Microbial processes and monitoring

Lecture 2

Learning outcomes

- Microbial kinetics
- Metabolism
- Microbial monitoring

Microbial kinetics

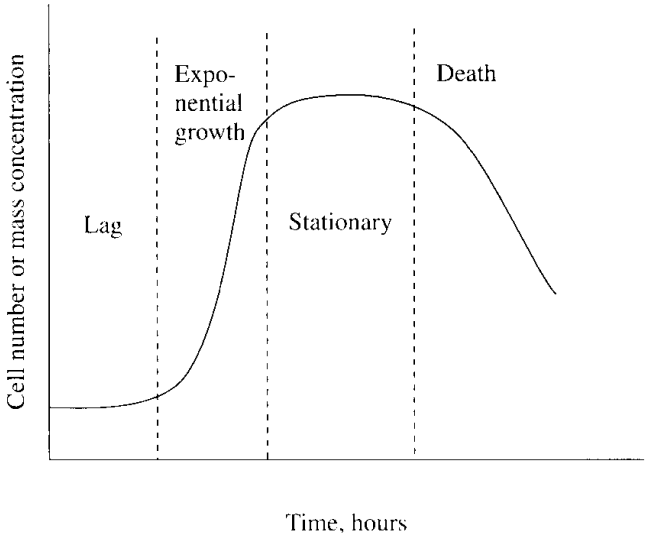
Biomass production

Bacterial biomass production is often accounted for with empirical cell ratios:

$C_5H_7O_2N$

or (more complex)

$C_{60}H_{87}O_{23}N_{12}P$



$N_t=N_0e^{kt}$ (first order kinetics)

N_t= number of microorganisms at time t

N_0= number of microorganisms at time 0

k=growth constant

for one doubling time: $N=2N_0 \Rightarrow t_d=\frac{\ln(2)}{k}$

Maximum recorded growth rates for some bacteria, measured at or near their respective optimal temperature, in complex media

Organism	Temperature, °C	Doubling time, h
Vibrio natriegens	37	0.16
Bacillus stearothermophilus	60	0.14
Escherichia coli	40	0.38
Bacillus subtilis	40	0.43
Pseudomonas putida	30	0.75*
Vibrio marinus	15	1.35
Rhodobacter sphaeroides	30	2.2
Mycobacterium tuberculosis	37	≈ 6
Nitrobacter agilis	27	≈ 20*

Source: Stanier et al., 1986.
*Grown in synthetic media.

Specific growth rate

• More generally, the **specific growth rate** (μ) defined as rate at which cells divide (generations per unit time or inverse of doubling time)

$$\frac{dX}{dt} = \mu X \qquad \text{(eq. 1)}$$

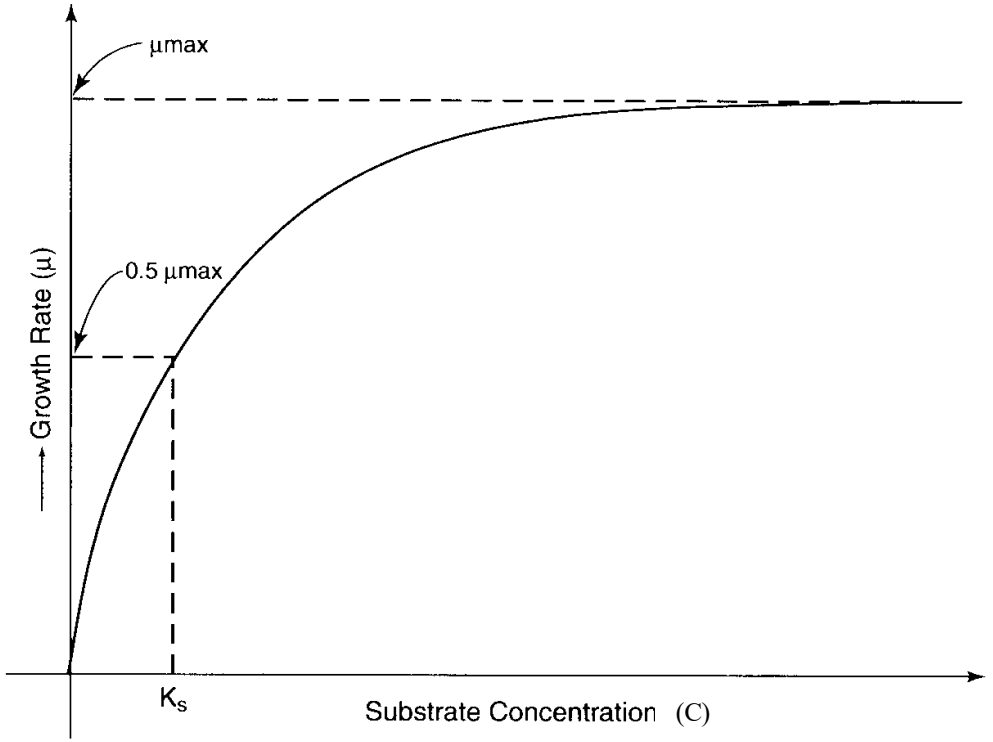
Where:

μ= specific growth rate ($time^{-1}$)
t= time
X= biomass concentration (mass/unit volume)

• A classic relationship exists between bacterial growth rate and substrate concentration (Monod model)

Monod model

$$\mu = \mu_{max} \frac{C}{K_S + C} \qquad \text{(eq. 2)}$$



Where:

μ= specific growth rate (time^{-1})

μ_{max}= maximum specific growth rate (time^{-1})

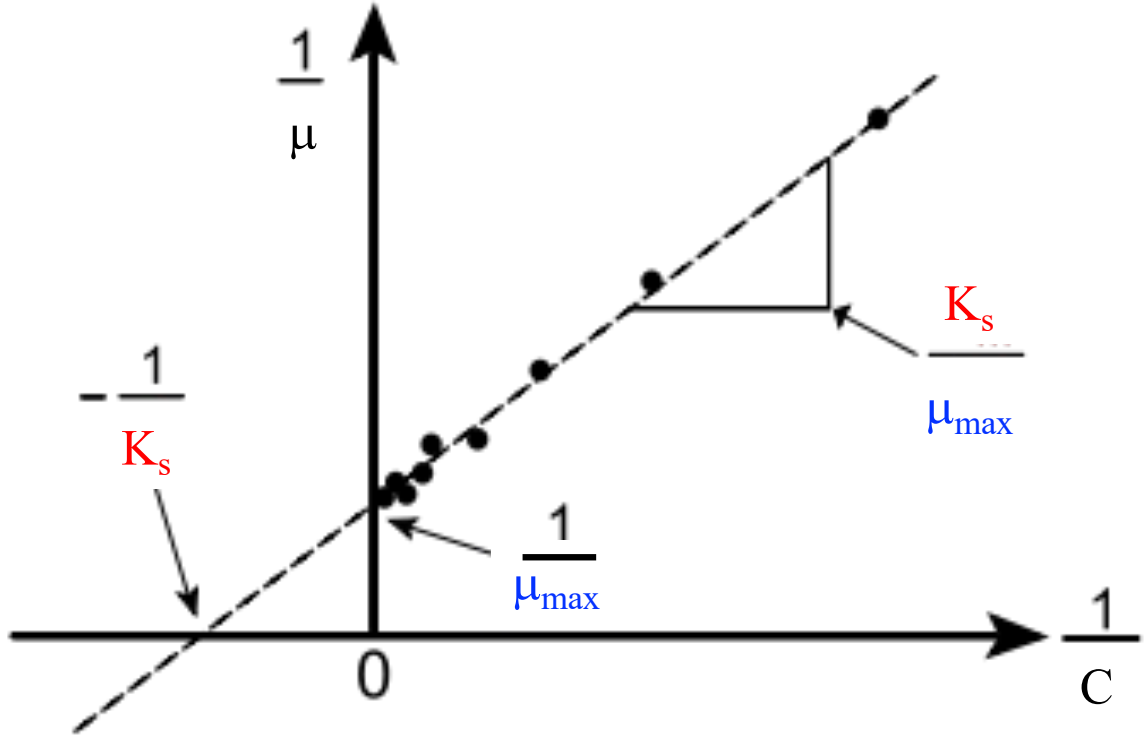
C= concentration of substrate in solution (mass/unit volume)

K_S= half-velocity constant (substrate concentration at which μ is one half of μ_{max}) (mass/unit volume)

Lineweaver-Burk plot

$$\mu = \frac{\mu_{max}\ C}{K_S + C}$$

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \times \frac{1}{C} + \frac{1}{\mu_{max}}$$

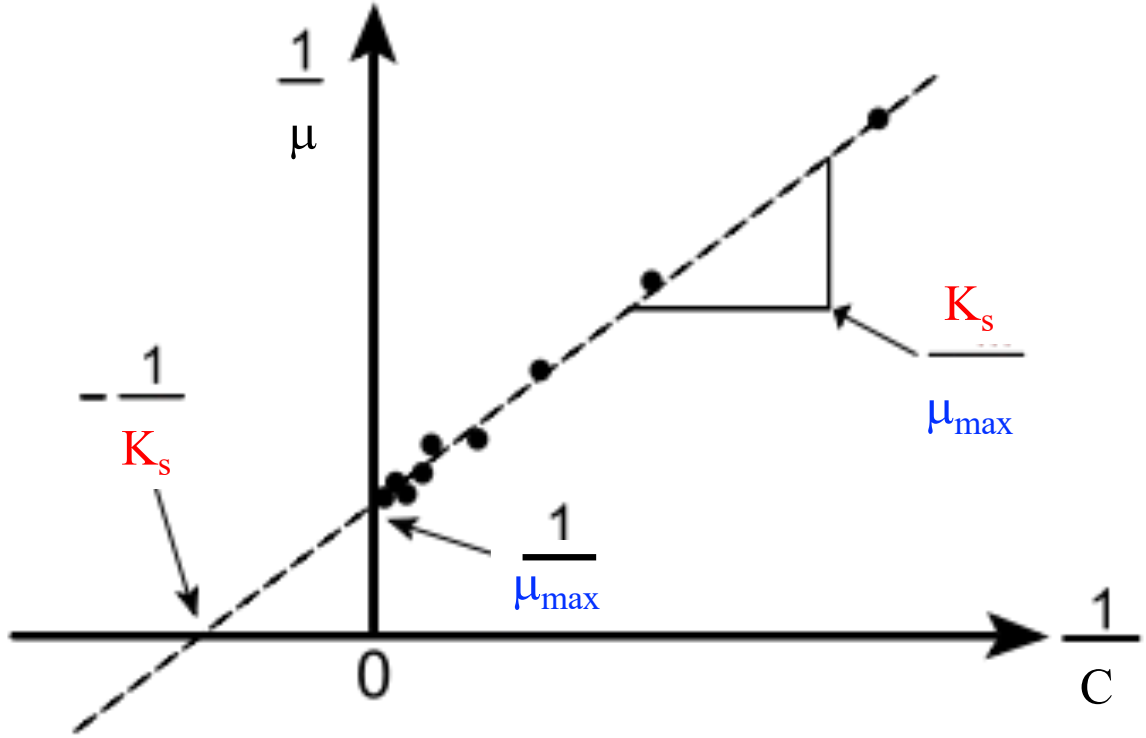


Lineweaver-Burk plot

$$\mu = \frac{\mu_{max}\ C}{K_S + C} \Rightarrow \frac{1}{\mu} = \frac{K_S + C}{\mu_{max} C} = \frac{K_S}{\mu_{max} C} + \frac{C}{\mu_{max} C}$$

$$\Rightarrow \frac{1}{\mu} = \frac{K_S}{\mu_{max} C} + \frac{1}{\mu_{max}}$$

$$\Rightarrow \boxed{\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \times \frac{1}{C} + \frac{1}{\mu_{max}}}$$



Modeling growth

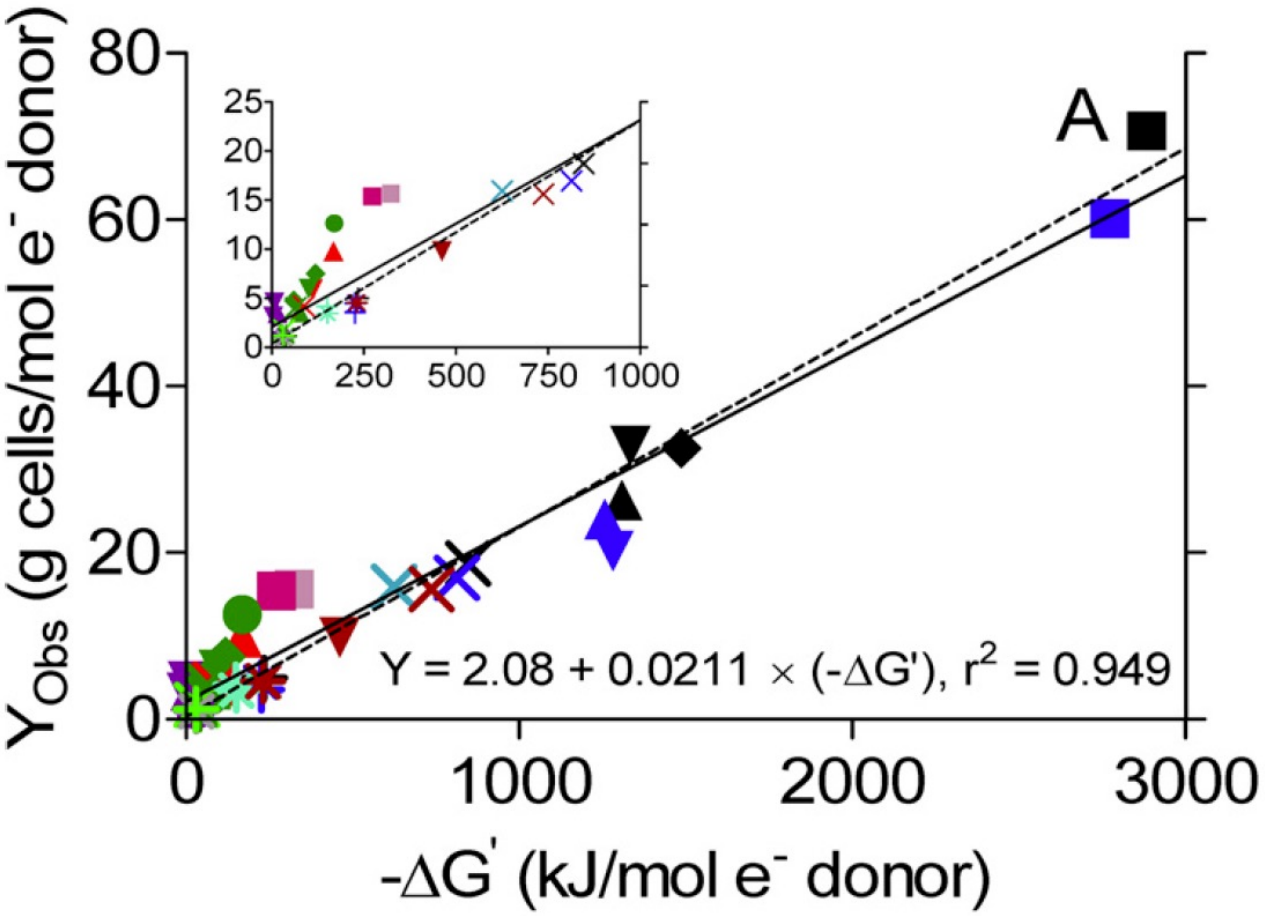
• For organic contaminants that serve as the primary substrate (the sole source of carbon and energy), biomass production is linked to substrate degradation (combine eqs. 1 & 2):

$$\frac{dX}{dt} = \left(\frac{\mu_{max} C}{K_S + C}\right) X \qquad \text{(eq. 3)}$$

• The mass of new cells synthesized per unit mass of substrate removed is constant for a given bacterium and a given substrate. Define the growth yield coefficient (Y= mass biomass/unit mass of substrate)

$$Y = \frac{dX/dt}{dC/dt} \qquad \text{(eq. 4)}$$

Growth yield coefficient (Y)



Roden and Jin, 2011

Y => related to the Gibbs free energy of the reaction

Y => Can be estimated

Modeling growth

Combining eqs. 3 & 4

$$\frac{dC}{dt} = \left(\frac{\mu_{max} C}{Y * (K_S + C)} \right) X \qquad \text{(eq. 5)}$$

The term $\boldsymbol{\mu_{max}/Y}$ can be replaced by the term **k**: degradation rate constant (or maximum rate of substrate removal per unit weight of biomass) (unit mass substrate/ (unit mass biomass . time))

$$\boxed{\frac{\mu_{max}}{Y} = \text{cst} = k}$$

[mass substrate/(mass biomass.time)]

$$\frac{dC}{dt} = \left(\frac{kC}{K_S + C} \right) X \qquad \text{(eq. 6)}$$

and

$$\frac{dX}{dt} = \left(\frac{YkC}{K_S + C} \right) X \qquad \text{(eq. 7)}$$

Modeling growth

In any biological treatment, a portion of the cells are not proliferating but rather in maintenance or dead phase. Need to take death of cells into account

$$\frac{dX}{dt} = \left(\frac{YkC}{K_s + C} - b \right) X \qquad \text{(eq. 8)}$$

Where b= endogenous decay constant (time^{-1})

Minimum substrate concentration

In many cases, the concentration of contaminants is low and does not support viable biomass. At low concentrations, a threshold will be reached where energy needs are not met and biomass loss is observed
This minimum substrate concentration occurs when

$$bX = \left(\frac{YkC_{min}}{K_S + C_{min}} \right) X \qquad \text{(eq. 9)}$$

Where
C_{min} = minimum concentration of substrate to support growth (mass/unit volume)

$$C_{min} = \frac{bK_S}{Yk - b} \qquad \text{(eq. 10)}$$

Typically, C_{min} is in the range of 0.1 to 1.0 mg/L. Some contaminants need to be removed to below 0.01 mg/L. In such cases, it may be necessary to induce cometabolism by adding a primary substrate.

Modeling growth

It is difficult to measure growth in subsurface, so make approximations:

- $C << K_S$

$$\frac{dC}{dt} = \frac{kC}{K_S} X \qquad \text{(eq. 11)}$$

- There is little growth, so X is constant

$$\ln \frac{C}{C_0} = - \frac{k}{K_S} Xt \qquad \text{(eq. 12)}$$

Where:

t= duration of treatment (time)

C_0= initial substrate concentration (mass/unit volume)

$$k' = \frac{k}{K_S} X \qquad \text{(eq. 13)}$$

Where:

k' = first-order degradation rate constant ($time^{-1}$)

Modeling growth

It is difficult to measure growth in subsurface, so make approximations

$$C = C_0 e^{-k' t} \qquad \text{(eq. 14)}$$

- Typically, half-lives of contaminants is reported:

$$k' = \frac{0.693}{t_{1/2}} \qquad \text{(eq. 15)}$$

Where:

$t_{1/2}$ = half-life (time)

In the subsurface

Use the solid phase concentration of cells (attached to soil particles) rather than solution phase cell conc.

(from eq. 3)

$$\frac{dX}{dt} = \left(\frac{\mu_{max} C}{K_S + C}\right) B \qquad \text{(eq. 16)}$$

Where B= solid phase concentration of cells mg cells/g soil

and $B = B_0 + Y * (C_0 - C)$ (eq. 17)

Example: Y = 0.5 g/g for aerobic bacteria or <0.1 g/g in some soils

Full equation

Putting the three equations together : eq. 5, 21, 22:

$$\frac{dC}{dt} = \frac{\mu_{max}}{Y} \frac{C\,[B_0 + Y\,(C_0\text{-}C)]}{K_s + C} \qquad \text{(eq. 18)}$$

In practice, (eq. 18) is often simplified:

Model	Necessary conditions	Differential form	Integral form
Zeroth-order	$C_0 >> K_S$ $B_0 >> YC_0$	$\frac{dC}{dt} = \frac{\mu_{max}}{Y} B_0$	$C = C_0 + \frac{\mu_{max} B_0}{Y} t$
Monod-no growth	B= constant	$\frac{dC}{dt} = \frac{\mu_{max} B_0 C}{Y(K_S + C)}$	$K_S \ln\frac{C}{C_0} + C - C_0 = \frac{B_0}{Y} \mu_{max} t$
First-order-no growth	$C_0 << K_S$ B_0= constant	$\frac{dC}{dt} = \frac{\mu_{max} B_0 C}{YK_S}$	$C = C_0 \exp\left(\frac{\mu_{max} B_0}{YK_S} t\right)$

Important kinetic parameters

Term	Definition	Significance
k	Degradation rate constant ($time^{-1}$)	Indication of degradability of an organic compound; for biological treatment to be effective high values are preferred
K_s	Half-velocity constant (mass/unit volume)	Indication of efficiency of the degradation; to degrade organics to low concentrations, low values are needed.
Y	Yield of biomass generated per unit mass of substrate removed (mass/unit mass)	Indication of utilization of substrate for the production of biomass
b	Endogenous decay constant ($time^{-1}$)	Indication of the rate of loss of biomass due to natural death and decay

Microbially-catalyzed degradation

Microbial growth requirements

- Electron acceptors: O_2 (aerobic) or NO_3^-, Fe(III), SO_4^{2-}, etc..
- Moisture: absolute minimum needed for biological treatment is 40% saturation of soil /sediment
- pH: most bacteria grow best at pH 6-8. Most bacteria die off at pH<4 and pH>9.5
- Inorganic nutrients: empirical equation for cellular material is $C_{60}H_{87}O_{23}N_{12}P$ (or simply $C_5H_7O_2N$). Rule of thumb TOC:N:P mass ratio of 20:5:1.
- Micronutrients: S, K, Ca, Mg, Fe, Ni, Cu, Zn, vitamins

Energetics

- Microbial metabolism is based on thermodynamics: If there is energy available in a reaction, a microbe may catalyze that reaction
- Most biologically-mediated transformations rely on redox transformations
- The relationship between energy and redox potential is captured in the Nernst equation:

$\Delta G^0 = -nF\ \Delta E_0$

where n= number of electrons transferred

F=faraday's constant 96,630 J/V

$\Delta E_0 = E_0$ (e- accepting couple)- E_0(e- donating couple)

TABLE 5.3

The electron tower: standard reduction potentials at 25°C and pH 7 for selected environmentally important redox couples

Half reaction	E_0, V
$6CO_2 + 24H^+ + 24e^- = C_6H_{12}O_6 + 6H_2O$	–0.43
$2H^- + 2e^- = H_2$	–0.41
$CO_2 + 6H^- + 6e^- = CH_3OH + H_2O$	–0.38
$NAD^+ + 2H^+ + 2e^- = NADH + H^+$	–0.32
$CO_2 + HCO_3^- + 8H^- + 8e^- = CH_3COO^- + 3H_2O$	–0.29
$CO_{2(g)} + 8H^+ + 8e^- = CH_{4(g)} + 2H_2O$	–0.25
$S_{(s)} + 2H^- + 2e^- = H_2S_{(g)}$	–0.24
$SO_4^{-2} + 9H^- + 8e^- = HS^- + 4H_2O$	–0.22
Pyruvate + $2H^+ + 2e^-$ = lactate	–0.19
$FeOOH_{(s)} + HCO_3^- + 2H^+ + e^- = FeCO_{3(s)} + 2H_2O$	–0.05*
$CH_3SOCH_3 + 2H^- + 2e^- = CH_3SCH_3 + H_2O$	0.16
$NO_3^- + 10H^+ + 8e^- = NH_4^+ + 3H_2O$	0.36
$NO_3^- + 2H^- + 2e^- = NO_2^- + H_2O$	0.42
$MnO_{2(s)} + HCO_3^- + 3H^- + 2e^- = MnCO_{3(s)} + 2H_2O$	0.52*
$CHCl_3 + H^- + 2e^- = CH_2Cl_2 + Cl^-$	0.56
$CCl_4 + H^+ + 2e^- = CHCl_3 + Cl^-$	0.67
$2NO_3^- + 12H^+ + 10e^- = N_2 + 6H_2O$	0.74
$Fe^{3+} + e^- = Fe^{2-}$	0.76
$O_{2(g)} + 4H^+ + 4e^- = 2H_2O$	0.82
$CCl_3CCl_3 + 2e^- = CCl_2CCl_2 + 2Cl$	1.13
$2HOCL + 2H^- + 2e^- = Cl_2 + 2H_2O$	1.18

*Based on $[HCO_3^-] = 10^{-3}$ M.

Problem:

A groundwater is contaminated with hexachloroethane Cl_3CCCl_3.

An environmental engineer is concerned that this compound will be transformed to tetrachloroethylene (Cl_2CCCl_2), an even more toxic compound, by reacting with nitrite in the groundwater.

Do you think this is likely to happen? Why or why not?

Degradation strategies vary depending on the compound

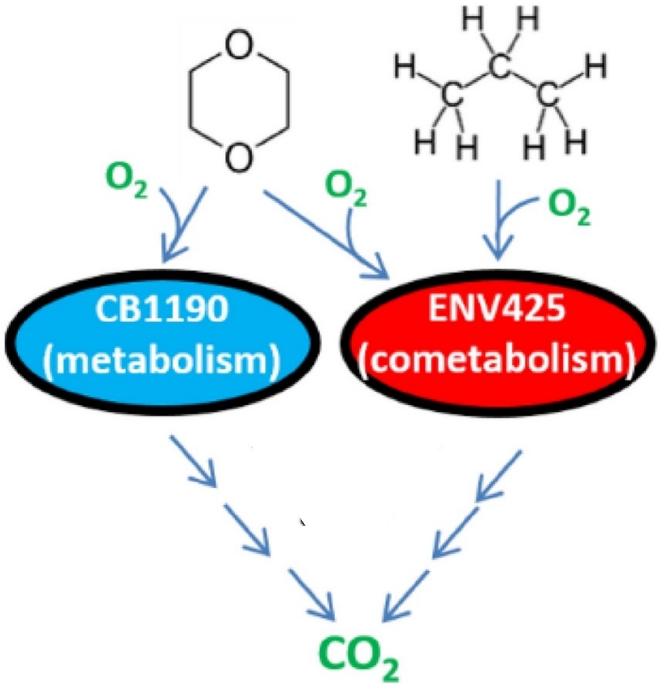
- Aerobic conditions:
  - Contaminant as an e^- donor and C source and O_2 as e^- acceptor- couple **growth** to the oxidation of contaminant
  - **Cometabolism**: organism grows on another primary substrate and also oxidizes the contaminant but obtains no benefit-O_2 as e^- acceptor

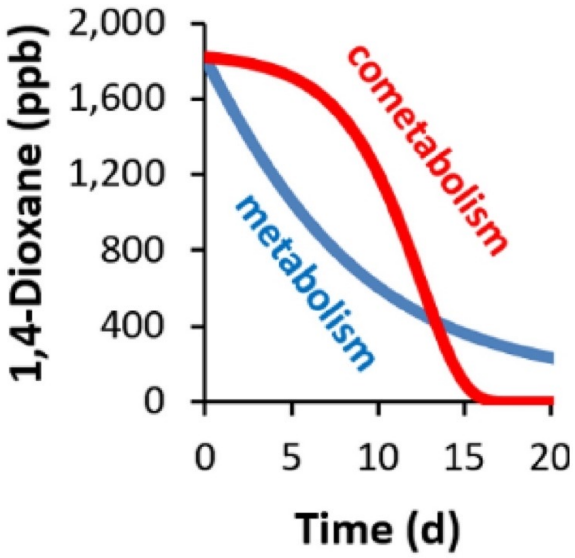
- Anaerobic conditions:

  - Contaminant as an e^- donor and C source and NO_3^-, SO_4^{2-} or Fe(III) as e^- acceptor- couple **growth** to the oxidation of contaminant
  - Contaminant as an e^- acceptor and e.g., H_2 as e^- donor: **Halorespiration**
  - **Cometabolism**: organism grows on another primary substrate and also oxidizes or reduces the contaminant (serves as e^- donor or acceptor but not coupled to growth)

Metabolism vs. cometabolism

- Organic compounds used as source of energy are most likely to be degraded
- Some compounds not used as energy source can be transformed through cometabolism
- Cometabolism: fortuitous transformation of a compound by enzymes designed for other purposes





1,4-dioxane metabolism by *Pseudonocardia dioxanivorans* (CB1190) compared to cometabolism by the propanotroph *Rhodococcus ruber (*ENV425)

Barajas-Rodriguez et al., 2018, JHM

Cometabolism

Transformation of a substrate by a microorganism that is unable to use the substrate as an energy source

Many enzymes can carry out cometabolism:

oxygenases

- Methane monooxygenase: methylotrophs can transform DCE
- Toluene dioxygenase: incorporates both atoms of molecular oxygen into toluene. Also transforms TCE
- Ammonia monoxygenase (*Nitrosomonas europaea*): chemoautotroph that cometabolizes TCE, DCE

CO dehydrogenase

- CO dehydrogenase of acetoclastic methanogens
- CO dehydrogenase pathway (oxidative) sulfate reducers
- CO dehydrogenase pathway (reductive) in homoacetogens

Co-factors

- Cometabolic reductive dehalogenation catalyzed by coenzyme B12 that is present in many anaerobic bacteria such as Clostridia, propionic acid bacteria
- Cytochrome P_{450} from *Pseudomonas* spp.
- F_{430} in methyl-CoM reductase in methanogens

Types of substrate

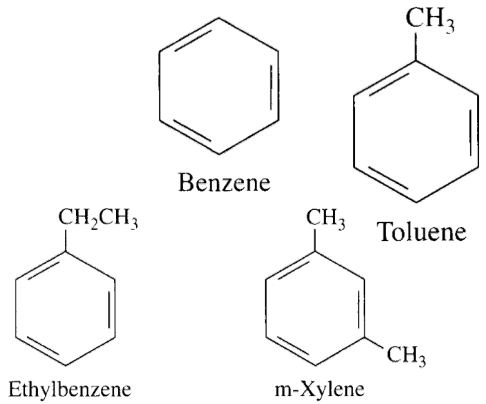
- Primary substrate: sole source of C and energy
- Secondary substrate: for cometabolism the substrate being degraded is not the same as that supporting growth
- Limiting substrate: one substrate (often O_2) can be limiting

	Cometabolic Bioremediation Conditions				
	Aerobic	**Aerobic**	**Aerobic**	**Anaerobic**	**Anaerobic**
Contaminants	• TCE • DCE • VC • PAHs • PCBs • MTBE • Creosote • >300 other compounds	• TCE • DCE • VC • TNT	• TCE • DCE • VC • 1,1-DCE • 1,1,1-TCA • MTBE	• PCE • TCE • DCE • VC • Hexachlorocyclohexane	• BTEX • PCE • PAHs • Atrazine • TNT
Substrates	• Methane • Methanol • Propane • Propylene	• Ammonia • Nitrate	• Toluene • Butane • Phenol • Citral • Cumin Aldehyde • Cumene • Limonene	• Methanol	• Glucose • Acetate • Lactate • Sulfate • Pyruvate
Microoganism(s)	• *Methylosinus*	• *Nitrosomonas* • *Nitrobacter*	• *Rhodococcus* • *Pseudomonas* • *Arthrobacter*	• *Pseudomonas* • *Streptomyces* • *Corynebacterium*	• *Dehalococcoides* • *Methanogens* • *Desulfovibrio* • *Clostridium* • *Geobacter* • *Clavibacter*
Enzyme(s) produced	• Methane monooxygenase • Methanol dehydrogenase • Alkene mono-oxygenase • Catechol dioxygenase	• Ammonia monooxygenase	• Toluene monooxygenase • Toluene dioxygenase	• Alcohol dehydrogenases	• Dehalogenase • AtzA • Dichloromethane Dehalogenase

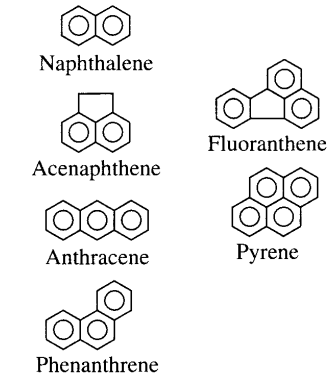
Modified from Hazen (2010)

Petroleum hydrocarbons

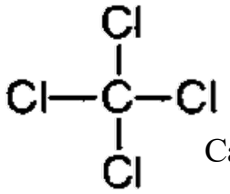
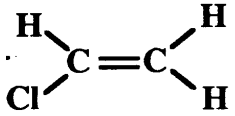
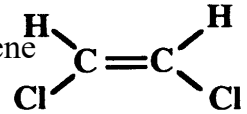
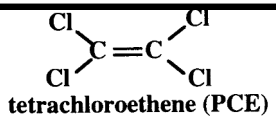
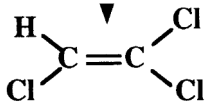
Benzene- Toluene- Ethylbenzene- Xylene (BTEX)

compound	aerobic		anaerobic		
	e- donor	process	e^- donor	e^- acceptor	process
			BTEX	nitrate	Anaerobic addition of O (from fumarate)
	Methane, toluene, NH_4^+	Cometabolism (oxygenase)			
	BTEX	Respiration (O_2 e^- acceptor)			

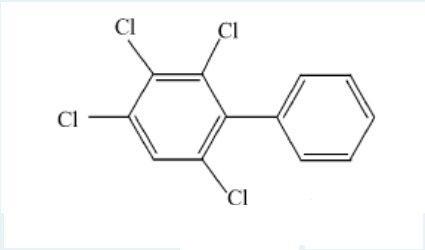
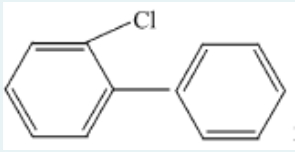
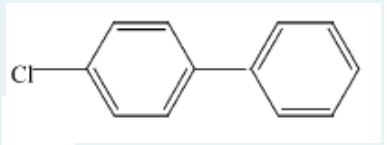
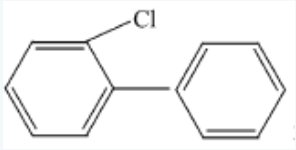
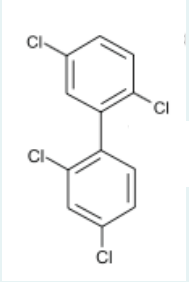
Polycyclic aromatic hydrocarbon (PAH)

compound	aerobic		anaerobic		
	e- donor	process	e^- donor	e^- acceptor	process
			PAH	nitrate	Denitrification
	Biphenyl or m-xylene	Cometabolism (dioxygenase)			
	Naphthalene	Respiration (O_2 e^- acceptor)			

Halogenated hydrocarbons

compound	aerobic		anaerobic		
	e- donor	process	e$^-$ donor	e$^-$ acceptor	process
 Carbon tetrachloride			H_2	CO_2 to CH_4	cometabolism
			H_2	CO_2 to acet.	cometabolism
			Acetate	SO_4^{2-}	cometabolism
			Lactate	NO_3^-	PDTC secretion
 Vinyl chloride	VC or DCE	respiration			
dichloroethylene 	Methane, toluene, NH_4^+	cometabolism			
			H_2	VC, DCE	halorespiration
			H_2	CO_2	cometabolism
 tetrachloroethene (PCE)			Methanol	Methanol	cometabolism
			H_2	PCE, TCE	halorespiration
 trichloroethene (TCE)	Methane, toluene, NH_4^+	Cometabolism of TCE, PCE			

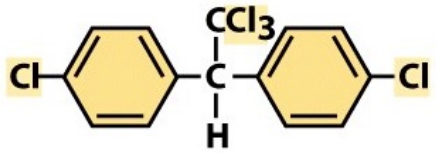
Polychlorinated biphenyls

Compound	aerobic	anaerobic
 2,3,4,6-tetrachlorobiphenyl		Dechlorination to: 4-chlorobiphenyl  2-chlorobiphenyl 
	Carbon source	
 2,2',4,5'-tetrachlorobiphenyl	2,3-dioxygenase attack *Pseudomonas* sp. 2	

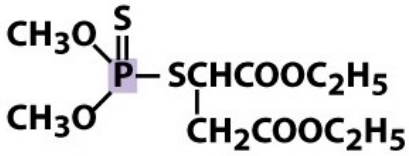
Pesticides

Persistence of herbicides and insecticides in soil

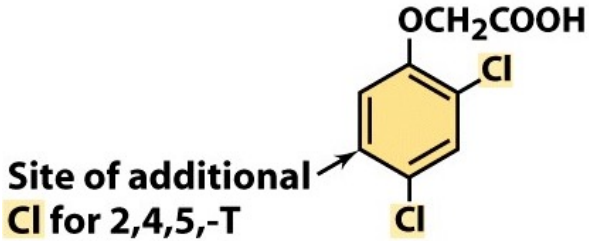
Substance	Time for 75–100% disappearance
Chlorinated insecticides	
DDT [1,1,1-trichloro-2,2-bis-(ρ-chlorophenyl)ethane][a]	4 years
Aldrin	3 years
Chlordane	5 years
Heptachlor	2 years
Lindane (hexachlorocyclohexane)	3 years
Organophosphate insecticides	
Diazinon	12 weeks
Malathion[a]	1 week
Parathion	1 week
Herbicides	
2,4-D (2,4-dichlorophenoxyacetic acid)	4 weeks
2,4,5-T (2,4,5-trichlorophenoxyacetic acid)	20 weeks
Dalapon	8 weeks
Atrazine[a]	40 weeks
Simazine	48 weeks
Propazine	1.5 years



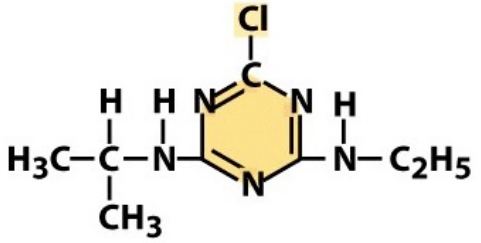
DDT; dichlorodiphenyltrichloro-ethane (an organochlorine)



Malathion; mercaptosuccinic acid diethyl ester (an organophosphate)



2,4-D; 2,4-dichlorophenoxy acetic acid (a chlorophenoxy acetic acid derivative)



Atrazine, 2-chloro-4-ethylamino -6-isopropylaminotriazine (a triazine derivative)

Biological monitoring

Characterization of microbial community and activity

- Culture-dependent approaches
- DNA-based approaches
- RNA-based approaches

Culture-dependent characterization

- All culture-dependent methods have a major limitation: < 0.5% of soil microorganisms are cultivable.
- **Enrichment culture**: a medium and set of incubation conditions are established that are selective for the desired organism
- Limitations:
  - Negative result does not guarantee absence of organism/ Can prove a positive but not a negative
  - No information on ecological importance or abundance of organism
  - Bias: liquid enrichment results different from plating results
  - Bias: rapidly growing ('weed') species appear quantitatively important (combat with dilution of inoculum)

Dark-chemolithotrophic bacteria: main C source, CO_2 (medium must lack organic C)			
Incubation in air: aerobic respiration			
Electron donor	***Electron acceptor***	***Organisms enriched***	***Inoculum***
NH_4^+	O_2	Ammonia-oxidizing bacteria (*Nitrosomonas*)	Soil, mud; sewage effluent
NO_2^-	O_2	Nitrite-oxidizing bacteria (*Nitrobacter, Nitrospira*)	
H_2	O_2	Hydrogen bacteria (various genera)	
H_2S, S^0, $S_2O_3^{2-}$	O_2	*Thiobacillus* spp.	
Fe^{2+}, low pH	O_2	*Acidithiobacillus ferrooxidans*	
Anoxic incubation			**Inoculum**
S^0, $S_2O_3^{2-}$	NO_3^-	*Thiobacillus denitrificans*	Mud, lake sediments, soil
H_2	NO_3^- + yeast extract	*Paracoccus denitrificans*	

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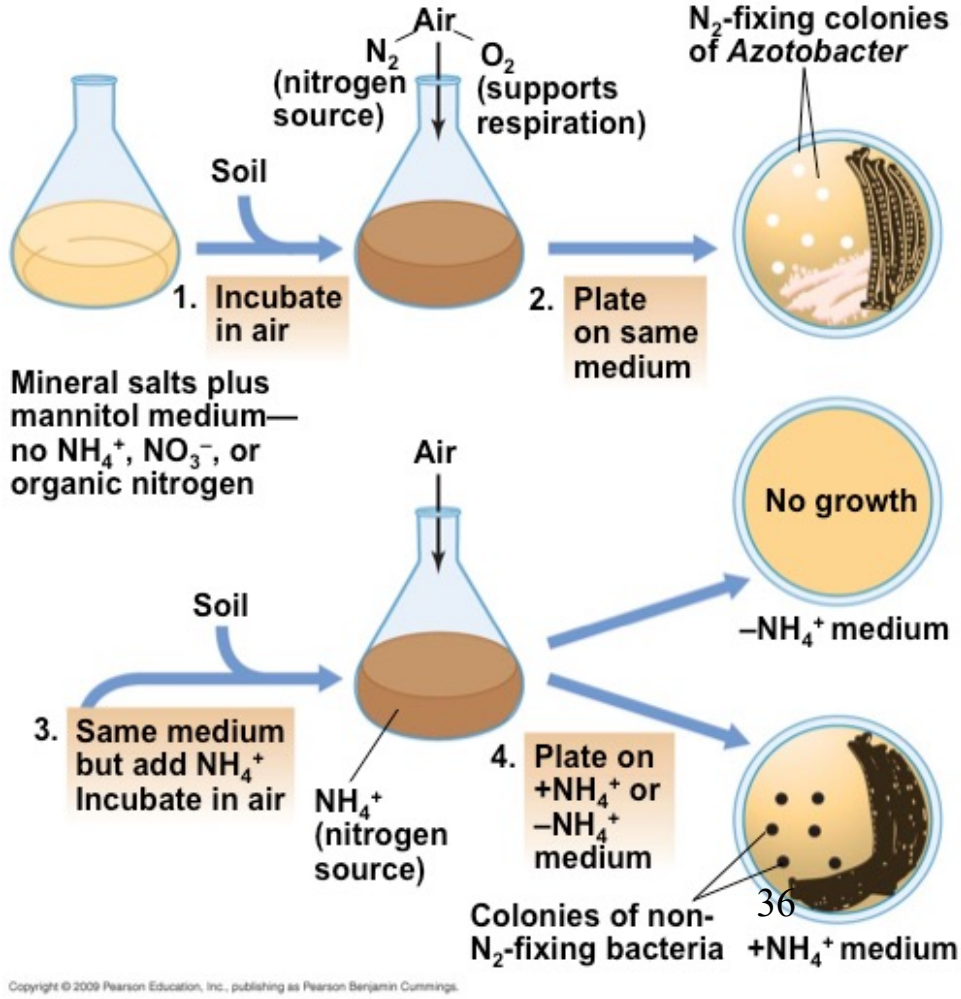
Culture-dependent characterization

- **Isolation**: obtaining a pure culture (a single kind of microorganism)
  - Streak plate: for organisms that form colonies on agar
  - Agar shake: mixed culture diluted in tubes of molten agar (colonies embedded in agar). Useful to isolate anaerobic microbes. Successive dilutions into molten agar medium.
  - Liquid dilution: serially dilute an inoculum into a liquid medium. Most probable number (MPN) method.



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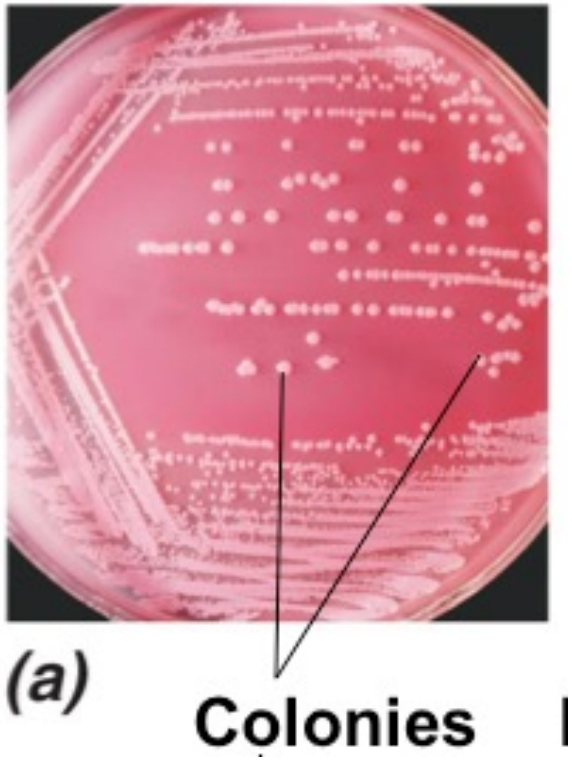
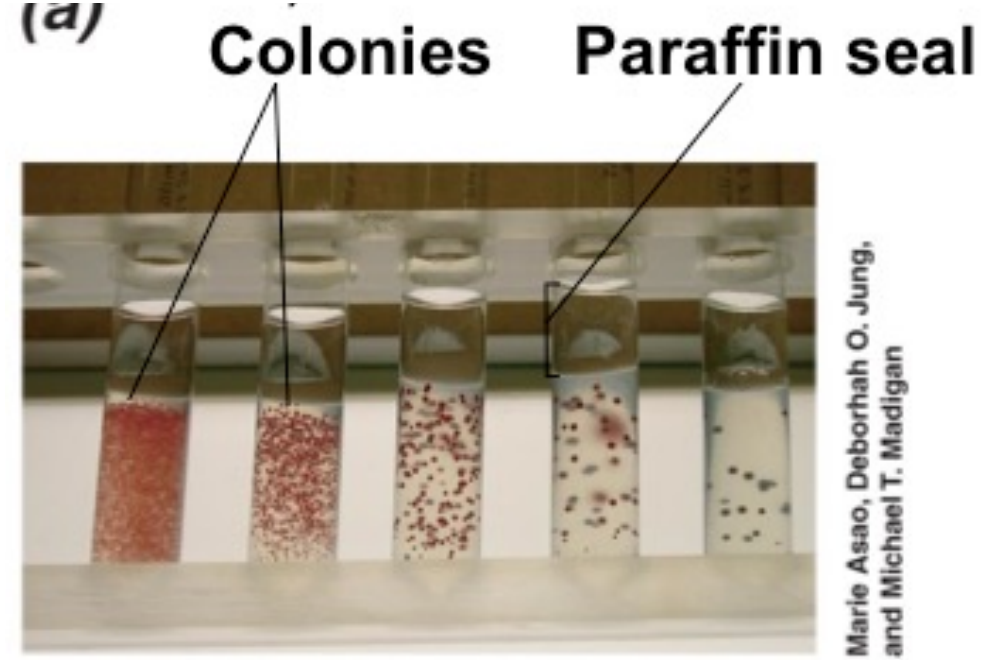
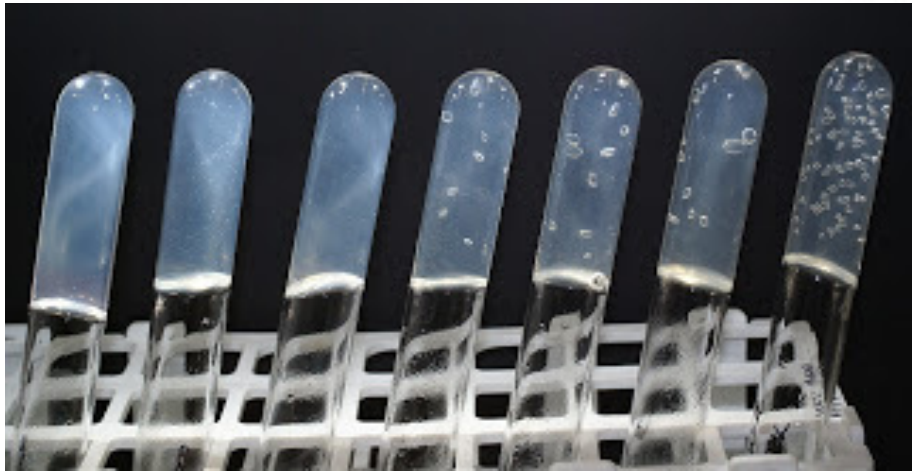
Culture-dependent characterization



Streak plate

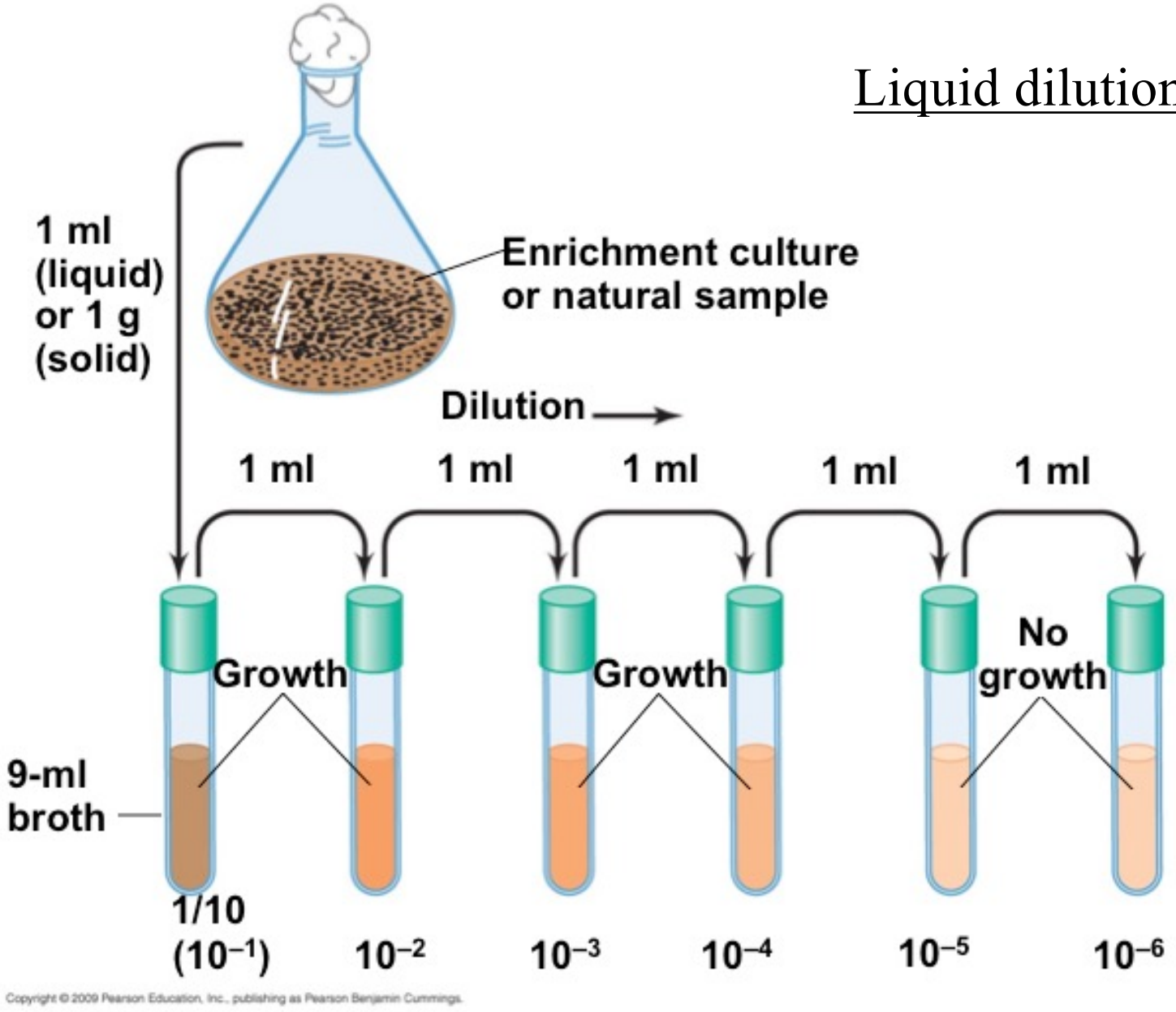




Agar shakes

Culture-dependent characterization

Liquid dilutions



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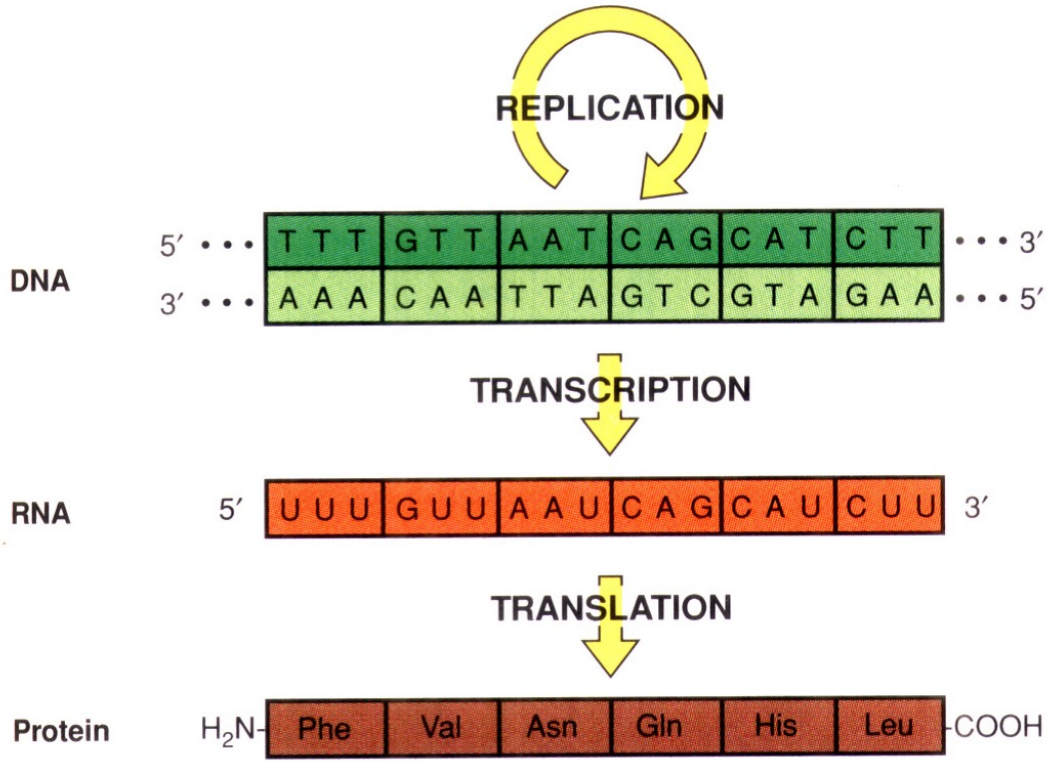
Culture-independent approaches

Culture-independent genetic analysis of microbiomes

- 16S rRNA amplicon sequencing
- Metagenomics

Molecular biology primer

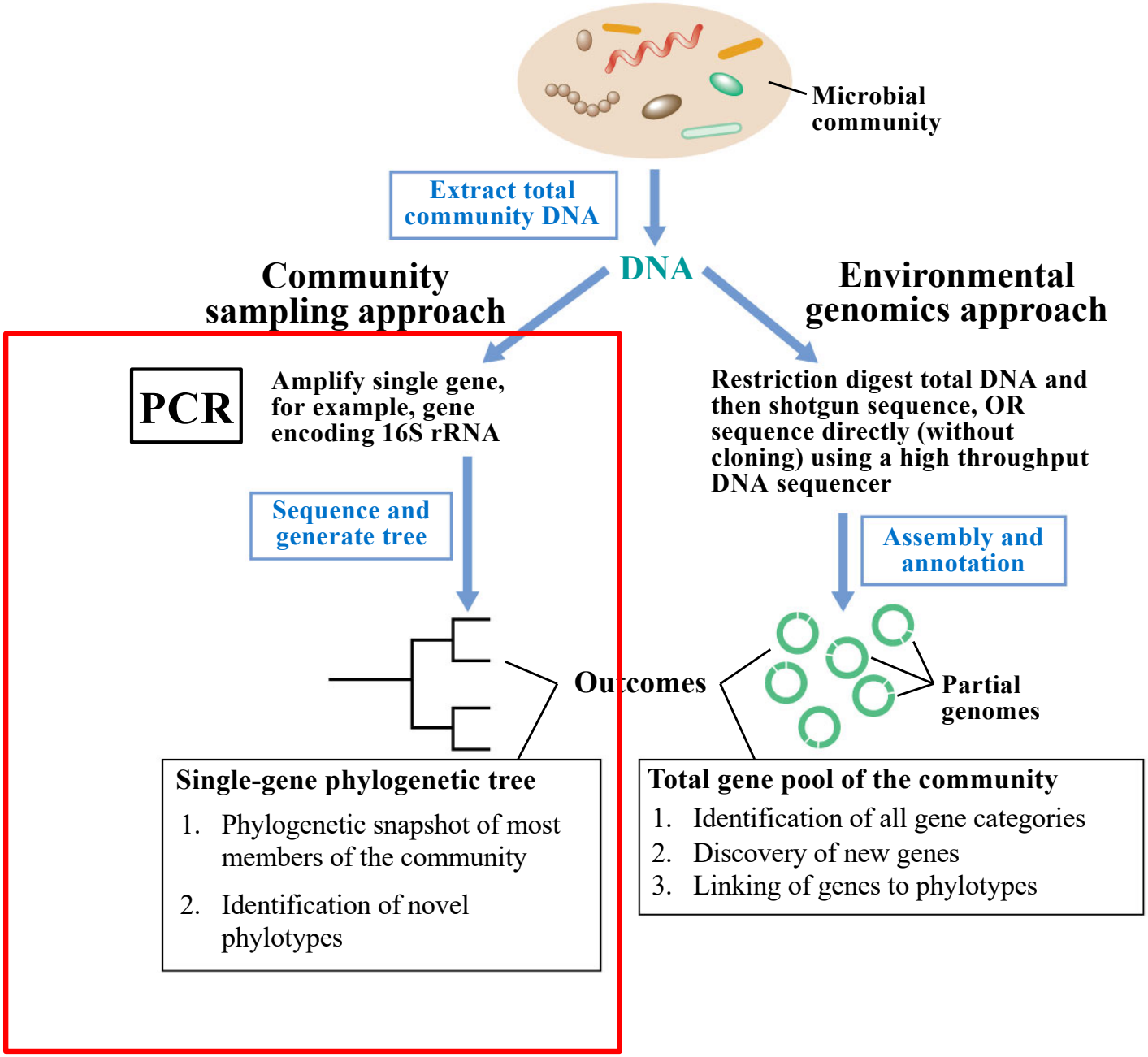


- DNA is somewhat stable even if microbe is dead
- DNA-based methods identify the presence of a microorganism but not necessarily activity

- RNA only transcribed when protein is needed
- messenger RNA (mRNA) is degraded quickly
- mRNA-based methods probe active population

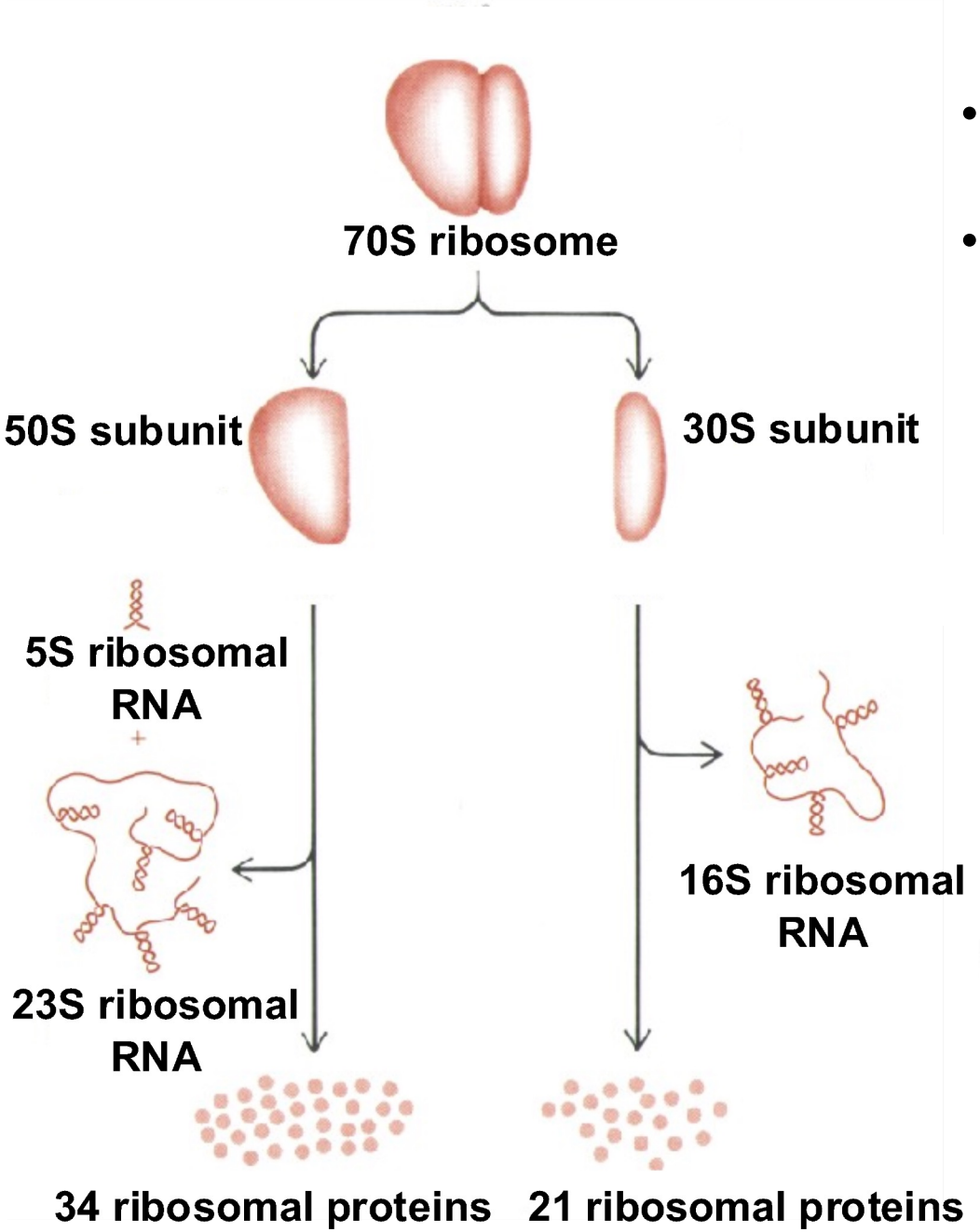
- proteins only present when need for specific activity
- proteins fairly stable
- few protein-based techniques because difficult to obtain protein from soil /sediment

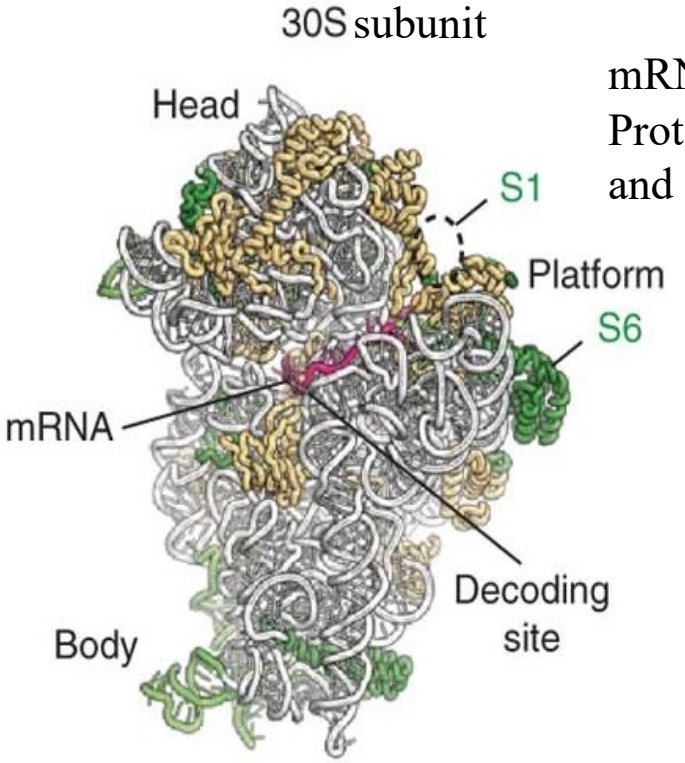
Single-gene *vs*. genomic approaches



Ribosomes and ribosomal RNA

- Ribosomes are responsible for the translation of mRNA to protein
- They themselves consist of ribosomal RNA and protein
- Bacteria/Archaea
  5S rRNA: 120 bp
  16S rRNA: 1500 bp
  23S rRNA: 3000 bp
  + 55 Proteins

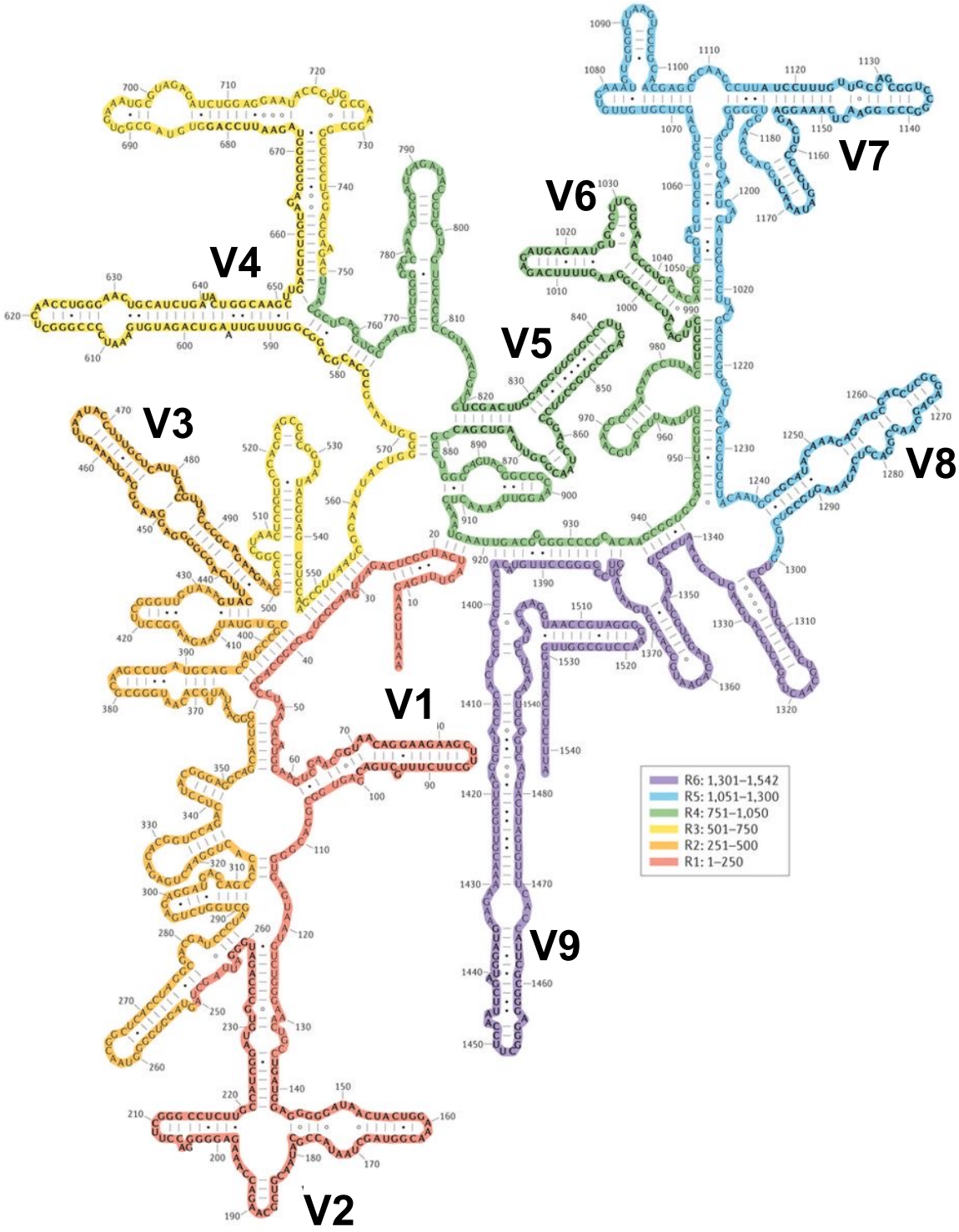




mRNA in white
Protein in orange and green

EPFL

16S rRNA – variable regions



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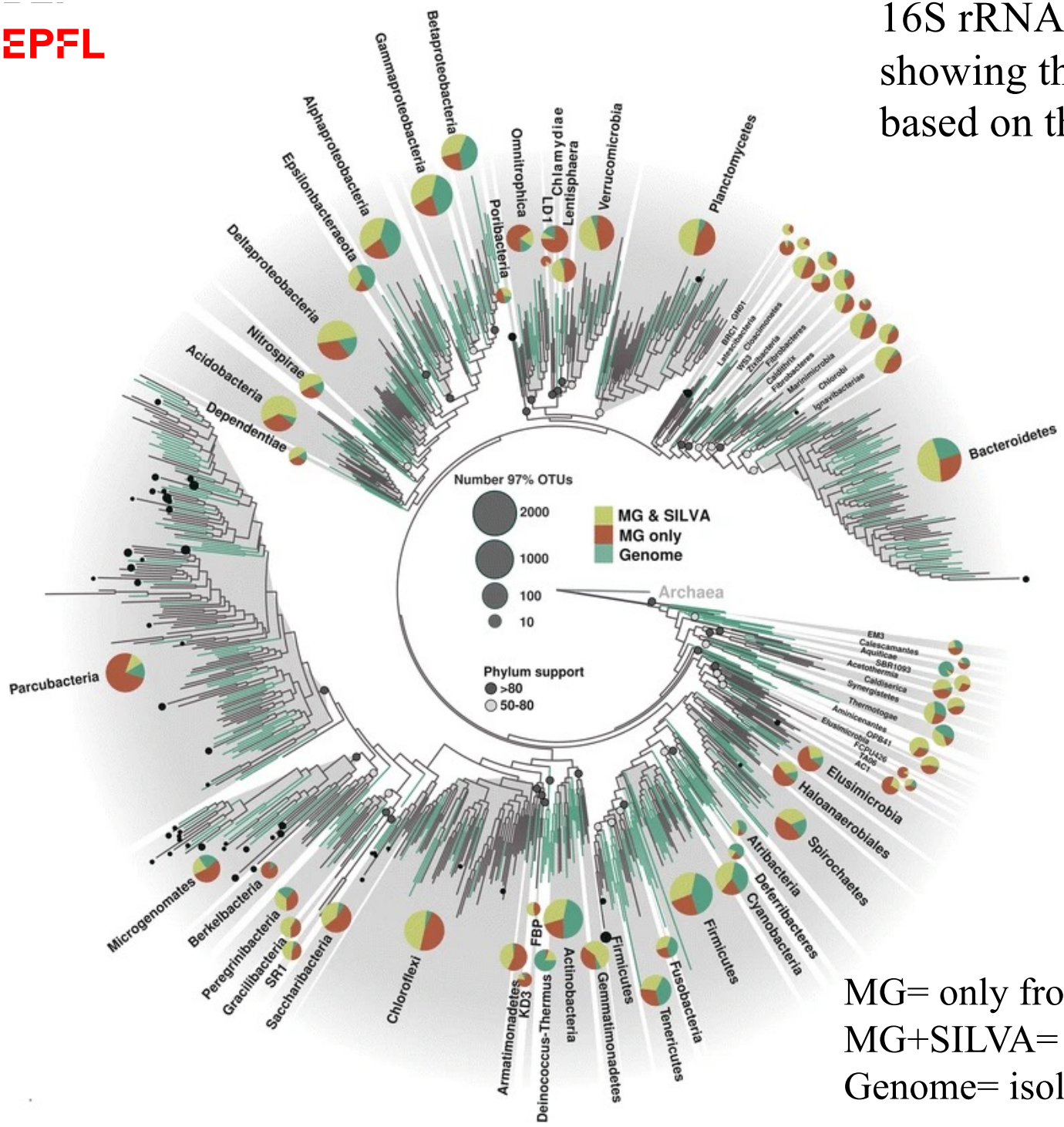
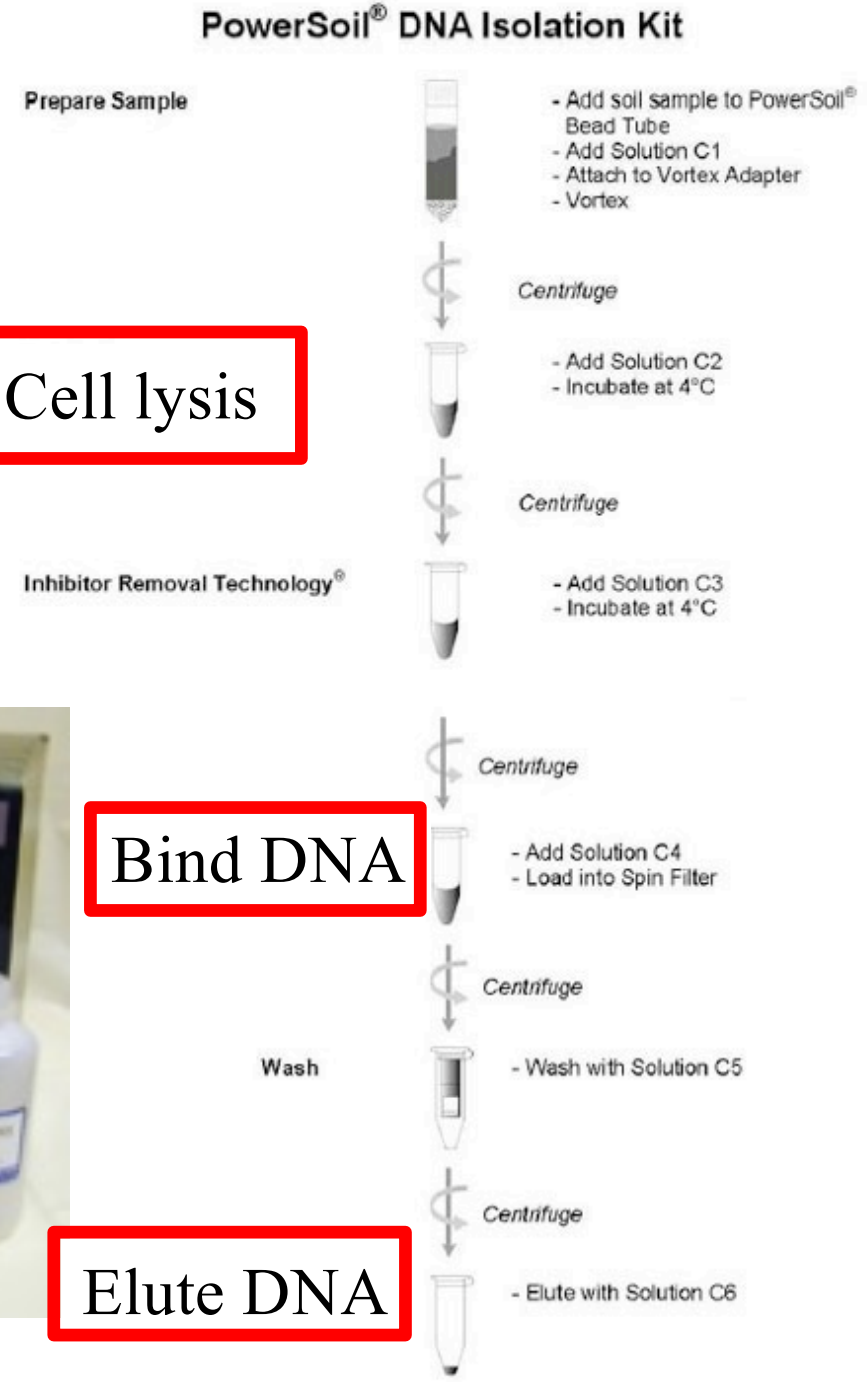
16S rRNA-based phylogenetic tree showing the distance between taxa based on this gene.



MG= only from amplicons, no isolates
MG+SILVA= at least one isolate
Genome= isolated and sequenced genome

DNA isolation from environmental sample

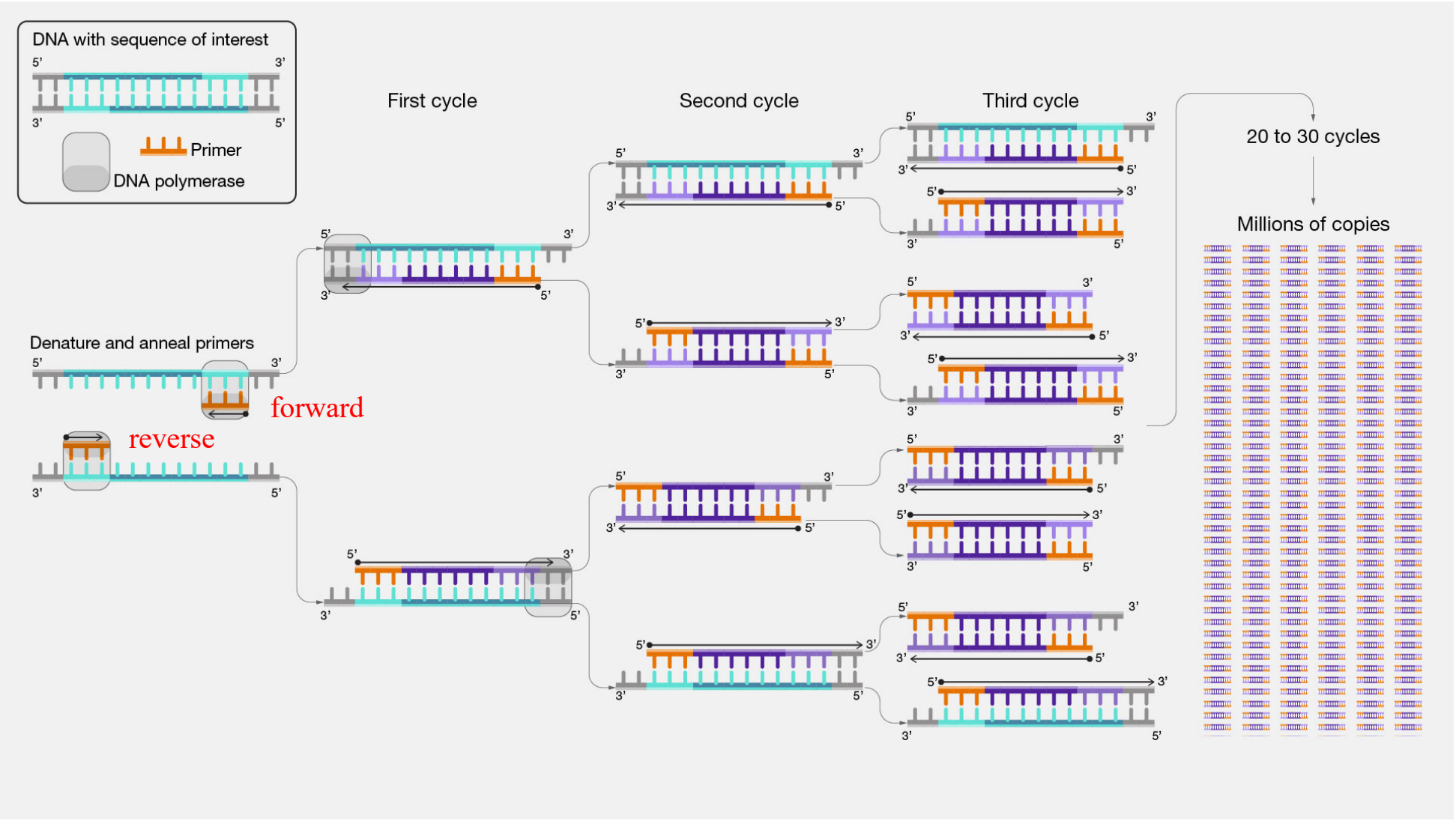


Cell lysis

Bind DNA

Elute DNA

Polymerase-chain reaction (**PCR**)

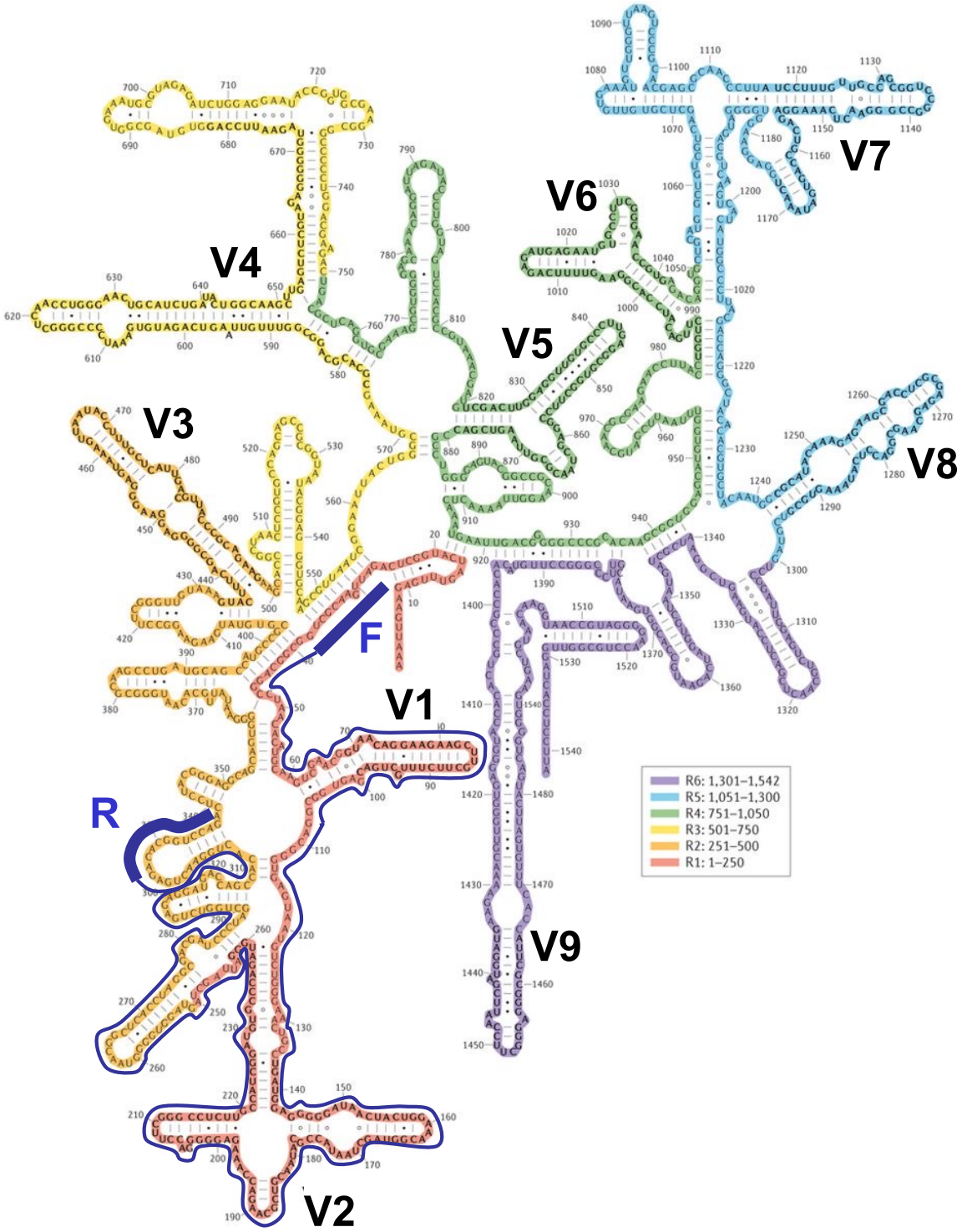


Repetitive amplification of target genes from DNA using specific primers

→ 2^n copies after n cycles

EPFL

16S rRNA – variable regions



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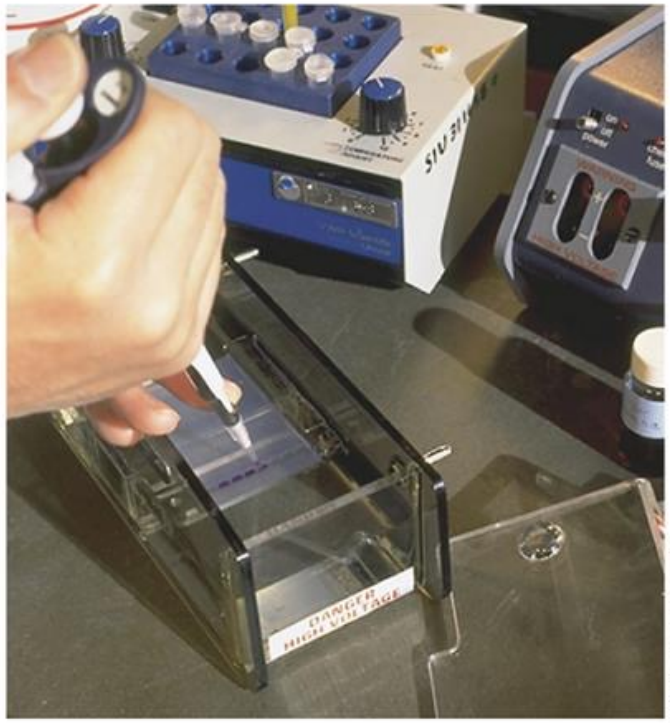
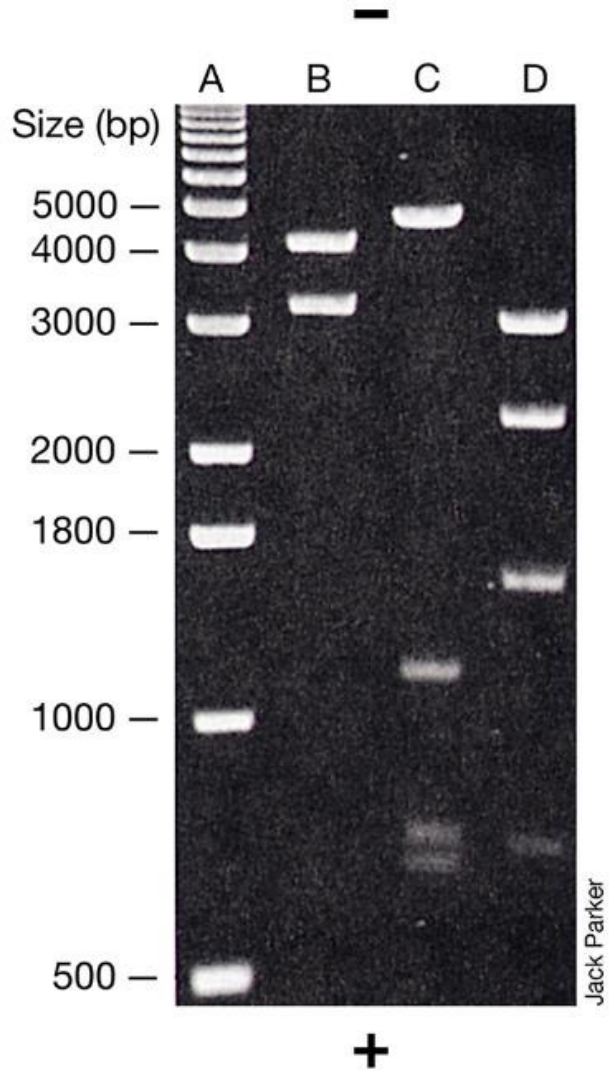
Agarose gel electrophoresis



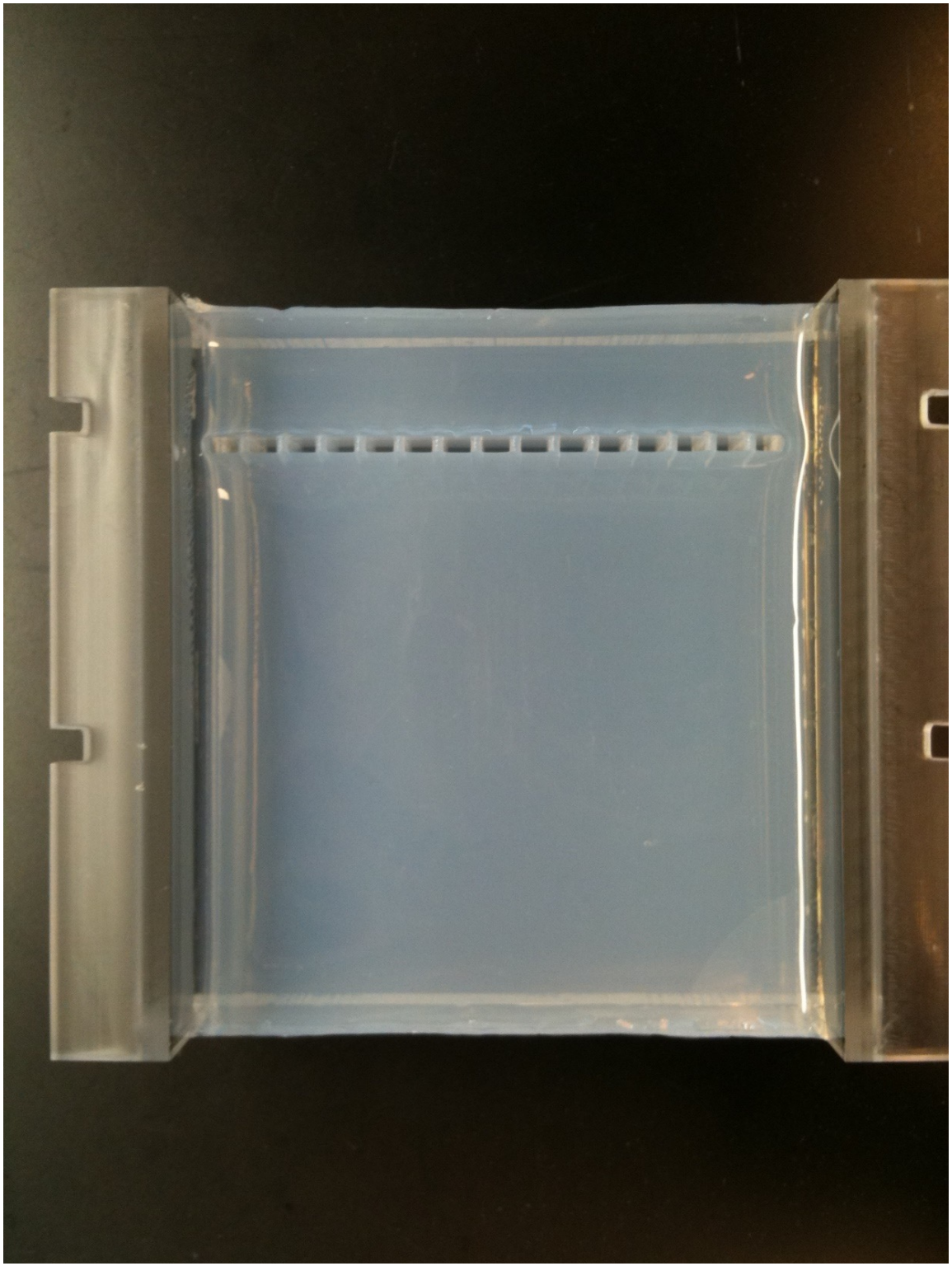
Elizabeth Parker

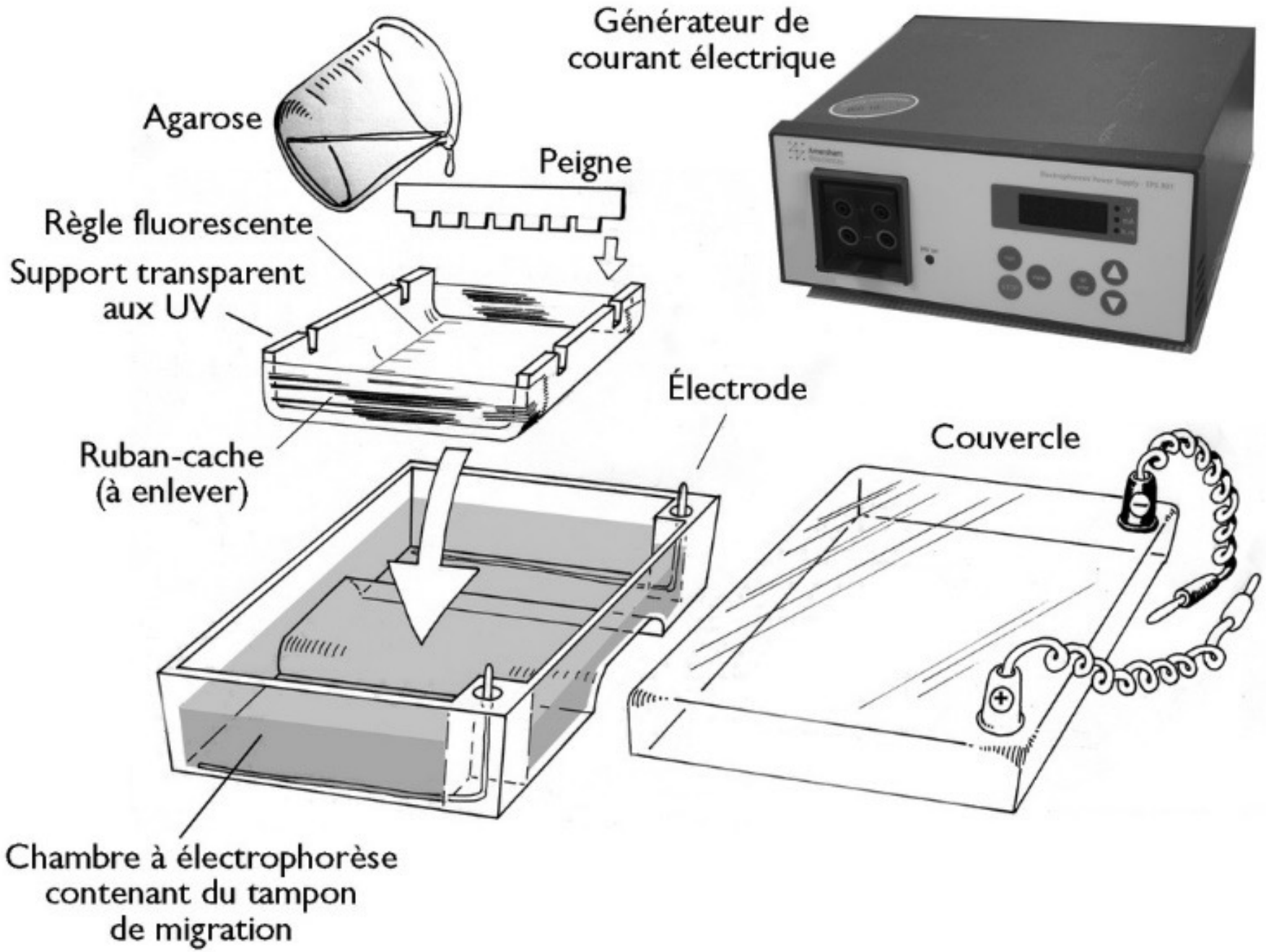
(a)

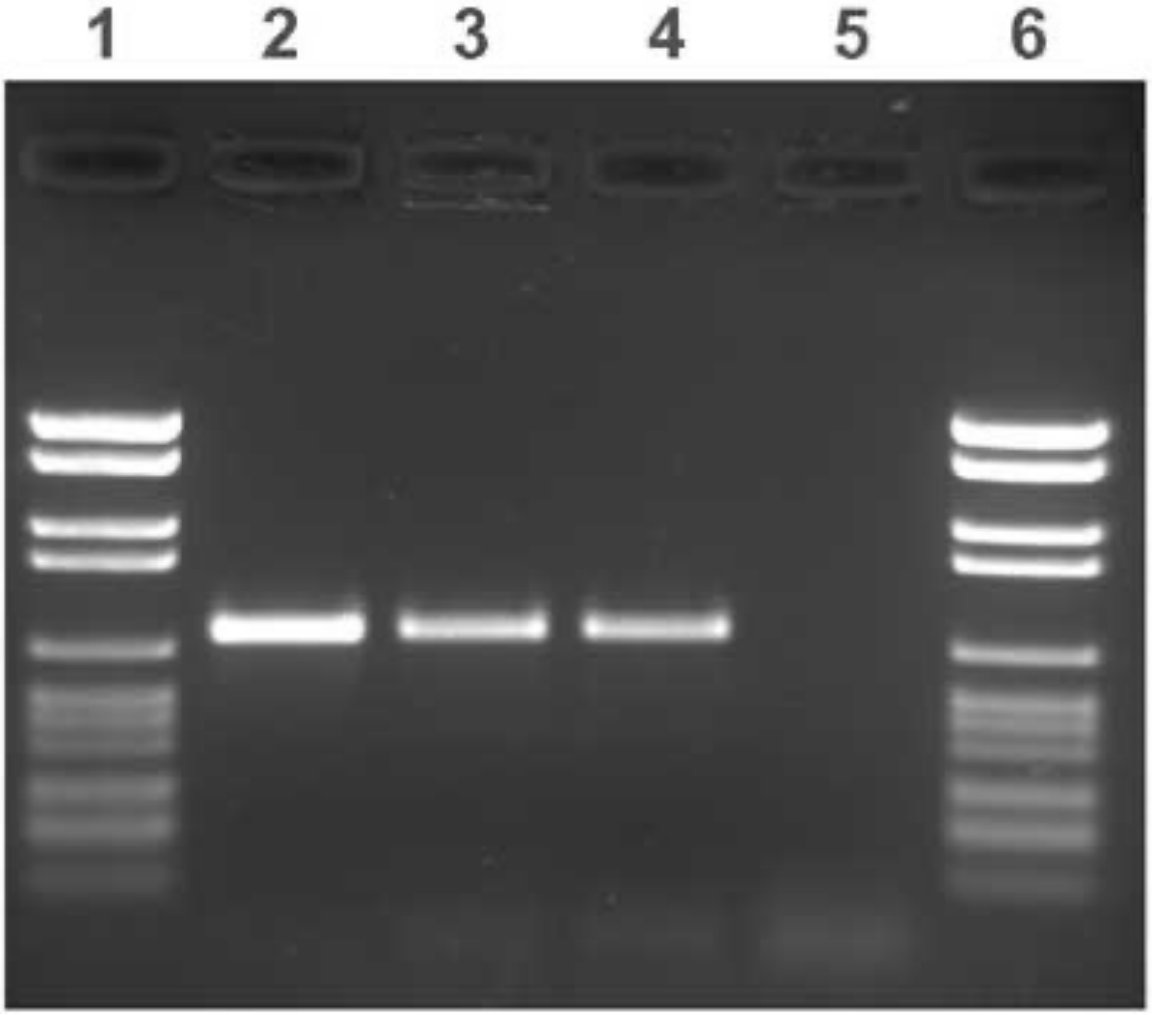


Jack Parker

(b)



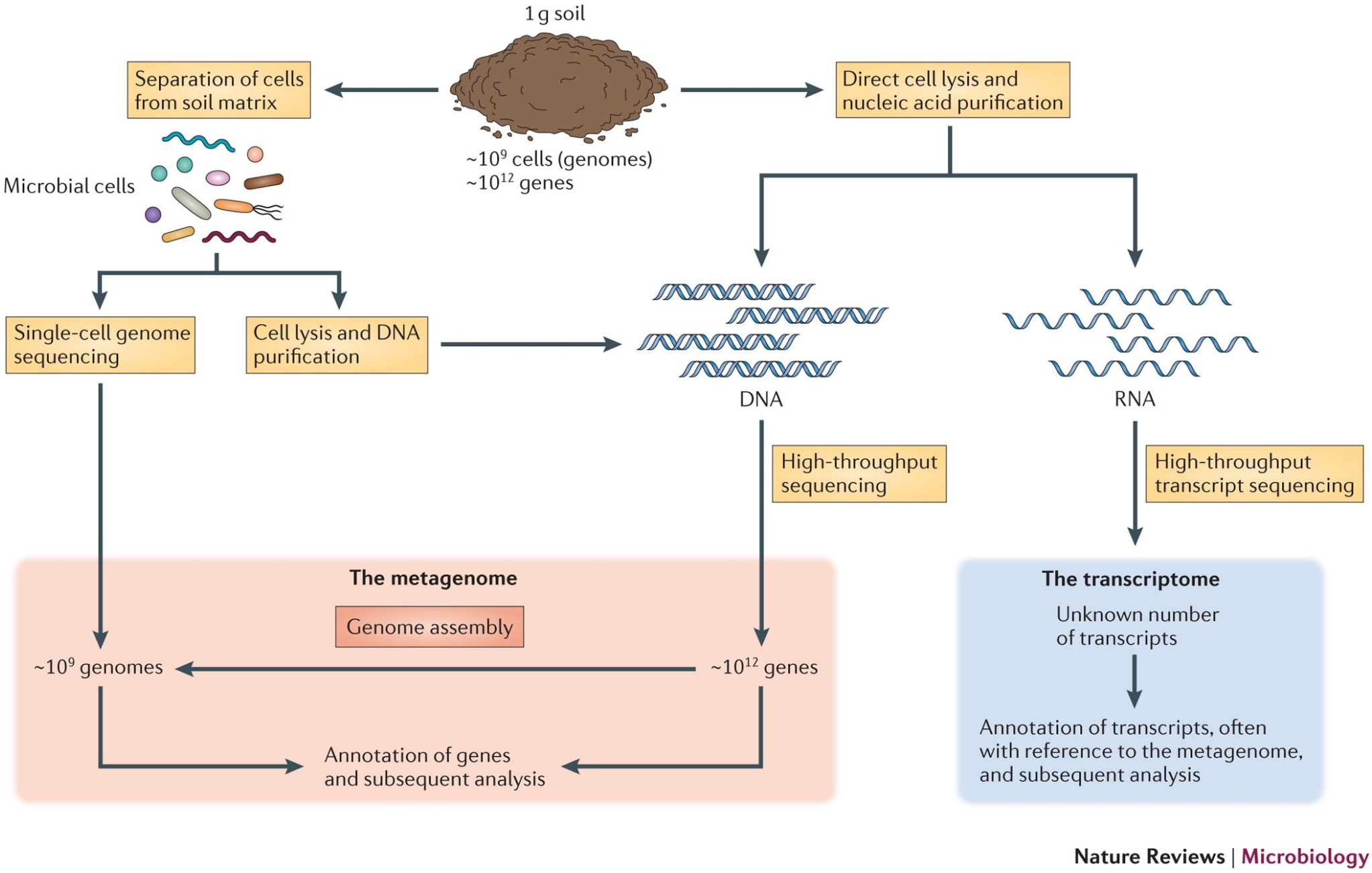

Générateur de
courant électrique
Agarose
Peigne
Règle fluorescente
Support transparent
aux UV
Électrode
Couvercle
Ruban-cache
(à enlever)
Chambre à électrophorèse
contenant du tampon
de migration


1
2
3
4
5
6

Ribosomal RNA as Evolutionary marker

E. coli	**GCGGTAAT** ACGGAGGGTG**CAAGCGTT**AATCGGAATTACTGGGCG **TAAAGCG**
A. nidulans	**GCGGTAAT** ACGGGAGAGG**CAAGCGTT**ATCCGGAATTATTGGGCG **TAAAGCG**
T. maratima	**GCGGTAAT** ACGTAGGGGG**CAAGCGTT**ACCCGGATTTACTGGGCG **TAAAGGG**
M. vannielii	**GCGGTAAT** ACCGACGGCC**CGAGTGGT**AGCCACTCTTATTGGGCC **TAAAGCG**
T. celer	**GCGGTAAT** ACCGGCGGCC**CGAGTGGT**GGCCGCTATTATTGGGCC **TAAAGCG**
S. sulfotaricus	**GCGGTAAT** ACCAGCTCCG**CGAGTGGT**CGGGGTGATTACTGGGCC **TAAGCGG**

Microbial characterization



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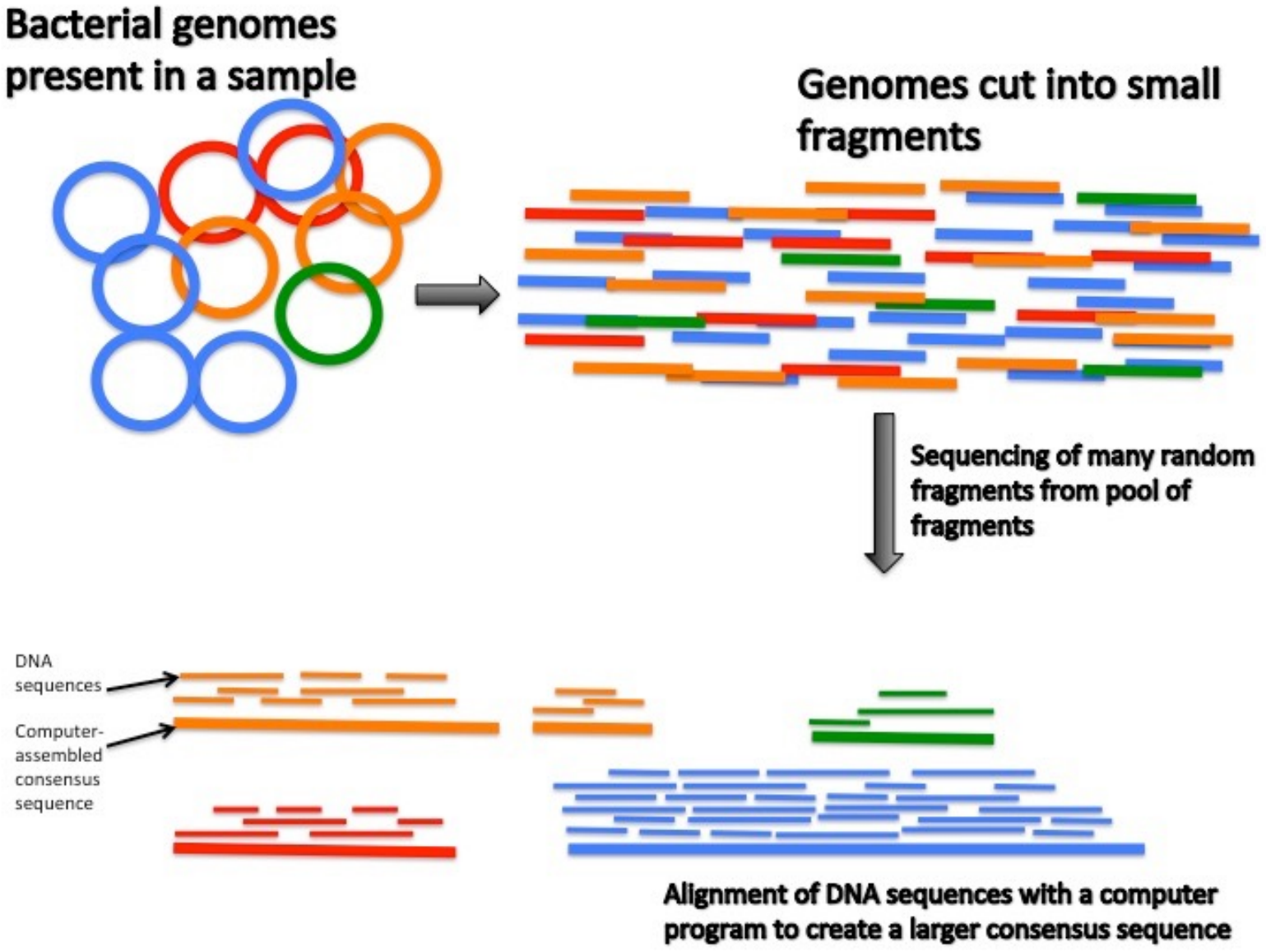
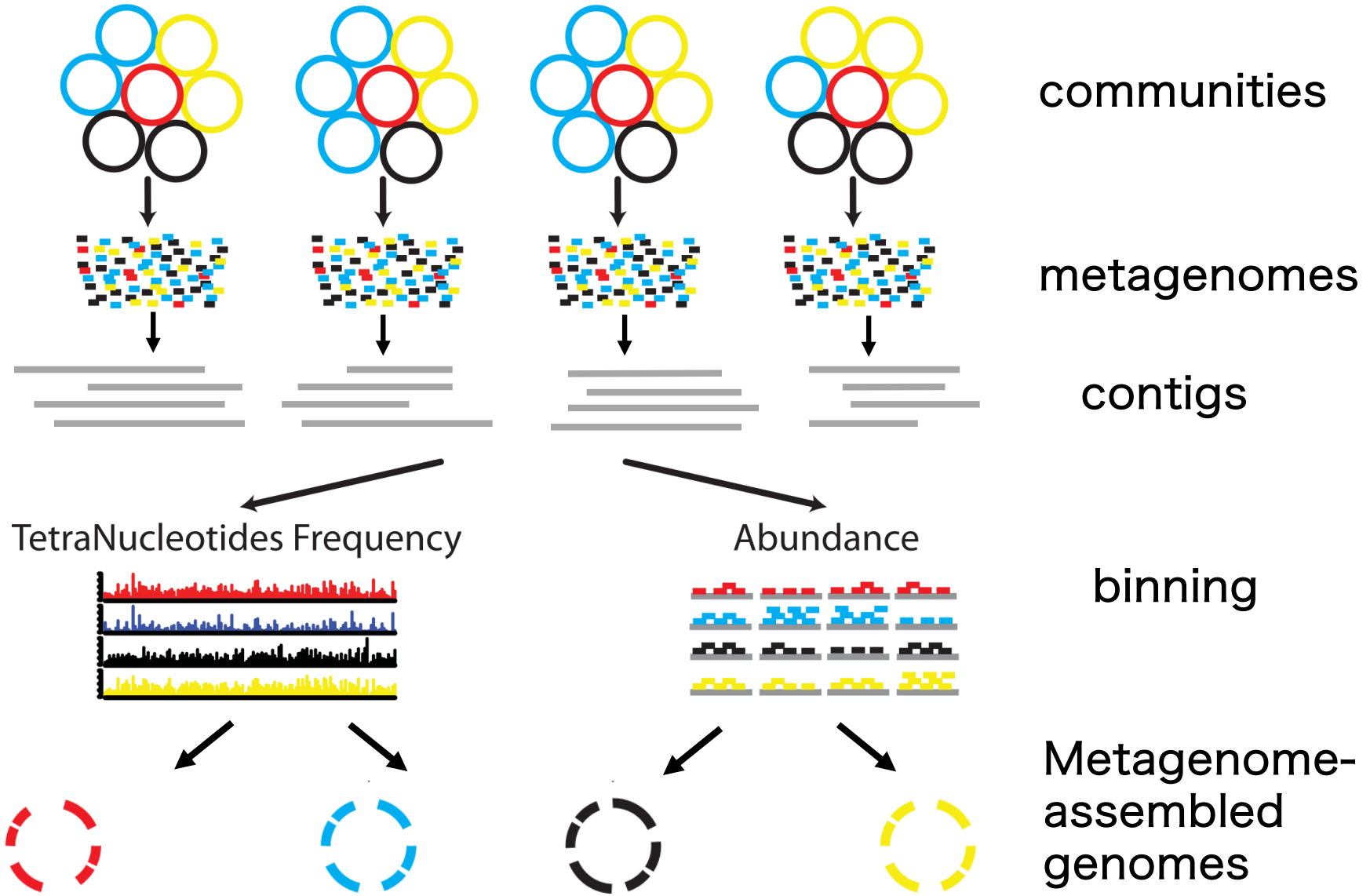
Illumina sequencing



Metagenome-assembled genomes



Modified from Kang et al., 2015