Morphological and physiological characteristics used in classification

- Colony morphology
- Colony color
- Cell shape
- Staining results (Gram)
- Endospore
- Motility
- Flagella
- Size
- Cellular inclusions
- Ultrastructure

- Oxygen relationship
- Energy sources
- Electron sources
- Carbon sources
- Fermentation products
- Cell wall components
- Temp and pH optima
- Osmotic tolerance
- Photosynthetic mechanism
- Secondary metabolites
- Sensitivity to antibiotics

Oxygen requirements

- Aerobes
- Anaerobes
- Microaerophiles
- Obligate aerobes
- Obligate anaerobes
- Facultative anaerobes

Table 5.1

Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 202–3*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 190–96*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 188–90*)
Organotrophs	Organic molecules (*chapter 9*)

---

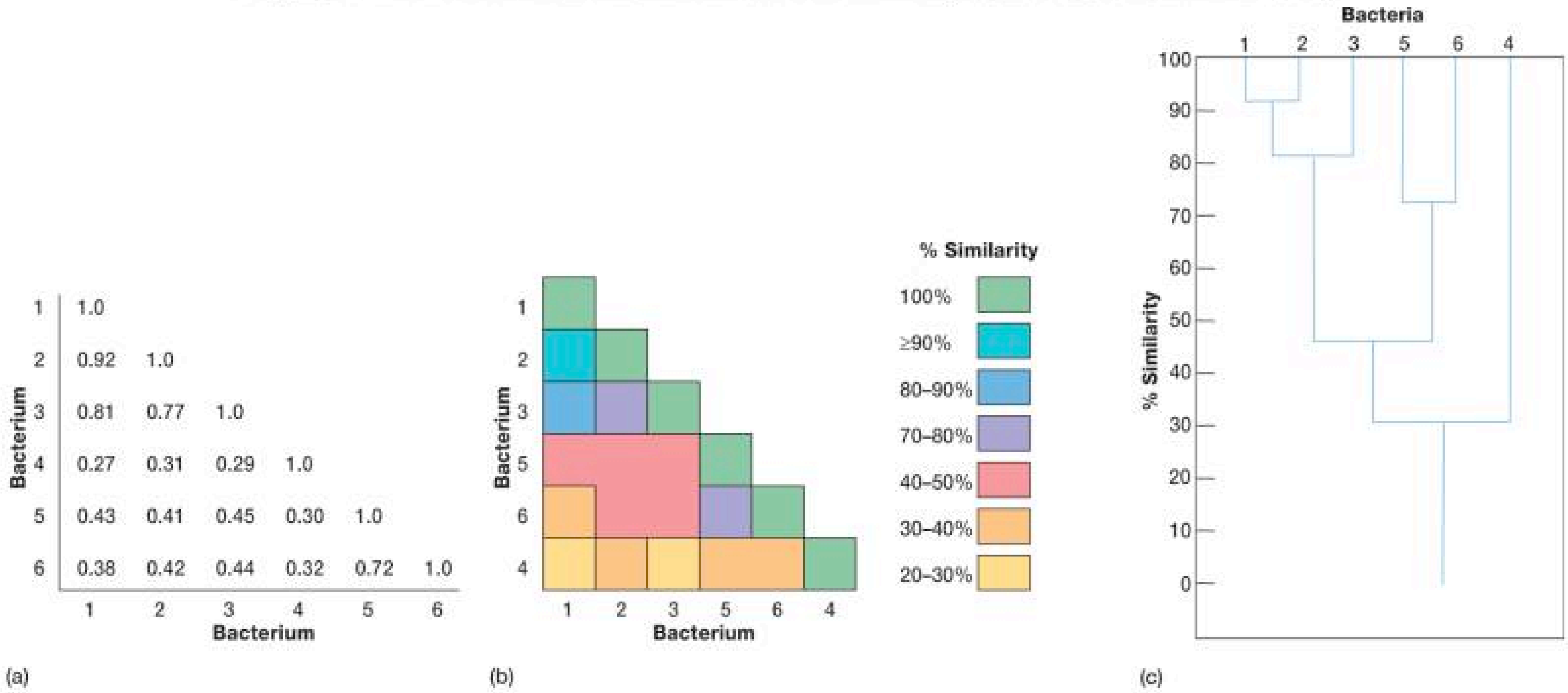
[a] For each category, the location of material describing the participating metabolic pathways is given within the parentheses.

Table 5.2
Major Nutritional Types of Microorganisms

Major Nutritional Types[a]	Sources of Energy, Hydrogen/Electrons, and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e$^-$) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e$^-$ donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e$^-$ donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e$^-$ donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

[a] Bacteria in other nutritional categories have been found. The categories are defined in terms of energy, electron, and carbon sources. Condensed versions of these names are given in parentheses.

Numerical Taxonomy



Bacterium	1	2	3	4	5	6
1	1.0					
2	0.92	1.0				
3	0.81	0.77	1.0			
4	0.27	0.31	0.29	1.0		
5	0.43	0.41	0.45	0.30	1.0	
6	0.38	0.42	0.44	0.32	0.72	1.0

Fig. 19.5

Molecular characteristics used in classification

- Nucleic acid base composition (G+C %)
- Nucleic acid hybridzation
- Nucleic acid sequencing
  - 16S rRNA
  - Signature sequences
- Nucleic acid/protein sequence comparisons
- Complete genome sequencing

DNA melting curve (G+C %)

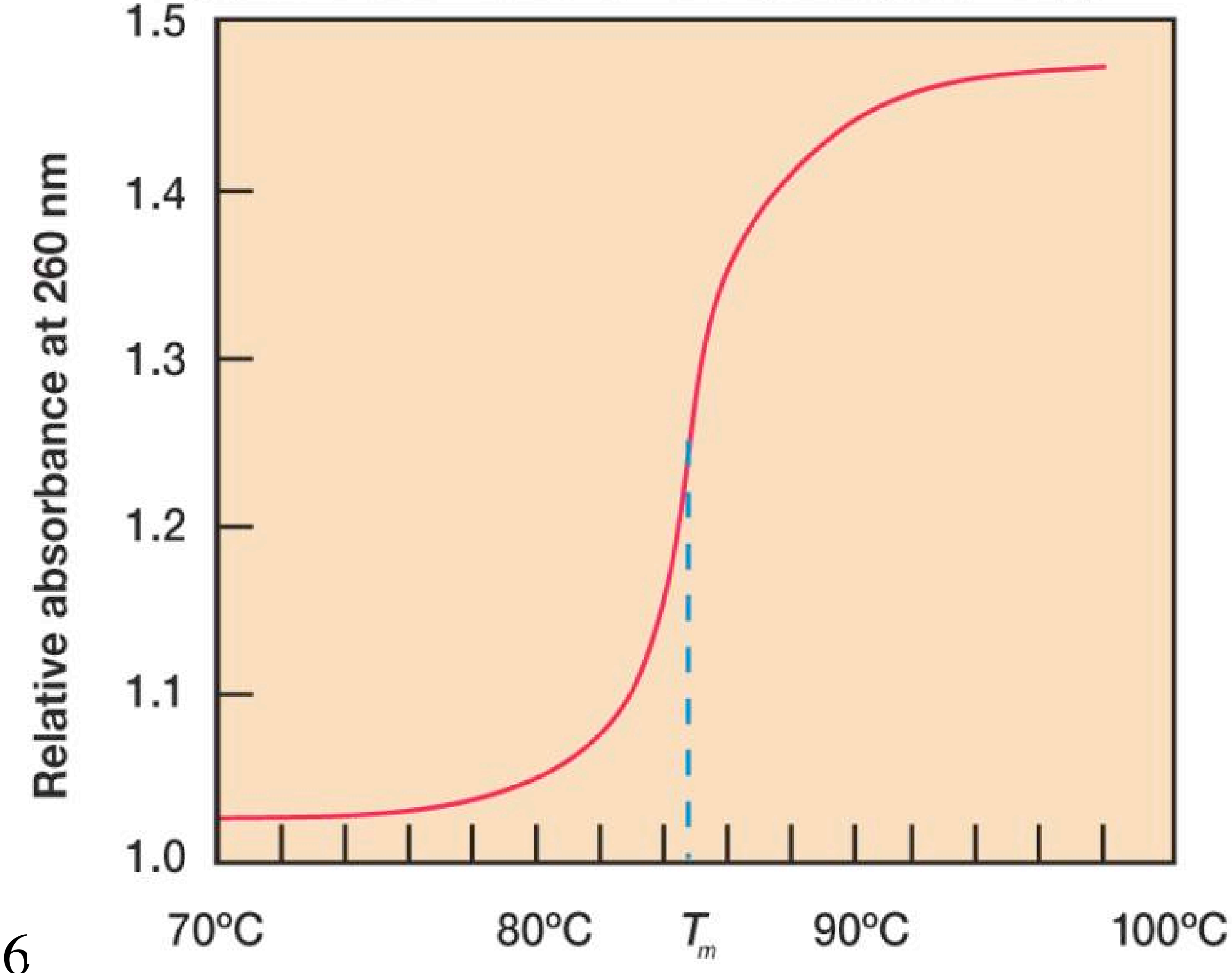


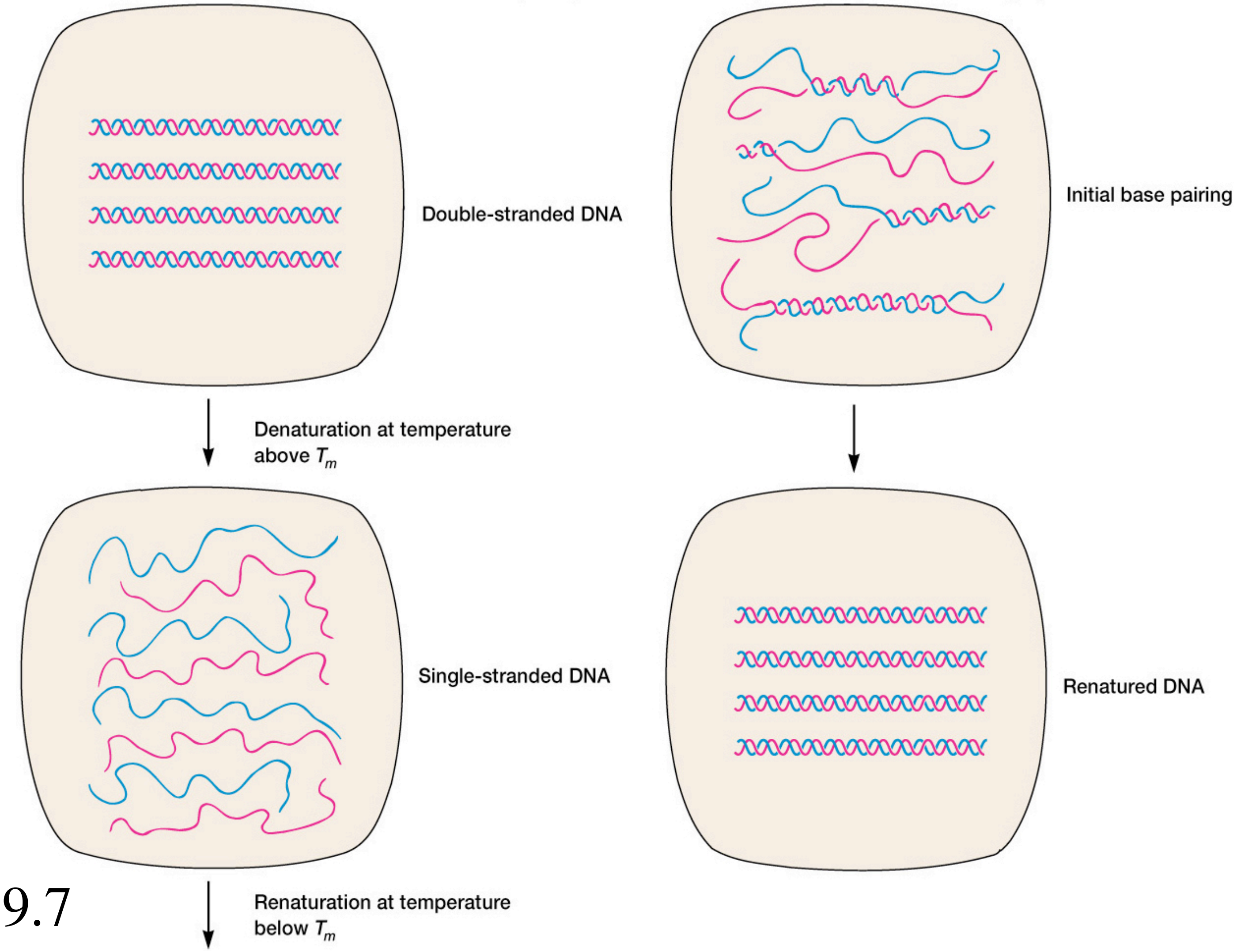
Fig. 19.6

Table 19.5

Representative G + C Contents of Microorganisms

Organism	Percent G + C
Bacteria	
Actinomyces	59–73
Anabaena	38–44
Bacillus	32–62
Bacteroides	28–61
Bdellovibrio	33–52
Caulobacter	63–67
Chlamydia	41–44
Chlorobium	49–58
Chromatium	48–70
Clostridium	21–54
Cytophaga	33–42
Deinococcus	62–70
Escherichia	48–52
Halobacterium	66–68
Hyphomicrobium	59–67
Methanobacterium	32–50
Micrococcus	64–75
Mycobacterium	62–70
Mycoplasma	23–40
Myxococcus	68–71
Neisseria	47–54
Nitrobacter	60–62
Oscillatoria	40–50
Prochloron	41
Proteus	38–41
Pseudomonas	58–70
Rhodospirillum	62–66
Rickettsia	29–33
Salmonella	50–53
Spirillum	38
Spirochaeta	51–65
Staphylococcus	30–38
Streptococcus	33–44
Streptomyces	69–73
Sulfolobus	31–37
Thermoplasma	46
Thiobacillus	52–68
Treponema	25–54
Algae	
Acetabularia mediterranea	37–53
Chlamydomonas	60–68
Chlorella	43–79
Cyclotella cryptica	41
Euglena gracilis	46–55
Nitella	49
Nitzschia angularis	47
Ochromonas danica	48
Peridinium triquetrum	53
Scenedesmus	52–64
Spirogyra	39
Volvox carteri	50
Protozoa	
Acanthamoeba castellanii	56–58
Amoeba proteus	66
Paramecium spp.	29–39
Plasmodium berghei	41
Stentor polymorphus	45
Tetrahymena	19–33
Trichomonas	29–34
Trypanosoma	45–59
Slime Molds	
Dictyostelium	22–25
Lycogala	42
Physarum polycephalum	38–42
Fungi	
Agaricus bisporus	44
Amanita muscaria	57
Aspergillus niger	52
Blastocladiella emersonii	66
Candida albicans	33–35
Claviceps purpurea	53
Coprinus lagopus	52–53
Fomes fraxineus	56
Mucor rouxii	38
Neurospora crassa	52–54
Penicillium notatum	52
Polyporus palustris	56
Rhizopus nigricans	47
Saccharomyces cerevisiae	36–42
Saprolegnia parasitica	61

Nucleic Acid Hybridization



Double-stranded DNA

Denaturation at temperature above T_m

Single-stranded DNA

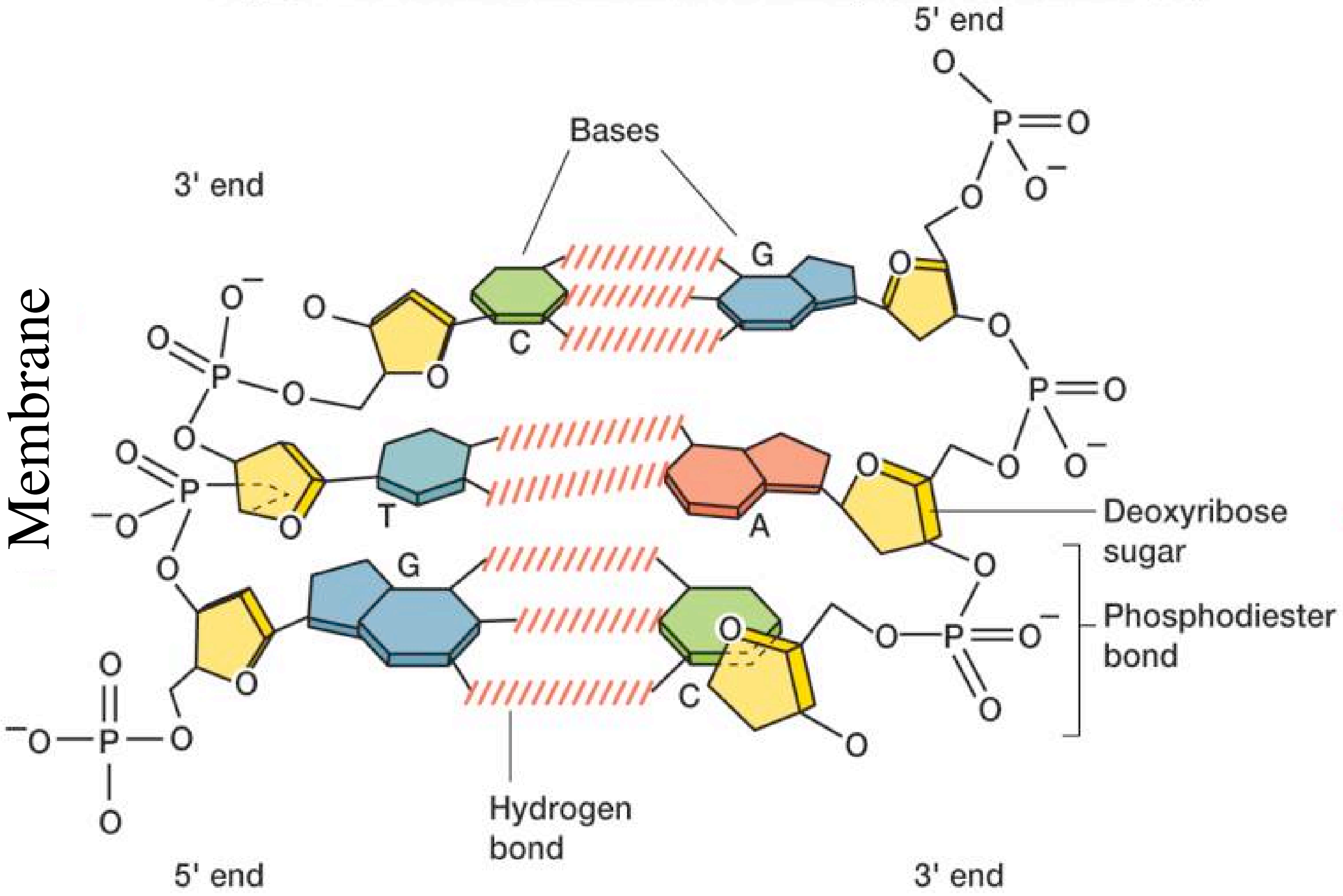
Renaturation at temperature below T_m

Initial base pairing

Renatured DNA

Fig. 19.7

Hybridization based on complementarity of DNA sequences



Membrane

Test DNA

Table 19.6

Comparison of *Neisseria* Species by DNA Hybridization Experiments

Membrane-Attached DNA[a]	Percent Homology[b]
Neisseria meningitidis	100
N. gonorrhoeae	78
N. sicca	45
N. flava	35

Source: Data from T. E. Staley and R. R. Colwell, "Applications of Molecular Genetics and Numerical Taxonomy to the Classification of Bacteria" in *Annual Review of Ecology and Systematics,* 8: 282, 1973.

[a] The experimental membrane-attached nonradioactive DNA from each species was incubated with radioactive *N. meningitidis* DNA, and the amount of radioactivity bound to the membrane was measured. The more radioactivity bound, the greater the homology between DNA sequences.

[b] $$\frac{\textit{N.meningitidis}\text{ DNA bound to experimental DNA}}{\text{Amount bound to membrane attached }\textit{N.meningitidis}\text{ DNA}} \times 100$$

Small ribosomal subunit RNA

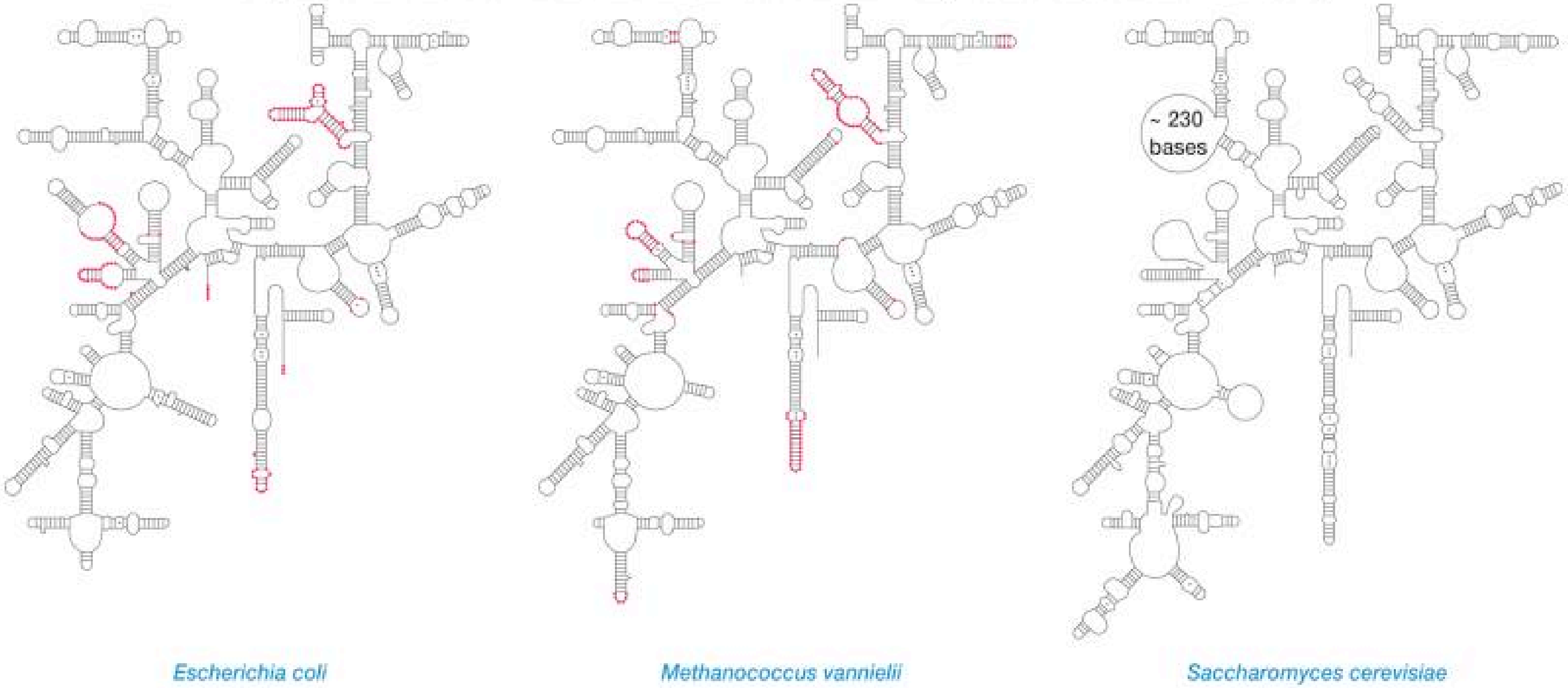


Fig. 19.9

Table 19.7

Selected 16S rRNA Signature Sequences for Some Bacterial Groups[a]

Position in rRNA	Consensus Composition	γ-Proteobacteria	Cyanobacteria	Spirochetes	*Bacteroides*	Green Sulfur	Green Nonsulfur	*Deinococcus*	Gram Positive (Low GC)	Gram Positive (High GC)	*Planctomyces*
47	C	+	+	U	+	+	+	+	+	+	G
53	A	+	+	G	+	+	G	+	+	+	G
570	G	+	+	+	U	+	+	+	+	+	U
812	G	c	+	+	+	+	+	C	+	+	+
906	G	Ag	+	+	+	+	A	+	+	A	+
955	U	+	+	+	+	+	+	+	+	AC	C
1,207	G	+	C	+	+	+	+	+	C	C	+
1,234	C	+	+	a	U	A	+	+	+	+	+

---

[a] A plus sign in a column means that the group has the same base as the consensus sequence. If the letter is given in upper case, it is changed in more than 90% of the cases. A lowercase letter signifies a minor occurrence base (<15% of the cases).

DNA sequencing

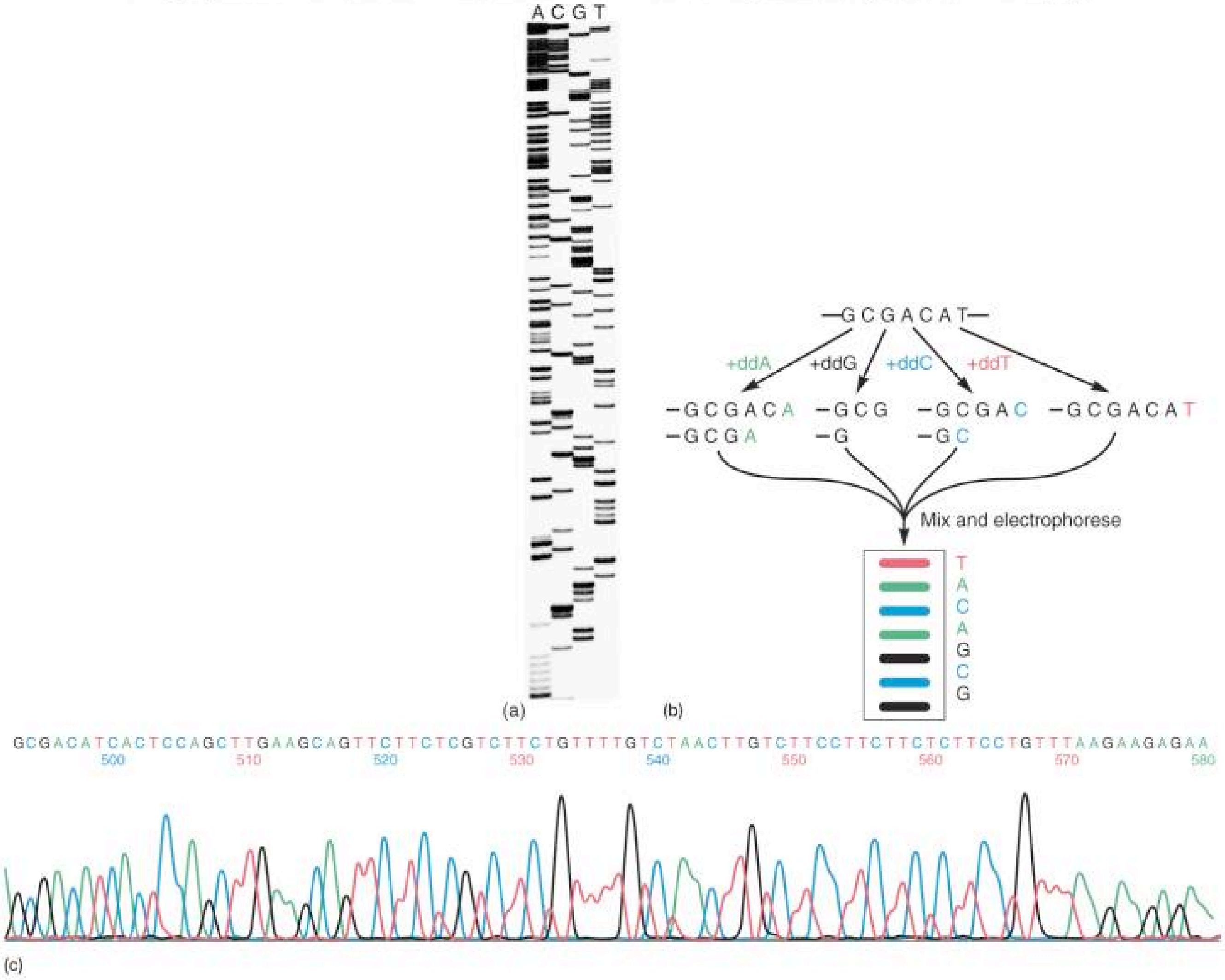


Fig. 15.2

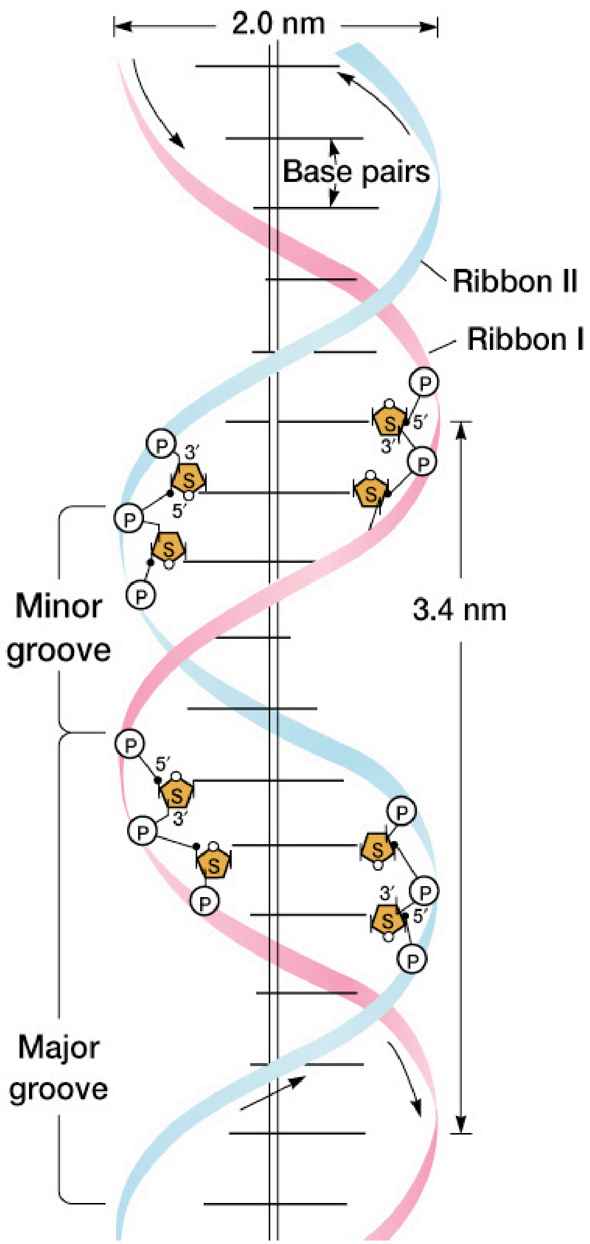

2.0 nm
Base pairs
Ribbon II
Ribbon I
P
S
3′
5′
Minor groove
3.4 nm
Major groove

Fig. 11.6

DNA polymerase reaction

$$n[\text{dATP, dGTP, dCTP, dTTP}] \xrightarrow[\text{DNA template}]{\text{DNA polymerase}} \text{DNA} + n\text{PP}_i$$

The mechanism of chain growth

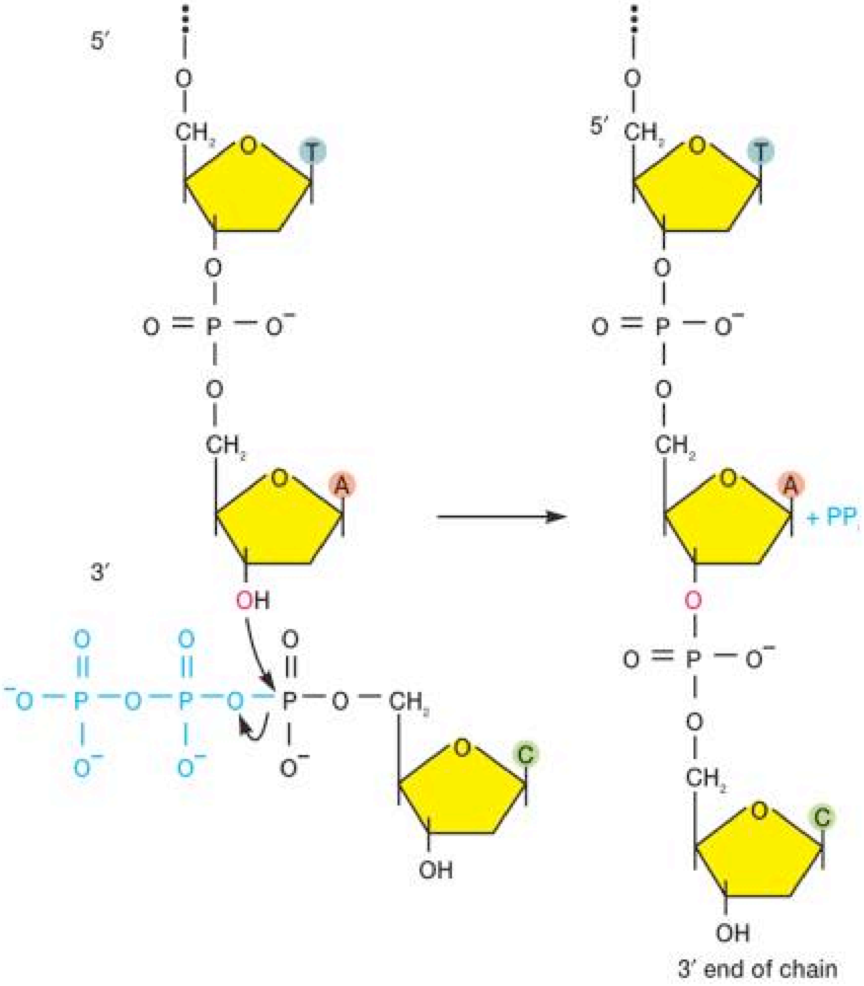


Fig. 11.15

ddATP= stops DNA elongation

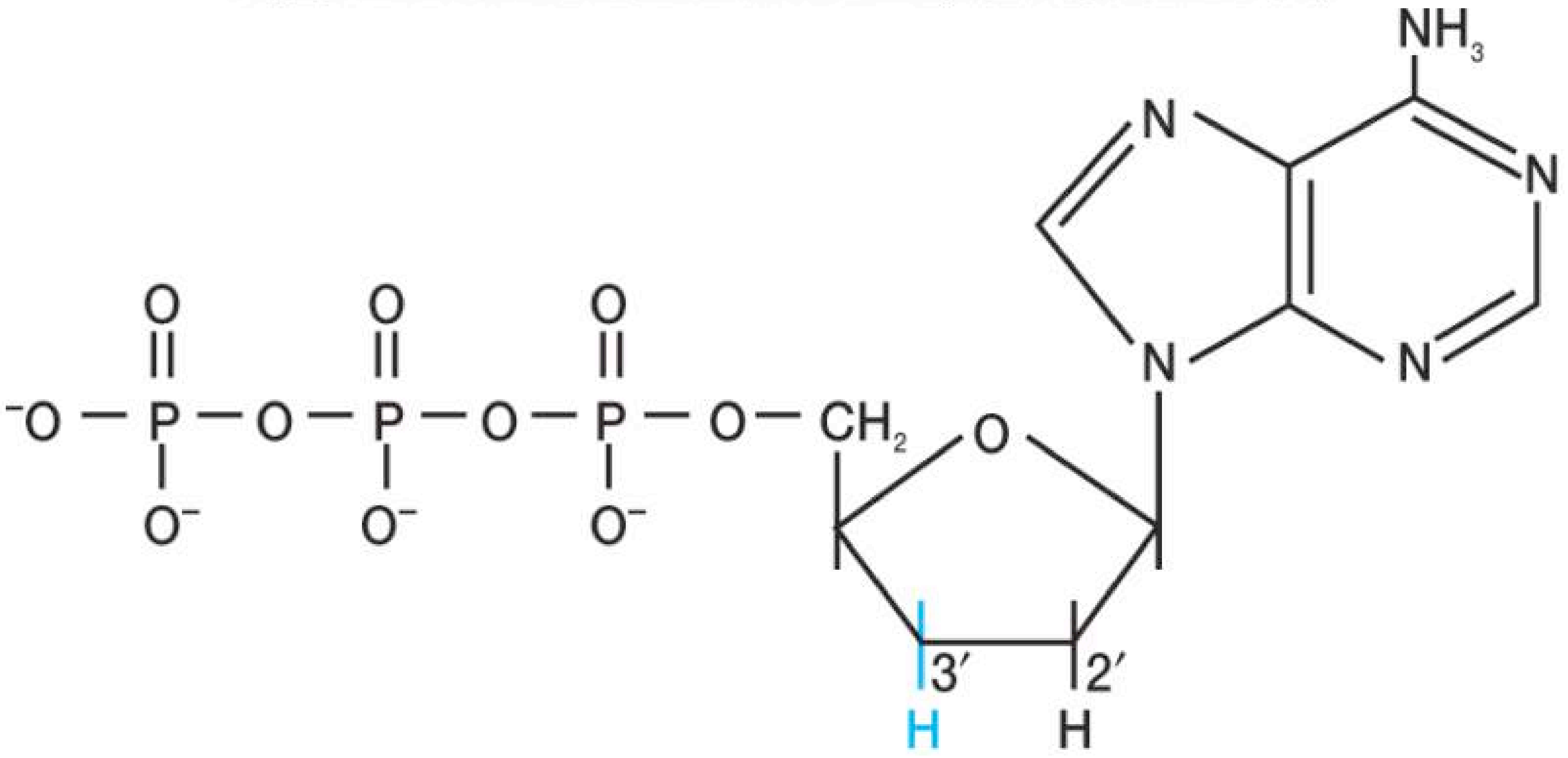


Fig. 15.1

DNA polymerase reaction

$$n[\text{dATP, dGTP, dCTP, dTTP}] \xrightarrow[\text{DNA template}]{\text{DNA polymerase}} \text{DNA} + n\text{PP}_i$$

The mechanism of chain growth

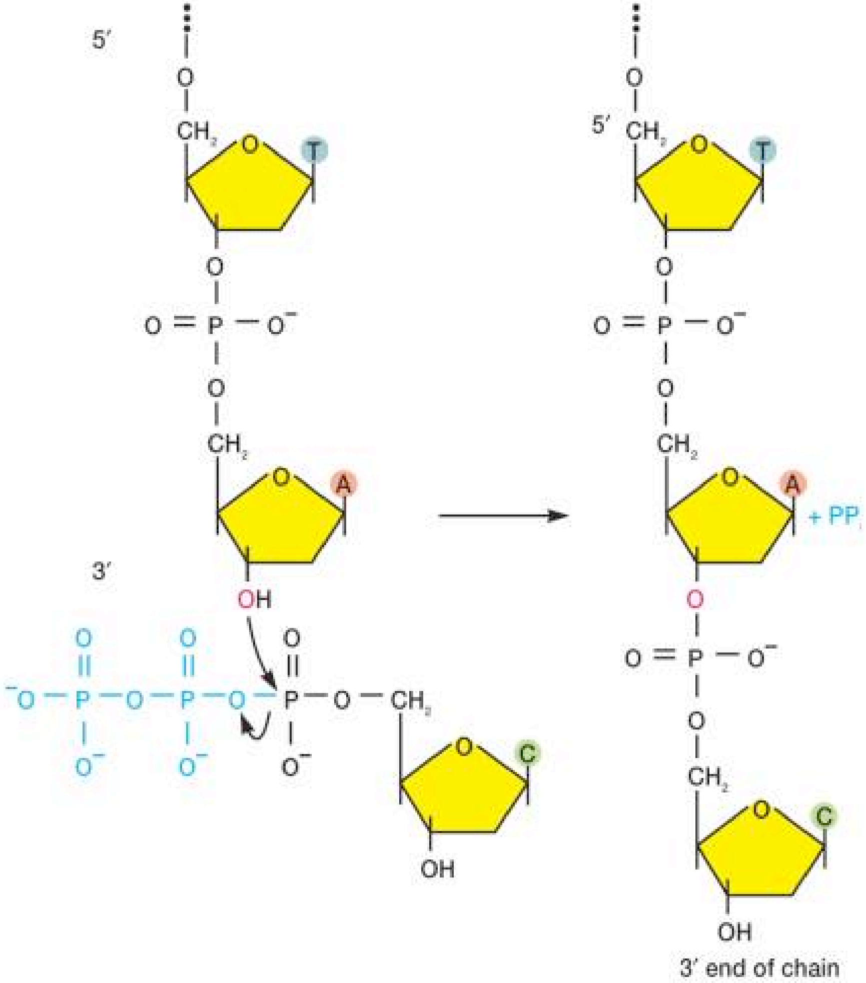


Fig. 11.15

DNA sequencing

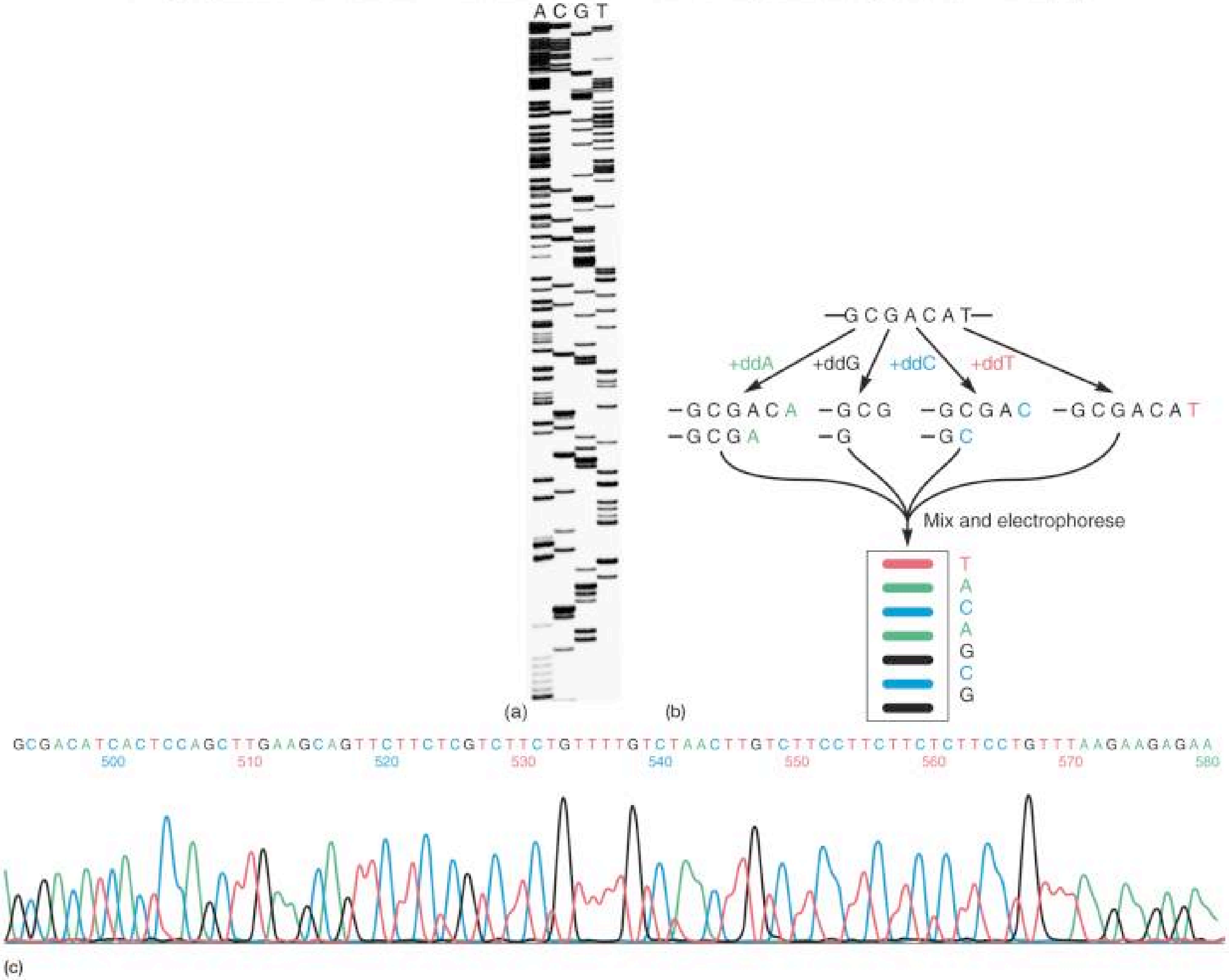
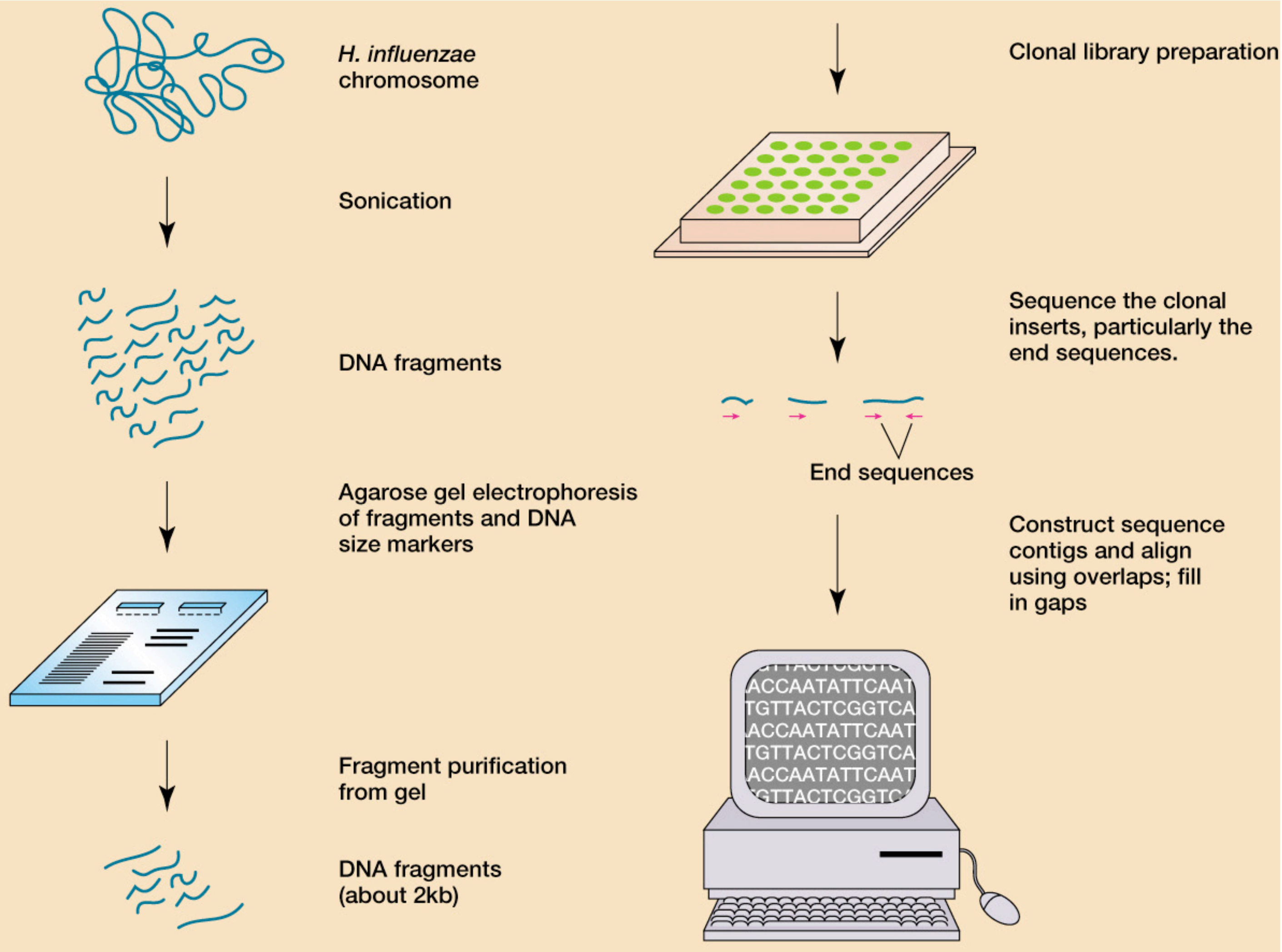


Fig. 15.2

Whole genome sequencing



H. influenzae chromosome

Sonication

DNA fragments

Agarose gel electrophoresis of fragments and DNA size markers

Fragment purification from gel

DNA fragments (about 2kb)

Clonal library preparation

Sequence the clonal inserts, particularly the end sequences.

End sequences

Construct sequence contigs and align using overlaps; fill in gaps

Fig. 15.3

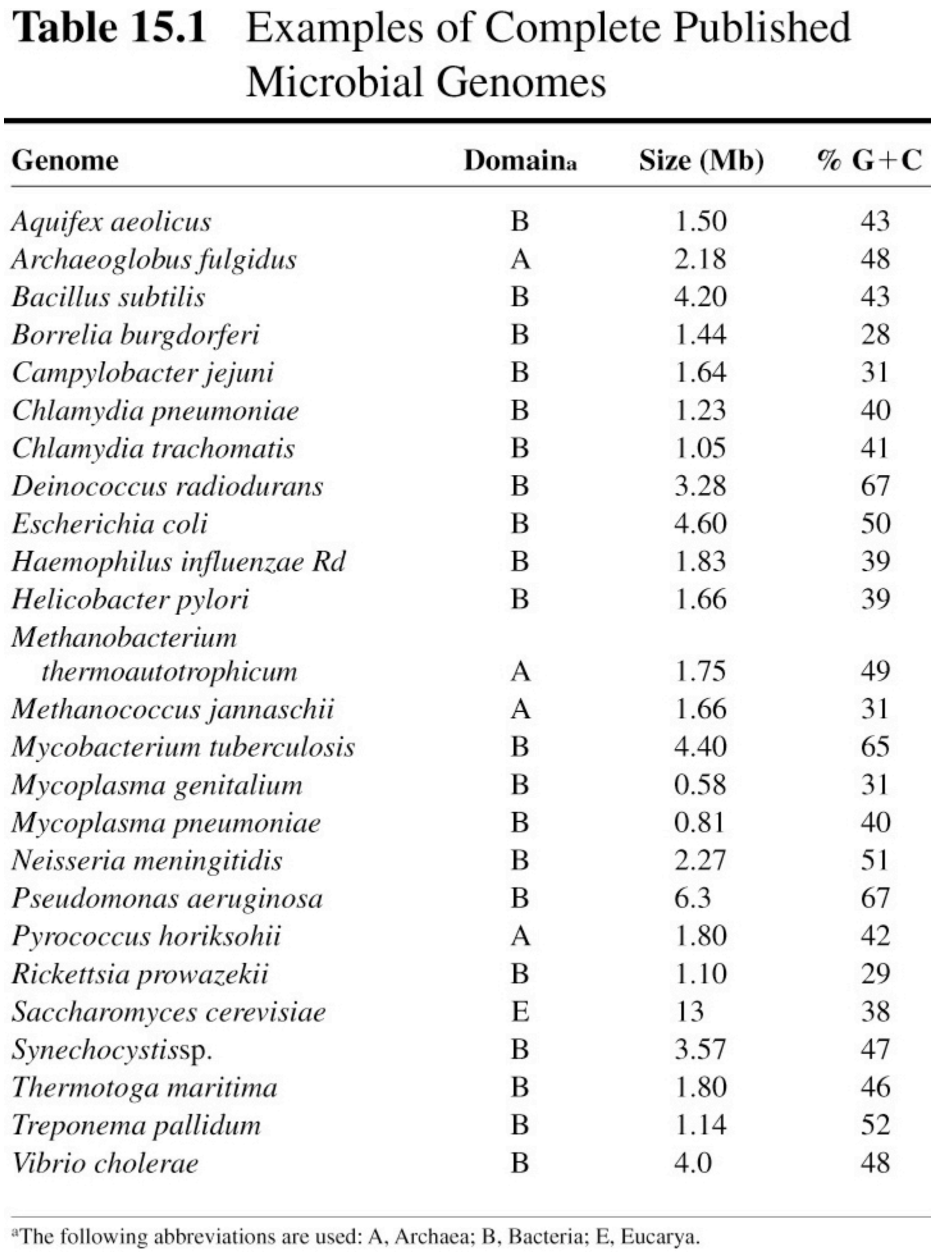


Table 15.1 Examples of Complete Published Microbial Genomes

Genome	Domain[a]	Size (Mb)	% G+C
Aquifex aeolicus	B	1.50	43
Archaeoglobus fulgidus	A	2.18	48
Bacillus subtilis	B	4.20	43
Borrelia burgdorferi	B	1.44	28
Campylobacter jejuni	B	1.64	31
Chlamydia pneumoniae	B	1.23	40
Chlamydia trachomatis	B	1.05	41
Deinococcus radiodurans	B	3.28	67
Escherichia coli	B	4.60	50
Haemophilus influenzae Rd	B	1.83	39
Helicobacter pylori	B	1.66	39
Methanobacterium thermoautotrophicum	A	1.75	49
Methanococcus jannaschii	A	1.66	31
Mycobacterium tuberculosis	B	4.40	65
Mycoplasma genitalium	B	0.58	31
Mycoplasma pneumoniae	B	0.81	40
Neisseria meningitidis	B	2.27	51
Pseudomonas aeruginosa	B	6.3	67
Pyrococcus horiksohii	A	1.80	42
Rickettsia prowazekii	B	1.10	29
Saccharomyces cerevisiae	E	13	38
Synechocystissp.	B	3.57	47
Thermotoga maritima	B	1.80	46
Treponema pallidum	B	1.14	52
Vibrio cholerae	B	4.0	48

---

[a] The following abbreviations are used: A, Archaea; B, Bacteria; E, Eucarya.

69 Complete Microbial Genomes (as of 12/17/01)

[A] - Archaea - 12 species

[B] - Bacteria - 57 species

224 Complete Microbial Genomes (as of 2/17/05)

[A] - Archaea - 21 species

[B] - Bacteria - 203 species

Map of *Mycoplasma genitalium*

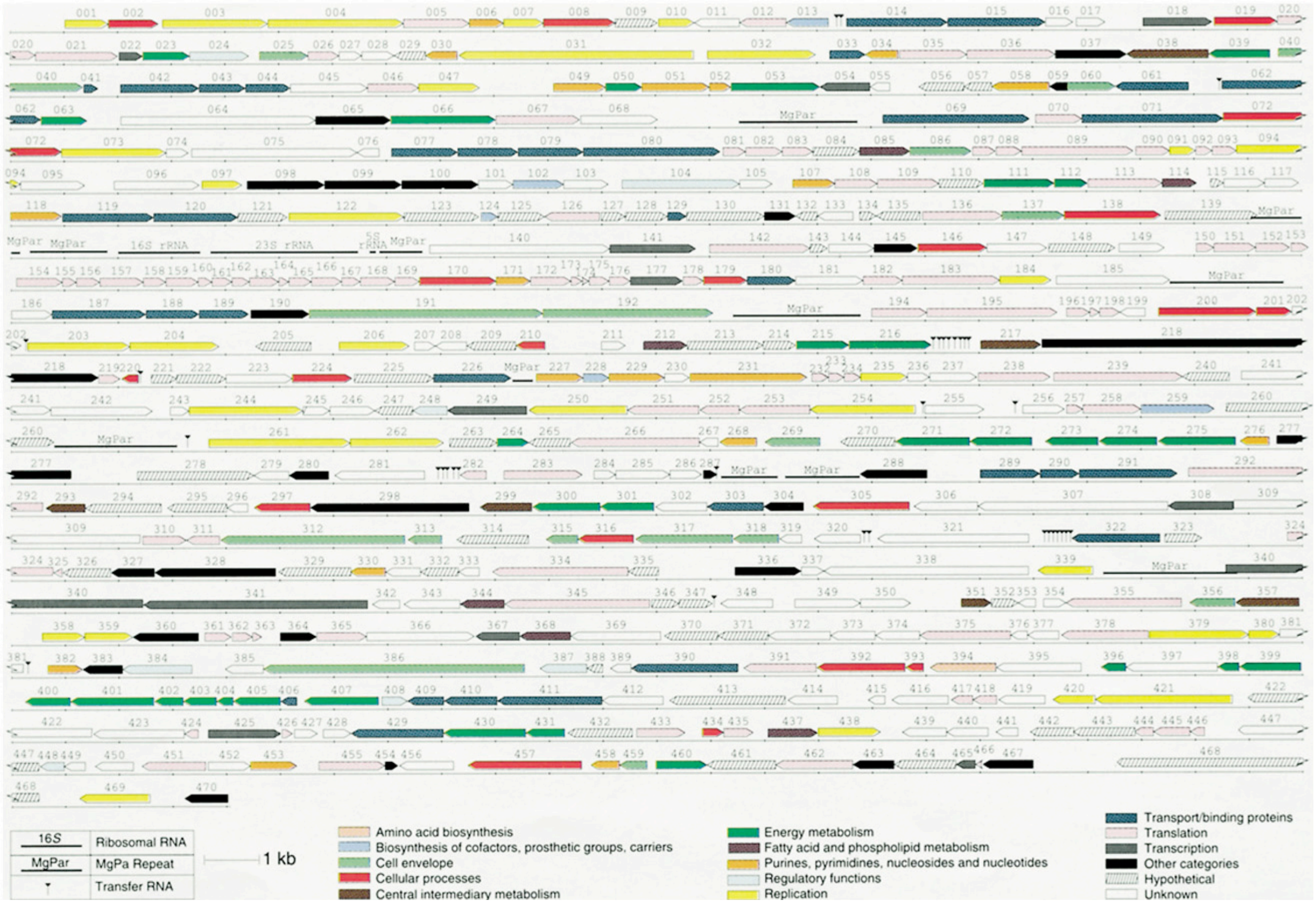


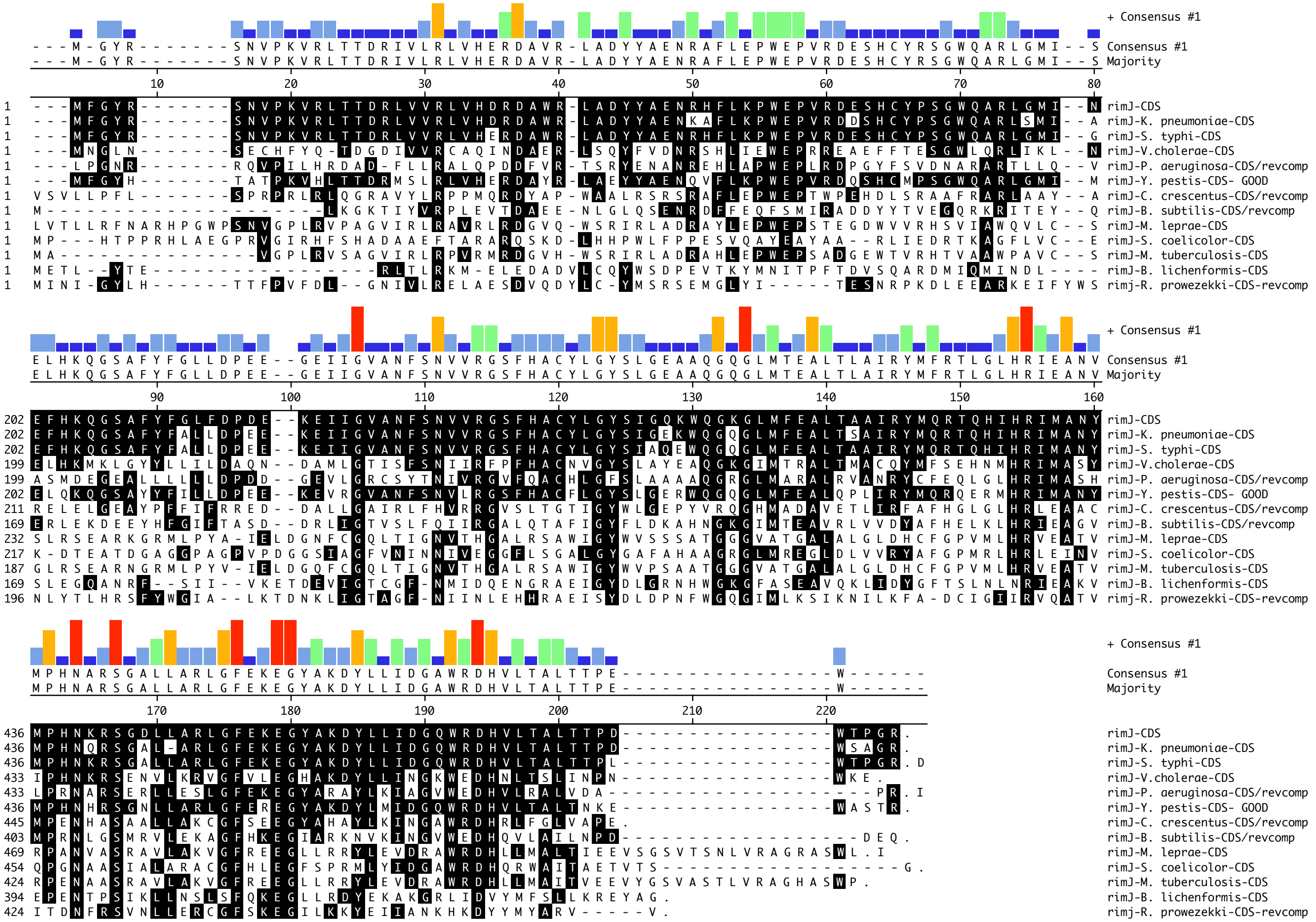
Fig. 15.5

What do you get from sequence data?

- Evolutionary relatedness based on several loci

- Infer function of proteins encoded by genes

Multiple sequence alignment



```
                                                                                      + Consensus #1
     ---M-GYR-------SNVPKVRLTTDRIVLRLVHERDAVR-LADYYAENRAFLEPWEPVRDESHCYRSGWQARLGMI--S Consensus #1
     ---M-GYR-------SNVPKVRLTTDRIVLRLVHERDAVR-LADYYAENRAFLEPWEPVRDESHCYRSGWQARLGMI--S Majority
             10        20        30        40        50        60        70        80
1    ---MFGYR-------SNVPKVRLTTDRLVVRLVHDRDAWR-LADYYAENRHFLKPWEPVRDESHCYPSGWQARLGMI--N rimJ-CDS
1    ---MFGYR-------SNVPKVRLTTDRLVVRLVHDRDAWR-LADYYAENKAFLKPWEPVRDDSHCYPSGWQARLSMI--A rimJ-K. pneumoniae-CDS
1    ---MFGYR-------SNVPKVRLTTDRLVVRLVHERDAWR-LADYYAENRHFLKPWEPVRDESHCYPSGWQARLGMI--G rimJ-S. typhi-CDS
1    ---MNGLN-------SECHFYQ-TDGDIVVRCAQINDAER-LSQYFVDNRSHLIEWEPRREAEFFTESGWLQRLIKL--N rimJ-V.cholerae-CDS
1    ---LPGNR-------RQVPILHRDAD-FLLRALQPDDFVR-TSRYENANREHLAPWEPLRDPGYFSVDNARARTLLQ--V rimJ-P. aeruginosa-CDS/revcomp
1    ---MFGYH-------TATPKVHLTTDRMSLRLVHERDAYR-LAEYYAENQVFLKPWEPVRDQSHCMPSGWQARLGMI--M rimJ-Y. pestis-CDS- GOOD
1    VSVLLPFL-------SPRPRLRLQGRAVYLRPPMQRDYAP-WAALRSRSRAFLEPWEPTWPEHDLSRAAFRARLAAY--A rimJ-C. crescentus-CDS/revcomp
1    M---------------------LKGKTIYVRPLEVTDAEE-NLGLQSENRDFFEQFSMIRADDYYTVEGQRKRITEY--Q rimJ-B. subtilis-CDS/revcomp
1    LVTLLRFNARHPGWPSNVGPLRVPAGVIRLRAVRLRDGVQ-WSRIRLADRAYLEPWEPSTEGDWVVRHSVIAWQVLC--S rimJ-M. leprae-CDS
1    MP---HTPPRHLAEGPRVGIRHFSHADAAEFTARARQSKD-LHHPWLFPPESVQAYEAYAA--RLIEDRTKAGFLVC--E rimJ-S. coelicolor-CDS
1    MA---------------VGPLRVSAGVIRLRPVRMRDGVH-WSRIRLADRAHLEPWEPSADGEWTVRHTVAAWPAVC--S rimJ-M. tuberculosis-CDS
1    METL--YTE-----------------RLTLRKM-ELEDADVLCQYWSDPEVTKYMNITPFTDVSQARDMIQMINDL---- rimJ-B. lichenformis-CDS
1    MINI-GYLH------TTFPVFDL--GNIVLRELAESDVQDYLC-YMSRSEMGLYI-----TESNRPKDLEEARKEIFYWS rimj-R. prowezekki-CDS-revcomp

                                                                                      + Consensus #1
     ELHKQGSAFYFGLLDPEE--GEIIGVANFSNVVRGSFHACYLGYSLGEAAQGQGLMTEALTLAIRYMFRTLGLHRIEANV Consensus #1
     ELHKQGSAFYFGLLDPEE--GEIIGVANFSNVVRGSFHACYLGYSLGEAAQGQGLMTEALTLAIRYMFRTLGLHRIEANV Majority
             90       100       110       120       130       140       150       160
202  EFHKQGSAFYFGLFDPDE--KEIIGVANFSNVVRGSFHACYLGYSIGQKWQGKGLMFEALTAAIRYMQRTQHIHRIMANY rimJ-CDS
202  EFHKQGSAFYFALLDPEE--KEIIGVANFSNVVRGSFHACYLGYSIGEKWQGQGLMFEALTSAIRYMQRTQHIHRIMANY rimJ-K. pneumoniae-CDS
202  EFHKQGSAFYFALLDPEE--KEIIGVANFSNVVRGSFHACYLGYSIAQEWQGQGLMFEALTAAIRYMQRTQHIHRIMANY rimJ-S. typhi-CDS
199  ELHKMKLGYYLLILDAQN--DAMLGTISFSNIIRFPFHACNVGYSLAYEAQGKGIMTRALTMACQYMFSEHNMHRIMASY rimJ-V.cholerae-CDS
199  ASMDEGEALLLLLLDPDD--GEVLGRCSYTNIVRGVFQACHLGFSLAAAAQGRGLMARALRVANRYCFEQLGLHRIMASH rimJ-P. aeruginosa-CDS/revcomp
202  ELQKQGSAYYFILLDPEE--KEVRGVANFSNVLRGSFHACFLGYSLGERWQGQGLMFEALQPLIRYMQRQERMHRIMANY rimJ-Y. pestis-CDS- GOOD
211  RELELGEAYPFFIFRRED--DALLGAIRLFHVRRGVSLTGTIGYWLGEPYVRQGHMADAVETLIRFAFHGLGLHRLEAAC rimJ-C. crescentus-CDS/revcomp
169  ERLEKDEEYHFGIFTASD--DRLIGTVSLFQIIRGALQTAFIGYFLDKAHNGKGIMTEAVRLVVDYAFHELKLHRIEAGV rimJ-B. subtilis-CDS/revcomp
232  SLRSEARKGRMLPYA-IELDGNFCGQLTIGNVTHGALRSAWIGYWVSSSATGGGVATGALALGLDHCFGPVMLHRVEATV rimJ-M. leprae-CDS
217  K-DTEATDGAGGPAGPVPDGGSIAGFVNINNIVEGGFLSGALGYGAFAHAAGRGLMREGLDLVVRYAFGPMRLHRLEINV rimJ-S. coelicolor-CDS
187  GLRSEARNGRMLPYV-IELDGQFCGQLTIGNVTHGALRSAWIGYWVPSAATGGGVATGALALGLDHCFGPVMLHRVEATV rimJ-M. tuberculosis-CDS
169  SLEGQANRF--SII--VKETDEVIGTCGF-NMIDQENGRAEIGYDLGRNHWGKGFASEAVQKLIDYGFTSLNLNRIEAKV rimJ-B. lichenformis-CDS
196  NLYTLHRSFYWGIA--LKTDNKLIGTAGF-NIINLEHHRAEISYDLDPNFWGQGIMLKSIKNILKFA-DCIGIIRVQATV rimj-R. prowezekki-CDS-revcomp

                                                                                      + Consensus #1
     MPHNARSGALLARLGFEKEGYAKDYLLIDGAWRDHVLTALTTPE----------------W------              Consensus #1
     MPHNARSGALLARLGFEKEGYAKDYLLIDGAWRDHVLTALTTPE----------------W------              Majority
            170       180       190       200       210       220
436  MPHNKRSGDLLARLGFEKEGYAKDYLLIDGQWRDHVLTALTTPD----------------WTPGR.               rimJ-CDS
436  MPHNQRSGAL-ARLGFEKEGYAKDYLLIDGQWRDHVLTALTTPD----------------WSAGR.               rimJ-K. pneumoniae-CDS
436  MPHNKRSGALLARLGFEKEGYAKDYLLIDGQWRDHVLTALTTPL----------------WTPGR.D              rimJ-S. typhi-CDS
433  IPHNKRSENVLKRVGFVLEGHAKDYLLINGKWEDHNLTSLINPN----------------WKE.                 rimJ-V.cholerae-CDS
433  LPRNARSERLLESLGFEKEGYARAYLKIAGVWEDHVLRALVDA--------------------PR.I              rimJ-P. aeruginosa-CDS/revcomp
436  MPHNHRSGNLLARLGFEREGYAKDYLMIDGQWRDHVLTALTNKE----------------WASTR.               rimJ-Y. pestis-CDS- GOOD
445  MPENHASAALLAKCGFSEEGYAHAYLKINGAWRDHRLFGLVAPE.                                    rimJ-C. crescentus-CDS/revcomp
403  MPRNLGSMRVLEKAGFHKEGIARKNVKINGVWEDHQVLAILNPD------------------DEQ.               rimJ-B. subtilis-CDS/revcomp
469  RPANVASRAVLAKVGFREEGLLRRYLEVDRAWRDHLLMALTIEEVSGSVTSNLVRAGRASWL.I                 rimJ-M. leprae-CDS
454  QPGNAASIALARACGFHLEGFSPRMLYIDGAWRDHQRWAITAETVTS------------------G.              rimJ-S. coelicolor-CDS
424  RPENAASRAVLAKVGFREEGLLRRYLEVDRAWRDHLLMAITVEEVYGSVASTLVRAGHASWP.                  rimJ-M. tuberculosis-CDS
394  EPENTPSIKLLNSLSFQKEGLLRDYEKAKGRLIDVYMFSLLKREYAG.                                 rimJ-B. lichenformis-CDS
424  ITDNFRSVNLLERCGFSKEGILKKYEIIANKHKDYYMYARV-----V.                                 rimj-R. prowezekki-CDS-revcomp
```

Consensus 'Consensus #1': When all match the residue of the Consensus show the residue of the Consensus, otherwise show '.'.

Decoration 'Decoration #1': Shade (with solid black) residues that match rimJ-CDS exactly.

The RimJ protein

MFGYRSNVPKVRLTTDRLVVRLVHDRDAWRLADYYAENRHFLKPWEPVRD

Motif C

L

ESHCYPSGWQARLGMINEFHKQGSAFYFGLFDPDEKEIIGVANFSNVVRG

Motif D

stop

SFHACYLGYSIGQKWQGKGLMFEALTAAIRYMQRTQHIHRIMANYMPHNK

Motif A

rimJ-2::m*Tn*10

RSGDLLARLGFEKEGYAKDYLLIDGQWRDHVLTALTTPDWTPGR.

Motif B

rimJ-1::m*Tn*10

Red= conserved in RimJ and 13 putative prokaryotic acetylases
Yellow=conserved in RimJ and 11 putative prokaryotic acetylases
Blue= *rimJ* mutations that cause a loss of *papBA* thermoregulation
Green= motifs conserved in N-acetyltransferase (GNAT) superfamily

Map of *Haemophilus influenzae*

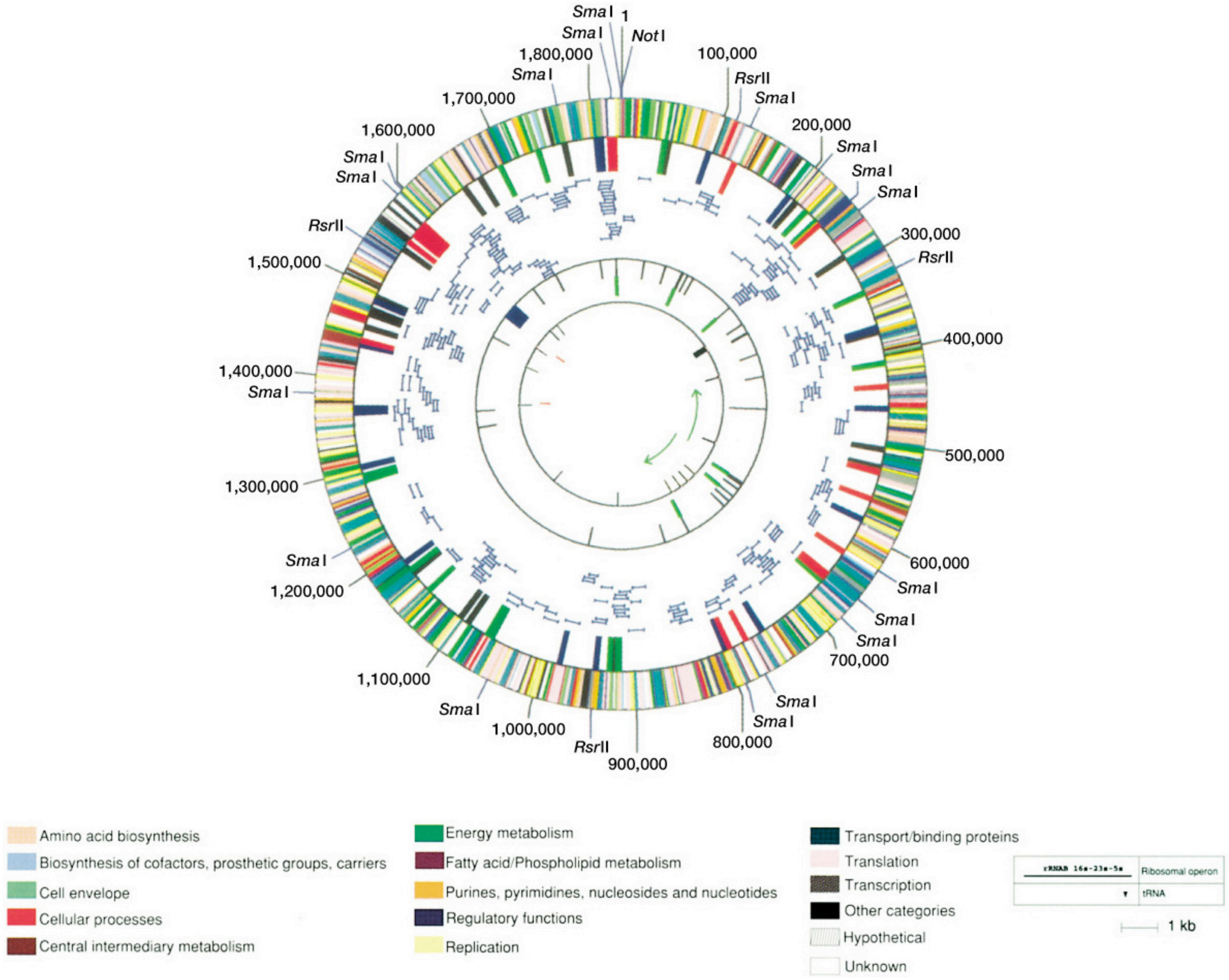
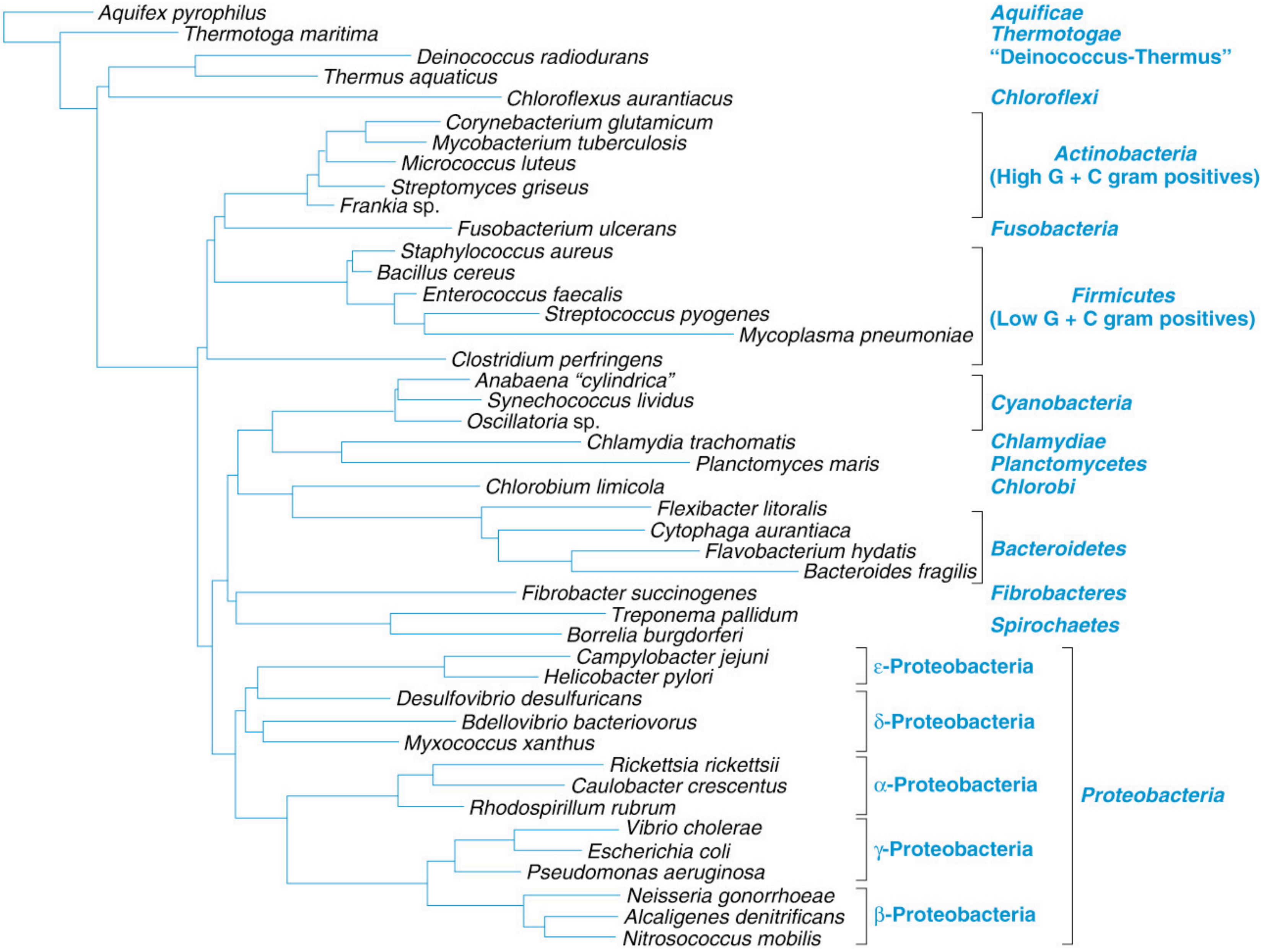


Fig.15.6


Aquifex pyrophilus
Thermotoga maritima
Deinococcus radiodurans
Thermus aquaticus
Chloroflexus aurantiacus
Corynebacterium glutamicum
Mycobacterium tuberculosis
Micrococcus luteus
Streptomyces griseus
Frankia sp.
Fusobacterium ulcerans
Staphylococcus aureus
Bacillus cereus
Enterococcus faecalis
Streptococcus pyogenes
Mycoplasma pneumoniae
Clostridium perfringens
Anabaena "cylindrica"
Synechococcus lividus
Oscillatoria sp.
Chlamydia trachomatis
Planctomyces maris
Chlorobium limicola
Flexibacter litoralis
Cytophaga aurantiaca
Flavobacterium hydatis
Bacteroides fragilis
Fibrobacter succinogenes
Treponema pallidum
Borrelia burgdorferi
Campylobacter jejuni
Helicobacter pylori
Desulfovibrio desulfuricans
Bdellovibrio bacteriovorus
Myxococcus xanthus
Rickettsia rickettsii
Caulobacter crescentus
Rhodospirillum rubrum
Vibrio cholerae
Escherichia coli
Pseudomonas aeruginosa
Neisseria gonorrhoeae
Alcaligenes denitrificans
Nitrosococcus mobilis
Aquificae
Thermotogae
"Deinococcus-Thermus"
Chloroflexi
Actinobacteria
(High G + C gram positives)
Fusobacteria
Firmicutes
(Low G + C gram positives)
Cyanobacteria
Chlamydiae
Planctomycetes
Chlorobi
Bacteroidetes
Fibrobacteres
Spirochaetes
ε-Proteobacteria
δ-Proteobacteria
α-Proteobacteria
γ-Proteobacteria
β-Proteobacteria
Proteobacteria

Bergey's Manuals

- *Manual of Determinative Bacteriology*
  - used for identification of bacterial species

- *Manual of Systematic Bacteriology*
  - more thorough taxonomic classification
  - 1st edition= phenetic classification
  - 2nd edition= phylogenetic classification