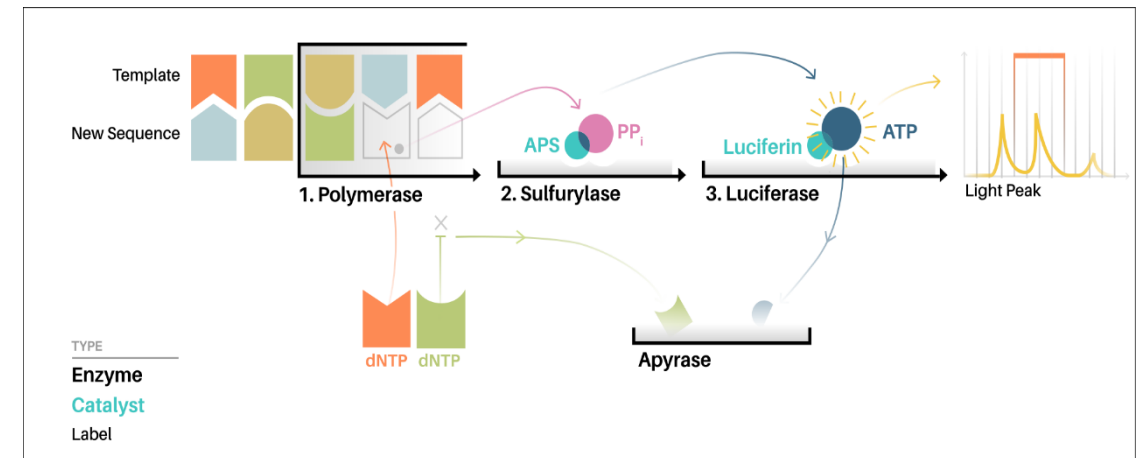
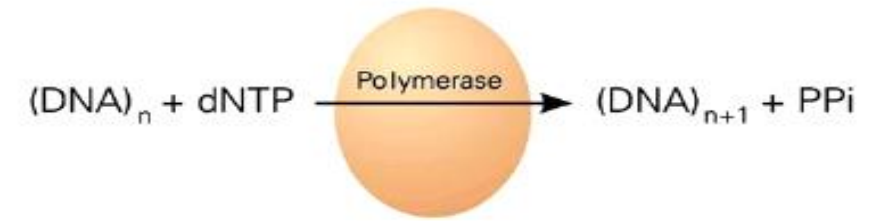


PYROSEQUENCING: THE FEATURES (PART 1)

1. PCR-BASED METHOD



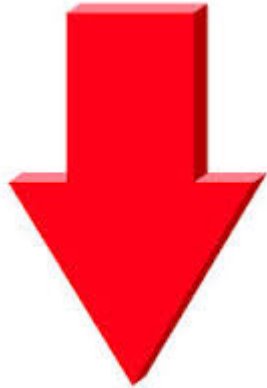
2. ENZYMATIC, SEQUENCE BY SINTESYS TECHNIQUE



PYROSEQUENCING: THE FEATURES (PART 2)

3. SHORT/MEDIUM RANGE SEQUENCE

NO MORE THAN 30-50 NUCLEOTIDE



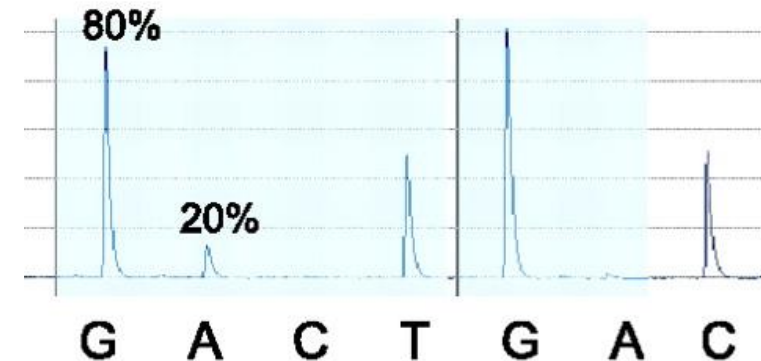
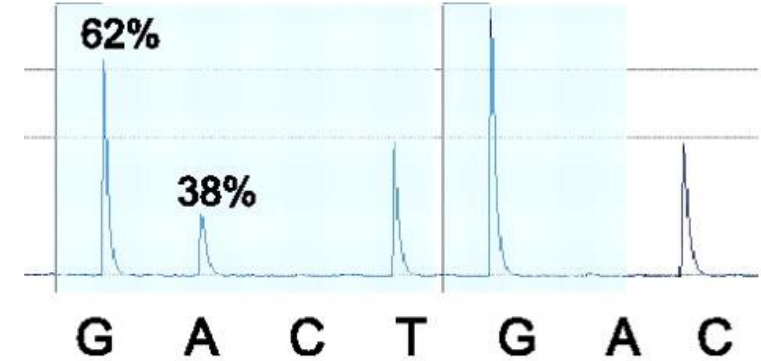
SENSIBILITY



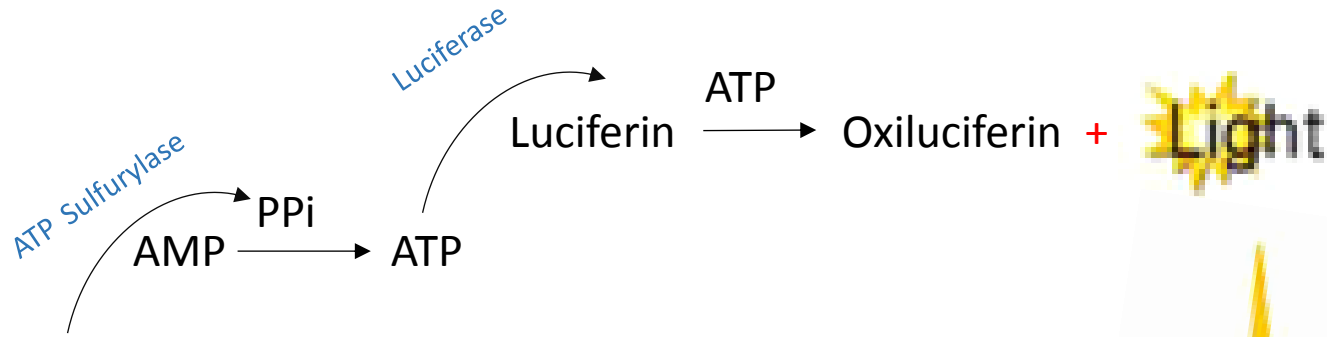
SPECIFICITY

4. QUANTITATIVE:

PERCENTAGE OF MUTATION



PYROSEQUENCING: THE CHEMISTRY

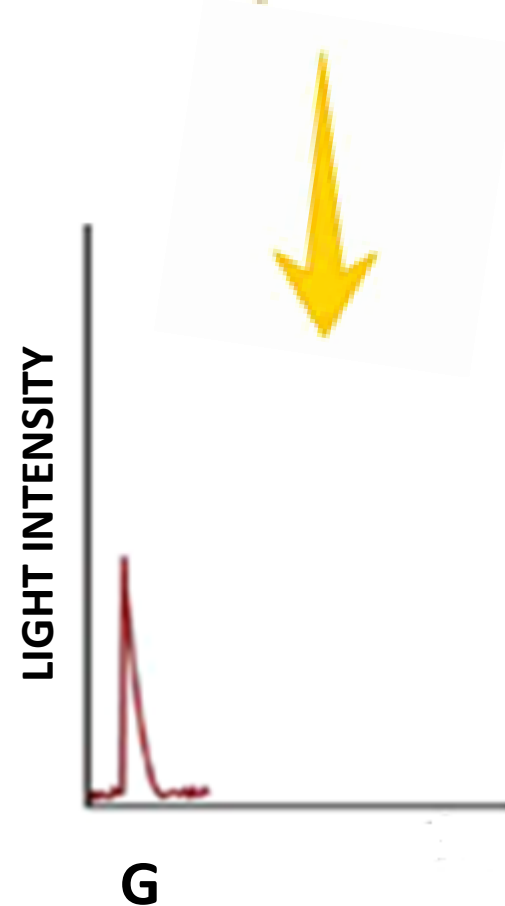


5'-TGGACCTGAGCT**G**
3'-ACCTGGACTCGA**C**GGAGGTGGCTAGATG-5'

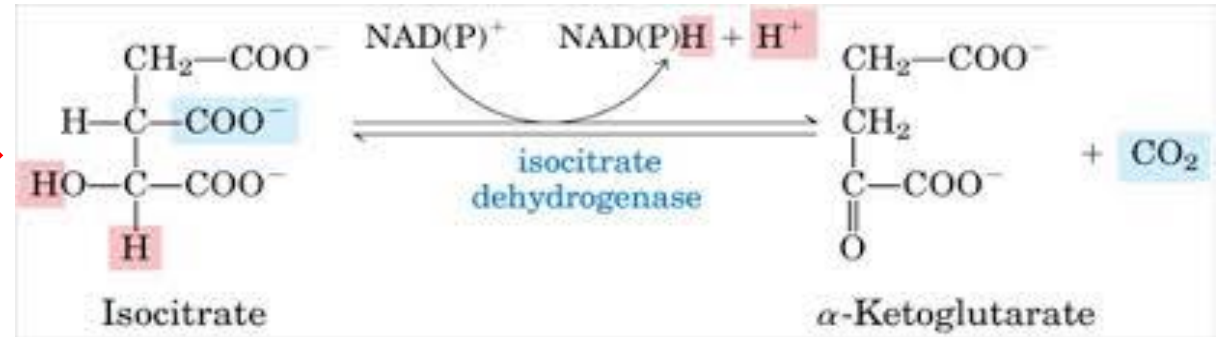
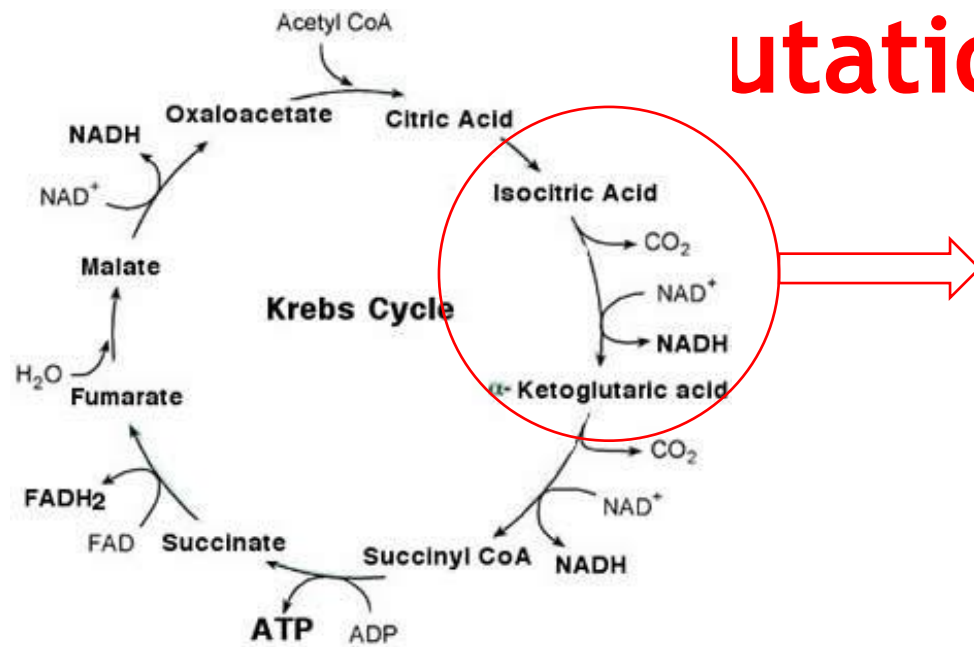
DNA polymerase

LIGHT INTENSITY

G



THE PYROSEQUENCING OUTPUT (1): *IDH1* mutation in Glioma



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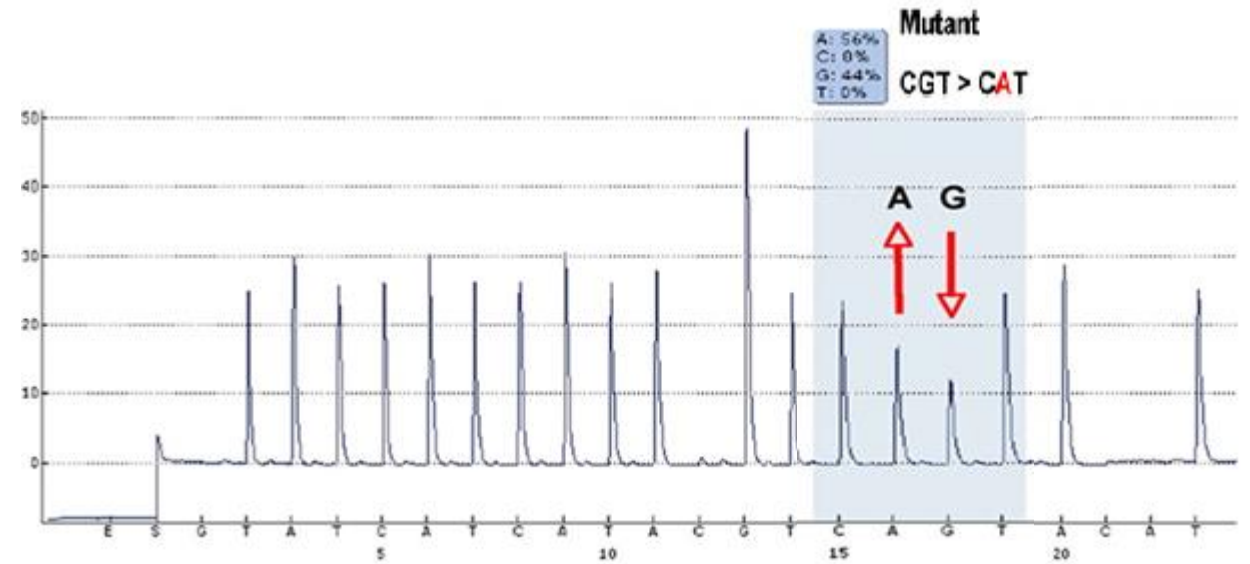
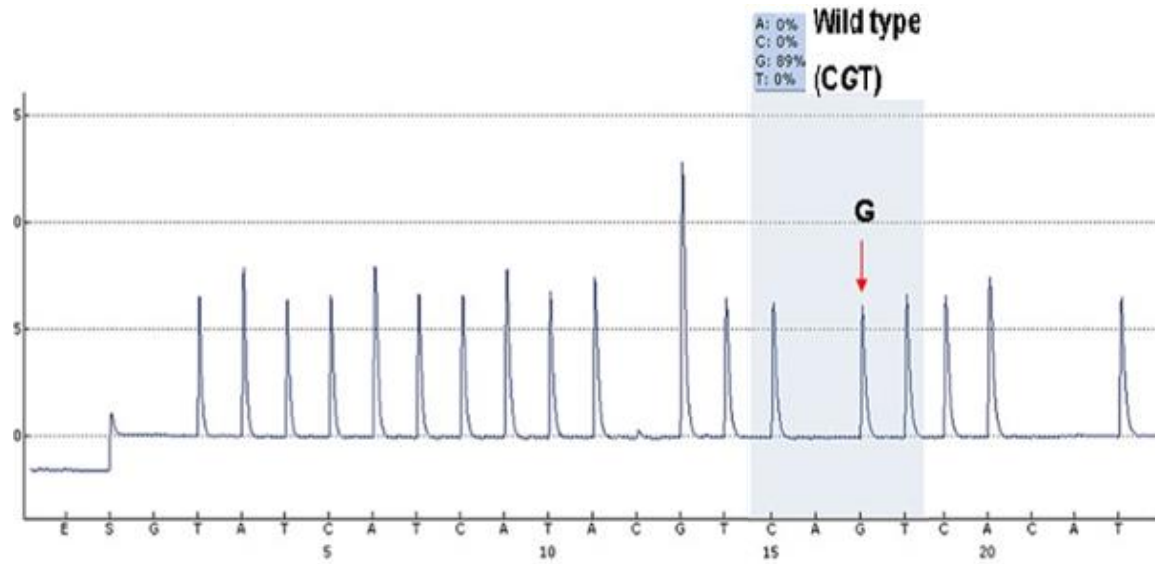
IDH1 and *IDH2* Mutations in Gliomas

Hai Yan, M.D., Ph.D., D. Williams Parsons, M.D., Ph.D., Genglin Jin, Ph.D., Roger McLendon, M.D., B. Ahmed Rasheed, Ph.D., Weishi Yuan, Ph.D., Ivan Kos, Ph.D., Ines Batinic-Haberle, Ph.D., Siân Jones, Ph.D., Gregory J. Riggins, M.D., Ph.D., Henry Friedman, M.D., Allan Friedman, M.D., David Reardon, M.D., James Herndon, Ph.D., Kenneth W. Kinzler, Ph.D., Victor E. Velculescu, M.D., Ph.D., Bert Vogelstein, M.D., and Darell D. Bigner, M.D., Ph.D.

Departments of Pathology (H.Y., G.J., R.M., B.A.R., D.D.B.), Radiation Oncology (I.K., I.B.-H.), Neuro-Oncology (H.F.), and Surgery (A.F., D.R.), the Pediatric Brain Tumor Foundation Institute and the Preston Robert Tisch Brain Tumor Center; and the Cancer Statistical Center (J.H.) — all at Duke University Medical Center, Durham, NC; the Ludwig Center for Cancer Genetics and Therapeutics and the Howard Hughes Medical Institute at Johns Hopkins Kimmel Cancer Center (D.W.P., S.J., K.W.K., V.E.V., B.V.) and the Department of Neurosurgery, Johns Hopkins Medical Institutions (G.J.R.) — all in Baltimore; the Department of Pediatrics, Baylor College of Medicine, Houston (D.W.P.); and the Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD (W.Y.)

80-90% of *IDH1* mutated Gliomas carrying the p.R132H (c.395G>A)

THE PYROSEQUENCING OUTPUT (2): *IDH1* c.395G>A (p.R132H)



THANKS FOR
YOUR
ATTENTION

