



LIVE RESEARCH FORUM:
TALENT OF TOMORROW

Microbiome Research and Therapeutics

- Introduction to microbiome research
- Fecal microbiota transplantation
- In vivo validation of the discovered probiotics
- Sequencing-based pathogen identification



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เจนเจริญพันธ์

วันที่ 29 เมษายน 2564 เวลา 12.00-13.30 น.

Online: by zoom

Onsite: ห้องประชุม 309 ชั้น 3 ตึกอำนวยการ (รับไม่เกิน 25 คน)

Register by scanning
QR code



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ศ. นพ. พิสิฐ ตั้งกิจวานิชย์

หัวหน้าศูนย์เชี่ยวชาญเฉพาะทาง
ด้านโรคตับอักเสบและมะเร็งตับ
คณะแพทยศาสตร์
จุฬาลงกรณ์มหาวิทยาลัย

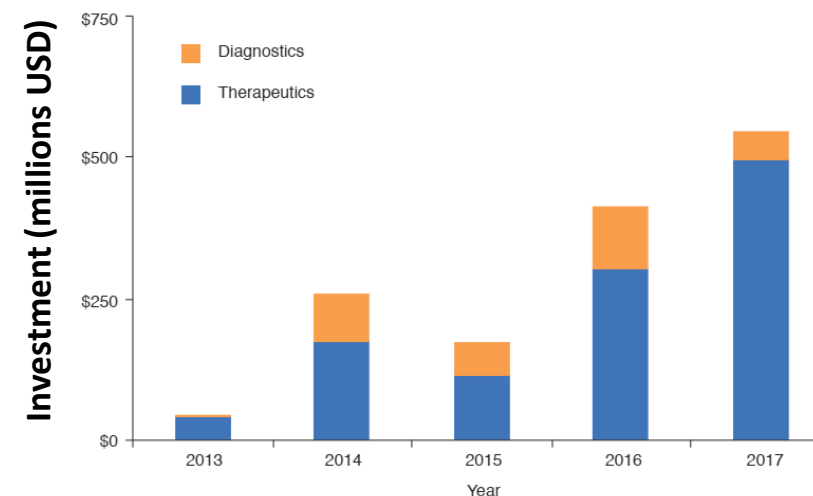
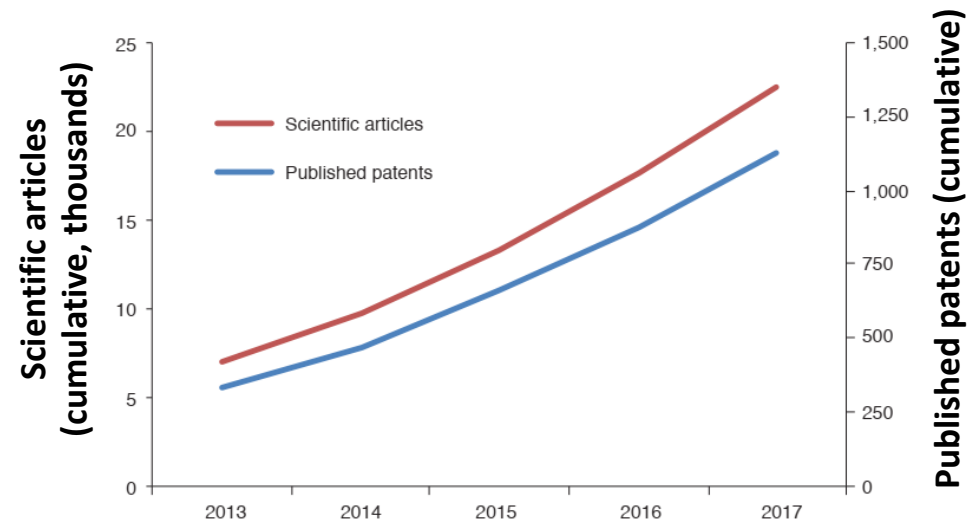


รศ. ดร. อินทวัฒน์ หนูแก้ว

Department of Biomedical
Informatics, College of Medicine,
University of Arkansas for
Medical Sciences, USA



WHY
WHO
WHEN
HOW ?





UAMS

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES



Mahidol University Faculty of Medicine Siriraj Hospital



Gut-Liver Axis

The Journal of Infectious Diseases

MAJOR ARTICLE



Improvement of Gut Diversity and Composition After Direct-Acting Antivirals in Hepatitis C Virus-Infected Patients With or Without Human Immunodeficiency Virus Coinfection

Nathaya Chuaypen,¹ Thananya Jinato,² Anchalee Avithingsanon,² Sakkarin Chirapongsathorn,³ Supapon Cheevadhanarak,^{3,4} Intawat Nookaew,^{5,6} Yasuhito Tanaka,⁷ and Pisit Tangkijvanich⁷



Fecal microbiota Transplantation (FMT)/ Antimicrobial resistance

Germ Line IgM Is Sufficient, but Not Required, for Antibody-Mediated Alphavirus Clearance from the Central Nervous System

Voraphoj Nilaratanakul,^{a,b*} Jie Chen,^a Oanh Tran,^{a*} Victoria K. Baxter,^{a,b*} Elizabeth M. Troisi,^a Jane X. Yeh,^a Diane E. Griffin^a

Probiotics/Prebiotics

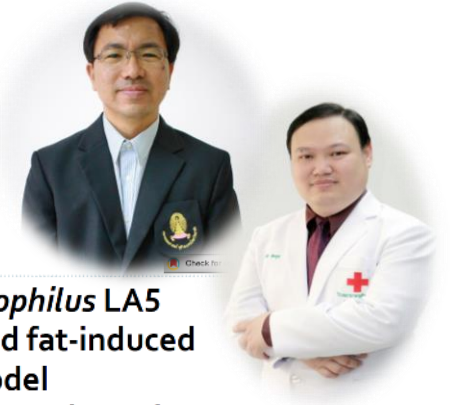
- Type 2 DM
- Obesity
- Probiotics
- Prebiotics

scientific reports

OPEN

Lactobacillus acidophilus LA5 improves saturated fat-induced obesity mouse model through the enhanced intestinal *Akkermansia muciniphila*

Thunnicha Ondee¹, Krit Pongpirul^{1,4,10}, Peerapat Visitchanakun⁵, Wilasinee Saisorn⁶, Suthicha Kanachoren⁷, Lampet Wongsaraj⁶, Chitrasak Kullapanich¹, Natharin Ngamwongsatit⁸, Sam Settachaimongkon^{9,10}, Naraporn Somboonna^{6,7} & Asada Leelahavanichkul^{1,11}*



Integration of wet lab and bioinformatics



Journal of Microbiological Methods 166 (2019) 105739

Contents lists available at ScienceDirect



Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

An assessment of Oxford Nanopore sequencing for human gut metagenome profiling: A pilot study of head and neck cancer patients

Thidathip Wongsurawat^a, Mayumi Nakagawa^b, Omar Atiq^{c,d}, Hannah N. Coleman^b, Piroon Jenjaroenpun^a, James I. Allred^e, Angela Trammel^e, Pantakan Puengrang^f, David W. Ussery^{a,g}, Intawat Nookaew^{a,h,*}

SCIENTIFIC REPORTS

nature research

Combining short and long read sequencing to characterize antimicrobial resistance genes on plasmids applied to an unauthorized genetically modified *Bacillus*

Bas Berbers^{1,2}, Assia Salytkova^{1,2}, Cristina Garcia-Graells³, Patrick Philipp³, Fabrice Arella⁴, Kathleen Marchal^{3,5}, Raf Winand³, Kevin Vanneste³, Nancy H. C. Roosens^{1,6} & Sigrid C. J. De Keersmaecker^{3,6*}

Introduction to microbiome Research

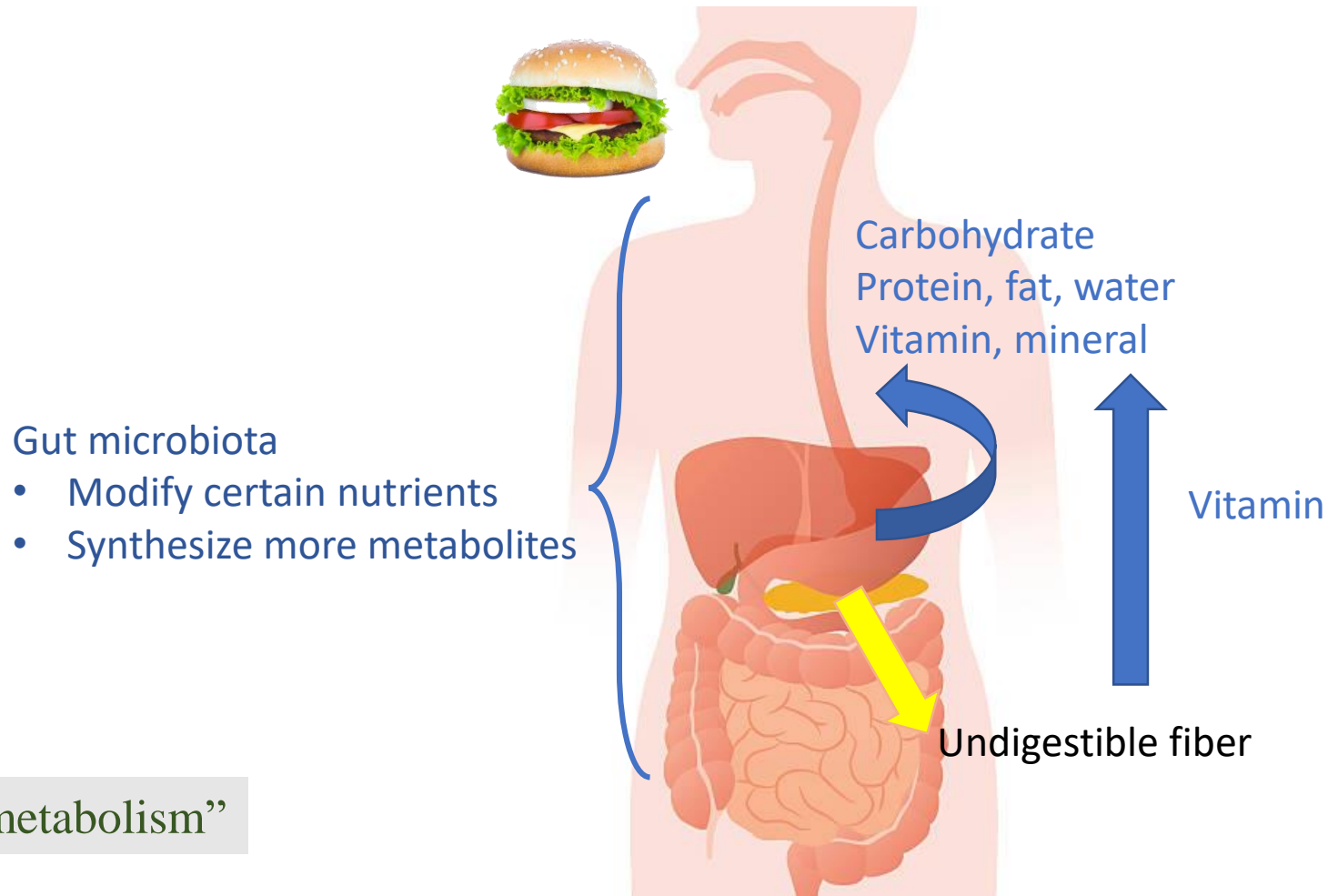
How the microbiome forms, How it can affect
your health and how to fix it???

Assoc. Prof. Thasinas Dissayabutra, M.D., PhD.

Natthaya Chuaypen, PhD.

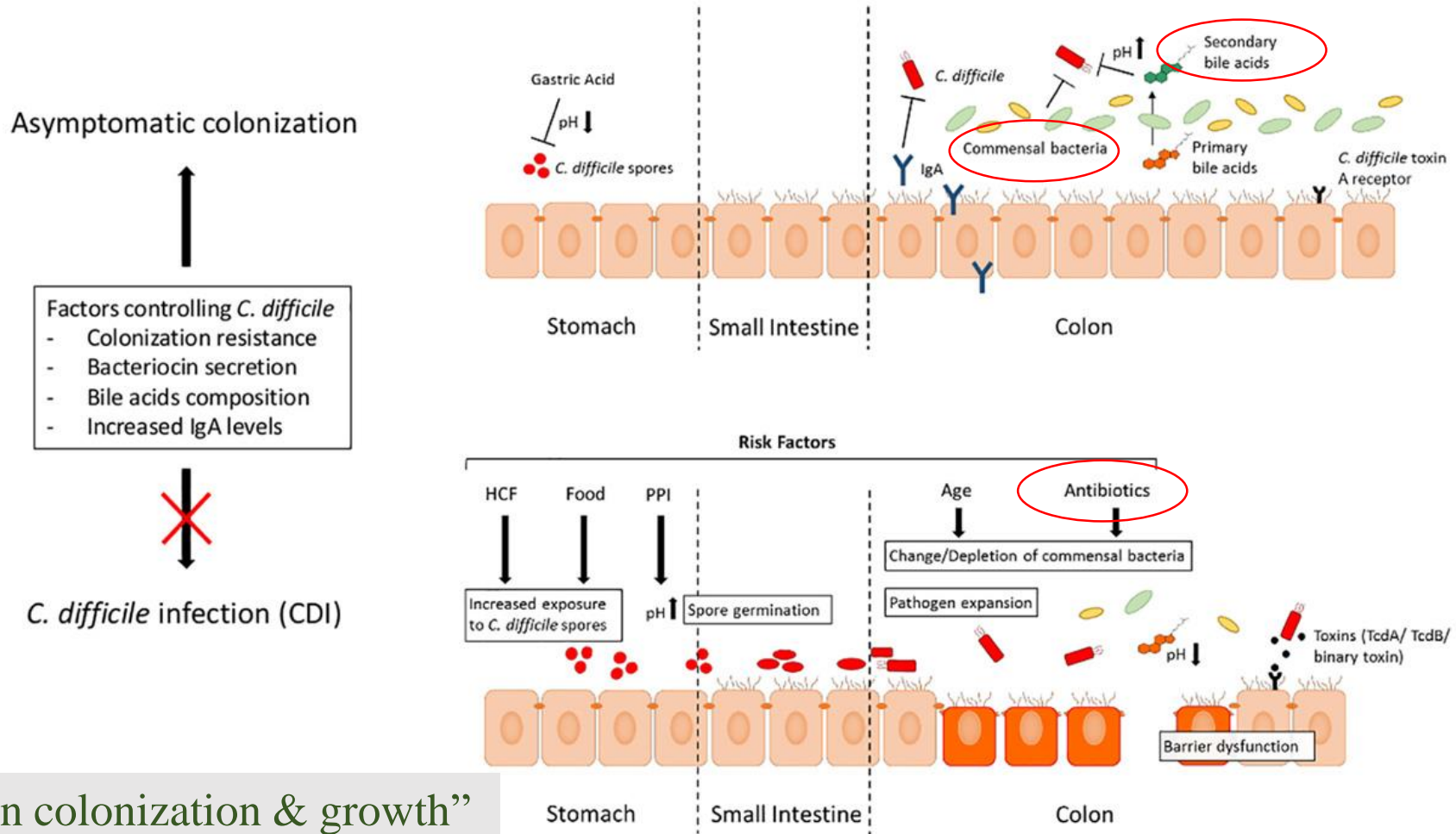
Department of Biochemistry, Faculty of Medicine, Chulalongkorn University

Gut microbiota: Old wine in new bottles



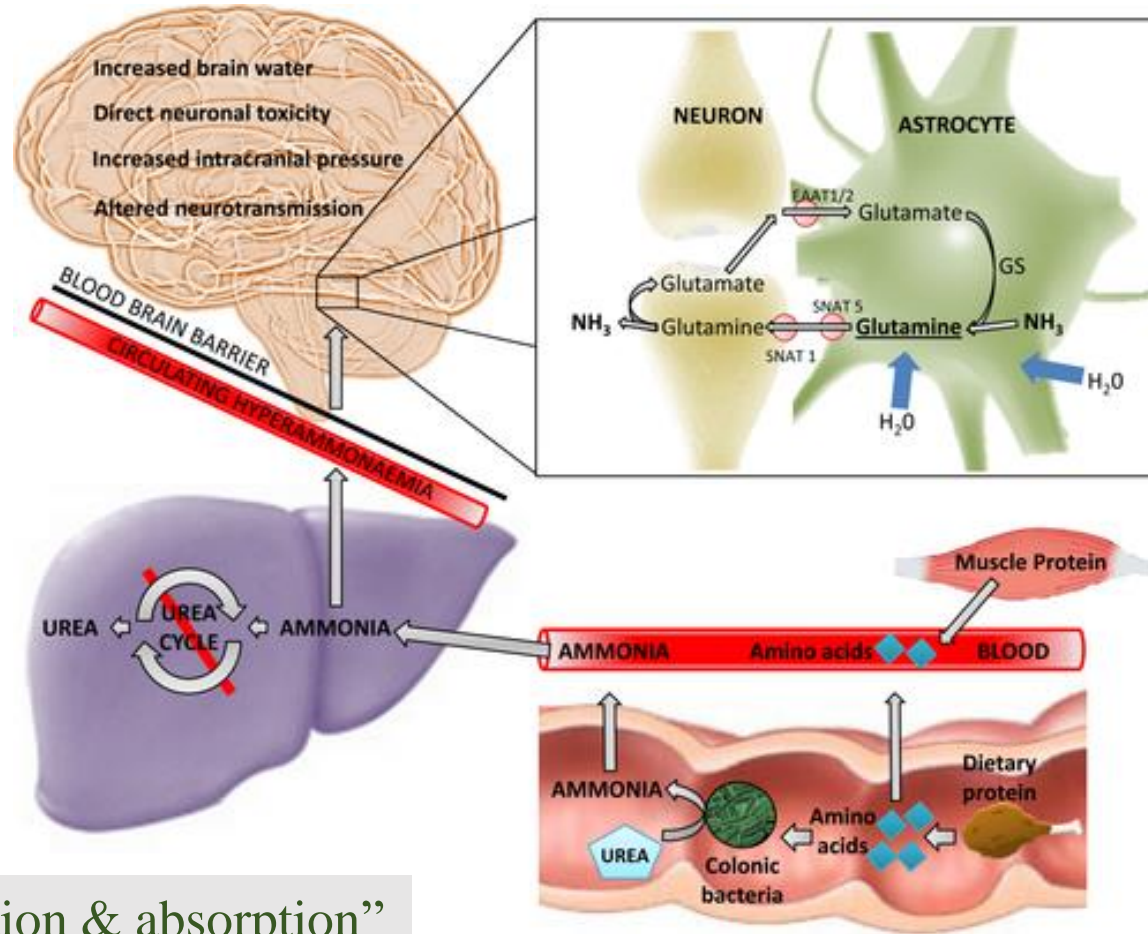
“Enhance metabolism”

Gut microbiota: Old wine in new bottles



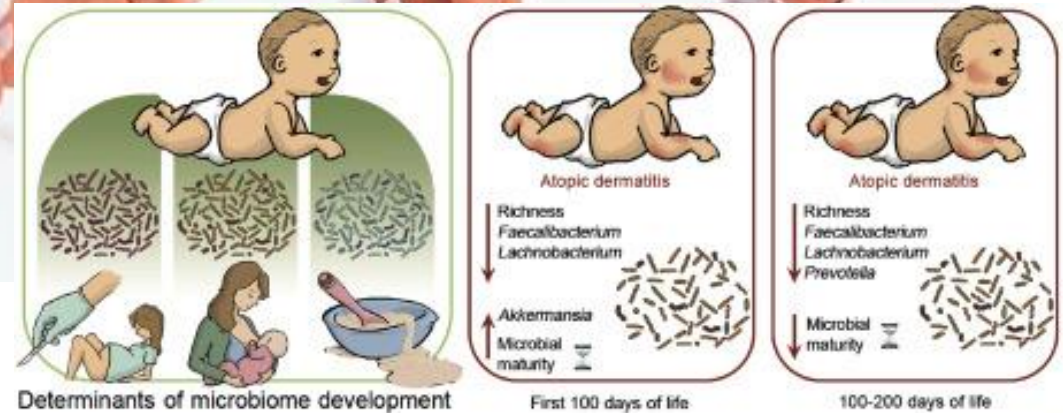
“Prevent pathogen colonization & growth”

Gut microbiota: Old wine in new bottles



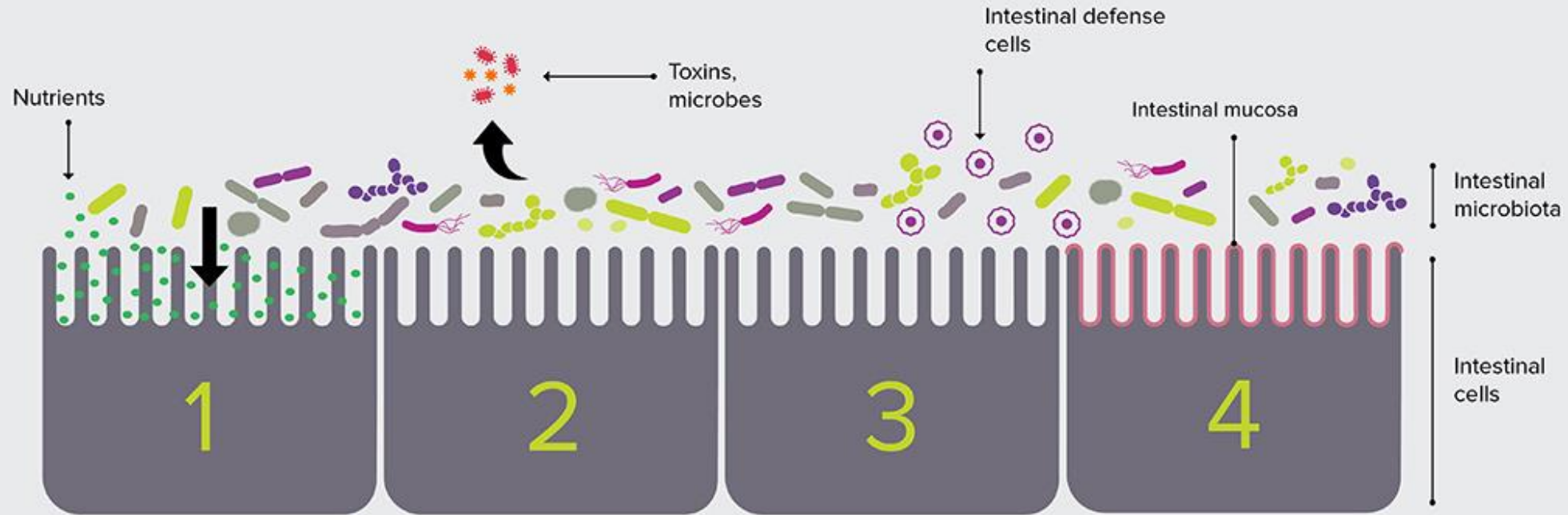
“Regulate toxin production & absorption”

Gut microbiota: Old wine in new bottles



“Immunomodulation”

THE ROLE OF THE INTESTINAL MICROBIOTA



1 Metabolic role

Promote digestion: fermentation of non-digestible foods, absorption of nutrients (amino acids, sugars, vitamins, etc.) by intestinal cells. Participation in the synthesis of metabolites (short-chain fatty acids, vitamins K, B12, B8)

2 Barrier role

Shield against microbes, toxins, etc. Production of protective mucus by intestinal cells.

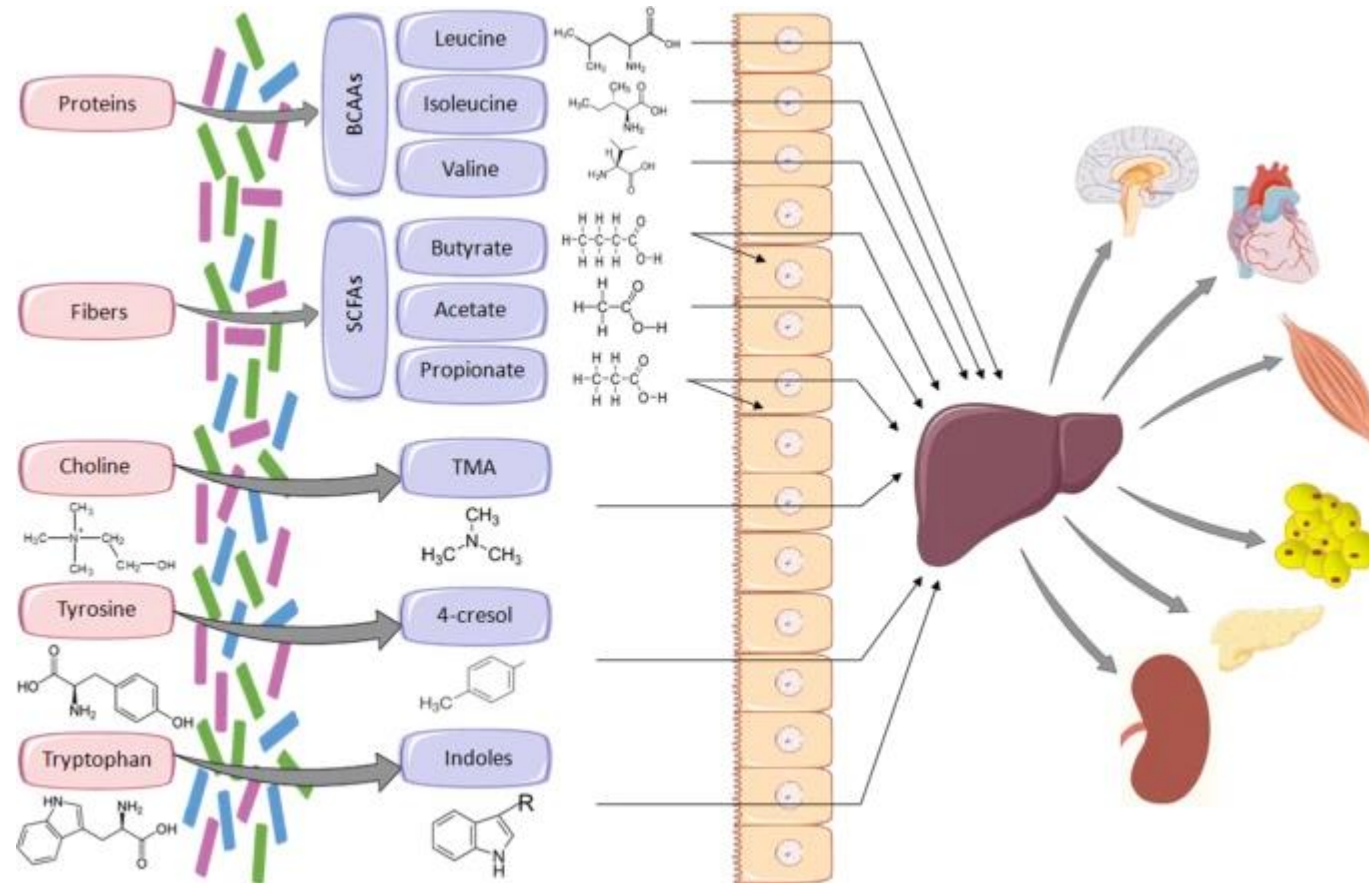
3 Defensive role

Development of the intestinal immune system

4 Maintenance role

Maturation of the gastrointestinal tract, maintenance of intestinal mucosa, production of mucus, enzymatic activity by the mucosa.

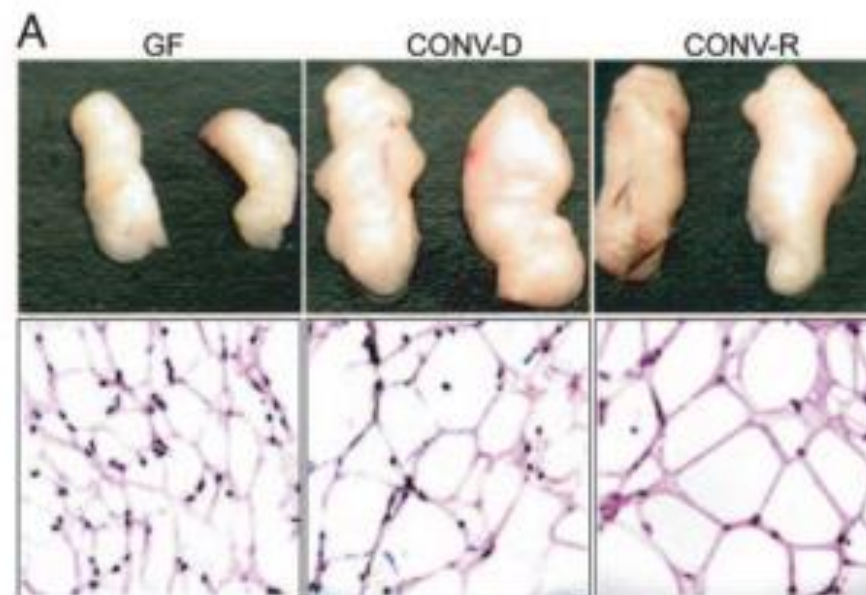
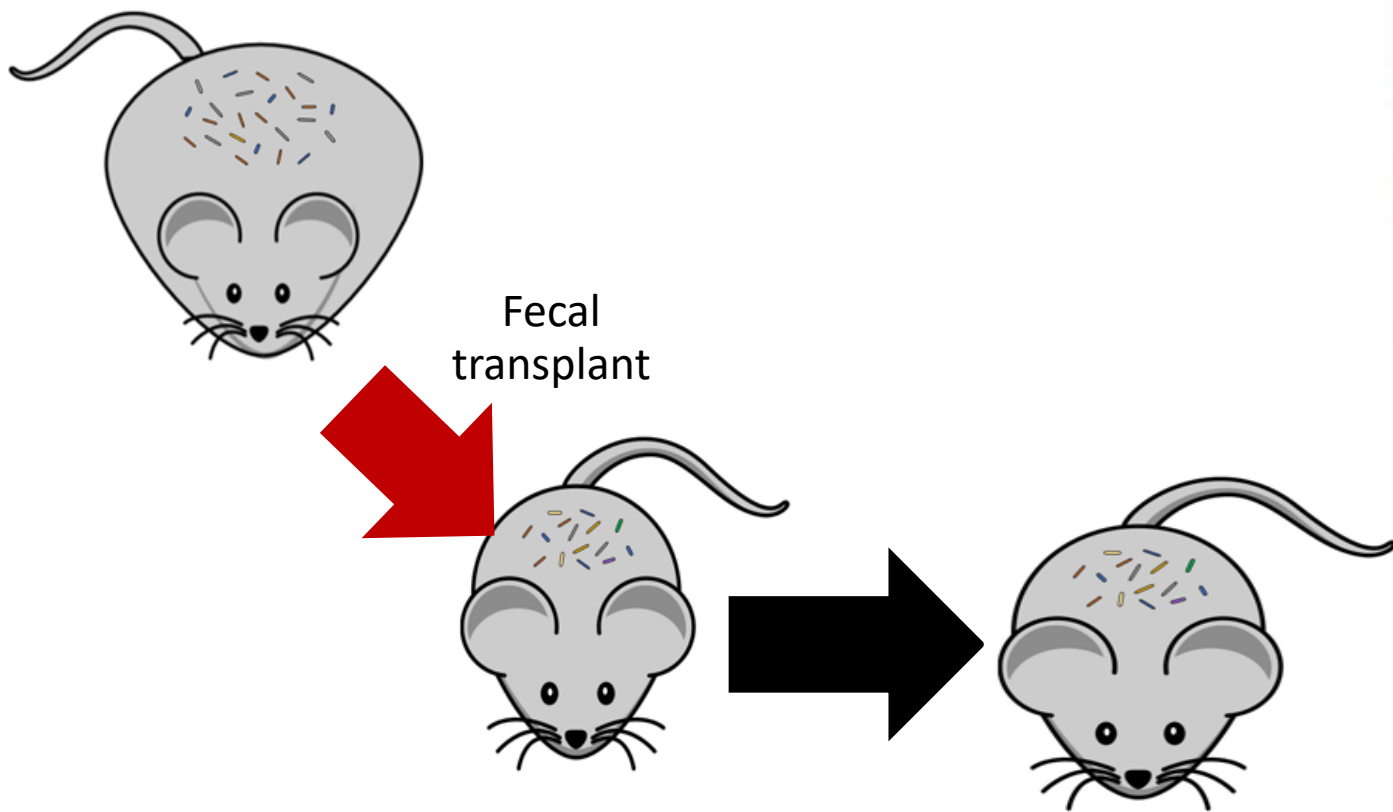
Gut microbiota metabolites and toxins



ARTICLES

An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh¹, Ruth E. Ley¹, Michael A. Mahowald¹, Vincent Magrini², Elaine R. Mardis^{1,2} & Jeffrey I. Gordon¹



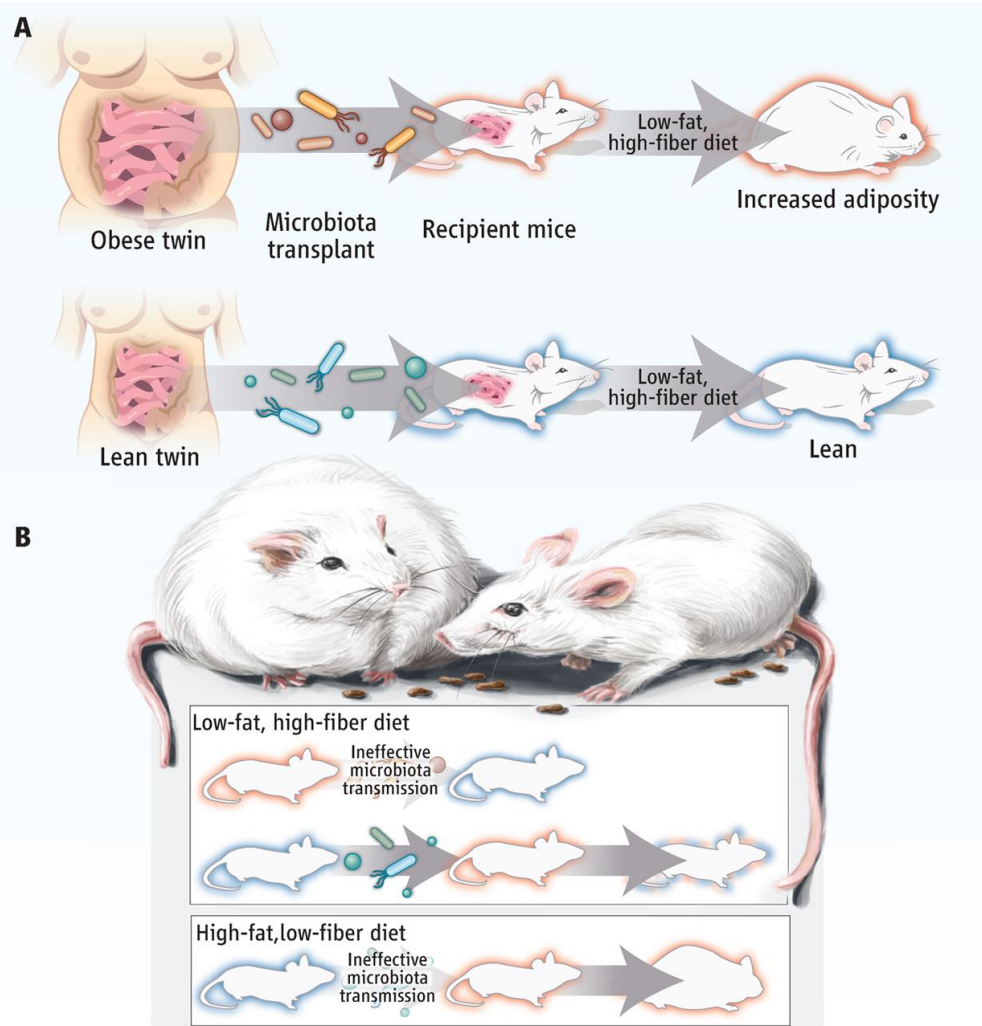
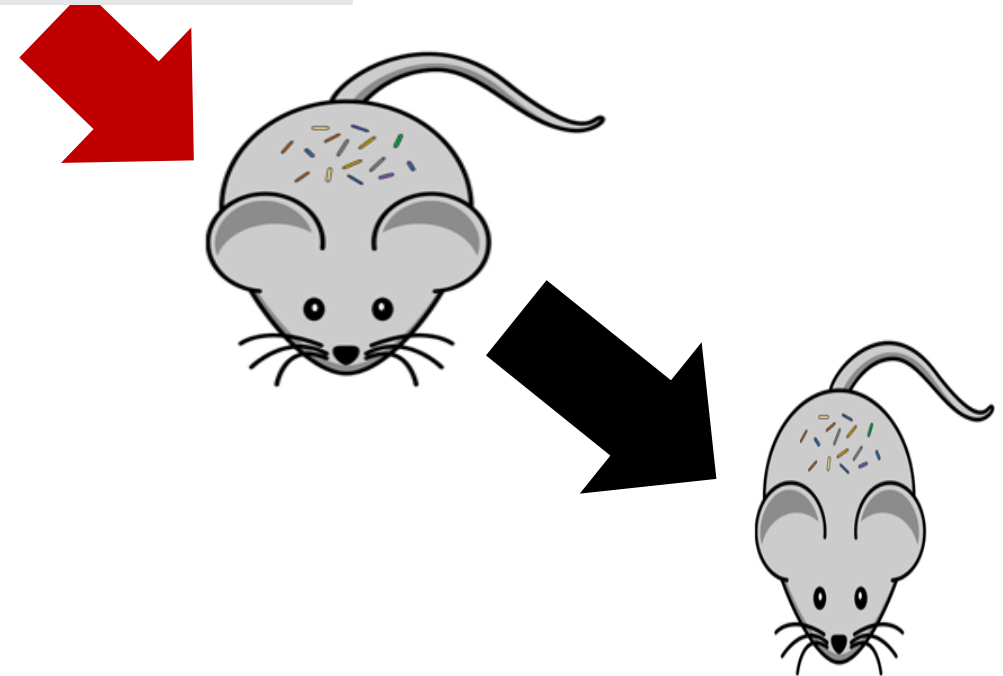
Obesity is transmittable

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Kwashiorkor and the Gut Microbiota

Wendy S. Garrett, M.D., Ph.D.

Fecal transplant from Kwashiorkor donor

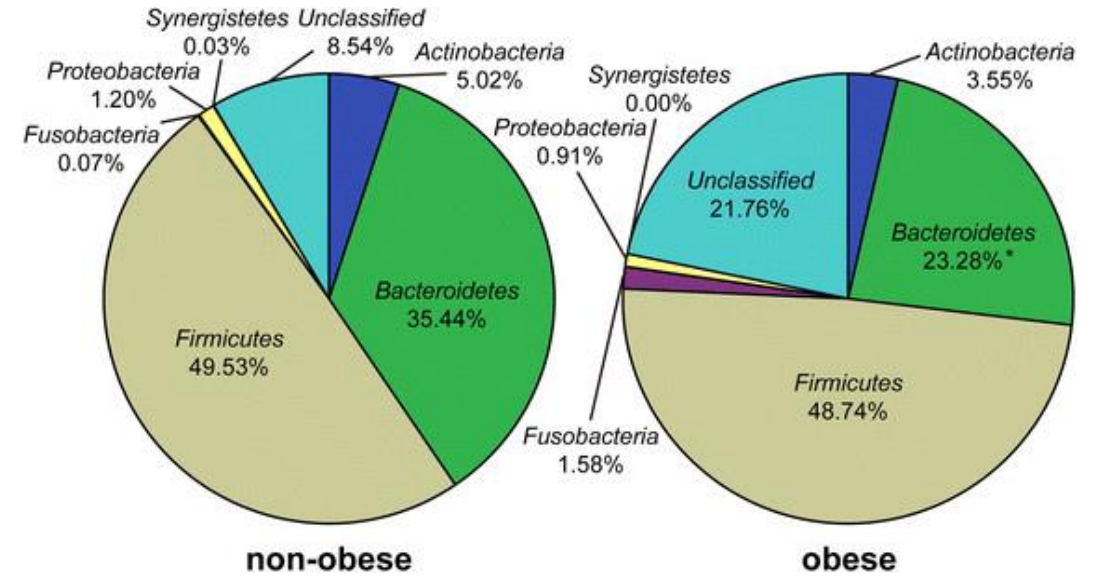


Alan W. Walker, and Julian Parkhill Science 2013;341:1069-1070
Copyright © 2013, American Association for the Advancement of Science

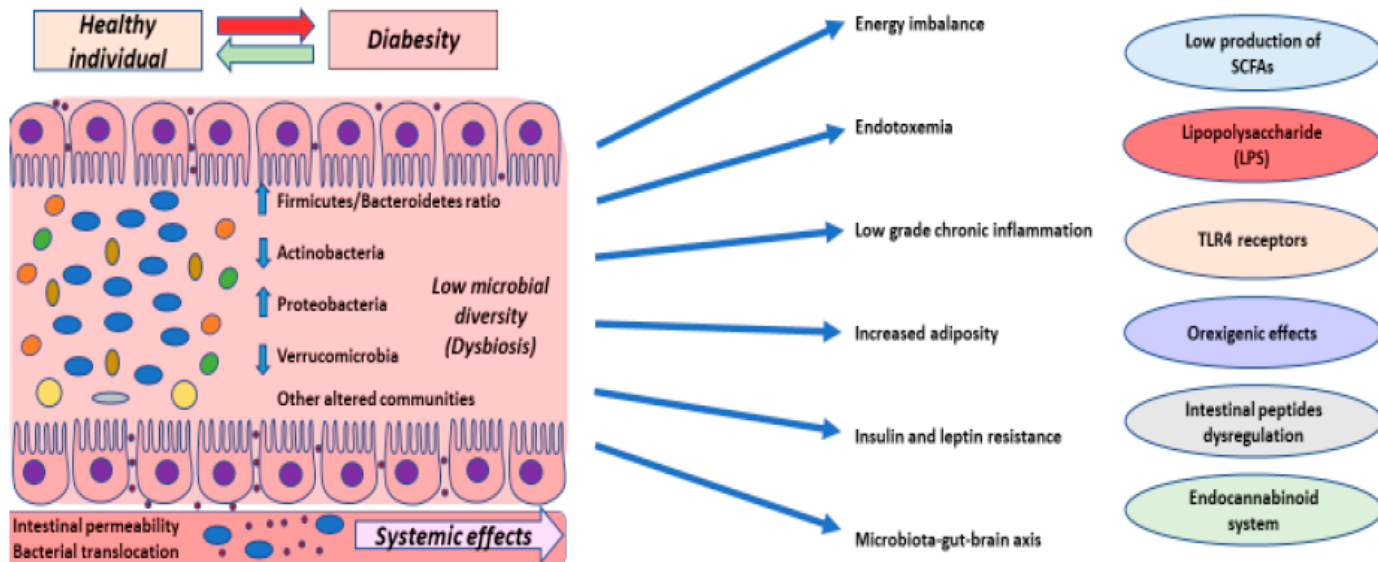
Review

Type 2 Diabetes Mellitus Associated with Obesity (Diabesity). The Central Role of Gut Microbiota and Its Translational Applications

Miguel A. Ortega ^{1,2,3,*}, Oscar Fraile-Martínez ^{1,†}, Irene Naya ¹, Natalio García-Hondurilla ^{1,2}, Melchor Álvarez-Mon ^{1,2,4,5}, Julia Buján ^{1,2,3}, Ángel Asúnsolo ^{6,7,†} and Basilio de la Torre ^{6,8,†}



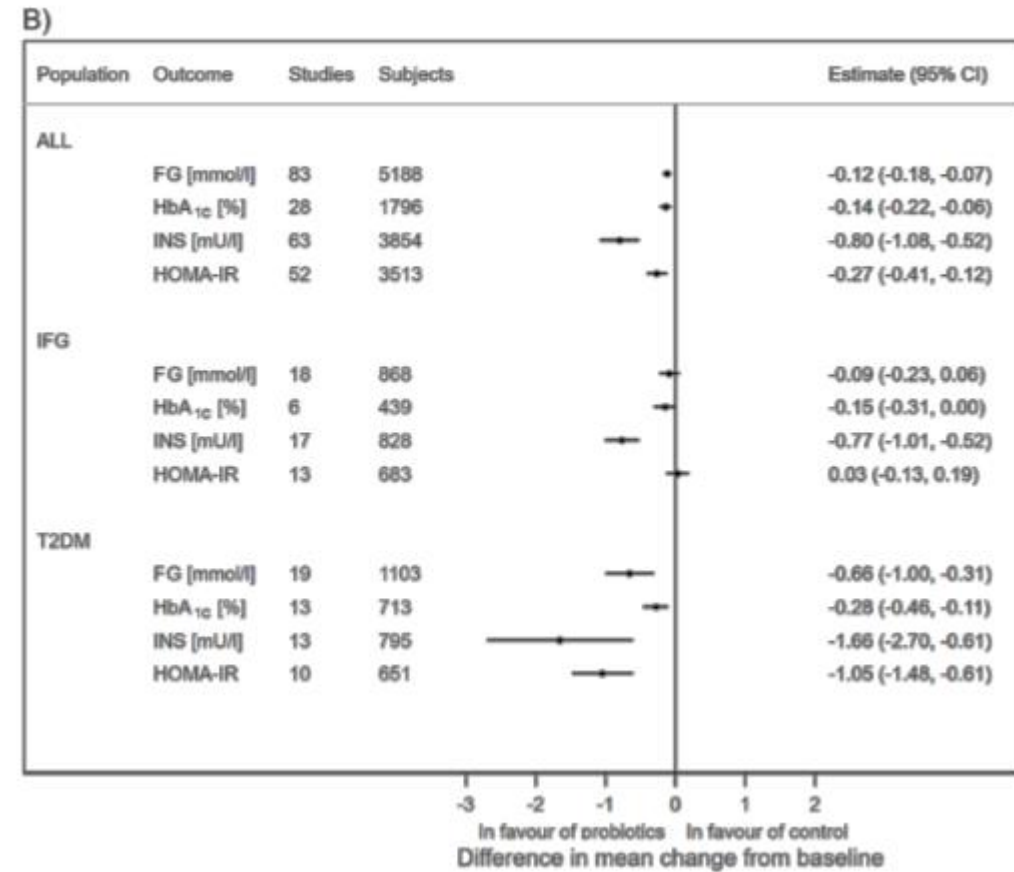
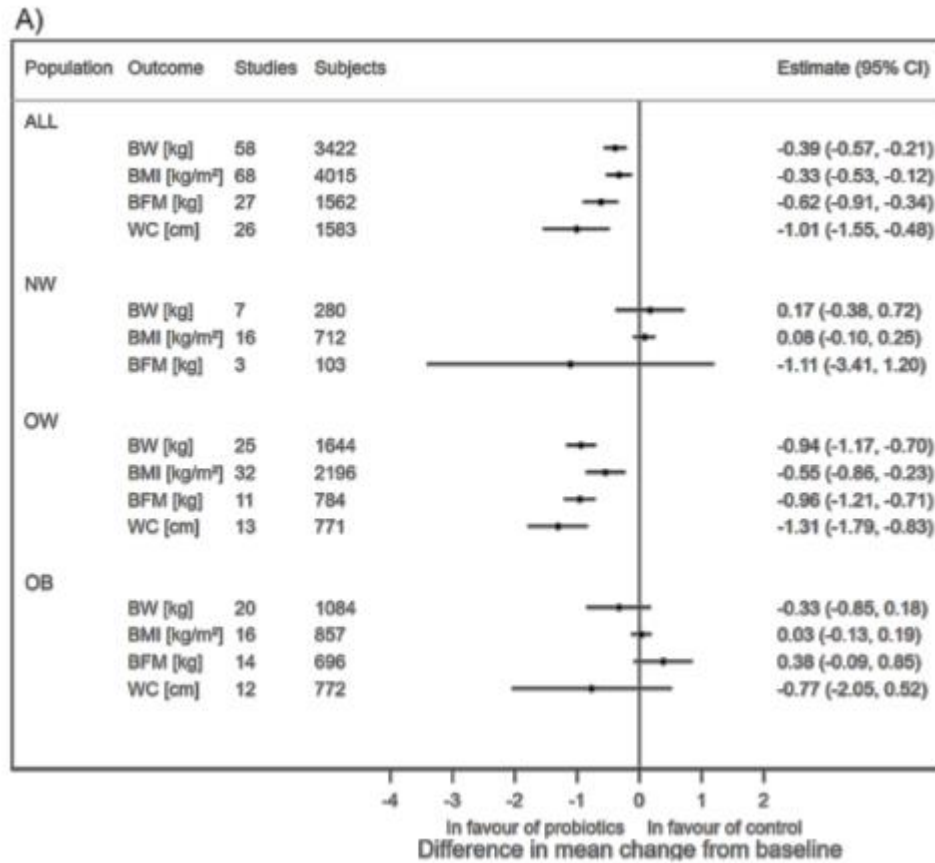
Kasai C. BMC Gastroenterology (2015)



Taxa features	Function features
• ↓ Bacteroidetes to Firmicutes ratio	• ↑ LGC: degradation of β-glucuronide and aromatic amino acids
• ↓ Akkermansia muciniphila	• ↑ HGC: production of organic acids and H ₂
• ↓ Bacteroides thetaiotaomicron	• ↑ Phosphotransferase system
• ↓ Clostridium histolyticum	• ↓ Glycosaminoglycan degradation
• ↓ Clostridium coccooides	• ↑ Glutamine or glutamate transport system
• ↑ Dorea longicatena	• ↑ Phenylalanine, tyrosine and tryptophan biosynthesis
• ↑ Eubacterium ventriosum	• ↓ Valine, leucine and isoleucine degradation
• ↓ Faecalibacterium prausnitzii	• ↓ Superoxide reductase
• ↓ Methanobrevibacter smithii	
• ↑ Roseburia intestinalis	
• ↑ Ruminococcus gnavus	
• ↑ Ruminococcus torques	

Yong F. Nature Review (2020)

Probiotic treatment on obesity and diabetes mellitus



Koutnikova H. BMJ (2019)
Marzloom K. Nutrient (2019)

Clinical cohorts consisted of relatively small sample size, and focused on short-term physical parameters, or inflammatory markers, making long-term follow up studies highly desired in future work

Drug factory probiotics for obesity and diabetes

Obesity

SCIENTIFIC REPORTS

OPEN

Administration of *N*-Acyl-Phosphatidylethanolamine Expressing Bacteria to Low Density Lipoprotein Receptor^{-/-} Mice Improves Indices of Cardiometabolic Disease

Linda S. May-Zhang¹, Zhongyi Chen¹, Noura S. Dosoky¹, Patricia G. Yancey², Kelli L. Boyd³, Alyssa H. Hasty⁴, MacRae F. Linton² & Sean S. Davies¹

Received: 31 May 2018
Accepted: 13 November 2018
Published online: 23 January 2019

Pharm Res (2014) 31:3404–3414
DOI 10.1007/s11095-014-1430-3

RESEARCH PAPER

T2DM

Oral Delivery of Glucagon Like Peptide-I by a Recombinant *Lactococcus lactis*

Payal Agarwal • Pulkit Khatri • Blasé Billack • Woon-Kai Low • Jun Shao

JCI The Journal of Clinical Investigation

T1DM

Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified *Lactococcus lactis* in mice

Tatiana Takiishi, ... , Conny Gysemans, Chantal Mathieu

J Clin Invest. 2012;122(5):1717-1725. <https://doi.org/10.1172/JCI60530>.



NAFL

RESEARCH ARTICLE
Therapeutics and Prevention



Secretion of Recombinant Interleukin-22 by Engineered *Lactobacillus reuteri* Reduces Fatty Liver Disease in a Mouse Model of Diet-Induced Obesity

Jee-Hwan Oh,^a Kathryn L. Schueler,^b Donnie S. Stapleton,^b Laura M. Alexander,^a Chi-Liang Eric Yen,^c Mark P. Keller,^b Alan D. Attie,^b Jan-Peter van Pijkeren^a

Research focus for today!

What and How?

- **Fecal Microbiota Transplantation (FMT)?**

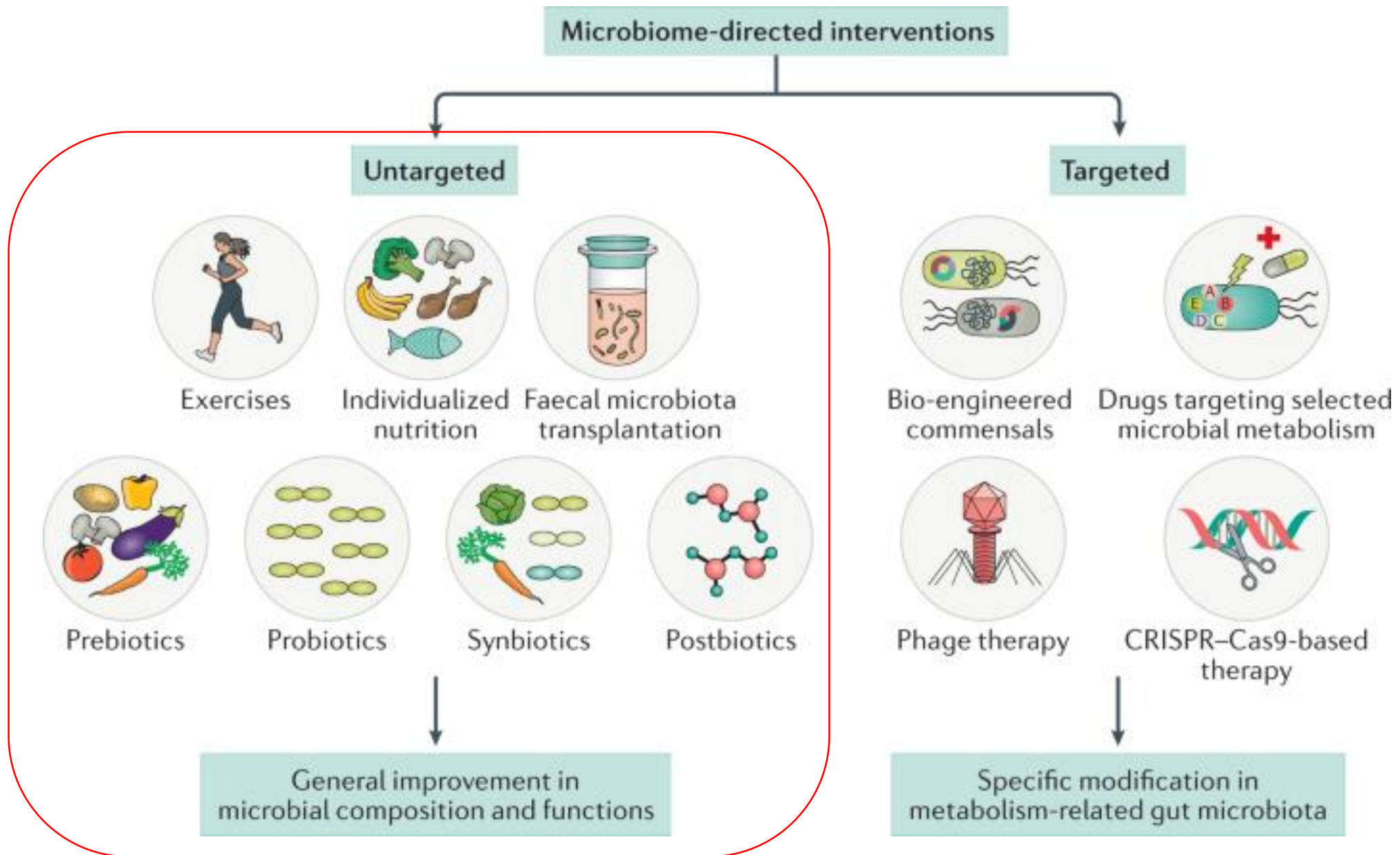
Voraphoj Nilaratanakul, MD, PhD

- **In vivo test for the probiotics?**

Assist. Prof. Asada Leelahavanichkul, MD., PhD.

- **Sequencing-based pathogen identification and clinical application?**

Thidathip Wongsurawat, PhD./ Piroon Jenjaroenpun, PhD.



Patients with *Clostridium difficile* infection (CDI) in Inflammatory Bowel Disease (IBD)



(a)



(b)

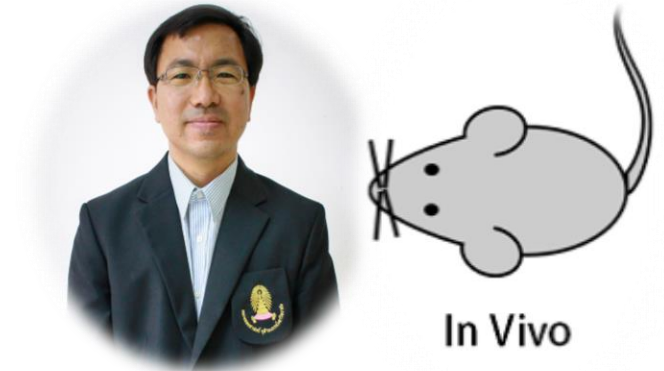
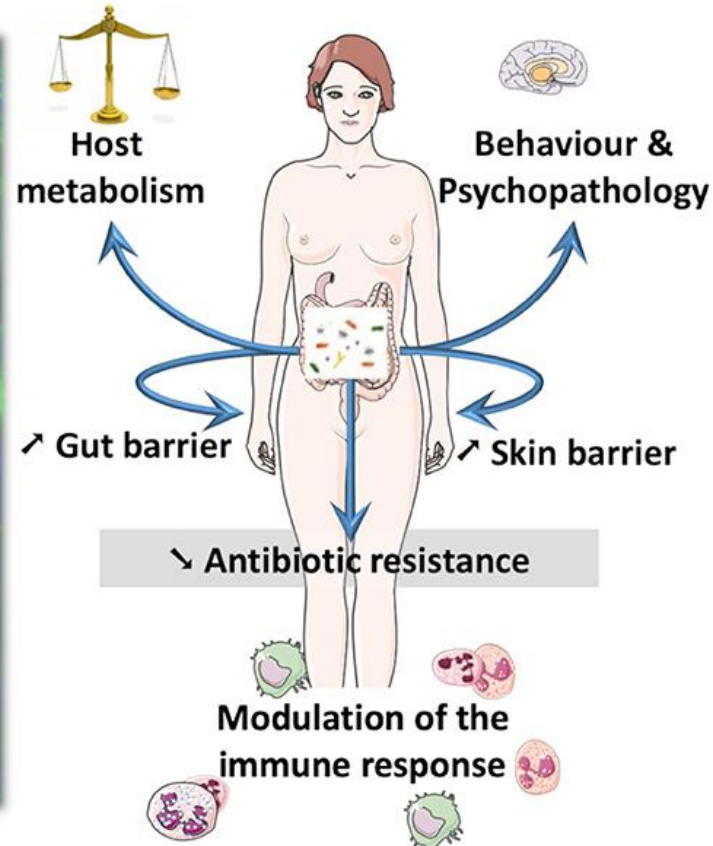
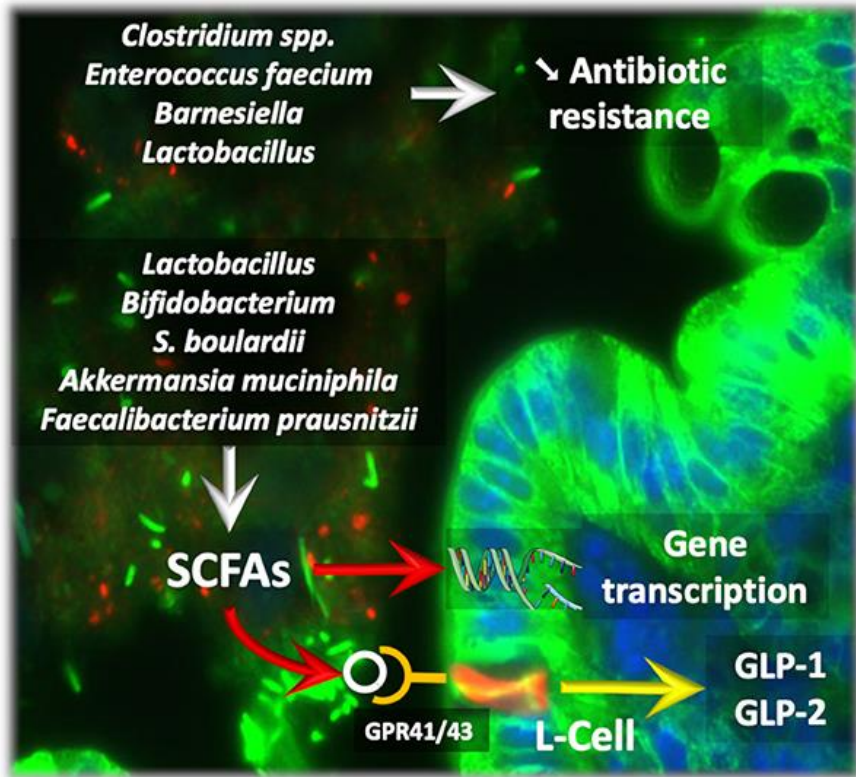


Remission of ulcerative colitis after 14 days of FMT



FMT in Thailand?

How Probiotics Affect the Microbiota and Health?

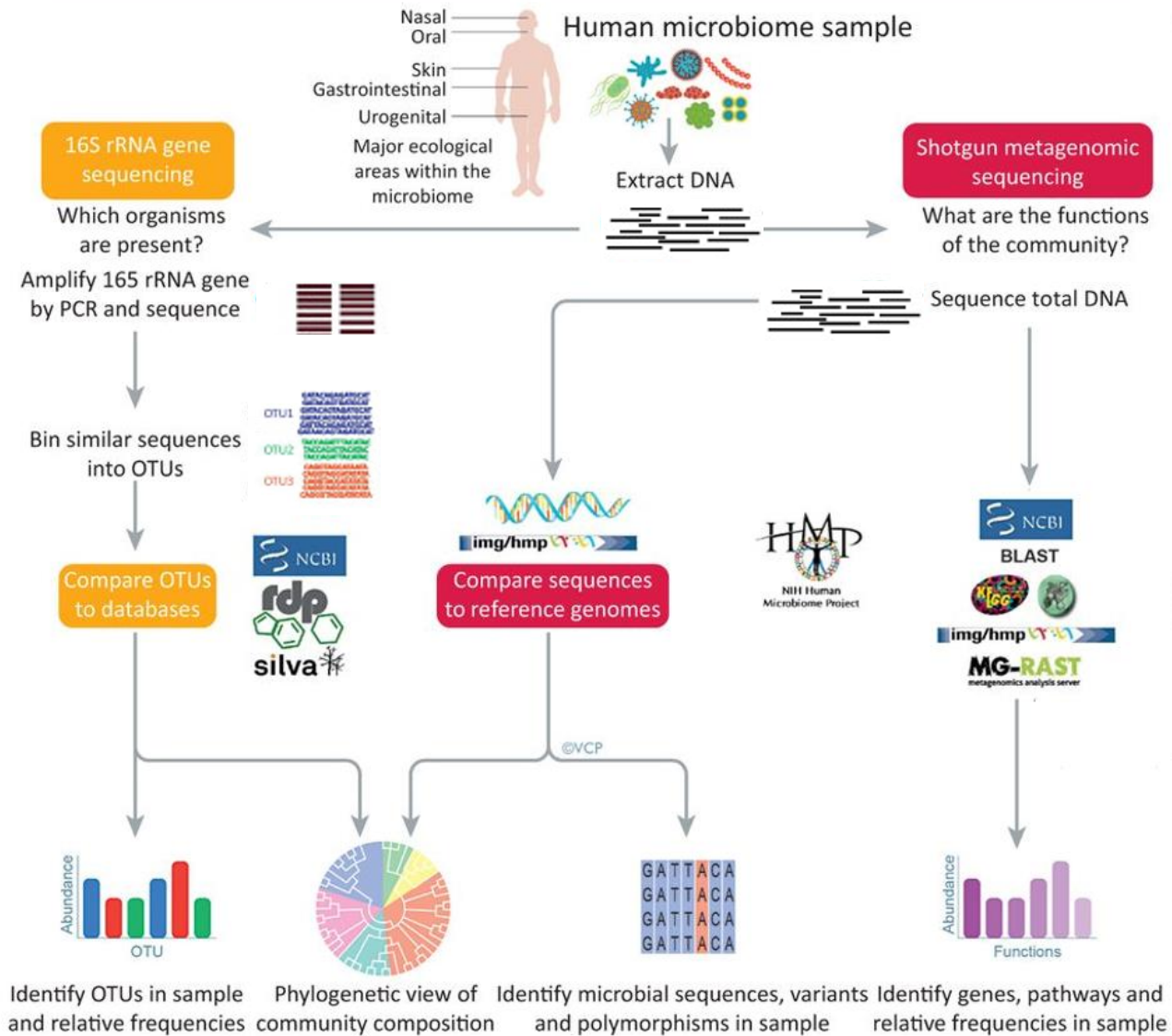


How to do *in vivo* test for the probiotics?

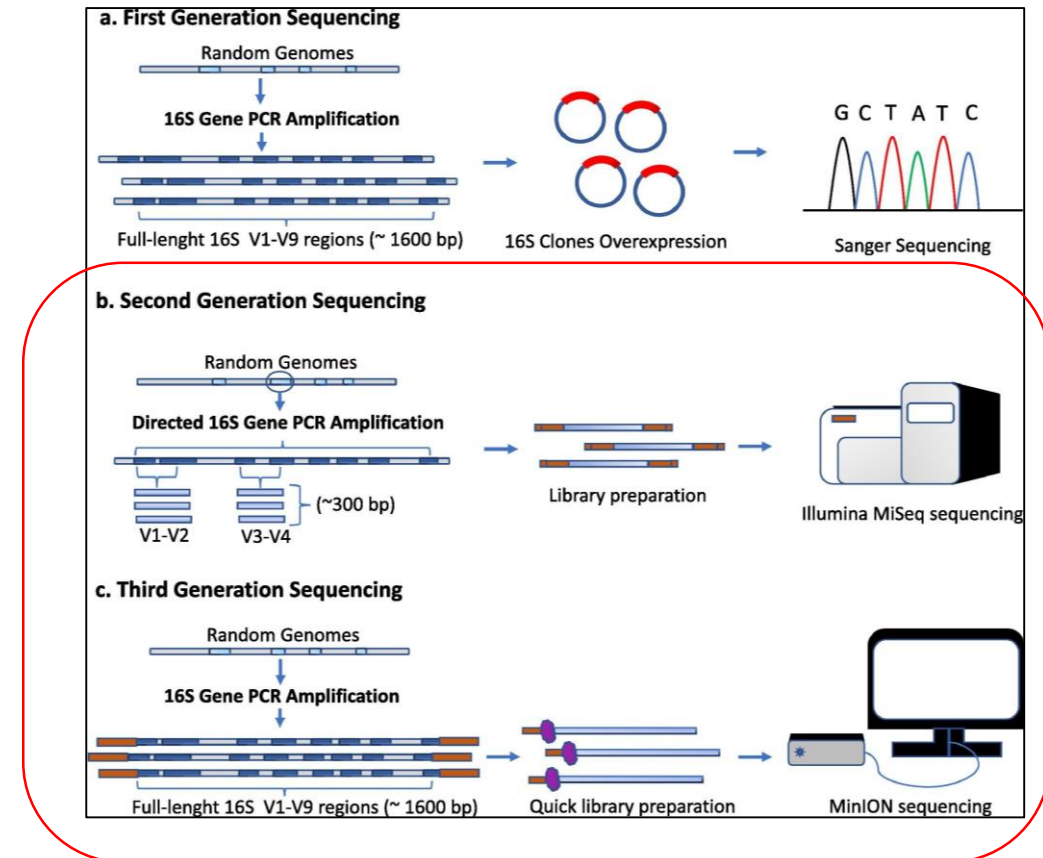


How to identify pathogen and AMR?

Current techniques in human microbiome profiling



How to identify pathogen based-on sequencing?





Fecal Microbiota Transplantation (FMT)

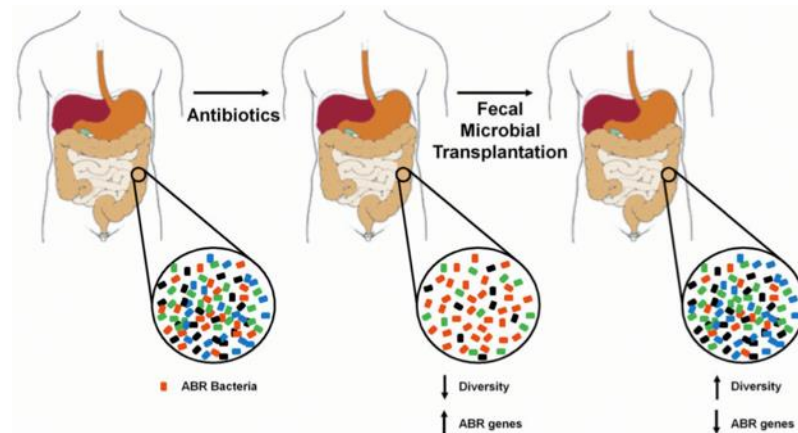
Voraphoj Nilaratanakul, MD, PhD

Dysbiosis

- Imbalance in the microbial community associated with diseases
 - ↓ beneficial flora (Firmicutes, Bacteroides)
 - ↑ pathobionts (Proteobacteria)
 - Loss of diversity
- Factors
 - Infections and illnesses
 - Food and drugs, esp. ATB
 - Stress, environment
 - Genetic, sex, age, BMI
 - Formula feeding, cesarean section, smoking, etc.
 - Hygiene hypothesis: worm therapy for allergy/autoimmune
- Sometimes imbalance can provide health benefits
 - Cyclophosphamide

Dysbiosis prevention & treatment

- Selective digestive decontamination
 - Aminoglycosides, colistin
- Protection
 - Ribaxamase
 - Activated charcoal
- Other manipulations
 - Phage
 - Parasite ova
 - MicroRNA



- Restoration
 - Waiting
 - 1-3 mo, but up to a year for MDR organisms to be eradicated
 - Appendix as a shelter for original microbiota
 - Probiotics (work in animal models but mostly inconclusive in humans)
 - Prebiotics: Human milk oligosaccharide
 - Probiotics: *Lactobacillus*, *Bifidobacterium*
 - Postbiotics: Butyrate, Indole
 - Synbiotics: Pre+Pro
 - Fecal microbiota transplantation (FMT)

1 probiotic for any diseases, any antibiotics, and any patients?

Same jobs, different kinds of microbes



<https://www.mintpressnews.com/wp-content/uploads/2014/11/AP450907056.jpg>



https://www.volunteeringsolutions.com/asset/uploads/img/photo_gallery/thailand/613__dsc5436.jpg

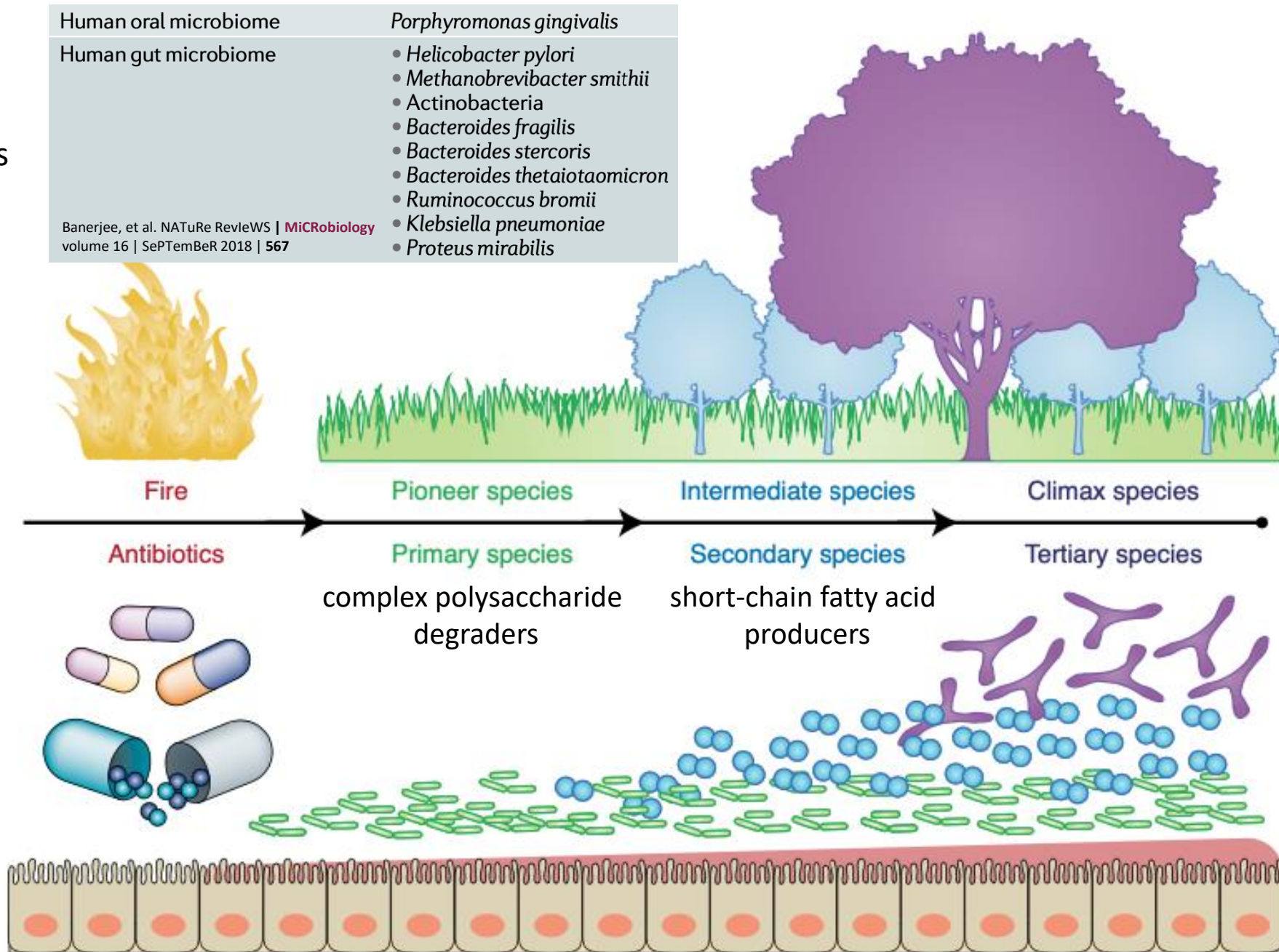


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<https://www.bragard.com/3047/chef-s-jackets-grand-chef-white.jpg>

Non-dominant keystone species

Phages?
Fungi?
Worms?



Fecal Microbiota Transplantation (FMT)

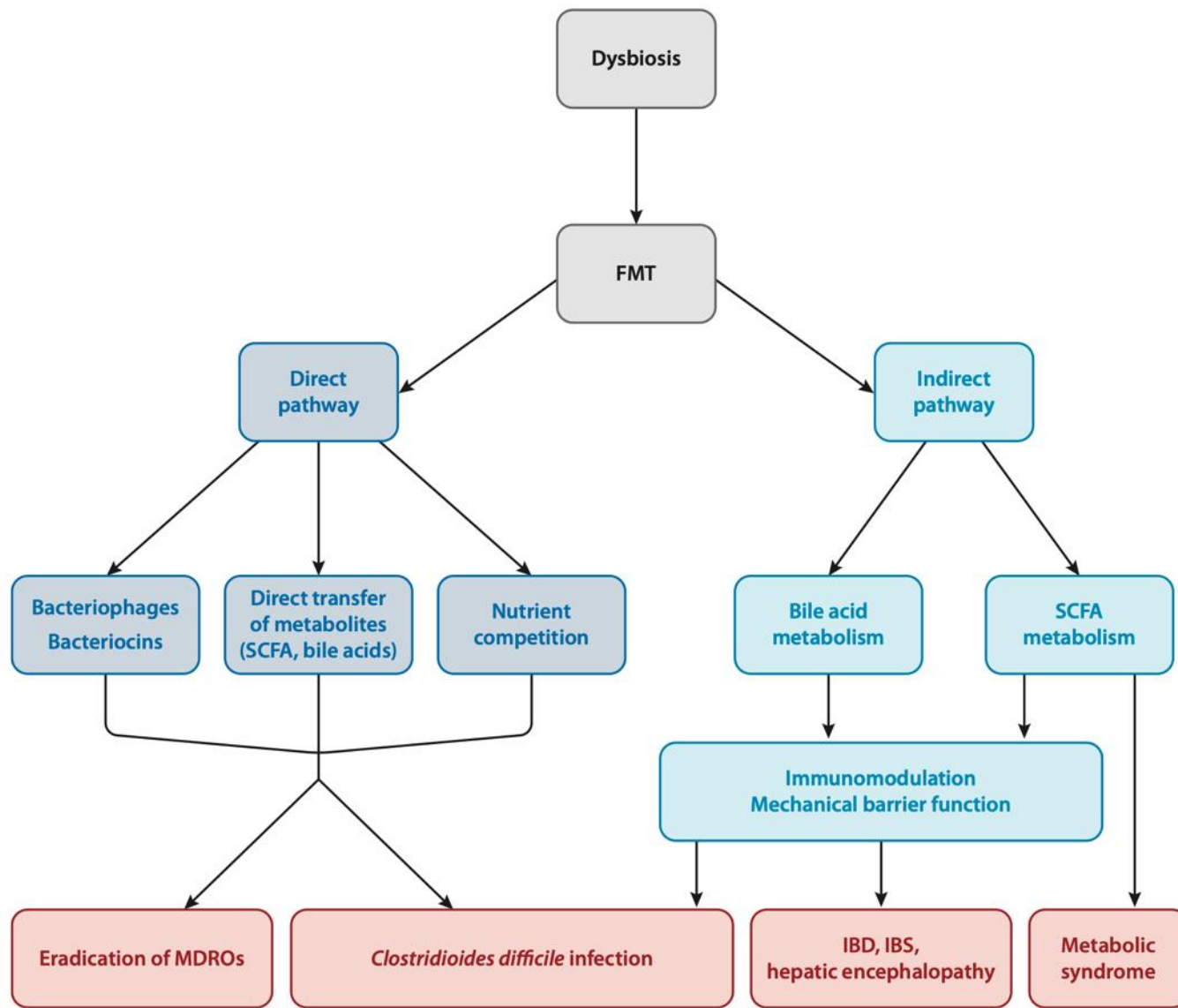
History

- 4th Century China – “Yellow Dragon Soup”
- 1958 1st report of FMT for pseudomembranous colitis
- Transfaunation – healthy animal donor → sick animal recipient
- 2013 1st RCT – FMT for recurrent CDI

FMT in Nature

- Mouse
- Panda
- Dog
- Bird
- Insect

Good source of B12!



Disorder	Type of study (references)	Outcome	Comments and important unresolved questions
Recurrent CDI	RCT (30–32) Meta-analysis (3)	Highly effective, cure rate single infusion >80%	Advised in guidelines for recurrent rCDI (1, 2)
Severe CDI	Case series (34)	Effective, probably safe	May be lifesaving
UC	RCT (47–50) Meta-analysis (51, 52)	Pooled response rate of 29% for achieving endoscopic remission	Optimization of protocol required: Is rational selection of donors required? Is it possible to select patients who are more likely to respond? Should FMT be offered as induction or maintenance treatment?
CD	Cohort studies (53, 54) Meta-analysis (52)	Pooled clinical response rate of 53%. No endoscopic remission achieved	RCT needed Rational donor selection needed
IBS	RCT (59)	Improvement of symptoms in 65% of patients after FMT versus 43% in controls No sustained effect after 1 year	Larger RCTs needed Which patients may benefit? Is repeated FMT required? How should patients be pre-treated before FMT?
HE	RCT (65)	Safe, no SAEs related to FMT, no new episodes of HE 150 days post-FMT	Confirmative study needed Rational donor selection needed
MDRO	Cohort studies (72–75)	Suggestive of some effectivity eradicating VRE and ESBL bacteria	Rational donor selection needed RCT needed
Metabolic syndrome/hepatic steatosis	RCT (78, 79)	No effect on clinical endpoints Transient increased insulin sensitivity	Strictly experimental
Autism	Open-label trial (84)	Effect noted on psychiatric and GI symptoms	Further studies are needed
GVHD	Case series (68, 69)	Steroid-refractory GVHD: decreased symptoms	Further studies are needed

Somewhat effective Highly effective Variedly effective Little or ineffective

Donor independent Donor dependent

Fecal transplantation in CDI

- 2050 CDI cases
- Overall efficacy 84%
- Safety
 - 42 AEs reported
 - No definitely related
 - 3 possibly related
 - 39 unrelated
- Capsule = NG = NJ = colonoscope > enema
- Non-related donor = related donor > auto
- Volume varies
- Frozen = fresh > spore
- Aerobic = anaerobic condition
- Sterile fecal filtrate? (no bacteria)

2120. Safety and Efficacy of Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection From An International Public Stool Bank: Results From a 2050-Patient Multicenter Cohort

เครดิตภาพ: Gregory Ginsberg, MD, University of Pennsylvania <https://emedicine.medscape.com/article/193031-workup#7>



No need for bacteria?

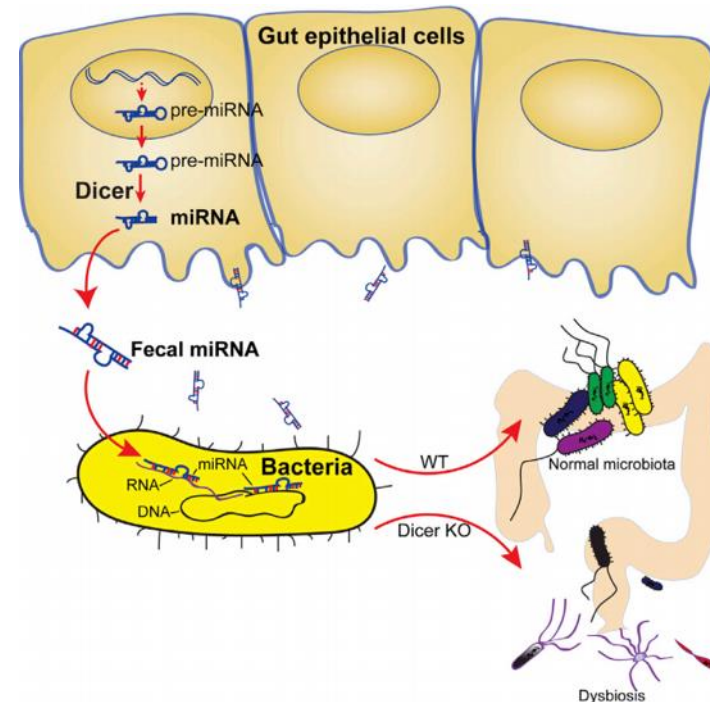
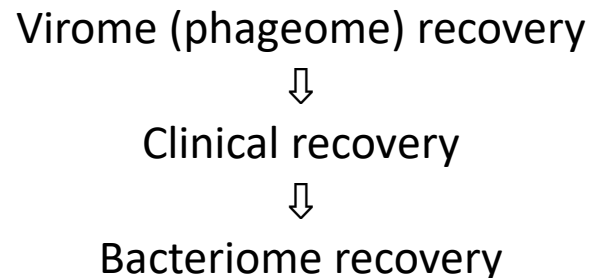


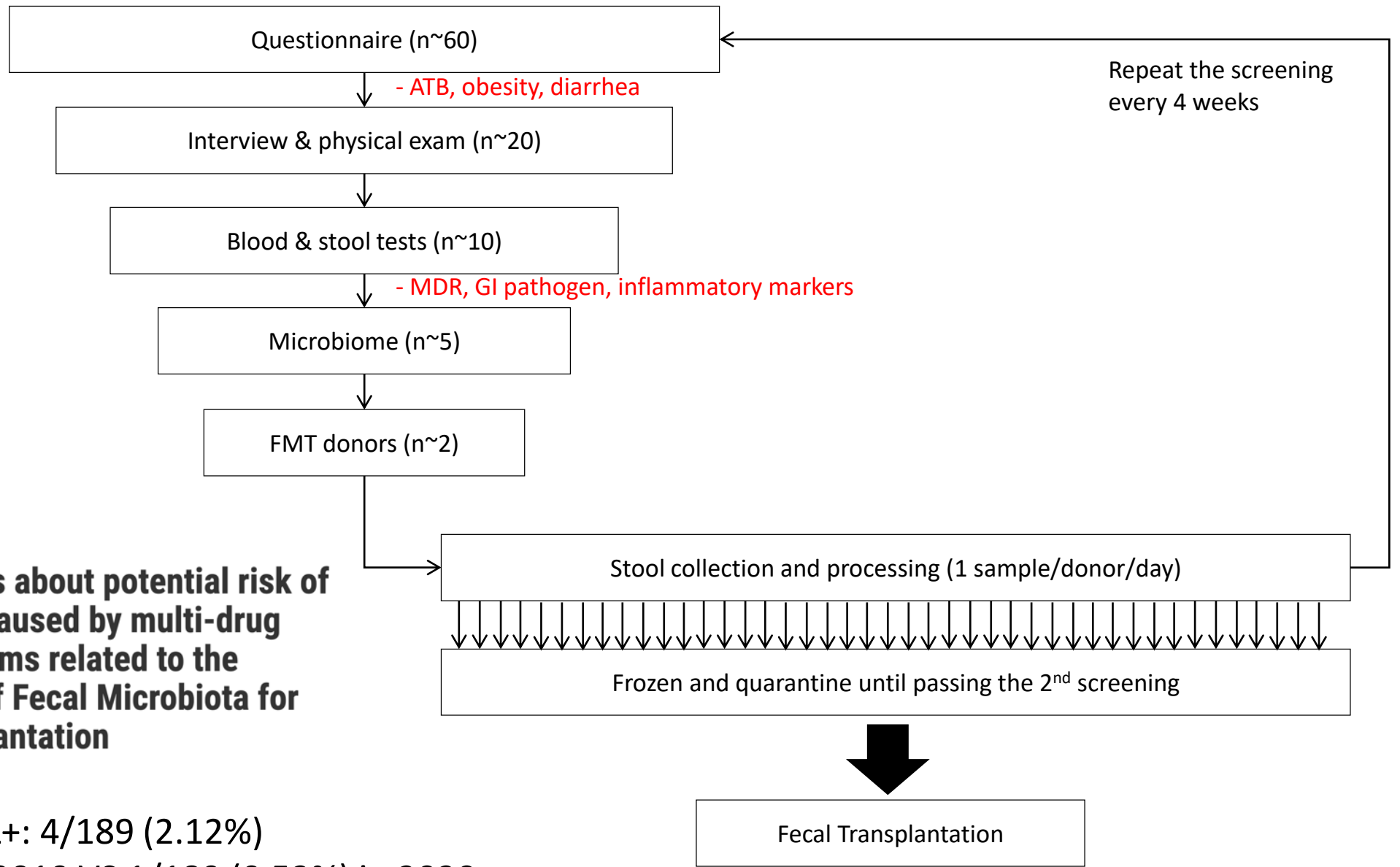
- Components of stool

- Bacteria, protozoa, fungus, epithelium
- **Phage, miRNA**, antibody, food, bile, host & microbial proteins, metabolite, etc.

FULL TEXT ARTICLE [↗](#)
Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With *Clostridium difficile* Infection [📄](#) [📡](#)
Stephan J. Ott, Georg H. Waetzig, Ateequr Rehman, Jacqueline Moltzau-Anderson, Richa Bharti, Juris A. Grasis, Liam Cassidy, Andreas Tholey, Helmut Fickenscher, Dirk Seegert, Philip Rosenstiel and Stefan Schreiber
Gastroenterology, 2017-03-01, Volume 152, Issue 4, Pages 799-811.e7, Copyright © 2017 AGA Institute

In some patients with CDI after FMT

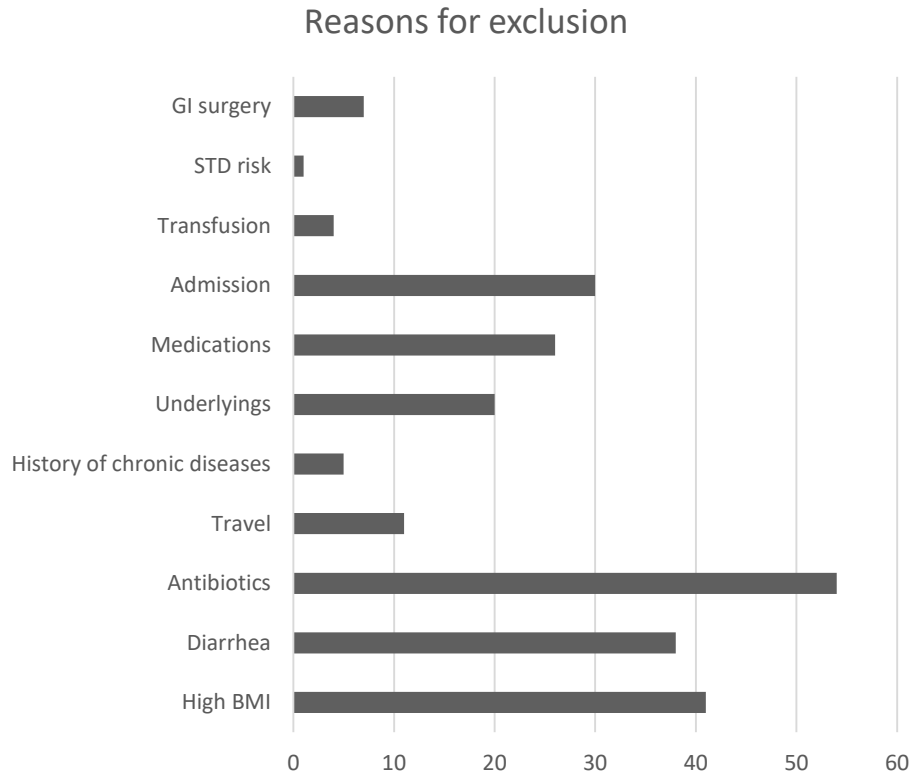




FDA In Brief: FDA warns about potential risk of serious infections caused by multi-drug resistant organisms related to the investigational use of Fecal Microbiota for Transplantation

Screen pass but ESBL+: 4/189 (2.12%)
 3/43 (6.98%) in 2019 VS 1/189 (0.53%) in 2020

FMT Donation: แบบสอบถาม



Pass 17.6%

- ไม่มีโรคประจำตัว
- ไม่มีประวัติของโรคทางเมตาบอลิก, เคยหรือกำลังเป็นมะเร็ง, โรคภูมิแพ้, โรคปวดเรื้อรัง, โรคลำไส้แปรปรวน (**irritable bowel syndrome**), ท้องอืด, ท้องผูกเรื้อรัง, ไข้ยาระบายเป็นประจำ, ท้องเสียเรื้อรัง, โรคทางเดินอาหารที่ไม่ทราบสาเหตุ
- ไม่ป่วยหรือมีสมาชิกครอบครัวป่วยด้วยโรค **inflammatory bowel disease**
- ไม่ใช้ยา ยกเว้นยาคุมกำเนิด
- ไม่ได้รับโปรไบโอติก หรือยาช่วยย่อย
- ไม่ได้ใช้ยาปฏิชีวนะในช่วง **6** เดือนก่อน
- ไม่ทานอาหารที่ทำให้เกิดอาการแพ้ได้บ่อย
- ไม่เป็นบุคลากรทางการแพทย์
- ไม่เดินทางไปยังประเทศที่ยังไม่พัฒนาภายใน **1** ปีที่ผ่านมา
- BMI ปกติ (**18.5-25**)

Initial labs (repeat at the end)

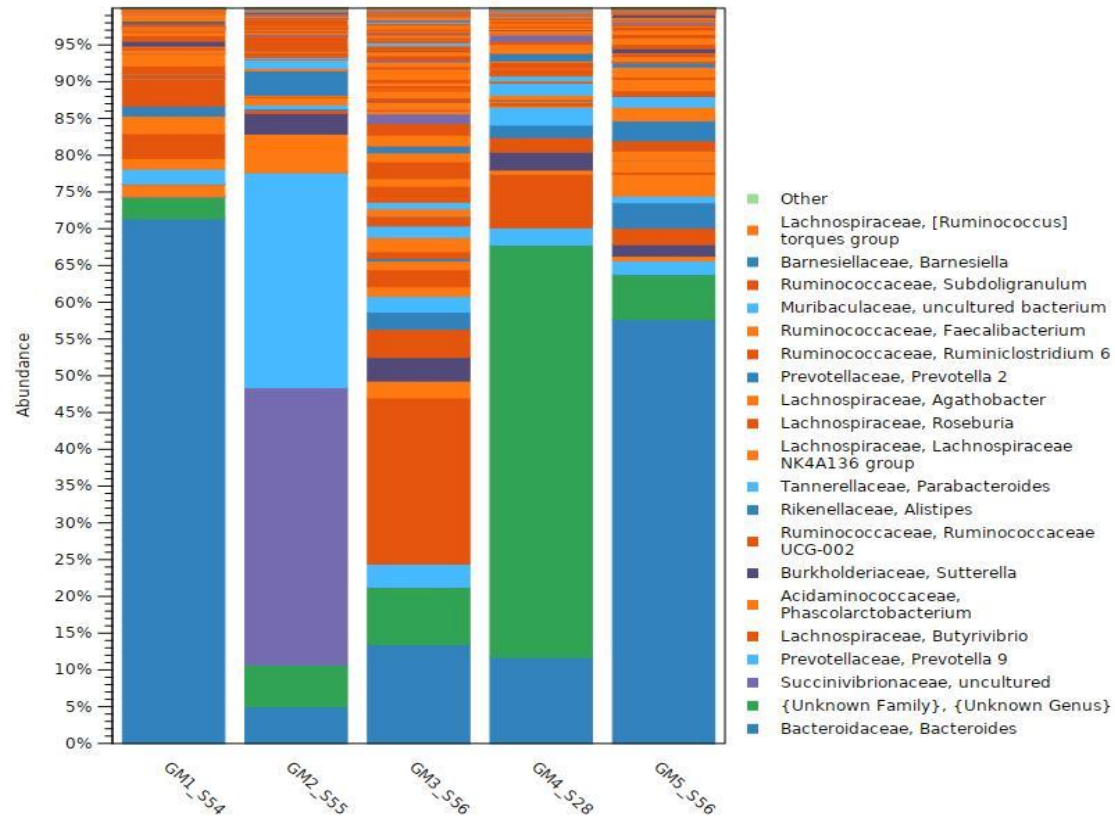
Tests	%found
Stool screen for ESBL+ bacteria	100%
Stool screen for CRE	40.74% (0% in 2019 VS 64.29% in 2020)
Stool culture (repeat every week)	30.77%
Stool exam for ova and parasites	<i>(Vibrio cholerae, Salmonella, Blastocystis hominis, Microsporidium, Pleisiomonas shigelloides)</i>
Stool stain for microsporidium	
Stool screen for VRE	3.7%
Nasal screen for MRSA	0%

- Repeat tests → more positive results

Further labs

General blood test

- High-sensitivity CRP, Lipid panel (Chol, HDL, LDL, TG)
- Comprehensive metabolic panel (BUN, Cr, Electrolyte, LFT)
- CBC, ANA
- HCG pregnancy test สำหรับผู้หญิง



Test for pathogens (repeat at the end)

- Stool *Clostridioides difficile* GDH test
- Stool AFB
- Stool stain for cryptosporidium
- Stool agar plate for *Strongyloides*
- Stool gastroenteritis panel
- Anti-HAV IgG/IgM
- HBsAg, Anti-HBS, Anti-HBc
- Anti-HEV IgG/IgM
- Anti-HCV
- Anti-HIV
- CMIA for syphilis
- Blood PCR for EBV viral load
- Blood PCR for CMV viral load
- Nasopharyngeal & throat swab for SARS

1	<i>E. coli</i>	ESBL
2	<i>E. coli</i>	ESBL
	<i>Acinetobacter baumannii</i>	Carbapenem-resistant
3	<i>E. coli</i>	ESBL
4	<i>E. coli</i>	ESBL
	<i>K. pneumoniae</i>	ESBL
	<i>Pseudomonas putida</i>	Carbapenem-resistant
5	<i>E. coli</i>	ESBL
6	<i>K. pneumoniae</i>	ESBL
	<i>Enterobacter cloacae</i> complex	CRE
	<i>Aeromonas sobria/veronii</i>	Carbapenem-resistant
	<i>Acinetobacter baumannii</i>	Carbapenem-resistant
7	<i>E. coli</i>	ESBL
	<i>Enterococcus faecalis</i>	Vancomycin-intermediate
8	<i>E. coli</i>	ESBL
9	<i>E. coli</i>	ESBL
	<i>E. coli</i>	CRE
	<i>Pseudomonas putida</i>	Carbapenem-resistant
10	<i>E. coli</i>	ESBL
	<i>E. coli</i>	CRE
11	<i>E. coli</i>	ESBL
	<i>E. coli</i>	CRE
12	<i>E. coli</i>	ESBL
	<i>E. coli</i>	CRE
13	<i>E. coli</i>	ESBL
	<i>K. pneumoniae</i>	CRE
	<i>Aeromonas sobria/veronii</i>	Carbapenem-resistant
	<i>Shewanella algae</i>	Carbapenem-resistant
14	<i>E. coli</i>	ESBL
	<i>K. pneumoniae</i>	ESBL
15	<i>E. coli</i>	ESBL
	<i>Pseudomonas putida</i>	Carbapenem-resistant

การตรวจหาเชื้อ ESBL ใน FMT donor ที่นำไปทำ transplant

Donor	Screen	1st collect									Last collect	
Date	6/10/20	19/10/20									23/11/20	
Stool	Fresh	Fresh	Glycerol	Frozen			FMT left over			Fresh	Glycerol	
ESBL+	KP	EC										
Screen	EC	<i>E. cloacae</i>	EC	EC	EC	EC	EC	EC	EC	KP	EC	EC
Ampicillin			R	R	R	R	R	R	R	R	R	R
Cefazolin			R	R	R	R	R	R	R	R	R	R
Amoxi/clav			R	R	S	I	I	R	I	S	R	R
Piper/tazo			S	S	S	S	S	S	S	S	S	S
Cefoxitin			S	S	I	S	S	R	S	S	S	S
Cefotaxime			R	R	R	R	R	R	R	R	R	R
Ceftriaxone			R	R	R	R	R	R	R	R	I	R
Ceftazidime			R	R	R	R	R	R	R	R	R	R
Cefepime			R	SDD	R	R	R	SDD	SDD	SDD	S	SDD
Doripenem			S	S	S	S	S	S	S	S	S	S
Ertapenem			S	S	S	S	S	S	S	S	S	S
Imipenem			S	S	S	S	S	S	S	S	S	S
Meropenem			S	S	S	S	S	S	S	S	S	S
Amikacin			S	S	S	S	S	S	S	S	S	S
Gentamicin			R	R	R	S	S	R	R	S	S	S
Cotrimox			S	S	R	R	R	S	S	I	S	S
Ciprofloxacin			R	S	S	S	S	S	S	I	S	I
Levofloxacin			R	R	R	R	R	R	R	R	S	I
Tetracycline			R	I	S	I	I	S	S	R	R	I
ESBL genes												
CTX-M			1	0	1	1	0	0	1	1		0
TEM			0	1	1	1	1	1	1	1		1
SHV			0	0	0	0	0	0	0	1		0

KP – *Klebsiella pneumoniae*, EC – *Escherichia coli*

พบว่าลักษณะการดื้อยาของเชื้อที่แยกได้แทบไม่ซ้ำกันเลยแม้จะเป็นเชื้อในตัวอย่างเดียวกันก็ตาม





Process

1. ผู้บริจาคนั่งบนเก้าอี้นั่งถ่ายสำเร็จรูป ถ่ายลงบนภาดฟอยล์สำหรับอาหารซึ่งผ่านการนึ่งความดัน ซ้อนอยู่ในถังรองพลาสติกซึ่งสามารถถอดเข้าออกจากเก้าอี้ได้โดยง่าย ในห้องน้ำแยกเฉพาะ สำหรับผู้บริจาค เมื่อถ่ายเสร็จ ผู้บริจาคทำการปิดฝาภาดฟอยล์และปิดฝาดังพลาสติกอีกชั้น แล้ว นำถังพลาสติกมาวางไว้หน้าห้องปฏิบัติการที่อยู่ติดกัน ตัวถังพลาสติก จะถูกเช็ดด้วย **70% ethanol** และรอให้แห้ง ก่อนนำเข้าสู่ห้องปฏิบัติการและ **biosafety cabinet** ตามลำดับ
2. ทำการเตรียม **fecal microbiota** ใน **biosafety cabinet class IIa2** ซึ่งมี **charcoal filter** สำหรับดูดซับป้องกันกลิ่นรบกวน ในห้องปฏิบัติการ **BSL-2** ซึ่งทั้ง **cabinet** และห้องนี้จะไม่นำไปใช้งานด้านอื่นเลย เพื่อความปลอดภัย ของผู้รับการปลูกถ่าย
3. แบ่ง **fresh stool** เล็กน้อยเก็บที่ **-80°C** เพื่อการตรวจสอบในอนาคต
4. ชั่งน้ำหนักและผสมอุจจาระกับ **sterile normal saline** ในอัตราส่วน **1:1**
5. กรองกากออกด้วยผ้าก๊อสปลอดเชื้อ **2** ชั้น
6. เก็บของเหลวที่ผ่านการกรองปริมาณ **45ml** ใน **conical tube** ขนาด **50ml** ชนิดทน ความเย็นจัดได้
7. เก็บ **conical tube** ที่มี **fecal microbiota** ที่ **-80°C** สำหรับใช้งานได้ไม่เกิน **6** เดือน และนำมาละลาย ที่ **37°C** เมื่อถึงเวลาที่จะใช้ปลูกถ่าย

Contraindication

Neutropenia

GI surgery

Aspiration risk (NG, NJ, oral capsule)

Perforation risk (Colonoscope, enema)

Pressure sore at sacrum/coccyx, loose anal sphincter (enema)

Recipient Preparation

- Hold offending antibiotics > 24-48 h
- Defecation (Colonoscopy, enema)
- Urination (enema)
- Antibiotic pretreatment (may improve engraftment of specific taxa)
- Bowel lavage
- Proton pump inhibitor (NG)

Conceal the Yuck factor!

- Example: Toilet-to-tap water → Adding an unnecessary reservoir makes people feel better.
- Encapsulated lyophilized FMT
- Fecal microbiota → probiotic cocktail

Commercial Fecal Microbiota

- US: OpenBiome
 - ฿30,000/treatment
 - ฿10,000/week for the donor
- UK: Taimount Clinic
- Thailand?



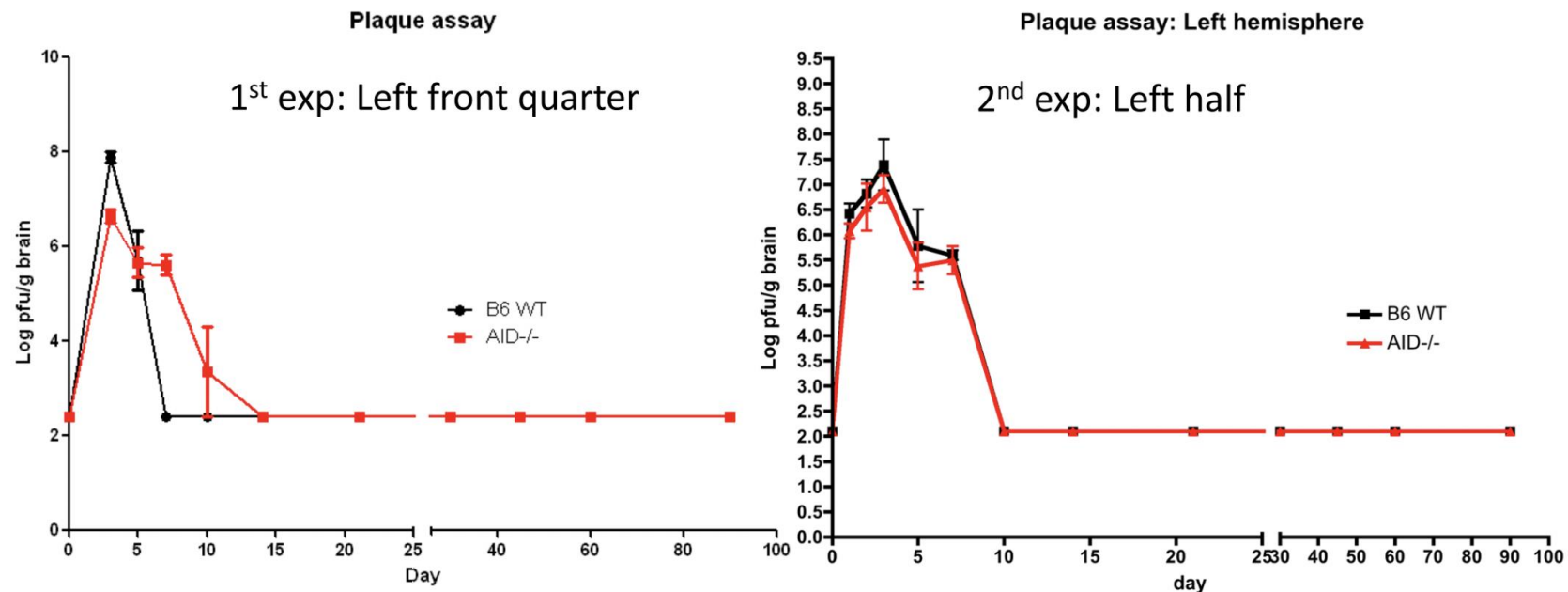
เครดิตภาพ: Dishant Bhasin



น้ำจากแหล่งเดียวกัน ในตู้ซ่ายที่ไม่มีหอยนางรม จะชุ่นกว่า
เมื่อเทียบกับตู้ซวางที่มีหอย ที่มา: www.mbl.edu

Germ Line IgM Is Sufficient, but Not Required, for Antibody-Mediated Alphavirus Clearance from the Central Nervous System

Voraphoj Nilaratanakul,^{a,b*} Jie Chen,^a Oanh Tran,^{a*} Victoria K. Baxter,^{a,b*} Elizabeth M. Troisi,^a Jane X. Yeh,^a Diane E. Griffin^a



Non-published data

Common confounders

Age

Sex

Genetics

Environments

Microbiota

E. coli to the rescue

- C57BL/6 mice from UC tolerated infection/gut leakage better than from Jackson Lab.
- During infection, *E. coli* O21:H⁺ in UC mice translocated from leaky gut to colonize on white adipose tissue.
- Flagella → inflammasome → Casp11 → IL-18 → IGF-1
- IGF-1/PI3K/AKT pathway inhibits *Atrogin-1* and *Murf1* expression, preventing muscle wasting

CLINICAL TRIALS

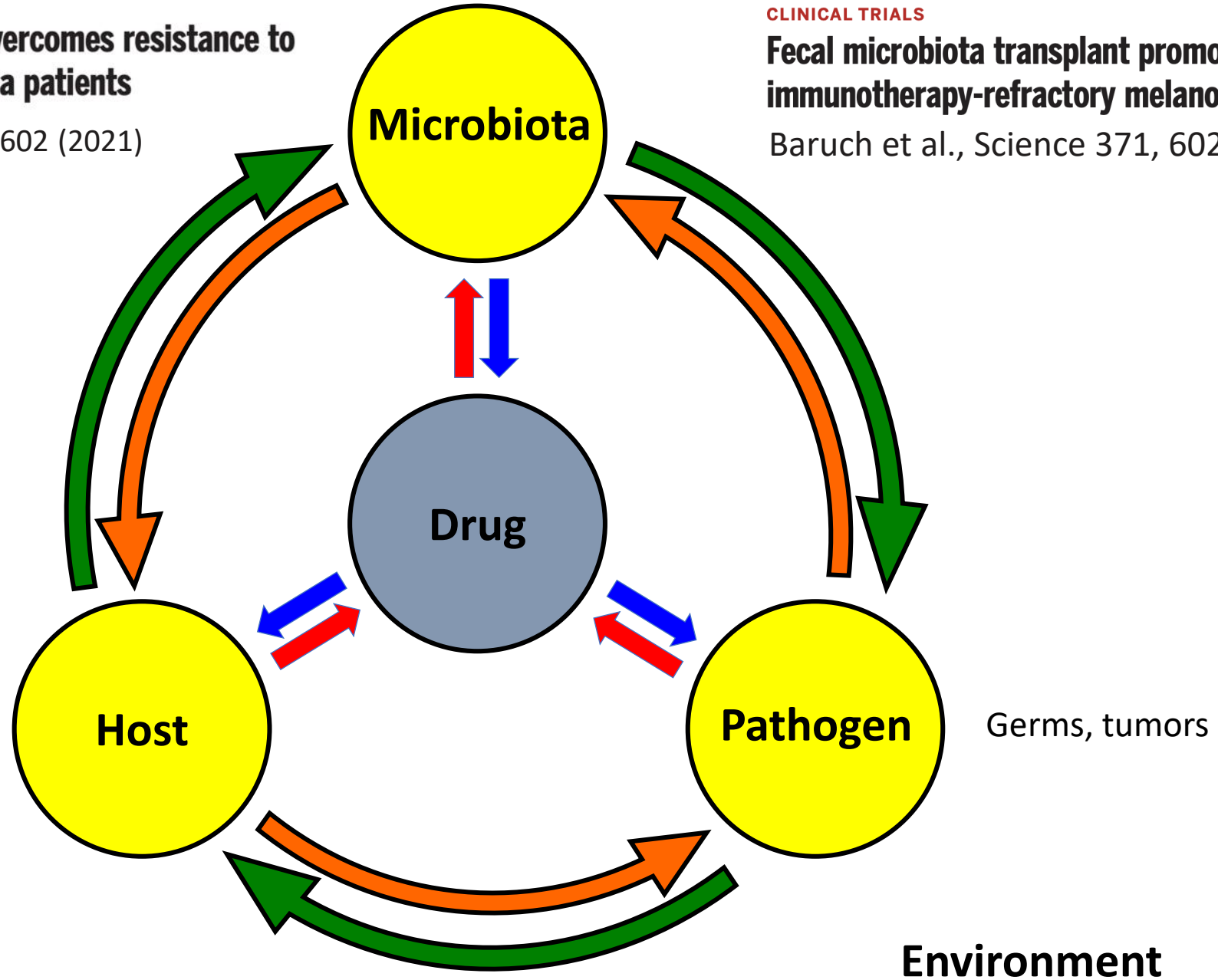
Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients

Davar et al., Science 371, 595–602 (2021)

CLINICAL TRIALS

Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients

Baruch et al., Science 371, 602–609 (2021)



Other sites of microbiota Tx

- Sinonasal → chronic rhinosinusitis
- Oral → gingivitis, dental caries, halitosis
- Vaginal → leukorrhea



<https://pantip.com/topic/38792689>

Take home messages

- Complex interactions between hosts, pathogens, microbiota, and treatments determine the outcomes
- Subdominant species may play a crucial role. (keystone species)
- Finding proper donors will be harder and harder.
- FMT is still the most effective way to manipulate microbiota.

- Future trend in the manipulation of microbiota.
 - AutoFMT
 - Probiotics – cocktail, sequential
 - FMT/probiotics in infants
 - Phage therapy
 - Herd Microbiota?
 - Precision microbiota
 - Modification of immunotherapy and other treatments



In vivo test for the probiotics

Asada Leelahavanichkul, MD., PhD.

รศ. นพ. ดร. อัษฎาศรี ลีฬหวนิชกุล

Department of Microbiology, Faculty of Medicine,
Chulalongkorn University

Objective

1. Introduction on studies of probiotics
2. Importance of in vivo test.
3. How to test?

PubMed.gov

leelahavanichkul A and probiotics

Advanced Create alert Create RSS User Guide

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MY NCBI FILTERS 13 results

RESULTS BY YEAR

Year	Number of Results
2016	1
2017	1
2018	1
2019	2
2020	5
2021	13

TEXT AVAILABILITY

Abstract

Free full text

Lactobacillus acidophilus LA5 improves saturated fat-induced obesity mouse model through the enhanced intestinal *Akkermansia muciniphila*.

1

Cite

Share

Ondee T, Pongpirul K, Visitchanakun P, Saisorn W, Kanacharoen S, Wongsaroj L, Kullapanich C, Ngamwongsatit N, Settachaimongkon S, Somboonna N, **Leelahavanichkul A**.
Sci Rep. 2021 Mar 18;11(1):6367. doi: 10.1038/s41598-021-85449-2.
PMID: 33737543 **Free PMC article**.

However, data on the correlation between a single administration of LA5 versus microbiota alteration might be helpful for the **probiotic** adjustment. LA5 was administered daily together with a high-fat diet (HFD) for 8 weeks in mice. ...In conclusion, LA5 attenuated obesity ...

Fluorometric Paper-Based, Loop-Mediated Isothermal Amplification Devices for Quantitative Point-of-Care Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA).

2

scientific reports

ORIGINAL RESEARCH published: 25 September 2021 doi: 10.1038/s41598-021-10107-7

Check for updates

frontiers
in Immunology

Candida Administration Worsens Cecal Ligation and Puncture-Induced Sepsis in Obese Mice Through Gut Dysbiosis Enhanced Systemic Inflammation, Impact of Pathogen-Associated Molecules From Gut Translocation and Saturated Fatty Acid

OPEN ACCESS

Edited by:
Akshay Taneja,
Massachusetts General Hospital
and Harvard Medical School,
United States

Reviewed by:
Henry D. Dawson,
Agricultural Research Service,
United States Department
of Agriculture, United States
Thomas Thirumani,
Orlando Met University, **Italy**

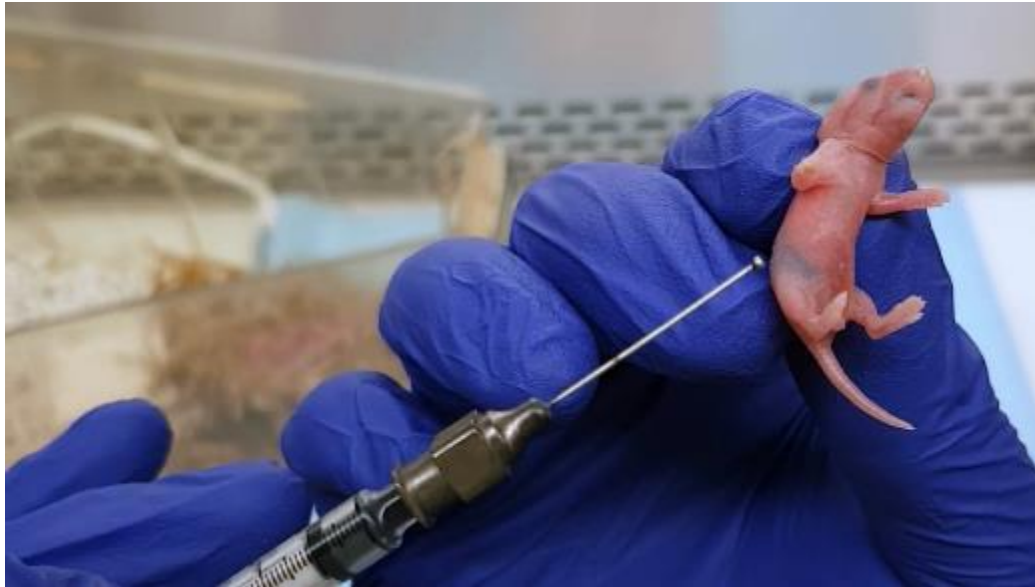
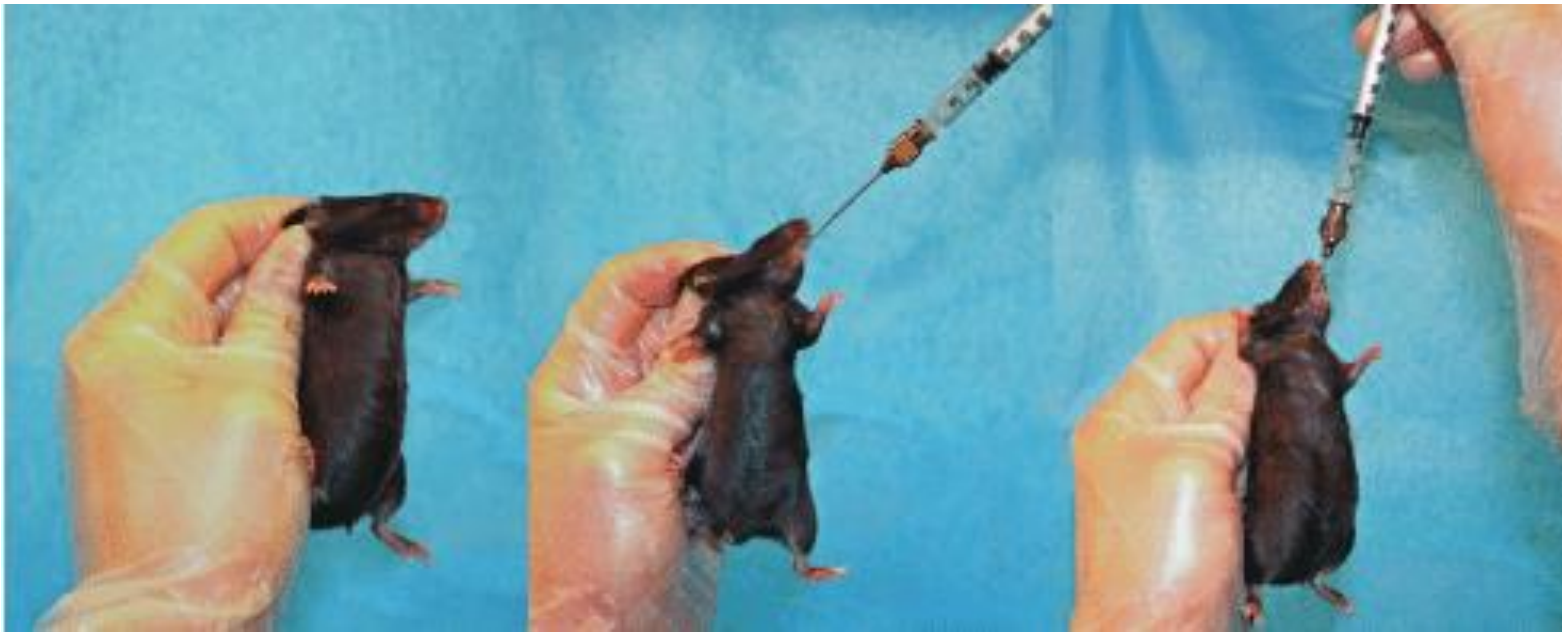
Wimonrat Panpetch¹, Vorthin Sawaswong^{2*}, Prangwalai Chanchaem^{3*},
Thunnicha Ondee⁴, Cong Phi Dang⁵, Sunchai Payungporn^{6,4} and
Asada Leelahavanichkul^{1,6*}

OPEN Lactobacillus acidophilus LA5 improves saturated fat-induced obesity mouse model through the enhanced intestinal *Akkermansia muciniphila*

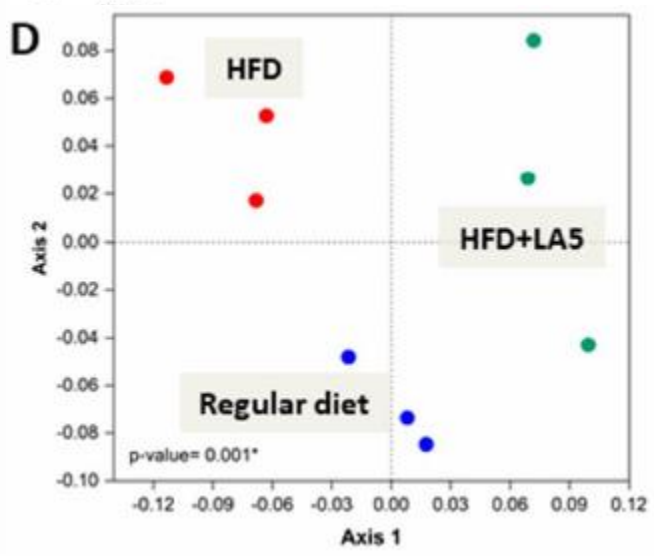
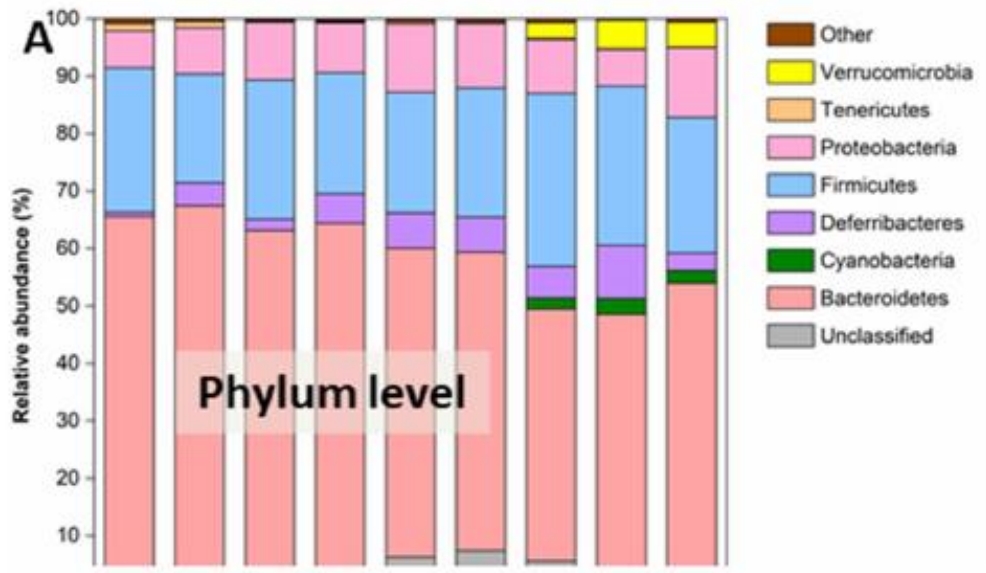
Thunnicha Ondee¹, Krit Pongpirul^{1,2,10*}, Peerapat Visitchanakun³, Wilasinee Saisorn⁴,
Suthicha Kanacharoen¹, Lampet Wongsaroj^{6,7}, Chitrasak Kullapanich^{4,7},
Natharin Ngamwongsatit⁸, Sam Settachaimongkon^{9,10}, Naraporn Somboonna^{6,7} &
Asada Leelahavanichkul^{6,11,12*}

Discovery of the new strains of probiotics

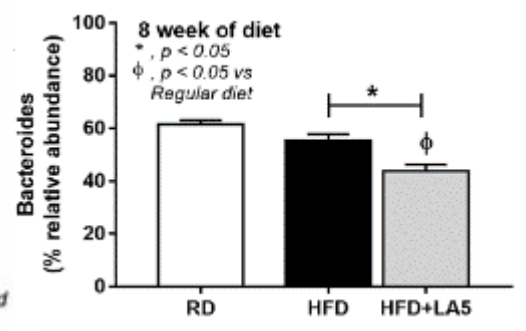
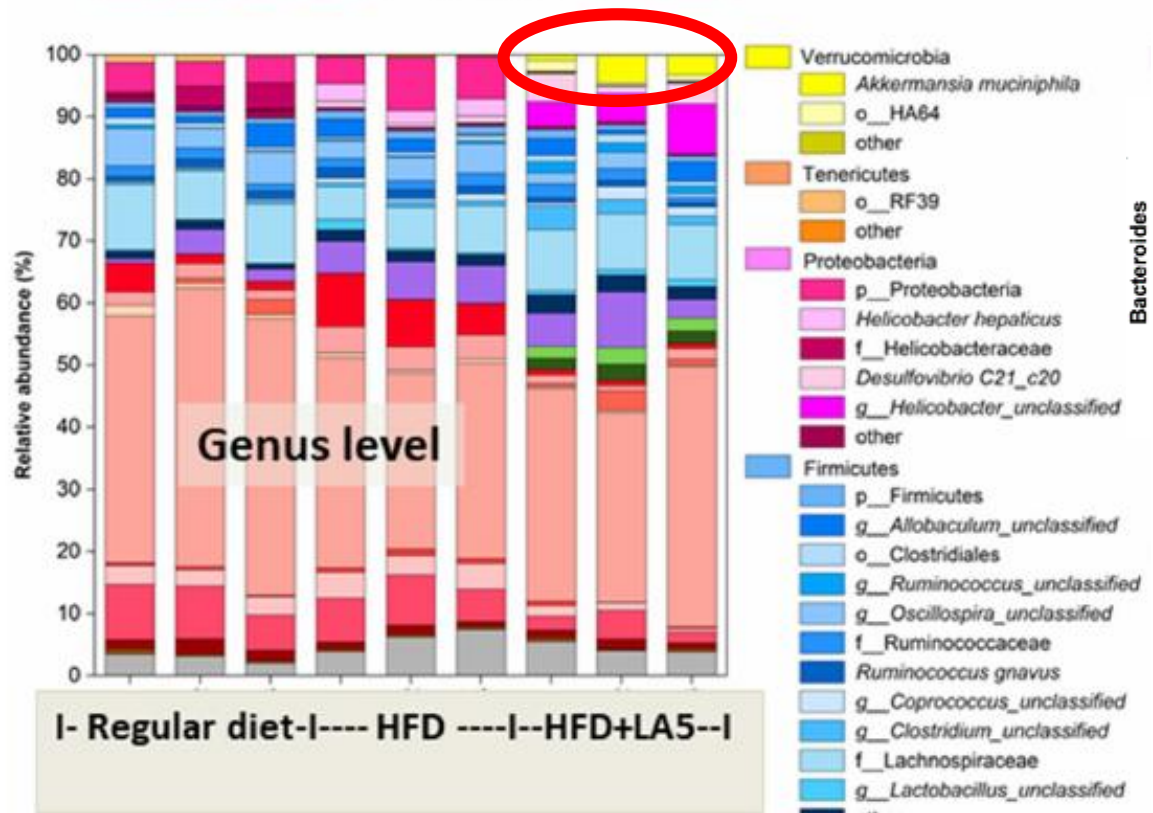
- Isolated interesting bacteria (mostly anaerobes) from healthy volunteers
- Test genome or not (new strains?)
- SELL! → health supplement without health claims! (หากอยู่ใน list อย.).
- TEST! → aim health claim
 - In **vitro test** (tolerability against acid, bile, attachment, antibiotics, hydrolase, lactate, toxin producing)
 - In **vivo test** (support the in vitro test) ±
 - **Human test**
- Microbiome analysis service: Omics center faculty of Science
(Should we have such a center in MDCU?)



Oral gavage vs
anal gavage

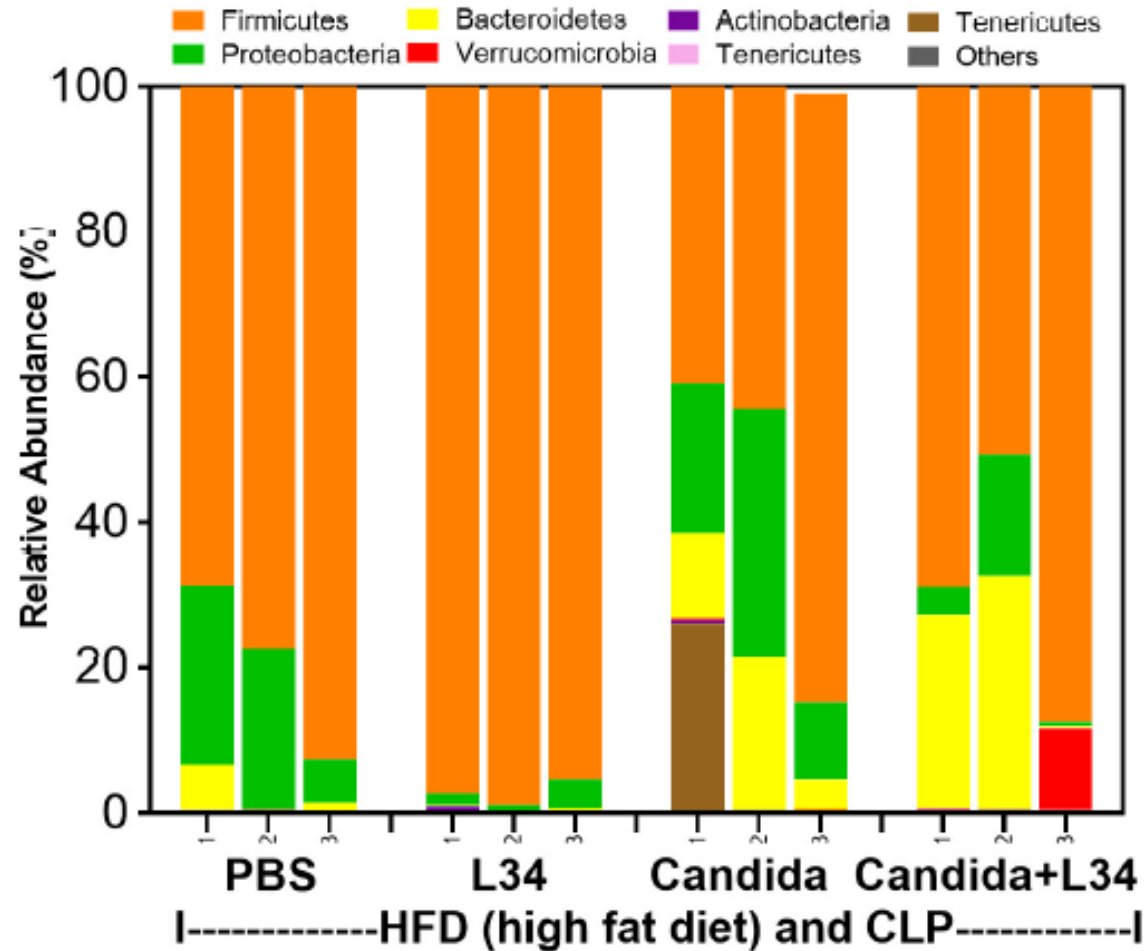


Old model with new mechanisms

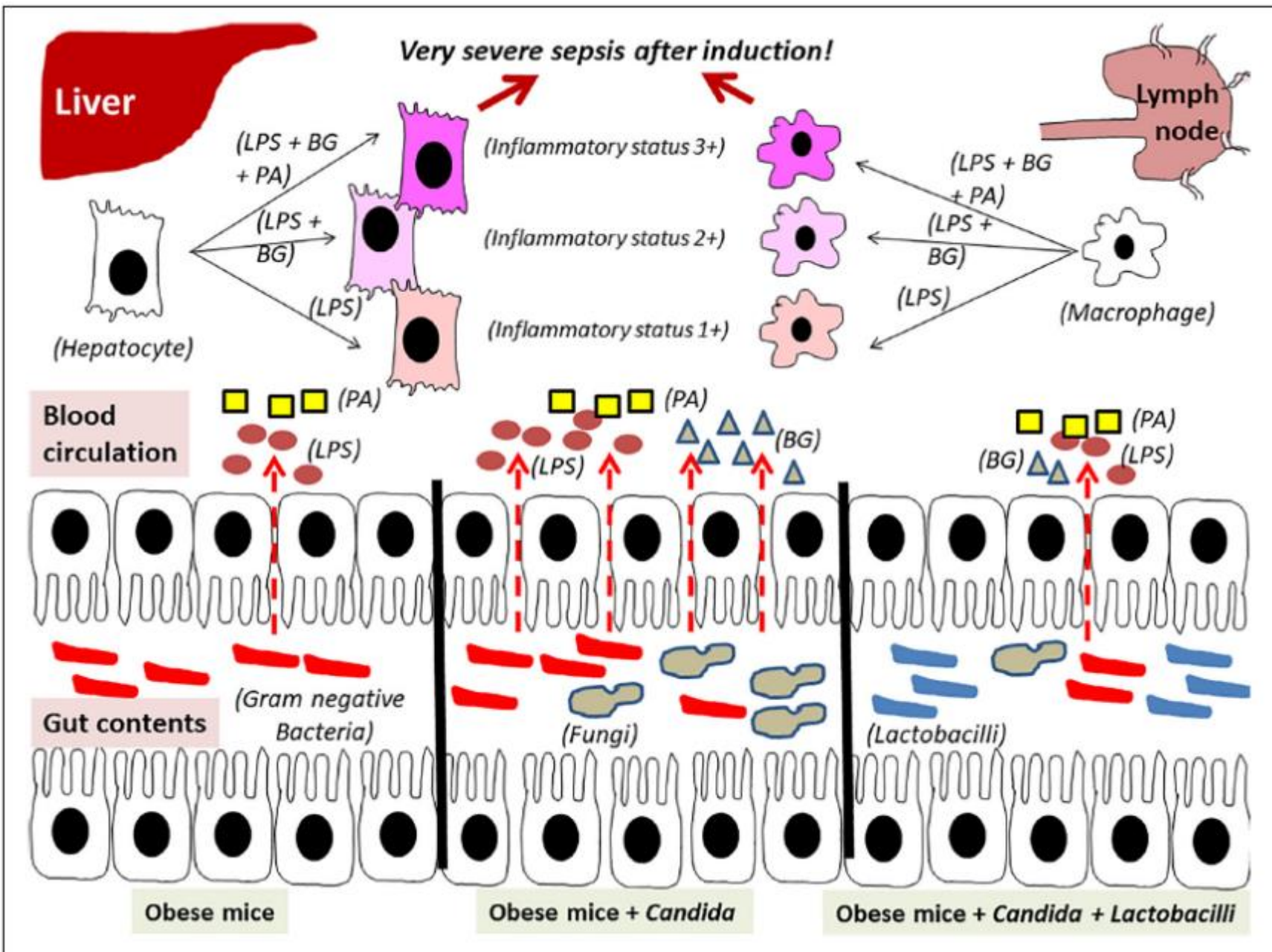


- Standard obese model
- Work through other bacteria

New model with old mechanisms



- Candida in a standard obese model
- Work through old mechanism (reduction of gut pathogenic bacteria) eg. total abundance of gram neg bacteria



Enhanced the model complexity

Conclusion

- Probiotics is an emerging topics that is very easy to get a product (following a direction of the current research grants)
- Test in animal model and in patients to put a health claim on the products will be helpful for the competition
- The specific property of some specific probiotics is controversial (class effect?).

Thank you for your attention



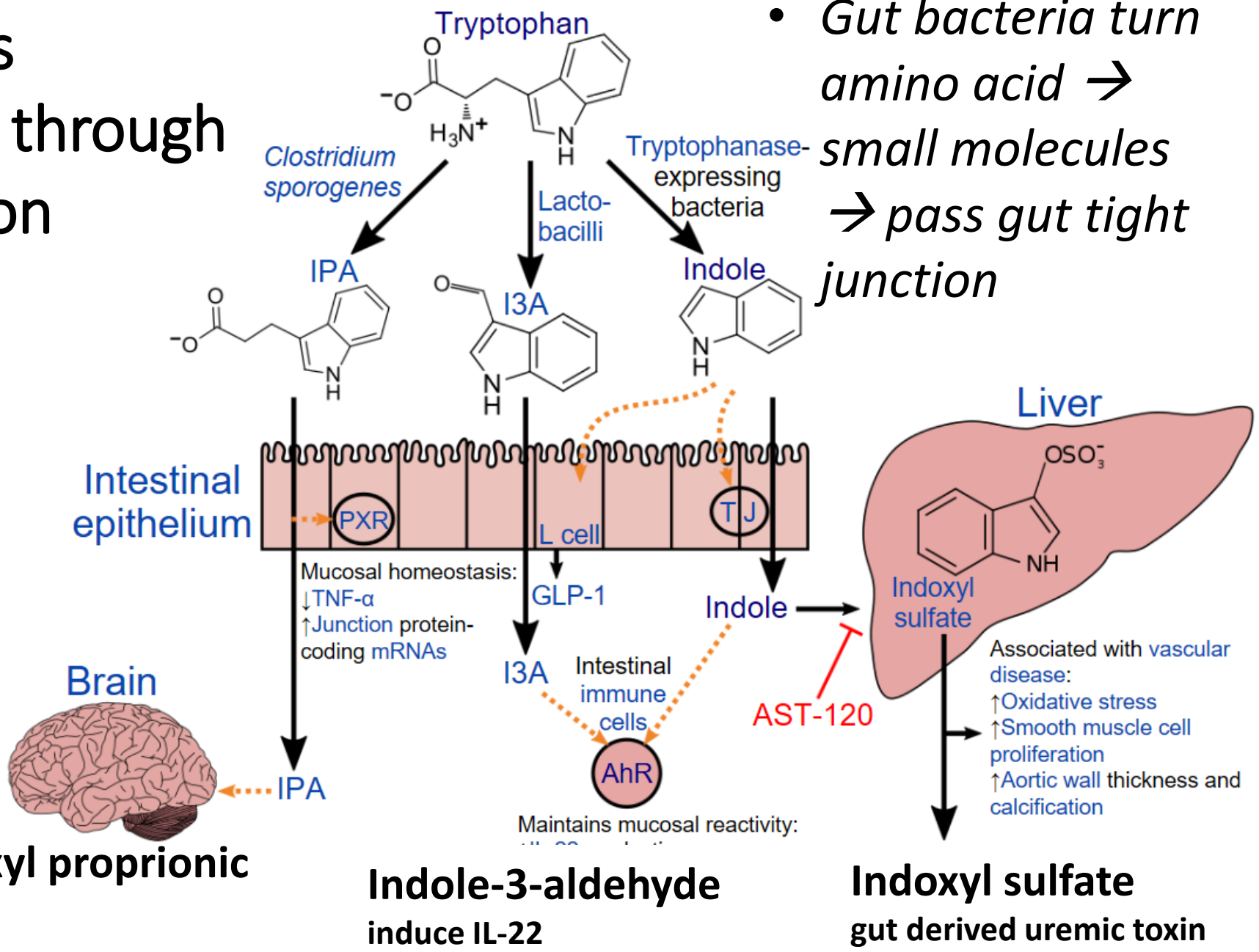
Translational Research in Inflammation and Immunology Research Unit (TRIRU)

Gut microbiota; a neglected organ

- In human = 10^{10} - 10^{14} cells (2kg) > liver wt;
 - i) nutrients digestion/ uptake, synthesis
 - ii) **intestinal integrity (permeability) vs gut leakage**
 - iii) enteric immunity (vs. hygiene hypothesis)
- Connect to host systems through
 - i) organism productions/ compositions (eg. LPS)-not TJ
 - ii) nutrient metabolites (eg. Indole from Tryptophan) -passTJ
- Controlled (in part) by “gut permeability”
- Molecules from Probiotics low+high MW

Metabolites
:freely pass through
tight junction

- Gut bacteria turn amino acid → small molecules → pass gut tight junction



Indoxyl proprionic acid
neuro-protection

Indole-3-aldehyde
induce IL-22

Indoxyl sulfate
gut derived uremic toxin

Sequencing-based pathogen identification

Thidathip Wongsurawat (Tip)

Piroon Jenjaroenpun (Man)

Instructor





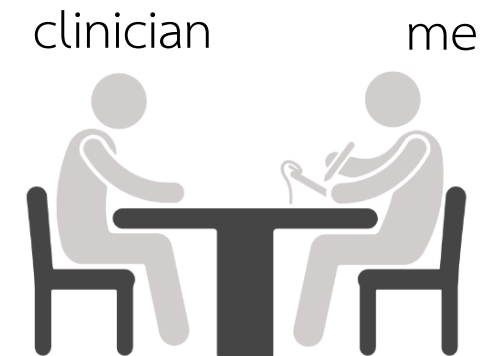
Assist.Prof. Prapat
Suriyaphol

11th SiMR



Goal

- 70% ของงานทั้งหมด -> ตอบโจทย์ clinician และ user
- ถูก (cost)
ดี (simple method)
เร็ว (fast turnaround time)



Sequencing-based pathogen identification

Microbiome

- genus level
- species level

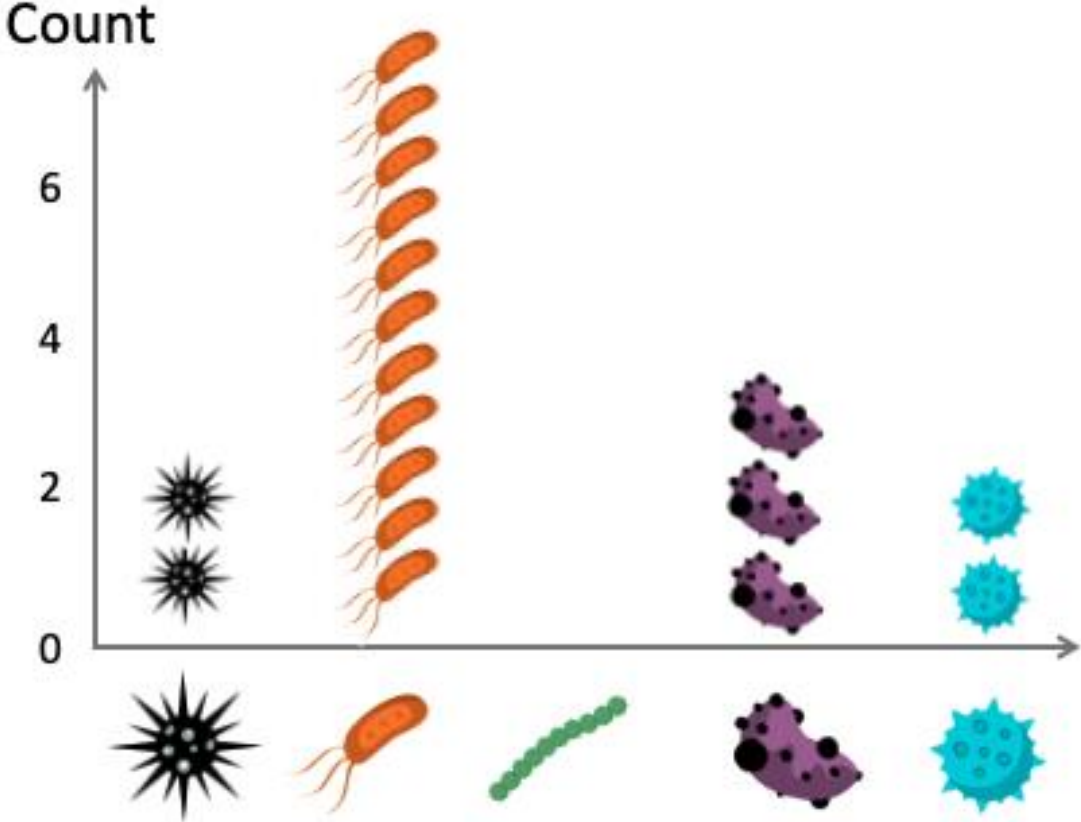
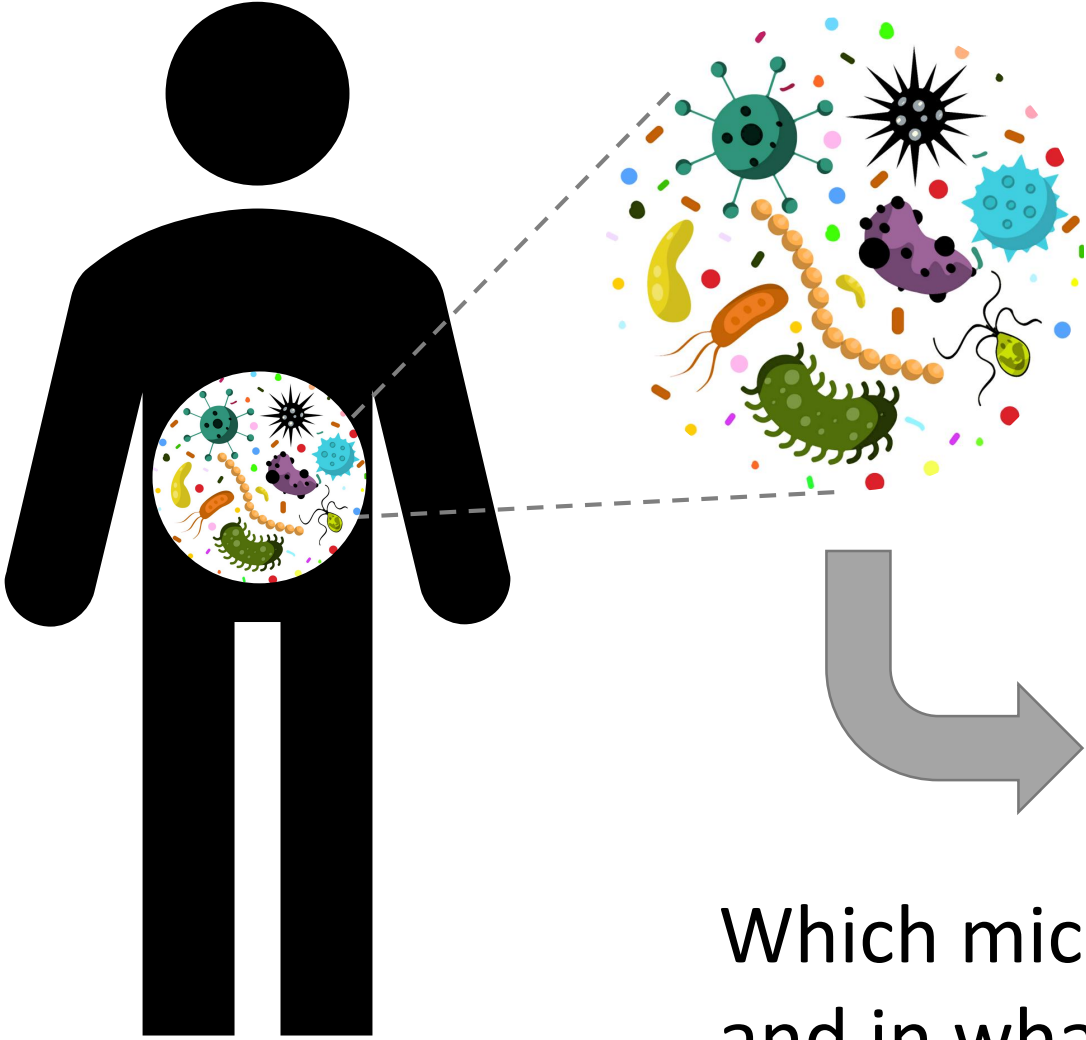


Metagenome

Whole genome sequencing

Targeted-seq

Microbiome



Which microbes are present and in what proportions?

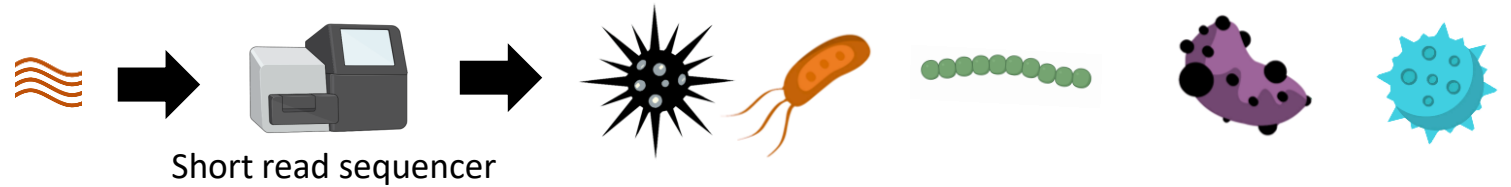
Microbiome

16S rRNA gene

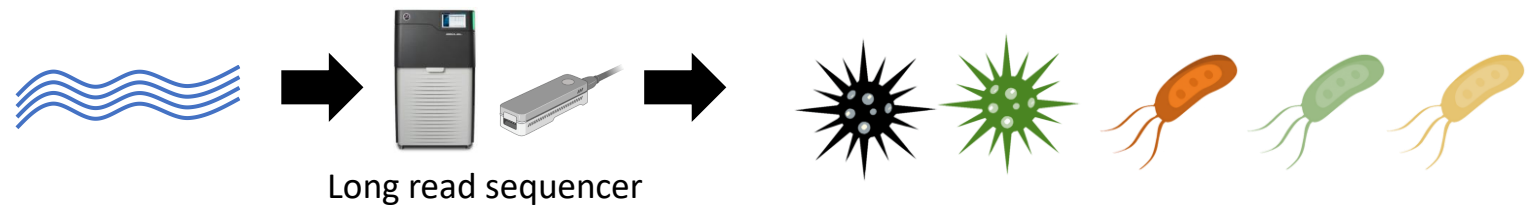
genome

16S gene sequencing

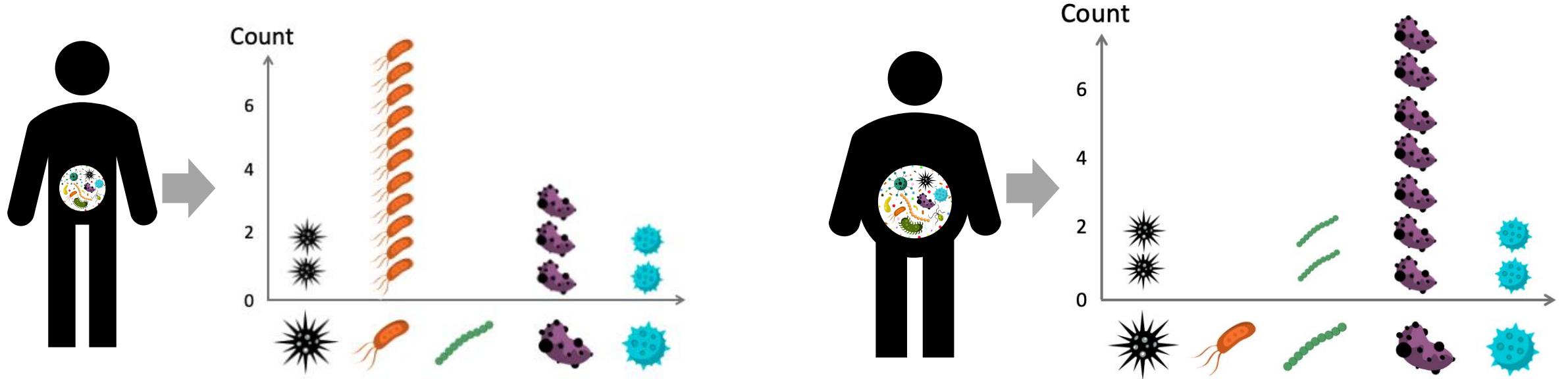
1) Partial 16S genes -> Genus level (< 1000 bp)



2) Full-length 16S genes -> Species/Strains level (~1500 bp)

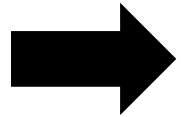


Microbiome



- Different microbial profile
- Pathogen identification

Metagenome (microbial-rich samples)



Metagenomic Next-Generation Sequencing (mNGS)



- Bacterial species/strains level
- Non-bacterial species
- Metabolic function profiling
- Antibiotic resistance gene profiling

Metagenome (pathogen identification)



Clinical applications

- Pathogen identification and discovery
- Diagnosis of infectious diseases
- Outbreak tracking

Clinical samples

- Blood
- Cerebrospinal fluid
- Respiratory samples
- Gastrointestinal fluid
- Ocular fluid



CHAN ZUCKERBERG BIOHUB

IDseq: An Open Source Platform for Infectious Disease Detectives



METADATA

Host	Human	2
Sample Type		
Bronchoalveolar Lavage	1	
Cerebrospinal fluid	1	
Mini	1	
Bronchoalveolar Lavage	1	
Not set	1	
SHOW MORE		
Location		
California, USA	2	

ต่างประเทศ

ในประเทศ

Red ocean



ZYMO RESEARCH S.M. CHEMICAL
5,300 – 5,900 (45 days)



OMICS
Omics Sciences & Bioinformatics Center
2,550-3,470 (varies)



Bumrungrad International
HOSPITAL
9,000 (?)

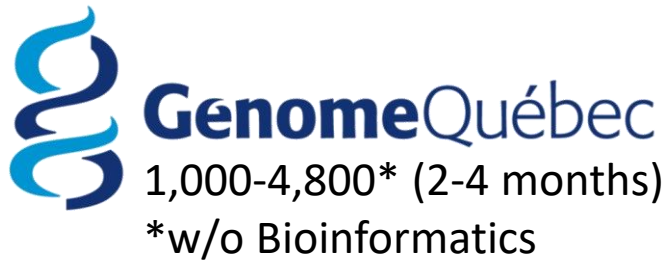


VIOME
4,800 – 10,000 (21-30days)



PANACURA
5,000-9,500 (21 days)

PHYATHAI
11,500 (?)



Genome Québec
1,000-4,800* (2-4 months)
*w/o Bioinformatics



MODGUT
3,600-4,800 (28 days)

KMUTT

Genus level

Blue ocean



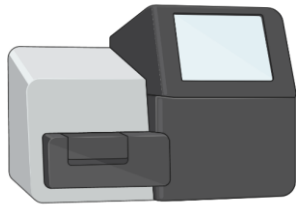
Full-length 16S / 16S-ITS-23S
Bacterial chromosome & plasmid

3,000-11,500
1-7 days

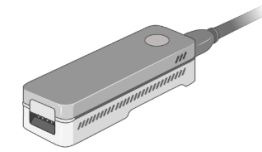
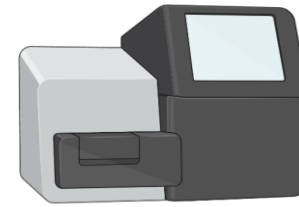
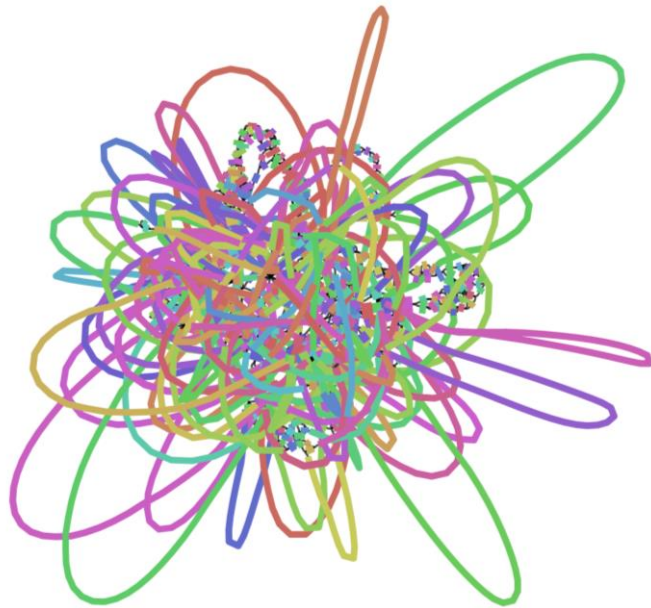
Gut, saliva, blood, wastewater, etc.

Species/ strain level

Whole genome sequencing



Illumina only



Illumina + Nanopore



Long reads improve de novo assemblies for large genomes

Bacterial chromosome and plasmid sequence



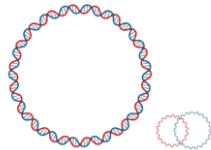
Dr. Sissades Tongsim
CEO National Biobank
of Thailand



Assoc. Prof. Chanwit
Tribuddharat



High quality
chromosome
& plasmid

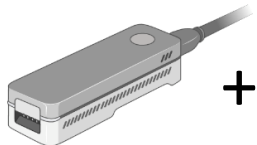


High quality DNA

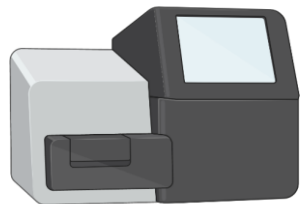
Probiotics



Library prep



+



NSTDA Supercomputer Center

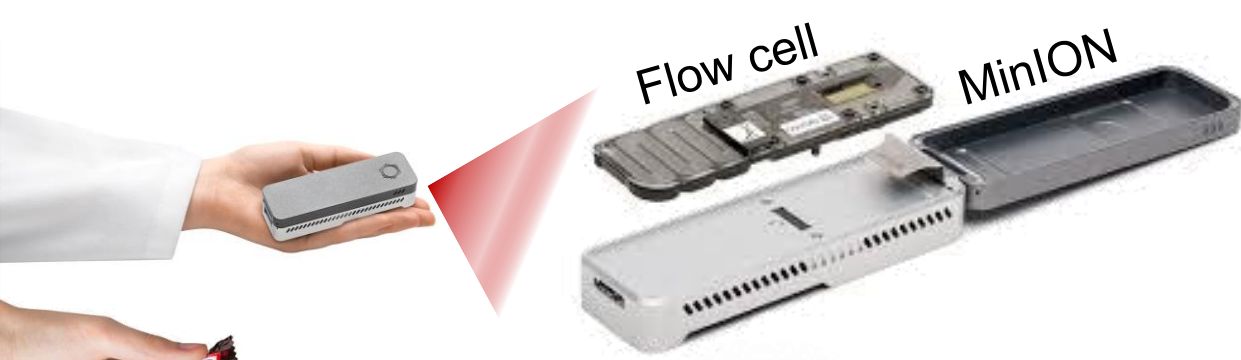


Bioinformatics + report

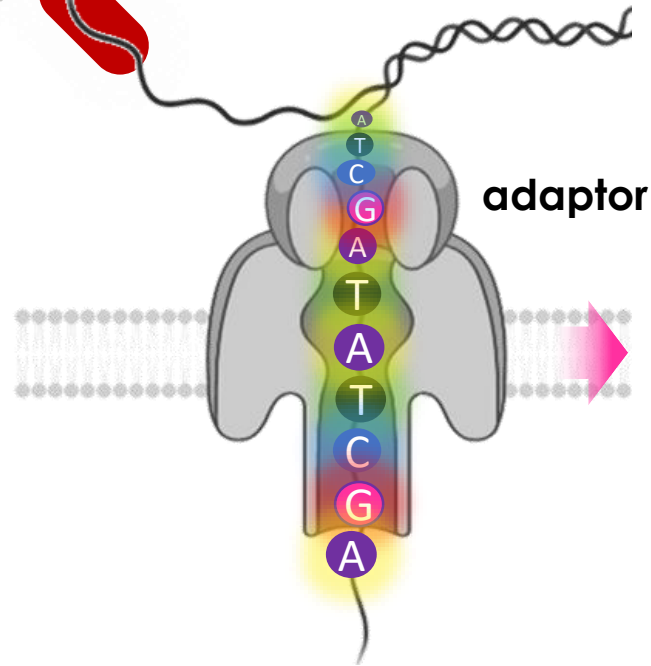
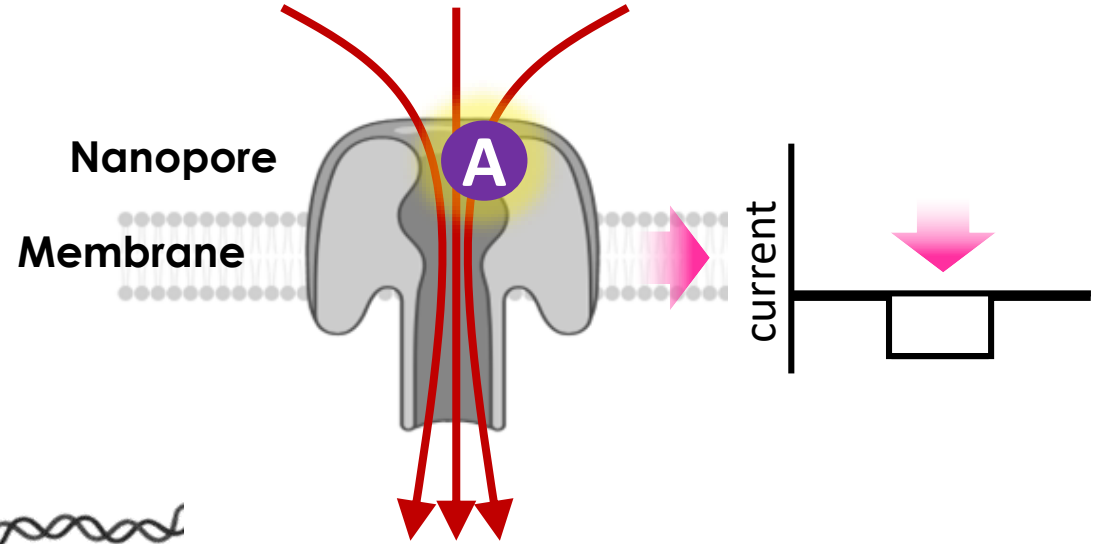


Targeted-seq

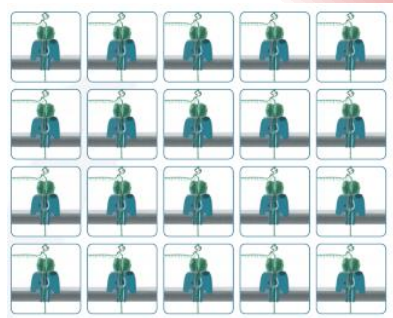
Cutting edge technology



Oxford Nanopore Technologies (ONT)






AGCTATAGCTA

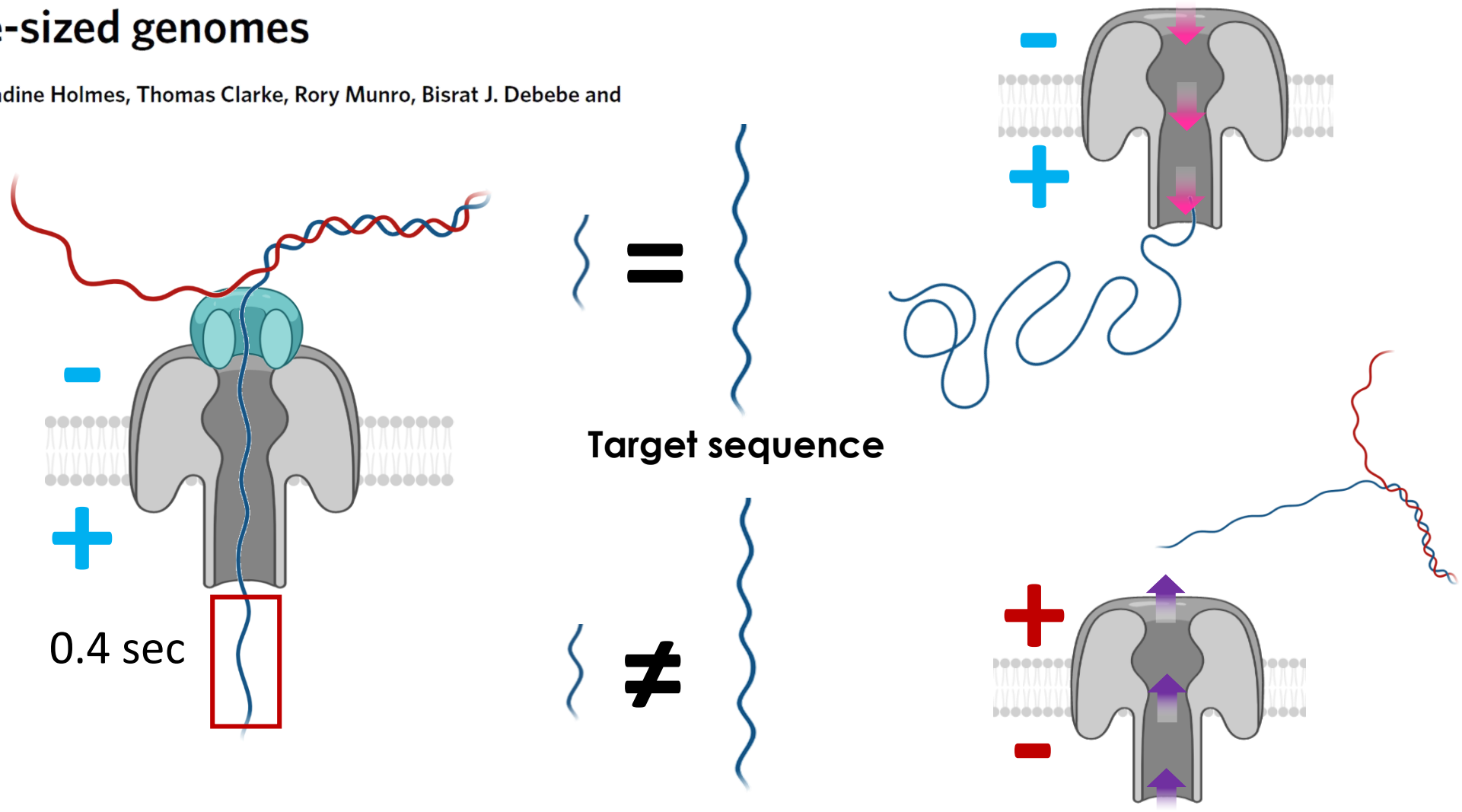


2048 nanopores

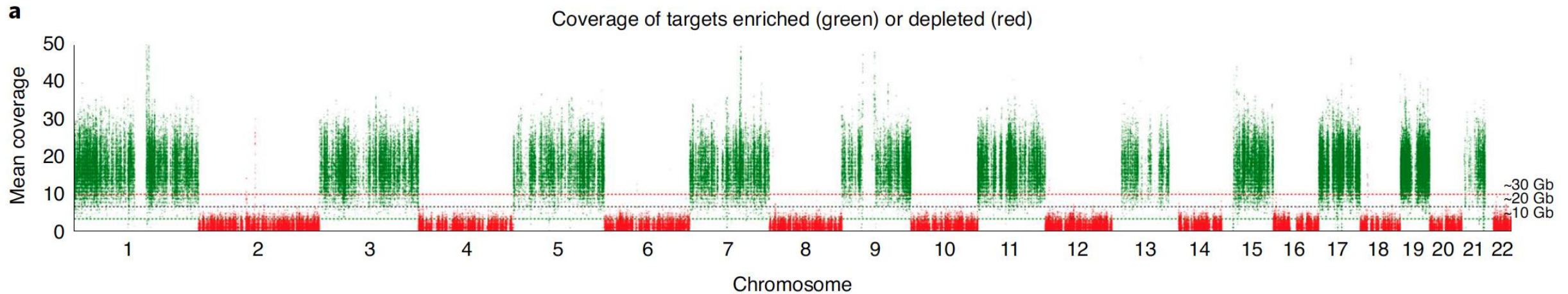


Readfish enables targeted nanopore sequencing of gigabase-sized genomes

Alexander Payne , Nadine Holmes, Thomas Clarke, Rory Munro, Bisrat J. Debebe and Matthew Loose  



Odd-numbered chromosomes are enriched (green) and even-numbered chromosomes are depleted (red)

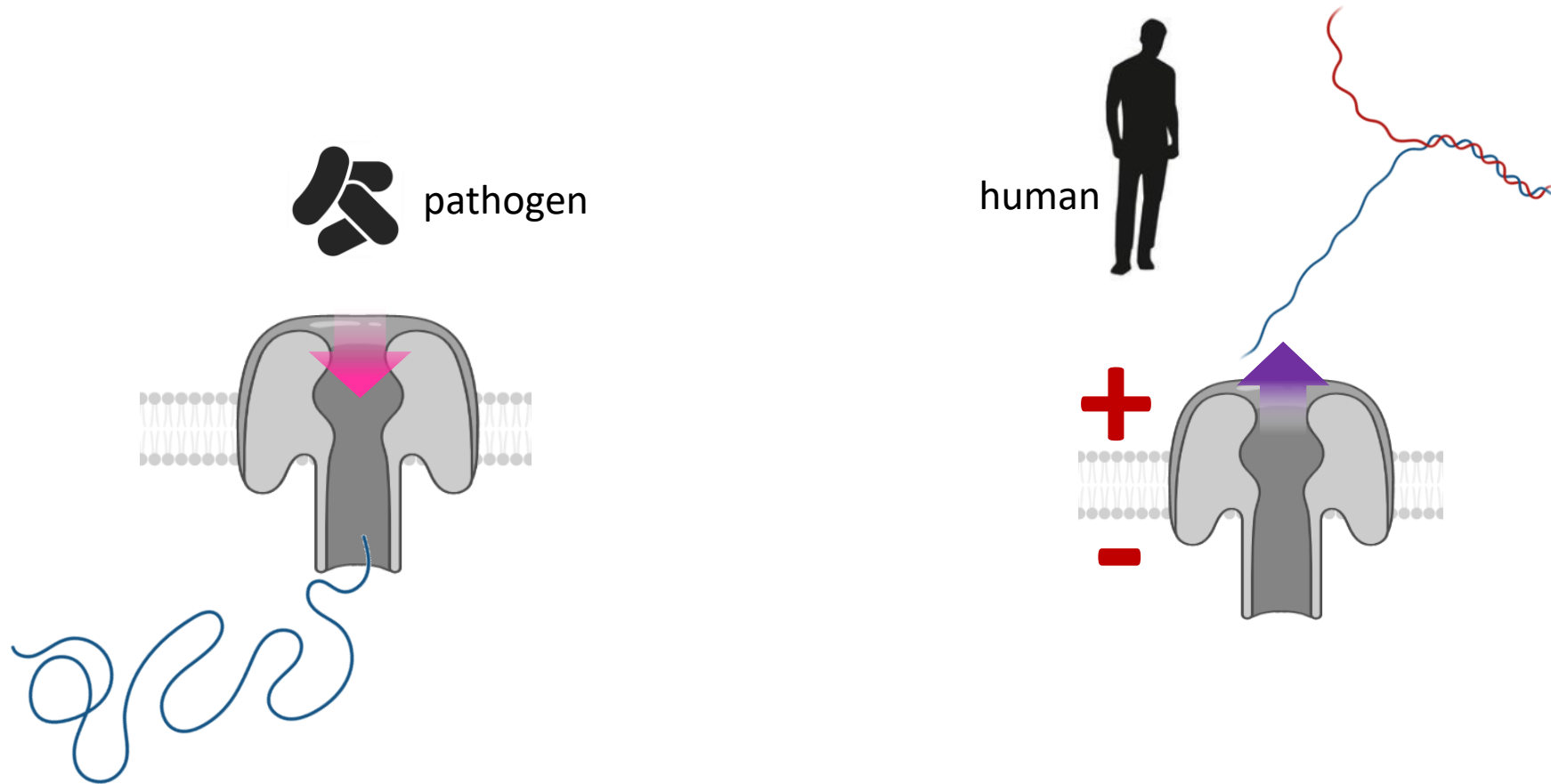


Pathogen DNA enrichment Human DNA depletion



Nay

Iyarace Khampakdee



Microbial DNA sample

Dr. Suganya
Yongkiettrakul



Assoc. Prof. Ariya
Chindamporn



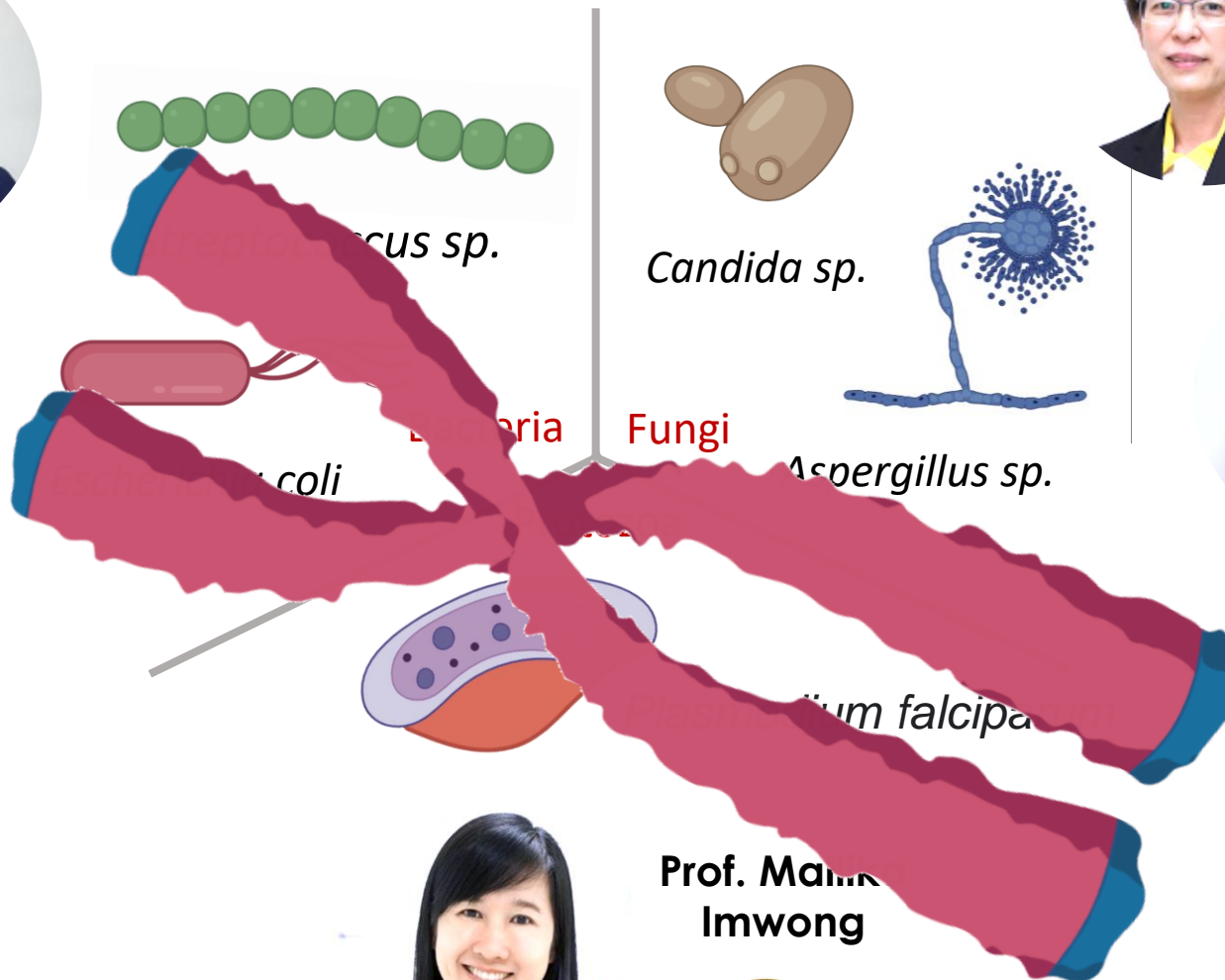
Dr. Navaporn
Worasilchai



Assist. Prof.
Anusak
Kerdsin



Prof. Manik
Imwong

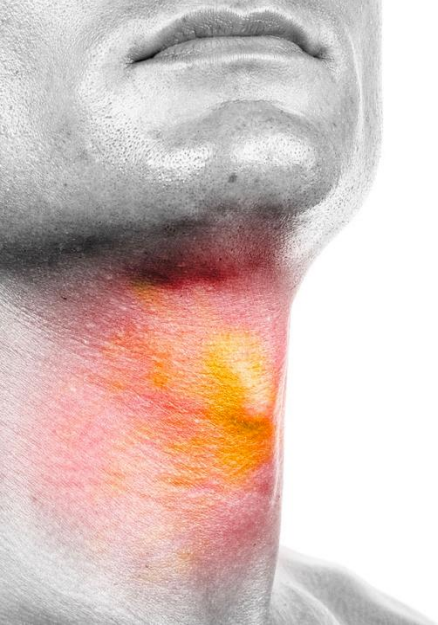


Q&A

**Microbiome
Metagenome
Whole genome sequencing
Targeted-seq**



Activity & Publications
longreadlab.com



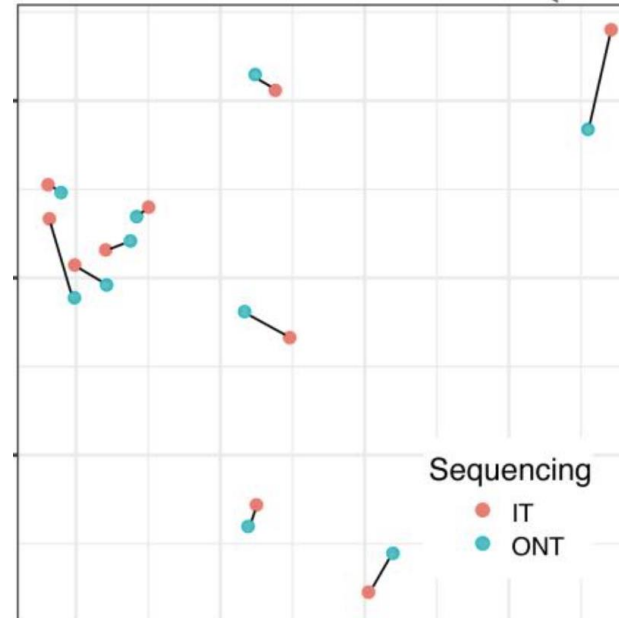
Metagenomic profiling of gut microbiota in head and neck cancers

Note

An assessment of Oxford Nanopore sequencing for human gut metagenome profiling: A pilot study of head and neck cancer patients

Thidathip Wongsurawat ^a, Mayumi Nakagawa ^b, Omar Atiq ^{c, d}, Hannah N. Coleman ^b, Piroon Jenjaroenpun ^a, James I. Allred ^e, Angela Trammel ^e, Pantakan Puengrang ^f, David W. Ussery ^{a, g}, Intawat Nookaew ^{a, g} ✉

The closer the dots,
the more similar profiling



“Taxonomic profiling using long-read and short-read sequencing are comparable”

“Potential usage of MinION on rapid clinical screening of metagenome”

The longer read lengths, the better classification recalled

OPEN

Combining short and long read sequencing to characterize antimicrobial resistance genes on plasmids applied to an unauthorized genetically modified *Bacillus*

Bas Berbers^{1,2}, Assia Saltykova^{1,2}, Cristina Garcia-Graells³, Patrick Philipp⁴, Fabrice Arella⁴, Kathleen Marchal^{2,5}, Raf Winand¹, Kevin Vanneste¹, Nancy H. C. Roosens^{1,6} & Sigrid C. J. De Keersmaecker^{1,6*}

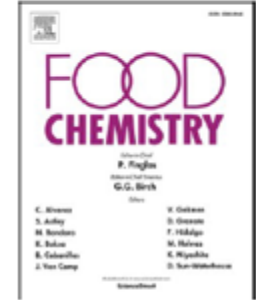
- Our findings would have been impossible using short reads only. We illustrated the added value of long read sequencing in addressing the challenges of plasmid reconstruction within the context of evaluating the risk of AMR spread.



Contents lists available at [ScienceDirect](#)

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



RE: Letter to the Editor – Analytical strategy for EU control laboratories to detect the presence of rDNA

Fraiture et al. (2020b) claim that “genetically modified microorganisms (GMM), harbouring *commonly* antimicrobial resistance (AMR) genes as selection markers, are frequently used to produce food and feed enzymes, additives and flavourings.” It is true that in the early days of genetic engineering, AMR genes were used as selection markers and were retained in the production strains. However, **state-of-the-art genetic engineering techniques now allow it either not to use AMR genes at all during strain construction or to remove the AMR gene(s) from the final production strains. Therefore, the majority of production strains on the EU market do not contain introduced AMR genes, and the remaining strains are in the process of being phased-out.**

EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

- EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain

Output metadata	
Output category	Statement
Date Endorsement	4 December 2019
Complete Author list	European Food Safety Authority
DOI	
Requestor	EFSA
Output number	
Question number	EFSA-Q-2019-00434
Correspondence	feedap@efsa.europa.eu
Short title [header]	/

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Annex A – List of information and data to be provided

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The below table lists the information and data that should be submitted by the applicants in the technical

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dossiers in those cases in which WGS-based data analysis needs to be submitted. This form should be

286

duly completed and signed by the applicants at the time of submission.

Section	Item	Provided		Comments
		Yes	NA	
	Reporting of methodologies and outcomes			
3.1	Microorganism and DNA extraction			
	Identifier for the microorganism subject of the application for authorisation (same used in other sections of the dossier)	<input type="checkbox"/>		
	Confirmation of the correspondence of the samples used, WGS and results reported with the microorganism subject of the application	<input type="checkbox"/>		
3.2	Sequencing and FASTQ data quality control			
3.2.1	Library construction			
	Library construction method including the fragmentation method	<input type="checkbox"/>		
	Threshold fragment size, where applied	<input type="checkbox"/>	<input type="checkbox"/>	
3.2.2	Sequencing strategy and quality control			
	Sequencing strategy and instrumentation used (base-calling method, where relevant)	<input type="checkbox"/>		
	Trimming and adaptor removal criteria applied, software, software version and parameters used	<input type="checkbox"/>		
	Average read depth coverage	<input type="checkbox"/>		
	Contamination in the sequencing data – Percent of reads assigned to the expected organism Tool used, the software version and parameters used, the genomes in the database used for classification should also be reported or database name and date of access	<input type="checkbox"/>		
3.3	Assembly and annotation			
	Software version and parameters (including those applied in post-assembly processing)	<input type="checkbox"/>	<input type="checkbox"/>	

3.5	Use of whole genome sequence data for the characterization of the microorganism			
3.5.1	Identification and characterization of the microorganism			
	<p>Methodology used, sequences used for comparison, results of the comparison including sequence identity or phylogenetic tree (where relevant)</p> <p>For read-mapping approach, the software used, including version number, and parameters related to the reads (including minimum length of mapping and percent coverage of the reference genome) should be reported.</p>	<input type="checkbox"/>	<input type="checkbox"/>	
3.5.2	Identification of genes of concern			
	Assembly and BLAST approach	<input type="checkbox"/>	<input type="checkbox"/>	
	Strategy, software and parameters used to identify genes of interest and database/s used (including version or accession date)	<input type="checkbox"/>	<input type="checkbox"/>	
	<p>For each hit:</p> <ul style="list-style-type: none"> - Subject sequence (including name accession and encoded protein) - E-value - BLAST score - Sequence similarity - Sequence coverage - Query sequence start and stop 	<input type="checkbox"/>	<input type="checkbox"/>	
	Reference-based read mapping approach	<input type="checkbox"/>	<input type="checkbox"/>	
	Strategy and software used and information on the subject sequences used	<input type="checkbox"/>	<input type="checkbox"/>	
	For each hit:	<input type="checkbox"/>	<input type="checkbox"/>	

• Q and A session??



Research Collaboration



- **Gut microbiota in elderly**
(Assoc. Prof. Kanitha Patarakul, M.D. PhD.)
- **Gut-Brain Axis**
(Assoc. Prof. Saknan Bongsebandhu-phubhakdi, PhD.)
- **Gut microbiota in patients with liver cancers**
(Assoc. Prof. Boonchoo Sirichindakul, M.D.)
- **Gut microbiota in patients with bariatric surgery**
(Assoc. Prof. Suthep Udomsawaengsup, M.D.)
- **Gut microbiota in pregnancy**
(Prof. Vorapong Phupong, M.D.)

Contact our poop lab:

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Faculty of Medicine, Chulalongkorn University
Tel: 02-256-4482 ex. 4724, Email: Nattaya.c@chula.ac.th