FRAGMENTS OF LIFE:

DETECTING MICROBES AND DISEASE BY MOLECULAR SIGNATURES

TAGGTCCATACTATGGT

AGGTCGCGCTTTGGT

GAGTTGCCATACACACC

JULIANA ANSARI, PHD

FAIRFIELD UNIVERSITY ROSS LECTURE

11-29-17

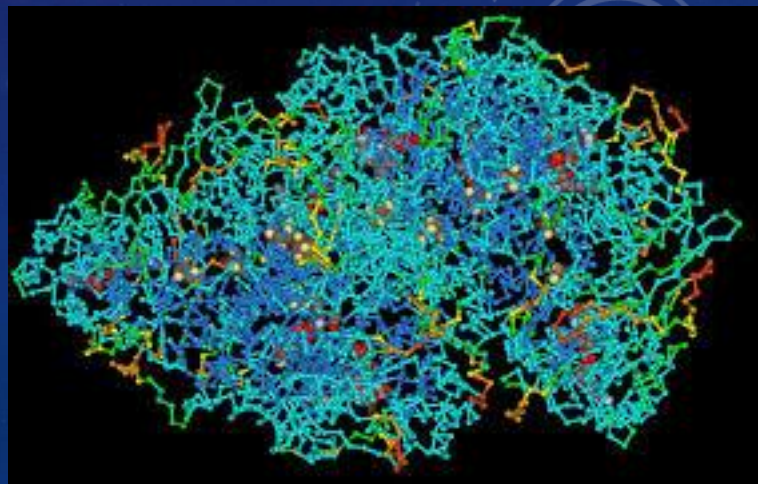
TOPICS FOR TODAY

- **Part I. Identifying & Characterizing Beneficial Microbes**
  - **Microbiome explosion & Future outlook for probiotics & therapies**
  - **3 approaches to identify bacterial strains**
    - **PCR**
    - **MALDI TOF**
    - **BIOLOG**
  - **Probiotics & unknowns**
  - **Carbon Sources & Prebiotics: What the microbes we eat, eat!**

- **Part II. Molecular signatures of Human Disease**
  - **Genetic markers vs. Circulating biomarkers of disease**
  - **miRNAs associated with cancer**

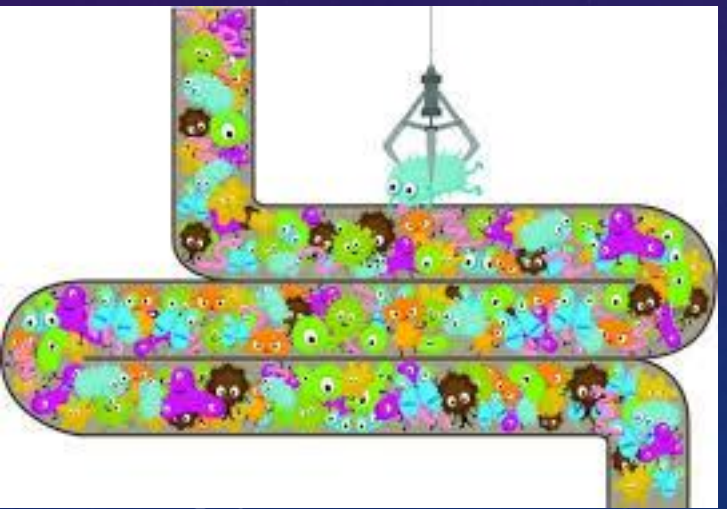
DNA
RNA (PART 2)
PROTEIN
PROTEIN ACTIVITY

(PHENOTYPE, BIOCHEMISTRY, METABOLISM)



MICROBIOME EXPLOSION…

- The Microbiome is a Hot Topic
- All the microbes (bacteria) in or on your body
- Linked to many chronic conditions, digestive & mental health



Articles from 1 week -
Microbiome connection to:
- PTSD
- Autoimmune disease
- Obesity
- Age-related
- How patients respond to cancer Immunotherapy drugs

THE GUARDIAN 11/6/17

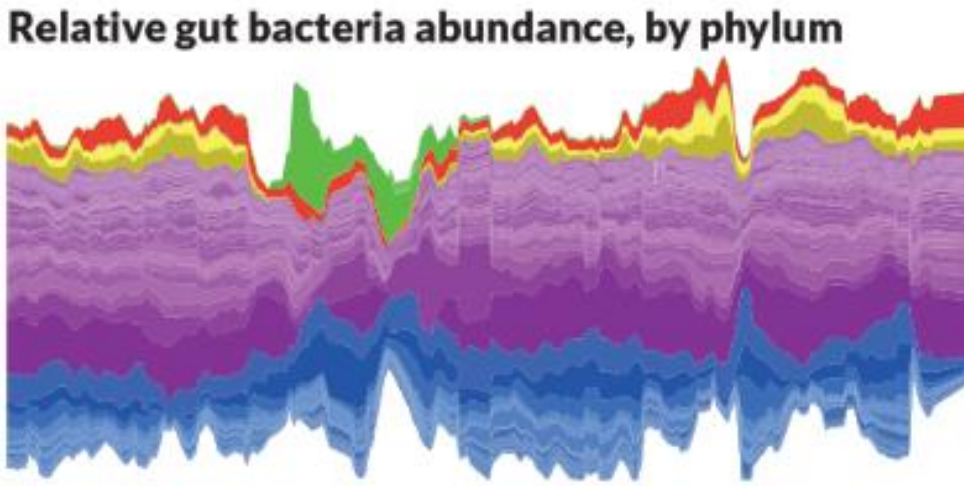
The past month alone has seen studies linking the gut microbiome with post-traumatic stress disorder (people with PTSD had lower than normal levels of three types of gut bacteria); fathoming its connection with autoimmune disease; finding that tea alters the gut microbiome in anti-obesogenic ways; showing that "ridiculously healthy" 90-year-olds have the gut microbiome of young adults; and how targeting mosquitos' gut flora could help beat malaria by increasing the malaria-attacking bacteria in their guts. And last week, two groundbreaking studies provided evidence that gut biodiversity influences whether or not immunotherapy drugs shrink tumours in cancer patients.

https://www.theguardian.com/lifeandstyle/2017/nov/06/microbiome-gut-health-digestive-system-genes-happiness

Is your gut microbiome the key to health and happiness?

Research suggests the vast ecosystem of organisms that lives in our digestive systems might be as complex and influential as our genes in everything from mental health to athleticism and obesity. But is 'poop doping' really the way ahead?

The gut microbiome weighs more than the brain. Illustration: Andy Goodman/Five Bar Gate

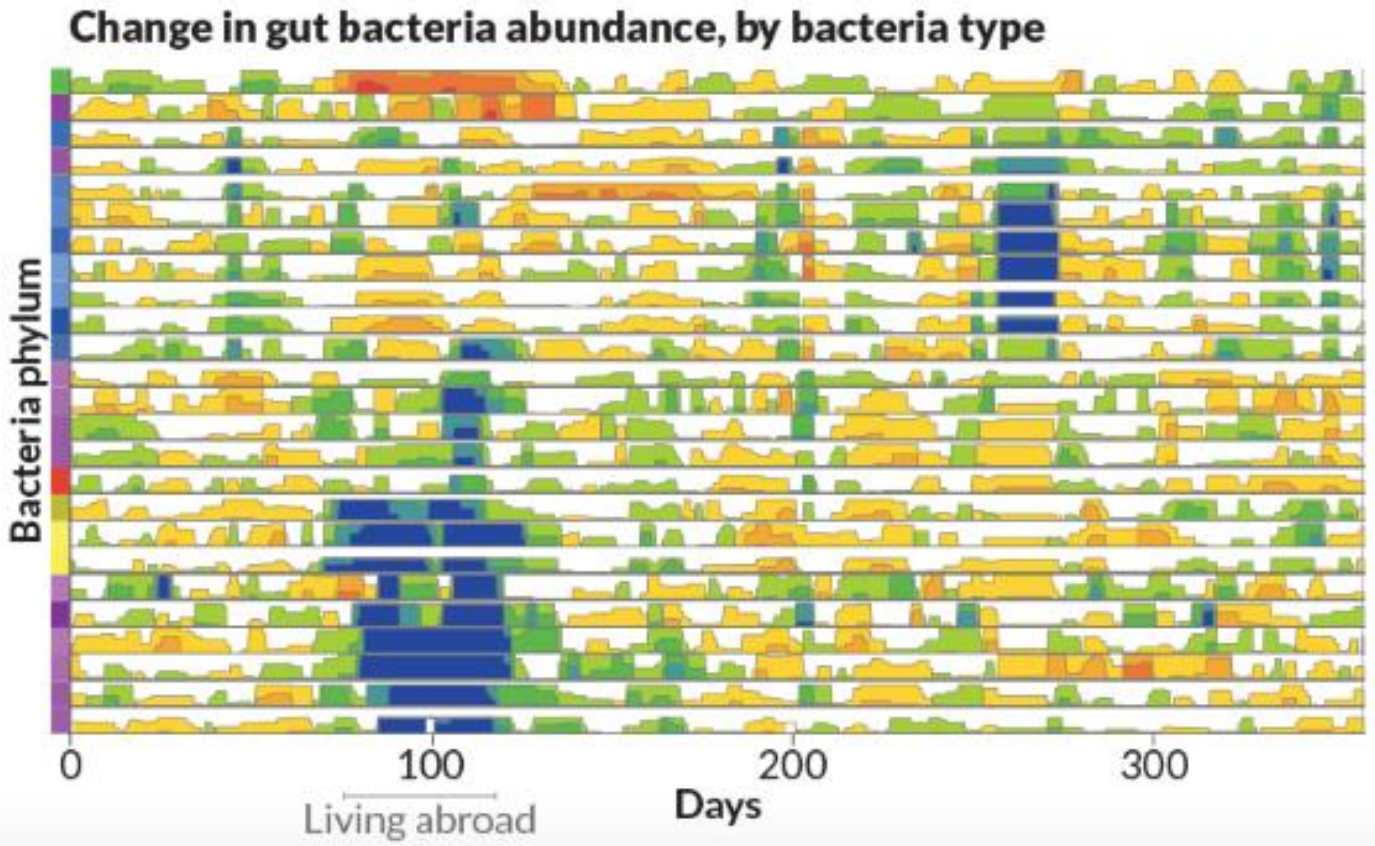


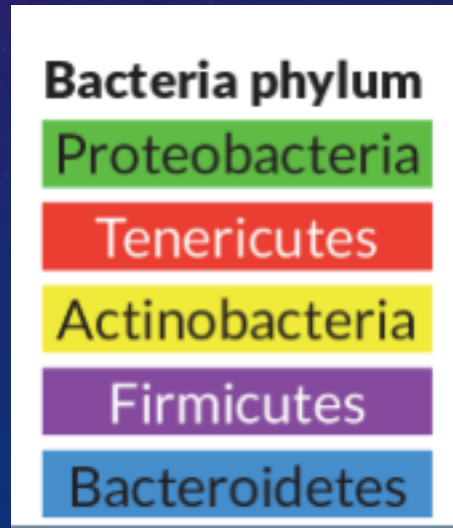
SCIENCE VISUALIZED MICROBIOLOGY, HEALTH

How one scientist's gut microbes changed over a year

Daily sampling allowed Lawrence David to track fluctuations in his microbiome

BY TINA HESMAN SAEY 11:03AM, SEPTEMBER 21, 2016





"UNLOCKING THE SECRETS OF THE MICROBIOME"
NEW YORK TIMES 11/6/17

For example, people with irritable bowel syndrome, inflammatory bowel disease, allergic disorders and infections with drug-resistant organisms may benefit from taking probiotics, though some probiotics sold in health food and drugstores may be ineffective. It may be necessary to tailor-make the remedy for each condition or even each patient.

20 COMMENTS

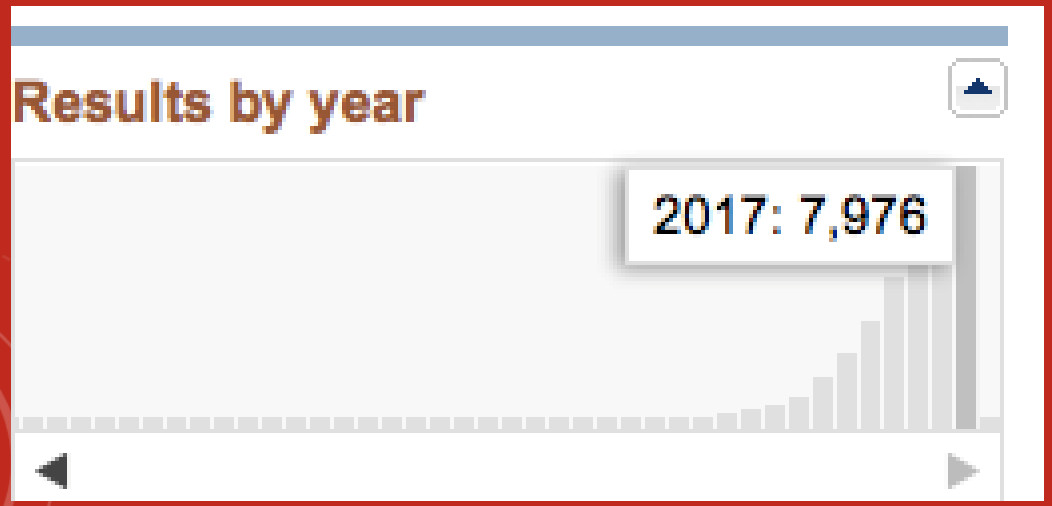
Meanwhile, people interested in fostering a health-promoting array of gut microorganisms should consider shifting from a diet heavily based on meats, carbohydrates and processed foods to one that emphasizes plants. As Dr. Jeffrey Gordon, a genomics specialist at Washington University School of Medicine, told The Times last year, "The nutritional value of food is influenced in part by the microbial community that encounters that food."

EAT FIBER, PLANTS!

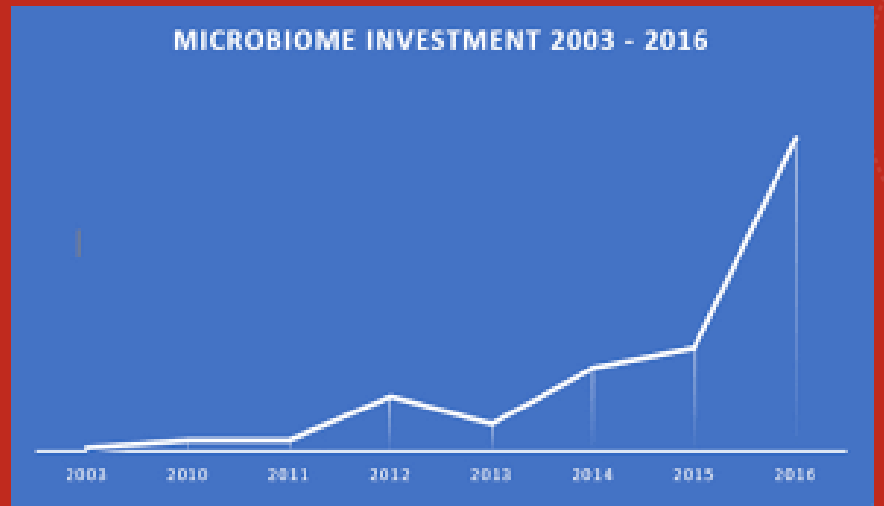
- https://www.nytimes.com/2017/11/06/well/live/unlocking-the-secrets-of-the-microbiome.html

MICROBIOME

RESEARCH PUBLICATIONS



INVESTMENT FUNDING



THE ECONOMIST 11/9/17

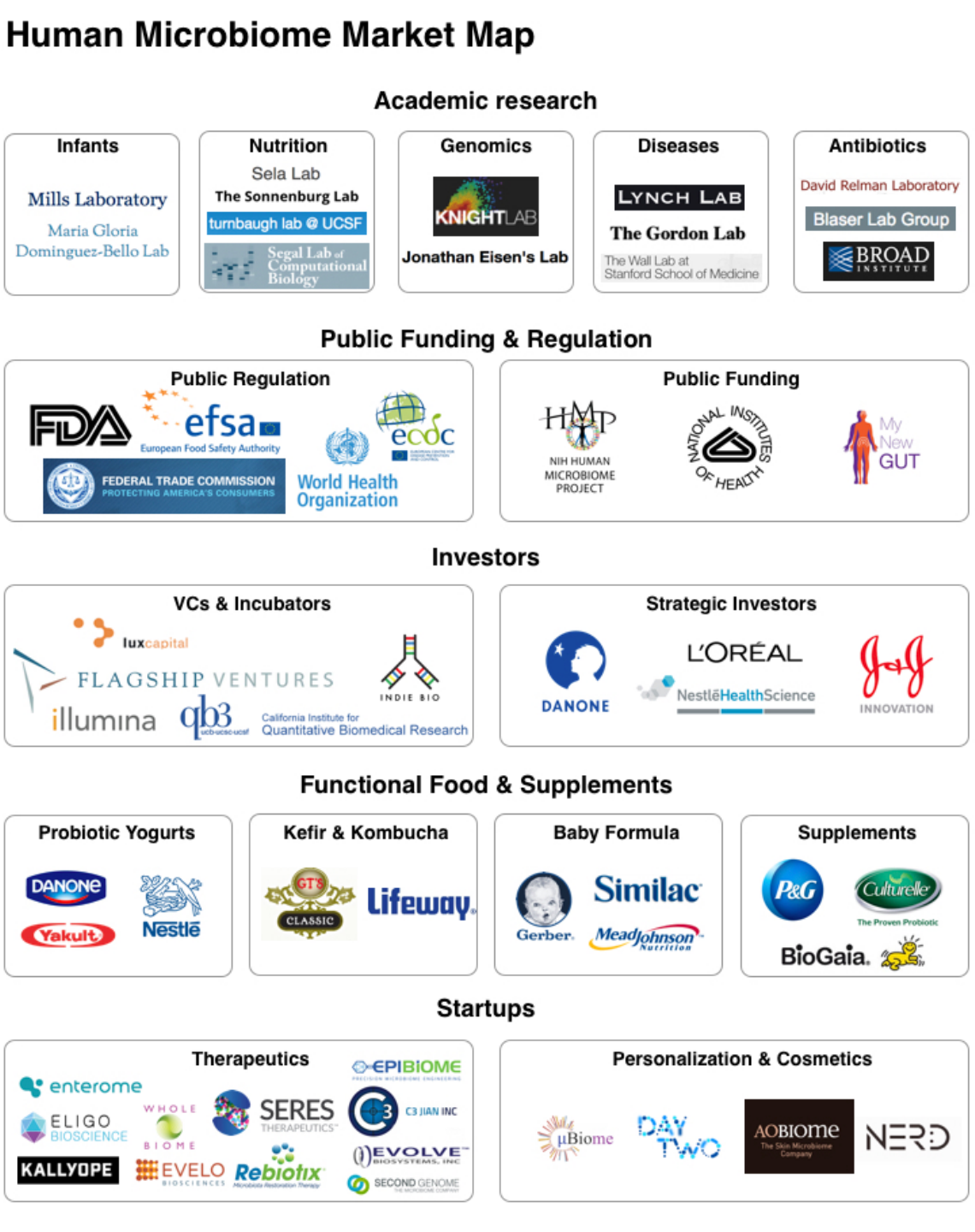
Companies mentioned:

Rebiotix
EpiBiome
Eligo Biosciences
Seres Therapeutics
C3J Therapeutics

https://www.economist.com/news/science-and-technology/21731109-no-guts-no-glory-enhanced-understanding-microbiome-helping-medicine

PROBIOTICS->

THERAPEUTICS->



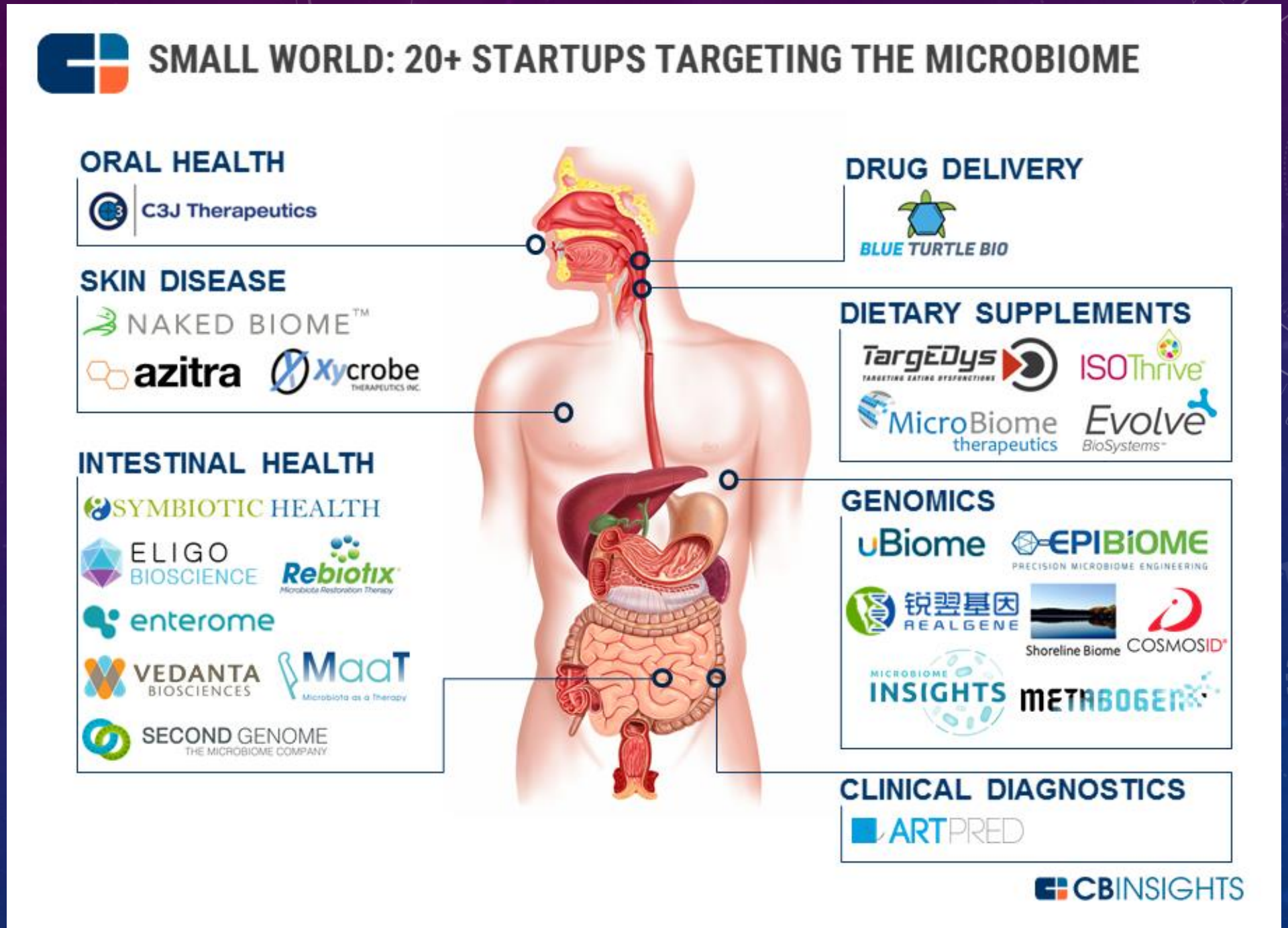
CT:

Azitra,

Shoreline Biome

MA:

Vedanta, SynLogic, Seres Therapeutics



CA:

Evolve Biosystems

EpiBiome

MICROBIOME THERAPEUTICS & PRECISION PROBIOTICS ARE ON THE HORIZON

Early Stage Companies

Seres Therapeutics – *Clostridium difficile (Cdiff)* microbiome-based therapy

GUSTO – Dr. Jack Gilbert of U Chicago using Computational modelling of bacterial interactions to design probiotics

trials. Most notably last year, a promising drug from Seres Therapeutics' meant to treat Clostridium difficile infection (or C. diff.) failed its Phase II clinical trials, sending their stock price tumbling and casting doubt on whether microbiome therapeutic are effective.

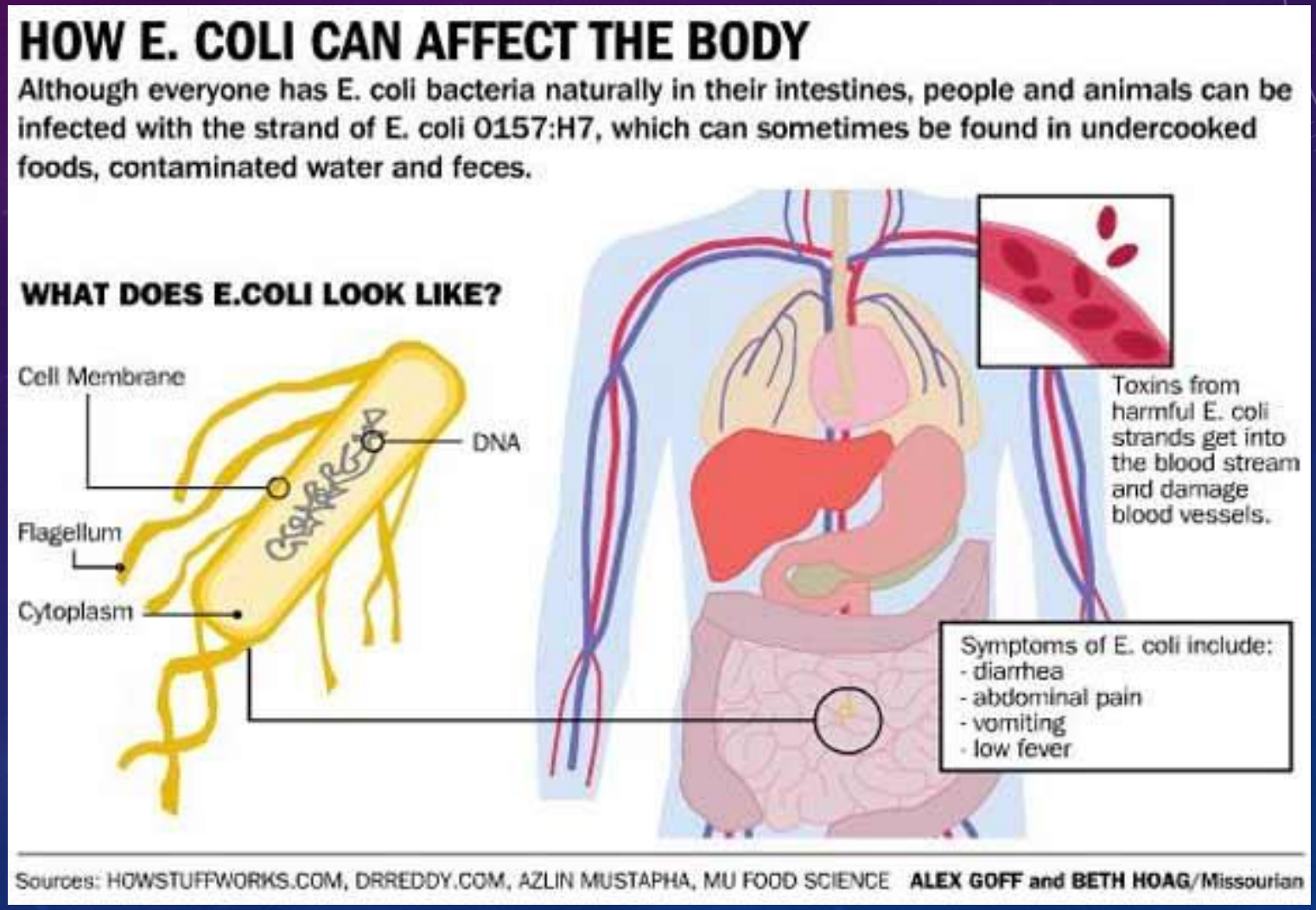
However, Gilbert believes that Gusto's systems biology approach will help avoid these unexpected failures in clinical trials.

"We want to build the car but we're going to build the car by redesigning it from the ground up," Gilbert said. "What Tesla did with electric vehicles...we're doing the same with probiotic formulations."

BACTERIAL POPULATIONS EVOLVE RAPIDLY & CAN EASILY BECOME CONTAMINATED, SO NEED STRICT QUALITY CONTROL!

- *You want to make sure the strain is the right one & maintains its beneficial effect!*

THE STRAIN MATTERS – *E. COLI* IS A COMMENSAL BUT SOME STRAINS CAUSE BLOODY DIARRHEA (0157:H7)

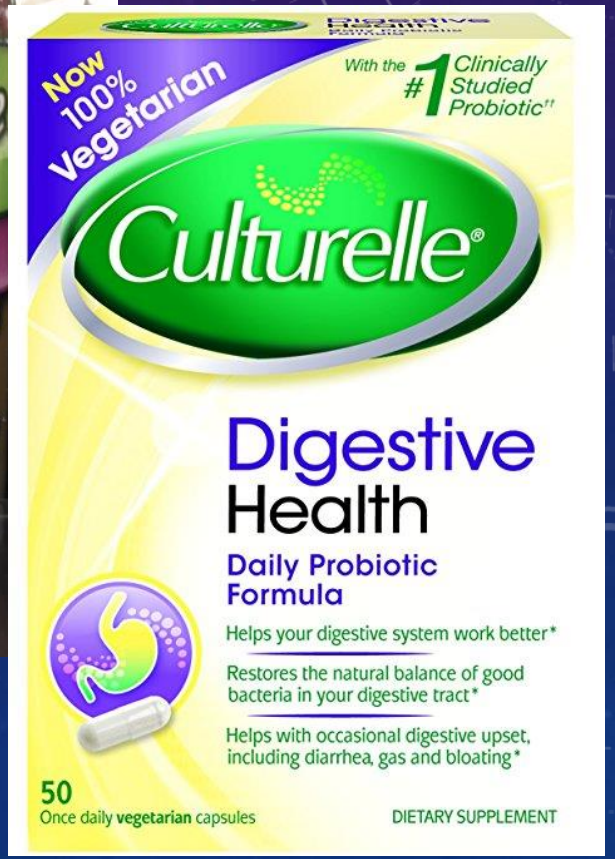


PROFILING PROBIOTIC MICROBES FROM FOOD & SUPPLEMENTS

- MORPHOLOGY
- BIOCHEMISTRY
- GENE SEQUENCING
- PHENOTYPIC PROFILING
- MASS SPECTROMETRY

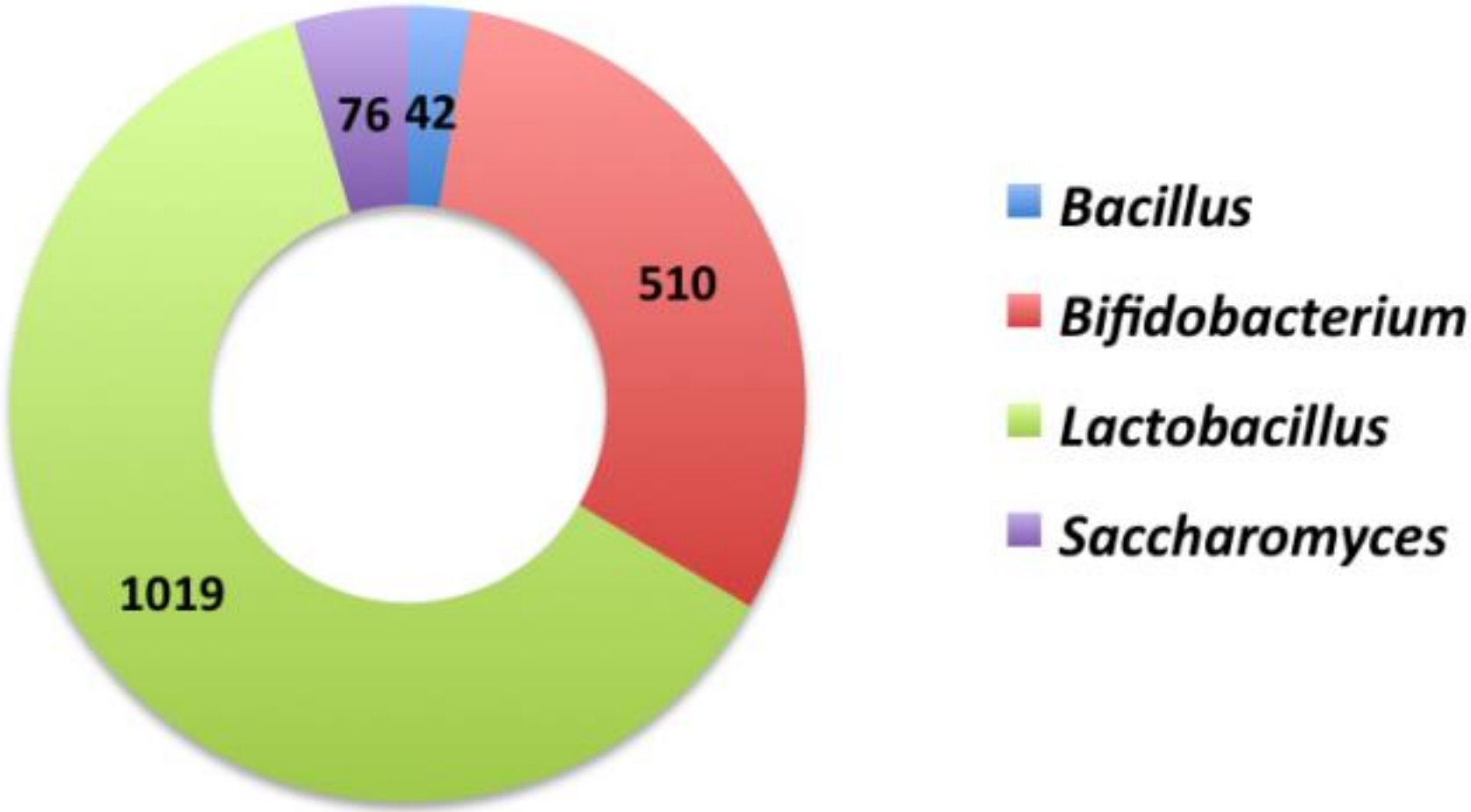
“WHAT’S THE DIFFERENCE BETWEEN ME & YOU?”

DR. DRE & EMINEM
2001



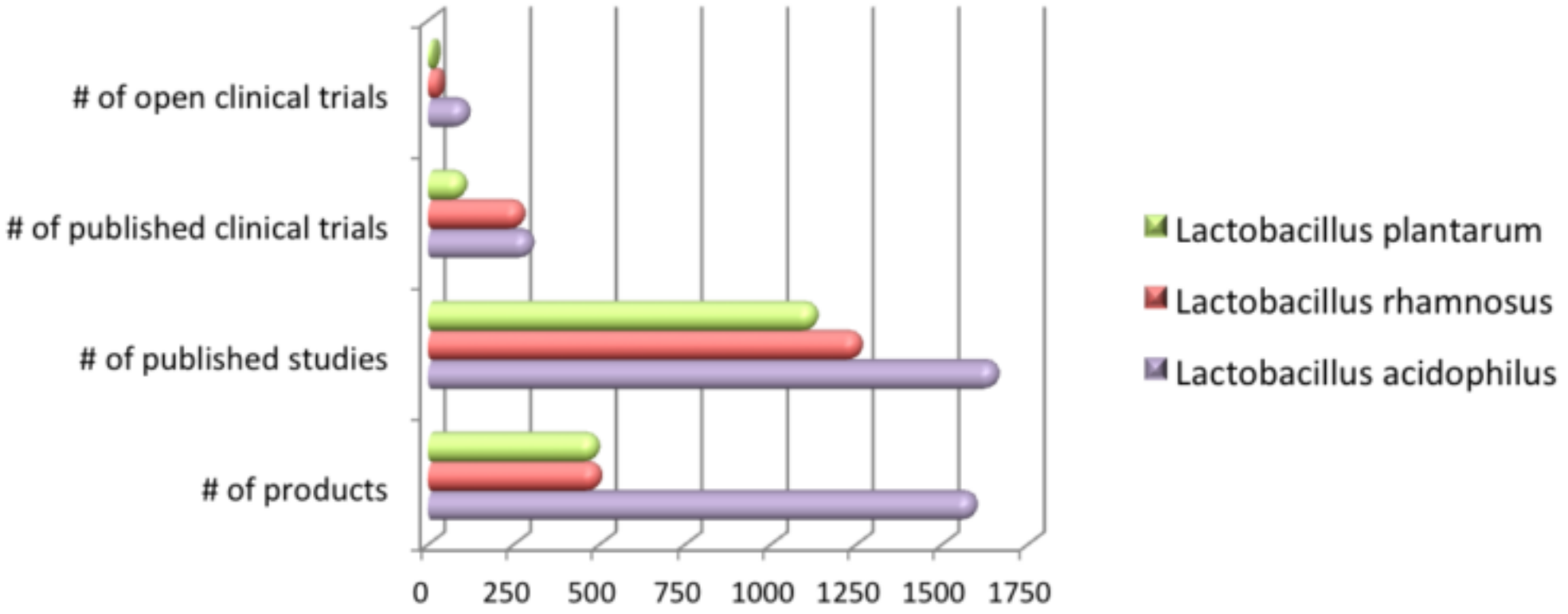
Clinical Trials on Probiotic Microbes

Sorce: PubMed.gov Clinical Trials published as of Mar. 2017



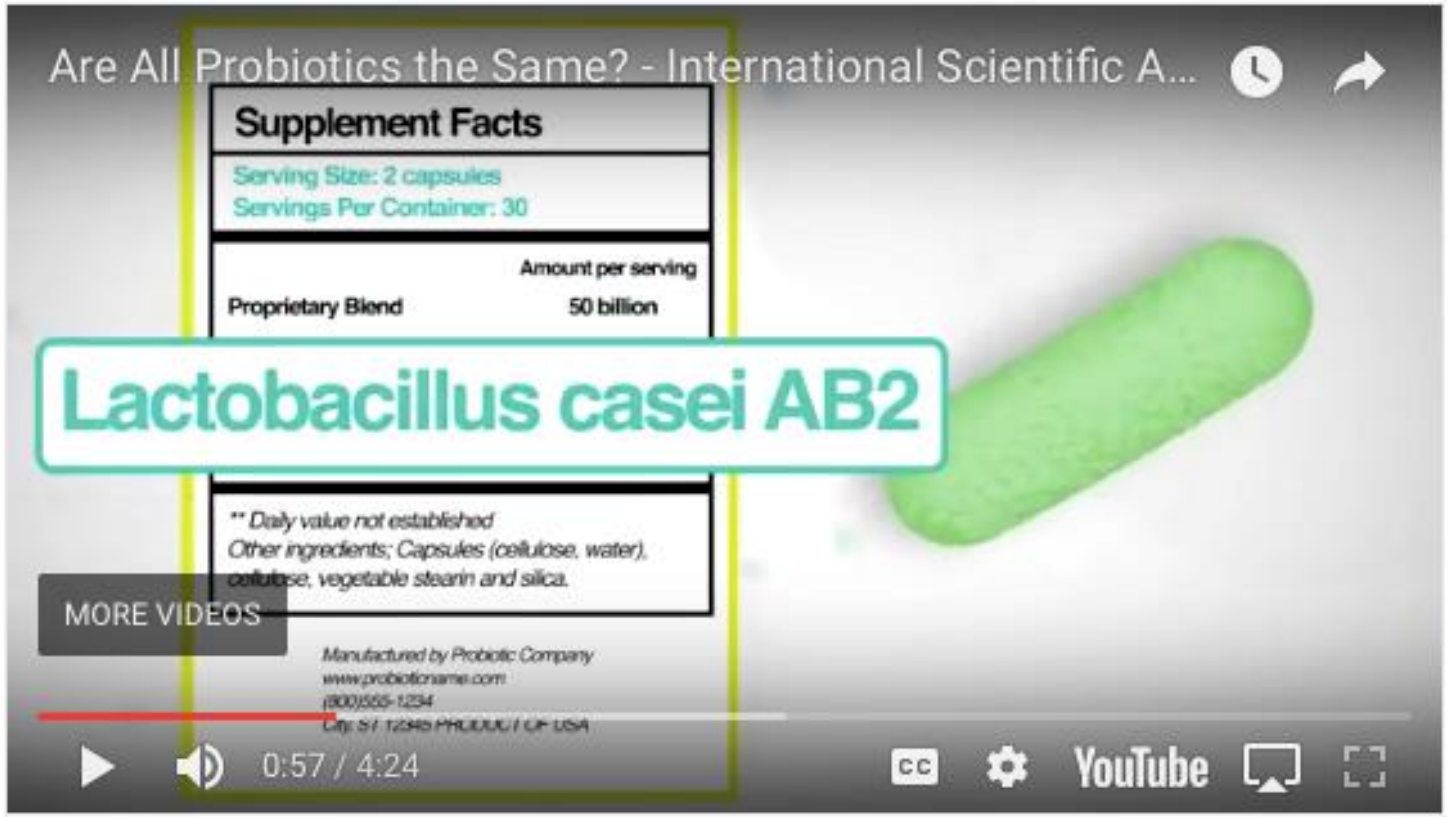
How well-studied are Lactobacillus strains?

(as of Mar 2017)



ISAPP VIDEO

Are all probiotics the same? Check out the answer here in our new informational video:



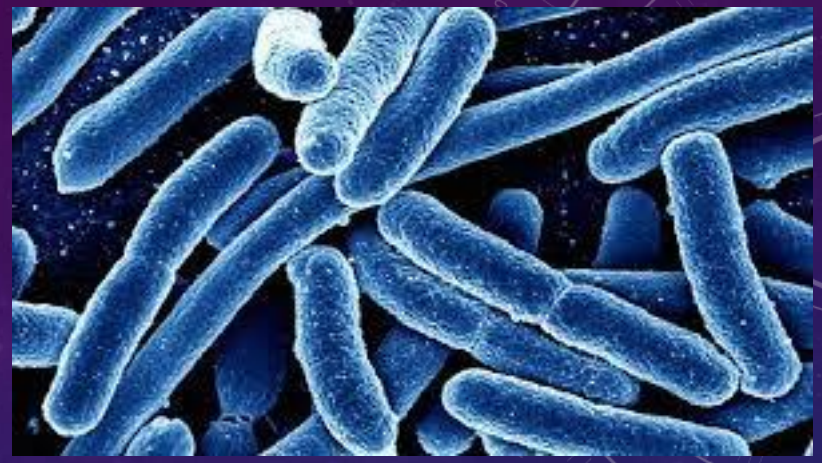
Are All Probiotics the Same? - International Scientific Association...

In this video you will learn more about probiotics and the important differences am…

youtube.com

2:17 PM - 9 Nov 2017

3 WAYS TO IDENTIFY BACTERIA



- 1. Polymerase Chain Reaction (PCR) of 16S rRNA gene
- 2. MALDI-TOF(Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry
- 3. BIOLOG Microbial ID system

GET SPECIES LEVEL ID BUT NOT ALWAYS STRAIN LEVEL

SOURCE OF ISOLATES: PROBIOTIC SUPPLEMENTS & DRINKS

Ansari Lab and Microbiology lab BI 352

STANDARD WORKFLOW TO IDENTIFY BACTERIA

SOURCE

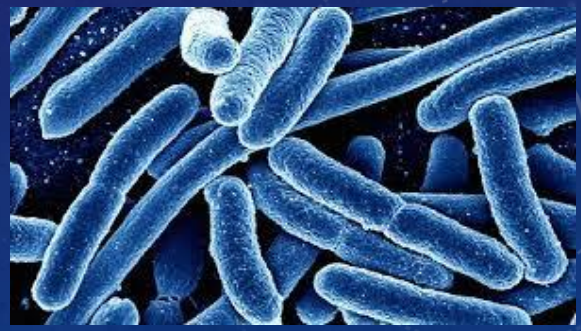
ISOLATED COLONY

APPROACHES:
PCR, BIOLOG, MALDI

ANALYZE (DNA, PROTEIN, METABOLISM)

- STEP 1
- STEP 2
- STEP 3

SEARCH DATABASE
&
GET TOP-SCORING ID!



Hi, My Name is _____

1

SEQUENCING OF 16S RRNA GENE AFTER PCR AMPLIFICATION

STANDARD WORKFLOW TO IDENTIFY BACTERIA

SOURCE

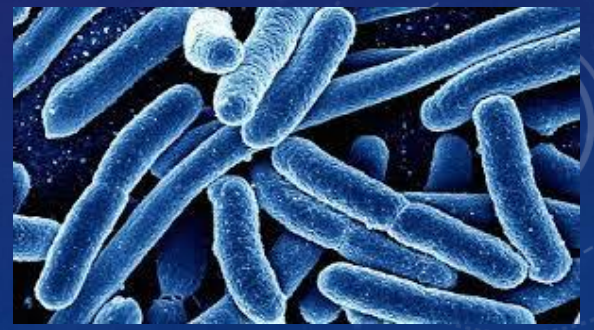
ISOLATED COLONY

ANALYZE DNA: PCR

- PCR amplification
- Gel electrophoresis
- Sequencing of PCR product
- Trim sequences

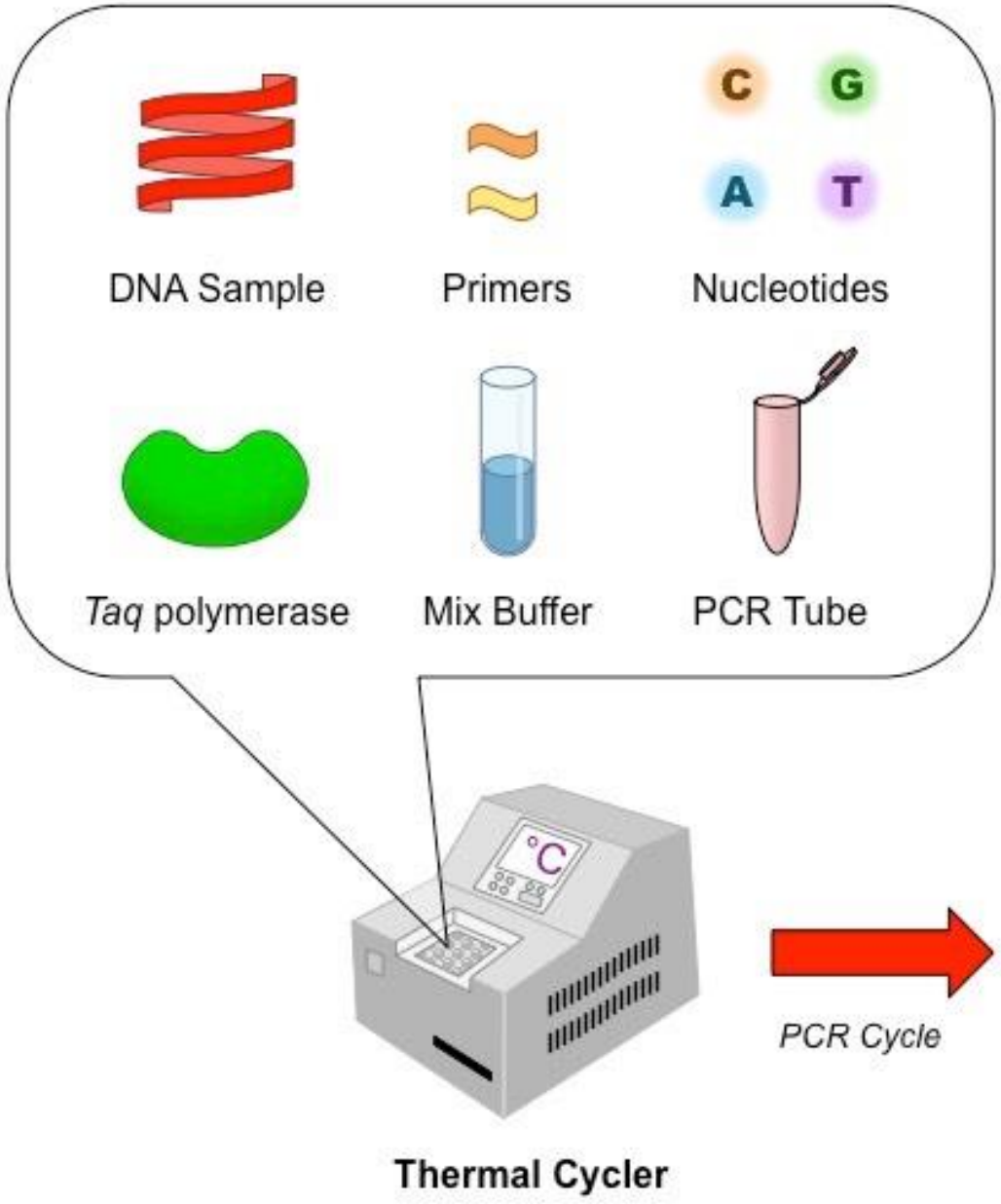
1 PCR

SEARCH DATABASE
(NCBI nr nt database)
NCBI BLASTN

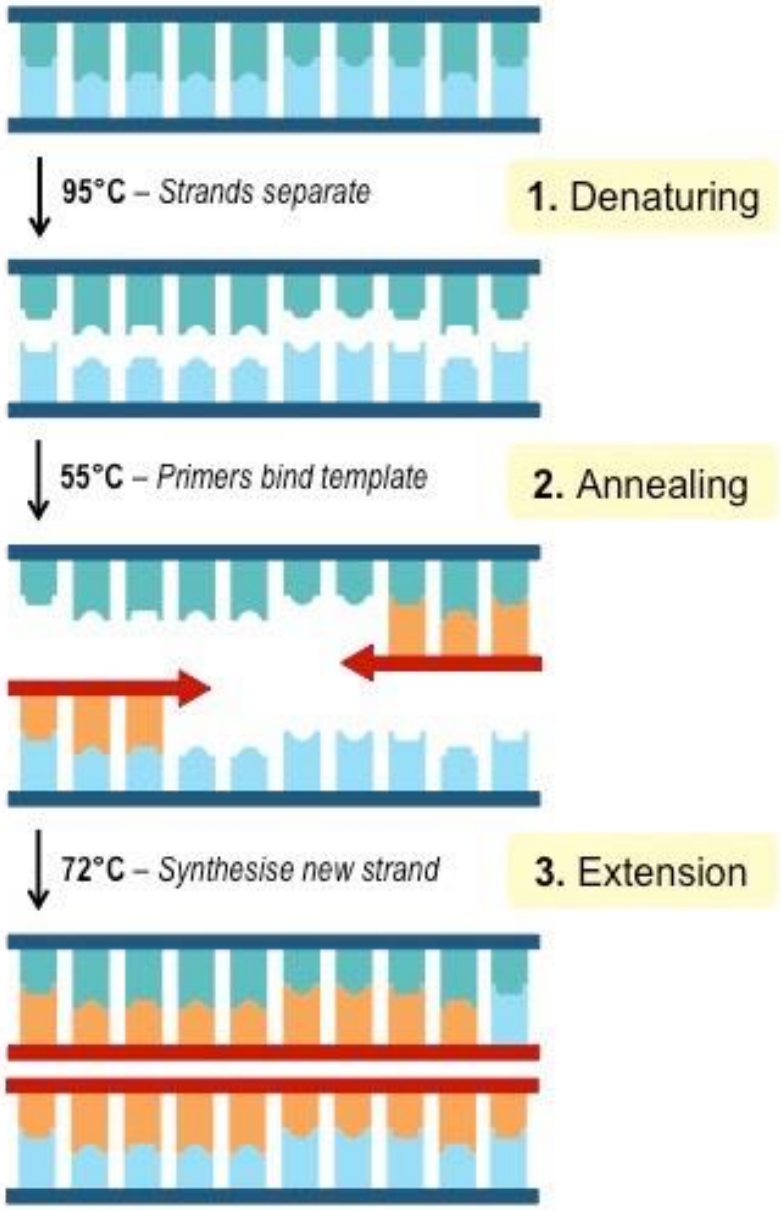


Hi, My Name is _____

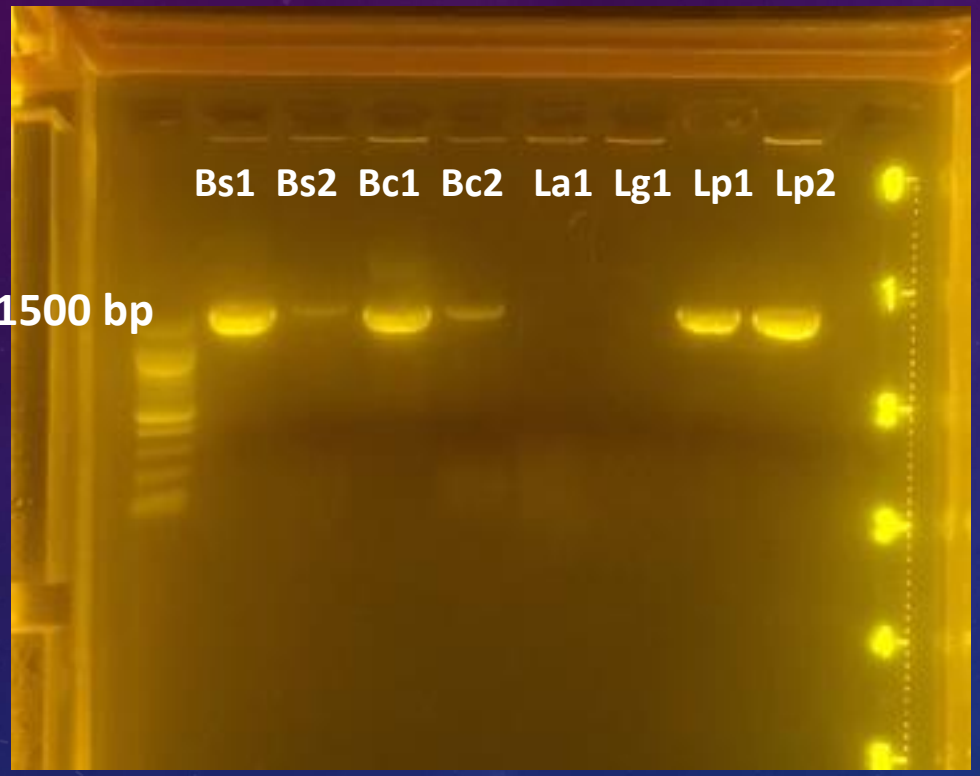
PCR Components

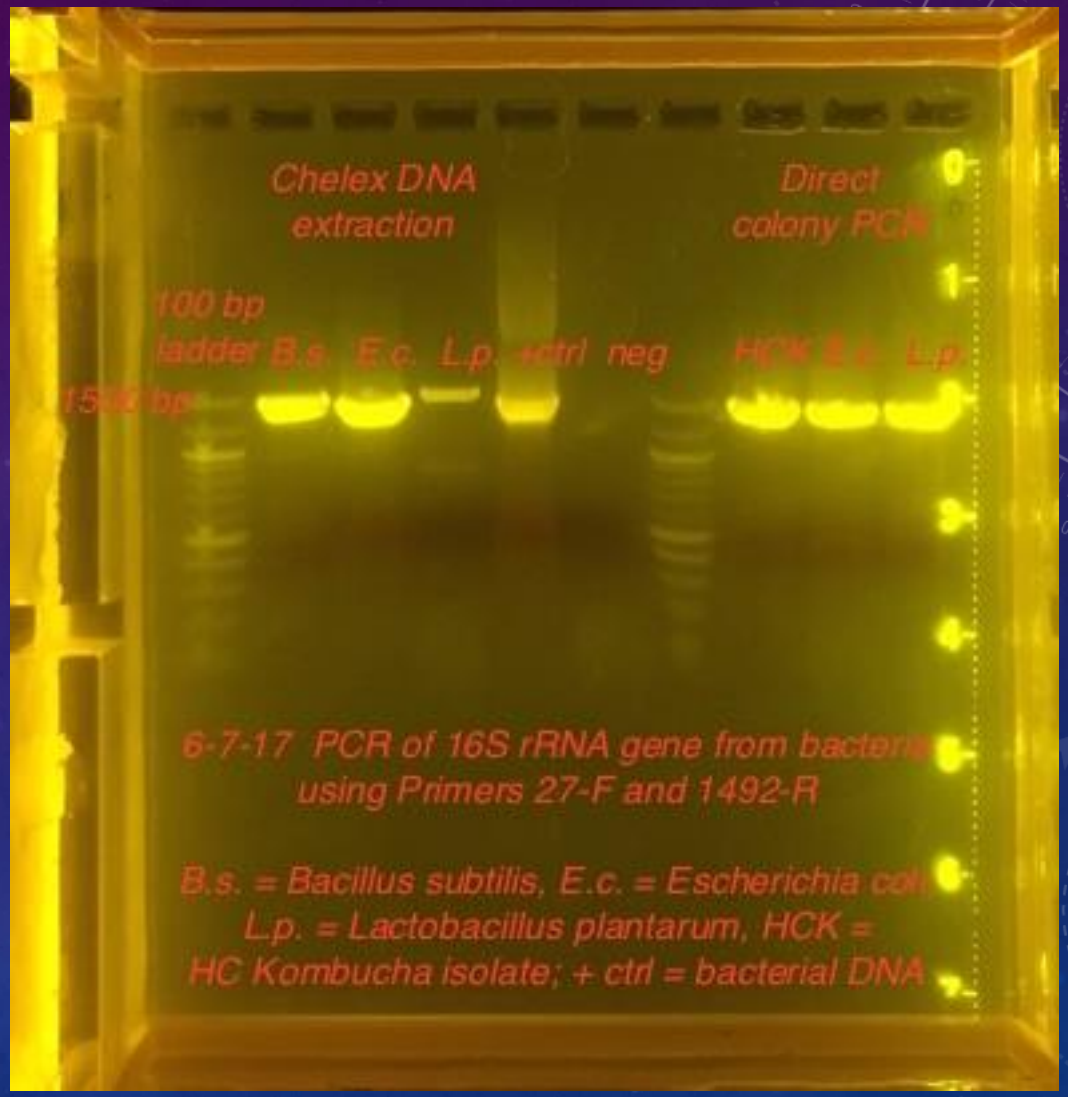


PCR Process (ONE Cycle)



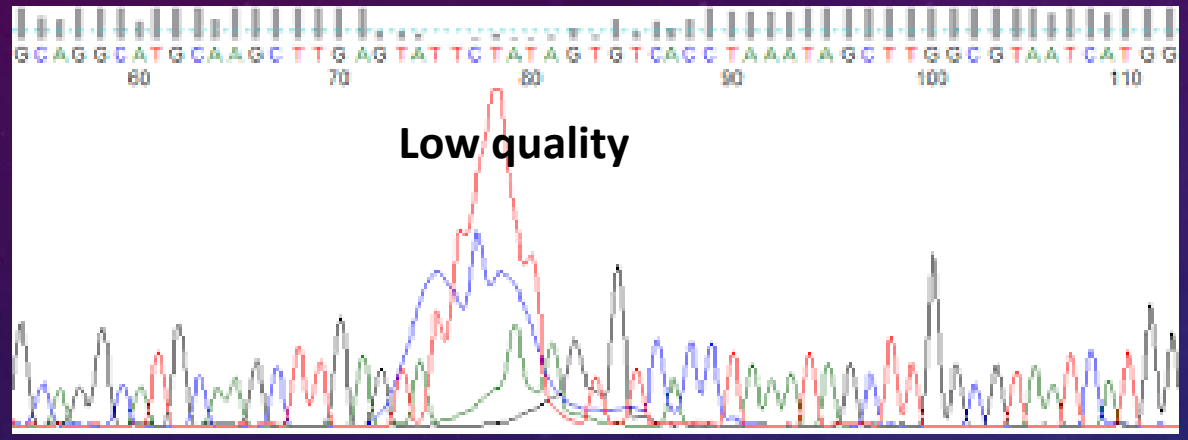
GEL ELECTROPHORESIS OF 16S GENE PCR PRODUCT

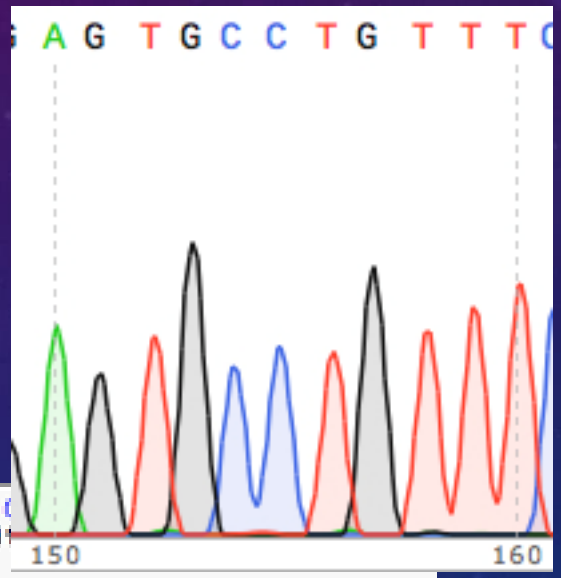


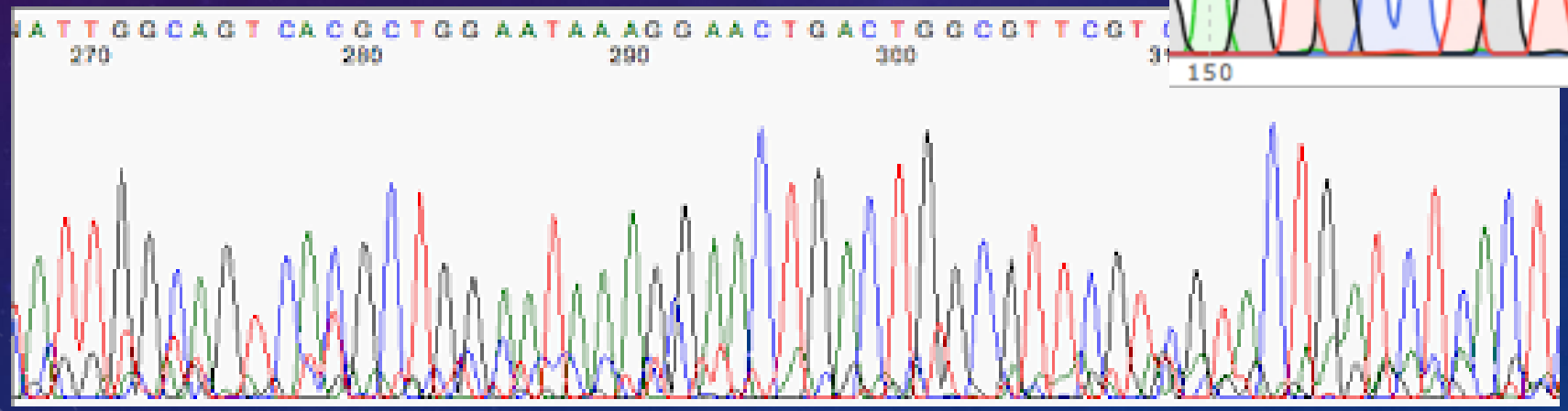


Fairfield U: Takeyce Powell, Eunsun Hong '19, Phil Strang '17

DNA CHROMATOGRAM (SANGER SEQUENCING)







Viewed with Geneious software, Trims low-quality sequences

DNA SEQUENCE OF L. RHAMNOSUS 16S GENE

>Lr-1F-27F Sample_Name=1925207 Chromat_id=1036974 Read_id=910298 Version=1 Length=1393
GTGCGGCAGCTAGACTGCAGTCGAGCGACAGACGAGGAGCTTGCTCCTCTGACGTTAGCGGCGGACGGGTGAGTA
ACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATATTGAACCG
CATGGTTCAATAGTGAAAGACGGTTTTGCTGTCACTTATAGATGGATCCGCGCCGCATTAGCTAGTTGGTAAGGTA
ACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCGGACGGAGCAACGCCGCGTGAGTGAT
GAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAGTGGCTATGCACGTCTTGACGGTA
CCTAAGCGGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTA
TTGGGCGTAAAGCGCGCGTAGGCGGTTTTTAAGTCTGATGTGAAAGCCCGCGGCTCATCCGTGGAGGGTCATTG
GAAACTGGAAAACTTGAGTGCGAAATAAGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCGAAGATATGGA
GGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGG
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GGTGGAGCATGTGGTTTAATTCGAAGCAACGTCGGAAAACCTTACCACATCGTTGACGTCCTCTTACCCCTCTAG
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TGGGTTAGTTCCCGCAACGATCGCAGCCCTTTAGCTTAGTTGCCATCATTAGTTTGGCACTCTAAGTTTACTGCCG
GTGACAACCCGGAGTAGGGTGGGATGACTTCAATCATCATGGCCCCGTTATGATTAGCTACGCCCGTGCTACATCG
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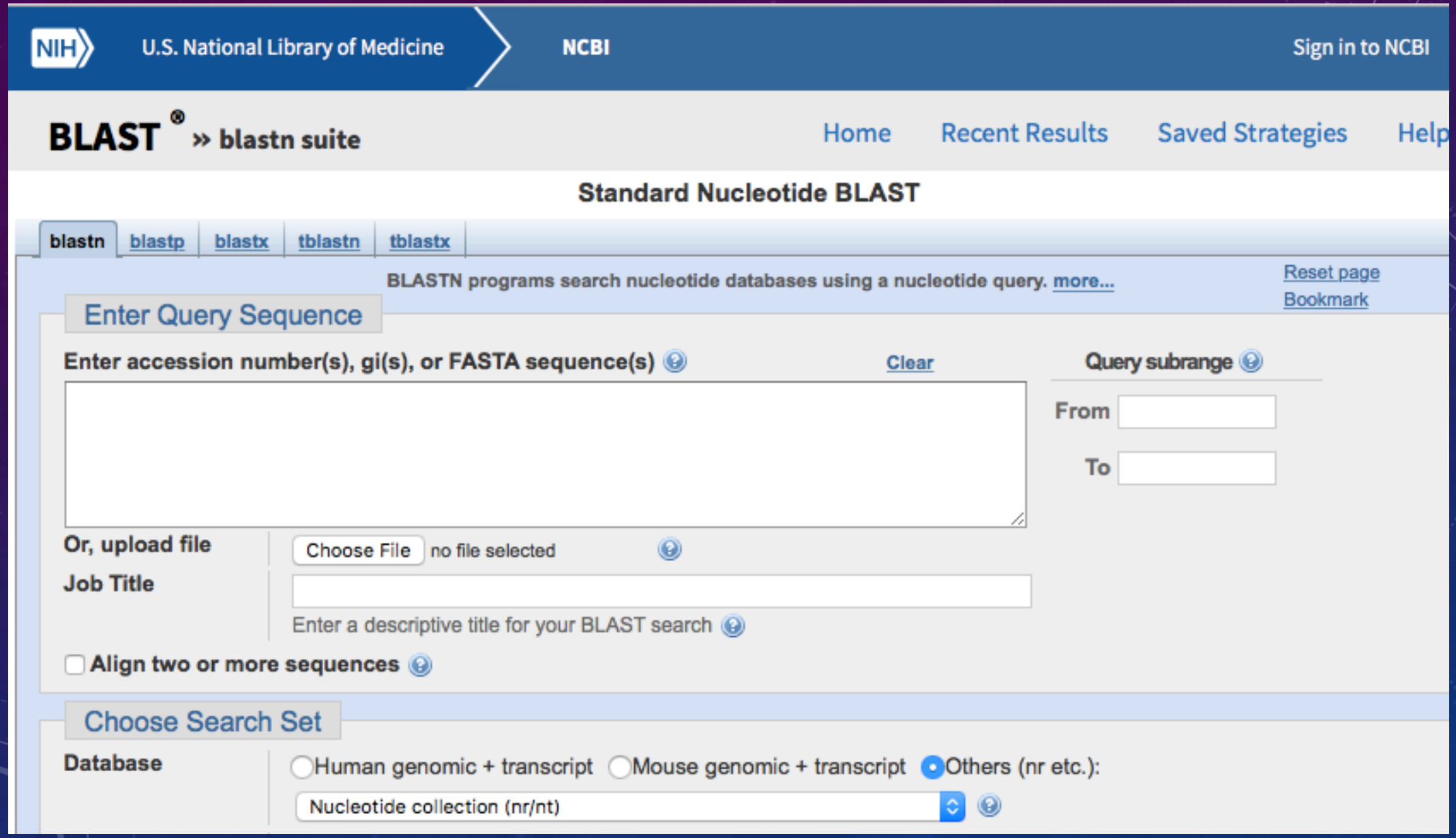
TCGCTATTATTGAGCTGGAAGTCCCGCTAGTATTCGTAGATTCAGCGATGCCTAGCGTGAGTACGGTTCCCGGAGT
CTTGGTTAAGCAGCAGGCGCCGAGCTCGCGTGTCGCG

BLAST SEARCH OF DNA SEQUENCE VS. NCBI NT DATABASE



16S GENE BLAST RESULTS: *L. RHAMNOSUS*

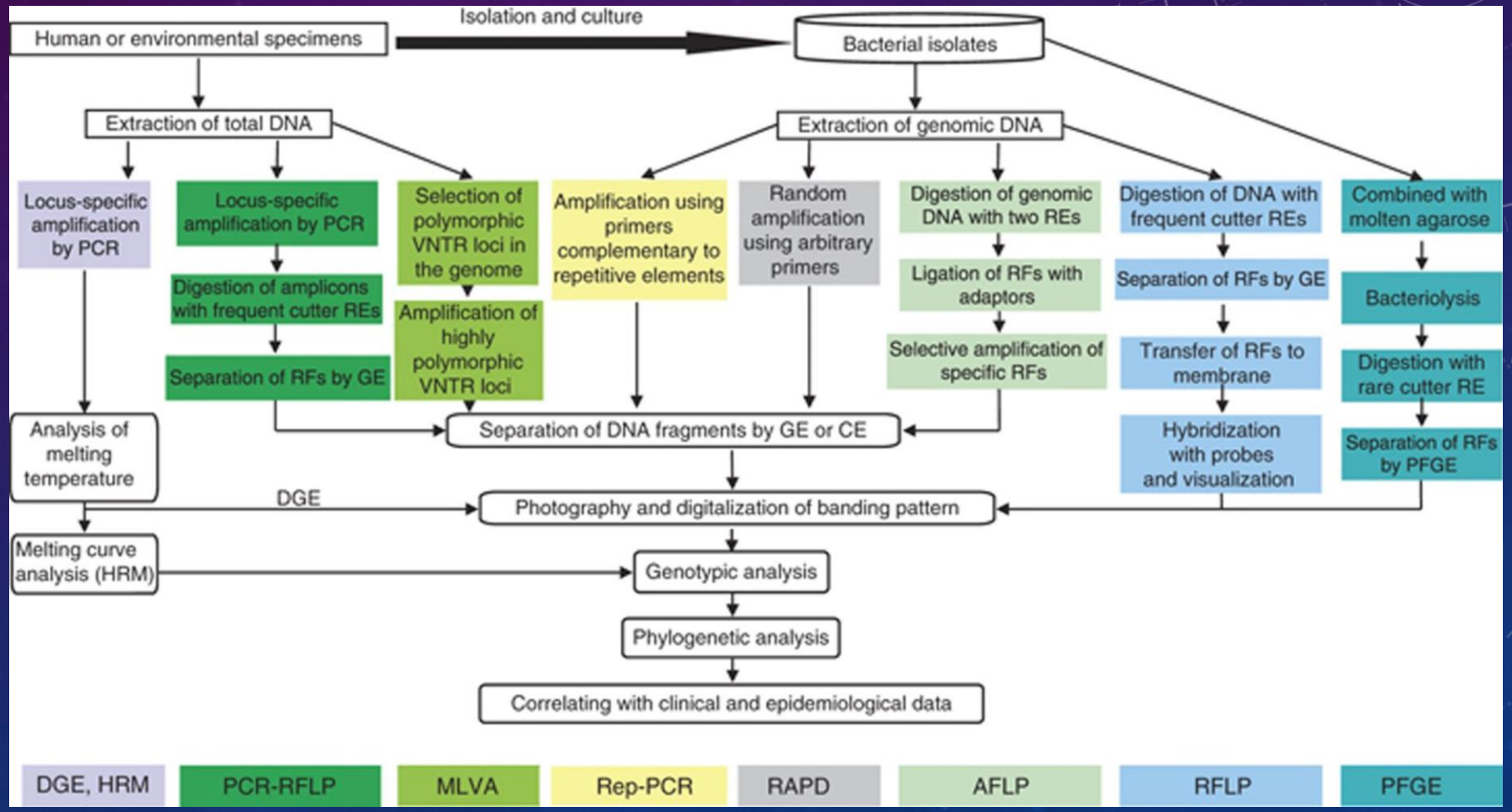
Database	Description	E Value	Free end gaps	Grade
nr	Lactobacillus rhamnosus GG whole genome sequence, strain GG (ATCC 53103)	0	TRUE	89.20%
nr	Lactobacillus rhamnosus strain WQ2 genome	0	TRUE	89.20%
nr	Lactobacillus rhamnosus strain LRB, complete genome	0	TRUE	89.10%
nr	Lactobacillus rhamnosus strain BFE5264, complete genome	0	TRUE	89.20%

16S SEQUENCE -> SPECIES ID BUT NOT SPECIFIC STRAIN

Bc-2F TRIMMED	Digestive Advantage Probiotic								
# Nucleotide Sequenc	# Nucleotides	# Sequences	% Identical Si	% Pairwise Id	%GC	Accession	Bit-Score	Cre	Dat	Description
0	2504	2	99.50%	99.50%	56.30%	JN366734.1	2279.89	We	nr	Bacillus coagulans strain 55N1-6 16S ribosomal RNA gene, partial sequence
0	2506	2	99.40%	99.40%	56.10%	FR727705.1	2272.5	We	nr	Bacillus sp. IMM05 partial 16S rRNA gene, strain IMM05
0	2504	2	99.40%	99.40%	56.20%	KU612257.1	2274.35	We	nr	Bacillus sp. BRTC-4 16S ribosomal RNA gene, partial sequence
0	2502	2	99.40%	99.40%	56.40%	KT351636.1	2272.5	We	nr	Bacillus coagulans 16S ribosomal RNA gene, partial sequence
Bc-2R TRIMMED	**Digestive Advantage Probiotic**								
# Nucleotide Sequenc	# Nucleotides	# Sequences	% Identical Si	% Pairwise Id	%GC	Accession	Bit-Score	Cre	Dat	Description
0	2613	2	99.10%	99.10%	56.30%	CP010525	2348.21	We	nr	Bacillus coagulans strain HM-08, complete genome
0	2612	2	98.90%	98.90%	56.60%	JX569800	2331.59	We	nr	Bacillus coagulans strain KM-1 16S ribosomal RNA gene, partial sequence
0	2612	2	99.30%	99.30%	56.20%	JF764794	2364.83	We	nr	Bacillus coagulans strain 001RC 16S ribosomal RNA gene, partial sequence
0	2611	2	98.90%	98.90%	56.60%	LC140744	2335.29	We	nr	Bacillus coagulans gene for 16S ribosomal RNA, partial sequence, strain: BA375
Bc-Kev-F TRIMMED	**Kevita Probiotic Drink**								
# Nucleotide Sequenc	# Nucleotides	# Sequences	% Identical Si	% Pairwise Id	%GC	Accession	Bit-Score	Cre	Dat	Description
0	813	2	94.10%	94.10%	57.20%	CP017888	617.901	We	nr	Bacillus coagulans strain BC-HY1, complete genome
0	813	2	94.10%	94.10%	57.50%	CP017888	617.901	We	nr	Bacillus coagulans strain BC-HY1, complete genome
0	813	2	94.30%	94.30%	57.50%	CP017888	623.441	We	nr	Bacillus coagulans strain BC-HY1, complete genome
0	813	2	94.30%	94.30%	56.80%	CP011939	623.441	We	nr	Bacillus coagulans strain S-lac, complete genome
Bc-Kev-R TRIMMED	**Kevita Probiotic Drink**								
# Nucleotide Sequenc	# Nucleotides	# Sequences	% Identical Si	% Pairwise Id	%GC	Accession	Bit-Score	Cre	Dat	Description
0	1122	2	98.40%	98.40%	56.70%	CP011939	987.231	We	nr	Bacillus coagulans strain S-lac, complete genome
0	1122	2	98.40%	98.40%	56.70%	CP010525	987.231	We	nr	Bacillus coagulans strain HM-08, complete genome
0	1122	2	98.40%	98.40%	56.70%	CP003056	987.231	We	nr	Bacillus coagulans 36D1, complete genome
0	1122	2	98.40%	98.40%	56.70%	CP003056	987.231	We	nr	Bacillus coagulans 36D1, complete genome

HOW CAN DNA BE USED TO GET STRAIN LEVEL ID? "STRAIN TYPING"

- Whole-genome sequencing
- PCR of a specific subset of genes
- Restriction fragment length analysis



Christine Colasacco '18

2

MICROBIAL ID USING MATRIX ASSISTED LASER DESORPTION IONIZATION- TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) AND THE AXIMA ID PLUS MICROBIAL ID SYSTEM

STANDARD WORKFLOW TO IDENTIFY BACTERIA

SOURCE

ISOLATED COLONY

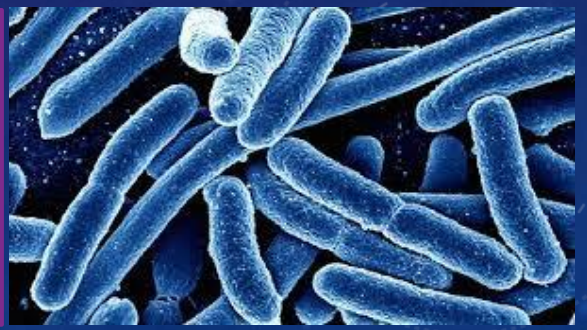
ANALYZE metabolism: BIOLOG

- Inoculate in 96well plate
- Incubate 24 hr
- Read + wells in Plate reader

2

MALDI-TOF

SEARCH DATABASE
(GenIII Bacterial DB)
BIOLOG SYSTEM



Hi, My Name is _____

MATRIX ASSISTED LASER DESORPTION IONIZATION- TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

- Microbial identification is applicable to many fields, including clinical diagnostics and food microbiology
- MALDI- TOF MS produces a mass fingerprint based on the composition of proteins in a sample
- In this study, MALDI was used for the identification of bacteria isolated from over the counter probiotics

Singhal, N., Manish K., Pawan K. K., & Jugsharan S. V. (2015). MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, 6(791), 1-16.

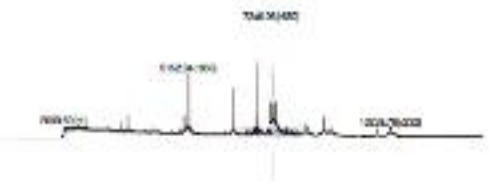
WORK FLOW

Christine Colasacco '18

Pure culture of bacterium/

- Spot biomass from a colony onto MALDI-TOF target plate
- Gram pos. bacteria – mix with 1µl 25% formic acid, air dry, then miix with 1 µl organic matrix (CHCA)
- Gram neg. bacteria – mix with 1 µl organic matrix (CHCA)

Load plate into MALDI-TOF MS

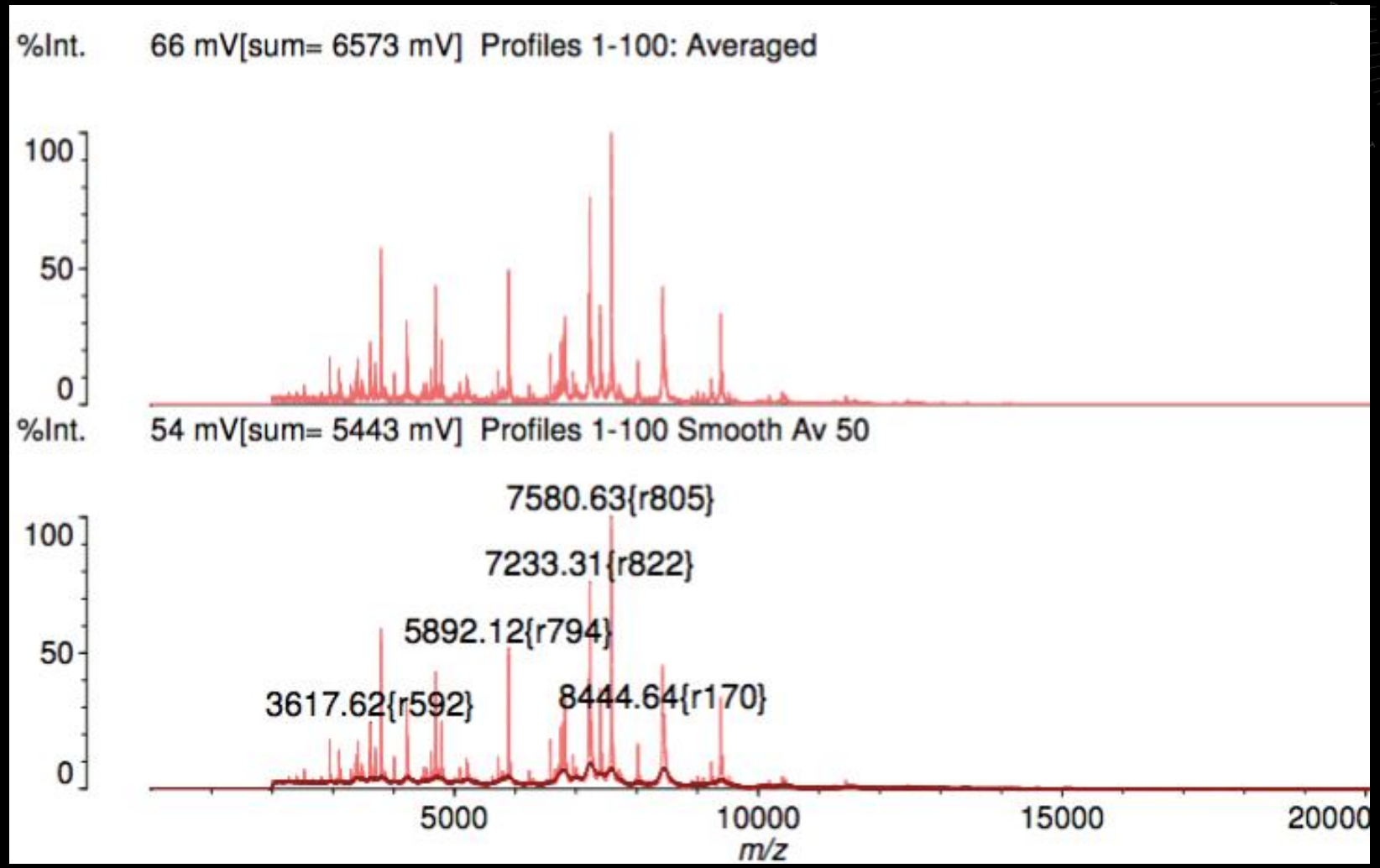


Mass fingerprint

- After loading plate into MALDI-TOF, a laser is used to ionize sample. Desorption & ionization create individual protonated
- ions. The positively charged ions are accelerated through a field of high voltage which separates the protonated ions
- based on a mass to charge ratio. Smaller ions hit a detector sooner than larger ions. **The difference in time of flight**
- **of ions in the sample is utilized to generate a spectrum known as a peptide mass fingerprint (PMF).**

Christine Colasacco '18

PEPTIDE MASS FINGERPRINT FOR STRAIN 1 (TUBE LABEL); MALDI IDENTIFICATION: *LACTOBACILLUS RHAMNOSUS*



MALDI IDENTIFICATION

Christine Colasacco '18

Tube Label	MALDI ID	Confidence Interval (%)	Label Identification	Method
Strain 13	Bacillus subtilis	96.9	Bacillus subtilis	Normal Protocol
Bacillus subtilis DE111	Bacillus subtilis	99.9	Bacillus subtilis	Normal Protocol
Strain 9	Bacillus subtilis	99.9	Bacillus subtilis	Normal Protocol
Bacillus coagulans SUJA	Bacillus coagulans	93.4	Bacillus coagulans	Normal Protocol
Bacillus coagluans Swanson	Bacillus coagulans	93.1	Bacillus coagulans	Normal Protocol
Strain 2	Lactobacillus pentous/plantarum	84.0	Lactobacillus pentous/plantarum	Normal Protocol
GG culturelle	Lactobacillus rhamnosus	99.9	Lactobacillus rhamnosus	Protein Extraction Method
CVS Lactobacillus rhamnosus	Lactobacillus rhamnosus	99.9	Lactobacillus rhamnosus	Protein Extraction Method
Chaas	Lactobacillus sp.	81.4	N/A	Protein extraction method

BIOLOG GENIII MICROBIAL ID SYSTEM (METABOLIC PROFILE)

3

STANDARD WORKFLOW TO IDENTIFY BACTERIA

SOURCE

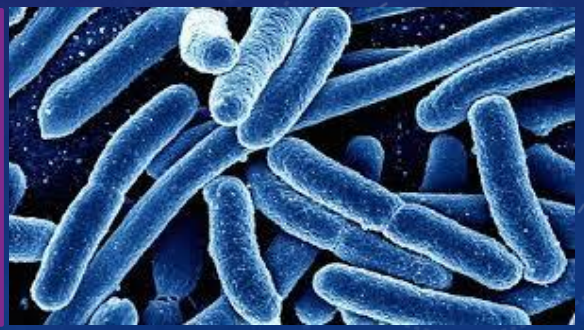
ISOLATED COLONY

ANALYZE metabolism: BIOLOG

- Inoculate in 96well plate
- Incubate 24 hr
- Read + wells in Plate reader

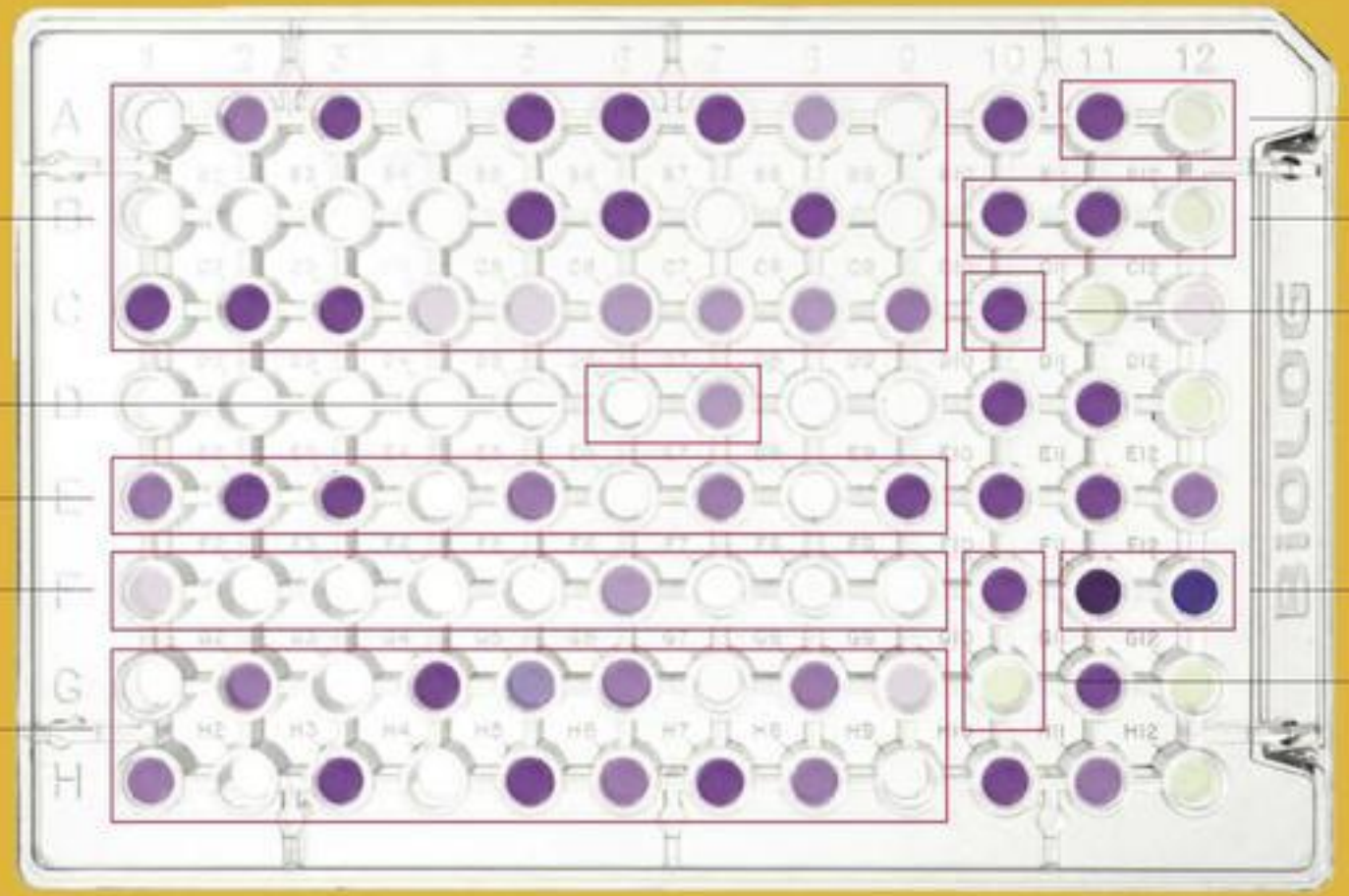
3 BIOLOG

SEARCH DATABASE
(GenIII Bacterial DB)
BIOLOG SYSTEM



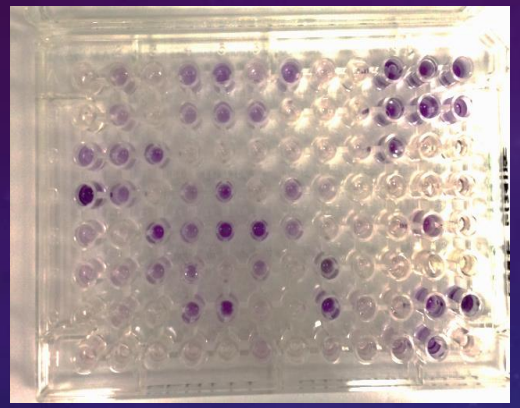
Hi, My Name is _____

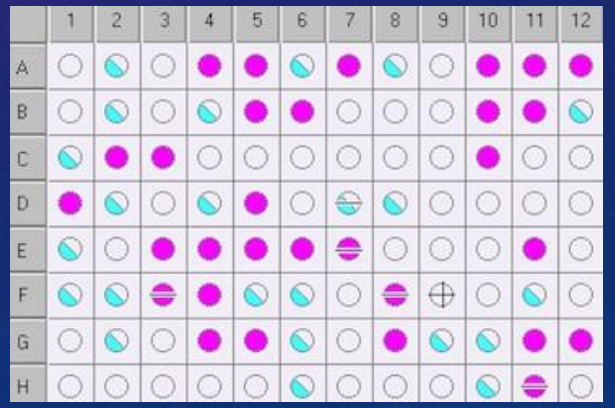
ID = *Stenotrophomonas maltophilia*



71 Carbon Source *plus* 23 Chemical Sensitivity Assays

BIOLOG RESULTS: PURPLE = POSITIVE GROWTH

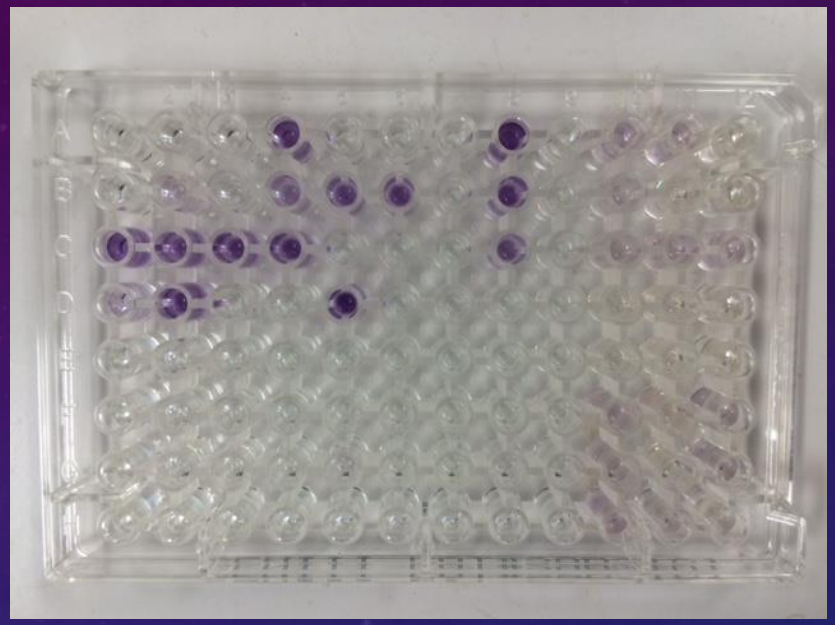




PROBIOTIC STRAINS CHARACTERIZED WITH BIOLOG

Species	# of Isolates
Bacillus coagulans	3
Bacillus subtilis	4
Lactobacillus plantarum	5
Lactobacillus rhamnosus	5
Saccharomyces (& other yeasts)	4

CVS Digestive Probiotic



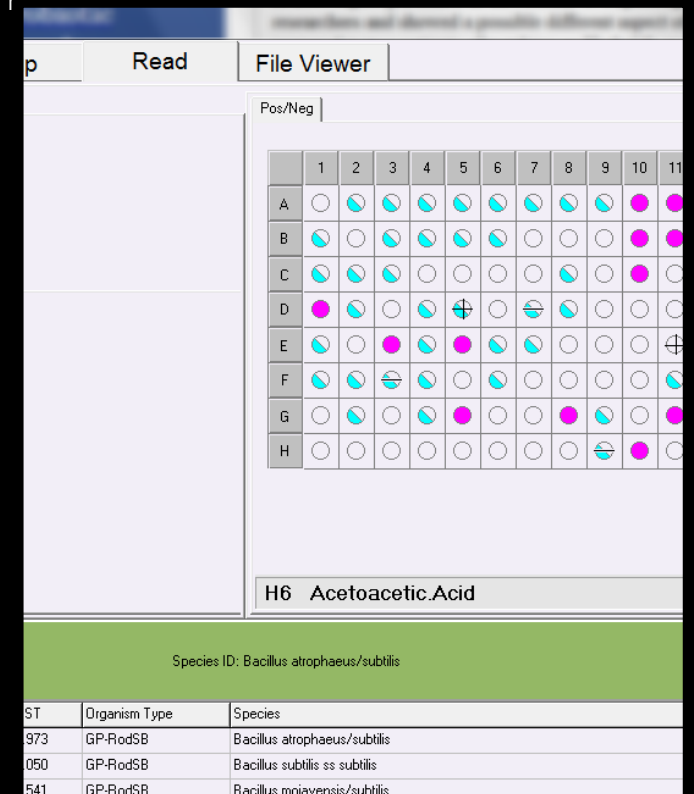
BIOLOG Phenotypic panel showing
Growth on different sugars
(purple wells = positive)

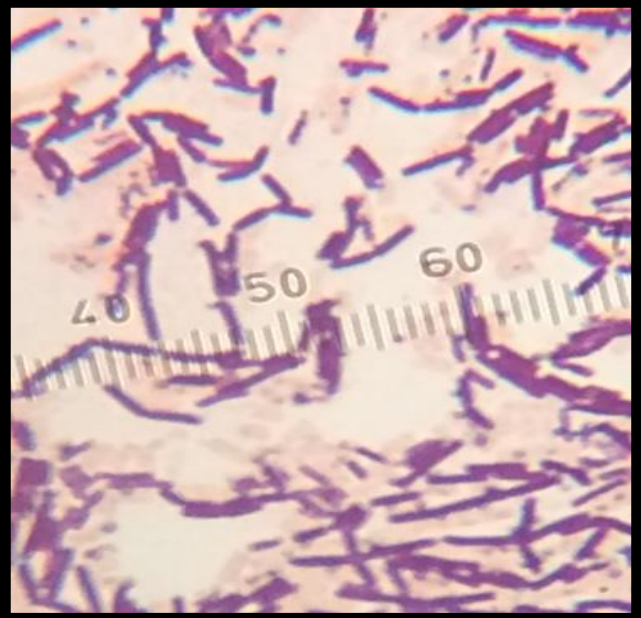
Lactobacillus rhamnosus from
“CVS Digestive Probiotic”

"Get Real" Probiotic with *Bacillus subtilis*

Streak plate of the probiotic powder

BIOLOG phenotypic panel results



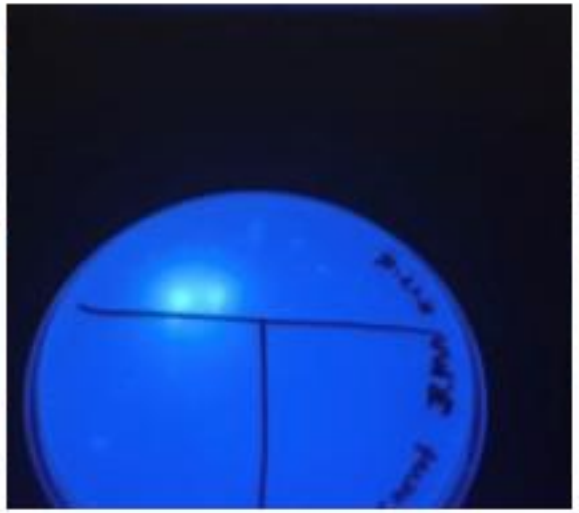


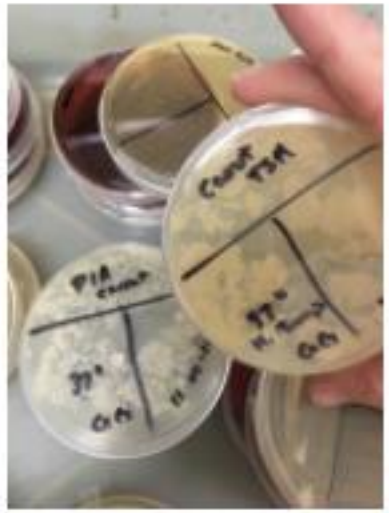
Bacillus

Gram positive

Rods viewed at 1000x

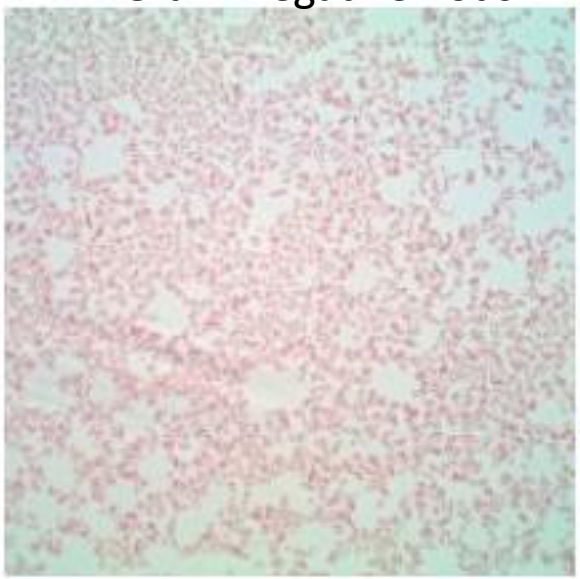
ISOLATE FROM A GARDEN CARROT





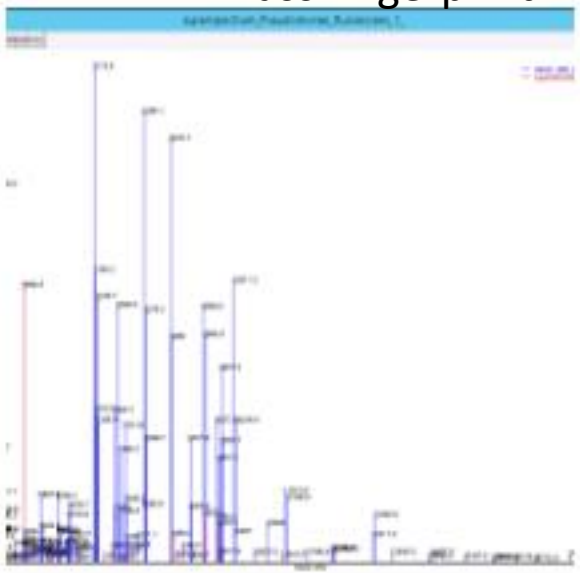
identity: Pseudomonas fluorescens 🙂 Yay.

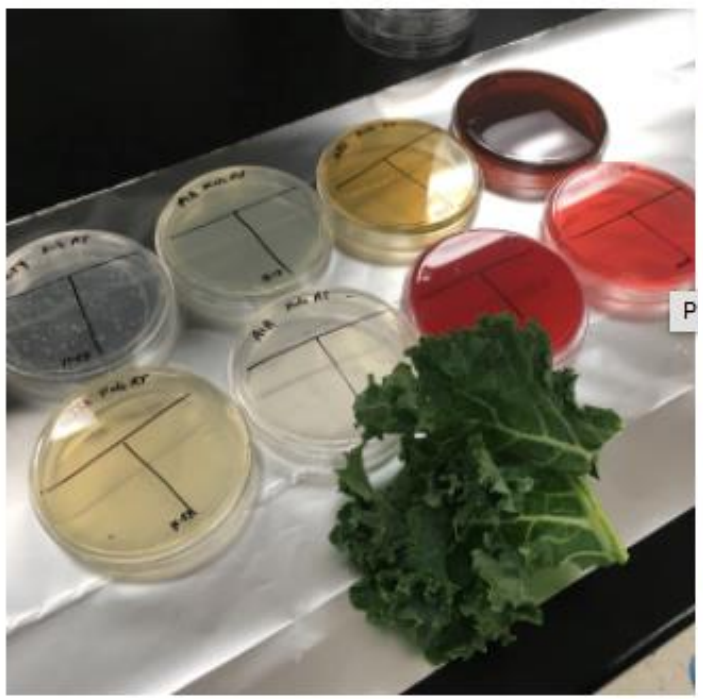
Gram Negative Rods

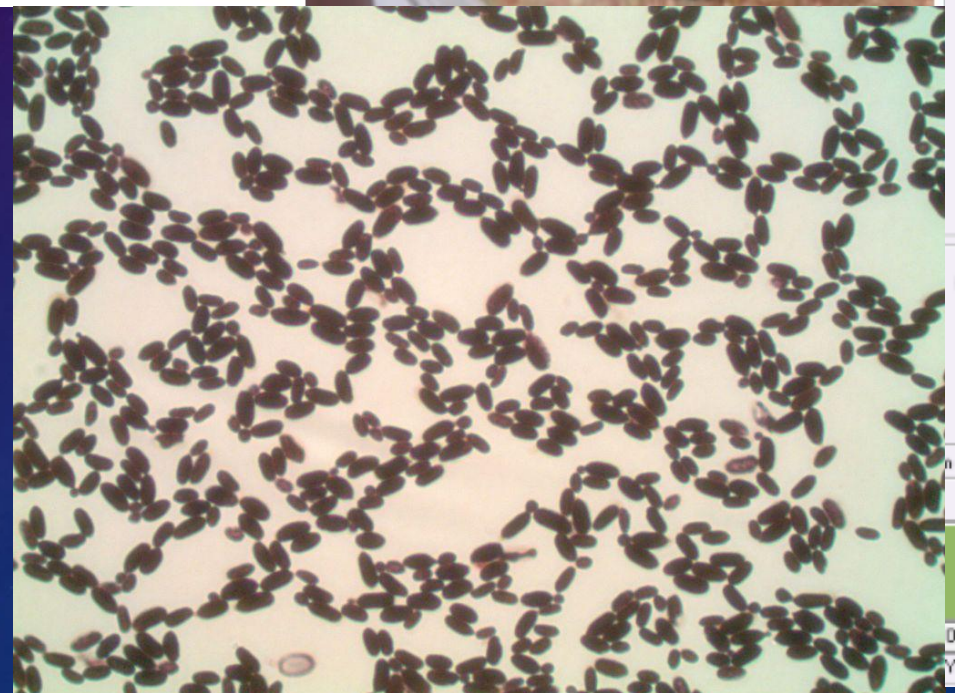


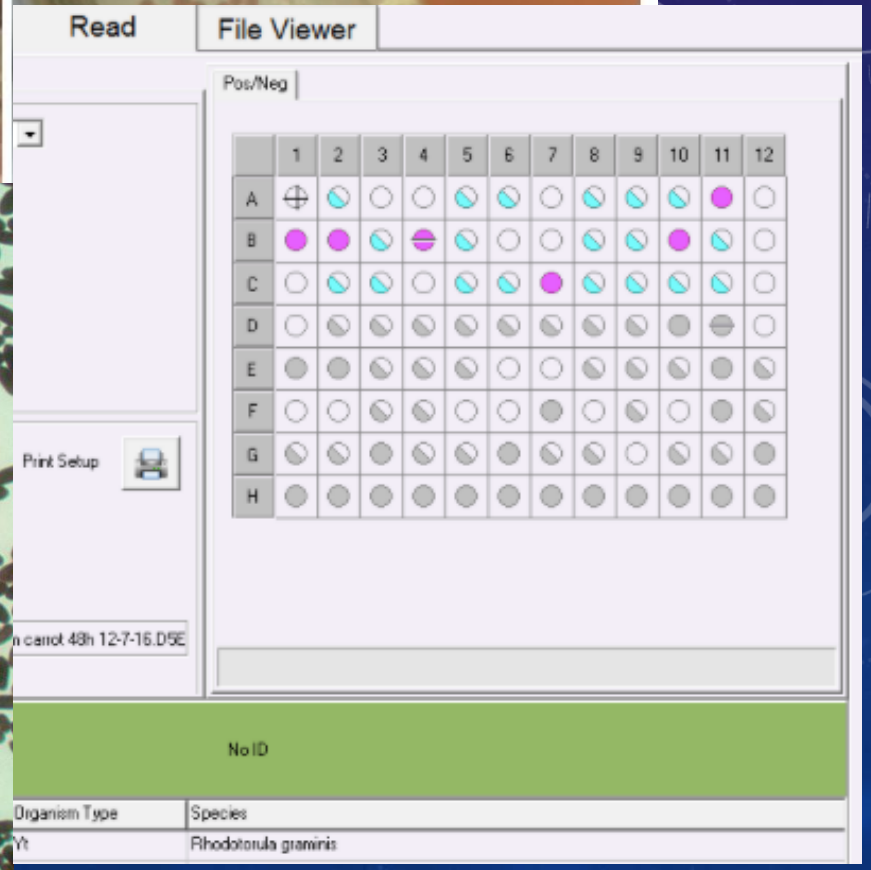
BIOLOG Plate

MALDI mass fingerprint



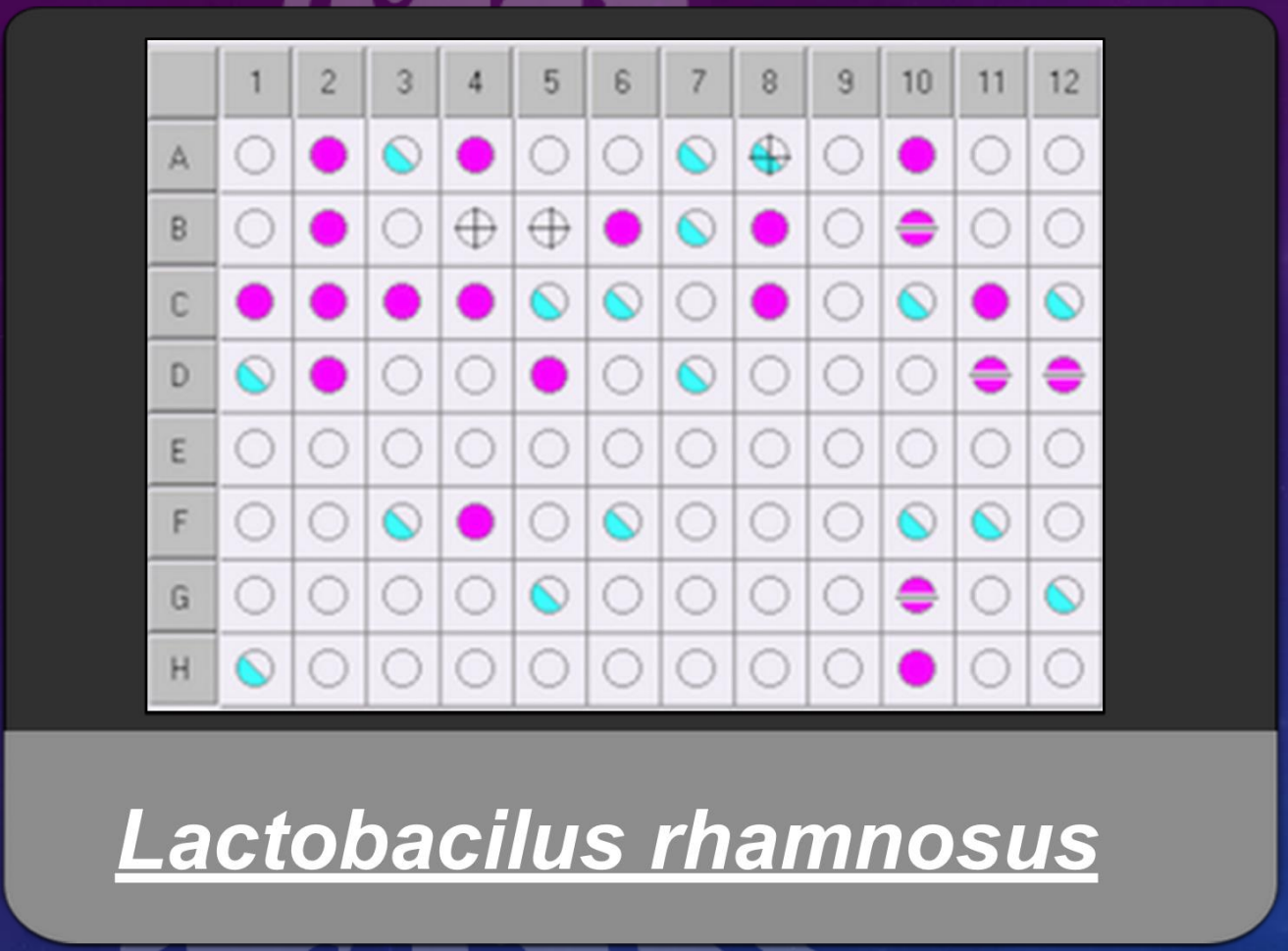






ISOLATE FROM KALE: PINK YEAST (NO ID, TOP HIT RHODOTORULA)

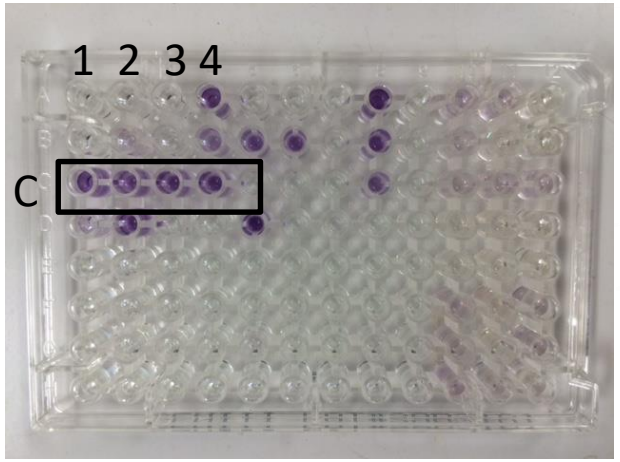
Scott Kohlhepp’18 & Elli Emmanouil ‘17



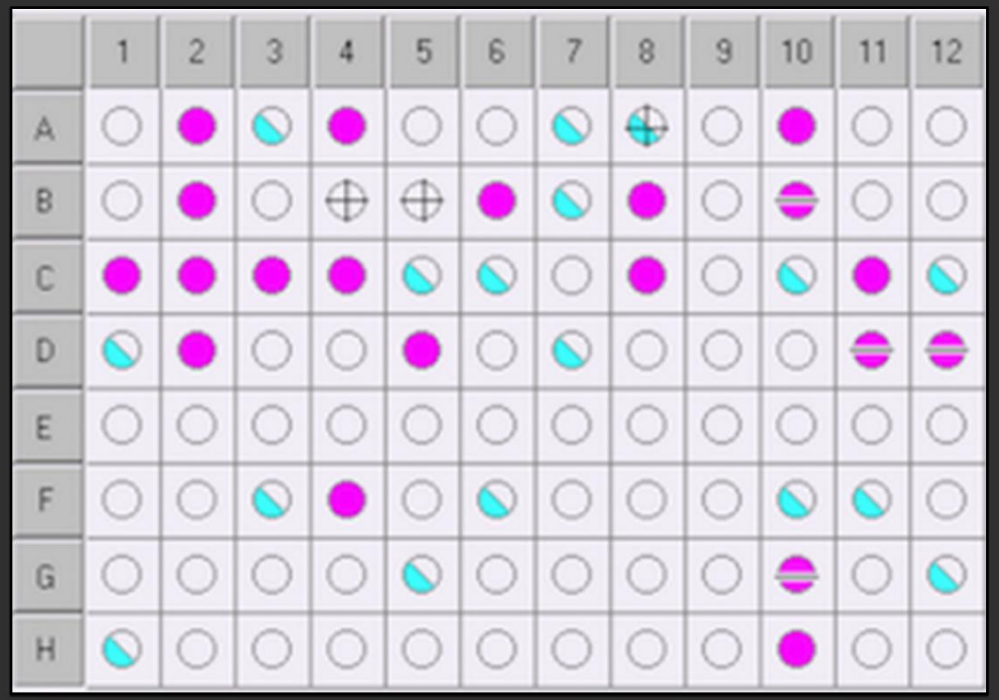
Lactobacilus rhamnosus

BIOLOG RESULTS: *LACTOBACILLUS RHAMNOSUS* STRAINS FROM 4 PROBIOTIC PRODUCTS

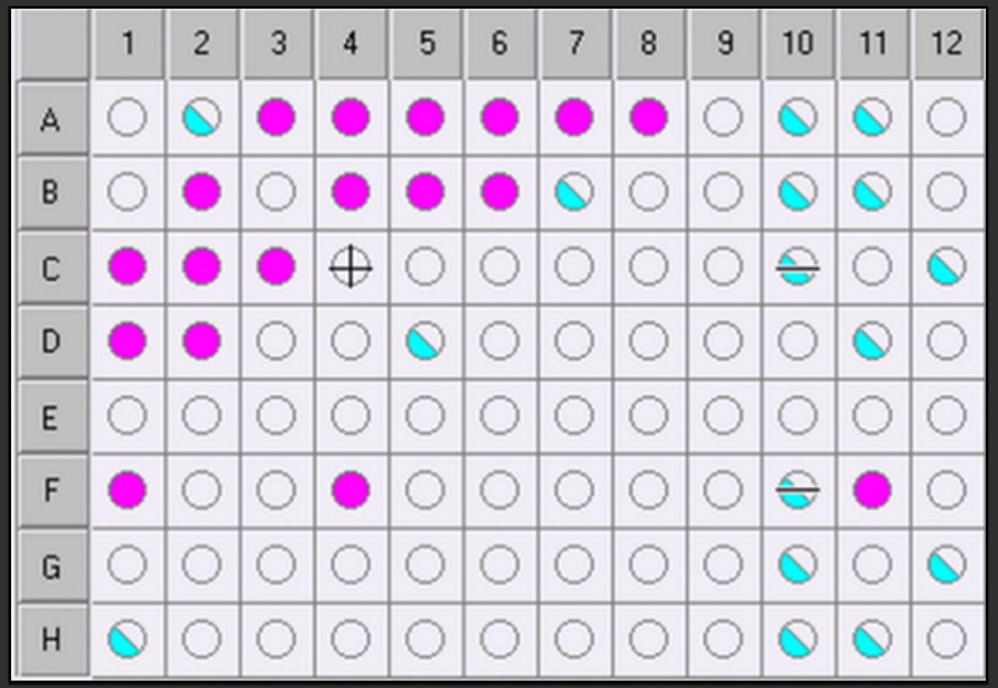
		L. rhamnosus			
		CVS	PrimKids1-EE	GG	WholeBiotics
		L. rhamnosus	Biolog Metabolic fingerprint (L. r		
Well	Carbon Source	CVS Digestive	EE Primkids	Culturelle	Wholebiotics
A1	Negative Control	-	-	-	-
A2	Dextrin	h	P	h	h
A3	D-Maltose	-	h	h	-
A4	D-Trehalose	P	P	P	P
A5	D-Cellobiose	-	-	h	h
A6	Gentiobiose	-	-	-	-
A7	Sucrose	-	h	h	-
A8	D-Turanose	P	h(+)	h	?
B1	D-Raffinose	-	-	-	-
B2	alpha D-Lactose	h	P	-	h
B3	D-Malibiose	-	-	-	-
B4	beta Methyl-D-Glucoside	P	?	-	P
B5	D-Salicin	P	?	h(+)	P
B6	N-Acetyl-D-Glucosamine	P	P	P	P
B7	N-Acetyl-D-Mannosamine	-	h	h	-
B8	N-Acetyl-D-Galactosamine	P	P	h	h
C1	alpha-D-Glucose	P	P	P	P
C2	D-Mannose	P	P	P	P
C3	D-Fructose	P	P	P	P
C4	D-Galactose	P	P	P	P
C5	3-Methyl Glucose	-	h	-	-
C6	D-Fucose	-	h	h	-
C7	L-Fucose	-	-	-	-
C8	L-Rhamnose	P	P	-	h



Scott Kohlhepp'18 & Elli Emmanouil '17



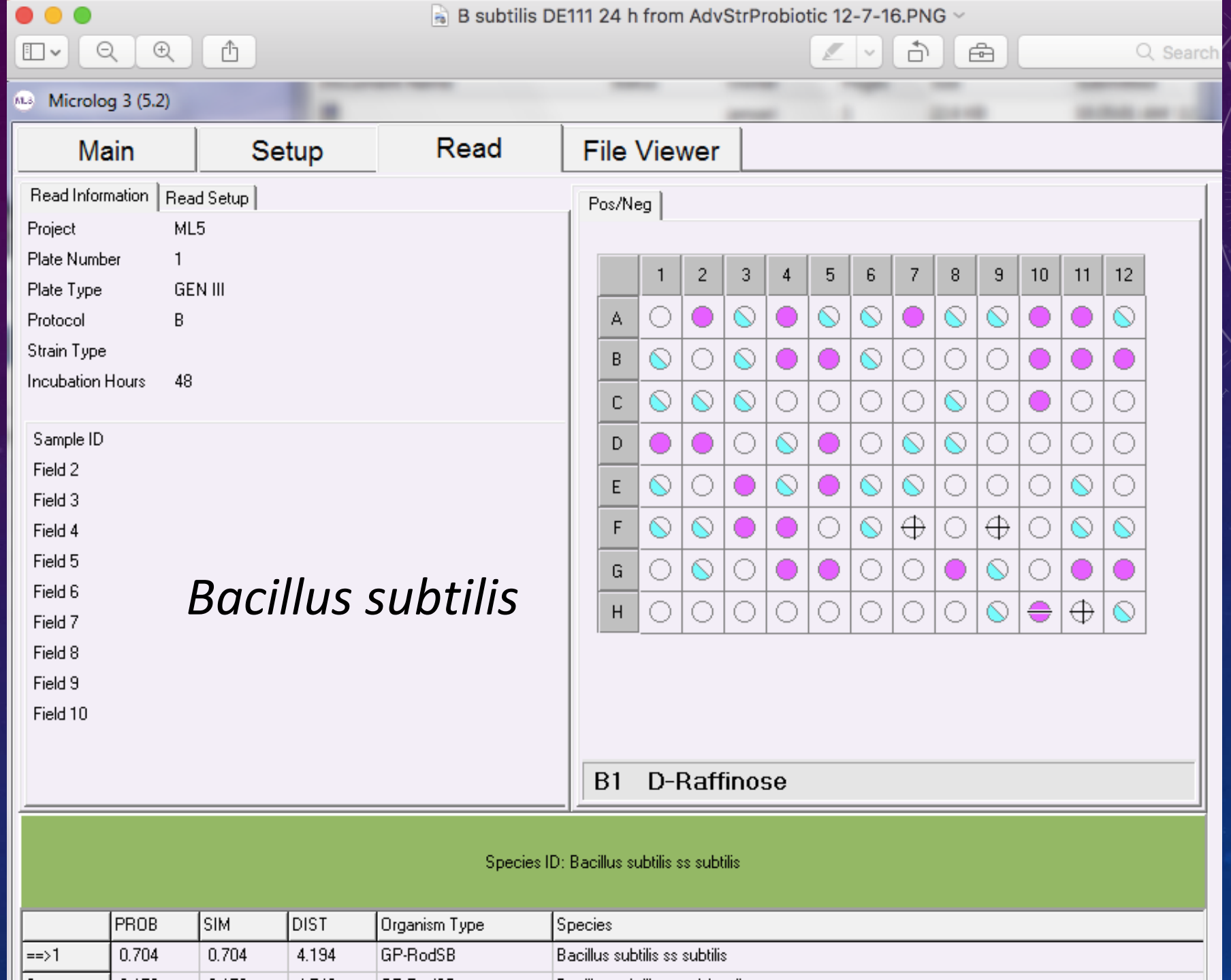
Lactobacilus rhamnosus



Lactobacillus plantarum

BIOLOG RESULTS: *L. PLANTARUM VS. L. RHAMNOSUS*

		L. plantaru								L. rhamnosus				
BIOLOG PLATE		FF	DrA	RUF	Swa	299V				CVS	Prim	Prim1	GG	Who
		Biolog Metabolic fingerprint							(L. rhamnosus Metabolic fingerprin					
Well	Carbon Source	Fruil	Dr.	Ren	Swa	299	REF	Well	Carb	Diges	Primk	rimkio	ulture	blebic
A1	Negative Control	-	-	-	-	-	-	A1	Nega	-	-	-	-	-
A2	Dextrin	h	h	h	(line	h	h	A2	Dext	h	P	h	h	h
A3	D-Maltose	P	P	P	P	P	h	A3	D-M	-	h	h	h	-
A4	D-Trehalose	P	P	P	P	P	h	A4	D-Tre	P	P	P	P	P
A5	D-Cellobiose	P	P	P	P	P	h	A5	D-Ce	-	-	-	h	h
A6	Gentiobiose	P	P	P	P	P	h	A6	Gent	-	-	-	-	-
A7	Sucrose	P	P	P	P	P	h	A7	Sucr	-	h	-	h	-
A8	D-Turanose	P	P	P	P	P	h	A8	D-Tu	P	h(+)	?	h	?
B1	D-Raffinose	-	-	-	-	-	-	B1	D-Ra	-	-	-	-	-
B2	alpha D-Lactose	?	h	P	P	P	h	B2	alph	h	P	P	-	h
B3	D-Malibiose	h	-	-	h	-	-	B3	D-M	-	-	-	-	-
B4	beta Methyl-D-Gluc	P	P	P	P	P	h	B4	beta	P	?	?	-	P
B5	D-Salicin	P	P	P	h	P	h	B5	D-Sal	P	?	?	h(+)	P
B6	N-Acetyl-D-Glucosan	P	P	P	P	P	h	B6	N-Ac	P	P	P	P	P
B7	N-Acetyl-D-Mannosa	(line	-	h	-	h	-	B7	N-Ac	-	h	-	h	-
B8	N-Acetyl-D-Galactos	-	-	-	-	-	-	B8	N-Ac	P	P	P	h	h
C1	alpha-D-Glucose	P	P	P	P	P	h	C1	alpha	P	P	P	P	P
C2	D-Mannose	P	P	P	P	P	h	C2	D-Ma	P	P	P	P	P
C3	D-Fructose	P	P	P	P	P	h	C3	D-Fru	P	P	P	P	P
C4	D-Galactose	h	P	?	h	?	h	C4	D-Ga	P	P	P	P	P
C5	3-Methyl Glucose	-	-	-	h	-	-	C5	3-Me	-	h	-	-	-
C6	D-Fucose	-	-	-	-	-	-	C6	D-Fu	-	h	h	h	-
C7	L-Fucose	-	-	-	-	-	-	C7	L-Fuc	-	-	-	-	-
C8	L-Rhamnose	-	-	-	-	-	-	C8	L-Rha	P	P	P	-	h


B subtilis DE111 24 h from AdvStrProbiotic 12-7-16.PNG
Search
Microlog 3 (5.2)
Main
Setup
Read
File Viewer
Read Information
Read Setup
Project ML5
Plate Number 1
Plate Type GEN III
Protocol B
Strain Type
Incubation Hours 48
Sample ID
Field 2
Field 3
Field 4
Field 5
Field 6
Field 7
Field 8
Field 9
Field 10
Bacillus subtilis
Pos/Neg
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
B1 D-Raffinose
Species ID: Bacillus subtilis ss subtilis
PROB SIM DIST Organism Type Species
==>1 0.704 0.704 4.194 GP-RodSB Bacillus subtilis ss subtilis

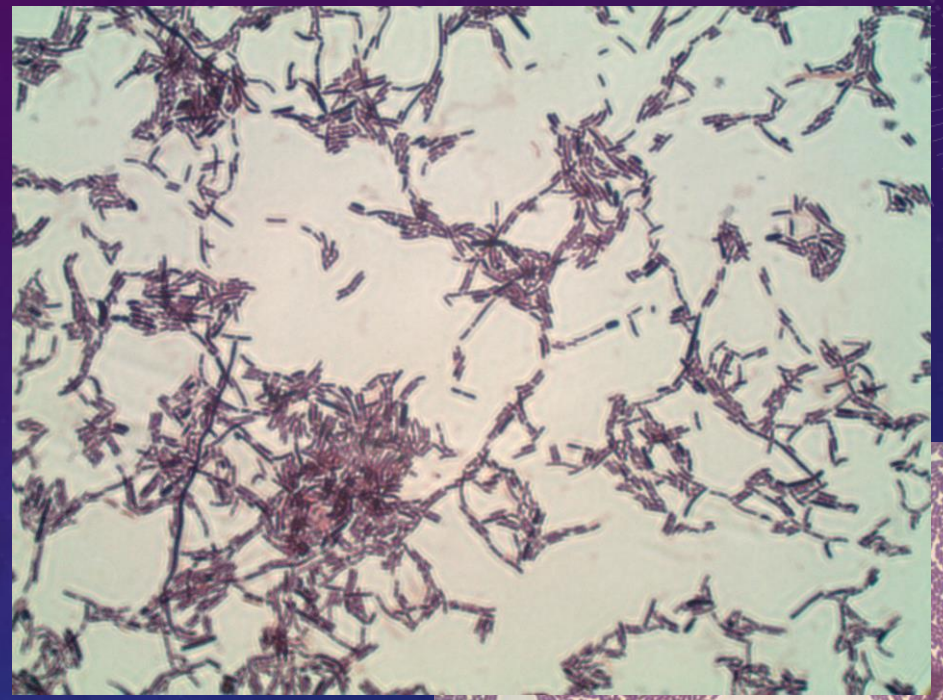
BIOLOG RESULTS:
L. PLANTARUM
VS.
B. SUBTILIS

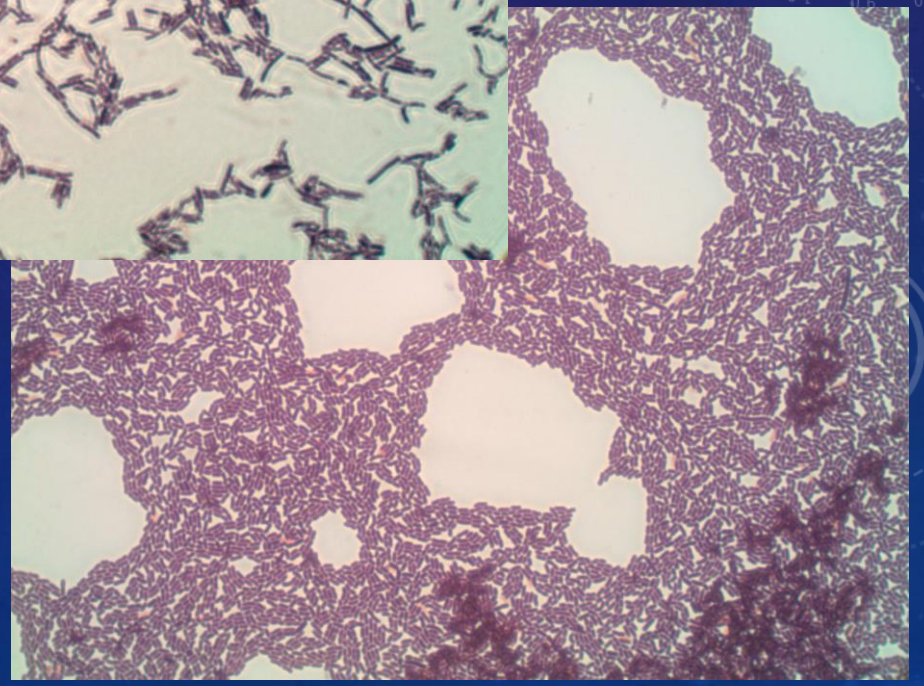
Amino acids!

	UTILIZATION OF CARBON SOURCES, cont.									
		L. plantarum					B. subtilis			
		FF	DrA	RUF	Swa	299V	DE11	GR	GoL	Swa
			Biolog Metabolic fingerprint (L. plantarum plates)				B. subtilis	B. subtilis	B. subtilis	B. subtilis
Well	Carbon Source	Fruit fly gut	Dr. Axe	Renew UltFlo	Swanson	299V GB	111-AdvStr P	GET REAL	Garden of Life	Swanson(cor
D1	D-Sorbitol	P	P	P	h	P	P	P	P	-
D2	D-Mannitol	P	P	P	P	P	P	h	h	h
D3	D-Arabitol	-	h	-	-	-	-	-	-	-
D4	myo-Inositol	-	-	-	-	-	h	h	h	h(?)
D5	Glycerol	P	h	h	h	h	P	h	P	P
D6	D-Glucose-6-PO4	-	-	-	-	-	-	-	-	-
D7	D-Fructose-6-PO4	-	-	-	h	-	h	h	h	P(line)
D8	D-Aspartic acid	-	-	-	-	-	h	h	-	-
E1	Gelatin	-	-	-	-	-	h	h	-	h
E2	Glycyl-L-Proline	-	-	-	-	-	-	-	-	-
E3	L-Alanine	-	-	-	-	-	P	P	P	P(line)
E4	L-Arginine	-	-	-	-	-	h	h	-	h
E5	L-Aspartic acid	-	-	-	-	-	P	P	h	h
E6	L-Glutamic acid	-	-	-	-	-	h	h	h	h
E7	L-Histidine	-	-	-	-	-	h	h	-	h
E8	L-Pyroglutamic acid	-	-	-	-	-	-	-	-	-
F1	Pectin	P	P	P	P	P	h	h	h	h
F2	D-Galacturonic acid	-	-	-	-	-	h	h	h	h
F3	L-Galactonic acid Lacton	-	-	-	-	-	P	h	h	h(line)
F4	D-Gluconic acid	P	P	P	P(line)	P	P	h	h	h

“WHAT’S THE DIFFERENCE BETWEEN ME & YOU?”

DR. DRE & EMINEM
2001





CONCLUSIONS FROM BIOLOG METABOLIC PROFILING

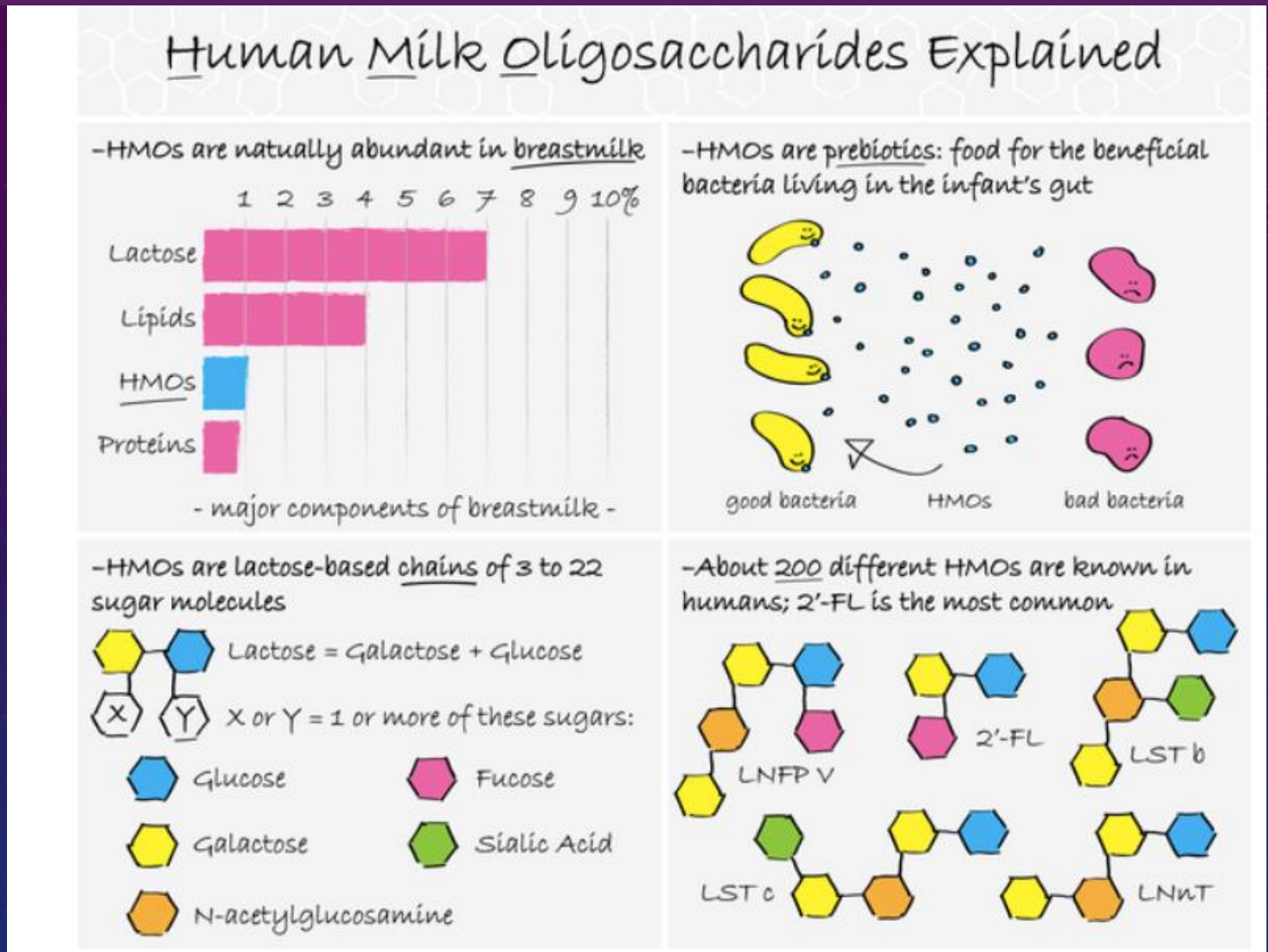
- Reveals Carbon source utilization by probiotic bacteria
a.k.a. **“what the microbes we eat, eat”**

- *Lactobacillus* strains primarily metabolize sugars, while *Bacillus* can use a wider range of Carbon sources

- Relates to probotic properties (i.e. bacterial end-product of lactic acid, short-chain fatty acids- production depend on preferred C sources being available in our diet)

- Limitation: Does not give all info about probiotic effect (antibiotic production, antibacterial, adhesion/colonization)

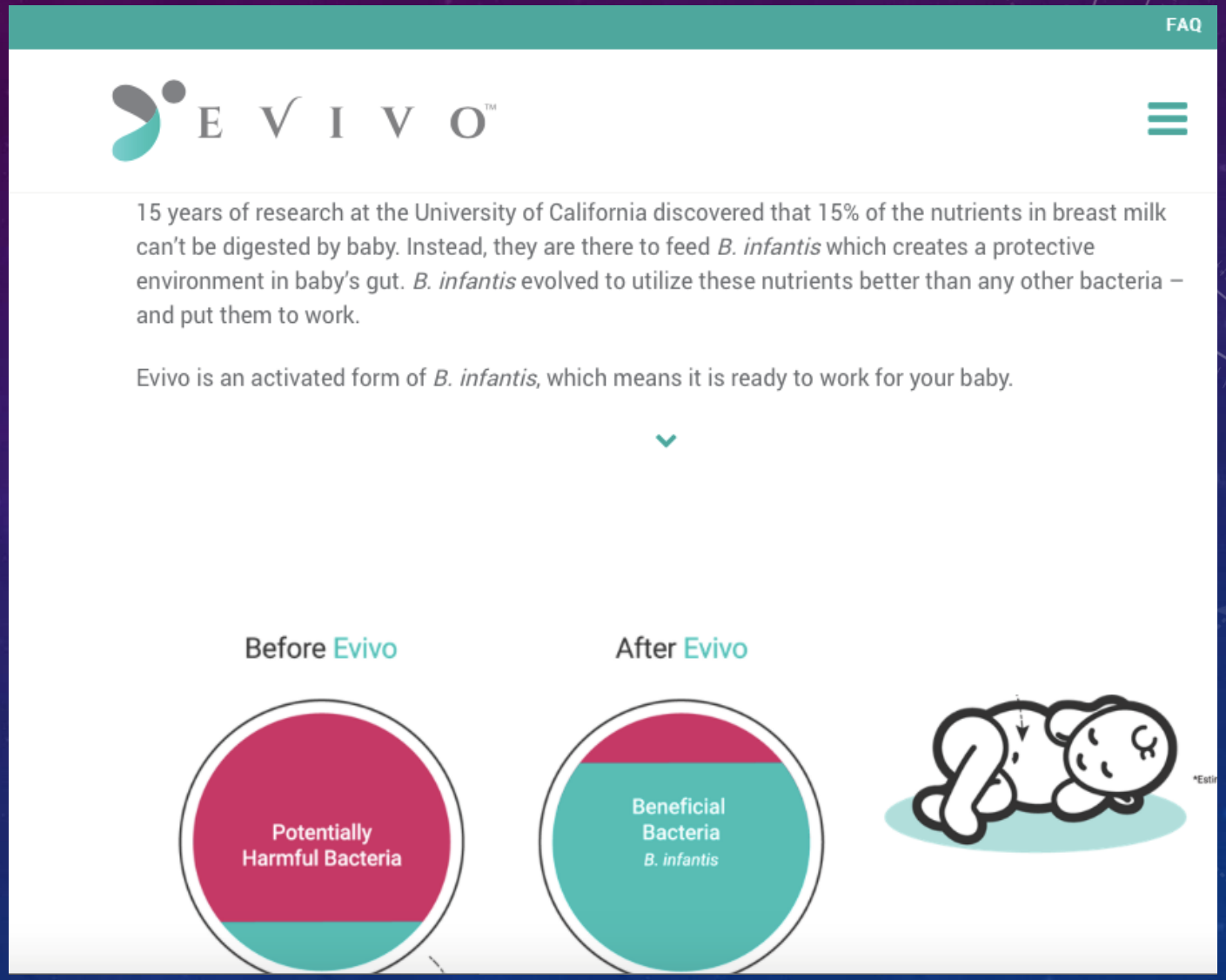
FEED THE MICROBES WHAT THEY LIKE TO EAT!



Graphic: Robert Lawrence

PREBIOTICS: FEED THE MICROBES WHAT THEY LIKE TO EAT!

Evivo sells an activated form of *B. infantis,* which is missing In microbiomes of many American infants! Adding this strain back in and breast-feeding can help restore a healthy microbiome.

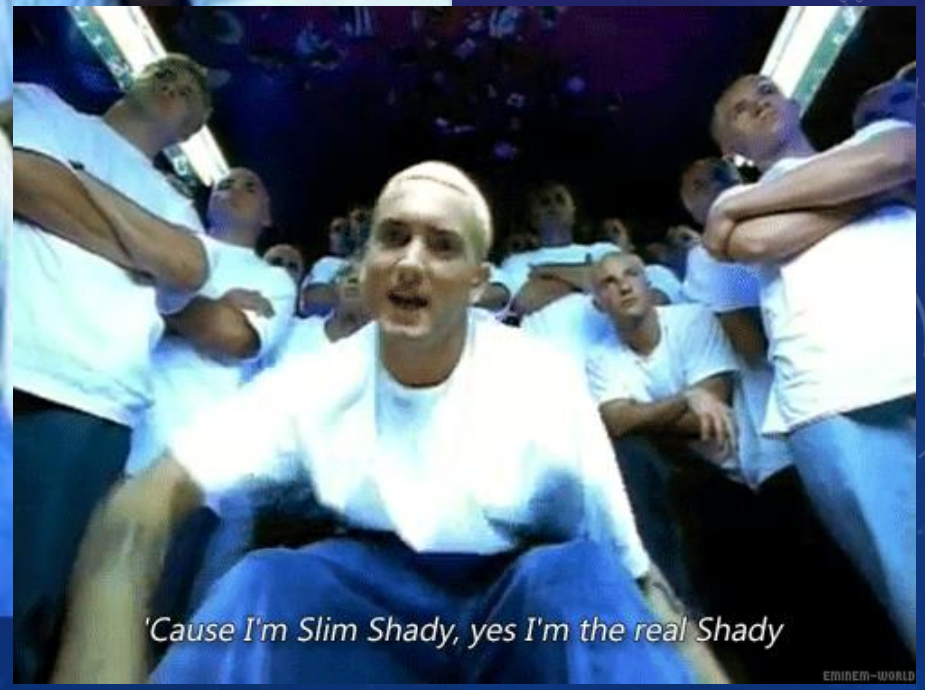


CONSISTENT RESULTS WITH 3 IDENTIFICATION METHODS

Protein activity DNA protein

Tube Label	Label Identification	BIOLOG Result	16S seq BLAST Result	MALDI Result	Confidence Interval	Method
Strain 13	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	96.9	Normal Protocol
Bacillus subtilis DE111	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	99.9	Normal protocol
Strain 9	*Bacillus subtilis*	*Bacillus atrophaeus/subtilis*	*Bacillus* sp. strain BCBT29	*Bacillus subtilis*	99.9	Normal Protocol
Bacillus coagulans SUJA	*Bacillus coagulans*	*No ID*	*Bacillus coagulans*	*Bacillus coagulans*	93.4	Normal Protocol
Bacillus coagulans Swanson	*Bacillus coagulans*	*Bacillus vallismortis/subtilis***	contam: *Bacillus subtilis* **	*Bacillus coagulans*	93.1	Normal Protocol
Strain 2	*Lactobacillus pentous/plantarum*	*Lactobacillus plantarum*	*Lactobacillus plantarum*	*Lactobacillus pentous/plantarum*	84	Normal Protocol
Strain 1	*Lactobacillus rhamnosus*	*Weissella viridescens*	*Lactobacillus rhamnosus*	*Lactobacillus rhamnosus*	99.9	Protein Extraction Method
CVS *Lactobacillus rhamnosus*	*Lactobacillus rhamnosus*	*Lactobacillus rhamnosus*	*Lactobacillus casei*	*Lactobacillus rhamnosus*	99.9	Protein Extraction Method
Chaas	N/A	*Lactobacillus rhamnosus*	*Lactobacillus rhamnosus*	*Lactobacillus sp.*	81.4	Protein Extraction Method

“WILL THE REAL “L. RHAMNOSUS GG” PLEASE STAND UP?”



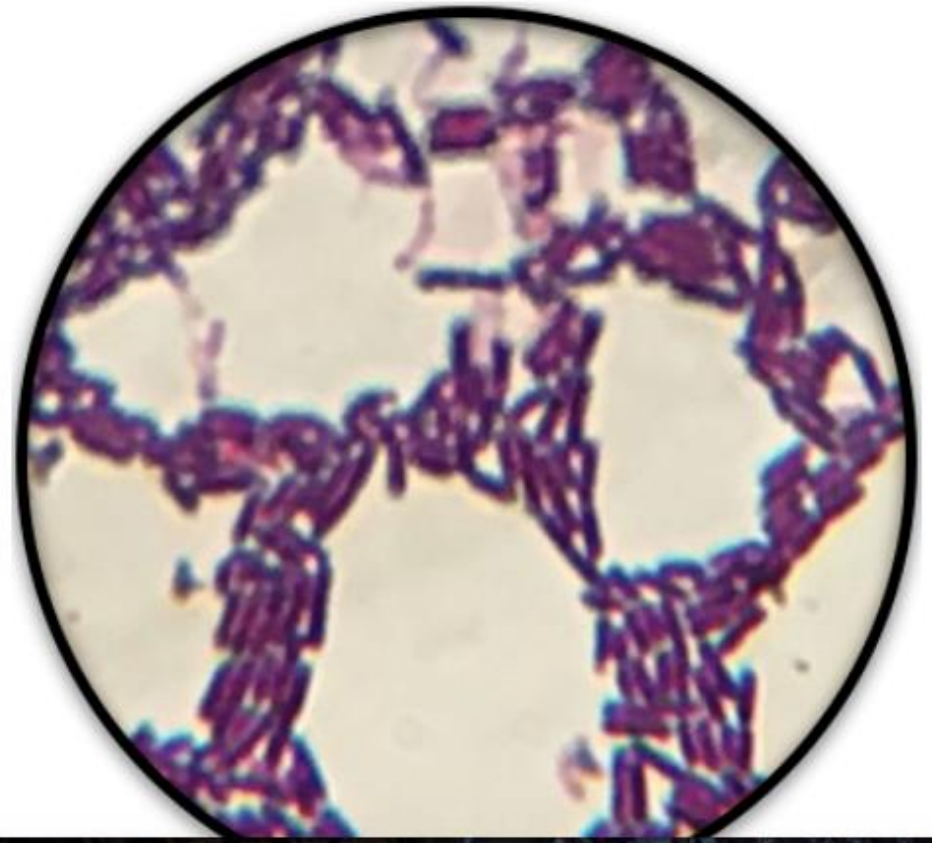
THE PROBIOTICS LAB

WWW.THEPROBIOTICSLAB.COM

LACTOBACILLUS RHAMNOSUS

Home / Lactobacillus / Lactobacillus rhamnosus

Lactobacillus rhamnosus



Well-studied probiotic with many clinical studies

Strains: *L. rhamnosus GG,*

TOPICS FOR TODAY

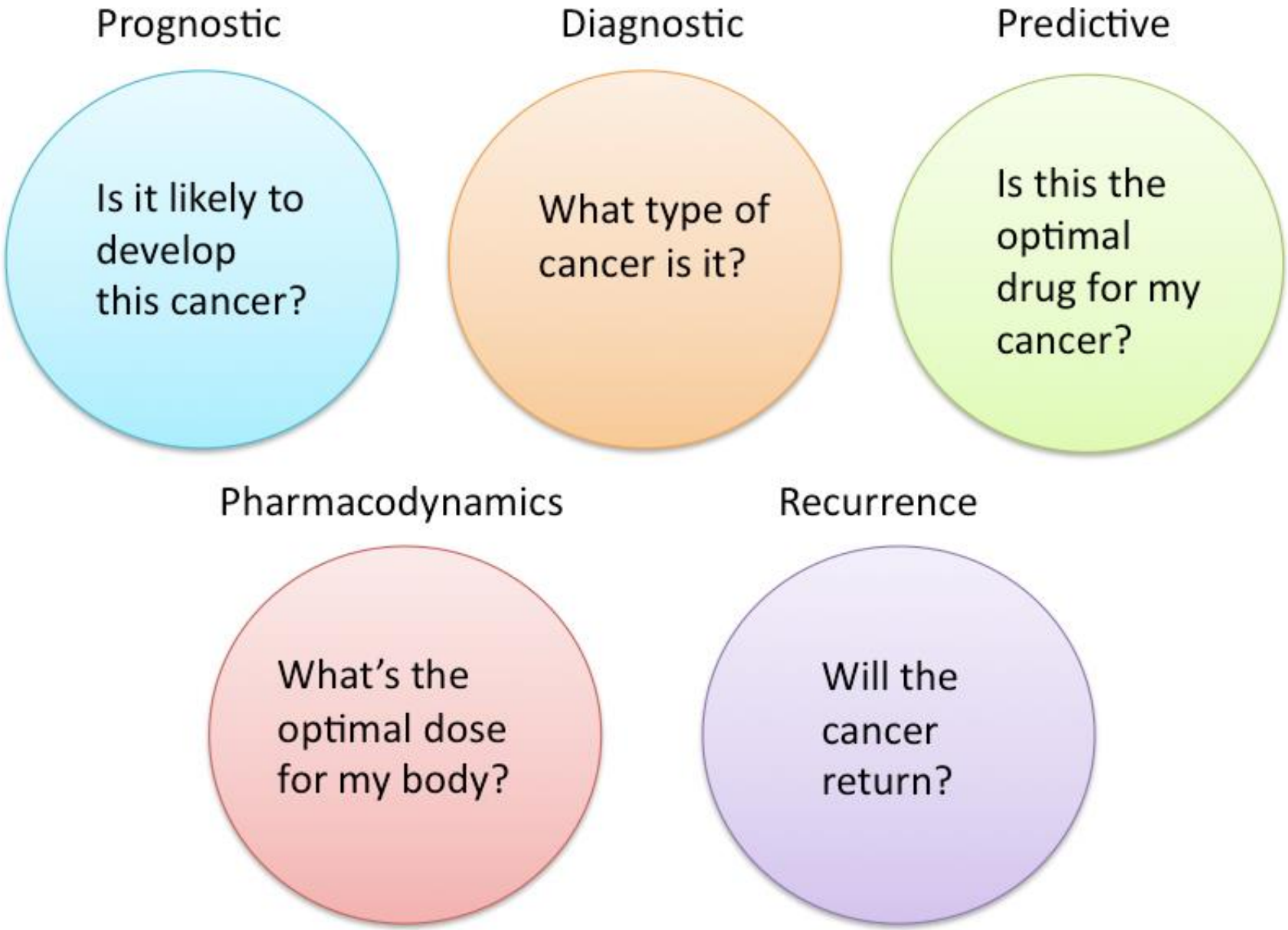
- Part I. Identifying & Characterizing Beneficial Microbes
  - Microbiome explosion & Future outlook for probiotics & therapies
  - 3 approaches to identify bacterial strains
    - PCR
    - MALDI TOF
    - BIOLOG
  - Probiotics & unknowns
  - Carbon Sources & Prebiotics: What the microbes we eat, eat!

- **Part II. Molecular signatures of Human Disease**
  - **Genetic markers vs. Circulating biomarkers of disease**
  - **miRNAs associated with cancer**

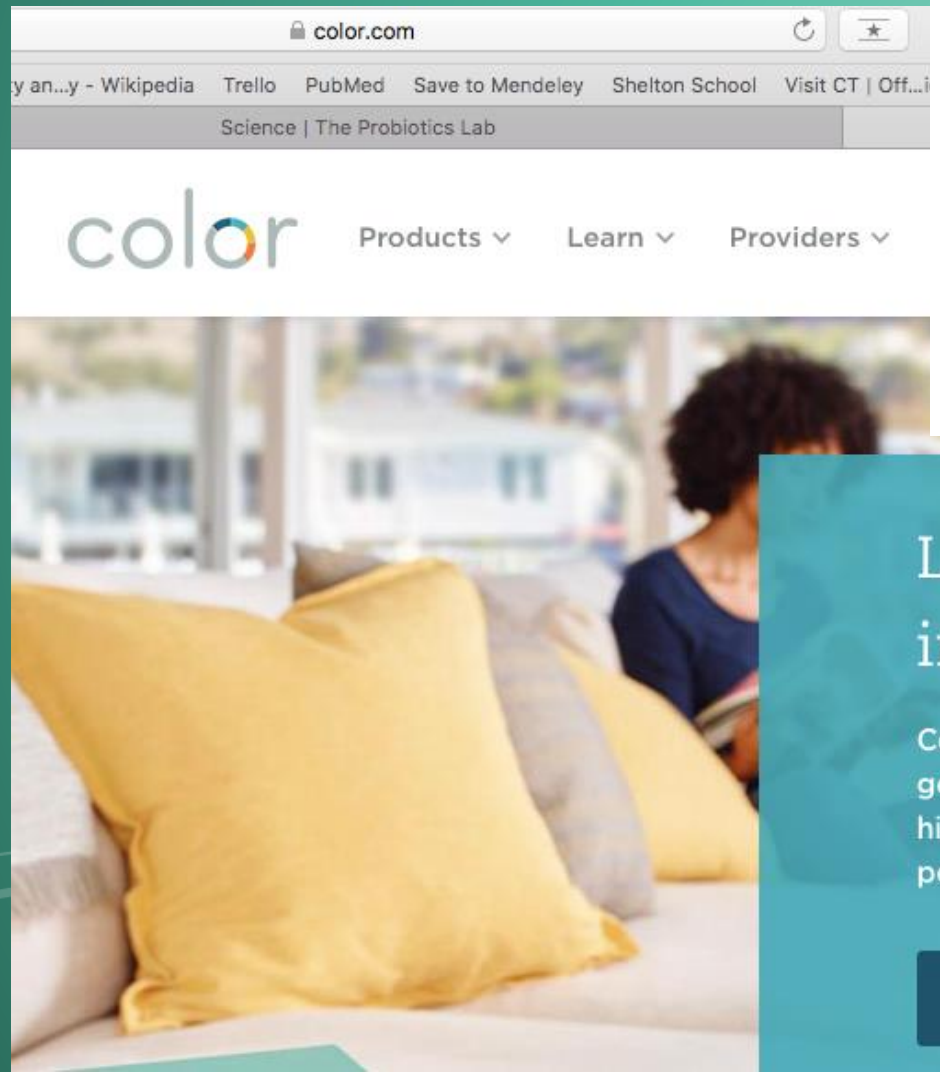
RNA

DNA

Questions that can be answered by cancer biomarkers



GENETIC RISK FOR HEREDITARY DISEASE (EX: COLOR GENOMICS)



color Products Learn Providers Employers

Hereditary Cancer Test

30 gene analysis, including *BRCA1* and *BRCA2*, to understand your risk for common hereditary cancers

Hereditary High Cholesterol Test

3 gene analysis to understand your risk for Familial Hypercholesterolemia

BRCA Test

BRCA1 and *BRCA2* gene analysis to start understanding your risk for hereditary breast and ovarian cancer

All Color Tests

Includes all genetic analysis currently offered by Color

Learn how your genes can impact your health

Color is a health service that helps you understand your genetic risk for common hereditary cancers and hereditary high cholesterol, and use this knowledge to create a personalized healthcare plan.

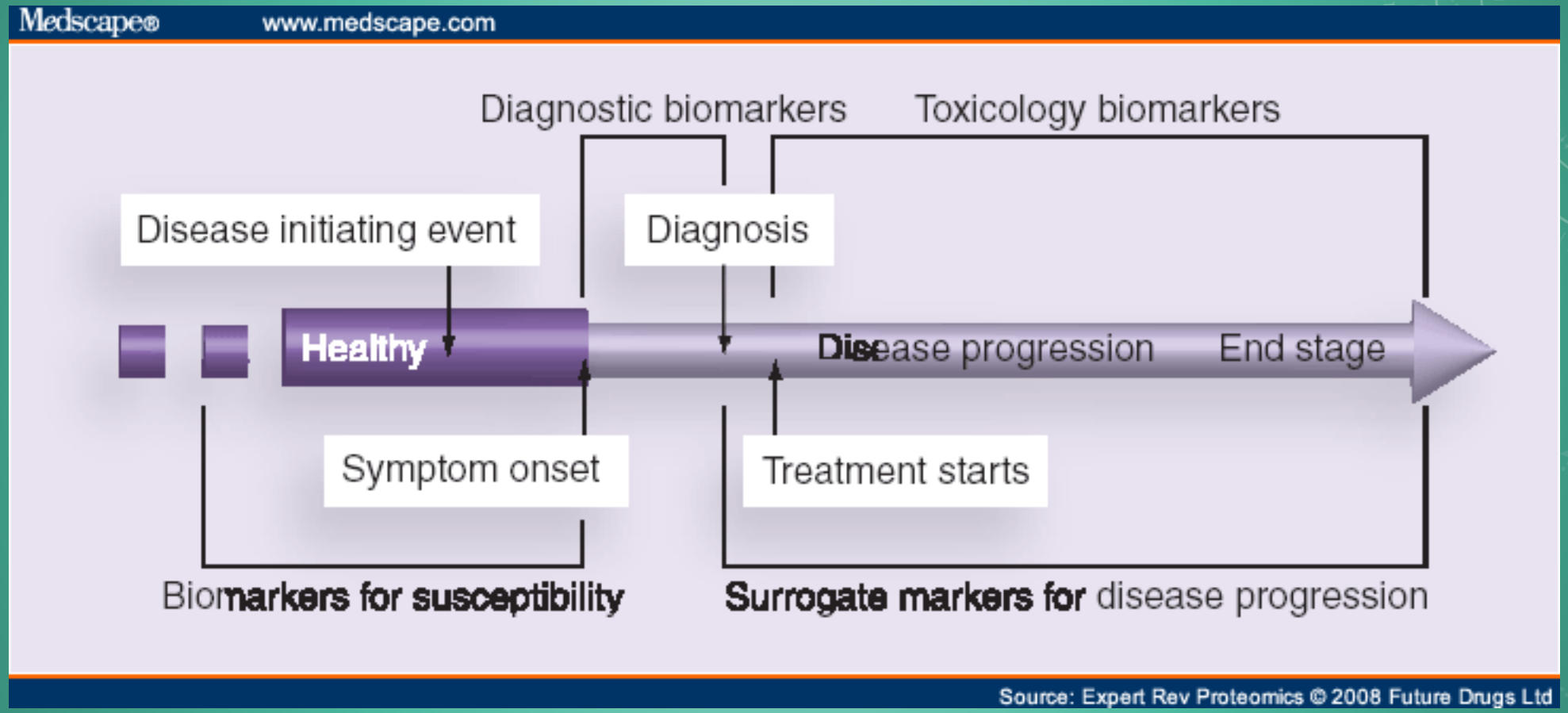
Get Started

DNA

GENETIC VARIANT SCREENING (EX: CELMATIX)

DNA

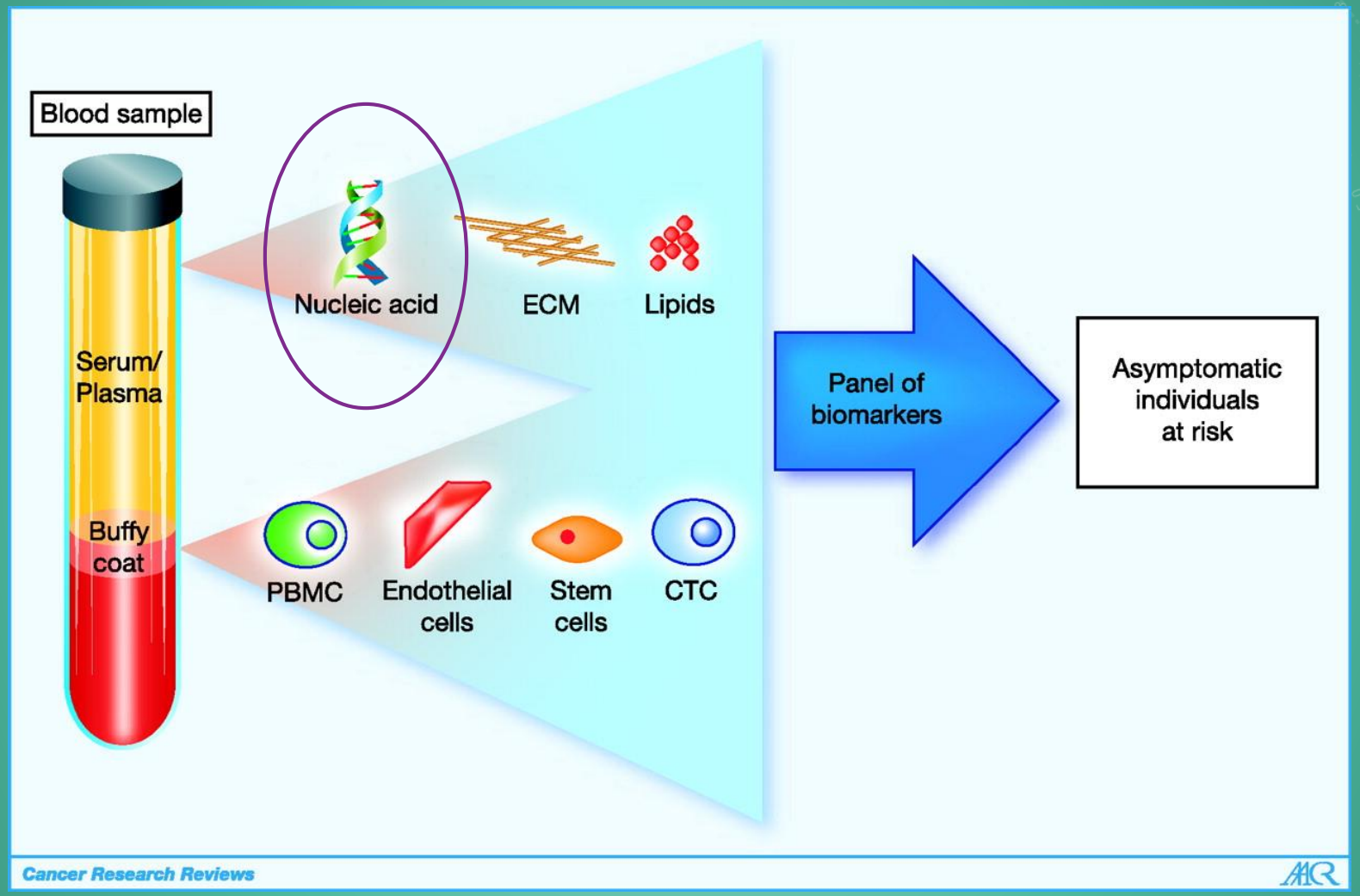
Biomarkers at different stages of disease



Characteristics of ideal biomarkers

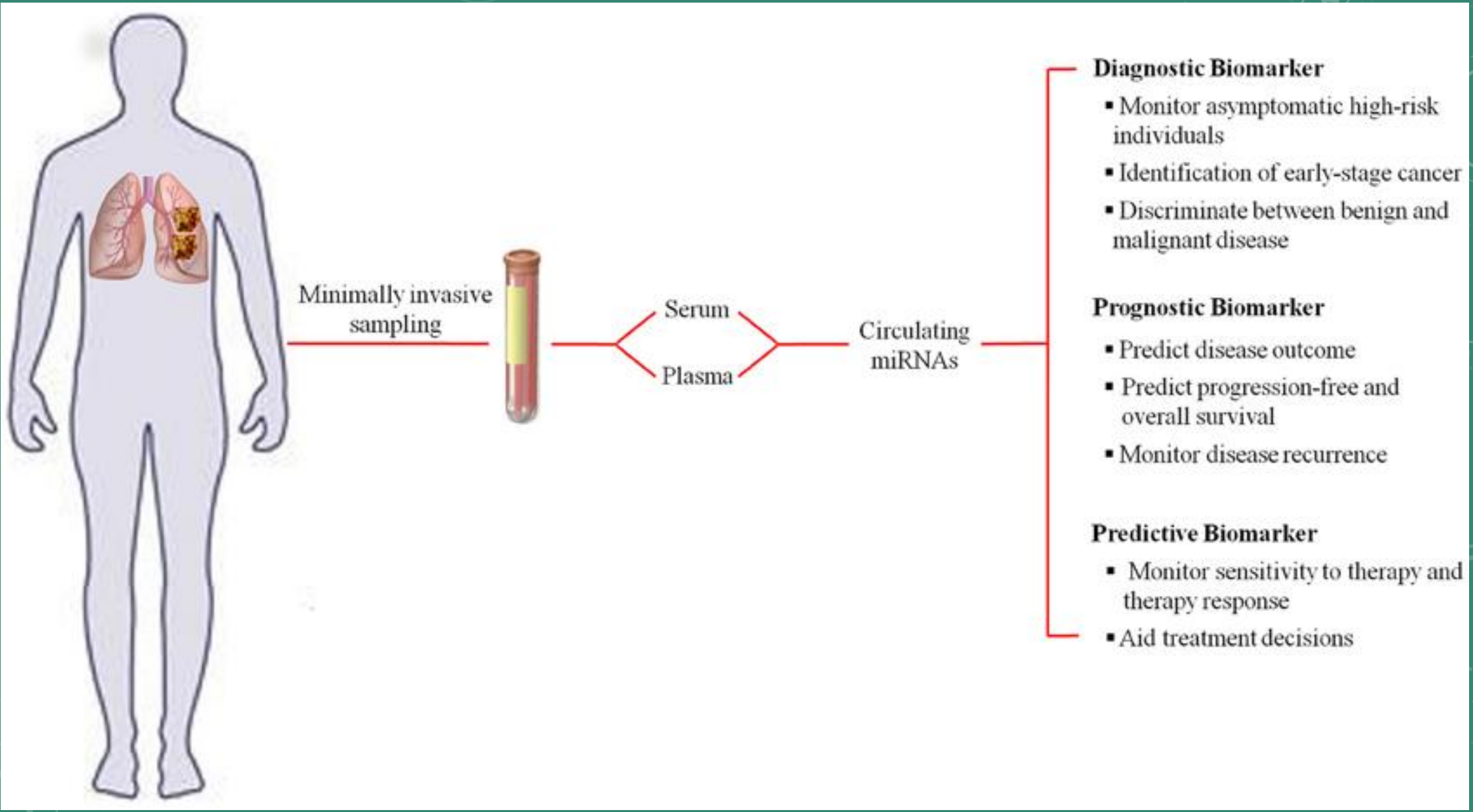
Characteristics of a Biomarker	
Specific	To a particular disease
Sensitive	Easily quantifiable
Predictive	Relevant to disease progression or treatment
Robust	Fast, simple, and cheap analysis
Stable	Equal concentrations at any time of day
Non-invasive	Samples easily acquired (blood, urine, etc)
Preclinical and clinical importance	Valid in animal/cell human models

Blood biomarkers for early cancer detection.



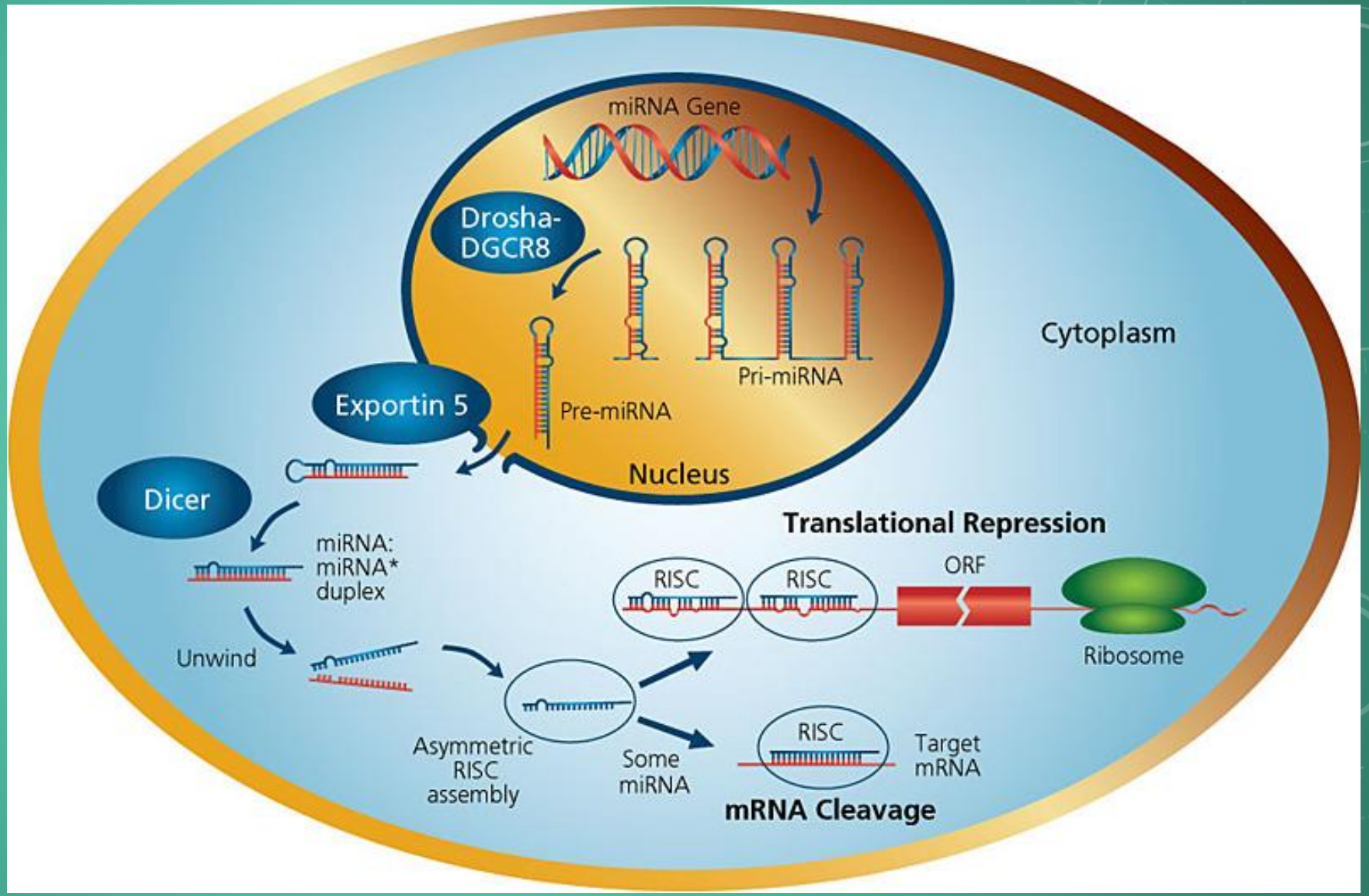
Katherine J. Martin et al. Cancer Res 2010;70:5203-5206

AACR American Association for Cancer Research
Cancer Research


Minimally invasive sampling
Serum
Plasma
Circulating miRNAs
Diagnostic Biomarker
▪ Monitor asymptomatic high-risk individuals
▪ Identification of early-stage cancer
▪ Discriminate between benign and malignant disease
Prognostic Biomarker
▪ Predict disease outcome
▪ Predict progression-free and overall survival
▪ Monitor disease recurrence
Predictive Biomarker
▪ Monitor sensitivity to therapy and therapy response
▪ Aid treatment decisions

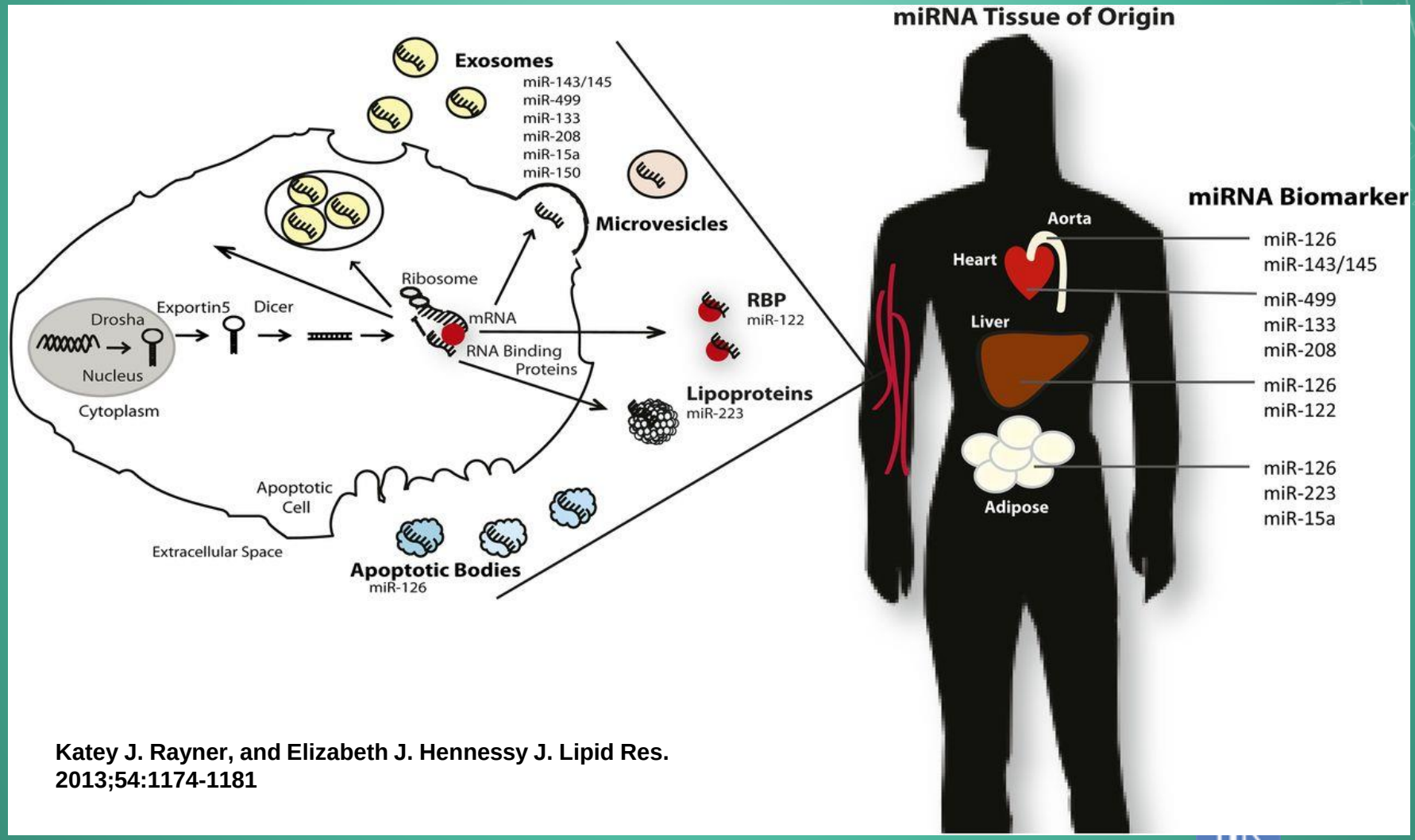
microRNA:

- non-coding
  - Regulate gene expression of mRNA targets Post-Transcriptionally
- >2500 miRNAs identified in humans



http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/mirna/learning-center/mirna-introduction.html

MicroRNAs are secreted into the circulation and are biomarkers for various diseases. miRNAs are secreted by various lipid-containing vesicles, including exosomes, microvesicles, and apoptotic bodies, and can be found outside of vesicles but bound to RNA-binding proteins



Katey J. Rayner, and Elizabeth J. Hennessy J. Lipid Res. 2013;54:1174-1181

Active area of research: microRNAs in various cancers

Plasma microRNA profiling: Exploring better biomarkers for lymphoma surveillance

Drirh Khare, Neta Goldschmidt, Aya Bardugo, Devorah Gur-Wahnon, Iddo Z. Ben-Dov, Batia Avni

Published: November 13, 2017 • https://doi.org/10.1371/journal.pone.0187722

Abstract

Early detection of relapsed lymphoma improves response and survival. Current tools lack power for detection of early relapse, while being cumbersome and expensive. We searched for sensitive biomarkers that precede clinical relapse, and serve for further studies on therapy response and relapse. We recruited 20 healthy adults, 14 diffuse large B-cell lymphoma (DLBCL) patients and 11 Hodgkin lymphoma (HL) patients at diagnosis. Using small-RNA sequencing we identified in DLBCL patients increased plasma levels of miR-124 and miR-532-5p, and decreased levels of miR-425, miR-141, miR-145, miR-197, miR-345, miR-424, miR-128 and miR-122. In the HL group, we identified miR-25, miR-30a/d, miR-26b, miR-182, miR-186, miR-140* and miR-125a to be up-regulated, while miR-23a, miR-122, miR-93 and miR-144 were down-regulated. Pathway analysis of potential mRNAs targets of these miRNA revealed in the DLBCL group potential up-regulation of STAT3, IL8, p13k/AKT and TGF-B signaling, and potential down-regulation of the PTEN and p53 pathways; while in the HL group we have found the cAMP-mediated pathway and p53 pathway to be potentially down-regulated. Survival analyses revealed that plasma levels of miR-20a/b, miR-93 and miR-106a/b were associated with higher mortality. In conclusion, we identified sets of dysregulated circulating miRNA that might serve as reliable biomarkers for relapsed lymphoma.

Active area of research: microRNAs in various cancers

Research | Open Access

Diagnostic and prognostic microRNAs in the serum of breast cancer patients measured by droplet digital PCR

Alessandra Mangolini, Manuela Ferracin, Maria Vittoria Zanzi, Elena Saccenti, Sayda Omer Ebnaof, Valentina Vultaggio Poma, Juana M. Sanz, Angela Passaro, Massimo Pedriali, Antonio Frassoldati, Patrizia Querzoli, Silvia Sabbioni, Paolo Carcoforo, Alan Hollingsworth and Massimo Negrini

Biomarker Research 2015 **3**:12
https://doi.org/10.1186/s40364-015-0037-0
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WRAP-UP

- MOLECULAR SIGNATURES
  - Measure various types of macromolecules (DNA, RNA, protein) or their activity (enzymes, phenotype)
  - Specific to the particular microorganism or human disease state
  - Exciting area of commercial development
    - Microbiome therapeutics/Precision Probiotics
    - Genetic screen & diagnostic tests for biomarkers

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  - Jenna Massaro
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