LGN0232 - Genética Molecular

Estudos das "Ômicas": Genômica *x* Transcritômica e Metagenômica

6ª aula

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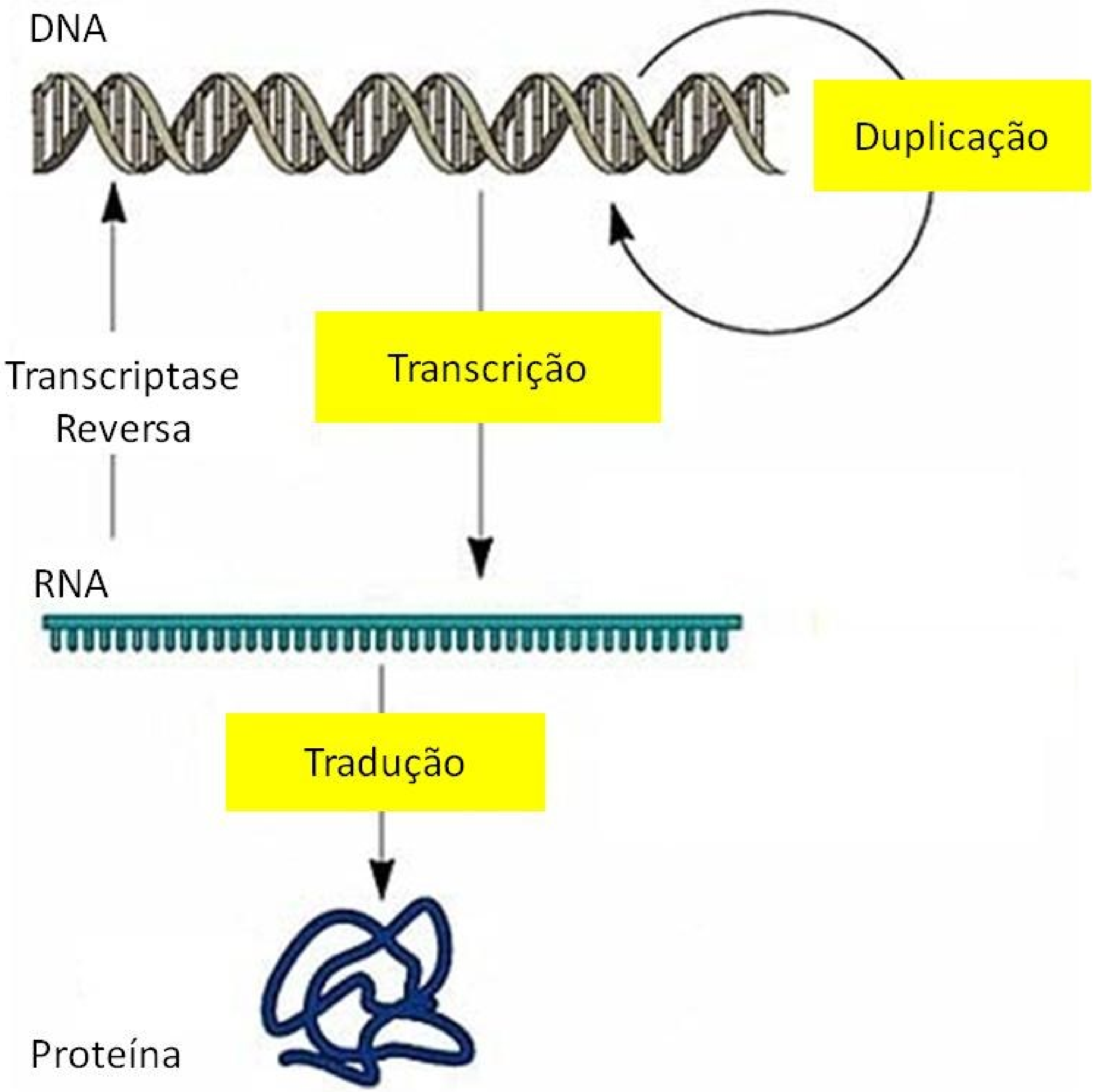
Dogma Central da Biologia Molecular

Genoma

Transcriptoma

Proteoma



O que seria Ômica?

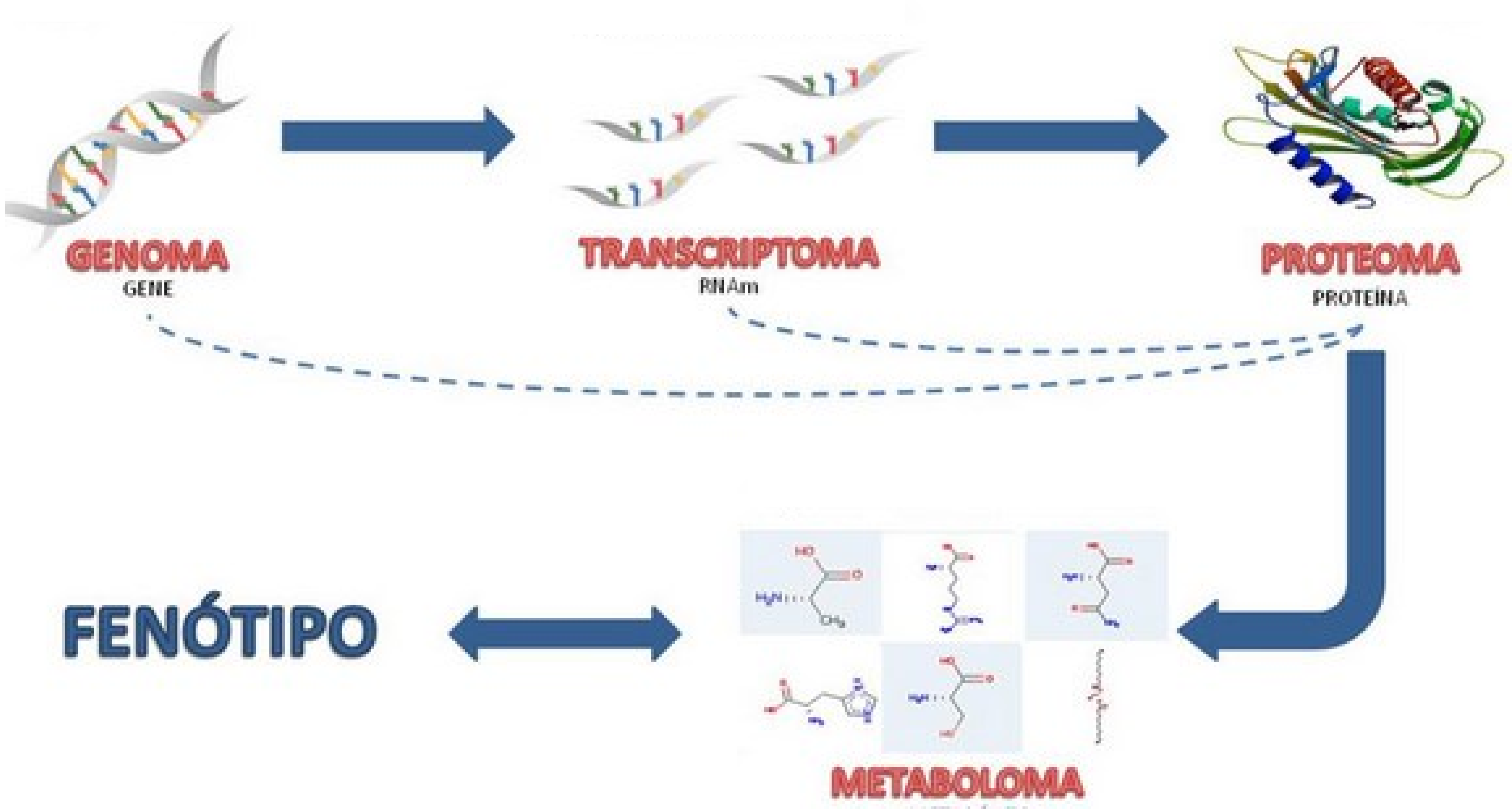
O que seria Ômica?

Todos os constituintes considerados coletivamente

Gene – Genoma (Gen<u>omic</u>)
Transcrito – Transcritoma (Transcript<u>omic</u>)
Proteína – Proteoma (Prote<u>omic</u>)
...

Estonian Biocentre

O que seriam Ômicas?



E há mais: epigenoma, lipidoma, interatoma, etc…

	Name	Target
Genomics		
	Genomics	Genes (DNA sequence)
	Epigenomics	Modification of DNA and DNA-binding proteins
Transcriptomics[1]		
	Transcriptomics	mRNA
	ncRNAomics	non-coding RNA (including microRNA)
Proteomics		
	Proteomics	Proteins
	Phosphoproteomics	Protein phosphorylation
	Localizomics[2]	Protein localization
	Fluxomics[2]	Protein flux
	Interactomics[2]	Protein-protein interaction
	Structural Proteomics	Protein structure
Metabolomics		
	Metabolomics	Metabolites
	Lipidomics	Lipids
	Aminomics	Amino acids
Others		
	Glycomics	Sugar chains
	Cytomics	Cells
	Populomics	Human population
	Exposomics	Environmental exposure[3]

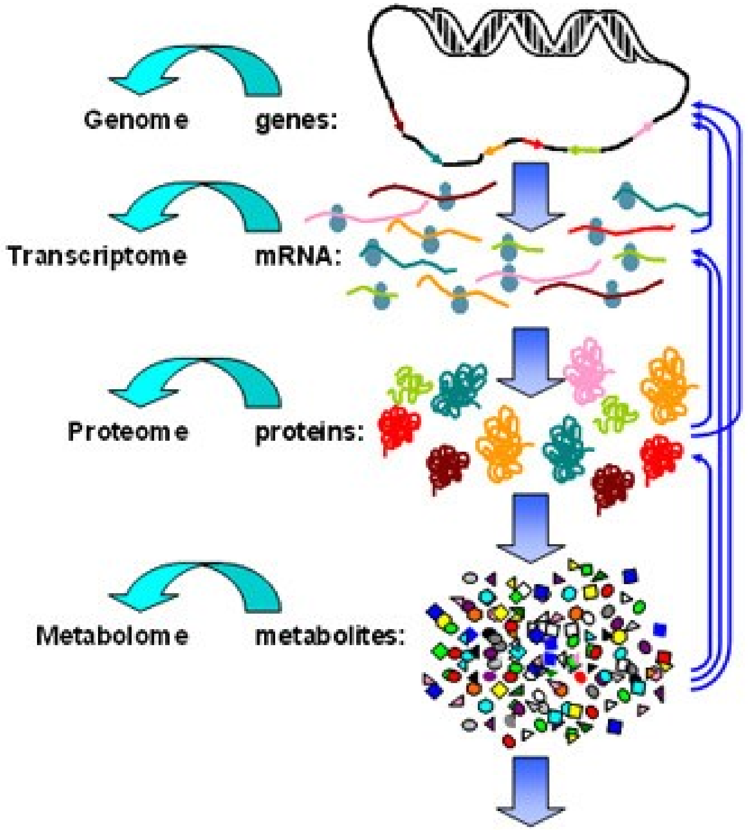
Four major categories and their subcategories are shown.

[1] Transcriptomics can be regarded as a subcategory of genomics.

[2] Corresponding omics of metabolites can also be the targets. In addition, omics analysis of protein-metabolite interaction may be possible.

[3] Borrell, 2011

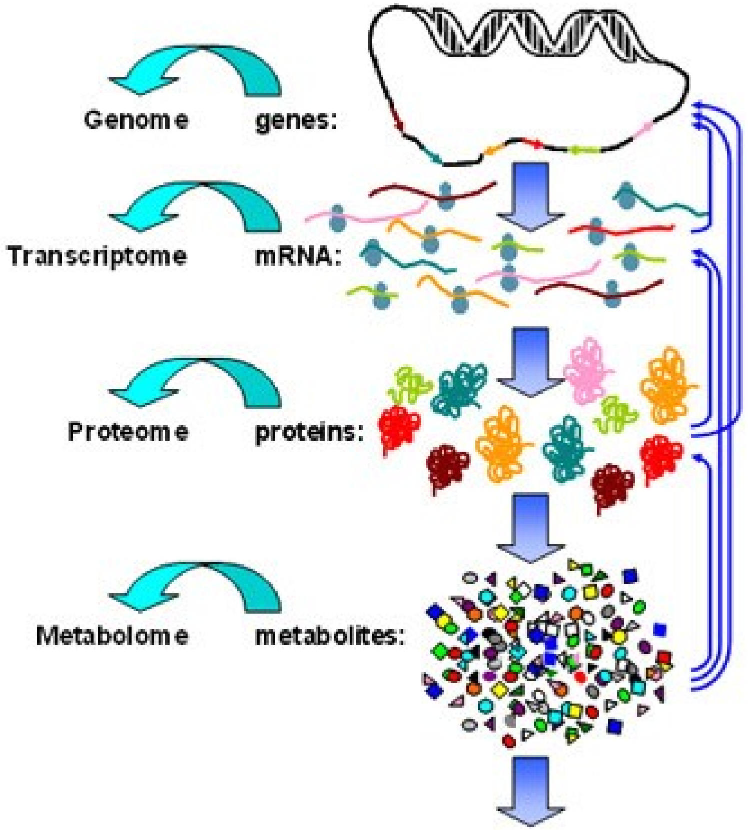
Avanços tecnológicos recentes permitiram o surgimento da Era das Ômicas



Ambiente

FENÓTIPO

Avanços tecnológicos recentes permitiram o surgimento da Era das Ômicas



Ambiente

FENÓTIPO

conjunto de todas as características observáveis – que são influenciadas tanto por seu genótipo quanto pelo ambiente

Em um organismo, somente o genoma permanece constante, independente do estágio de desenvolvimento, tecido e ou condição ambiental!





Diferentes estímulos podem afetar diretamente o transcritoma, o proteoma e o metaboloma.





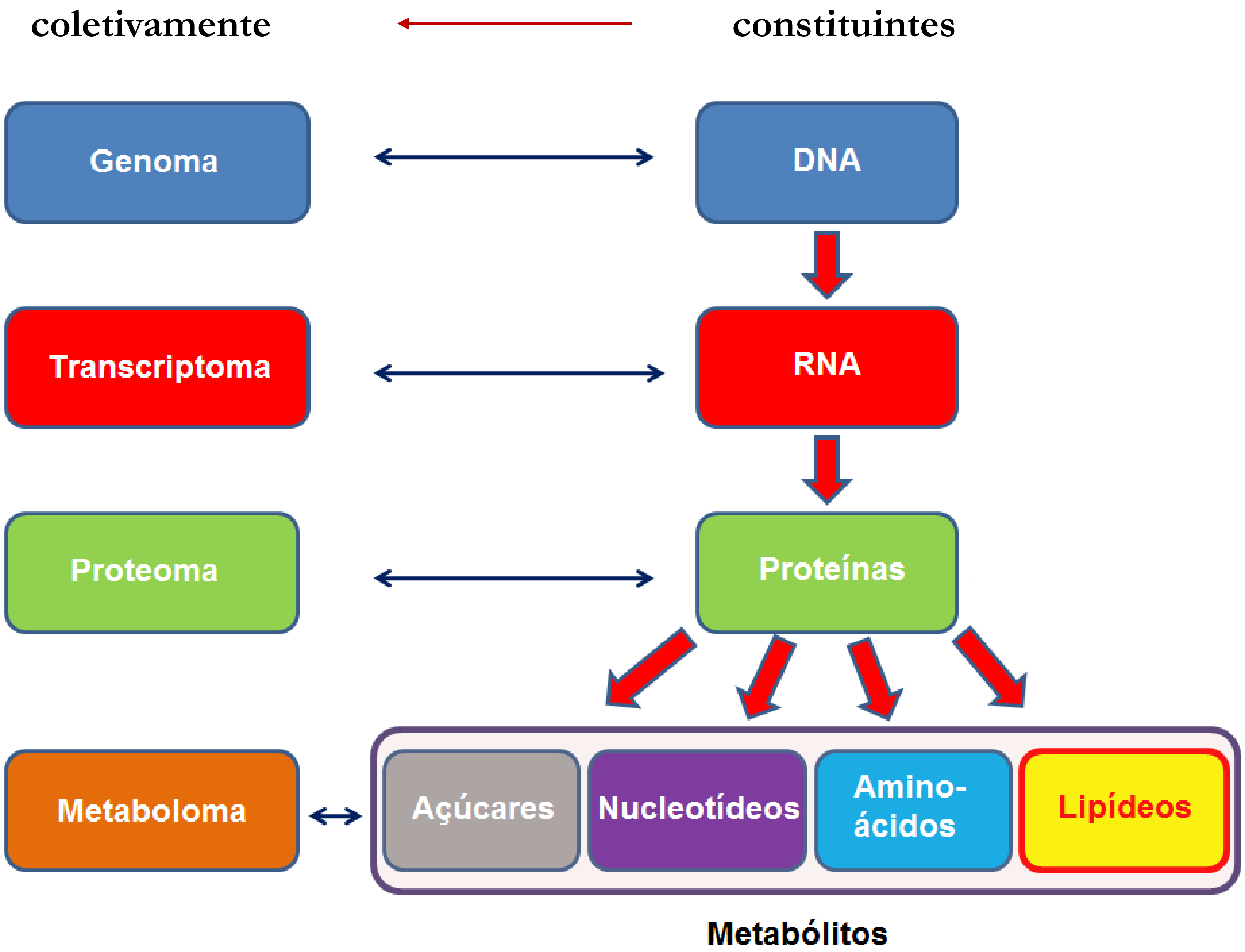
Definindo Conceitos

Genoma: toda a informação hereditária de um organismo que está codificada em seu DNA (ou, em alguns vírus no RNA). Isto inclui tanto os **genes** como as **sequências não-codificadoras e regiões regulatórias**.

Transcritoma: conjunto completo de **transcritos** (RNAs mensageiros, RNAs ribossômicos, RNAs transportadores e os microRNAs) de um dado organismo, órgão, tecido ou linhagem celular. Portanto, é o reflexo direto da expressão dos genes.

Proteoma: conjunto de todas as **proteínas** em uma célula, organela fluido biológico, tecido ou organismo em um dada condição biológica

Metaboloma: conjunto de todos os **metabólitos** em uma célula, fluido biológico, tecido ou organismo em dada condição biológica



coletivamente ← constituintes

Genoma ↔ DNA

Transcriptoma ↔ RNA

Proteoma ↔ Proteínas

Metaboloma ↔ Açúcares, Nucleotídeos, Amino-ácidos, Lipídeos

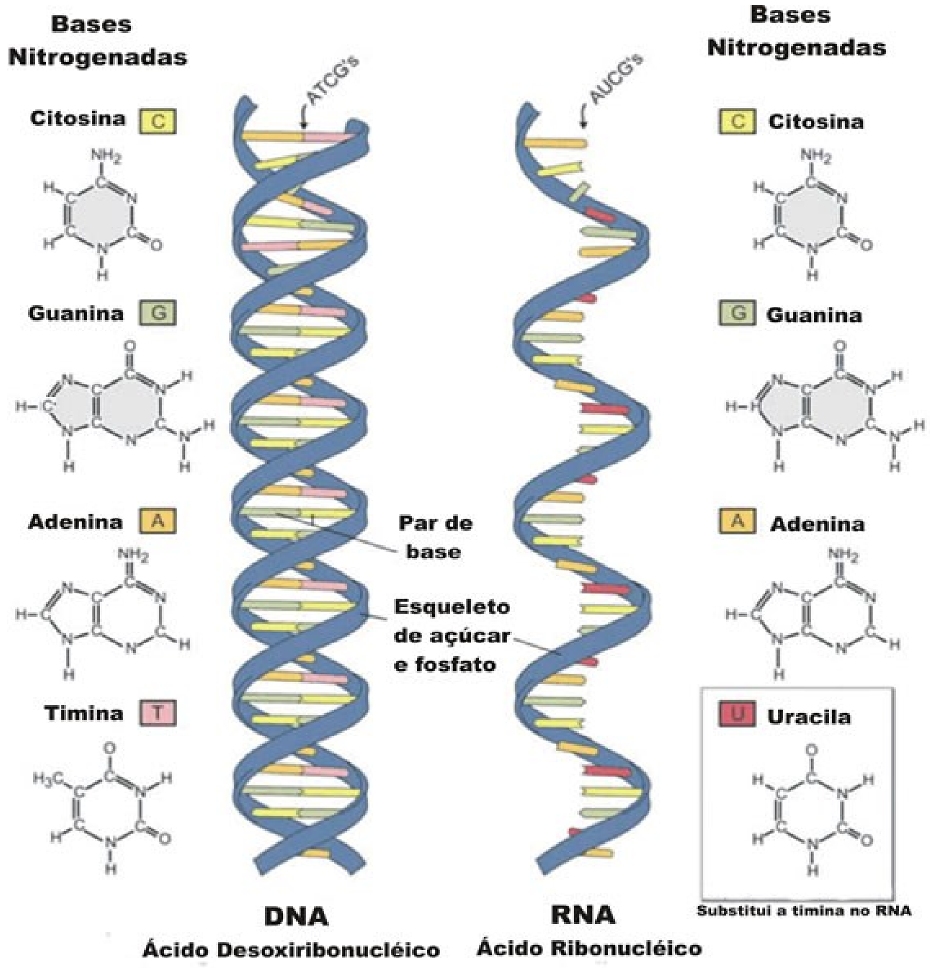
Metabólitos

DNA e RNA

1- O que são?

2- Qual a estrutura?

São **ácidos nucléicos**, encontrados em todas as células.
Estão envolvidos na transmissão de **caracteres hereditários** e na <u>produção</u> de proteínas

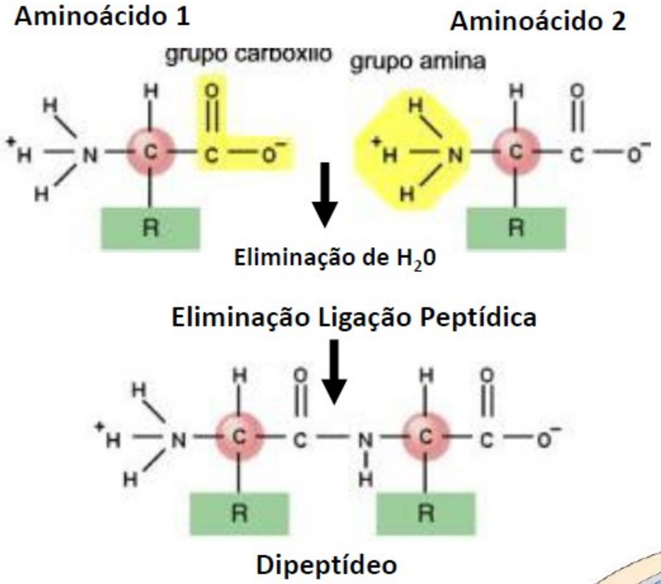


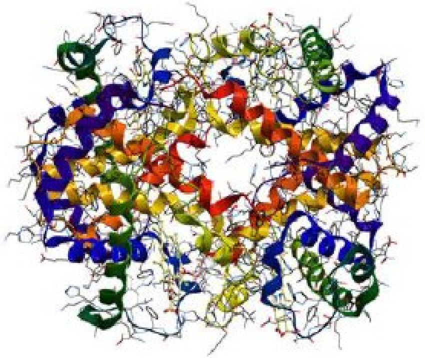
Proteínas

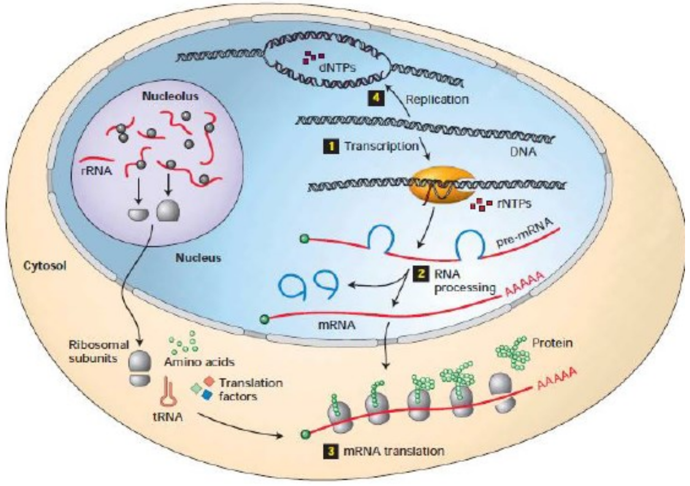
1- O que são?

2- Qual a estrutura?

São moléculas orgânicas de estrutura complexa e Massa Molecular elevada.
São sintetizads pelos organismos vivos através de ligações peptídicas covalentes entre aminoácidos.
Funções: enzimas, anticorpos, componentes estruturais







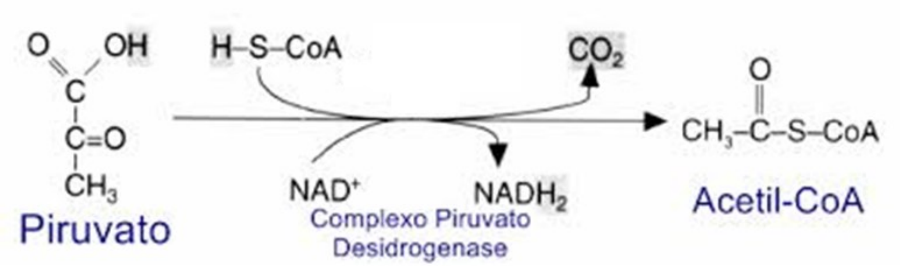
Metabólitos

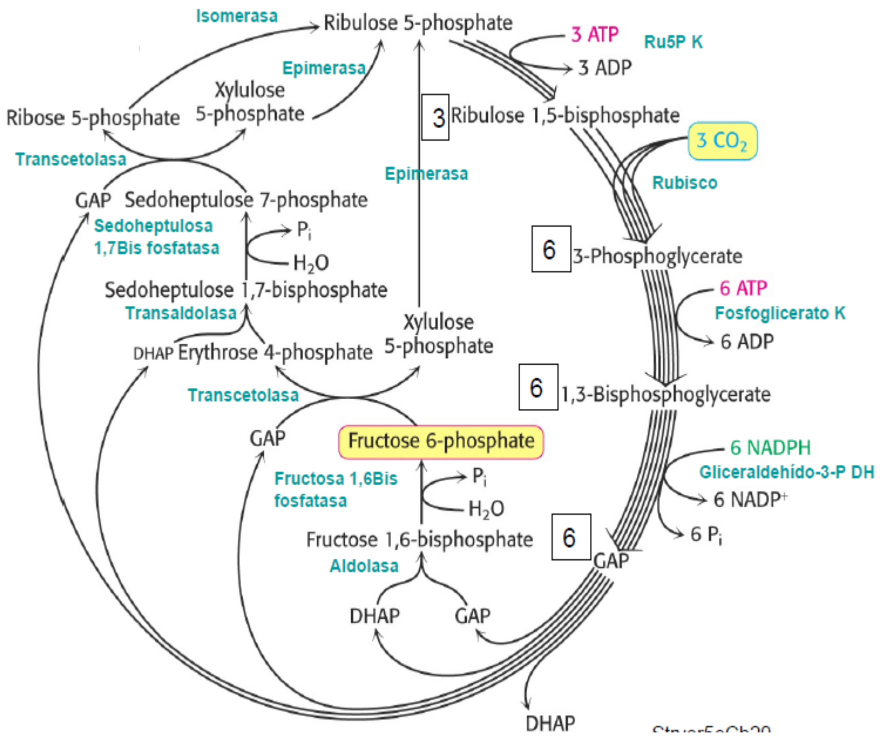
1- O que são?

2- Qual a estrutura?

Carboidratos
Álcoois
Aminoácidos
Ácidos orgânicos
Lipídios

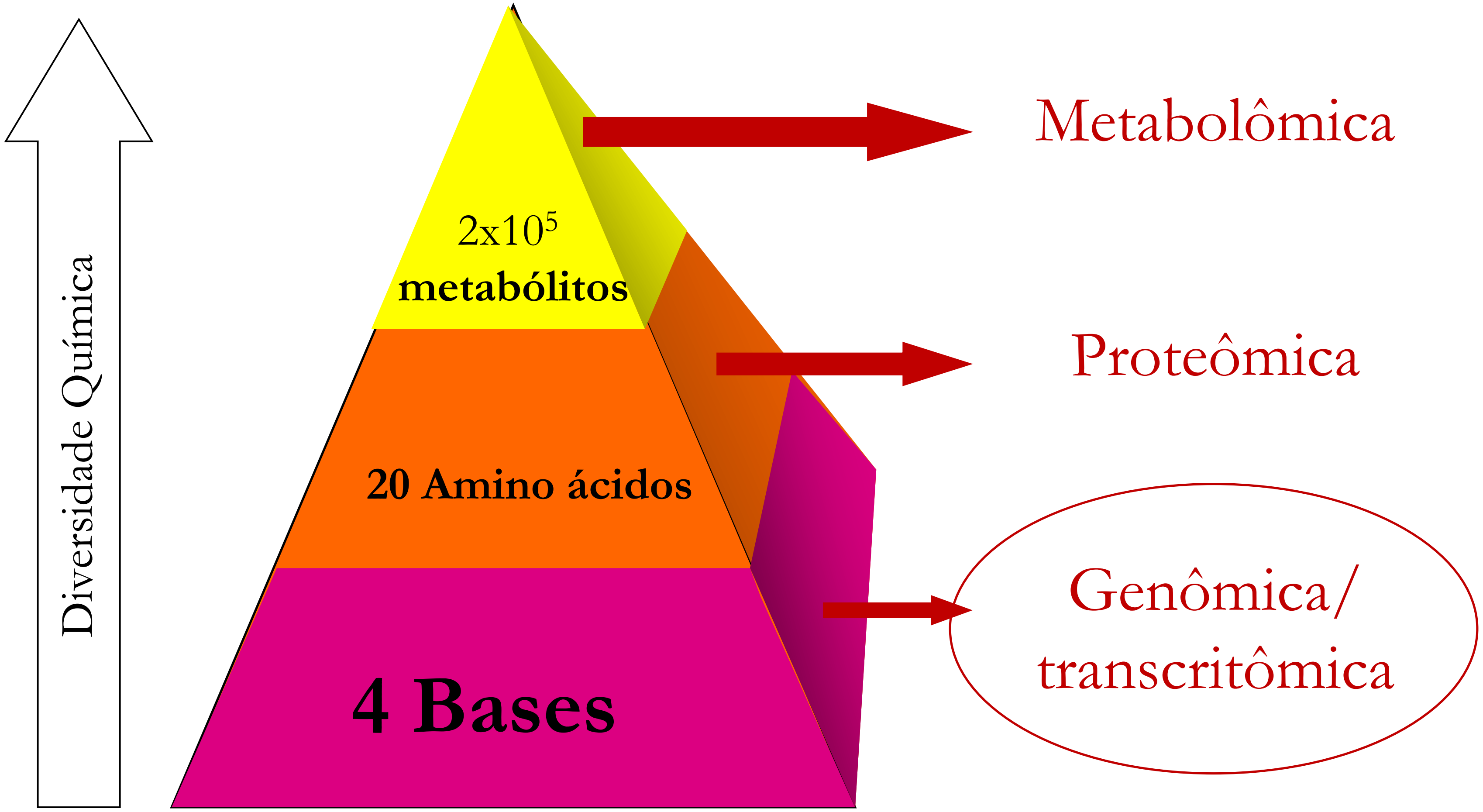
Metabólitos são os intermediários (substratos, cofatores) e produtos do metabolismo!





Ciclo de Calvin

Dificuldades do estudos de Ômicas



A pirâmide da vida

Vamos estudar somente como obter informação das 4 bases!

As ômicas:

Integrando a bioinformação

O papel da bioinformática em expansão

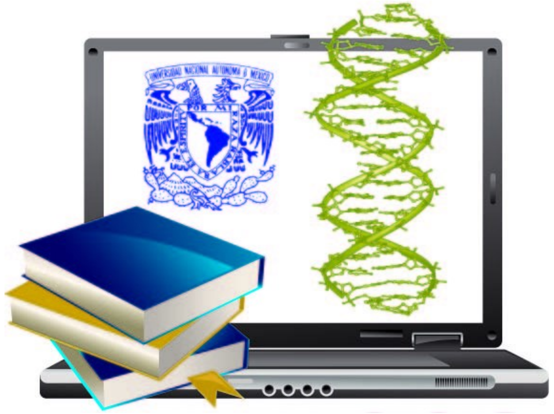
Dr. Eliseu Binneck
Consultor/Pesquisador na área de Bioinformática
Embrapa Soja, Londrina – PR.

binneck@cnpso.embrapa.br

Imagens cedidas pelo autor

Como estudar o
Genoma e o Transcritoma?

Sequenciamento de DNA e RNA

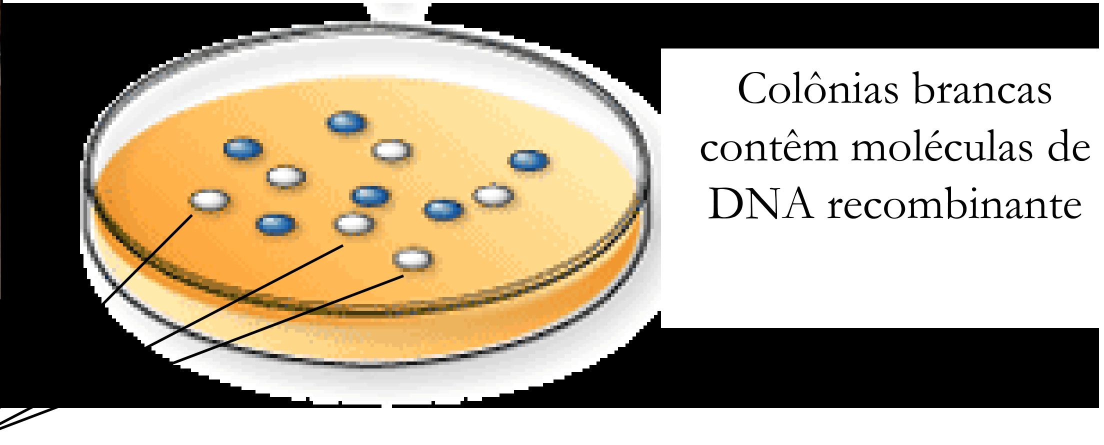


Método clássico:

Biblioteca Genômica: coleção de clones de DNA representando o genoma de um organismo

Biblioteca de cDNA: coleção de clones com insertos de DNA complementar (cDNA), sintetizados a partir de moléculas de mRNA de uma amostra (condição biológica)

Clone: uma coleção de moléculas ou células, todas idênticas a uma molécula ou célula original.

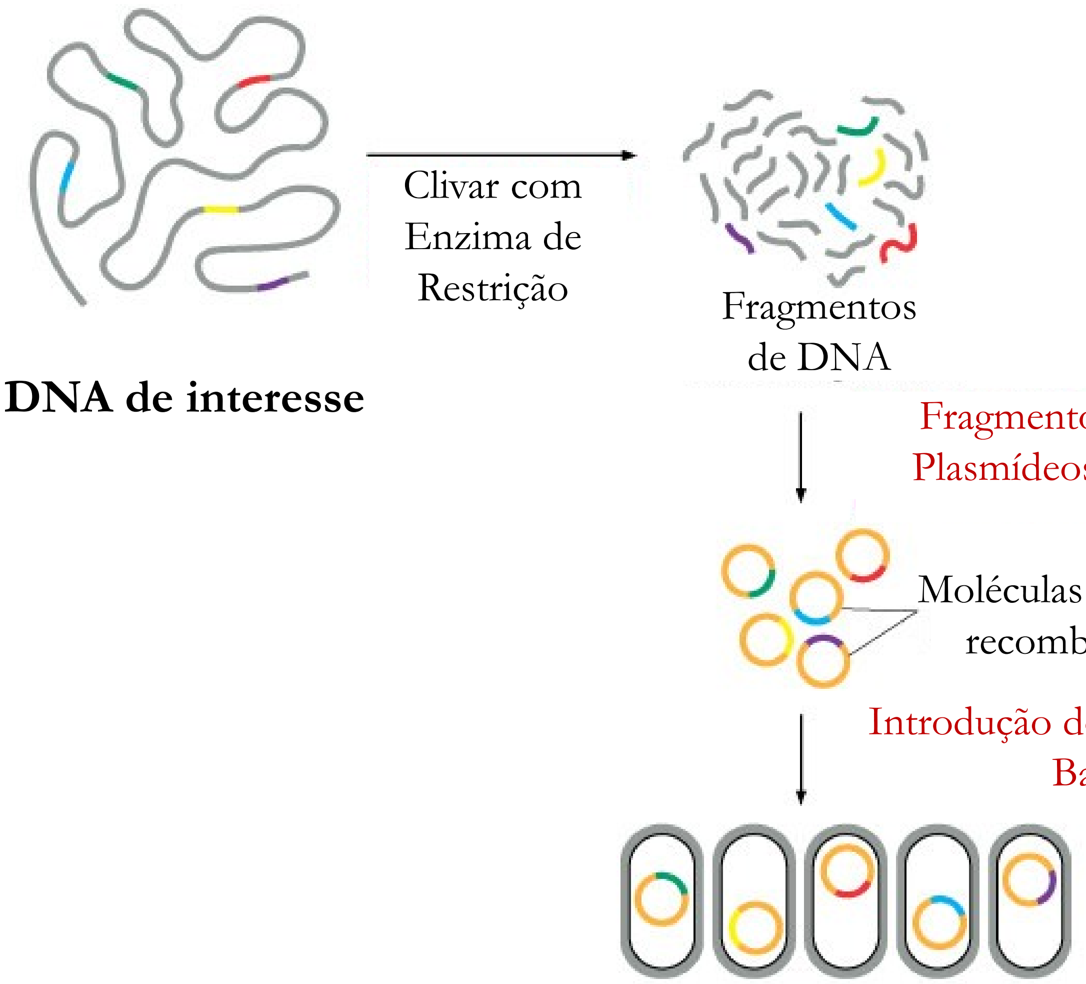


Colônias brancas contêm moléculas de DNA recombinante

Clones

Bibliotecas Genômicas

Construção de Bibliotecas Genômicas



DNA de interesse

Clivar com
Enzima de
Restrição

Fragmentos
de DNA

Fragmentos inseridos em
Plasmídeos usando Ligase

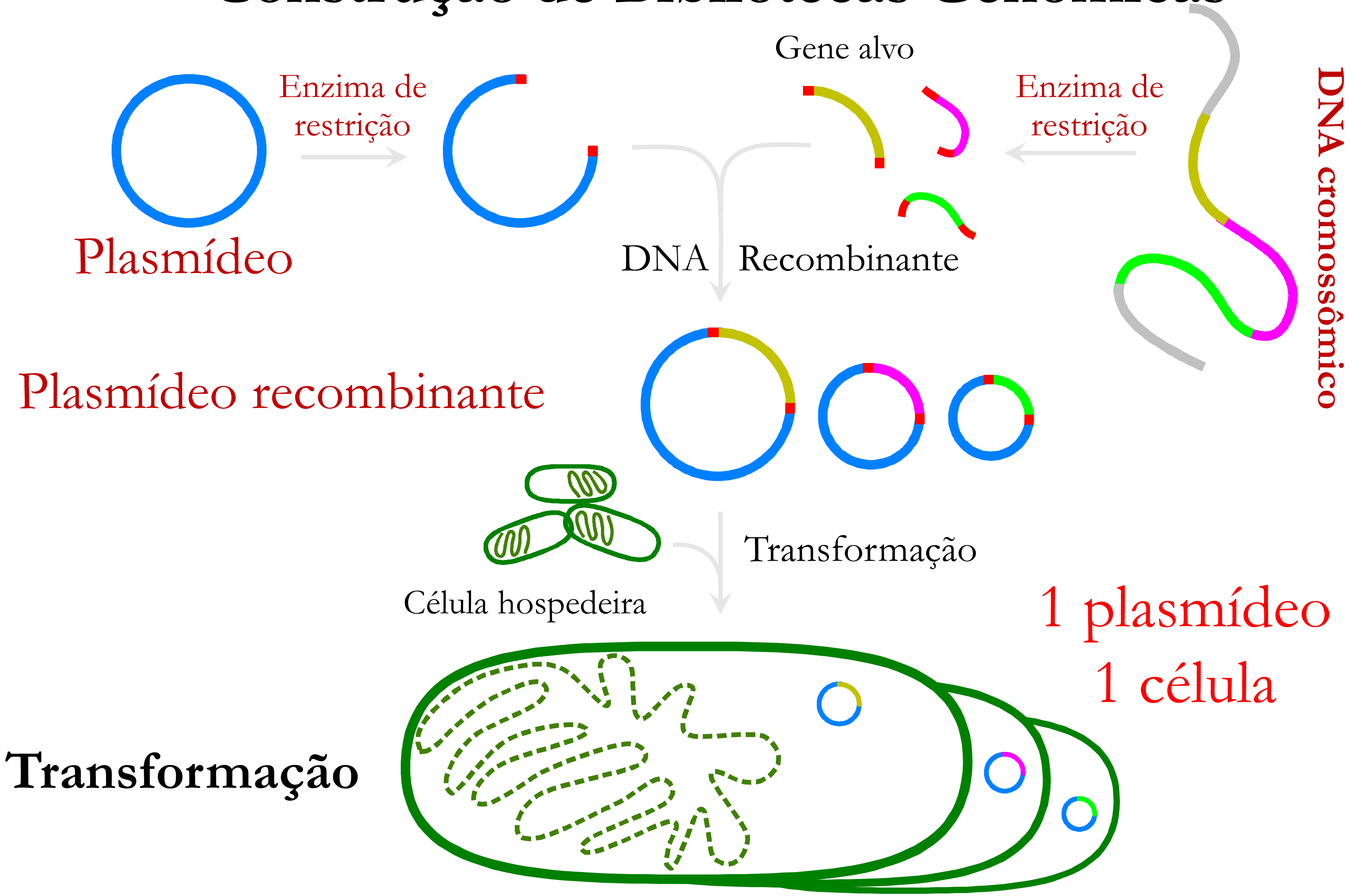
Moléculas de DNA
recombinante

Introdução dos Plasmídeos nas
Bactérias

Biblioteca genômica

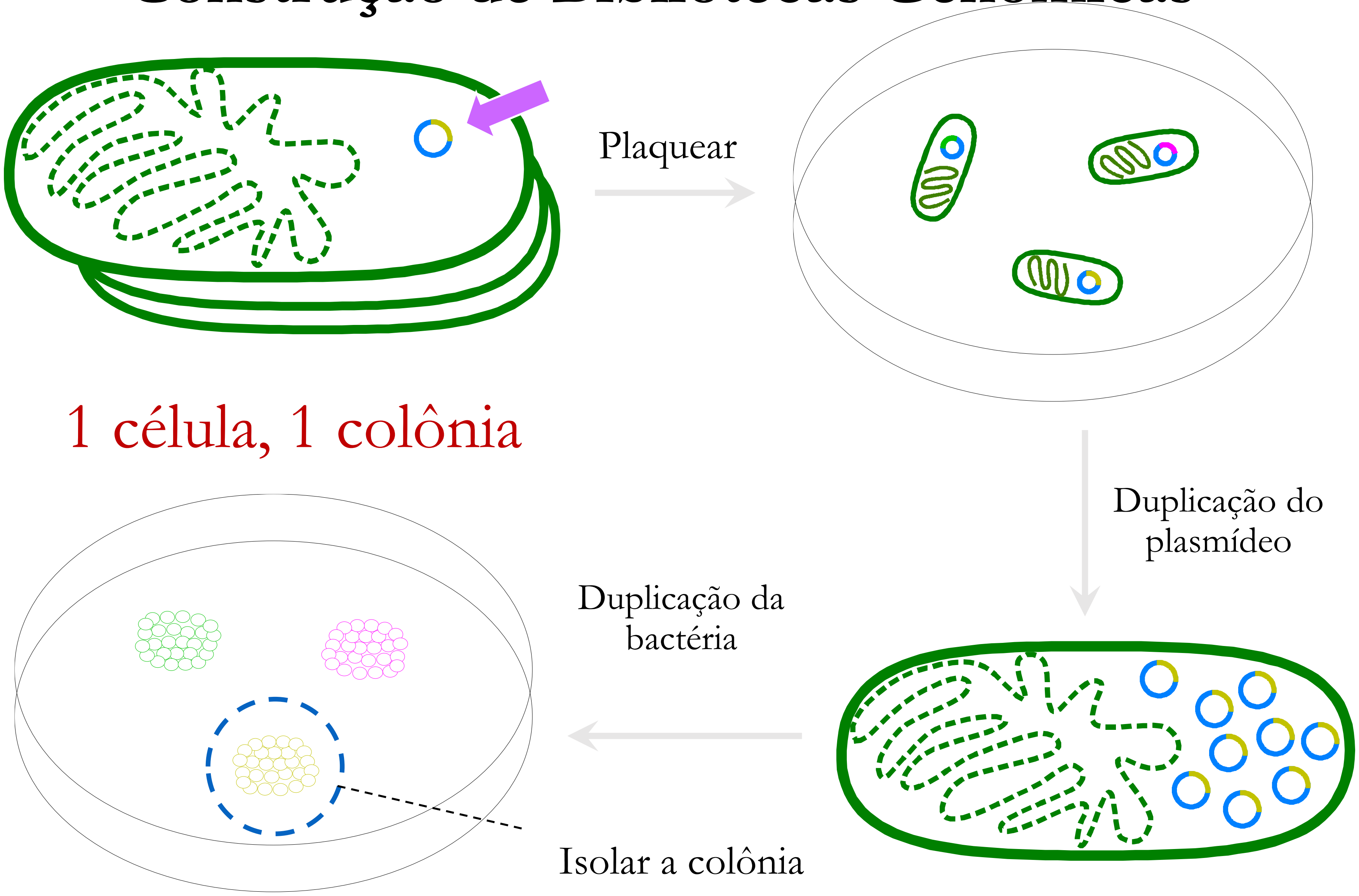
Figure 10-23 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Construção de Bibliotecas Genômicas



Juang RH (2004) BCbasics

Construção de Bibliotecas Genômicas



Plaquear

1 célula, 1 colônia

Duplicação do plasmídeo

Duplicação da bactéria

Isolar a colônia com o gene de interesse

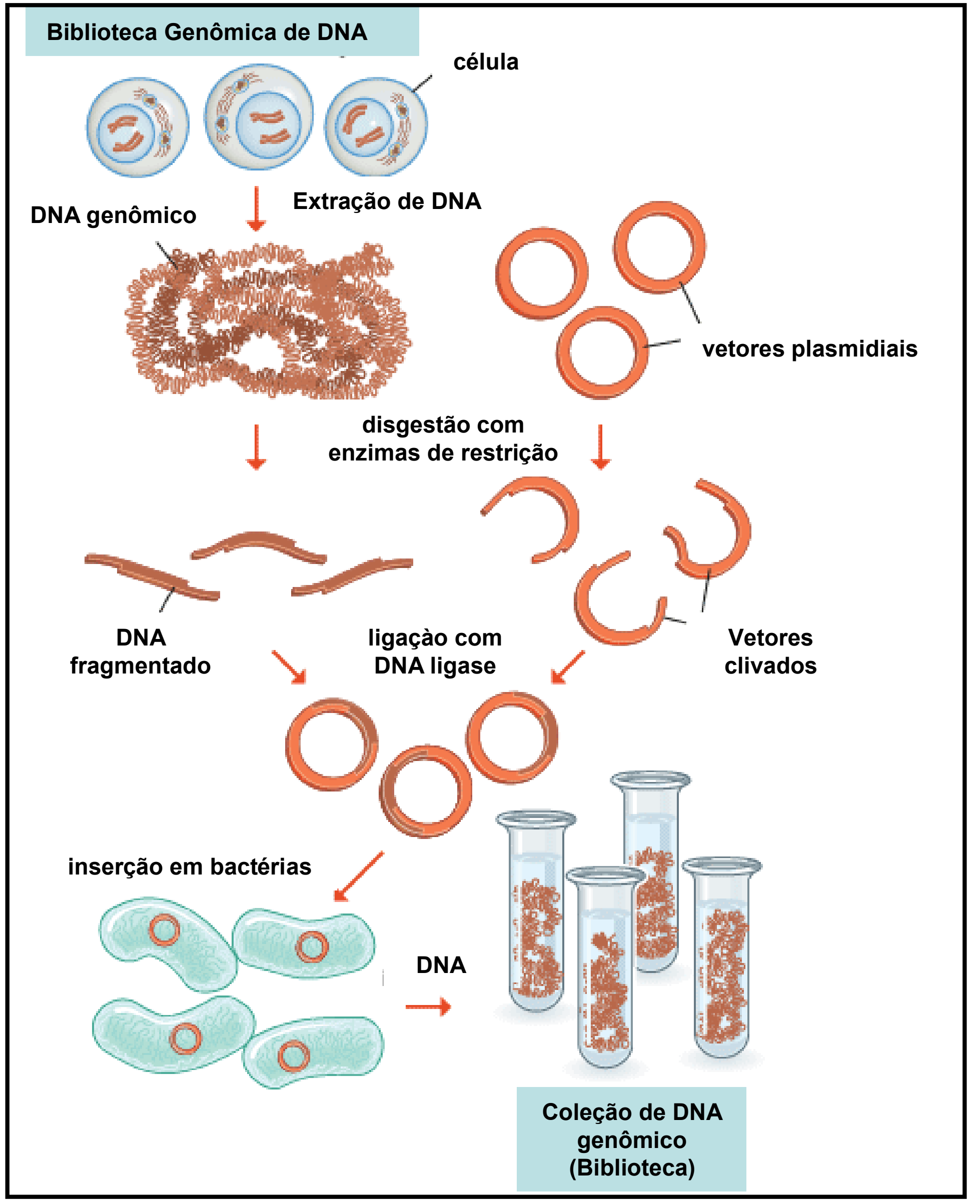
Juang RH (2004) BCbasics

Construção de Bibliotecas Genômicas

Esquema básico para construção de uma biblioteca genômica

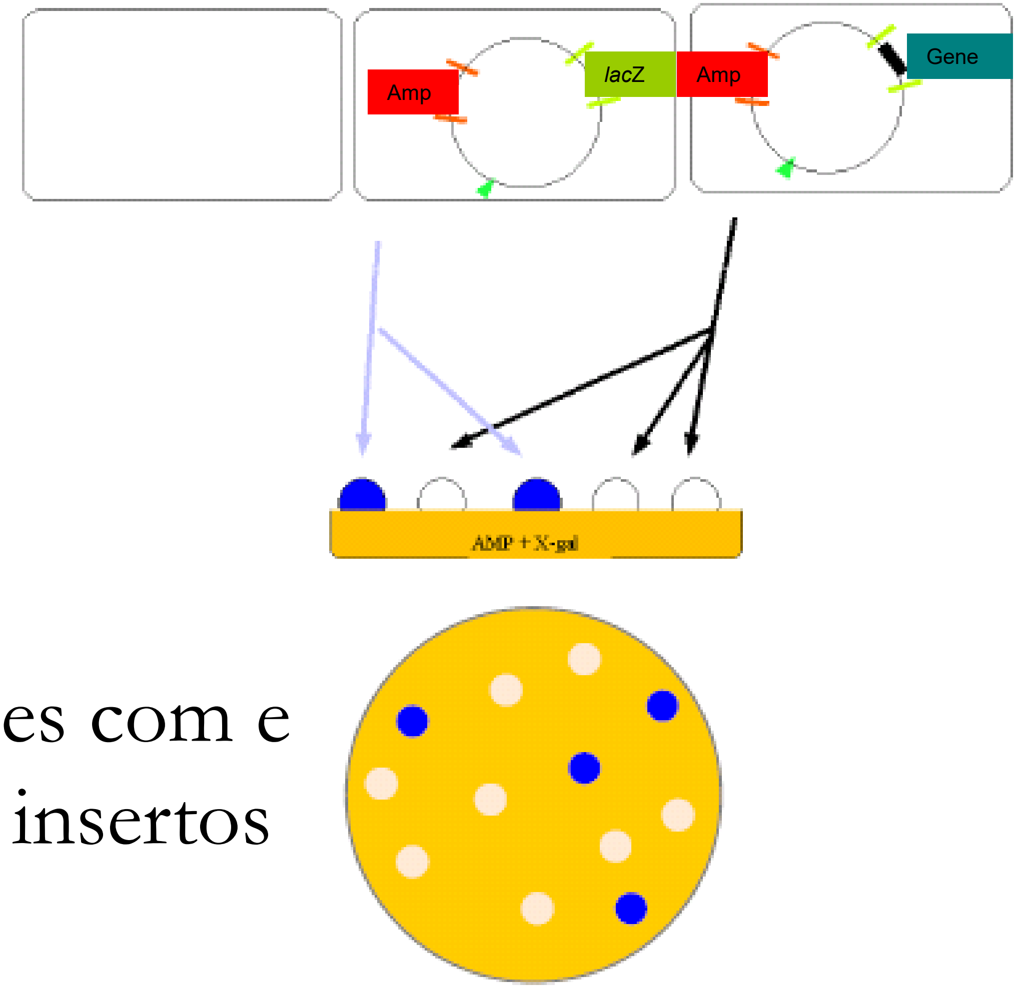
Etapas:

1. Extração de DNA
2. Fragmentação do DNA
3. Ligação em vetores
4. Inserção em bactérias
5. Multiplicação (clones)



Seleção de Clones Transformantes

Célula bacteriana

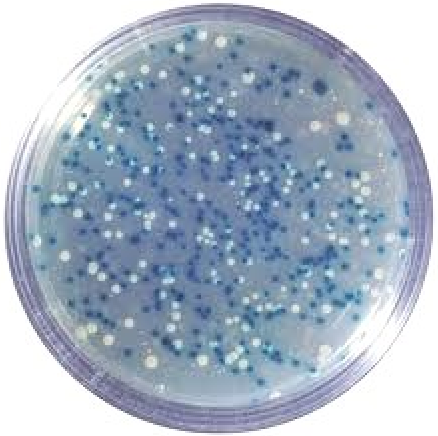


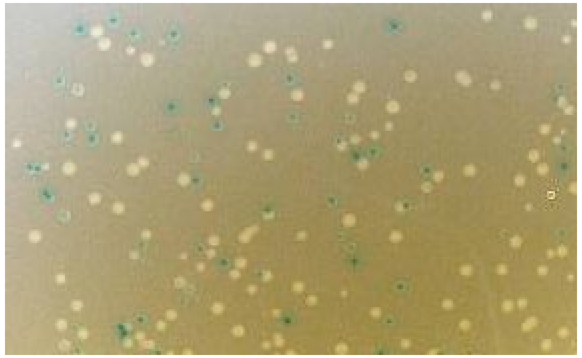
Transformação

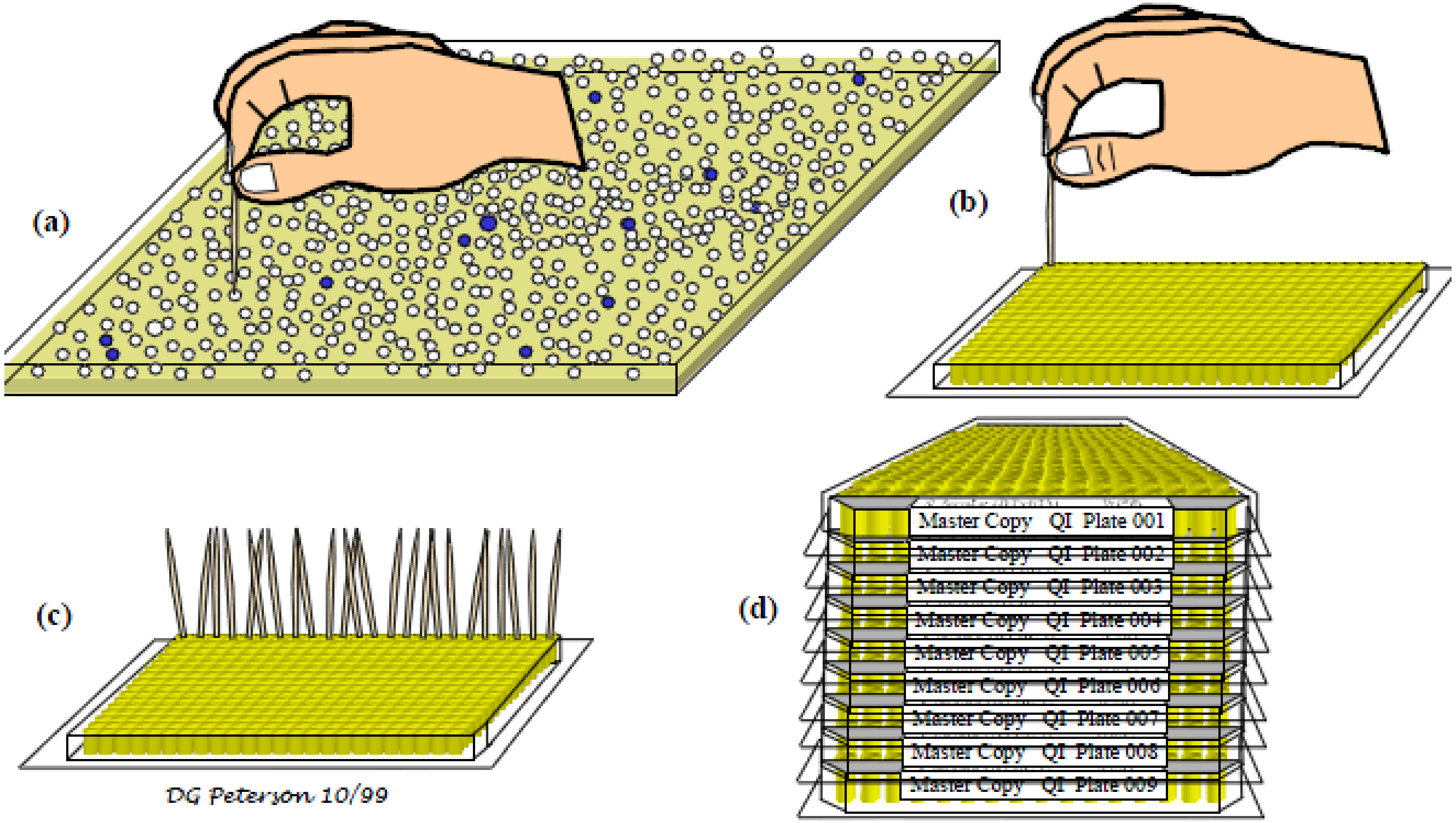
3 possibilidades

Clones com e sem insertos

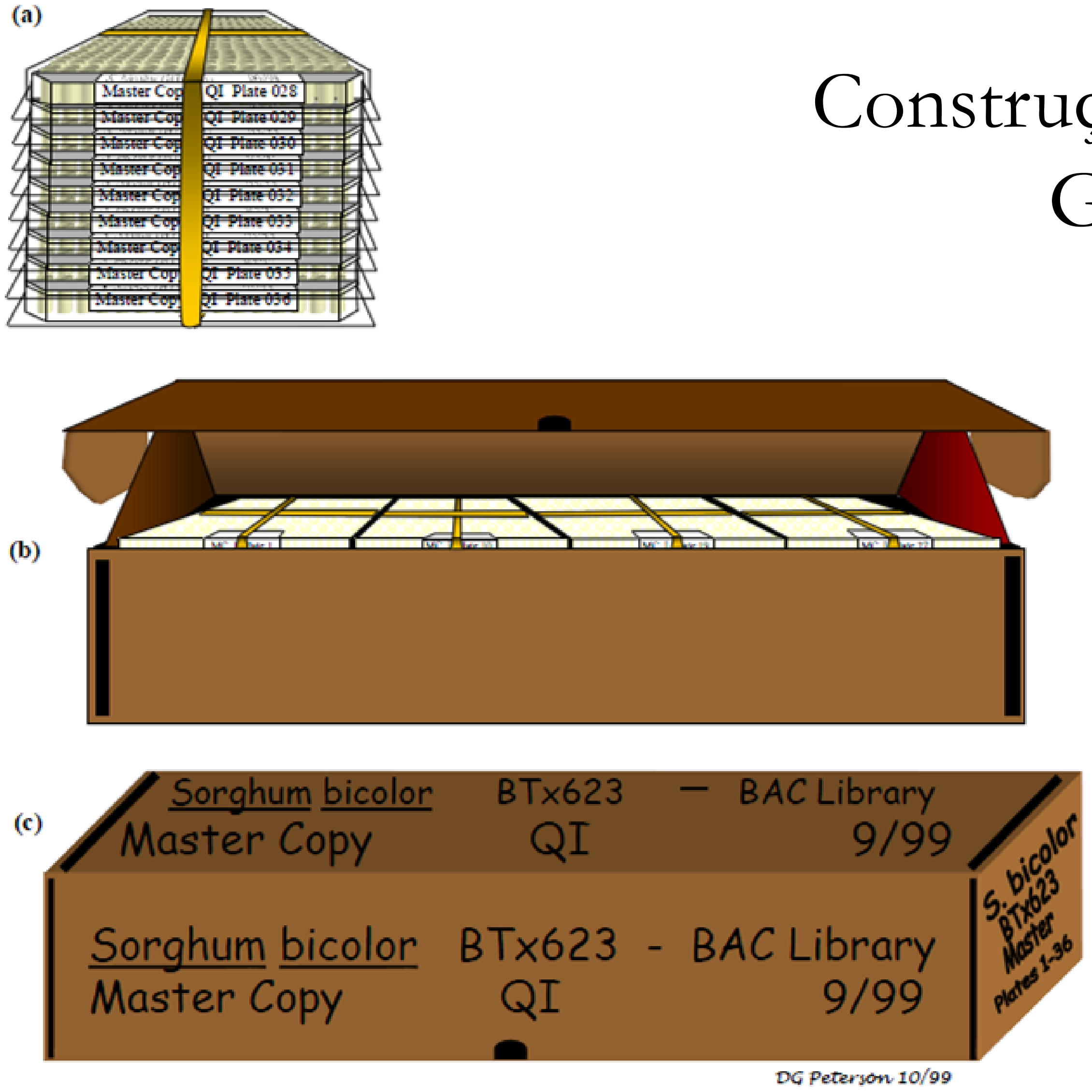
Construção de Bibliotecas Genômicas







Construção de Bibliotecas Genômicas



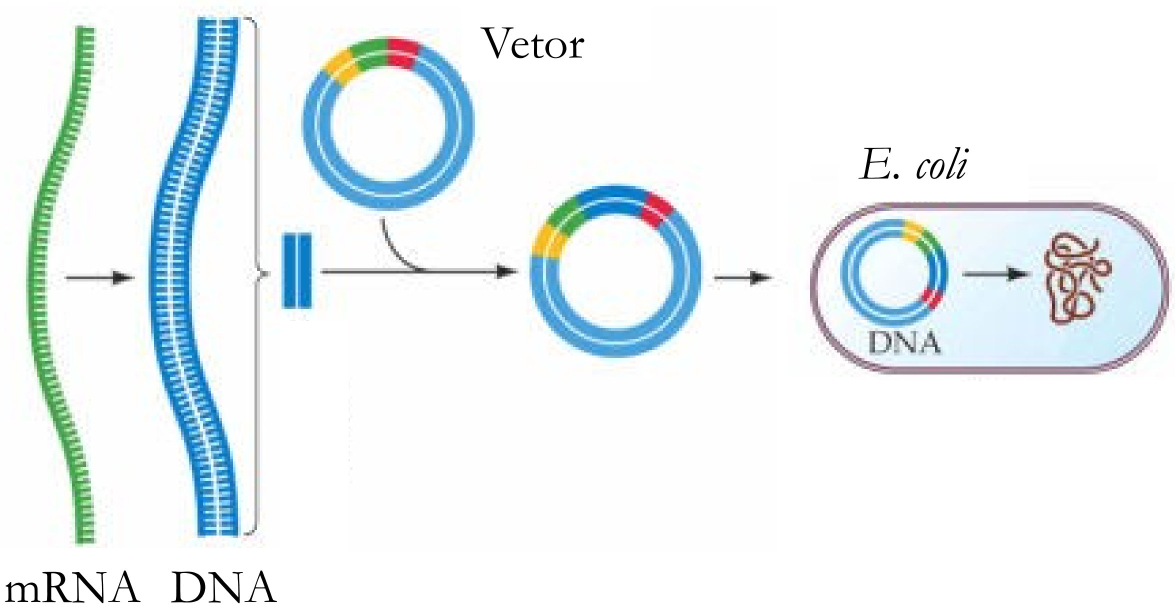
Freezer -80 °C

Bibliotecas de cDNA

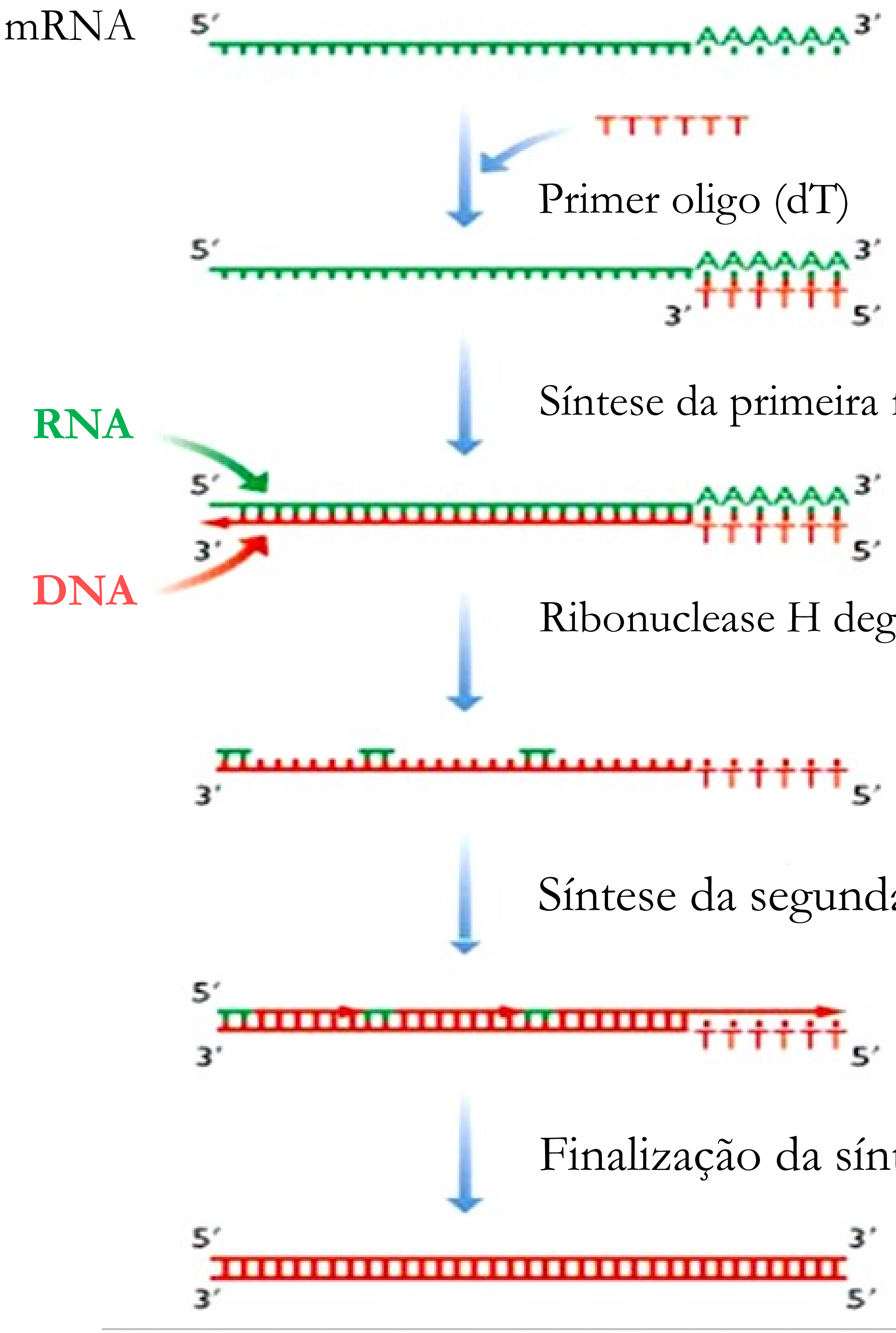
Construção de Bibliotecas de cDNA

cDNA : DNA sintetizado a partir do mRNA usando a enzima ***transcriptase reversa***.

***cDNA: DNA complementar ao mRNA**



SÍNTESE DE cDNA



mRNA

Cauda poli (A)

Primer oligo (dT)

Síntese da primeira fita de DNA pela transcriptase reversa

RNA

DNA

Ribonuclease H degrada o RNA

Síntese da segunda fita de DNA pela DNA polimerase I

Finalização da síntese da segunda fita de DNA

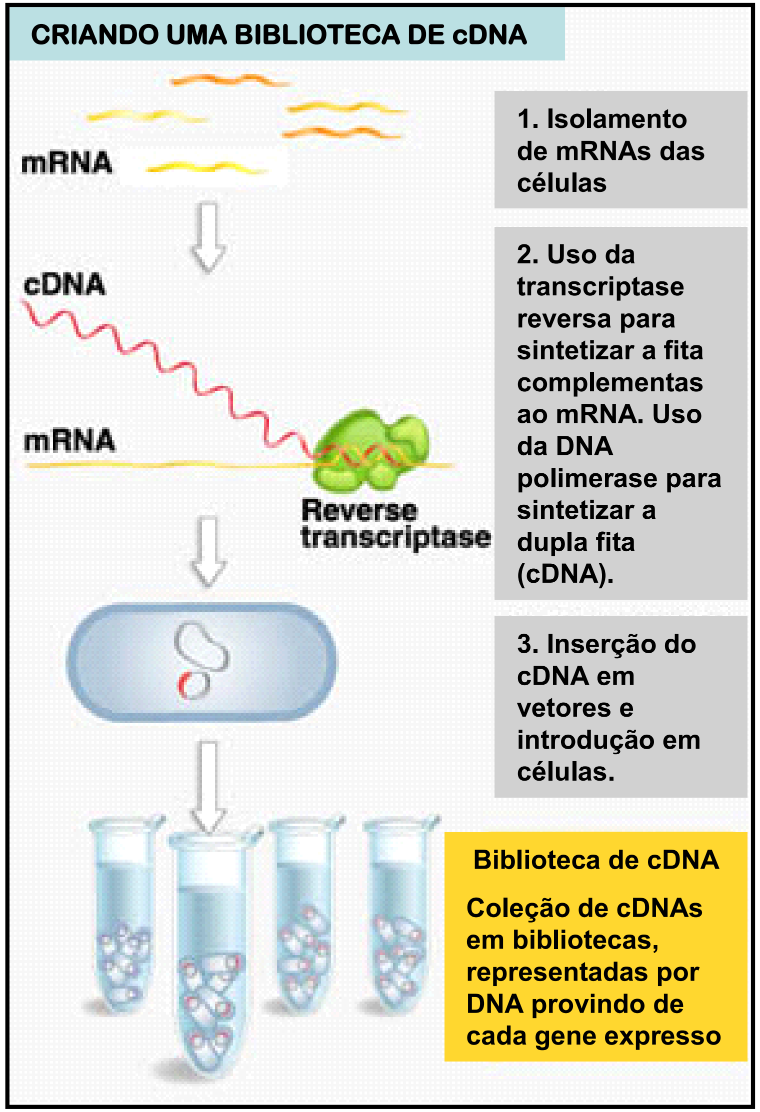
Construção de Bibliotecas de cDNA

Esquema básico para construção de uma biblioteca de cDNA

Etapas:

1. Extração de mRNA
2. Síntese de cDNA
3. Ligação em vetores
4. Inserção em bactérias
5. Multiplicação (clones)

Vantagem: somente os genes expressos são selecionados (clonados)!



CRIANDO UMA BIBLIOTECA DE cDNA

1. Isolamento de mRNAs das células

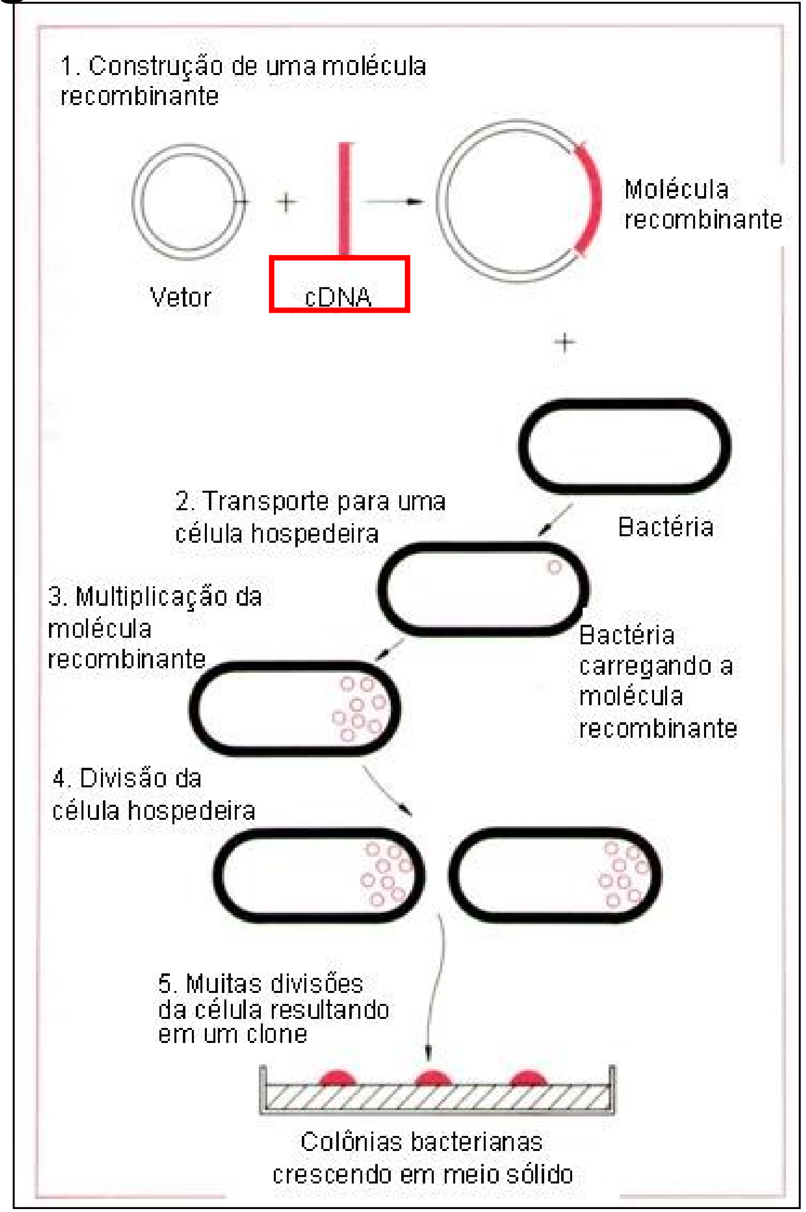
2. Uso da transcriptase reversa para sintetizar a fita complementas ao mRNA. Uso da DNA polimerase para sintetizar a dupla fita (cDNA).

3. Inserção do cDNA em vetores e introdução em células.

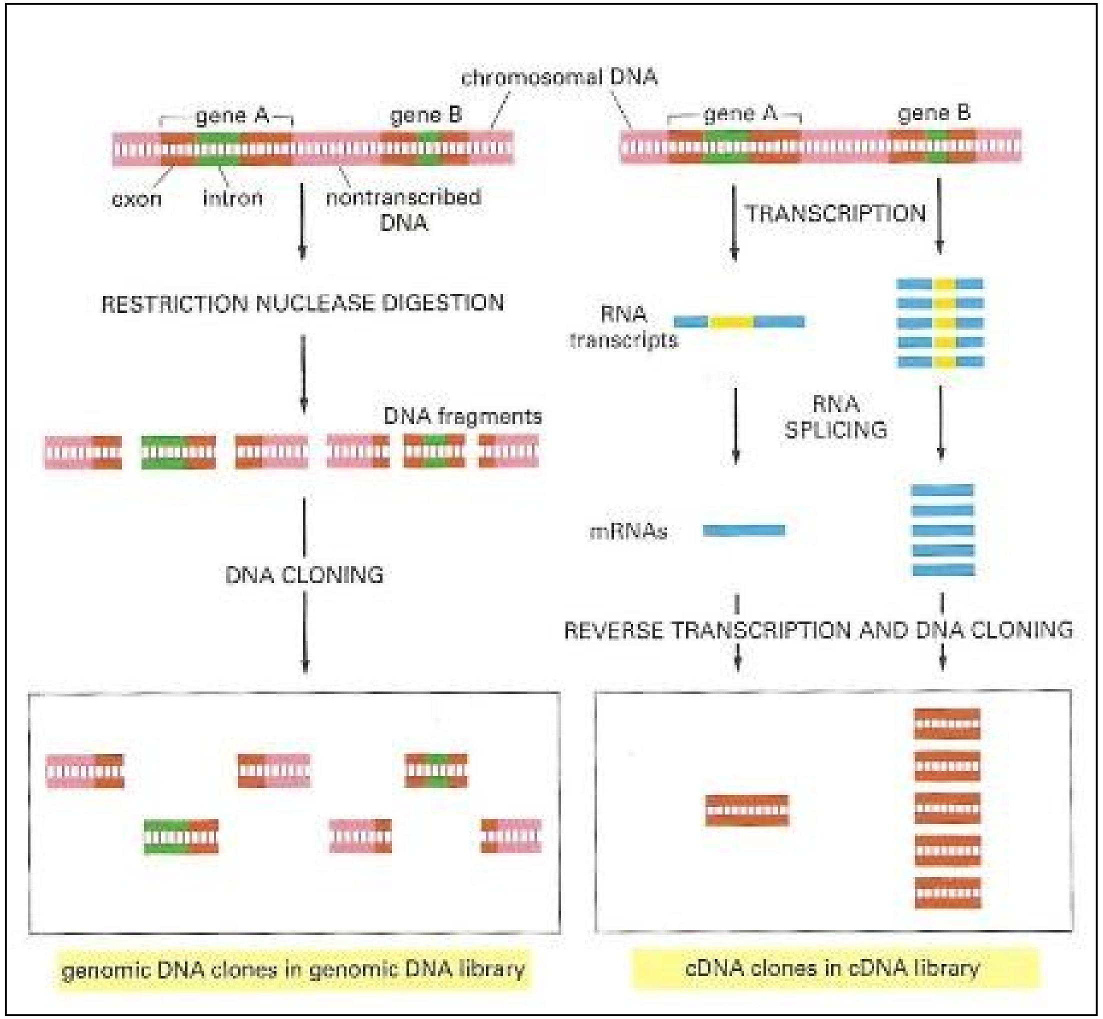
Biblioteca de cDNA

Coleção de cDNAs em bibliotecas, representadas por DNA provindo de cada gene expresso

Construção de Bibliotecas de cDNA



Biblioteca Genômica x Biblioteca de cDNA



← Problema!
Qual?

Qual é a melhor biblioteca de DNA ou de cDNA?

Depende de seu objetivo!

O que é METAGENÔMICA?

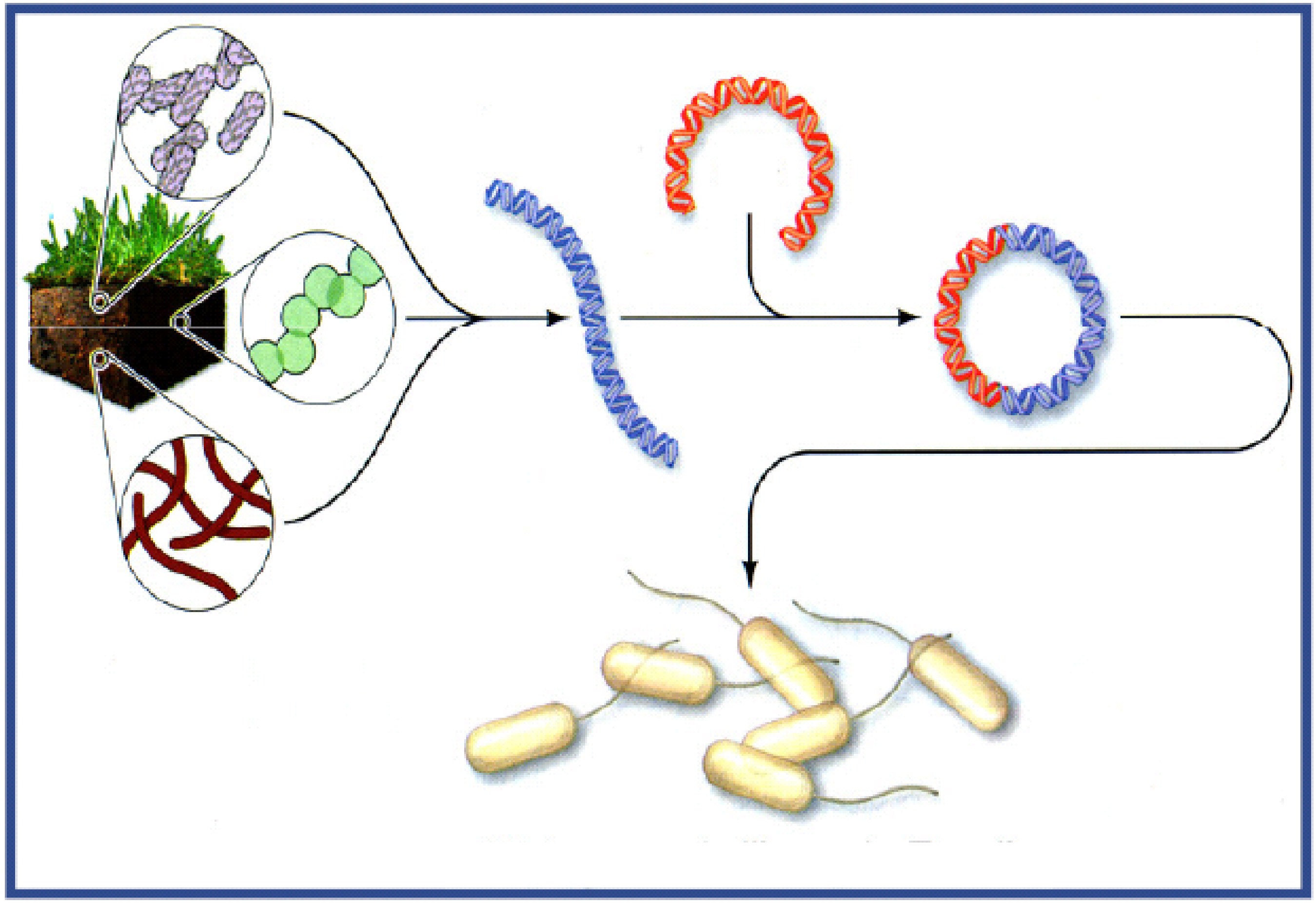
Metagenoma é o nome dado ao **genoma coletivo** da **microbiota** total encontrada em um determinado **habitat**

Específico para cada condição ambiental e biológica

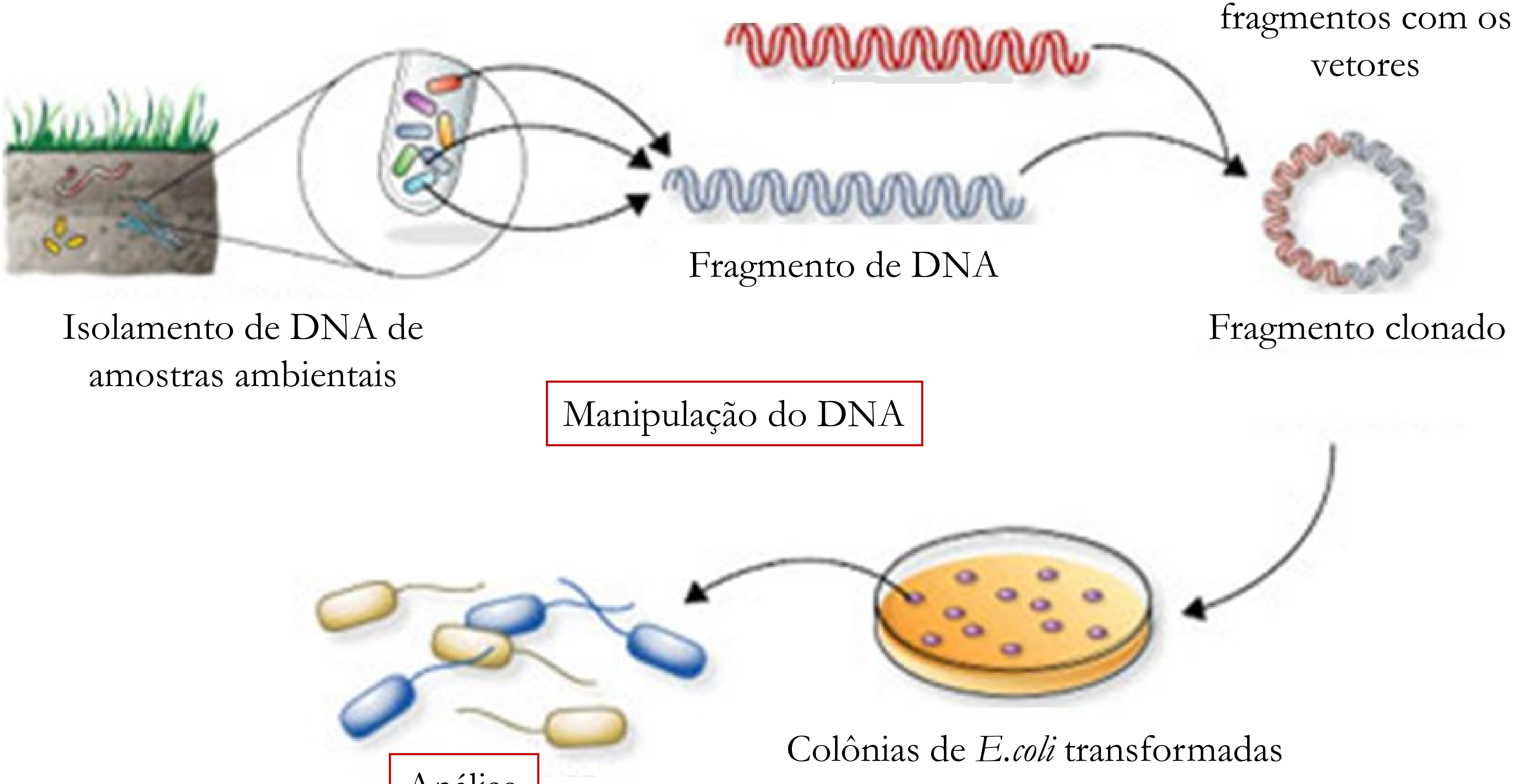
Meta – sentido de “além”, ‘transcender”

O que é METAGENÔMICA?

Metagenoma é o nome dado ao **genoma** **coletivo** da **microbiota** total encontrada em um determinado **habitat.**



Construção de Biblioteca METAGENÔMICA



Vetor de clonagem

Ligação dos
fragmentos com os
vetores

Isolamento de DNA de
amostras ambientais

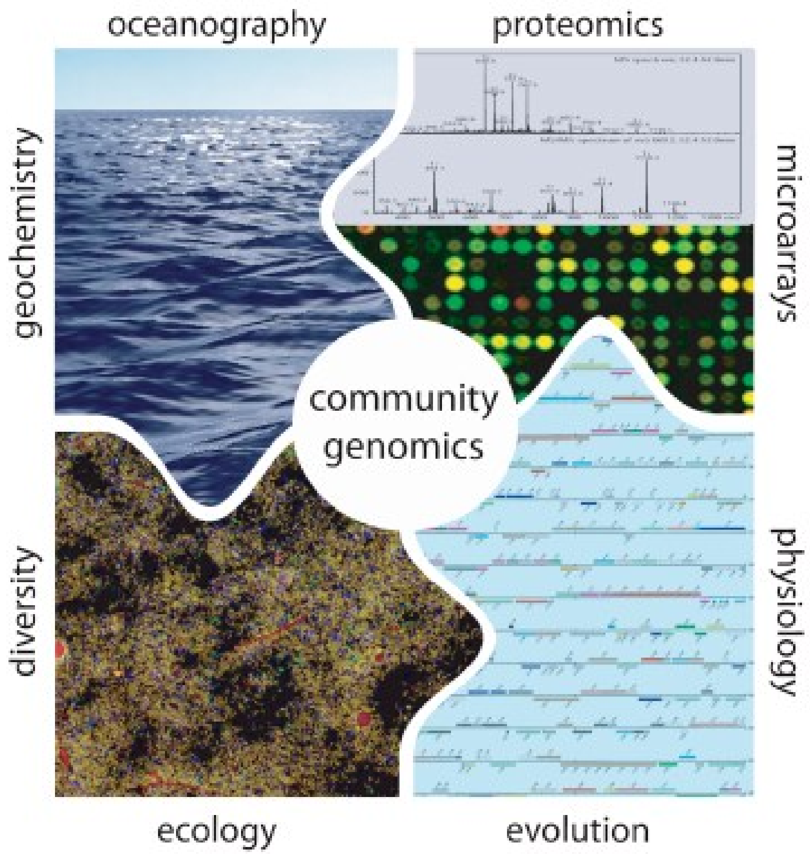
Fragmento de DNA

Fragmento clonado

Manipulação do DNA

Colônias de *E.coli* transformadas

Análise



Questões básicas

- Quem está na amostra?
- Que funções estão presentes?
- Avaliação quantitativa (abundância)
- Metagenômica comparativa

🔵 Projetos Genoma Tradicionais

🟢 Projetos em Metagenômica

🟦 Baseados em sequência

🟧 Baseados em função

Escolha de um metagenoma

Amostra

Descrever ambiente (coleta de metadados)

Avaliar diversidade

- Análises de rRNA 16S
- Fingerprinting com T-RFLP
- Análises baseadas em hibridização

Extração DNA/RNA

Novas tecnologias permitem o sequenciamento sem a construção de bibliotecas

Criar biblioteca

Sequenciamento

Montagem

Anotação

Geração de genomas completos

"Diagrama" completo de um organismo

Comparar com outras comunidades

Identificar genes

Identificar vias metabólicas

Depósito e curadoria de banco de dados

Bioinformática e Análise

Avaliar função

Expressão de genes em sistemas heterólogos

Detecção de clones positivos para a função de interesse

Sequenciamento de clones

QUAIS ORGANISMOS?

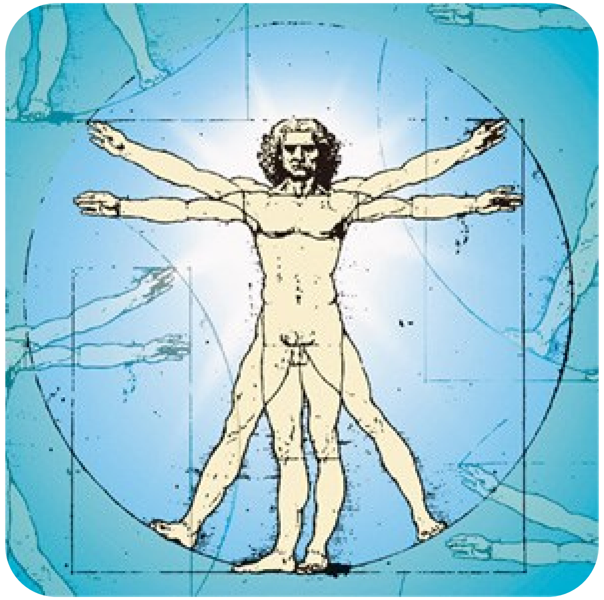
O QUE ELES ESTÃO FAZENDO?

Traduzido e adaptado de: RESEARCH COUNCIL (U.S.). COMMITTEE ON METAGENOMICS: CHALLENGES AND FUNCTIONAL APPLICATIONS, 2007

COMPLEXIDADE MICROBIANA

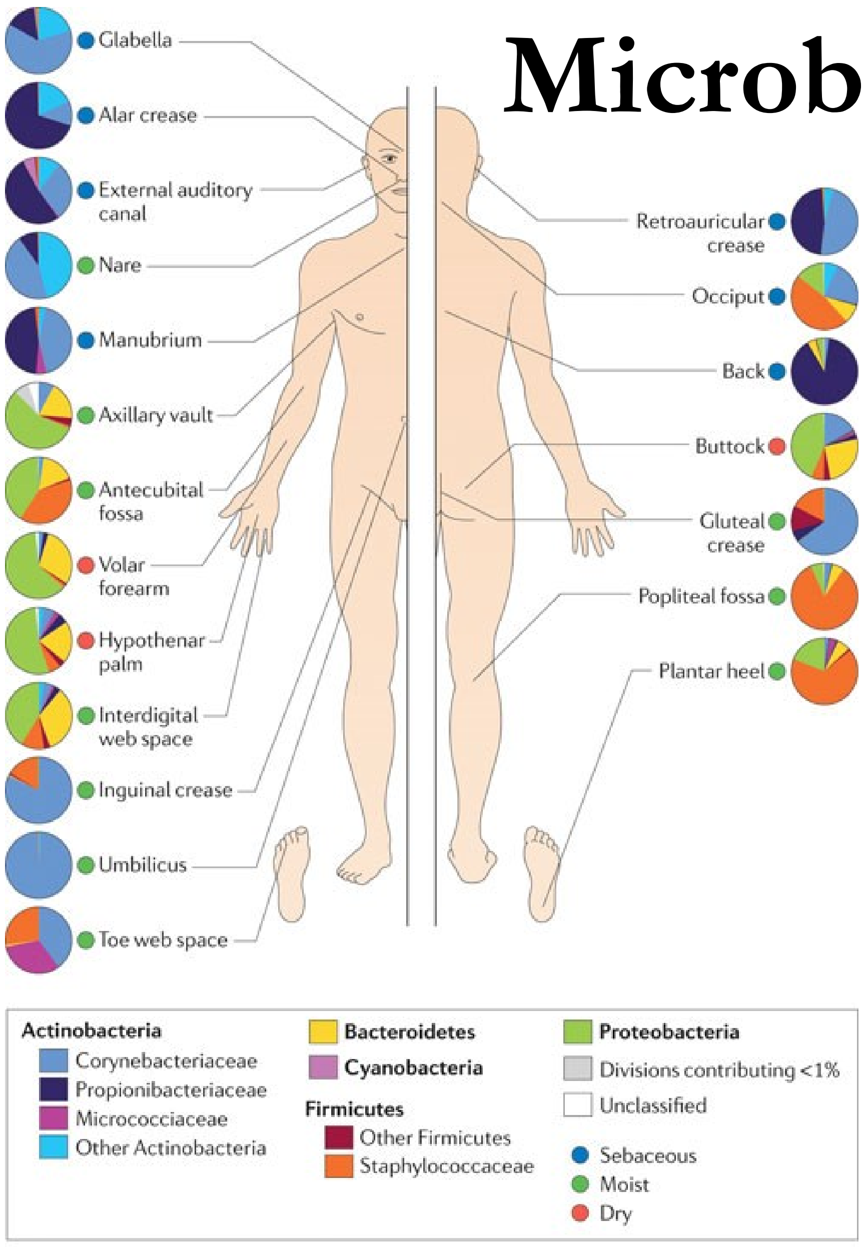
10^9 células microbianas por grama

10^8 células microbianas por mL

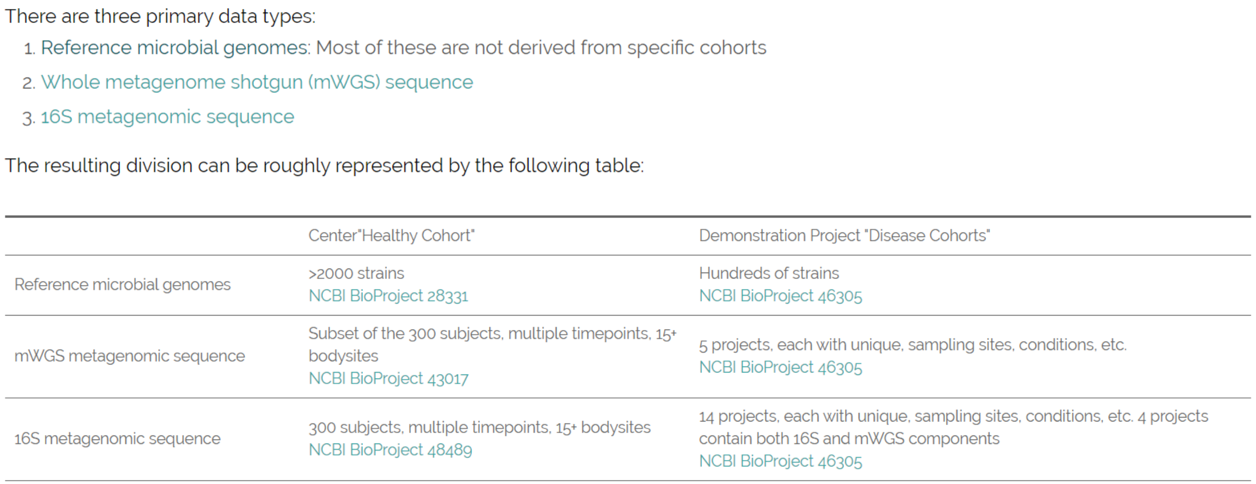


Mais células microbianas do que células humanas

Microbioma Humano



Nature Reviews | Microbiology



There are three primary data types:

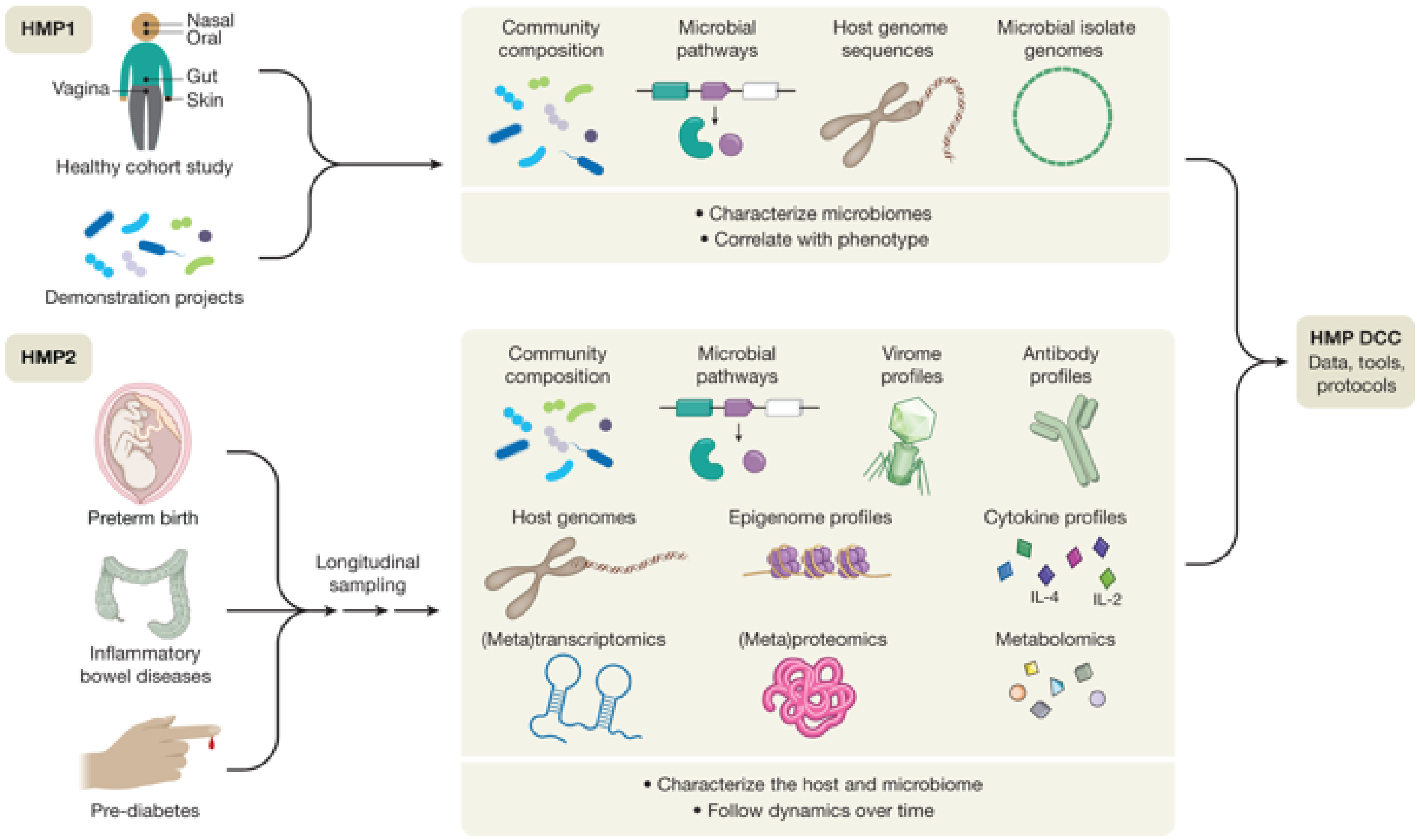
1. Reference microbial genomes: Most of these are not derived from specific cohorts
2. Whole metagenome shotgun (mWGS) sequence
3. 16S metagenomic sequence

The resulting division can be roughly represented by the following table:

	Center"Healthy Cohort"	Demonstration Project "Disease Cohorts"
Reference microbial genomes	>2000 strains NCBI BioProject 28331	Hundreds of strains NCBI BioProject 46305
mWGS metagenomic sequence	Subset of the 300 subjects, multiple timepoints, 15+ bodysites NCBI BioProject 43017	5 projects, each with unique, sampling sites, conditions, etc. NCBI BioProject 46305
16S metagenomic sequence	300 subjects, multiple timepoints, 15+ bodysites NCBI BioProject 48489	14 projects, each with unique, sampling sites, conditions, etc. 4 projects contain both 16S and mWGS components NCBI BioProject 46305

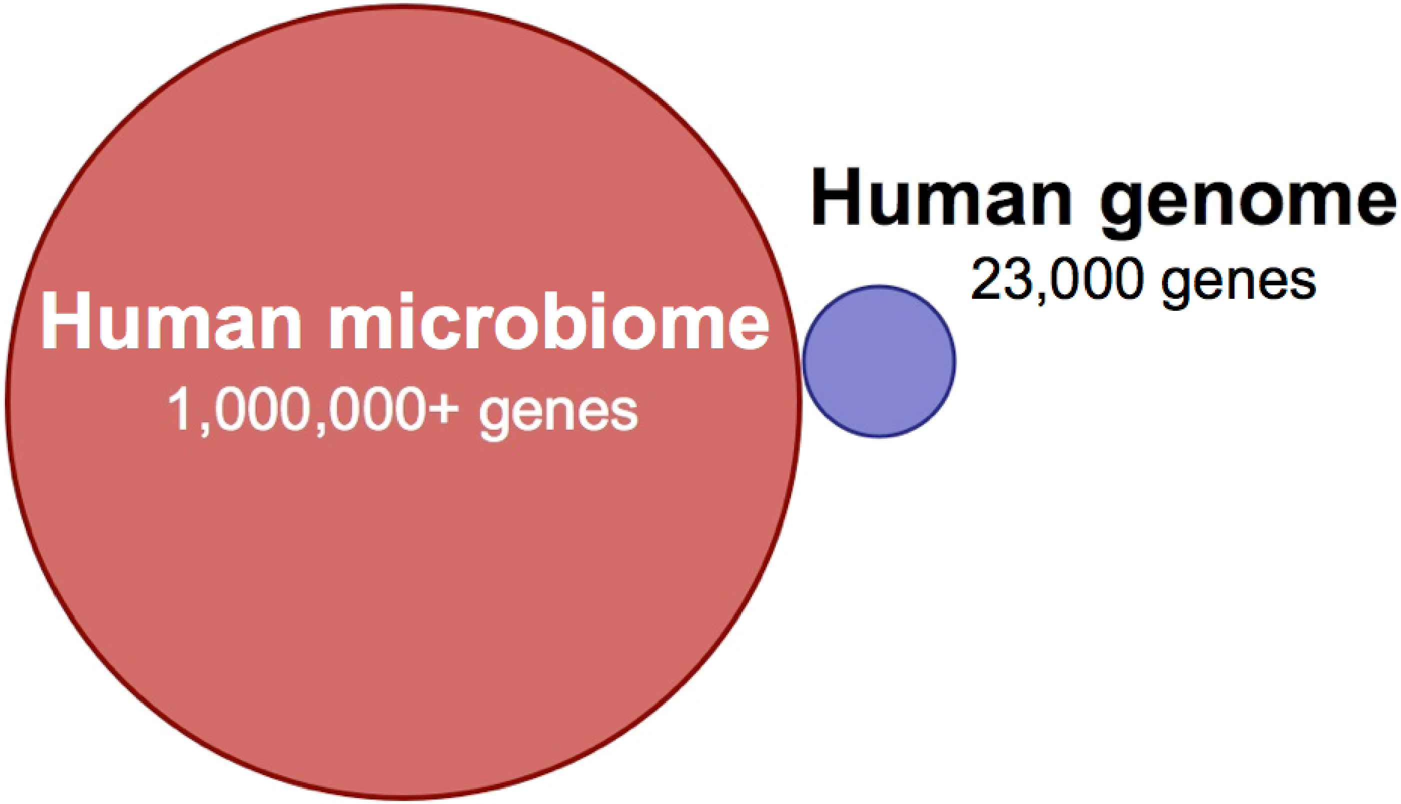
https://www.hmpdacc.org/

Microbioma Humano



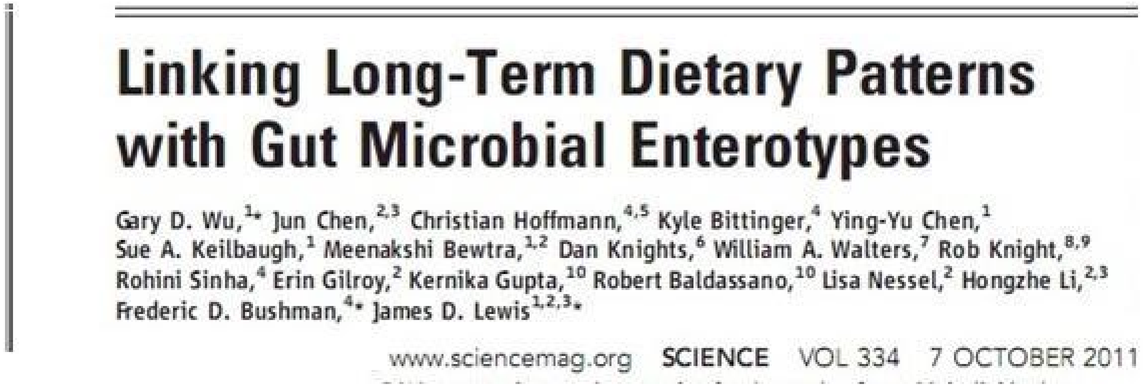
https://www.hmpdacc.org/health/projectdemos.php

Microbioma



Microbioma

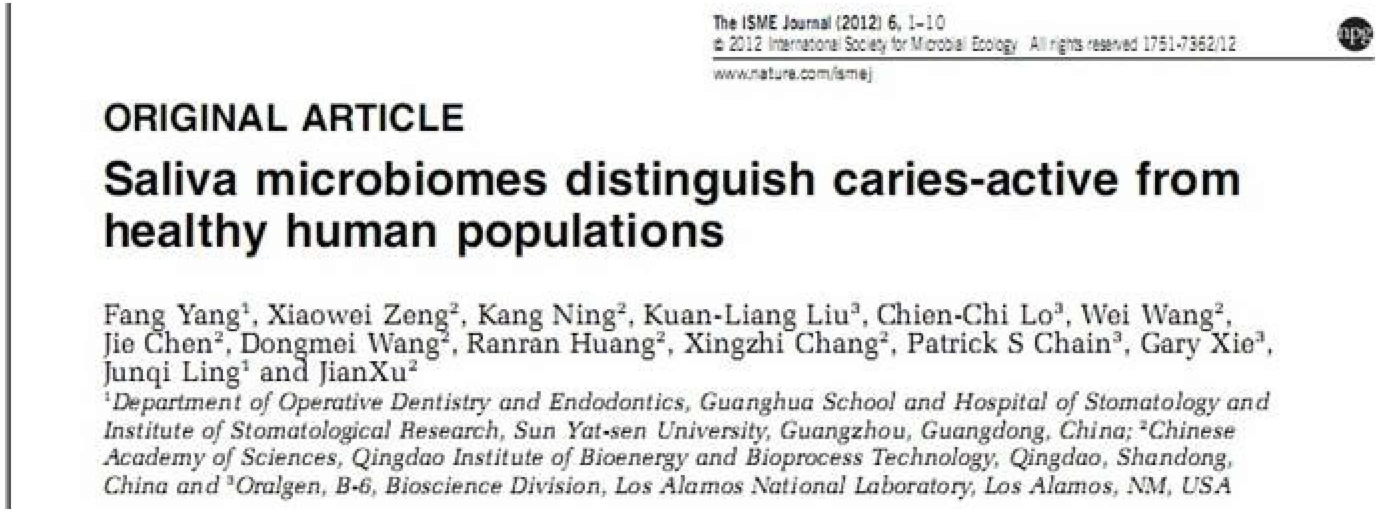
Microbioma - Aplicações



Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes

Gary D. Wu,[1*] Jun Chen,[2,3] Christian Hoffmann,[4,5] Kyle Bittinger,[4] Ying-Yu Chen,[1] Sue A. Keilbaugh,[1] Meenakshi Bewtra,[1,2] Dan Knights,[6] William A. Walters,[7] Rob Knight,[8,9] Rohini Sinha,[4] Erin Gilroy,[2] Kernika Gupta,[10] Robert Baldassano,[10] Lisa Nessel,[2] Hongzhe Li,[2,3] Frederic D. Bushman,[4*] James D. Lewis[1,2,3*]

www.sciencemag.org SCIENCE VOL 334 7 OCTOBER 2011



The ISME Journal (2012) 6, 1–10
© 2012 International Society for Microbial Ecology All rights reserved 1751-7362/12
www.nature.com/ismej

ORIGINAL ARTICLE

Saliva microbiomes distinguish caries-active from healthy human populations

Fang Yang[1], Xiaowei Zeng[2], Kang Ning[2], Kuan-Liang Liu[3], Chien-Chi Lo[3], Wei Wang[2], Jie Chen[2], Dongmei Wang[2], Ranran Huang[2], Xingzhi Chang[2], Patrick S Chain[3], Gary Xie[3], Junqi Ling[1] and JianXu[2]

[1]Department of Operative Dentistry and Endodontics, Guanghua School and Hospital of Stomatology and Institute of Stomatological Research, Sun Yat-sen University, Guangzhou, Guangdong, China; [2]Chinese Academy of Sciences, Qingdao Institute of Bioenergy and Bioprocess Technology, Qingdao, Shandong, China and [3]Oralgen, B-6, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM, USA

NEWS IN FOCUS

MYUNG J. CHUN/LOS ANGELES TIMES VIA GETTY

Babies born by caesarean section harbour different microbes than do those born vaginally.

HEALTH

Do C-section babies need mum's microbes?

Scientists begin trials to test whether swabbing infants with bacteria affects their health.

BY SARA REARDON

When a baby passes through its mother's birth canal, it is bathed in a soup of microbes. Those born by caesarean section (C-section) miss out on this bacterial baptism, and researchers are split on whether that increases the risk of chronic health problems such as obesity and asthma.

A wave of clinical trials now under way could help to settle the question — and feed into the debate over whether seeding babies born by C-section with their mother's vaginal bacteria is beneficial or potentially harmful. At least four sets of researchers — in the United States, Sweden and China — have begun separate experiments, in which they are swabbing hundreds of C-section babies with their mother's microbes, then comparing them against a control group.

Each team plans to monitor its study participants over several years in the hope of learning more about how the collection of microbes in their bodies might influence weight, allergy risk and other factors.

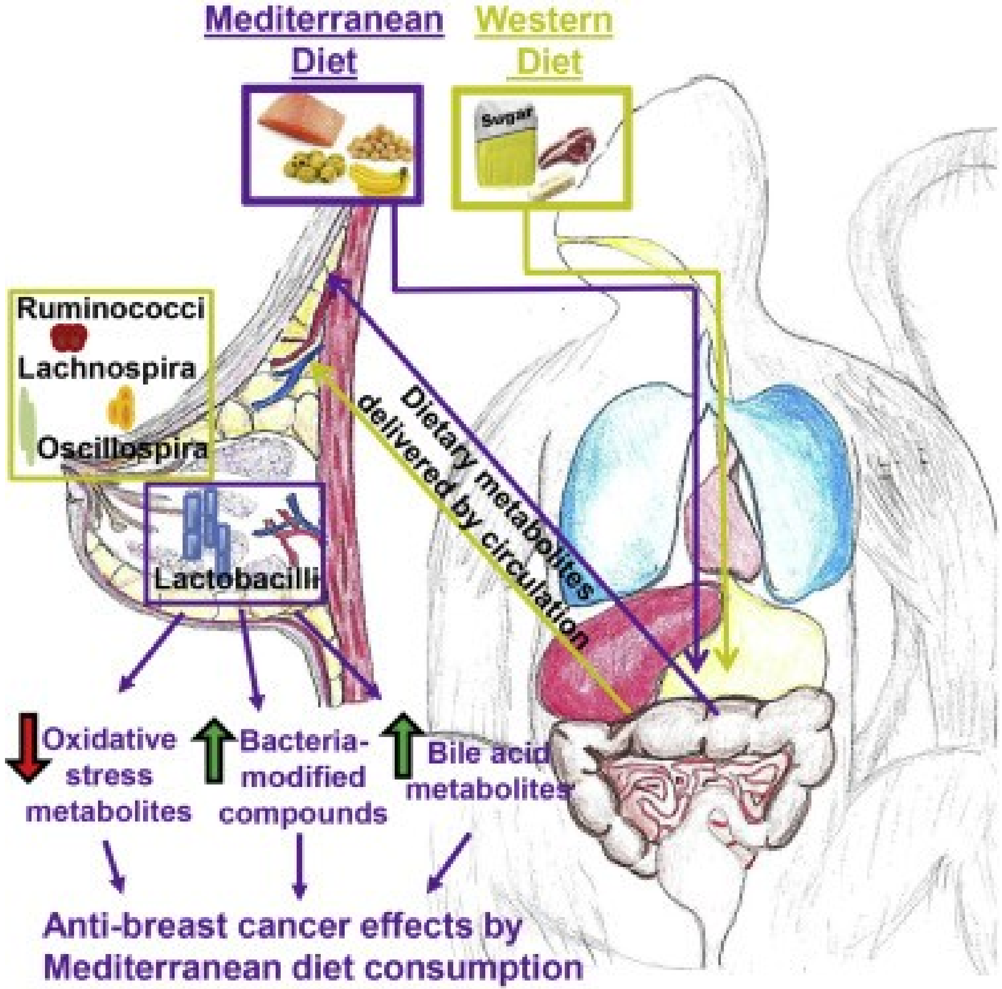
But some scientists say that the trials could expose C-section babies to infection, or encourage mothers to try do-it-yourself swabbing, without much evidence that there is a benefit. "It's not clear to me exactly what disease we're trying to prevent or treat," says Adam Ratner, a microbiologist at New York University in New York City. In the worst-case scenario, he says, "you've taken a kid with low risk of infection and you've rubbed herpes all over their face".

The idea that birth mode might affect health gained traction in 2010, when microbial ecologist Maria Gloria Dominguez Bello at Rutgers University in New Brunswick, New Jersey, found that babies delivered surgically harboured different collections of bacteria than ▶

Consumption of Mediterranean versus Western Diet Leads to Distinct Mammary Gland Microbiome Populations

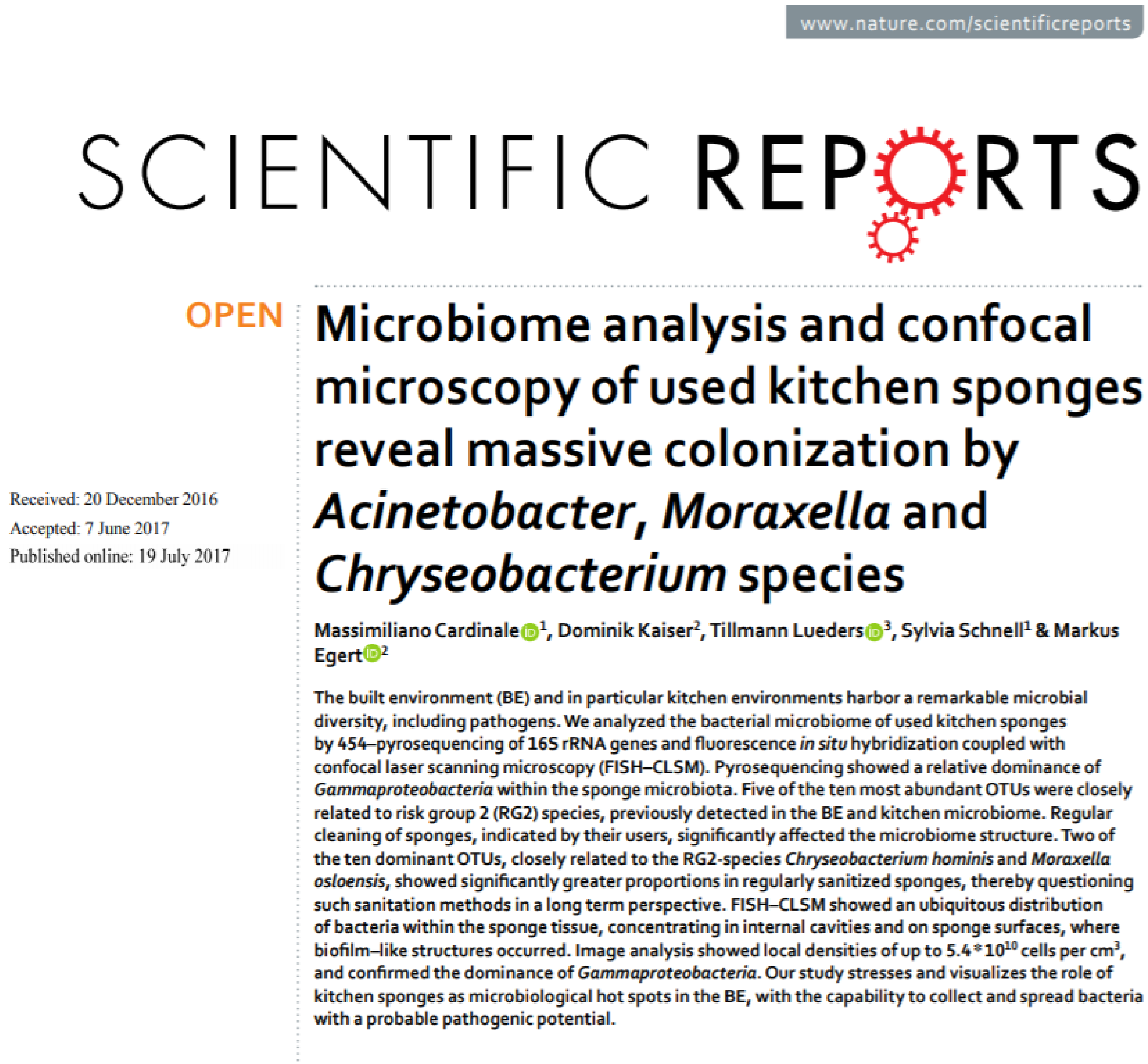
Cell Reports **Pub Date : 2018-10-02** , *DOI: 10.1016/j.celrep.2018.08.078*

Carol A. Shively, Thomas C. Register, Susan E. Appt, Thomas B. Clarkson, Beth Uberseder, Kenysha Y.J. Clear, Adam S. Wilson, Akiko Chiba, Janet A. Tooze, Katherine L. Cook



Microbioma

earth
microbiome project



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SCIENTIFIC REPORTS

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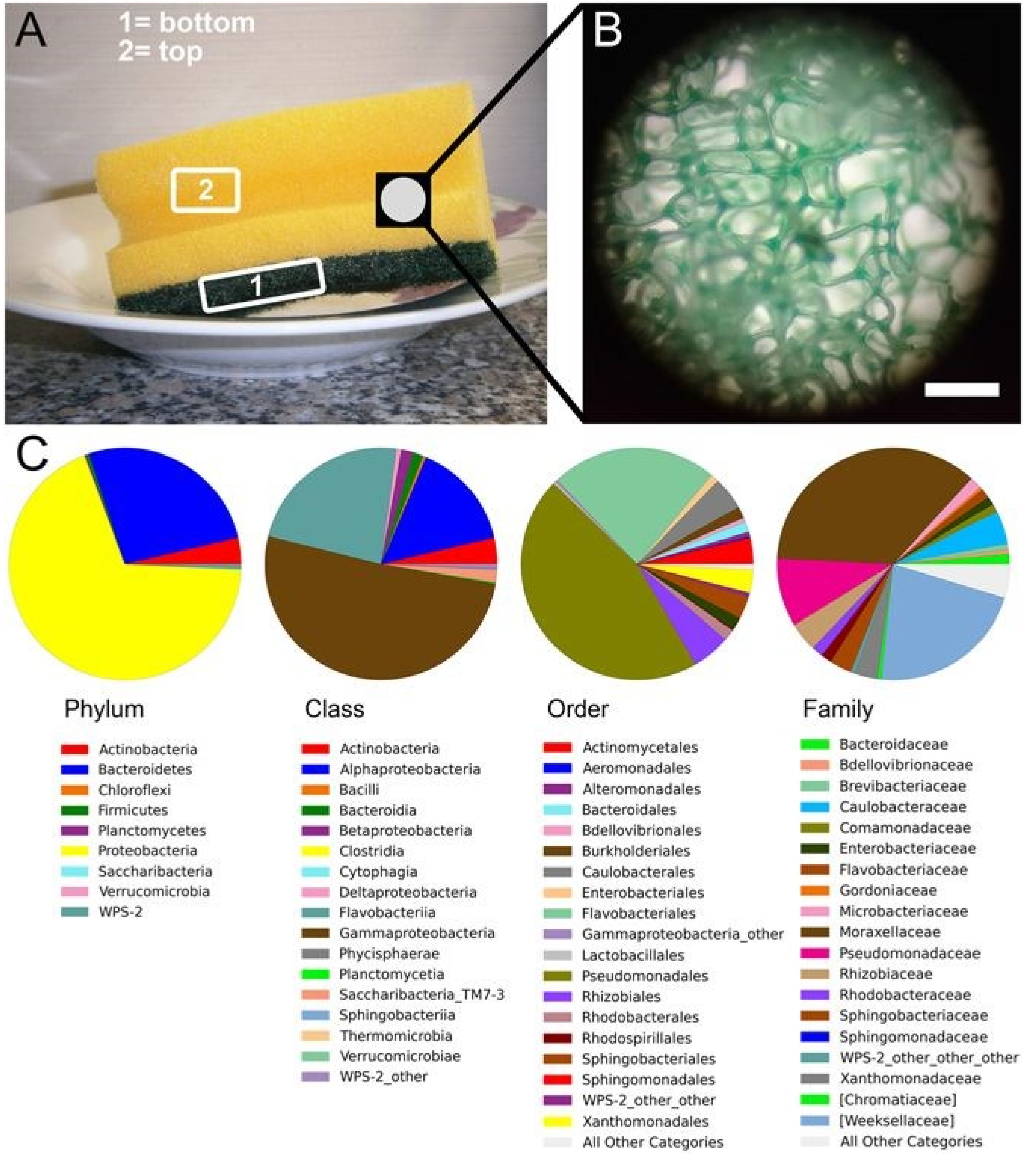
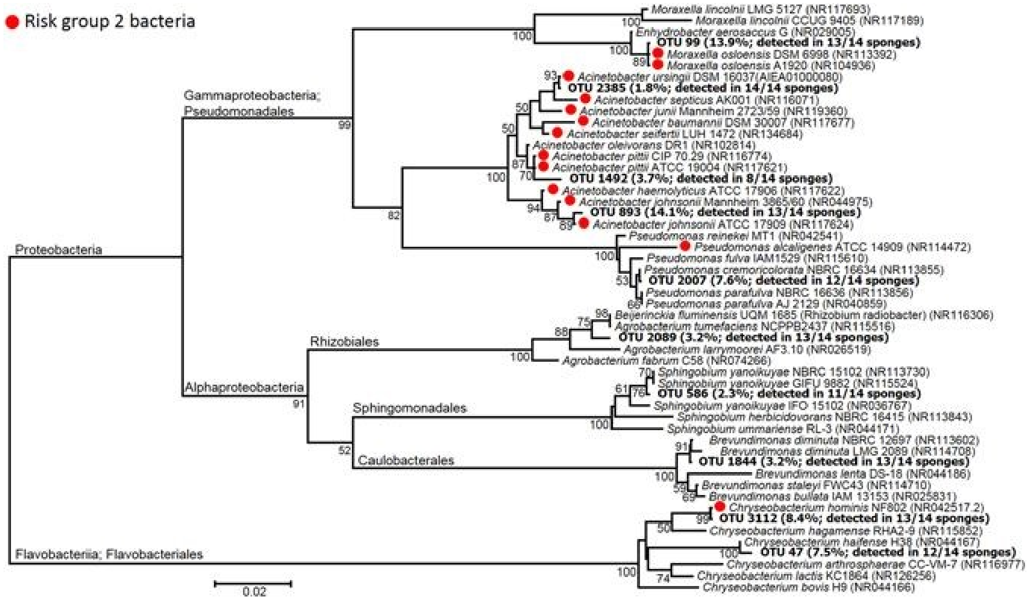
Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by *Acinetobacter*, *Moraxella* and *Chryseobacterium* species

Massimiliano Cardinale[1], Dominik Kaiser[2], Tillmann Lueders[3], Sylvia Schnell[1] & Markus Egert[2]

The built environment (BE) and in particular kitchen environments harbor a remarkable microbial diversity, including pathogens. We analyzed the bacterial microbiome of used kitchen sponges by 454–pyrosequencing of 16S rRNA genes and fluorescence *in situ* hybridization coupled with confocal laser scanning microscopy (FISH–CLSM). Pyrosequencing showed a relative dominance of *Gammaproteobacteria* within the sponge microbiota. Five of the ten most abundant OTUs were closely related to risk group 2 (RG2) species, previously detected in the BE and kitchen microbiome. Regular cleaning of sponges, indicated by their users, significantly affected the microbiome structure. Two of the ten dominant OTUs, closely related to the RG2-species *Chryseobacterium hominis* and *Moraxella osloensis*, showed significantly greater proportions in regularly sanitized sponges, thereby questioning such sanitation methods in a long term perspective. FISH–CLSM showed an ubiquitous distribution of bacteria within the sponge tissue, concentrating in internal cavities and on sponge surfaces, where biofilm–like structures occurred. Image analysis showed local densities of up to $5.4*10^{10}$ cells per cm^3, and confirmed the dominance of *Gammaproteobacteria*. Our study stresses and visualizes the role of kitchen sponges as microbiological hot spots in the BE, with the capability to collect and spread bacteria with a probable pathogenic potential.


A
1= bottom
2= top
2
1
B
C
Phylum
Actinobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Planctomycetes
Proteobacteria
Saccharibacteria
Verrucomicrobia
WPS-2
Class
Actinobacteria
Alphaproteobacteria
Bacilli
Bacteroidia
Betaproteobacteria
Clostridia
Cytophagia
Deltaproteobacteria
Flavobacteriia
Gammaproteobacteria
Phycisphaerae
Planctomycetia
Saccharibacteria_TM7-3
Sphingobacteriia
Thermomicrobia
Verrucomicrobiae
WPS-2_other
Order
Actinomycetales
Aeromonadales
Alteromonadales
Bacteroidales
Bdellovibrionales
Burkholderiales
Caulobacterales
Enterobacteriales
Flavobacteriales
Gammaproteobacteria_other
Lactobacillales
Pseudomonadales
Rhizobiales
Rhodobacterales
Rhodospirillales
Sphingobacteriales
Sphingomonadales
WPS-2_other_other
Xanthomonadales
All Other Categories
Family
Bacteroidaceae
Bdellovibrionaceae
Brevibacteriaceae
Caulobacteraceae
Comamonadaceae
Enterobacteriaceae
Flavobacteriaceae
Gordoniaceae
Microbacteriaceae
Moraxellaceae
Pseudomonadaceae
Rhizobiaceae
Rhodobacteraceae
Sphingobacteriaceae
Sphingomonadaceae
WPS-2_other_other_other
Xanthomonadaceae
[Chromatiaceae]
[Weeksellaceae]
All Other Categories


● Risk group 2 bacteria
Proteobacteria
Gammaproteobacteria; Pseudomonadales
Alphaproteobacteria
Rhizobiales
Sphingomonadales
Caulobacterales
Flavobacteriia; Flavobacteriales
Moraxella lincolnii LMG 5127 (NR117693)
Moraxella lincolnii CCUG 9405 (NR117189)
Enhydrobacter aerosaccus G (NR029005)
OTU 99 (13.9%; detected in 13/14 sponges)
Moraxella osloensis DSM 6998 (NR113392)
Moraxella osloensis A1920 (NR104936)
Acinetobacter ursingii DSM 16037(AIEA01000080)
OTU 2385 (1.8%; detected in 14/14 sponges)
Acinetobacter septicus AK001 (NR116071)
Acinetobacter junii Mannheim 2723/59 (NR119360)
Acinetobacter baumannii DSM 30007 (NR117677)
Acinetobacter seifertii LUH 1472 (NR134684)
Acinetobacter oleivorans DR1 (NR102814)
Acinetobacter pittii CIP 70.29 (NR116774)
Acinetobacter pittii ATCC 19004 (NR117621)
OTU 1492 (3.7%; detected in 8/14 sponges)
Acinetobacter haemolyticus ATCC 17906 (NR117622)
Acinetobacter johnsonii Mannheim 3865/60 (NR044975)
OTU 893 (14.1%; detected in 13/14 sponges)
Acinetobacter johnsonii ATCC 17909 (NR117624)
Pseudomonas reinekei MT1 (NR042541)
Pseudomonas alcaligenes ATCC 14909 (NR114472)
Pseudomonas fulva IAM1529 (NR115610)
Pseudomonas cremoricolorata NBRC 16634 (NR113855)
OTU 2007 (7.6%; detected in 12/14 sponges)
Pseudomonas parafulva NBRC 16636 (NR113856)
Pseudomonas parafulva AJ 2129 (NR040859)
Beijerinckia fluminensis UQM 1685 (Rhizobium radiobacter) (NR116306)
Agrobacterium tumefaciens NCPPB2437 (NR115516)
OTU 2089 (3.2%; detected in 13/14 sponges)
Agrobacterium larrymoorei AF3.10 (NR026519)
Agrobacterium fabrum C58 (NR074266)
Sphingobium yanoikuyae NBRC 15102 (NR113730)
Sphingobium yanoikuyae GIFU 9882 (NR115524)
OTU 586 (2.3%; detected in 11/14 sponges)
Sphingobium yanoikuyae IFO 15102 (NR036767)
Sphingobium herbicidovorans NBRC 16415 (NR113843)
Sphingobium ummariense RL-3 (NR044171)
Brevundimonas diminuta NBRC 12697 (NR113602)
Brevundimonas diminuta LMG 2089 (NR114708)
OTU 1844 (3.2%; detected in 13/14 sponges)
Brevundimonas lenta DS-18 (NR044186)
Brevundimonas staleyi FWC43 (NR114710)
Brevundimonas bullata IAM 13153 (NR025831)
Chryseobacterium hominis NF802 (NR042517.2)
OTU 3112 (8.4%; detected in 13/14 sponges)
Chryseobacterium hagamense RHA2-9 (NR115852)
Chryseobacterium haifense H38 (NR044167)
OTU 47 (7.5%; detected in 12/14 sponges)
Chryseobacterium arthrosphaerae CC-VM-7 (NR116977)
Chryseobacterium lactis KC1864 (NR126256)
Chryseobacterium bovis H9 (NR044166)
0.02

video

Microbioma - Aplicações

Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

Matthias Hess,[1,2*] Alexander Sczyrba,[1,2*] Rob Egan,[1,2] Tae-Wan Kim,[3] Harshal Chokhawala,[3]
Gary Schroth,[4] Shujun Luo,[4] Douglas S. Clark,[3,5] Feng Chen,[1,2] Tao Zhang,[1,2]
Roderick I. Mackie,[6] Len A. Pennacchio,[1,2] Susannah G. Tringe,[1,2] Axel Visel,[1,2] Tanja Woyke,[1,2]
Zhong Wang,[1,2] Edward M. Rubin[1,2]†

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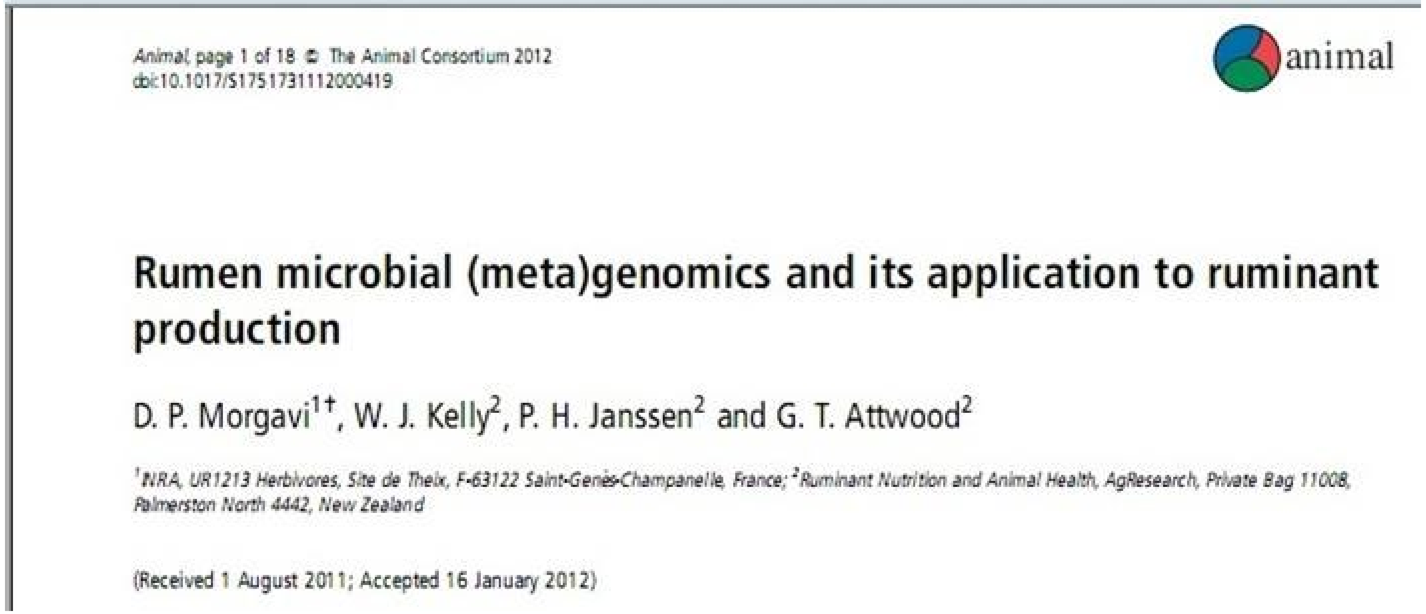

Animal, page 1 of 18 © The Animal Consortium 2012
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animal

Rumen microbial (meta)genomics and its application to ruminant production

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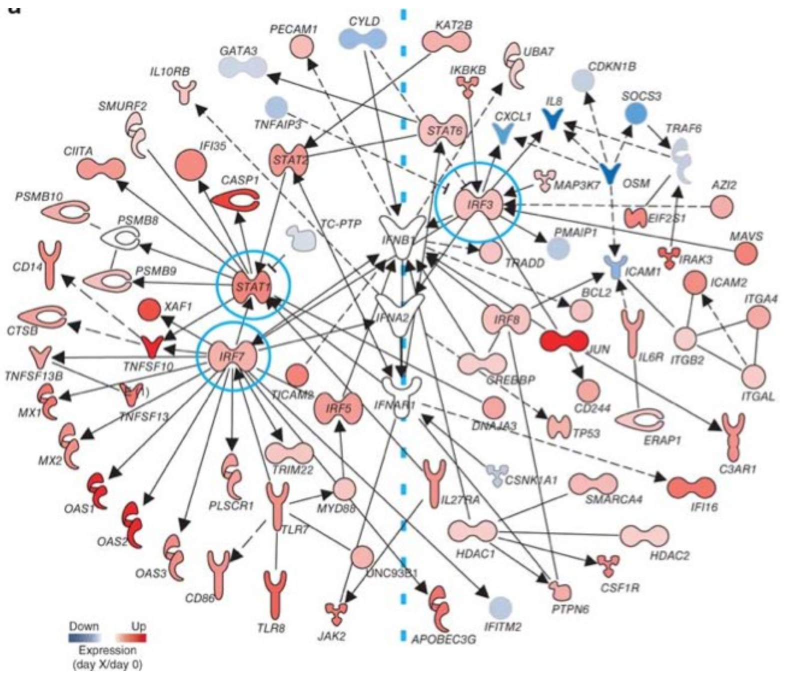
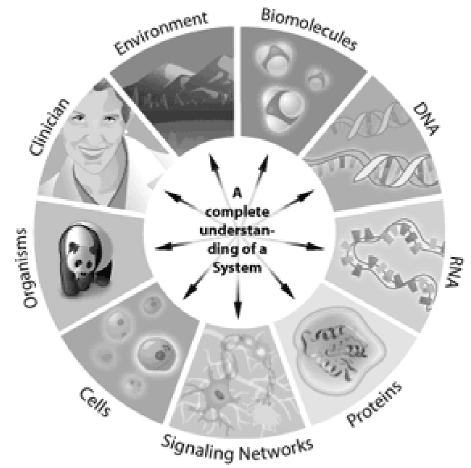
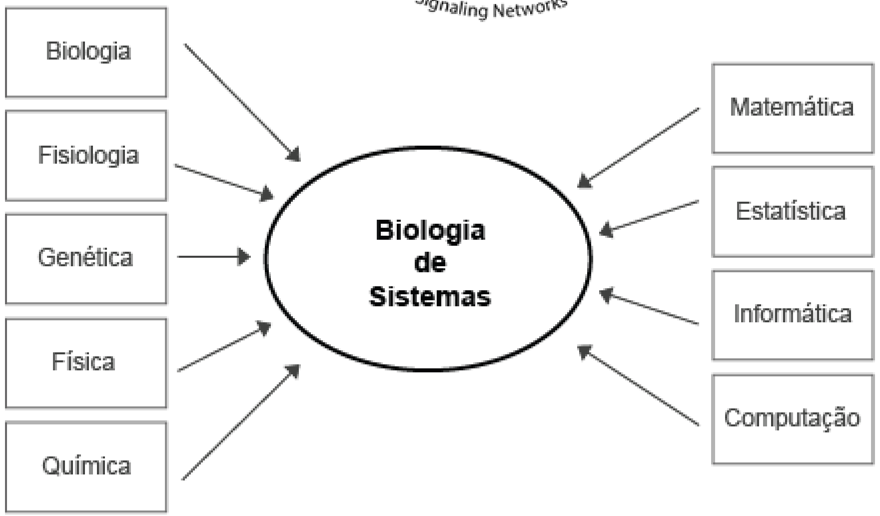
O grande desafio:
Visão Tradicional x Holística





Biologia de Sistemas!



Biologia

Fisiologia

Genética

Física

Química

Biologia de Sistemas

Matemática

Estatística

Informática

Computação

https://www.youtube.com/watch?v=9mUcScHdcus

ESTUDO DIRIGIDO

1. Conceito de ômicas;
2. Construção de biblioteca genômica;
3. Construção de biblioteca cDNA;
4. Conceito de metagenômica.