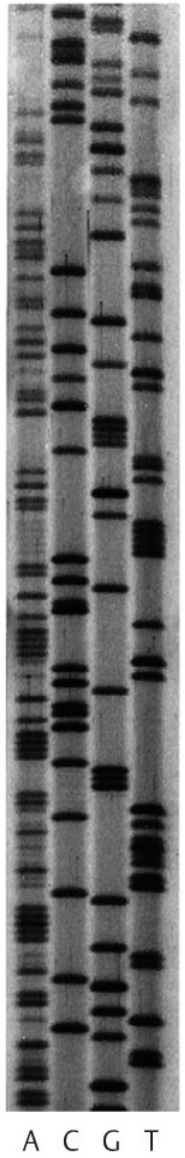


DNA-Analytik I

DNA

Desoxyribonukleinsäure

Friedrich Miescher
1844-1895

- beschrieb Abtrennung von Cytoplasma und Zellkernen
- isolierte aus Zellkernen

NUKLEIN

saure Verbindung mit ungewöhnlich viel Phosphor und - im Gegensatz zu Proteinen – ohne/wenig Schwefel

Nukleoproteide *dts*

Nucleoprotein *engl*

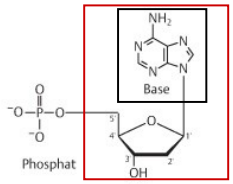
Nukleinsäure

= Zucker + Phosphat + Base

DNA

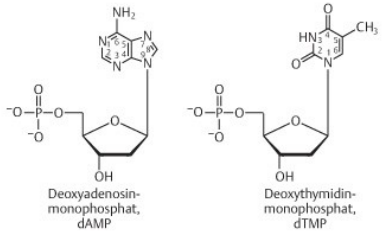
Nukleotide

Basen Adenin
Guanin
Cytosin
Thymin
Uracil



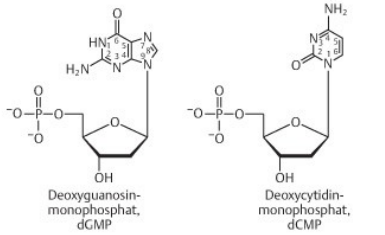
Zucker (Deoxyribose)
Nucleosid (Deoxyadenosin)
Nucleotid (Deoxyadenosin-5'-phosphat)

Nukleoside Adenosin
Guanosin
Cytidin
Thymidin
Uridin



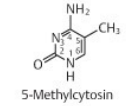
Deoxyadenosin-monophosphat, dAMP

Deoxythymidin-monophosphat, dTMP

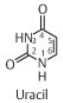


Deoxyguanosin-monophosphat, dGMP

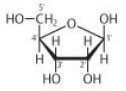
Deoxycytidin-monophosphat, dCMP



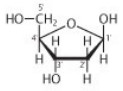
5-Methylcytosin



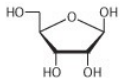
Uracil

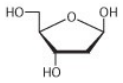


D-Ribose



2-Deoxy-D-ribose

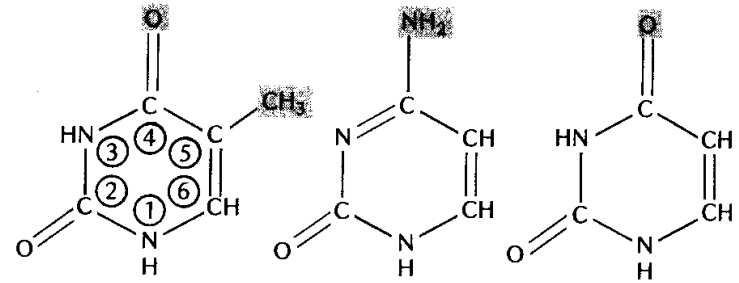




DNA

Basen

Pyrimidine, Basen mit einem Ring:



Purine, Basen mit zwei Ringen:

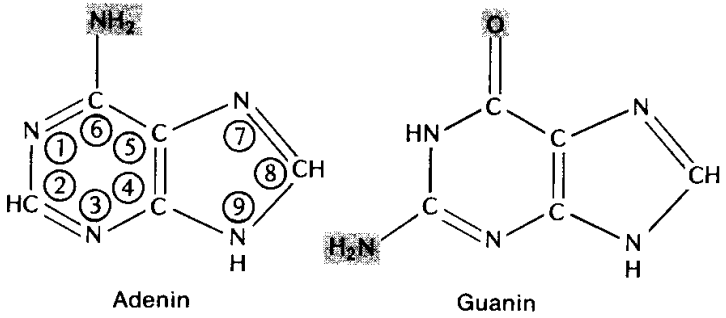


Abb. 4-3 Strickberger, 1985

DNA

Normale Basen und einige ihrer Analoga

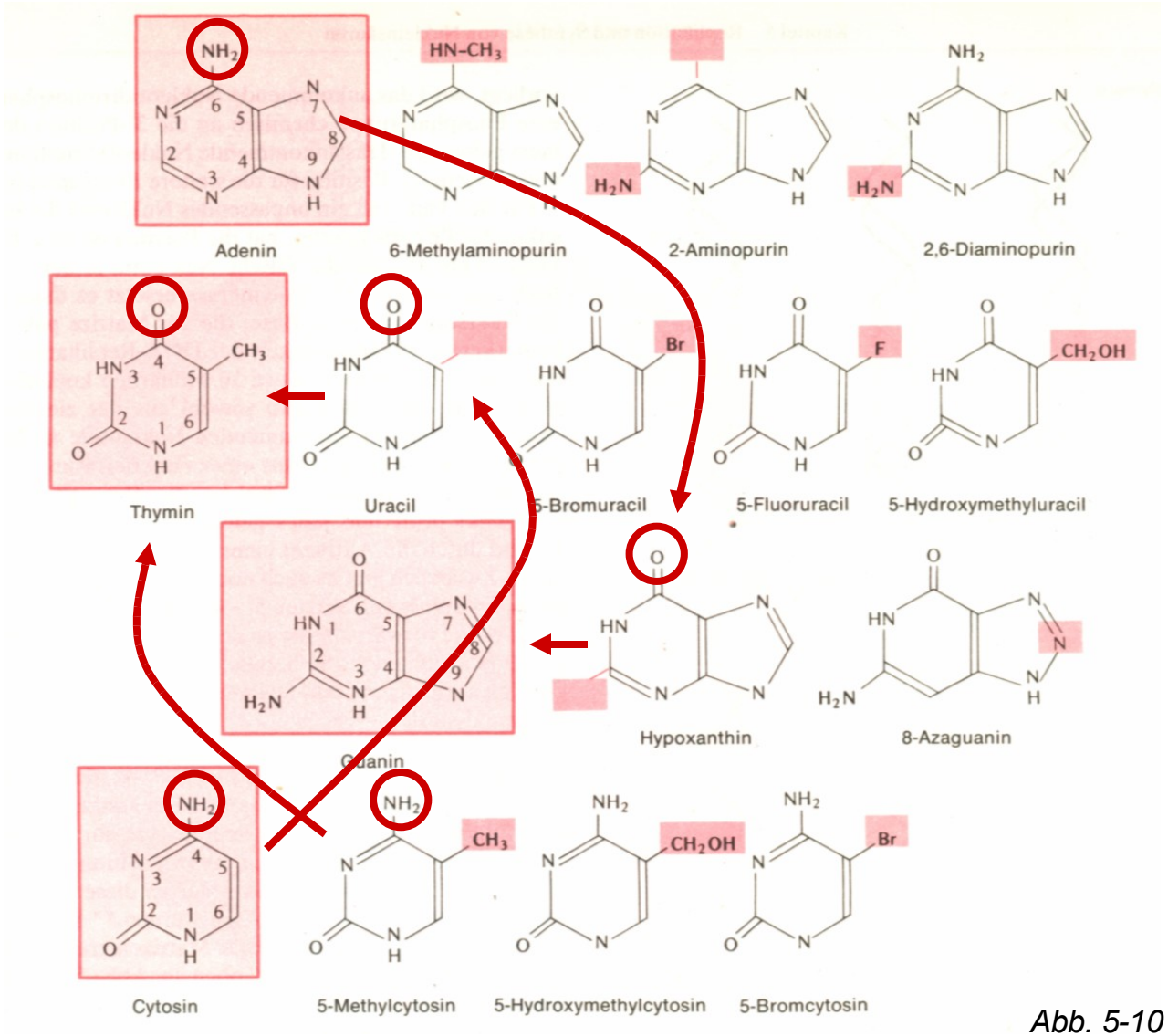
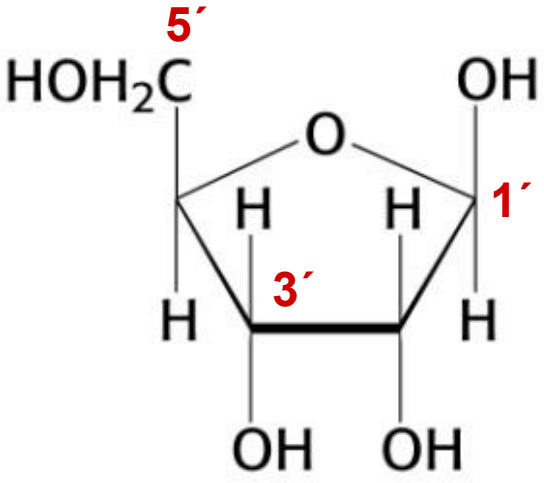


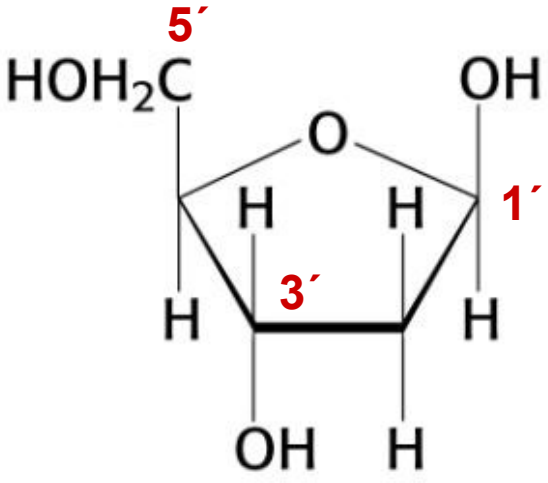
Abb. 5-10 Strickberger, 1985

DNA

Zucker



D-Ribose



2-Deoxy-D-ribose

DNA

Zuckerphosphat

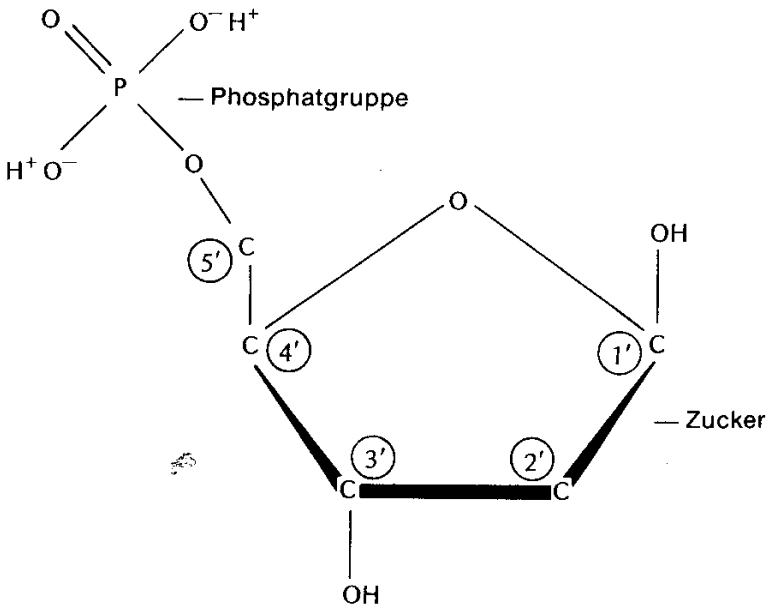


Abb. 4-2 Strickberger, 1985

DNA

Polynukleotid

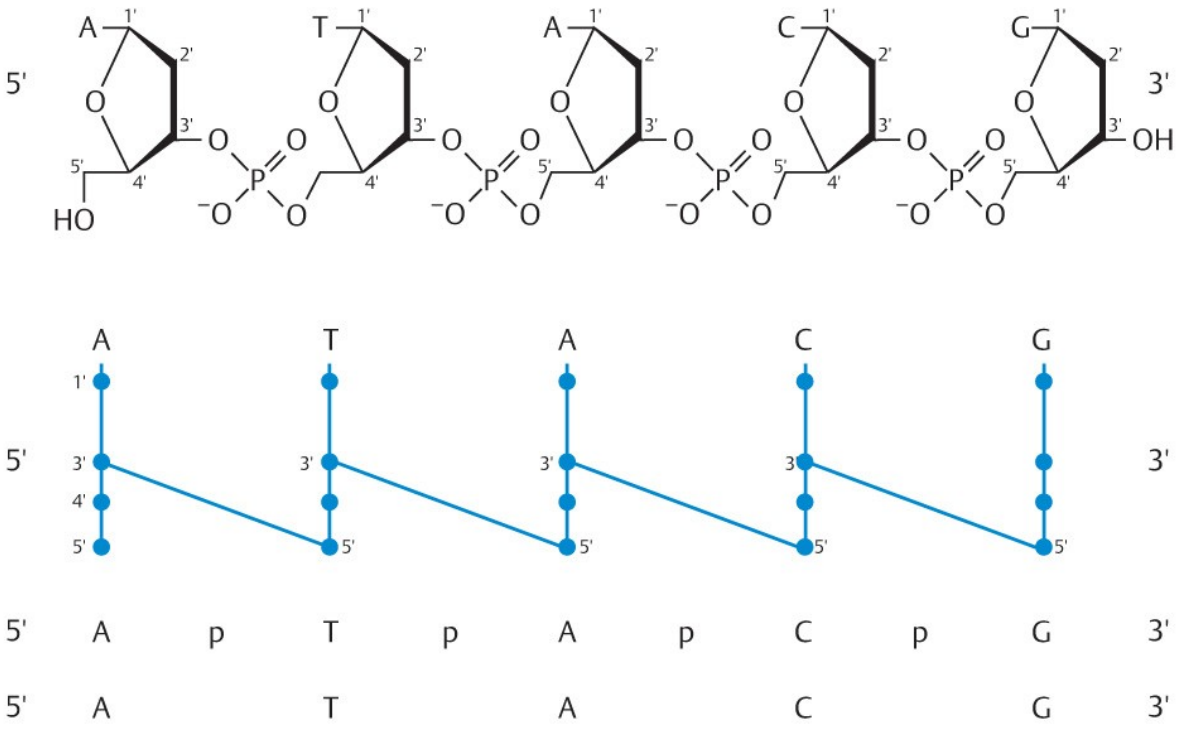
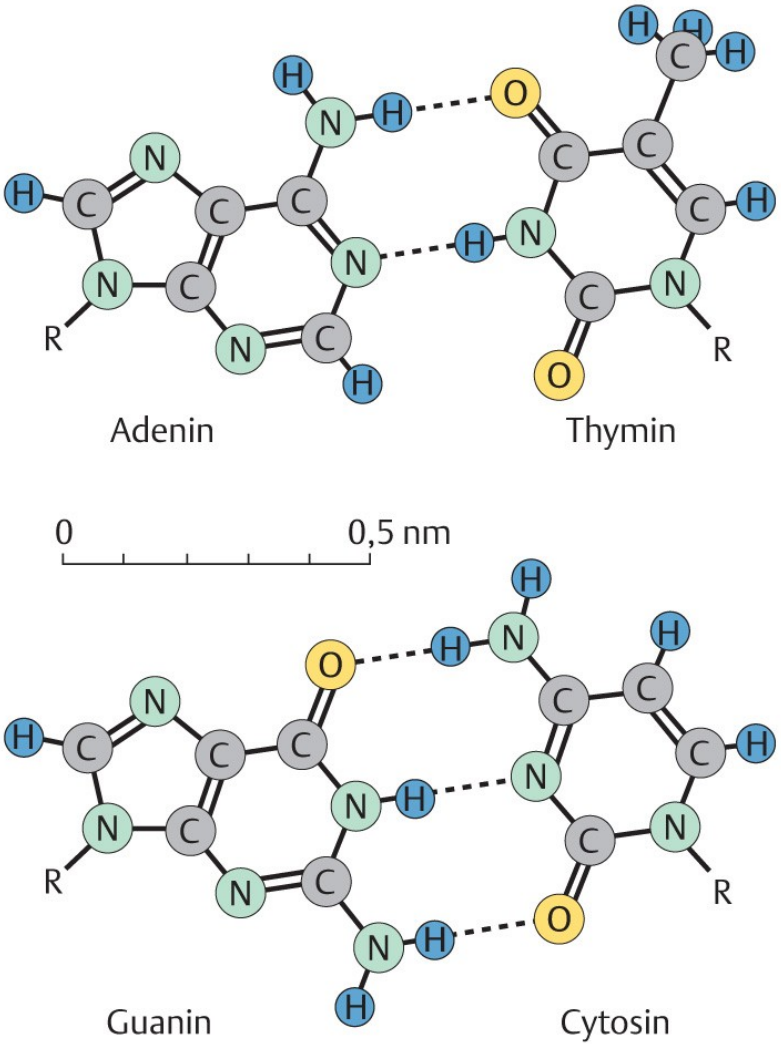


Abb. 2-02

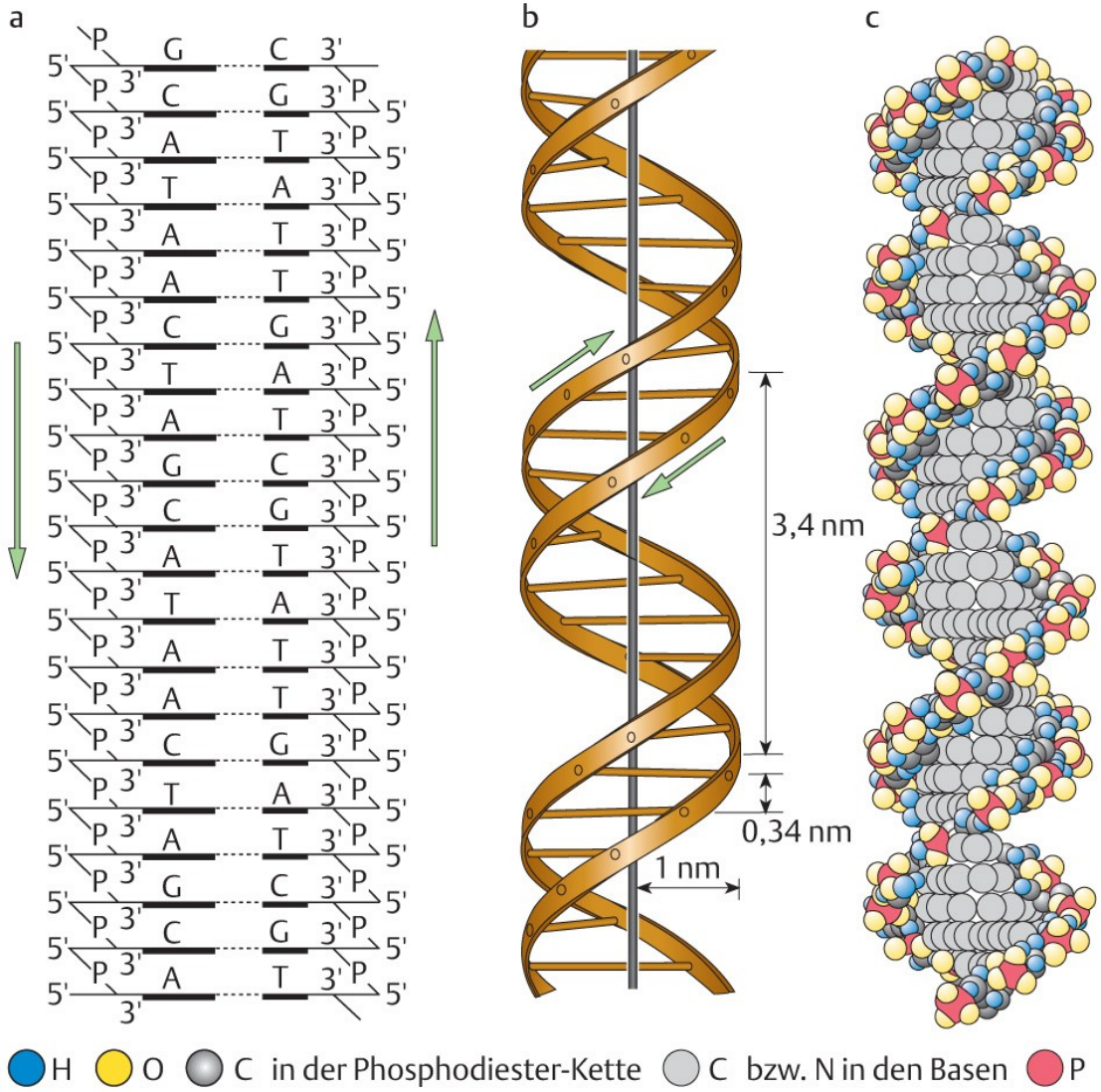
DNA

Wasserstoffbrückenbindung



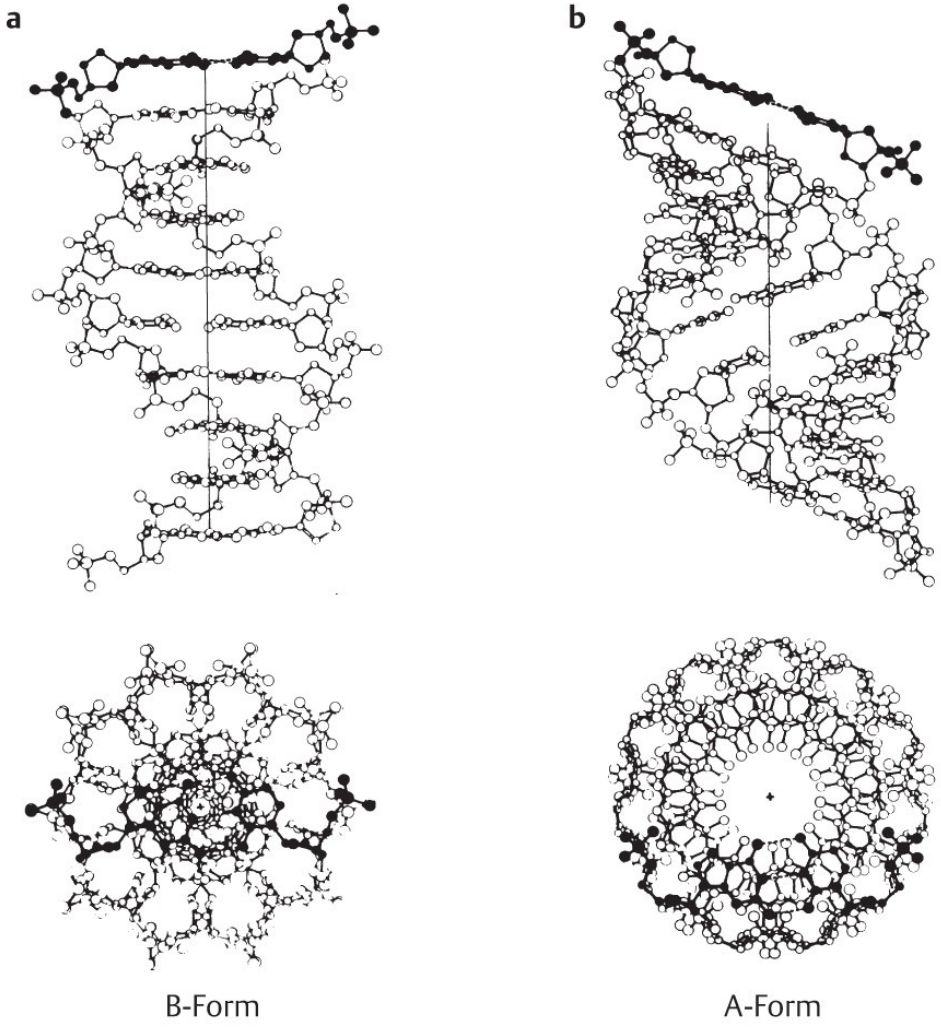
DNA

Polynukleotidkette & Doppelhelix



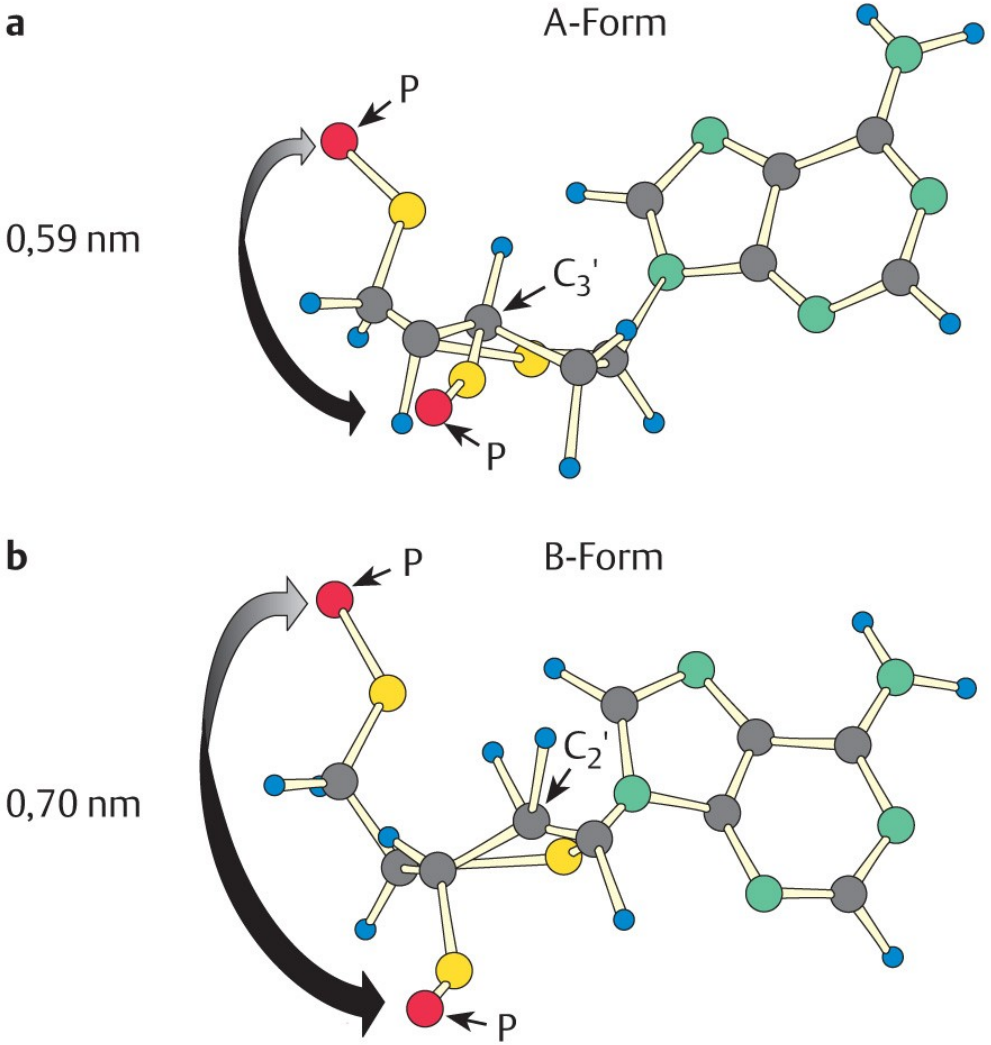
DNA

Doppelhelices, rechtsgängig



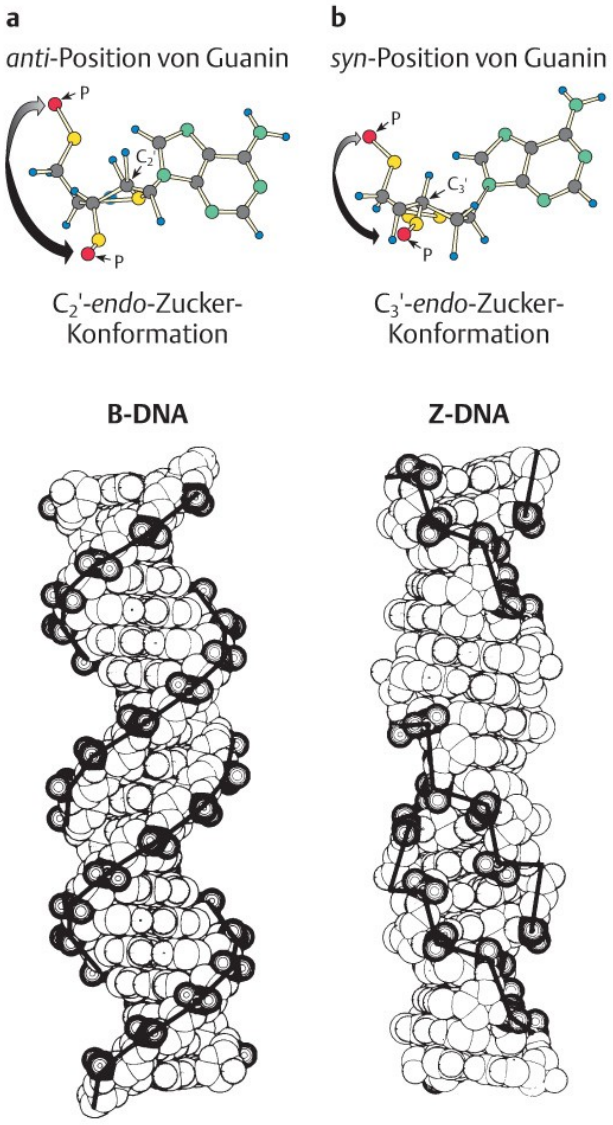
DNA

Doppelhelices, rechtsgängig



DNA

Doppelhelices, rechts- & linksgängig



DNA-Komplexität

Phylogenie

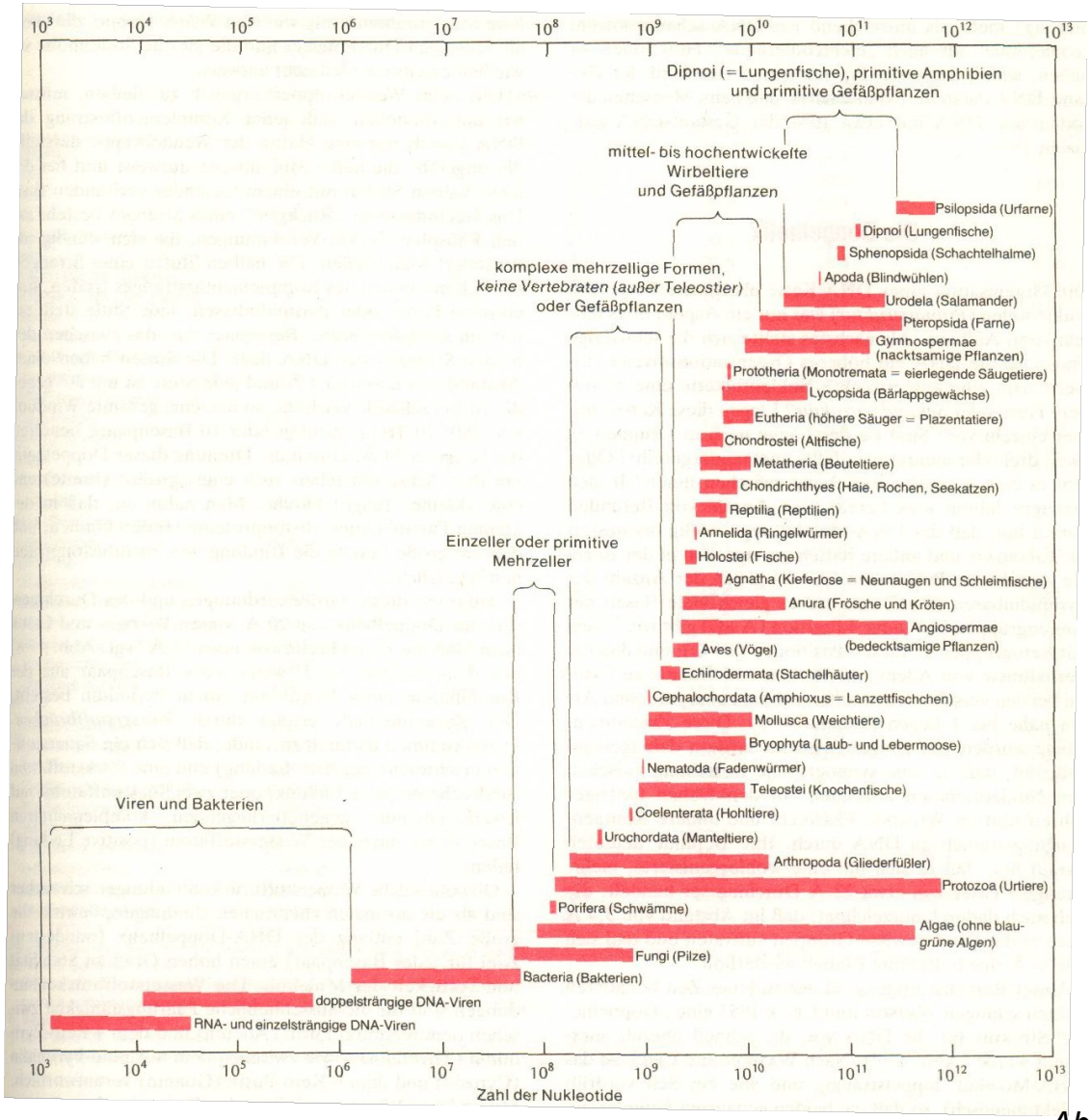
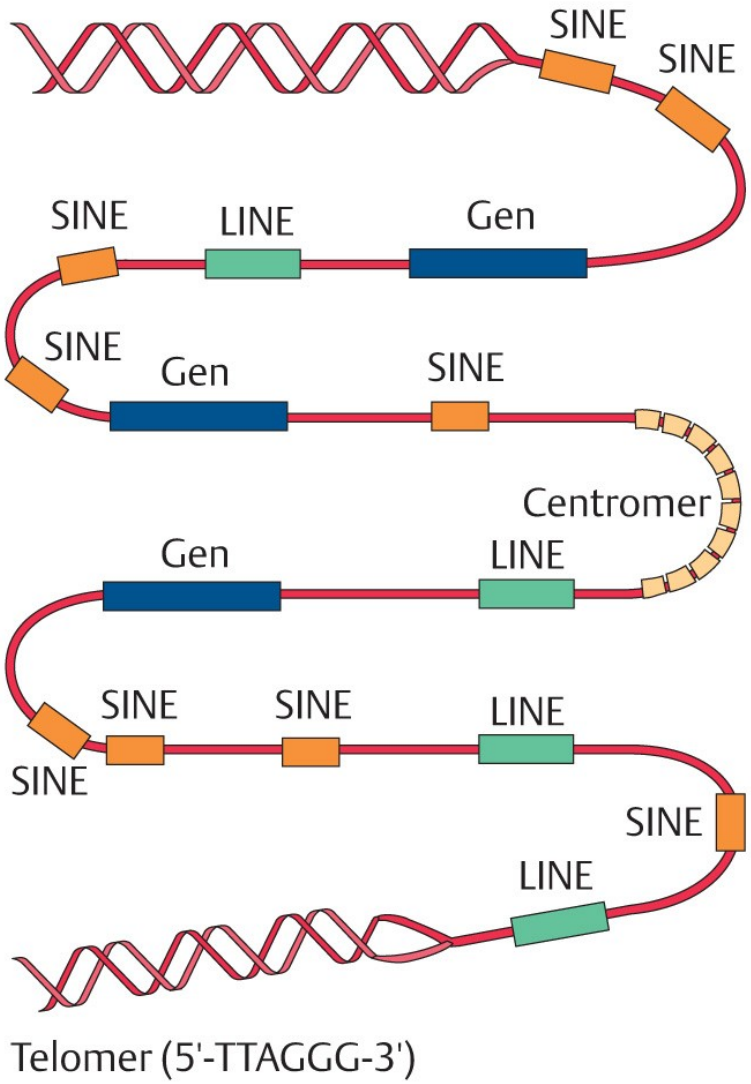


Abb. 4-6 Strickberger, 1985

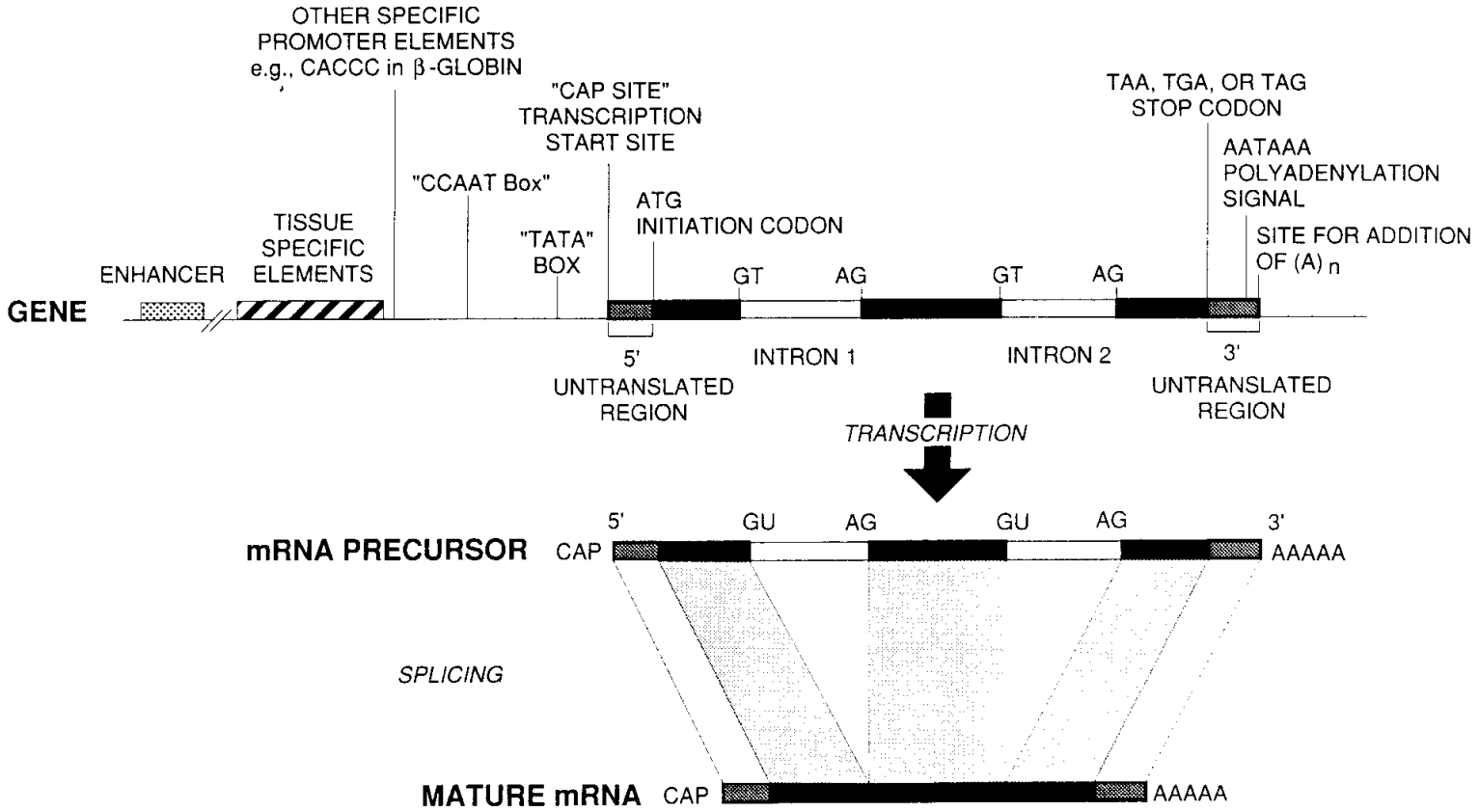
DNA-Komplexität

genomweite „Repeats“



DNA-Komplexität

eukaryontischen Genstruktur



DNA-Analytik

Stöchiometrie & Metrik

1 g DNA = 2×10^{21} Nukleotide
1 pg DNA = 2×10^{9} Nukleotide

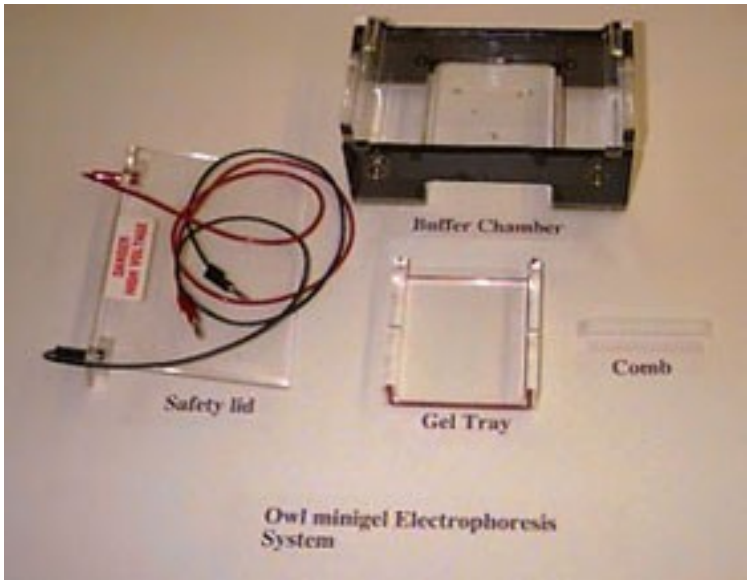
1 mm DNA = $2{,}9 \times 10^{6}$ Nukleotide

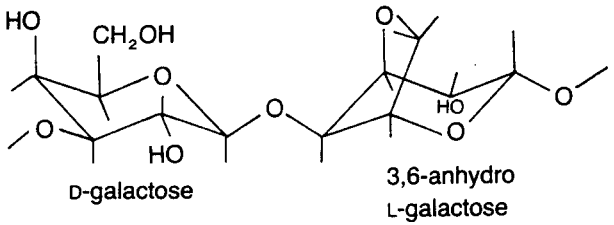
Humangenom = 3×10^{9} bp = 6×10^{9} Nukleotide

haploide Chromosomensatz = **3 pg** DNA **~ 1 m**

DNA-Analytik

Agarose-Gelelektrophorese





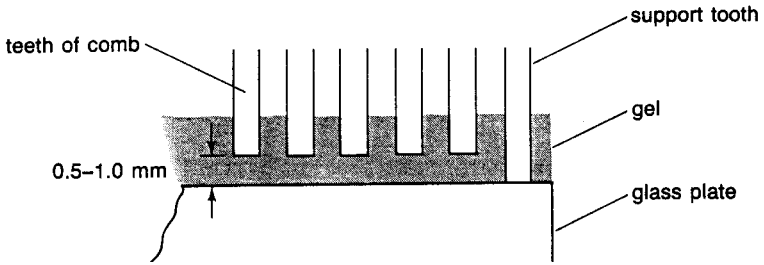


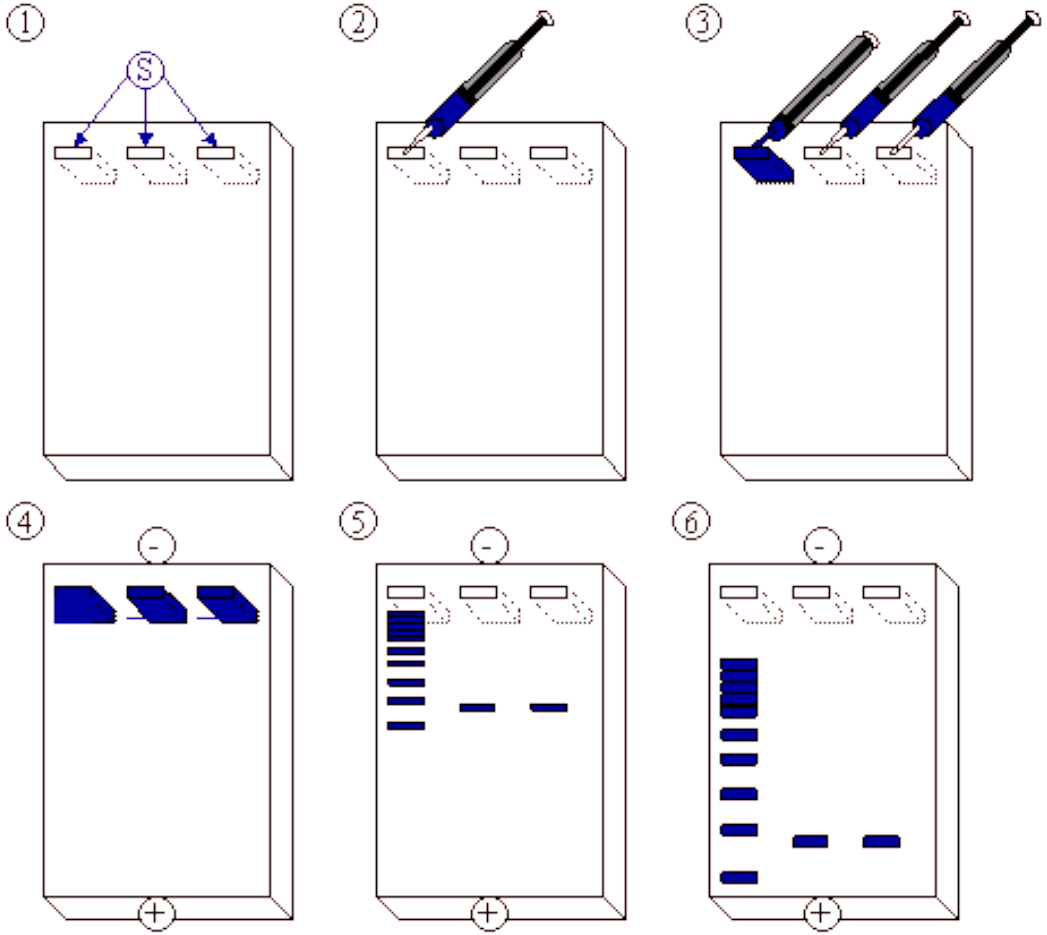
TABLE 6.1 Range of Separation in Gels Containing Different Amounts of Agarose

Amount of agarose in gel (% [w/v])	Efficient range of separation of linear DNA molecules (kb)
0.3	5–60
0.6	1–20
0.7	0.8–10
0.9	0.5–7
1.2	0.4–6
1.5	0.2–3
2.0	0.1–2

Maniatis, 1989

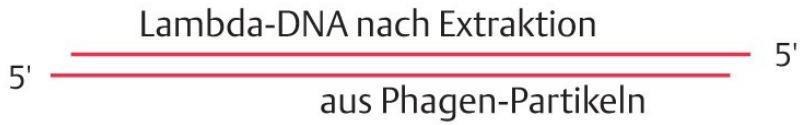
DNA-Analytik

Agarose-Gelelektrophorese

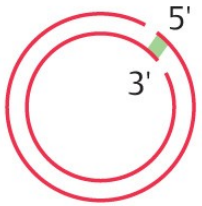


DNA-Analytik

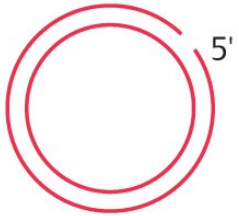
Agarose-Gelelektrophorese



Form III, linear



Lockerer Ringschluss durch Wasserstoffbrücken zwischen den komplementären Basen der einzelsträngigen Enden



Kovalente Bindung zwischen den Nucleotiden am 3'- und am 5'- Ende eines der Stränge durch das Enzym Polynucleotid-Ligase

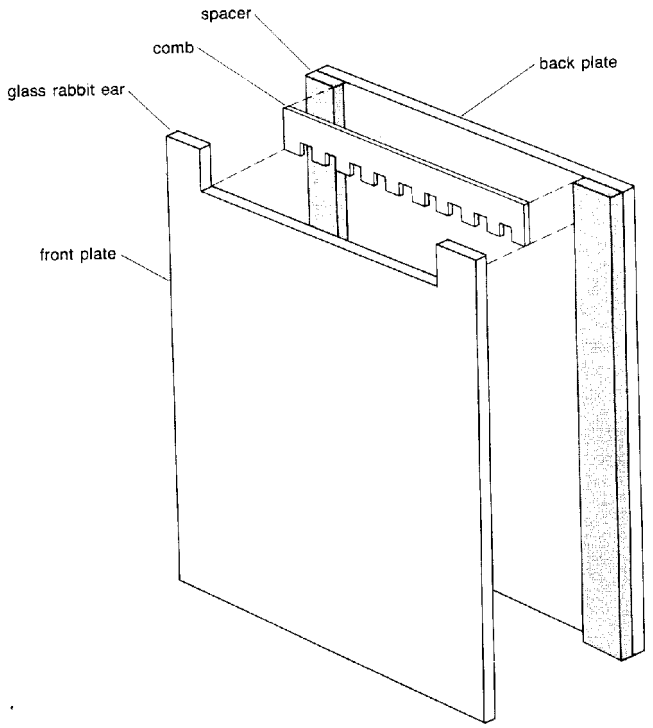
Form II, oc

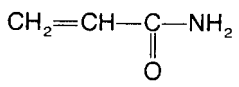
Ringförmige DNA-Moleküle, in denen beide Stränge geschlossen sind, sind nach der Extraktion aus der Zelle vielfach durch Verdrillungen gekennzeichnet. Man bezeichnet diese Verdrillungen als Supercoils

Form I, ccc

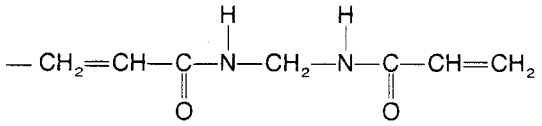
DNA-Analytik

Polyacrylamid(PA)-Gelelektrophorese





Acrylamid (AA)



N,N'-Methylen-Bis-AA

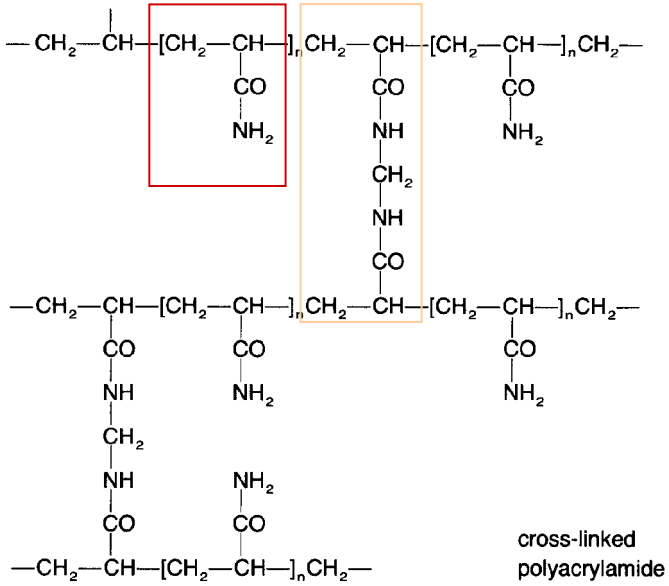


TABLE 6.4 Effective Range of Separation of DNAs in Polyacrylamide Gels

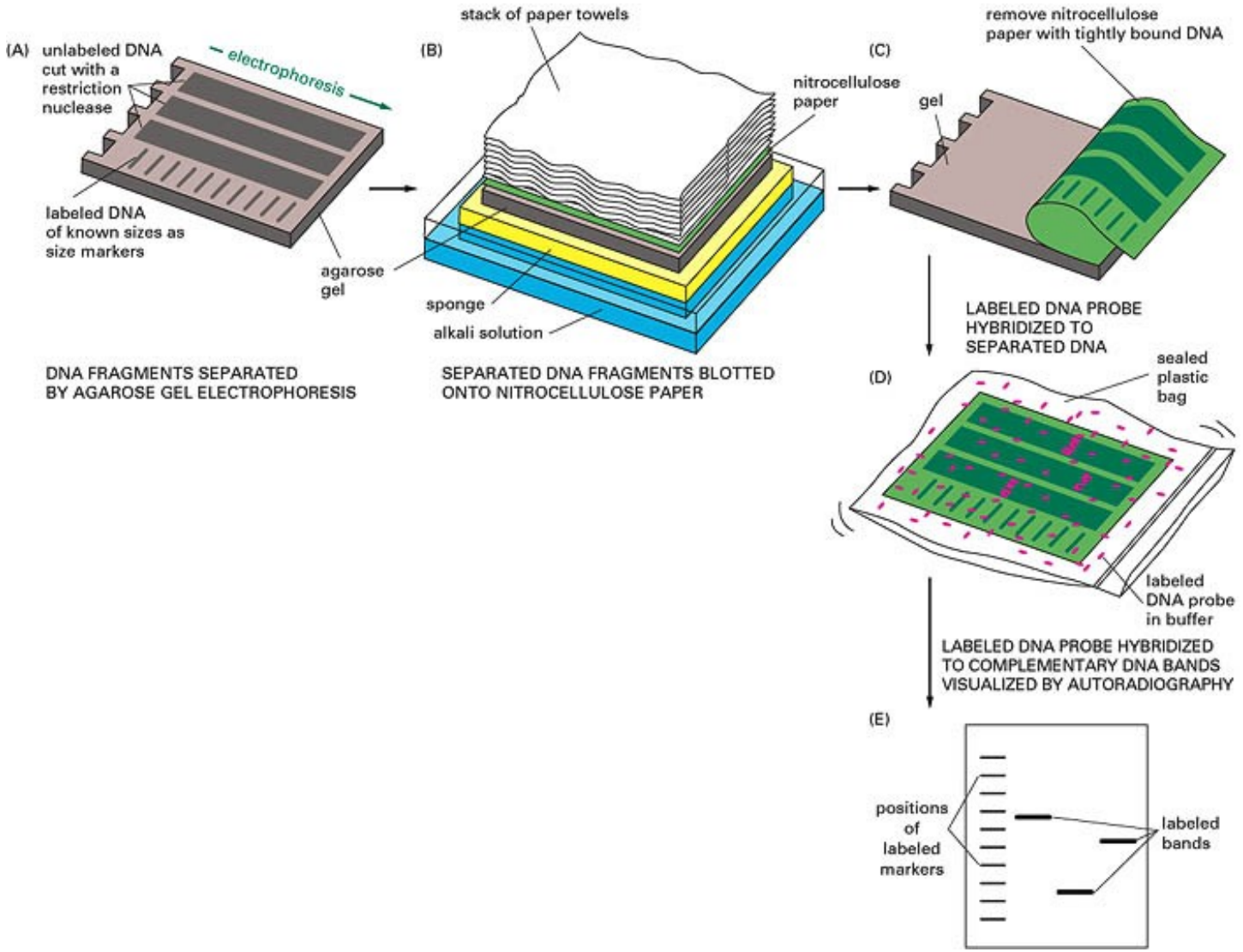
Acrylamide (% [w/v])[a]	Effective range of separation (bp)	Xylene cyanol FF[b]	Bromophenol blue[b]
3.5	1000–2000	460	100
5.0	80–500	260	65
8.0	60–400	160	45
12.0	40–200	70	20
15.0	25–150	60	15
20.0	6–100	45	12

[a] *N,N'*-methylenebisacrylamide is included at 1/30th the concentration of acrylamide.
[b] The numbers given are the approximate sizes (in nucleotide pairs) of fragments of double-stranded DNA with which the dye comigrates.

Maniatis, 1989

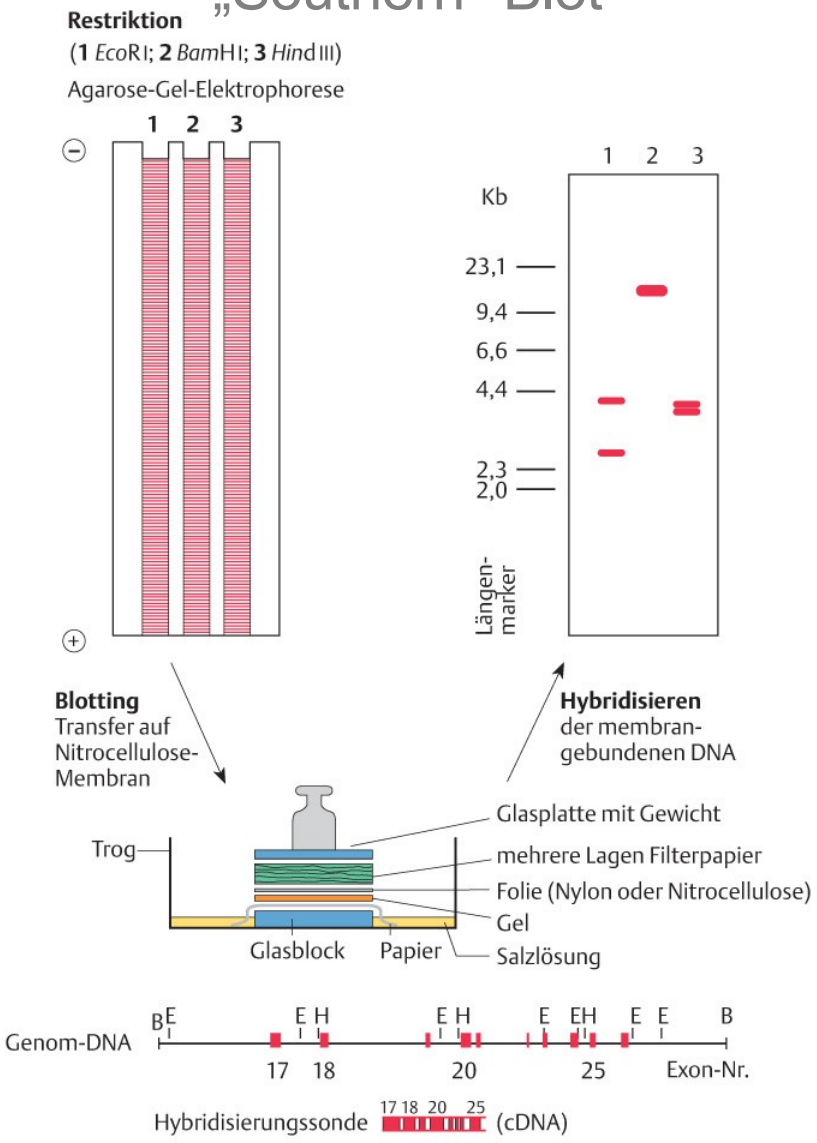
DNA-Analytik

„Southern“-Blot



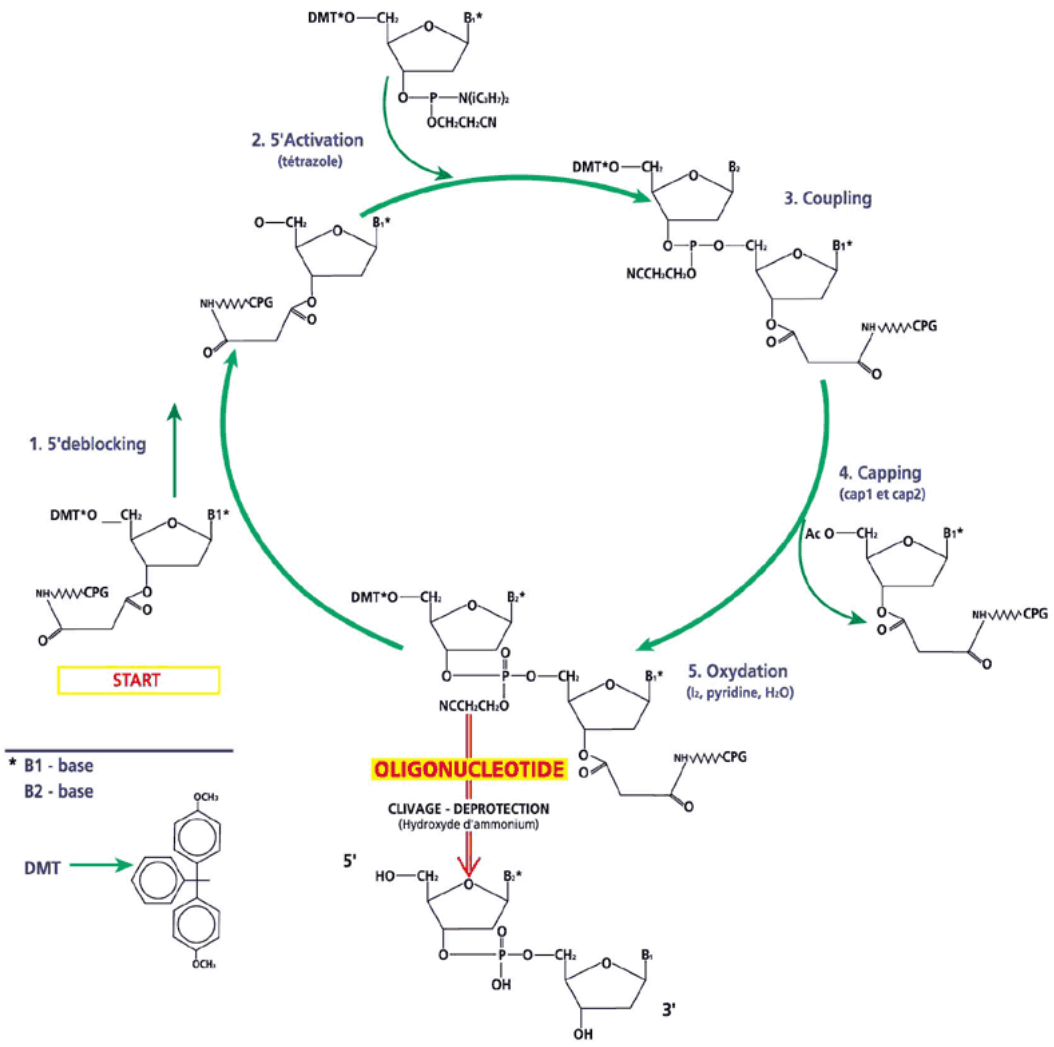
DNA-Analytik

„Southern“-Blot



DNA-Synthese

Phosphit-Methode



Umsetzungsrate & Ausbeute

$0{,}9^{20} = 0{,}12$

$0{,}99^{20} = 0{,}82$

$0{,}999^{20} = 0{,}98$

DNA-Synthese

Gensynthese aus Oligonukleotiden

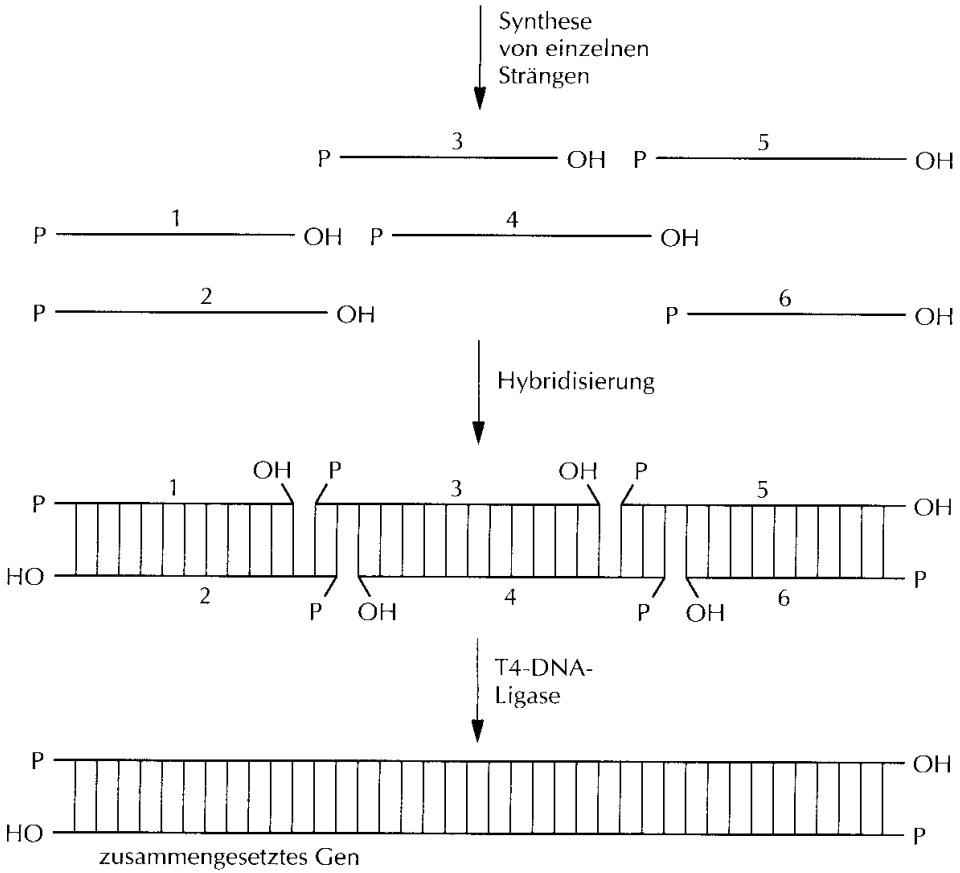


Abb. 3-9 Glick & Pasternak, 1995

DNA-Synthese

Gensynthese aus Oligonukleotiden

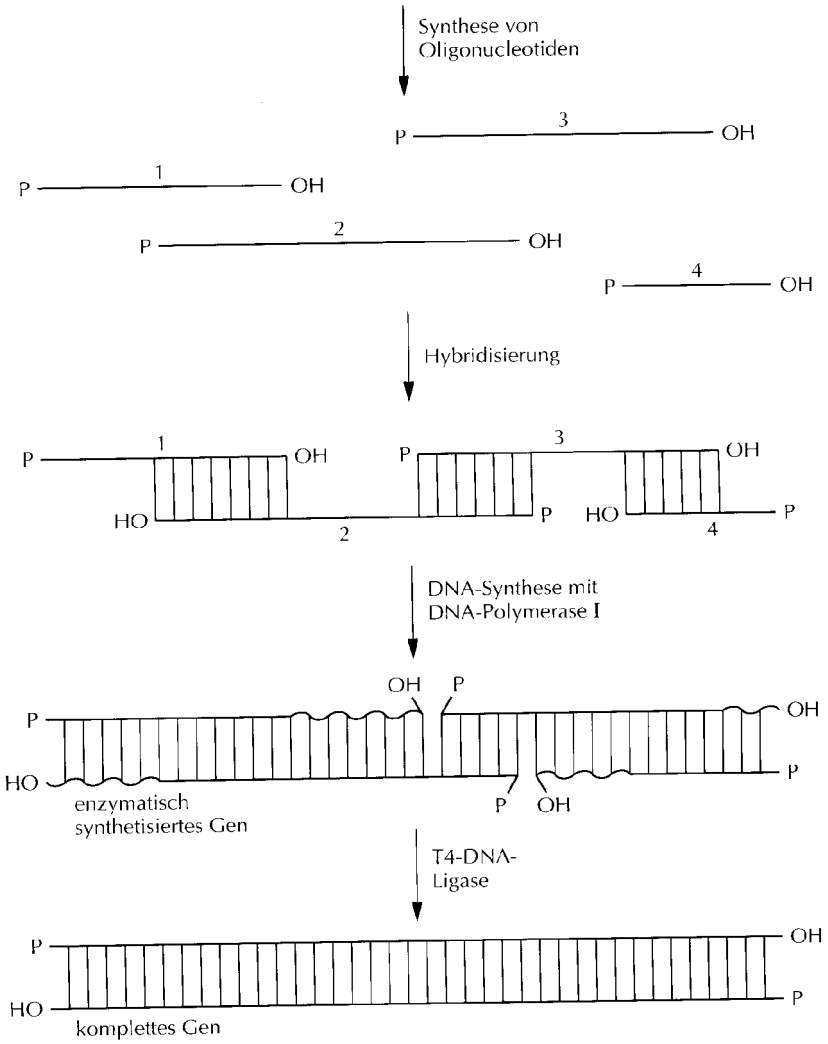
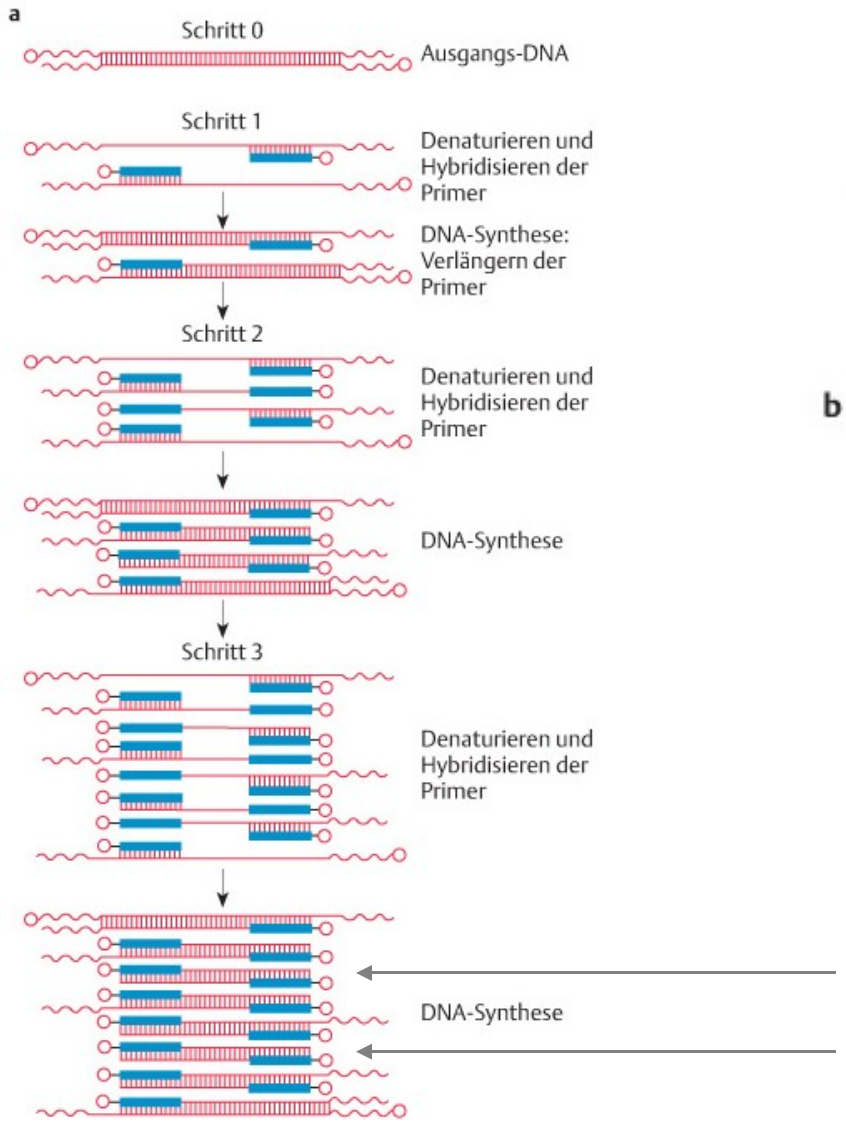
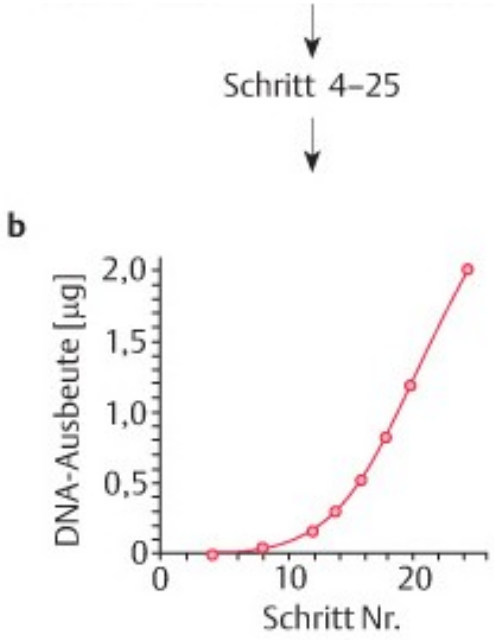


Abb. 3-10 Glick & Pasternak, 1995

DNA-Amplifikation

Polymerase Chain Reaction (PCR)





Zielmoleküle entstehen erst im 3. Schritt !

DNA-Amplifikation

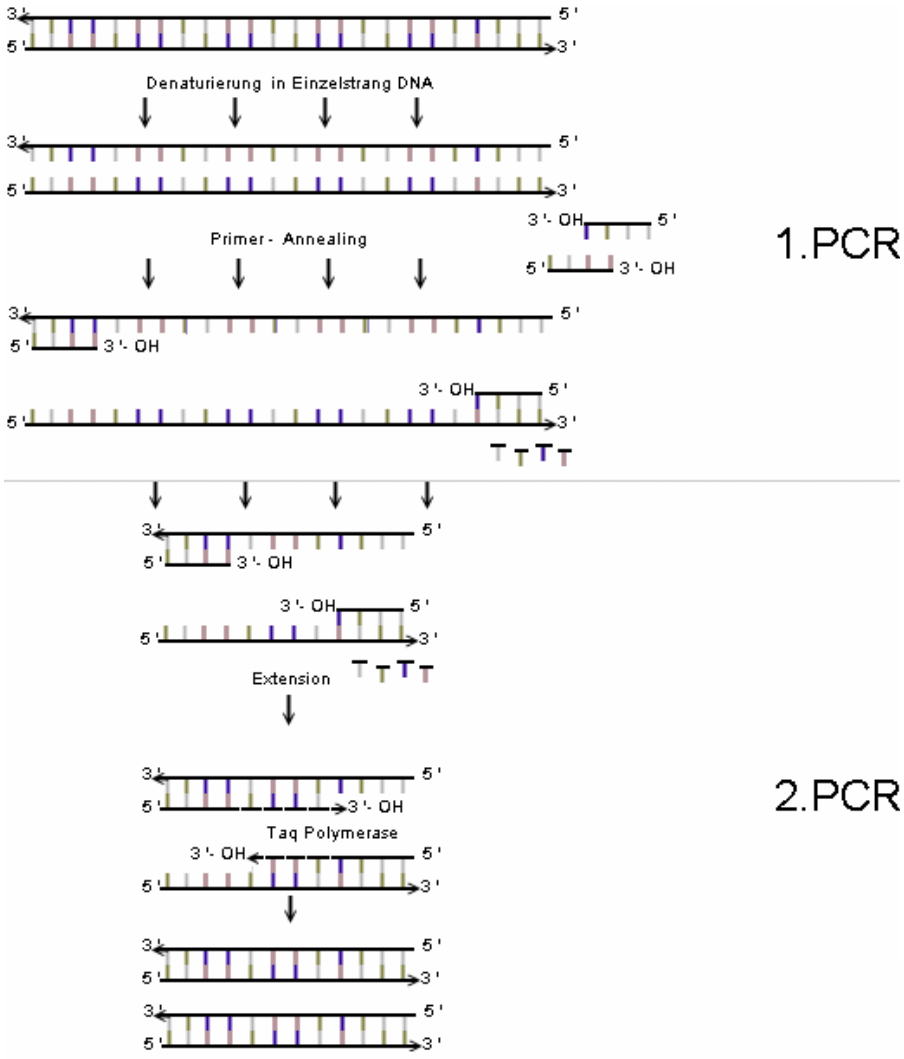
PCR, exponentielle Amplifikation

Zyklen n	**Zielmoleküle** N
1	0
2	0
3	2
4	4
5	8
…	
32	1.073.741.824

$$N = 2^{n-2} \quad (n>3)$$

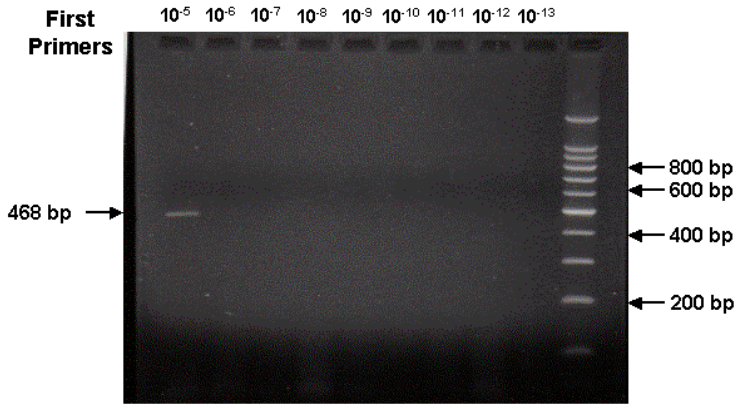
DNA-Amplifikation

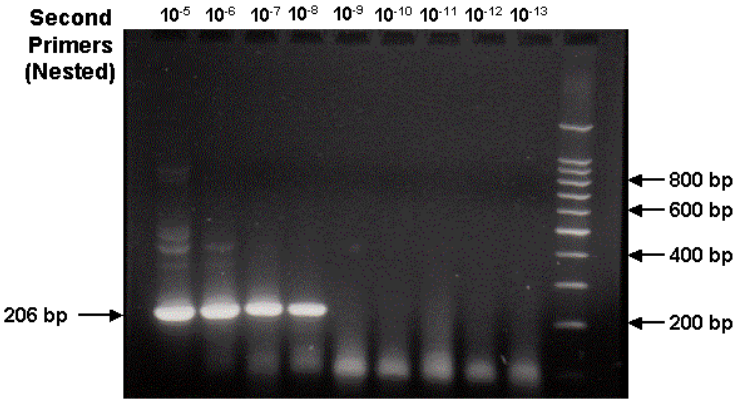
„nested“ / verschachtelte PCR



1.PCR

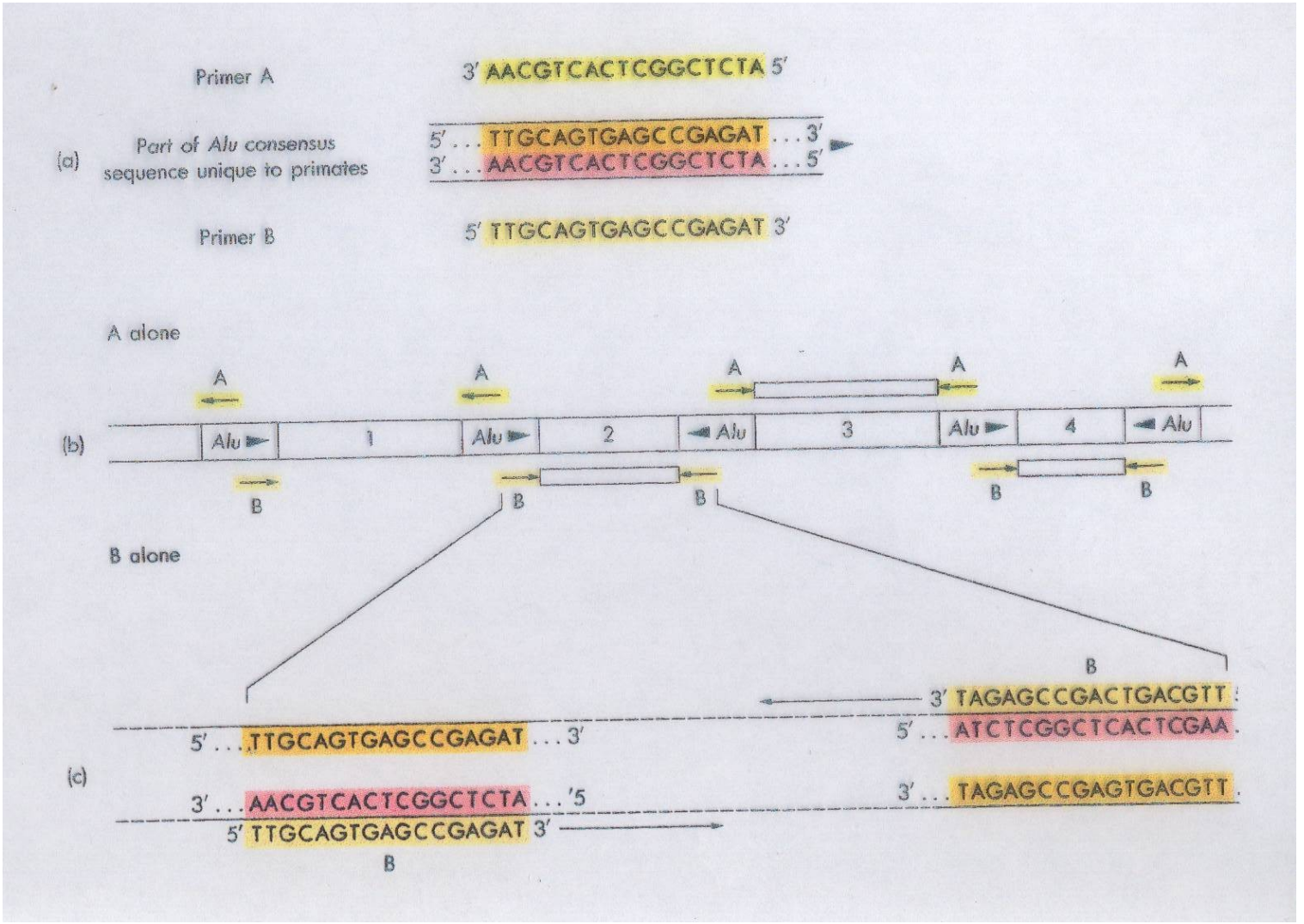
2.PCR





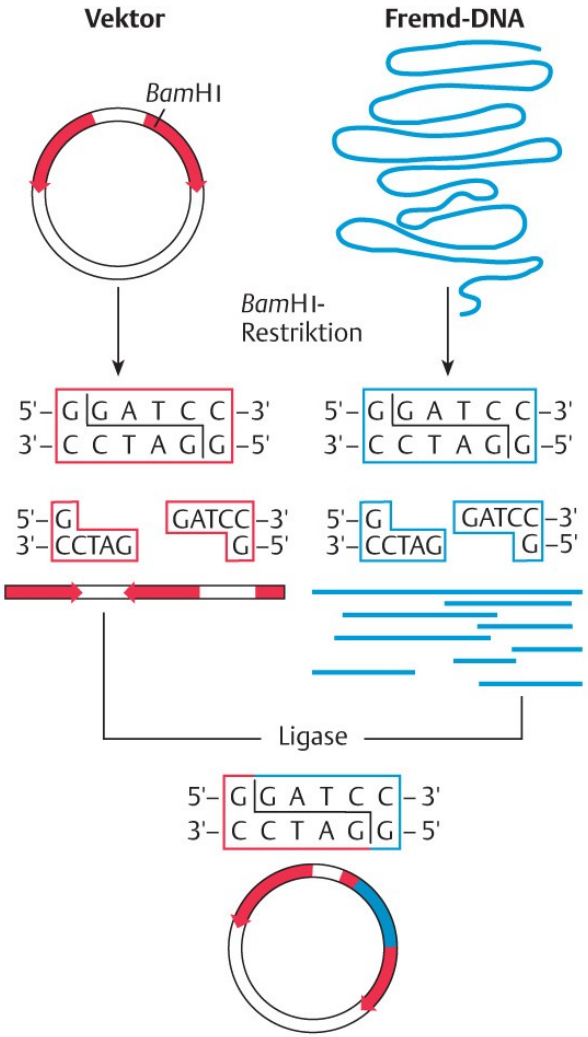
DNA-Amplifikation

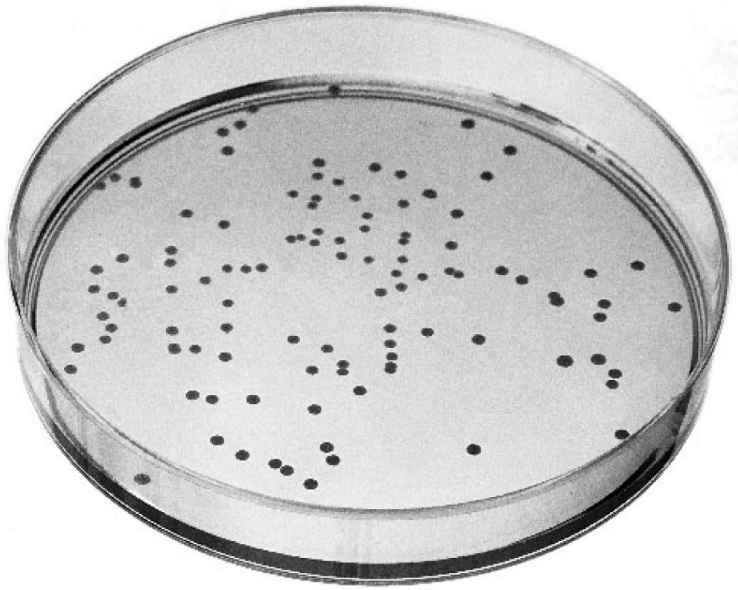
Alu-PCR



DNA-Amplifikation

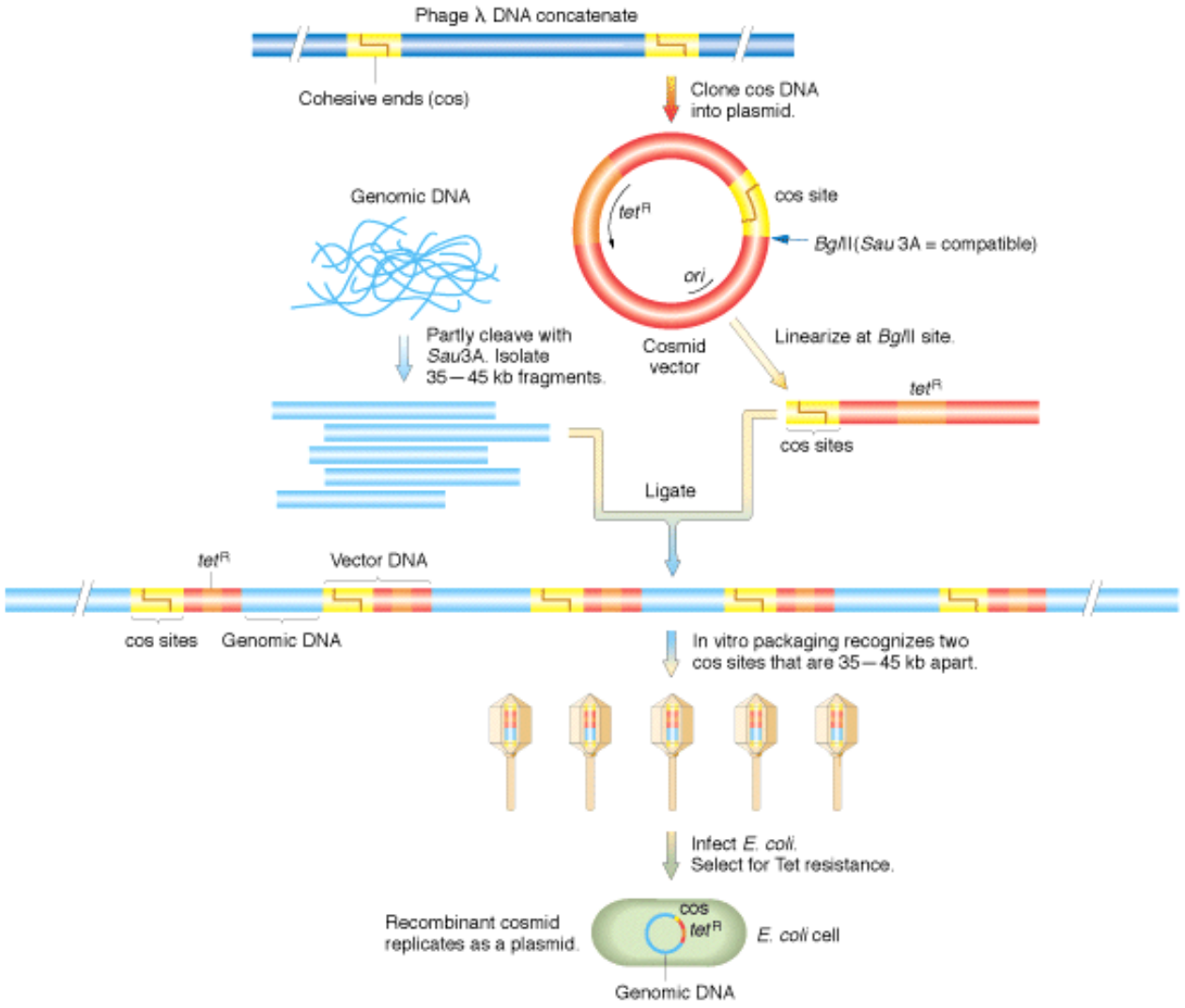
Bakterielle Klonierung





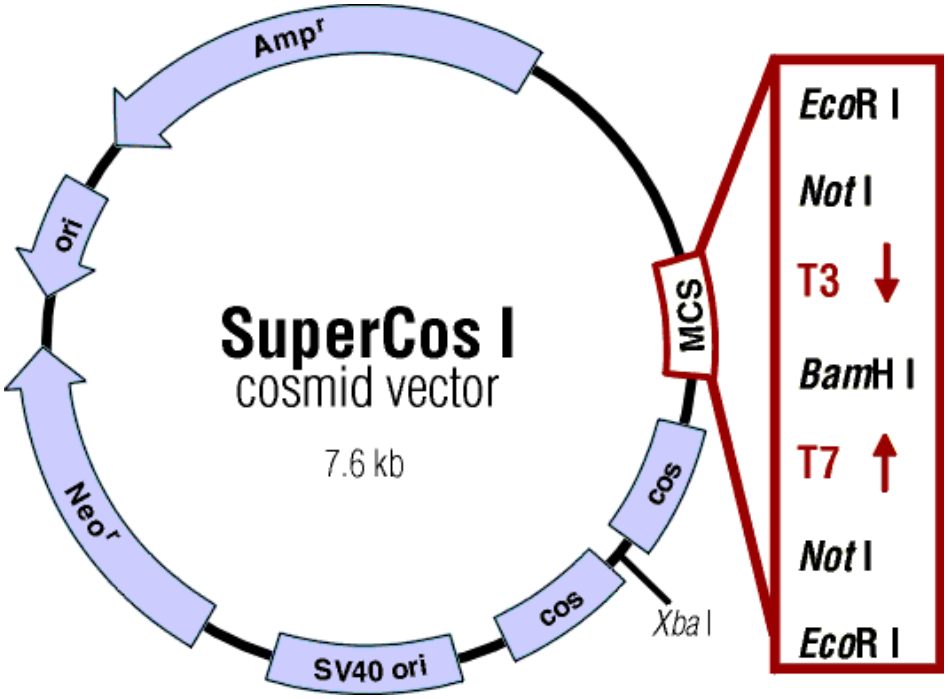
DNA-Amplifikation

Cosmid-Klonierungssystem



DNA-Amplifikation

Cosmid-Klonierungssystem

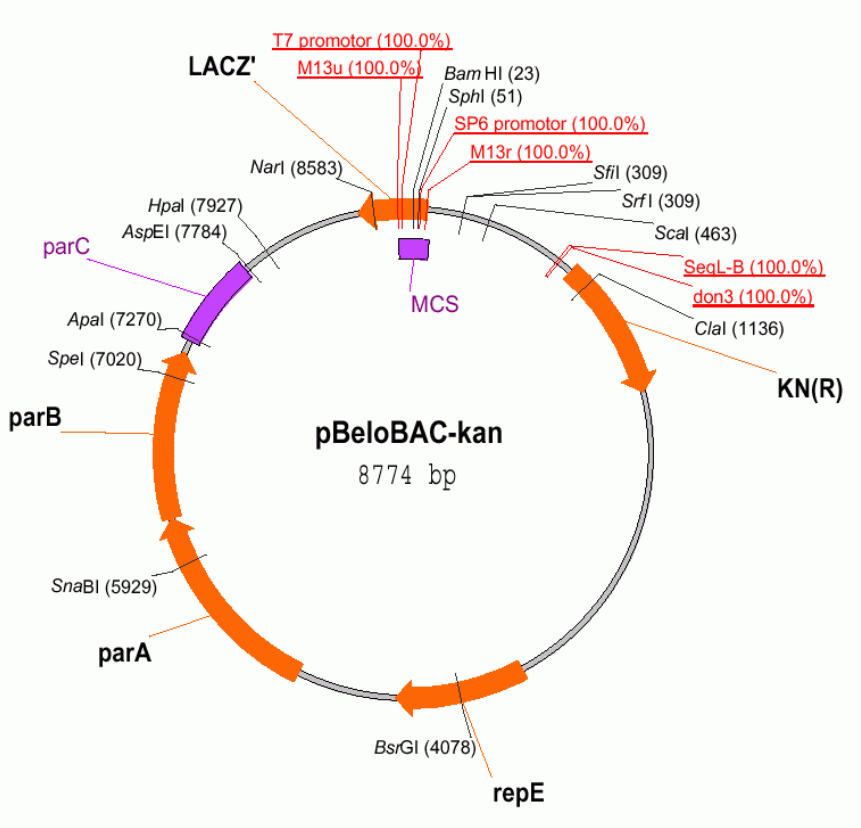


Klonierungskapazität

52 kb – 7,6 kb = 44,4 kb

DNA-Amplifikation

BAC/PAC-Klonierungssystem

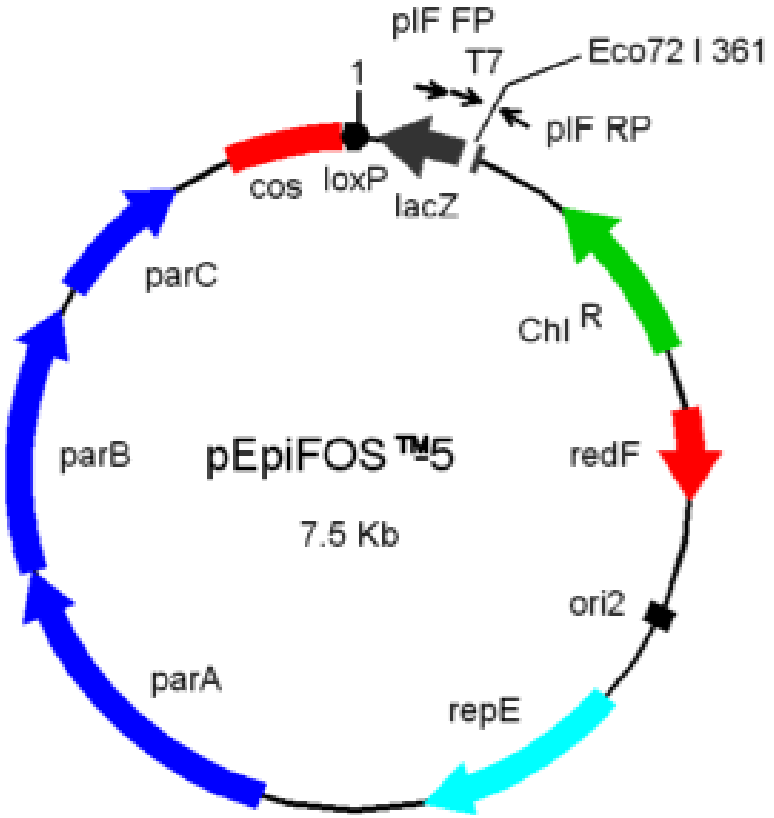


Klonierungskapazität

100 -250 kb

DNA-Amplifikation

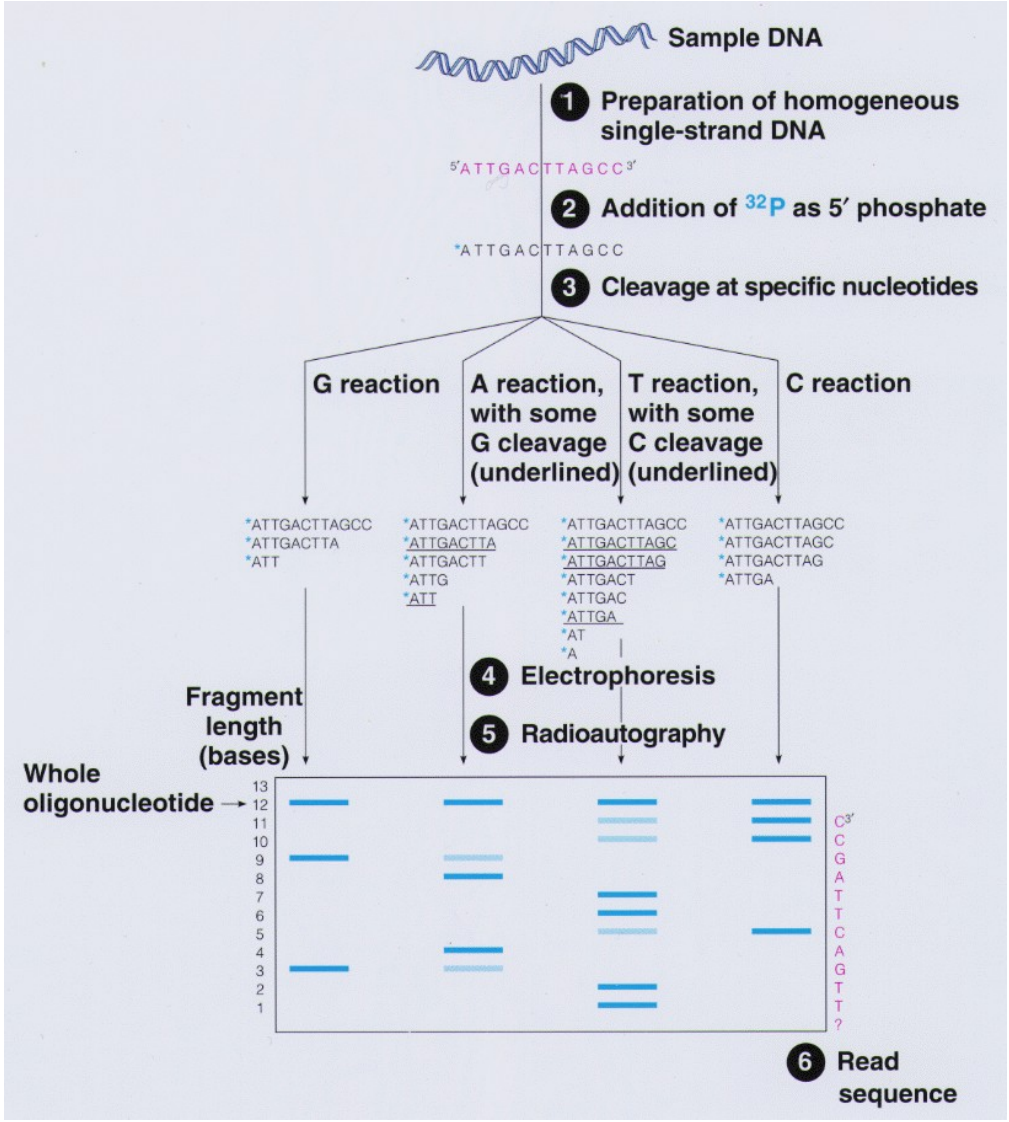
Fosmid-Klonierungssystem



Einzelkopie-Cosmid

DNA-Sequenzierung

Maxam-Gilbert-Methode, radioaktiv *1977



DNA-Sequenzierung

Maxam-Gilbert-Methode, radioaktiv *1977

TABLE 13.2 Chemical Modifications Used in the Maxam-Gilbert Method

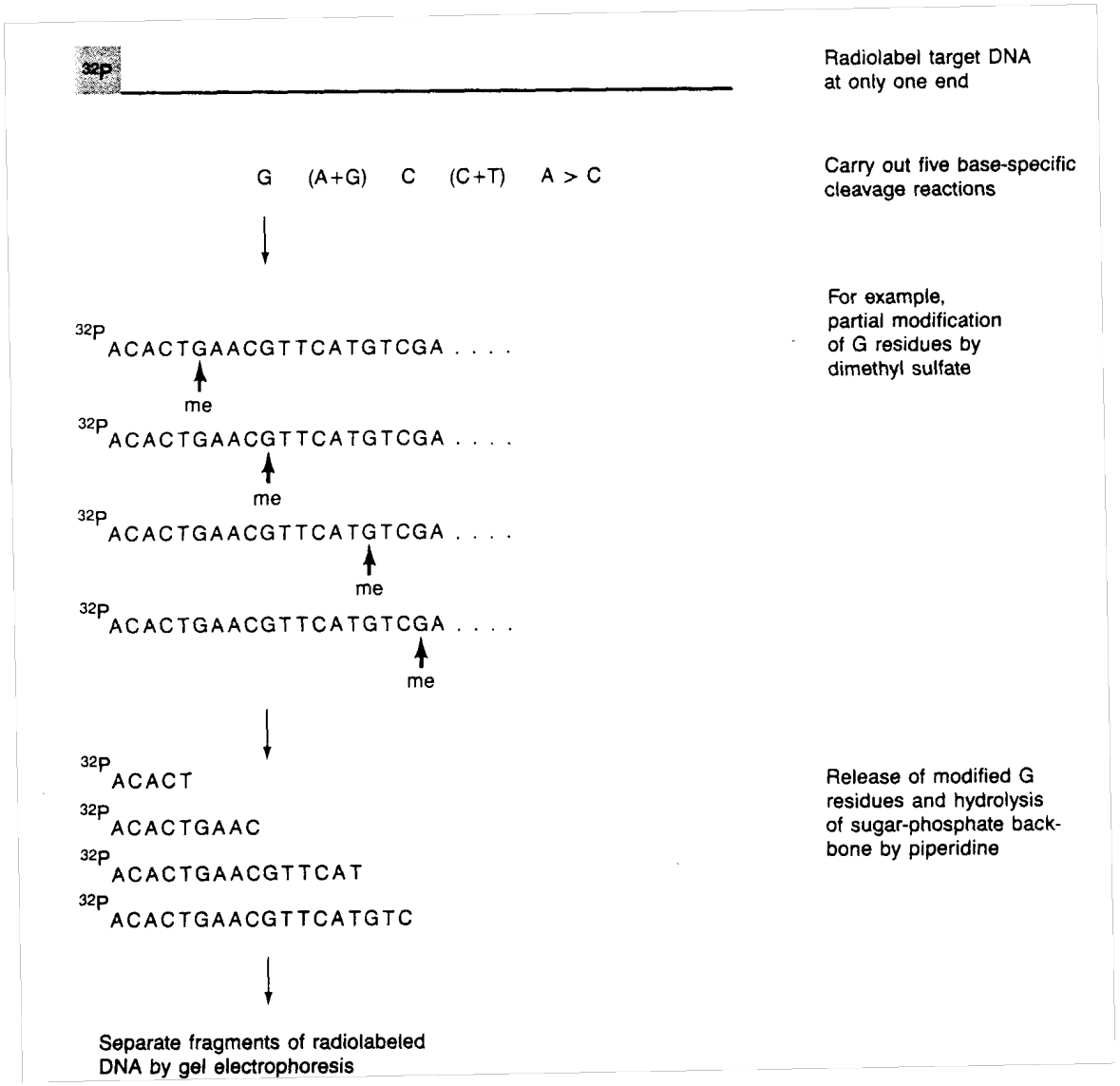
Base	Specific modification[a]
G	Methylation of N_7 with dimethyl sulfate at pH 8.0 makes the C_8—C_9 bond specifically susceptible to cleavage by base
A + G	Piperidine formate at pH 2.0 weakens the glycosidic bond of adenine and guanine by protonating nitrogen atoms in the purine rings resulting in depurination
C + T	Hydrazine opens pyrimidine rings, which recyclize in a five-membered form that is susceptible to removal
C	In the presence of 1.5 M NaCl, only cytosine reacts appreciably with hydrazine
A > C	1.2 N NaOH at 90°C results in strong cleavage at A and weaker cleavage at C

[a] Hot (90°C) piperidine (1 M in H_2O) is used to cleave the sugar-phosphate chain of DNA at the sites of chemical modifications.

Maniatis, 1989

DNA-Sequenzierung

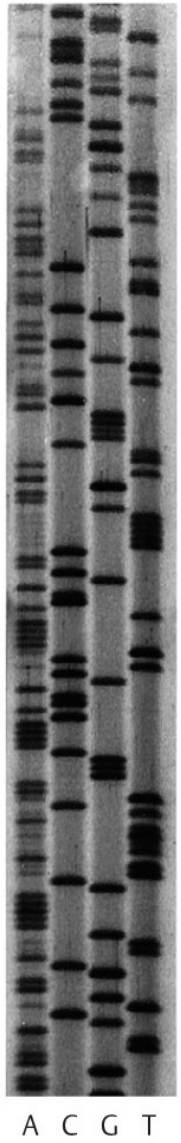
Maxam-Gilbert-Methode, radioaktiv *1977

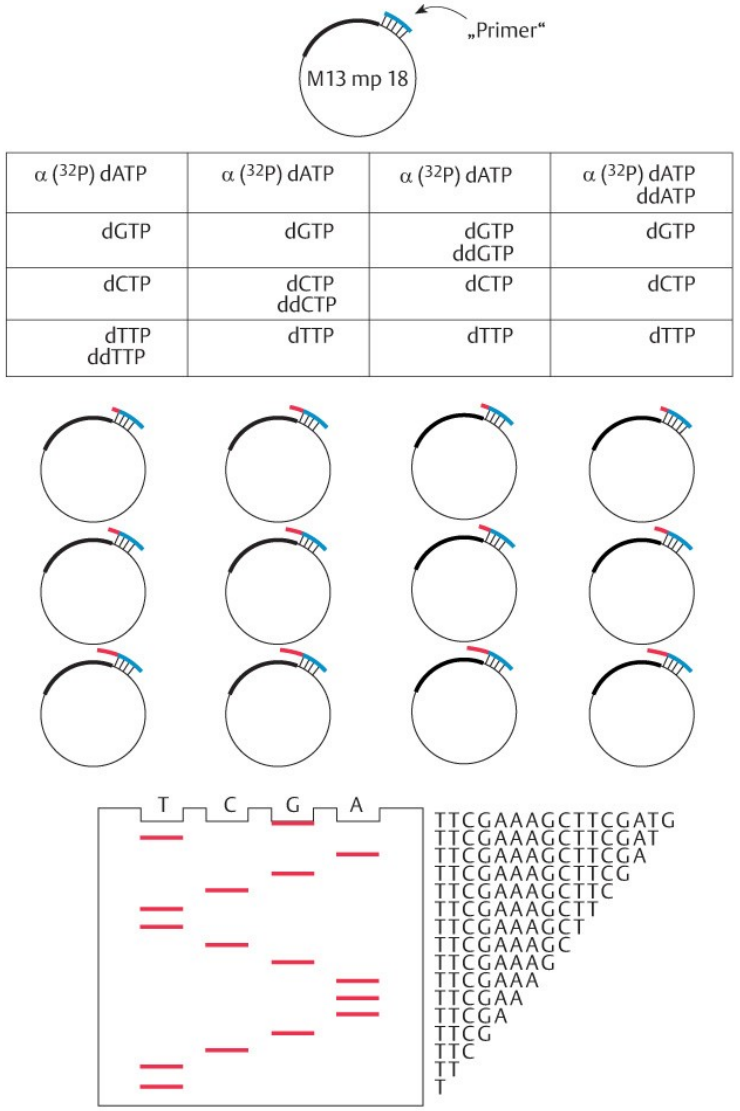


Maniatis, 1989

DNA-Sequenzierung

ddNTP-Kettenabbruch~, Didesoxy~, radioaktiv *1977



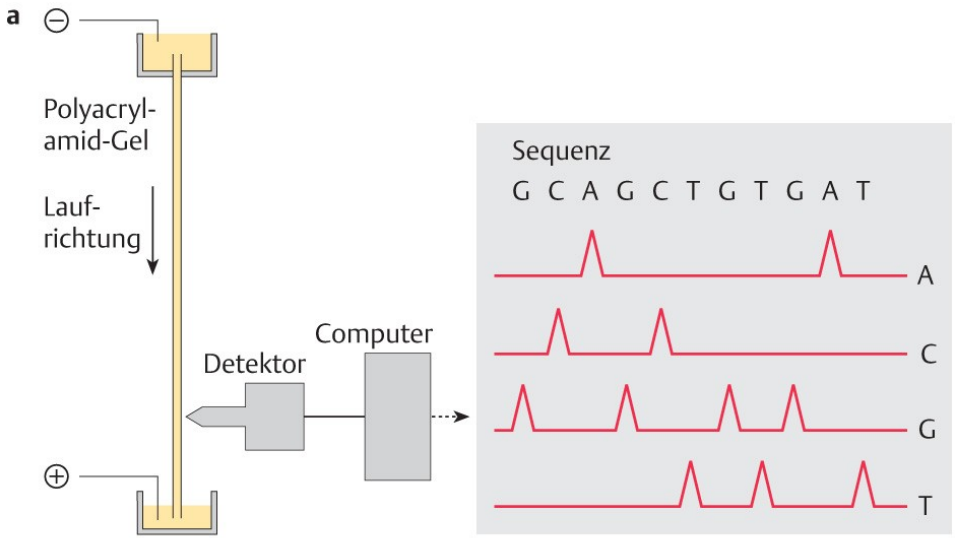


Fred Sanger

*Nobelpreise 1958, **1980***

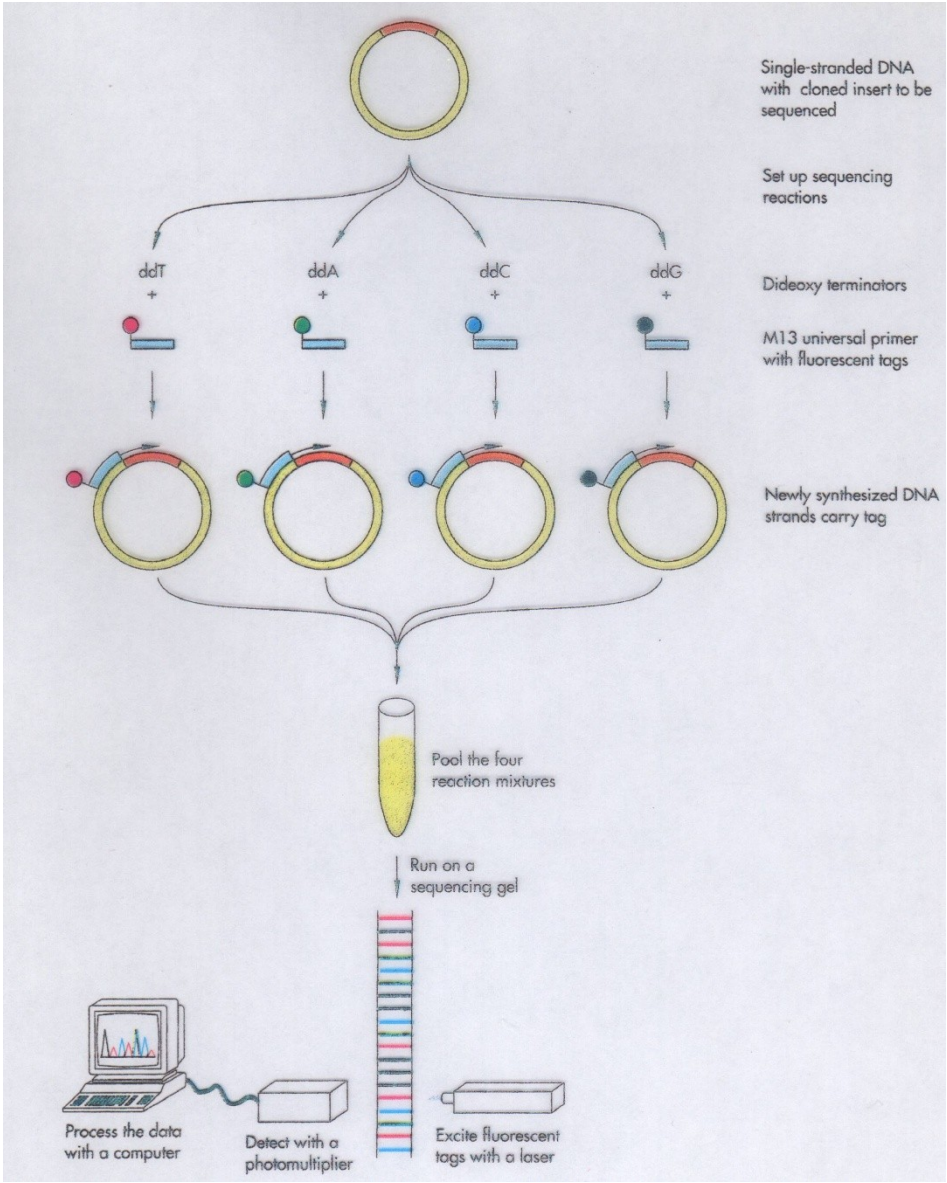
DNA-Sequenzierung

Didesoxy~, Fluoreszenz-markiert



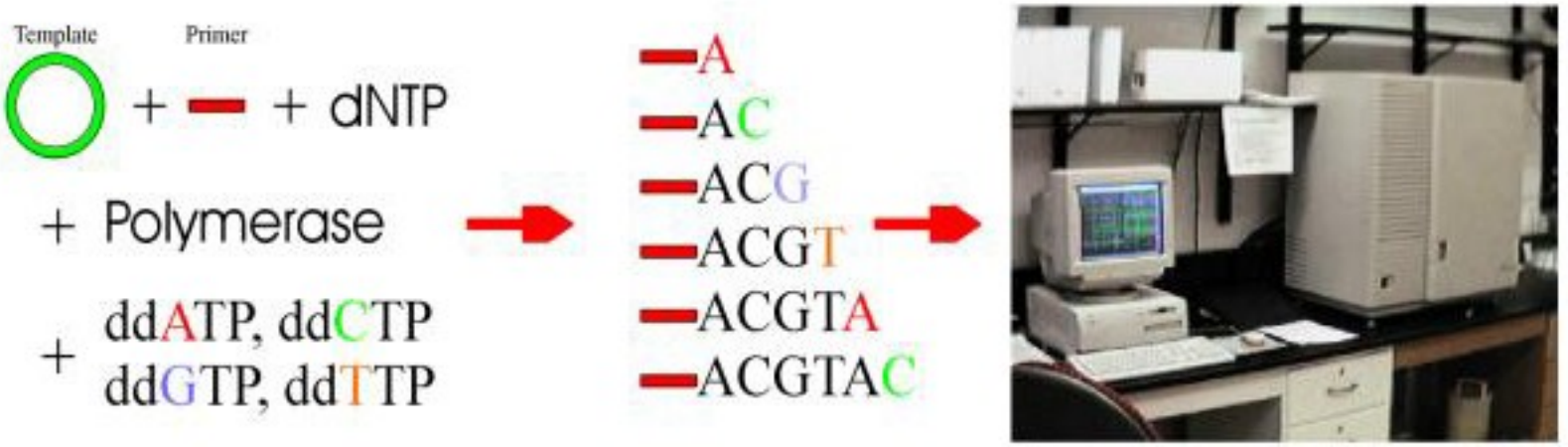
Sequenzier-Technologie

Dye-Primer



DNA-Sequenzierung

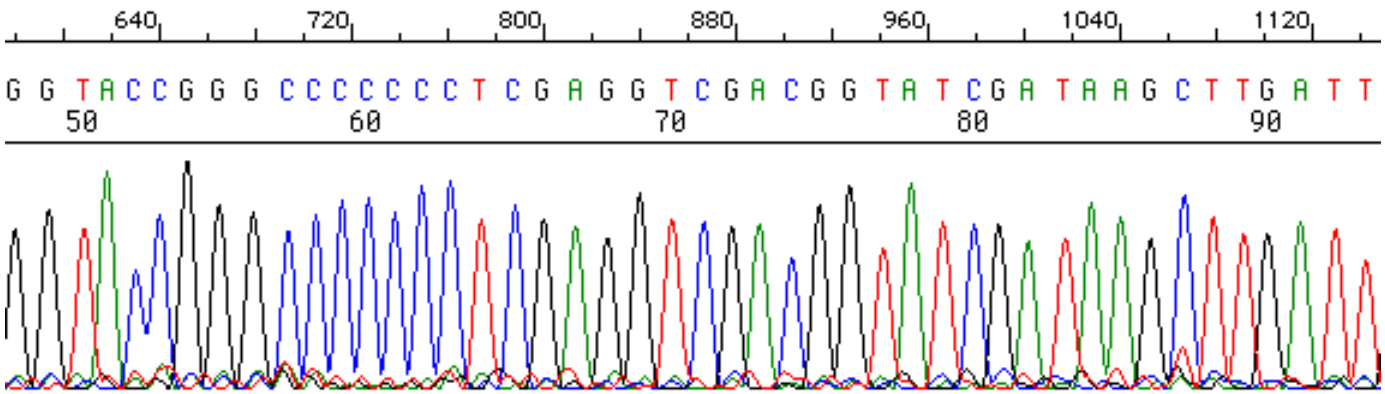
Dye-Terminator



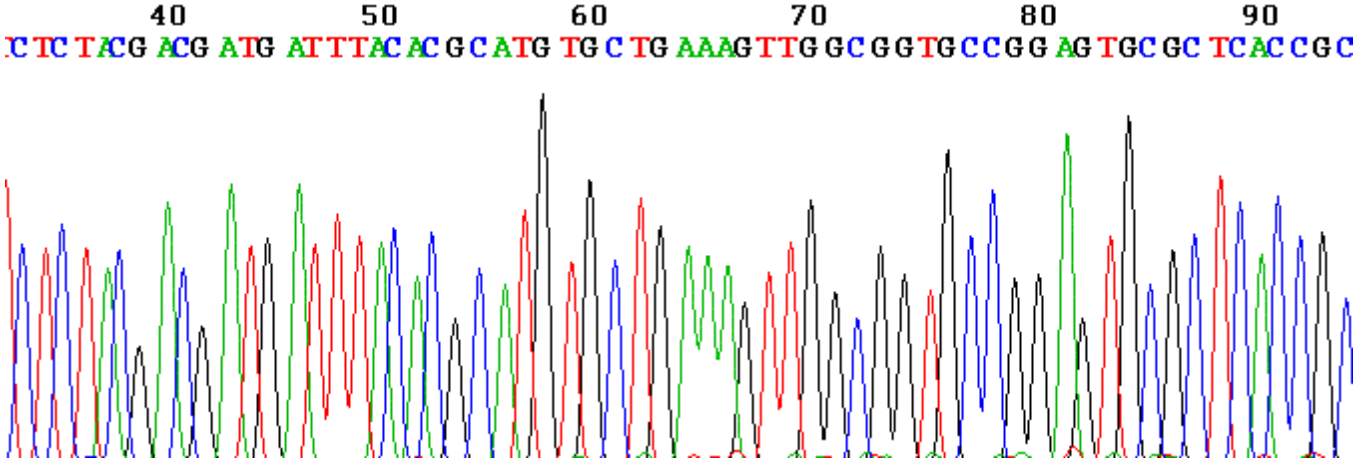
Sequenzier-Technologie

Dye-Primer vs. Dye-Terminator

DP



DT



Sequenzier-Technologie

„cycle“-Sequenzierung

Sequenzier-Technologie

Manipulierte Polymerasen

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6339–6343, July 1995
Biochemistry

A single residue in DNA polymerases of the *Escherichia coli* DNA polymerase I family is critical for distinguishing between deoxy- and dideoxyribonucleotides

(DNA sequencing/dideoxynucleotides/T7 DNA polymerase/*Taq* DNA polymerase/fidelity)

STANLEY TABOR AND CHARLES C. RICHARDSON

Department of Biological Chemistry and Molecular Pharmacology, 240 Longwood Avenue, Harvard Medical School, Boston, MA 02115

Contributed by Charles C. Richardson, March 28, 1995

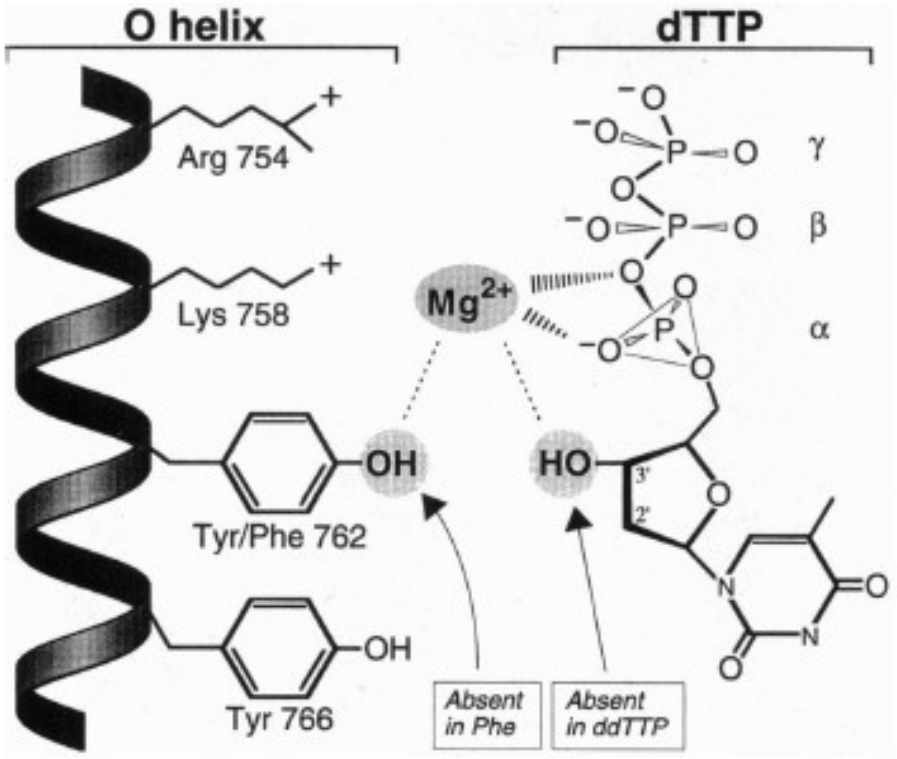
ABSTRACT **Bacteriophage T7 DNA polymerase efficiently incorporates a chain-terminating dideoxynucleotide into DNA, in contrast to the DNA polymerases from *Escherichia coli* and *Thermus aquaticus*. The molecular basis for this difference has been determined by constructing active site hybrids of these polymerases. A single hydroxyl group on the polypeptide chain is critical for selectivity. Replacing tyrosine-526 of T7 DNA polymerase with phenylalanine increases discrimination against the four dideoxynucleotides by >2000-fold, while replacing the phenylalanine at the homologous position in *E. coli* DNA polymerase I (position 762) or *T. aquaticus* DNA polymerase (position 667) with tyrosine decreases discrimination against the four dideoxynucleotides 250- to 8000-fold. These mutations allow the engineering of new DNA polymerases with enhanced properties for use in DNA sequence analysis.**

METHODS

Construction of Hybrid Genes. Hybrid genes were constructed by using synthetic oligonucleotides and the polymerase chain reaction. Hybrids of the T7 DNA polymerase gene were expressed under the control of the *lac* promoter in the vector pUC18; the parent vector (pGP5-12) contains the gene for T7 DNA polymerase with a deletion that encodes amino acid residues 118–145, inactivating the exonuclease (9). Hybrids of the *E. coli* DNA polymerase I gene were constructed in pKLEN-1, which encodes the large fragment of *E. coli* DNA polymerase I (beginning at residue 324) under the control of a T7 RNA polymerase promoter. Hybrids of *Taq* DNA polymerase were constructed in pTQΔ-1, which encodes a truncated fragment of *Taq* DNA polymerase (beginning at residue 289) under the control of a T7 RNA polymerase promoter. For characterization of the purified *Taq* hybrid polymerase C-Q5 (see Table 1), a

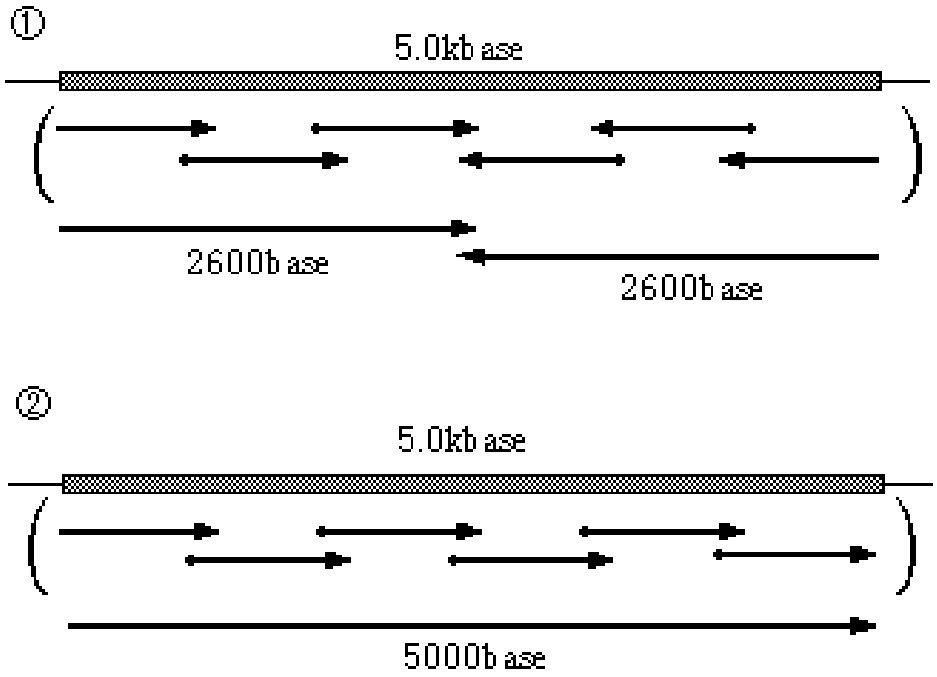
Sequenzier-Technologie

Manipulierte Polymerasen



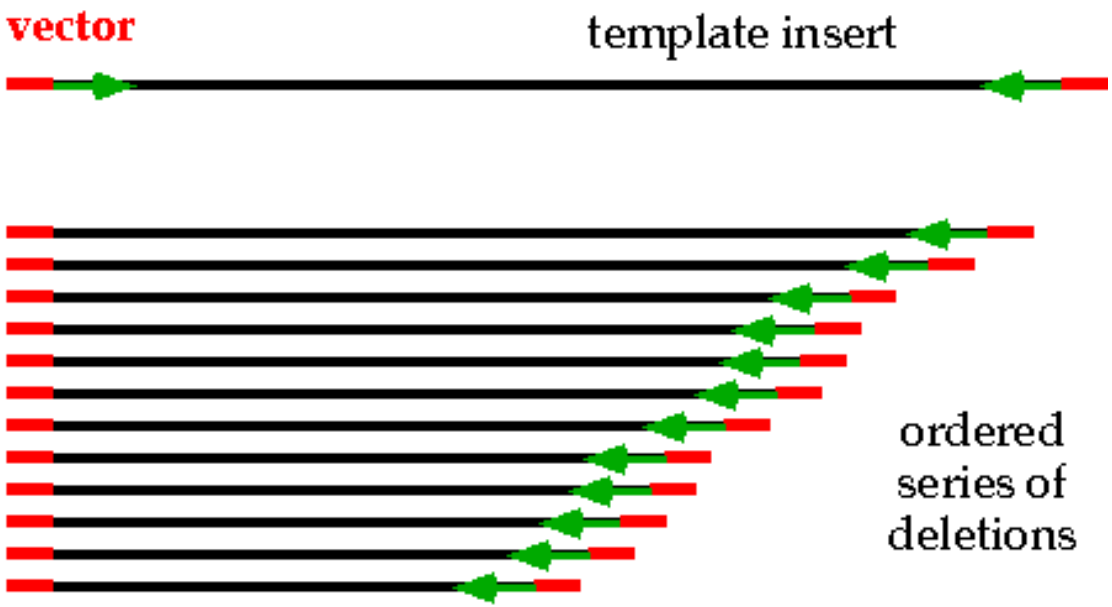
Sequenzier-Strategie

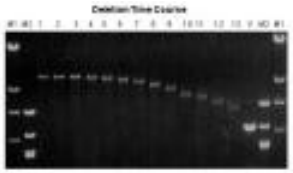
Primer-„walking“



Sequenzier-Strategie

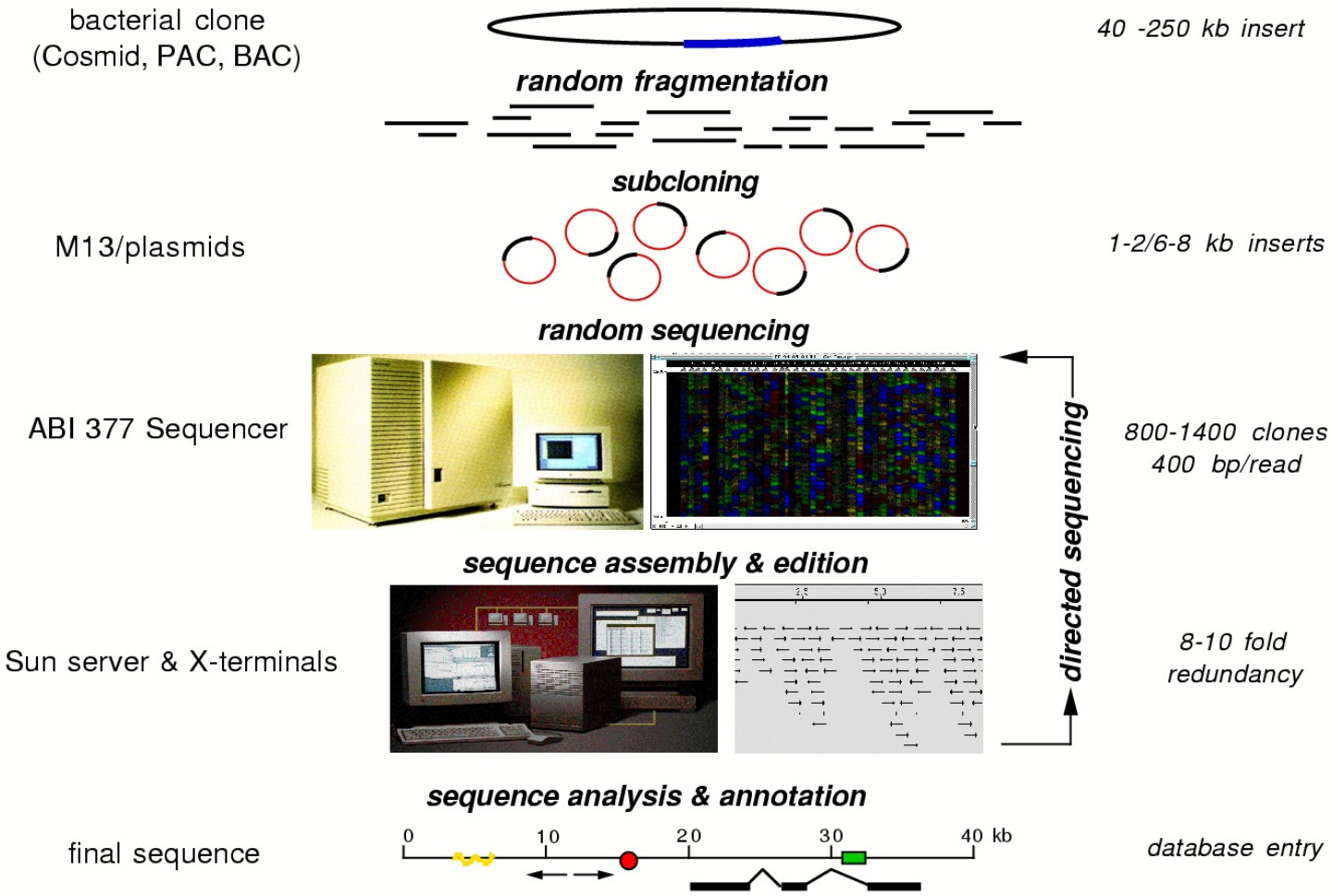
„nested“ / verschachtelte Deletionen





Sequenzier-Strategie

Schrotschuss („shotgun“)



“shotgun”-Theorie

Lander & Waterman (1988)

nicht sequenzierter Anteil : $U = e^{-(n*l)/g}$

verbleibende Lücken: $G = n*e^{-(n*l)/g}$

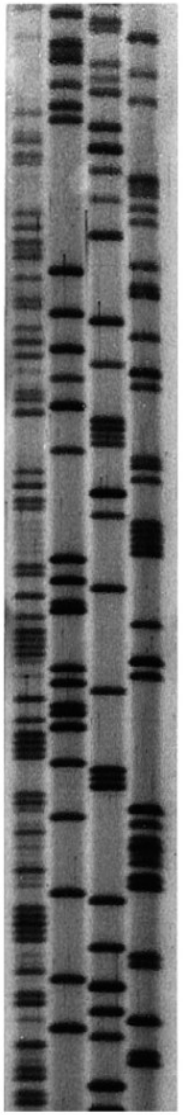
n: Anzahl der „shotgun“-Sequenzen

l : Länge einer „shotgun“-Sequenz

g: Größe des Genoms/Ausgangsmoleküls

Beispiel: g = 100.000 bp ; l = 400 bp

n	(n*l)/g	U	G
250	1	37%	92
1250	5	1%	12,5
2500	10	0,005%	0,125



genome.fli-leibniz.de
Teaching