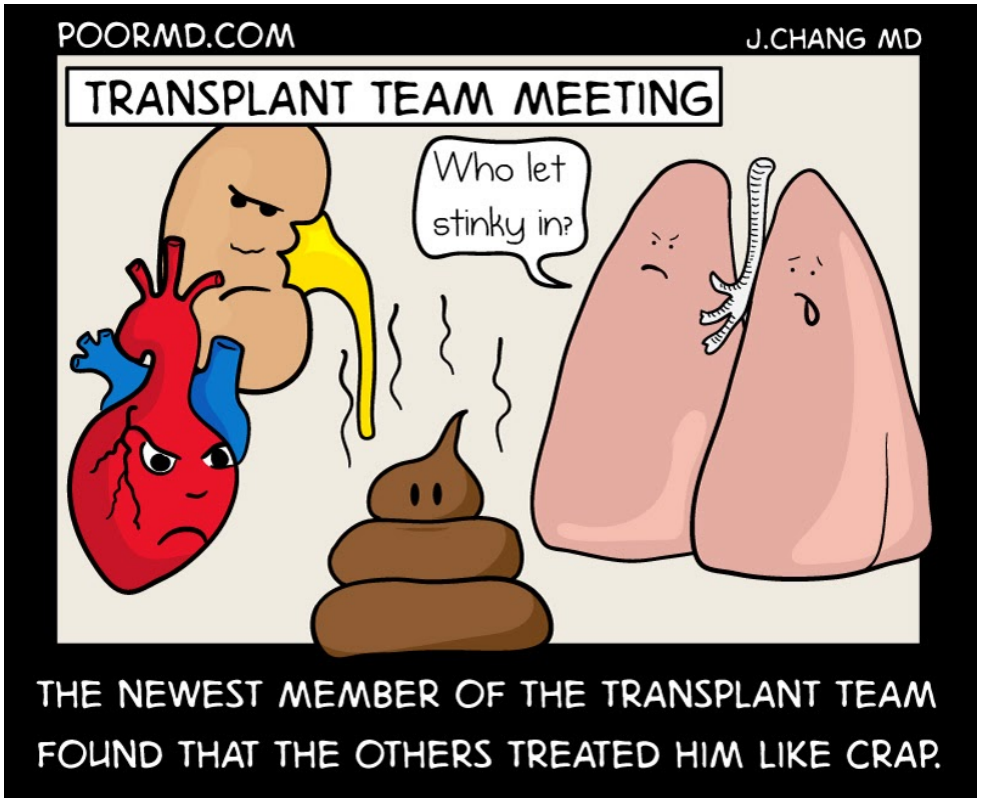
Mice as Translational Models:
Planning a Fecal Microbiota Transplantation Study

RANDI LUNDBERG, DVM
APPLICATIONS SCIENTIST

3rd Annual Translational Microbiome Conference
Boston, April 11-13, 2017

FMT – not so new after all

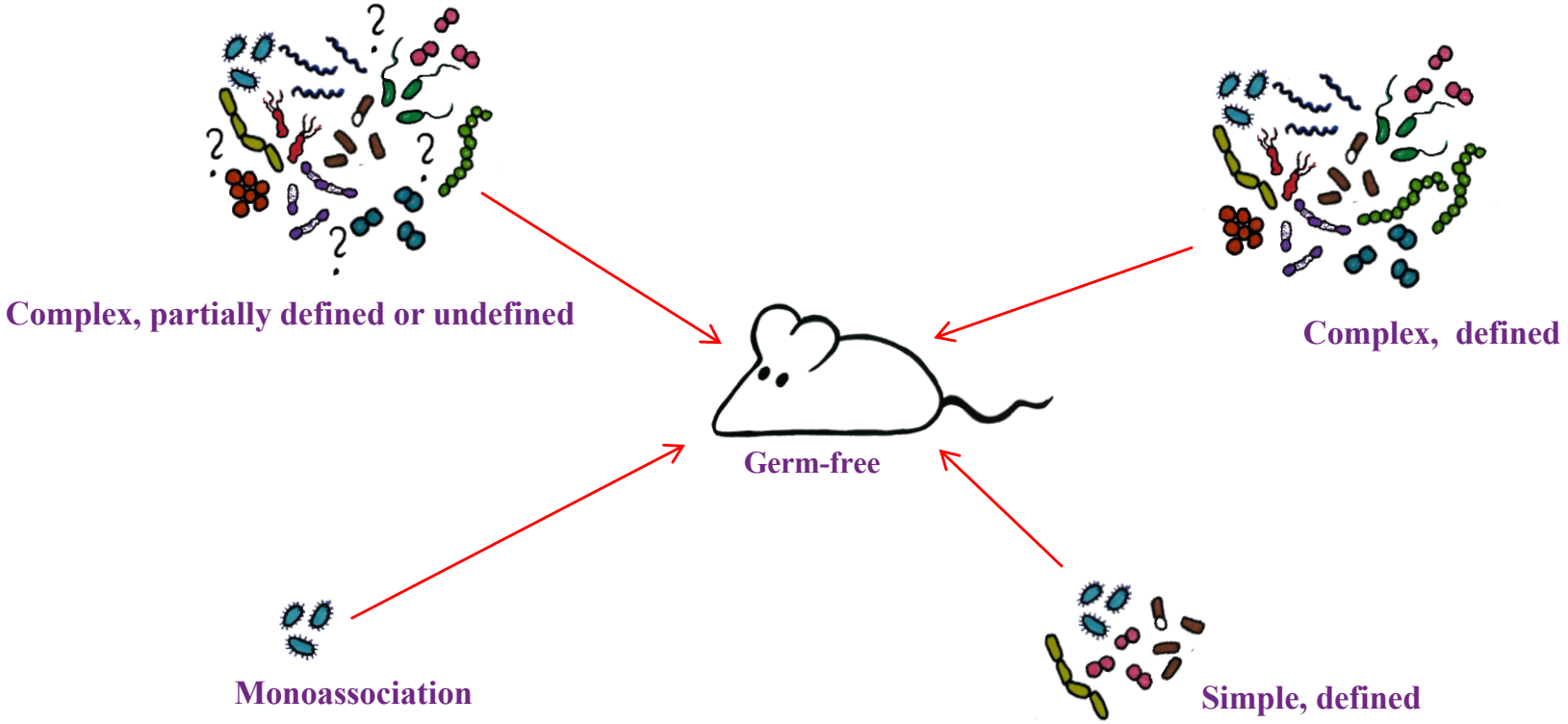


Earliest written report of FMT:
Ge Hong (283-343 C.E.), China

- Used oral FMT for food poisoning and severe diarrhea with success

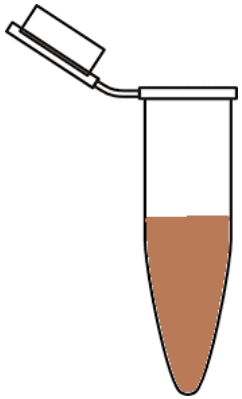
FMT in mice

- Effect of microbiome has been studied in germ-free mice since the late 1950's
- Was mostly monoassociations, simplistic defined consortia, conventionalization (typically by co-housing with conventional mice)



Illustrations © R. Lundberg 2015

FMT in mice - applications



Oral gavage with fecal slurry

http://www.procedureswithcare.org.uk/oral-gavage-in-the-mouse/

- Study host-microbiome interactions
- Phenotype transfer - prove causative role of microbiome
- Generate mouse cohorts with a relevant microbiome (e.g. human) for drug testing
- Generate mice with disease-prone microbiota for induction or acceleration of the model

FMT in mice - procedures

- Topical origin of transplant
- Fresh vs. frozen transplant
- Cryopreservation or not
- Anaerobic handling
- Administration (route, single vs. repeated dose)
- Recipient (health status, age)
- Housing and husbandry

May all affect FMT

FMT in mice - procedures

- Topical origin of transplant
- Fresh vs. frozen transplant
- Cryopreservation or not
- Anaerobic handling
- Administration (route, single vs. repeated dose)
- **Recipient (health status, age)**
- **Housing and husbandry**

May all affect FMT

Recipient

Germ-free

Antibiotic-treated

Ampicillin 1 g/L DW
Vancomycin 0.5 g/L DW
Neomycin 1 g/L DW
Metronidazole 1 g/L DW

The case of antibiotics

- Complete germ-free state not obtained (even after up to 3-4 weeks on antibiotics)
- Individual variation in surviving species, thus variation in baseline immunology
- Observed effect may happen through other pathways than the microbiota
- Usually mainly targets bacteria
- Not targeting microbiome outside the gut
- Risk of favoring bacteria with antibiotic resistance genes

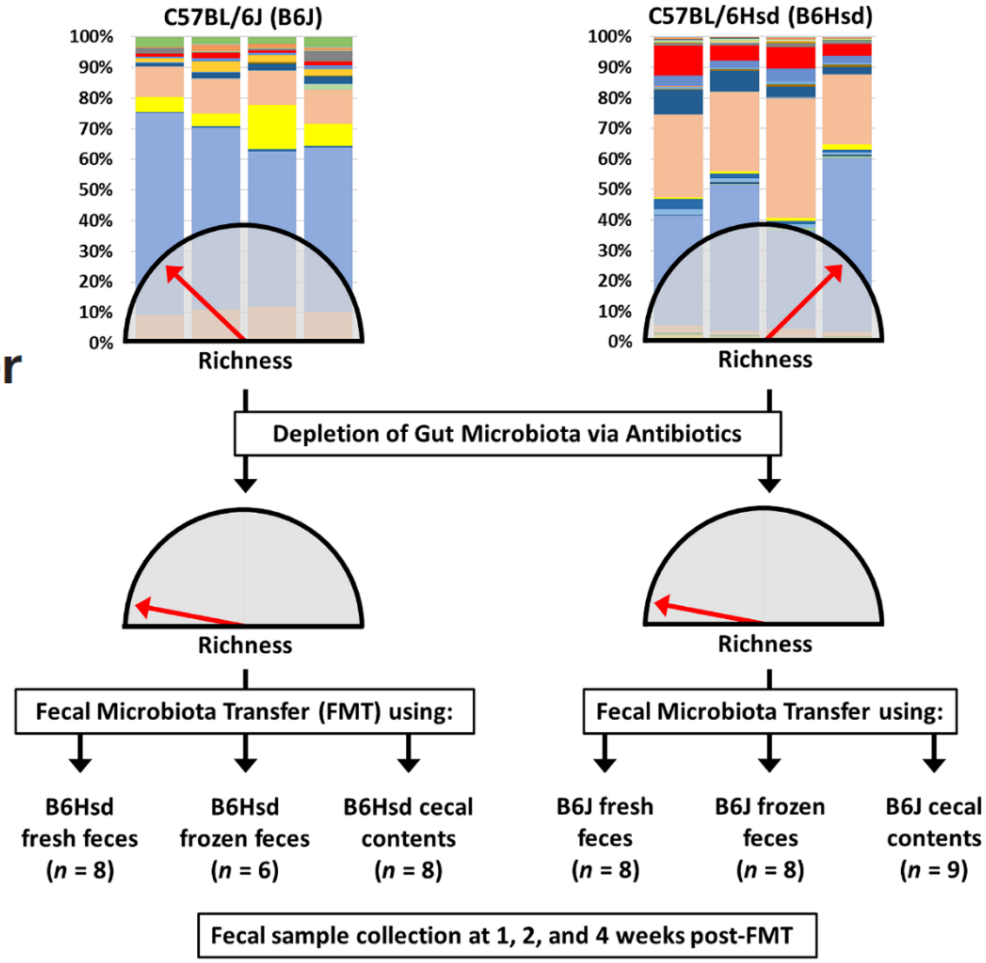
The case of antibiotics

FMT success inconsistent with antibiotics

Variable Colonization after Reciprocal Fecal Microbiota Transfer between Mice with Low and High Richness Microbiota

Aaron C. Ericsson[1,2,3], Alexa R. Personett[3], Giedre Turner[2,3], Rebecca A. Dorfmeyer[2,3] and Craig L. Franklin[1,2,3]*

Front. Microbiol. Feb 2017

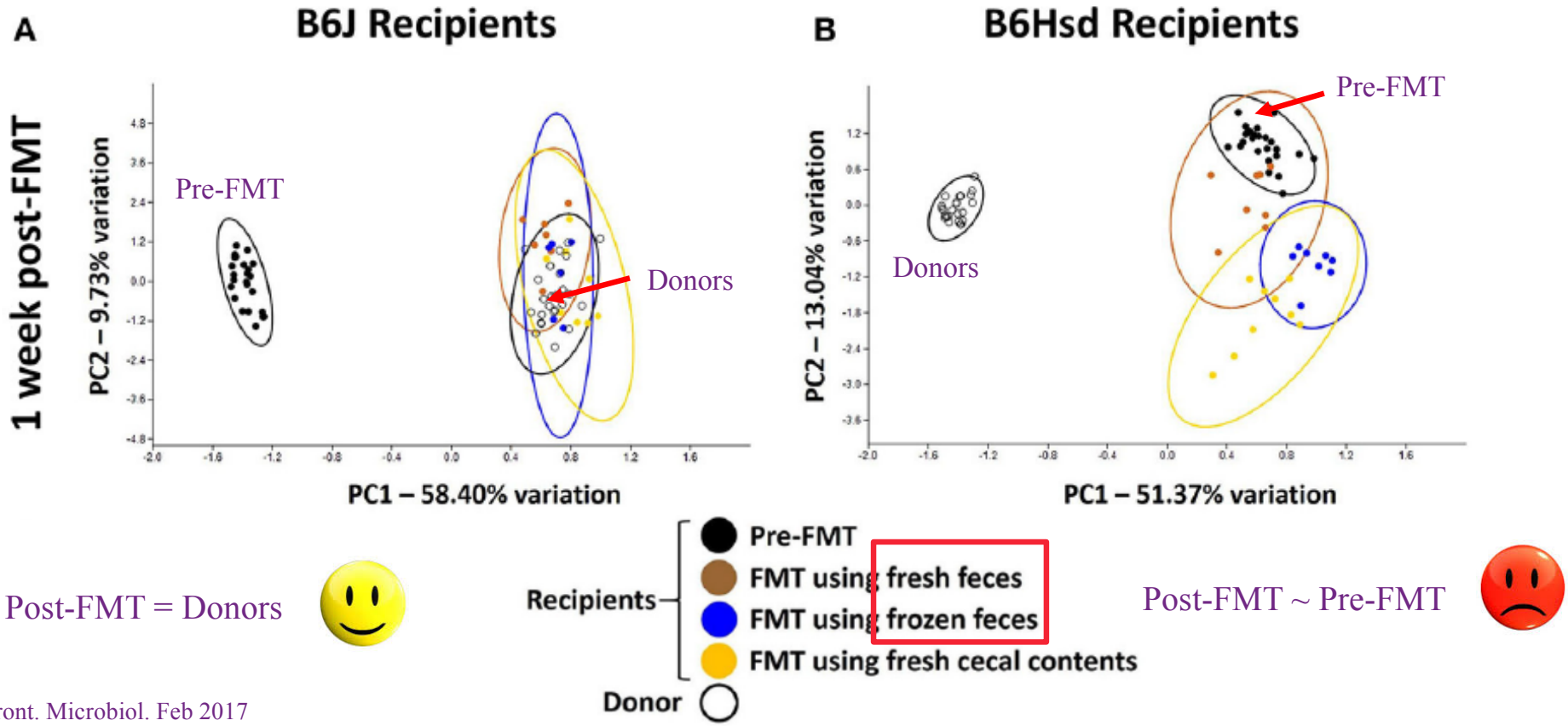


The case of antibiotics

FMT success inconsistent with antibiotics

High richness donors
Low richness recipients

Low richness donors
High richness recipients



Post-FMT = Donors

Post-FMT ~ Pre-FMT

Front. Microbiol. Feb 2017

The case of antibiotics

Direct effect of antibiotics on host tissue

- Ampicillin/neomycin/vancomycin/metronidazole-cocktail
- Performed gene expression microarrays
- Detected toxic effect in antibiotic-treated germ-free mice
  - Suppression of mitochondrial function
  - Suppression of gene expression related to ribosomes
  - Death of intestinal epithelium

Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks

Andrey Morgun,[1,7] Amiran Dzutsev,[2] Xiaoxi Dong,[1] Renee L Greer,[3] D Joseph Sexton,[4] Jacques Ravel,[5] Martin Schuster,[4] William Hsiao,[6] Polly Matzinger,[7] Natalia Shulzhenko[3,7]

Gut, Nov 2015

The case of antibiotics

47 page table with information on

- Genes affected by the antibiotic cocktail
- Why they change (microbiota effect, direct effect, effect of surviving species)
- In which tissue the changes occur

Description	Clone	Entrez gene ID	UG cluster	Gene symbol	fold change Control/ABx	p-value control/Abx	assignment: M=normal microbiota; ABx=direct effect of antibiotics; ABresM=antibiotics resistant microbiota	predominant expression
serum amyloid A 1 (Saa1), mRNA.	NM_009117	20208	Mm.148800	Saa1	28.805	0.0000021	M+ABx	Epith
fibroblast growth factor 15 (Fgf15), mRNA.	NM_008003	14170	Mm.3904	Fgf15	15.763	0.0005486	M	
hematopoietic cell transcript 1 (Hemt1), m	NM_010416	15202	Mm.17336	Hemt1	11.072	0.0000051	M+ABx	Epith
serum amyloid A 2 (Saa2), mRNA.	NM_011314	20209	Mm.200941	Saa2	9.758	0.0004239	M	Epith
mannose binding lectin (C) (Mbl2), mRNA.	NM_010776	17195	Mm.30045	Mbl2	9.534	0.0000283	M	
granzyme B (Gzmb), mRNA.	NM_013542	14939	Mm.14874	Gzmb	8.549	0.0000001	M	Epith
granzyme A (Gzma), mRNA.	NM_010370	14938	Mm.15510	Gzma	8.065	0.0000001	M	Epith~LP>Crypt
chemokine (C-C motif) ligand 5 (Ccl5), mRN	NM_013653	20304	Mm.284248	Ccl5	7.642	0.0000001	M	Epith~LP>Crypt
ubiquitin D (Ubd), mRNA.	NM_023137	24108	Mm.140210	Ubd	7.588	0.0000001	M	Epith~LP>Crypt
dual oxidase maturation factor 2 (Duoxa2),	NM_025777	66811	Mm.171333	Duoxa2	7.506	0.0000001	M+ABx	Epith

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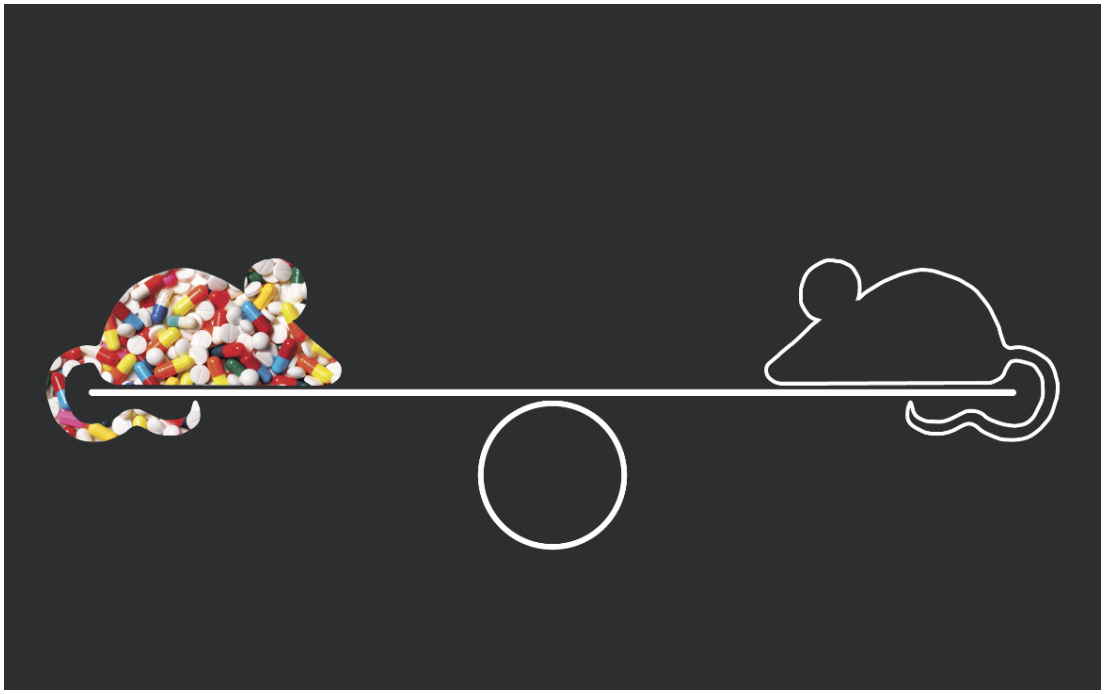


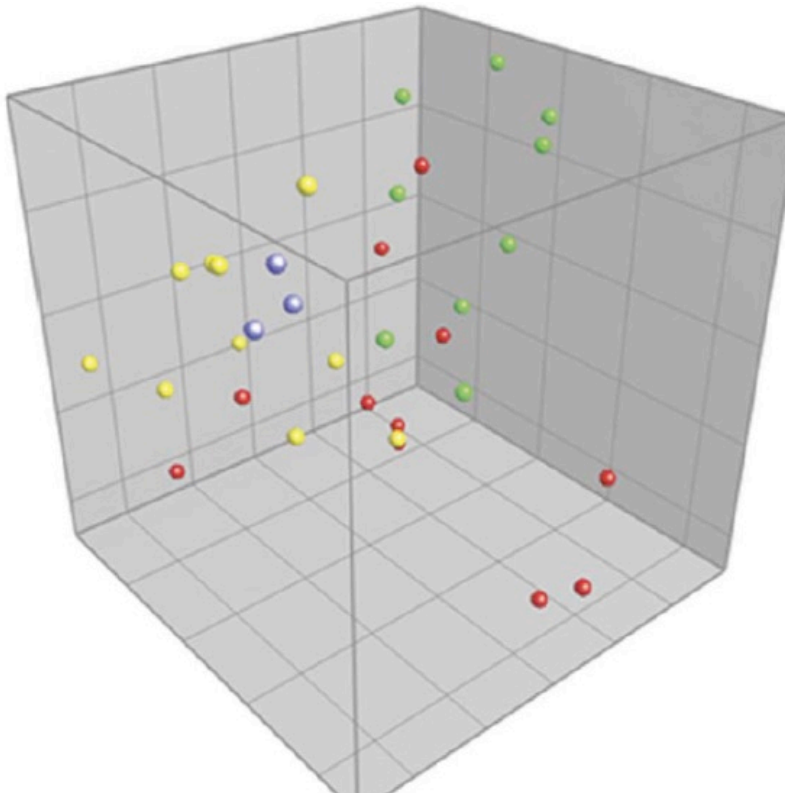
Gut Microbes, Jan 2016

Antibiotic-treated versus germ-free rodents for microbiota transplantation studies

Randi Lundberg[a,b], Martin F. Toft[b,†], Benjamin August[b,‡], Axel K. Hansen[a], and Camilla H. F. Hansen[a]

[a]Section of Experimental Animal Models, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark; [b]Internal Research and Development, Taconic Biosciences, Lille Skensved, Denmark

Does age affect FMT?



- FMT performed in 1 week (red balls) and 3 week (yellow balls) old mice
- Closest microbiota resemblance to donor (blue balls) when FMT done at 3 weeks

Patterns of Early Gut Colonization Shape Future Immune Responses of the Host PLOS ONE, Mar 2012

Camilla Hartmann Friis Hansen[1]*, Dennis Sandris Nielsen[2], Miloslav Kverka[3], Zuzana Zakostelska[3], Klara Klimesova[3], Tomas Hudcovic[3], Helena Tlaskalova-Hogenova[3], Axel Kornerup Hansen[1]

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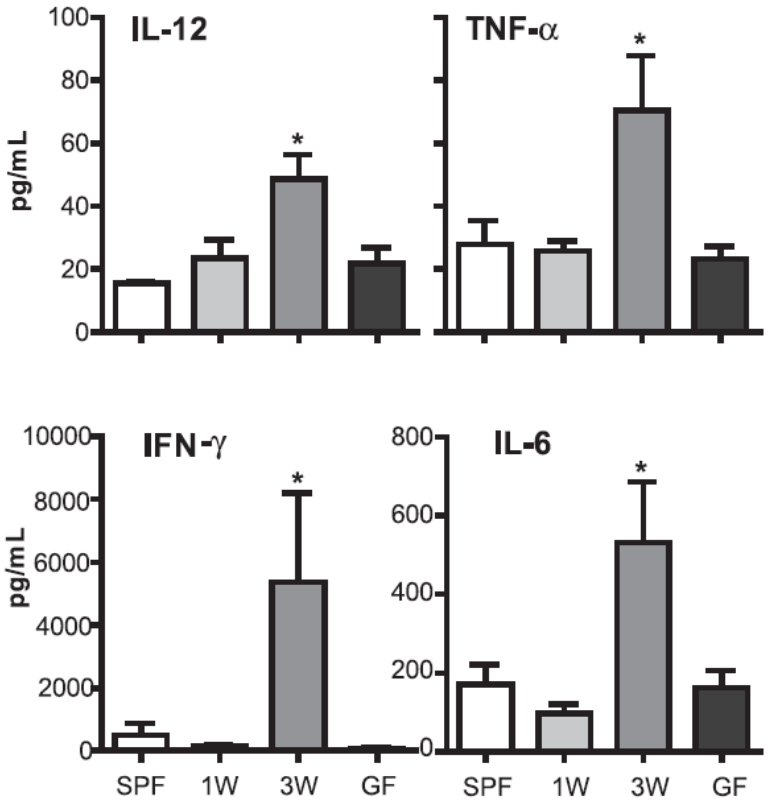

- FMT performed in 1 week (red balls) and 3 week (yellow balls) old mice
- Closest microbiota resemblance to donor (blue balls) when FMT done at 3 weeks
- FMT at 3 weeks → proinflammatory phenotype later in life (9 weeks of age)

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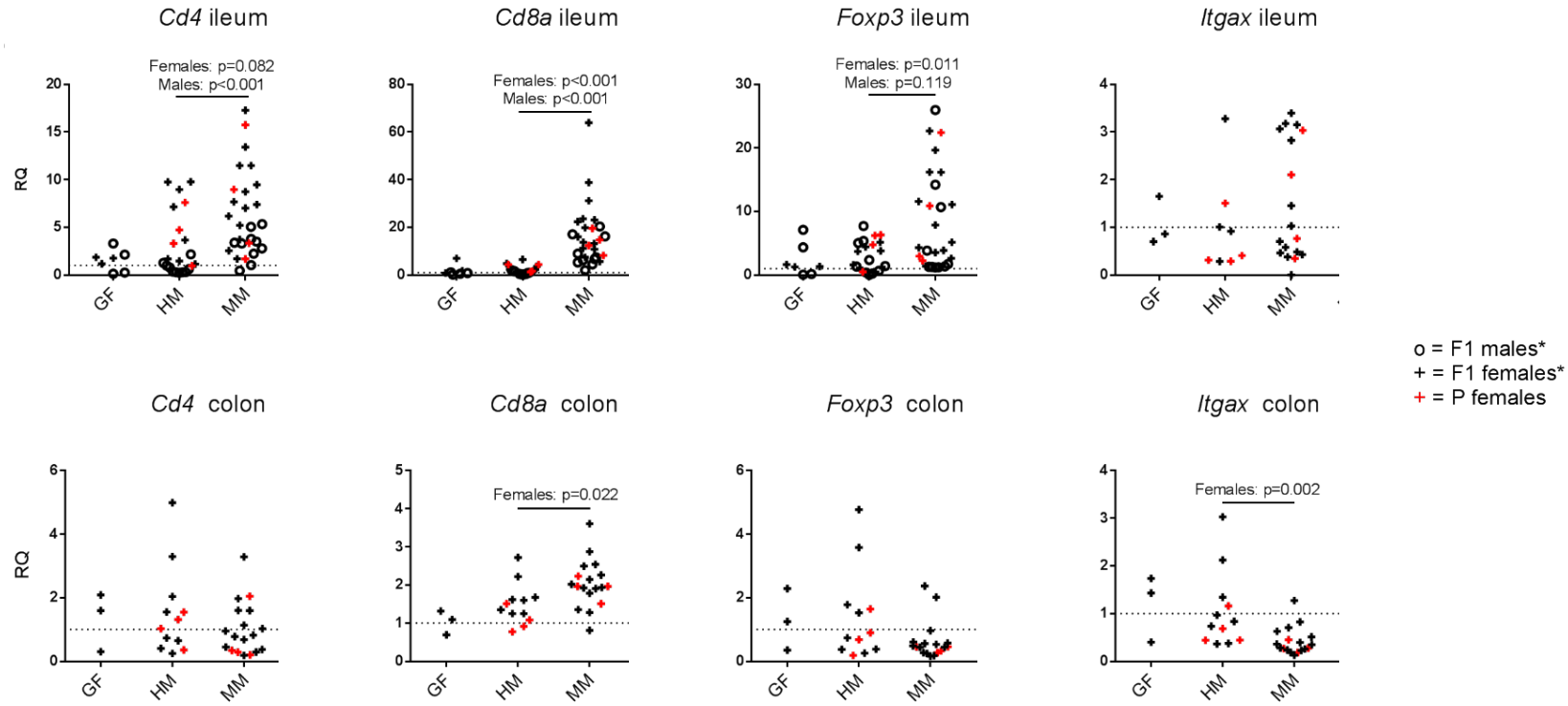
Does age affect FMT?

Ileum

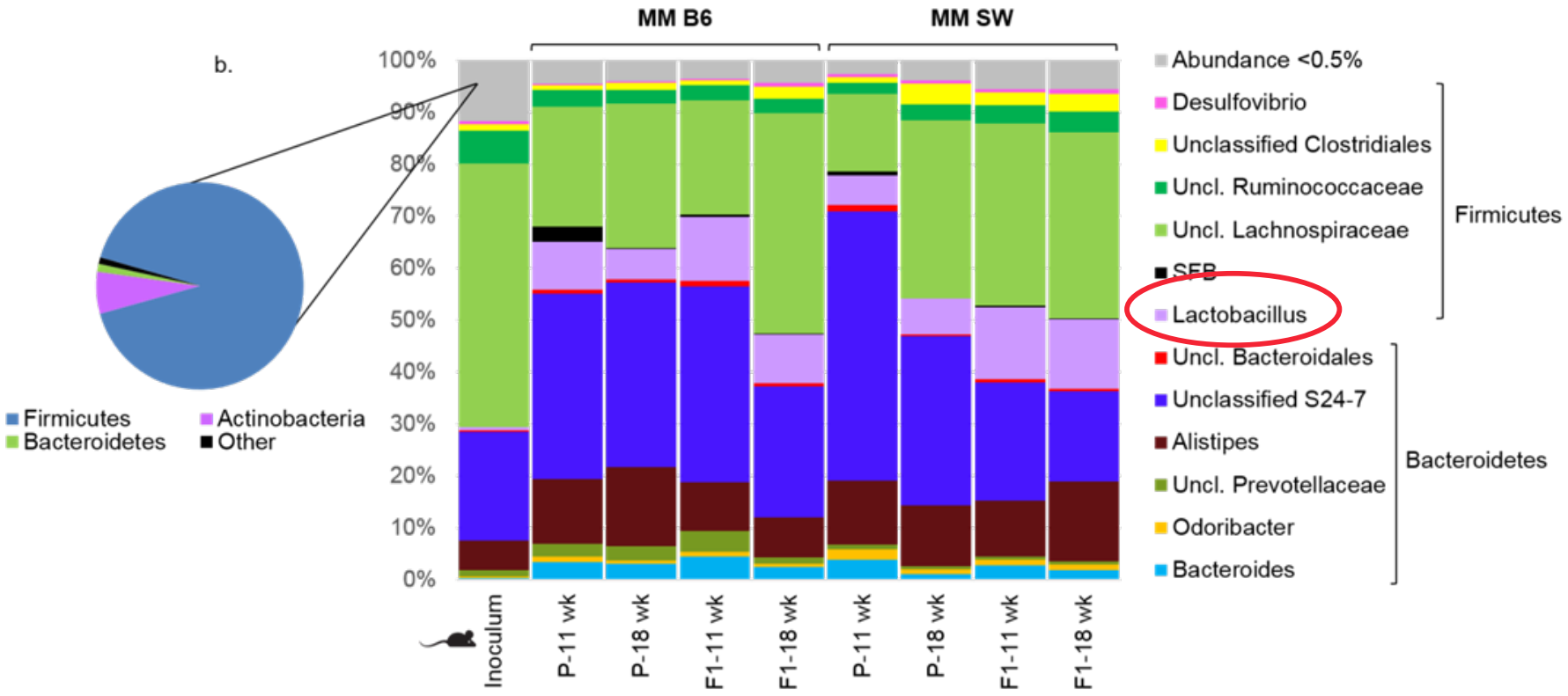
Colon



Lundberg et al. 2017 Unpublished data

- No difference in immunological phenotype at 18 weeks of age between colonized mice and their offspring born with the microbiota

Inoculation effect



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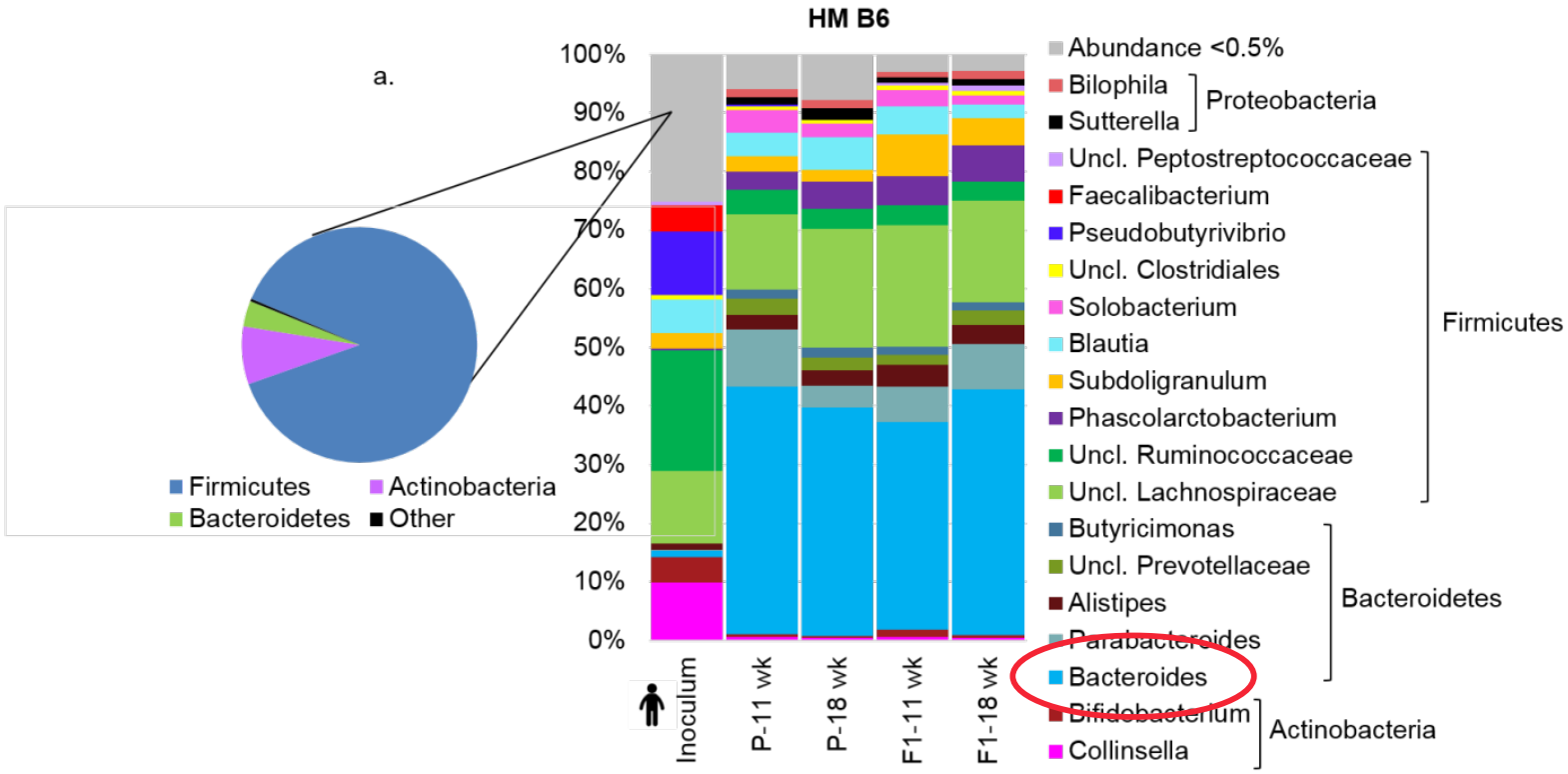

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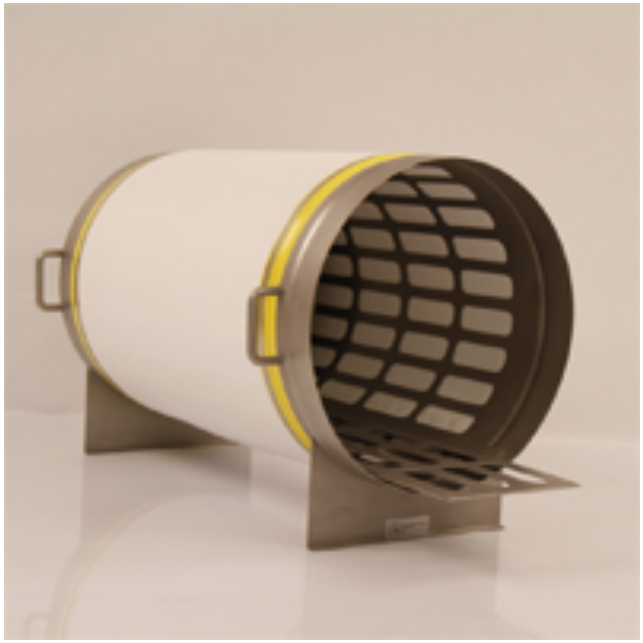
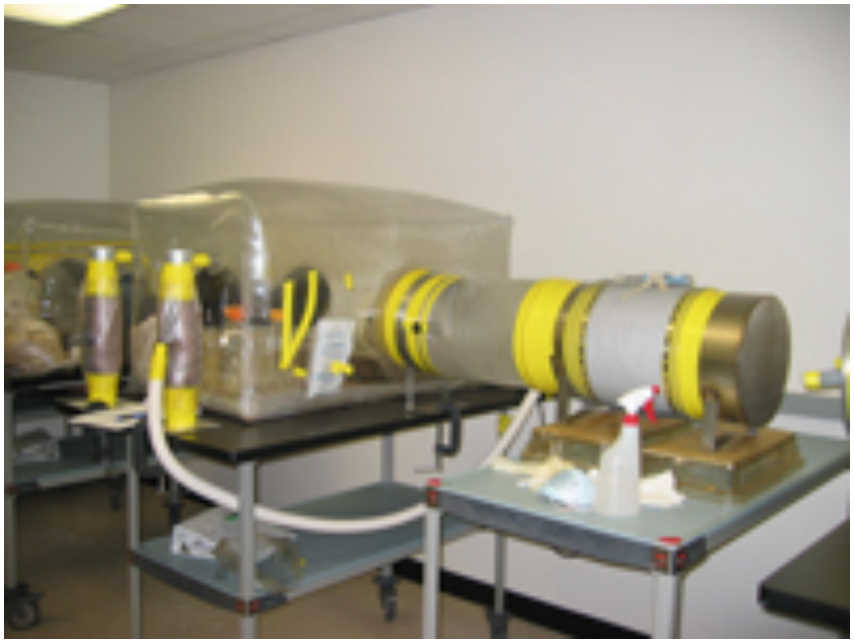
Housing

Isolator Housing



http://www.cbclean.com/isolator_starter_system.html



http://hooperlab.org/gnotobiotics/index.html

IVCs for gnotobiotic mice

- ISOCAGE P: Airtight cages with high positive pressure
- Weekly cage changes: Surgical approach
- Germ-free for at least 12 weeks

Potential for using a hermetically-sealed, positive-pressured isocage system for studies involving germ-free mice outside a flexible-film isolator Gut Microbes, July/Aug 2015

Jisun Paik[1,*], Olesya Pershutkina[1], Stacey Meeker[1], Jaehun J Yi[1], Susan Dowling[1], Charlie Hsu[1], Adeline M Hajjar[1], Lillian Maggio-Price[1], and David A C Beck[2,3]

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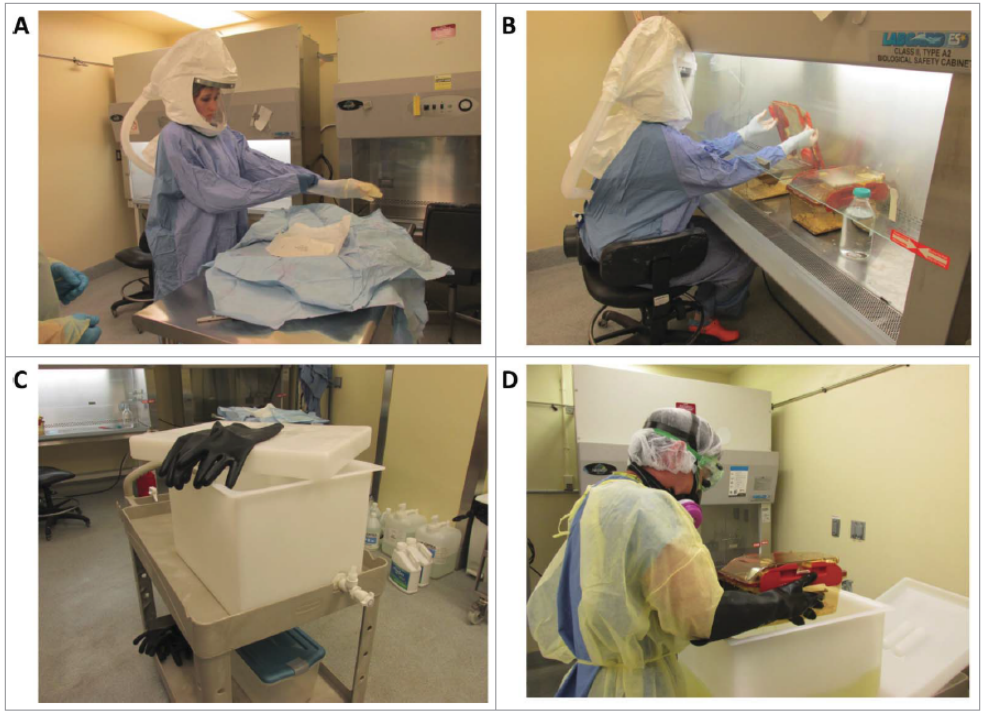

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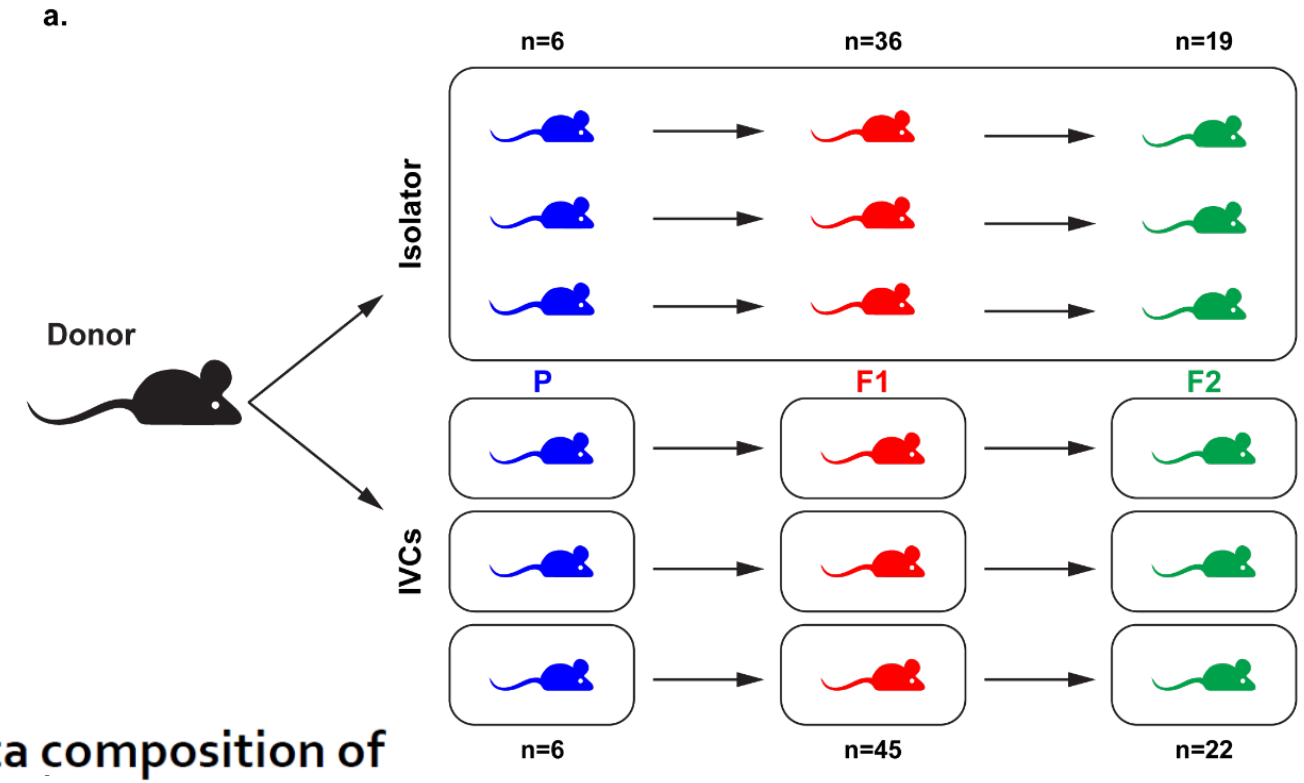


Microbiota composition of simultaneously colonized mice housed under either a gnotobiotic isolator or individually ventilated cage regime

Sci Rep, Feb 2017

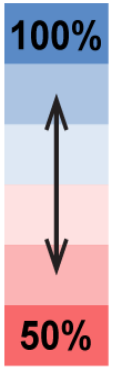
Randi Lundberg[1,2], Martin I. Bahl[3], Tine R. Licht[3], Martin F. Toft[2,†] & Axel K. Hansen[1]

- Weekly cage changes in biosafety cabinet
- Breeding two offspring generations
- 5 months timeline

Does housing affect FMT?

Lowest resemblance with donor 1 week post-colonization and in offspring generations around weaning

	Day of Study	Isolator	IVCs
Inoculum	0	100%	
P - 7 wk	7	48% ± 2	51% ± 9
P - 11 wk	38	62% ± 0	60% ± 4
F1 - 4 wk	56	56% ± 11	55% ± 8
P - 18 wk	84	62% ± 18	51% ± 19
F1 - 11 wk	98	65% ± 10	63% ± 7
F2 - 4 wk	121	49% ± 11	52% ± 8
F1 - 18 wk	154	68% ± 6	65% ± 6
F2 - 11 wk	168	65% ± 11	64% ± 7



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No difference in colonization efficiency between IVCs and isolator

Does housing affect FMT?

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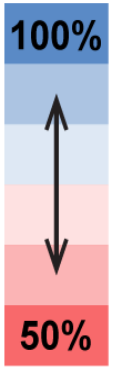

Highest resemblance with donor in audlt offspring

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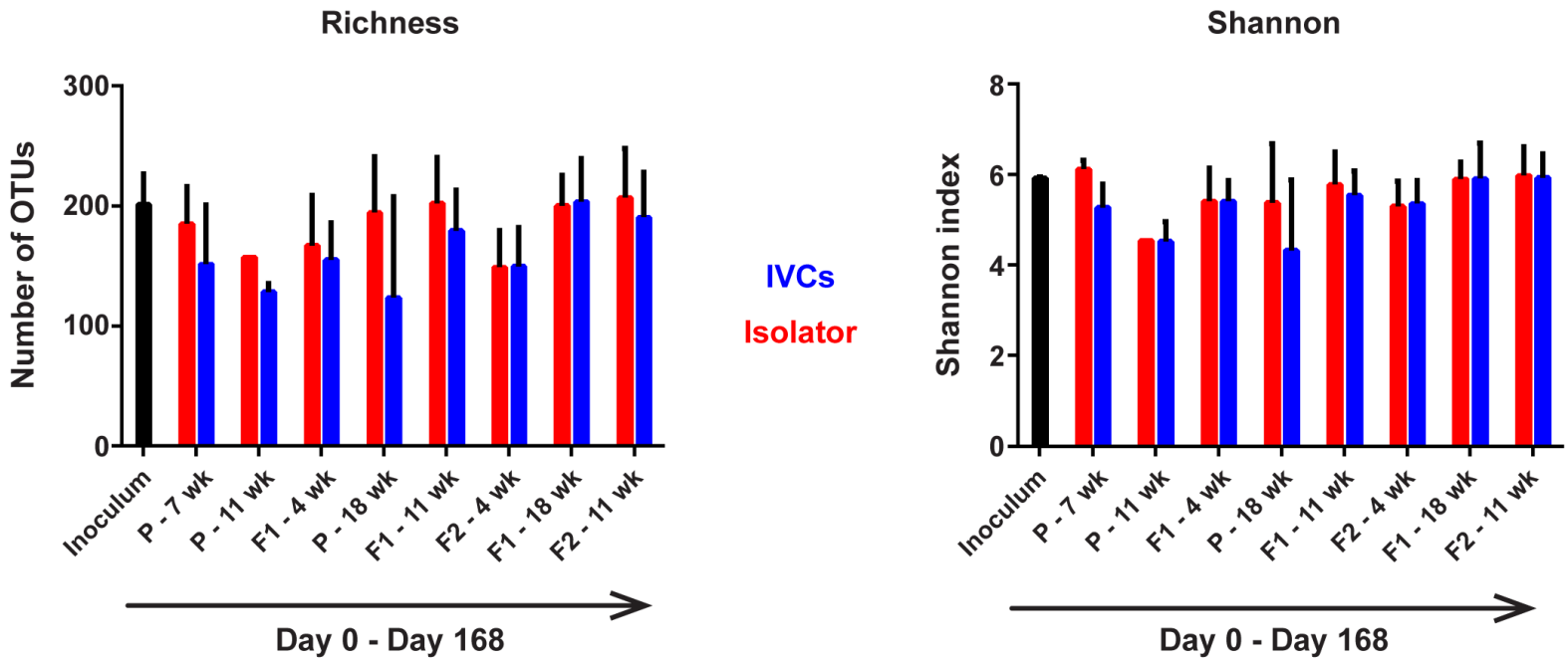

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IVC microbiota was not more diverse

Husbandry - water

Water treatment

- Chlorine and acid changes the gut microbiota
- Acidification (HCl) reported to increase *and* dicrease incidence of Type 1 diabetes in NOD mice – possibly explained by different baseline microbiotas
- Chlorination reported to affect topical formation of colonic tumours – possibly through changes in gut microbiota

pH of Drinking Water Influences the Composition of Gut Microbiome and Type 1 Diabetes Incidence

Diabetes, Feb 2014

M. Hanief Sofi,[1] Radhika Gudi,[2] Subha Karumuthil-Melethil,[3] Nicolas Perez,[3] Benjamin M. Johnson,[1] and Chenthamarakshan Vasu[1,2]

Consumption of Acidic Water Alters the Gut Microbiome and Decreases the Risk of Diabetes in NOD Mice

Kyle J. Wolf, Joseph G. Daft, Scott M. Tanner, Riley Hartmann, Ehsan Khafipour, and Robin G. Lorenz

J Histochem Cytochem, 2014

Chlorinated Water Modulates the Development of Colorectal Tumors with Chromosomal Instability and Gut Microbiota in *Apc*-Deficient Mice

Tatsunari Sasada[1], Takao Hinoi[1]*, Yasufumi Saito[1], Tomohiro Adachi[1], Yuji Takakura[1], Yasuo Kawaguchi[1], Yusuke Sotomaru[2], Kazuhiro Sentani[3], Naohide Oue[3], Wataru Yasui[3], Hideki Ohdan[1]

PLOS ONE, July 2015

Husbandry - diet

Diet

- Natural source vs. purified
- Composition (animal vs. vegetable sources, nutrient proportions)

Husbandry - diet

Diet

- **Natural source vs. purified**
- Composition (animal vs. vegetable sources, nutrient proportions)

Husbandry - diet

Diet

- Natural source vs. purified
- **Composition (animal vs. vegetable sources, nutrient proportions)**

Free of fish/meat/animal fat

altromin International

+ Fish/meat/animal fat

+ Fish meal & porcine fat

+ Fish meal

ssniff Spezialdiäten GmbH
www.ssniff.de / www.ssniff.com

Free of fish/meat/animal fat

Can diet improve FMT?

Diet

- Natural source vs. purified
- **Composition (animal vs. vegetable sources, nutrient proportions)**

Animal source
Customized rodent diet, animal and grain-based, low fat, high carb

Human profile
Customized rodent diet, grain-based, medium fat, medium carb

Chow
Standard rodent diet, grain-based, low fat, high carb

Macronutrient composition	AS diet (Animal Source)			HP diet (Human Profile)			C diet (Control)		
	Source	kcal%	gm%	Source	kcal%	gm%	Source	kcal%	gm%
Carbohydrates	Soy, corn, wheat	64	46.1	Soy, corn, wheat	55	48.3	Soy, corn, wheat	64	46.4
Proteins	Caseins, soy	24	19.2	Soy	20	19.1	Soy	24	19.2
Fats	Milk fat	12	4.3	Soybean oil	25	10.5	Soybean oil	12	4.3
Vitamins Minerals	Calcium carbonate, dicalcium phosphate, premixed vitamins, premixed minerals, trace elements								

Moreno-Indias, Lundberg et al. 2017 Unpublished data

Can diet improve FMT?

		Human Microbiota			Mouse Microbiota		
	Inoculum	100%			100%		
P generation	P - T1	42% ±16	50% ±23	37% ±11	68% ±7[b]	37% ±5	37% ±4
	P - T2	48% ±0	48% ±4	38% ±2	70% ±10	63% ±6	62% ±4
F1 generation	F1 - T1	30% ±6	37% ±12	33% ±3	65% ±3[c]	55% ±6	53% ±7
	F1 - T2	30% ±4	40% ±9[a]	36% ±4	68% ±8[(d)]	61% ±6	65% ±6
	Diet	↑ AS	↑ HP	↑ C	↑ AS	↑ HP	↑ C

100%
70%
50%
40%
30%

Grain-based diet w/ medium fat improved human-mouse FMT

Animal source-diet w/ low fat improved mouse-mouse FMT

Moreno-Indias, Lundberg et al. 2017 Unpublished data

Husbandry - miscellaneous

- Bedding type
- Enrichment items (nesting material, gnawing sticks, shelters etc.)
- Cage density
- Temperature and air draft
- Noise
- Timing (latest meal?)
- Caretakers
- …

If a variable can affect the microbiome → possible effect on FMT

If a variable can affect stress level → possible effect on FMT

FMT in mice – procedures

- Topical origin of transplant
- Fresh vs. frozen transplant
- Cryopreservation or not
- Anaerobic handling
- Administration (route, single vs. repeated dose)
- Recipient (health status, age)
- Housing and husbandry

May all affect FMT

Planning FMT in mice

White paper

Acknowledgments

University of Copenhagen
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Camilla H. F. Hansen
Stine B. Metzdorff
Lukasz Krych
Helene Farlov & Mette Nelander

Technical University of Denmark
Martin Iain Bahl
Li Zhang
Bodil Madsen
Tine Rask Licht

Taconic Biosciences
Martin Fitzner Toft
Benjamin August
James Vitale
Signe Borggreen & Animal Care Team

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