

Reducing group size in studies by consideration of the gut microbiota

Nordic 3 R Webinar 2021

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Open Ctrl+O

New Project Ctrl+Shift+N

Power and Sample Size for 2-Sample t

Specify values for any two of the following:

Sample sizes:

Differences:

Power values:

Standard deviation:

Options... Graph...

Help OK Cancel

Power and Sample Size for 2-Sample t: Options

Alternative Hypothesis

Less than

Not equal

Greater than

Significance level:

Help OK Cancel

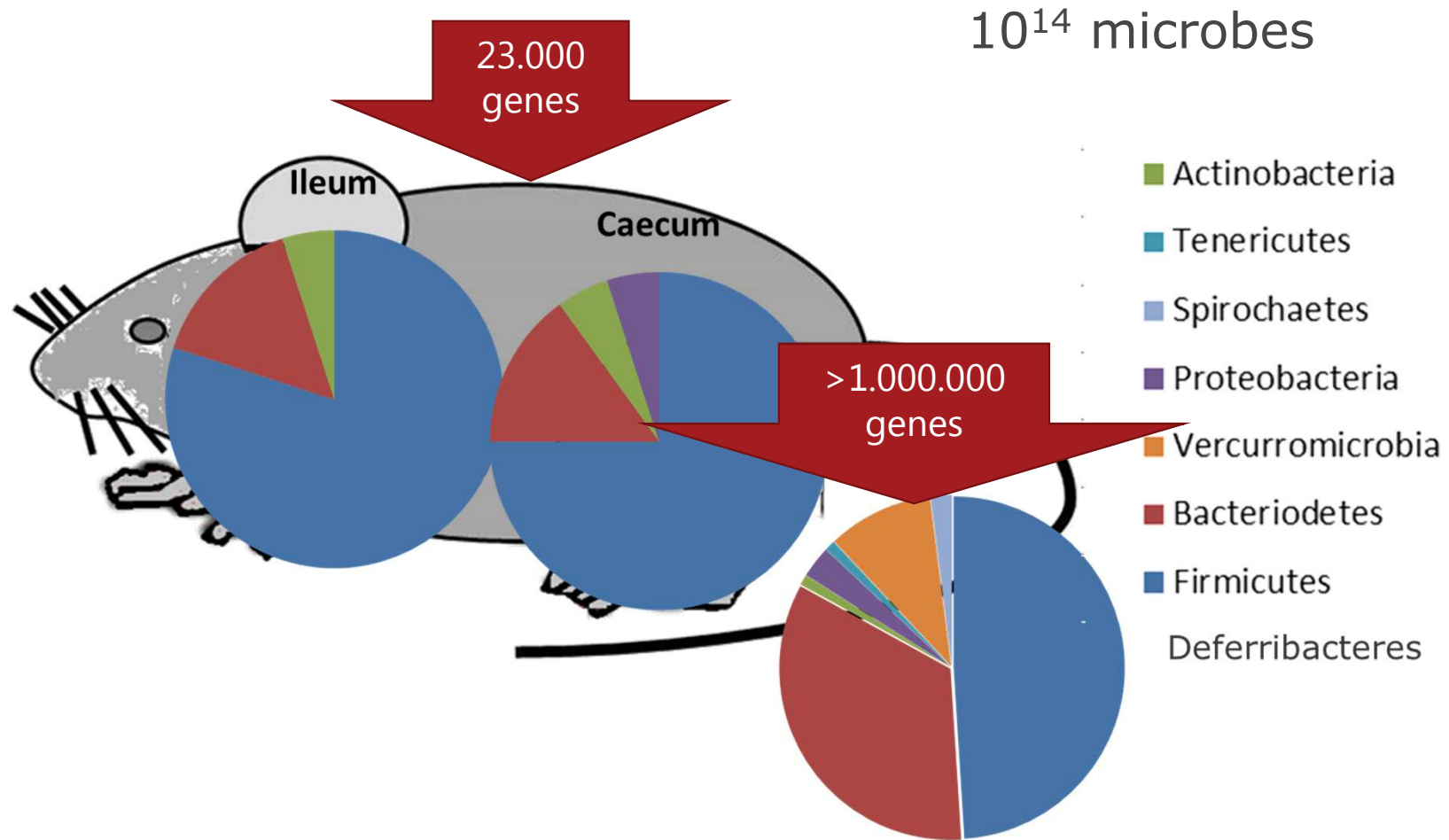
The smaller the difference
The more animals we need

The higher the variation
The more animals we need

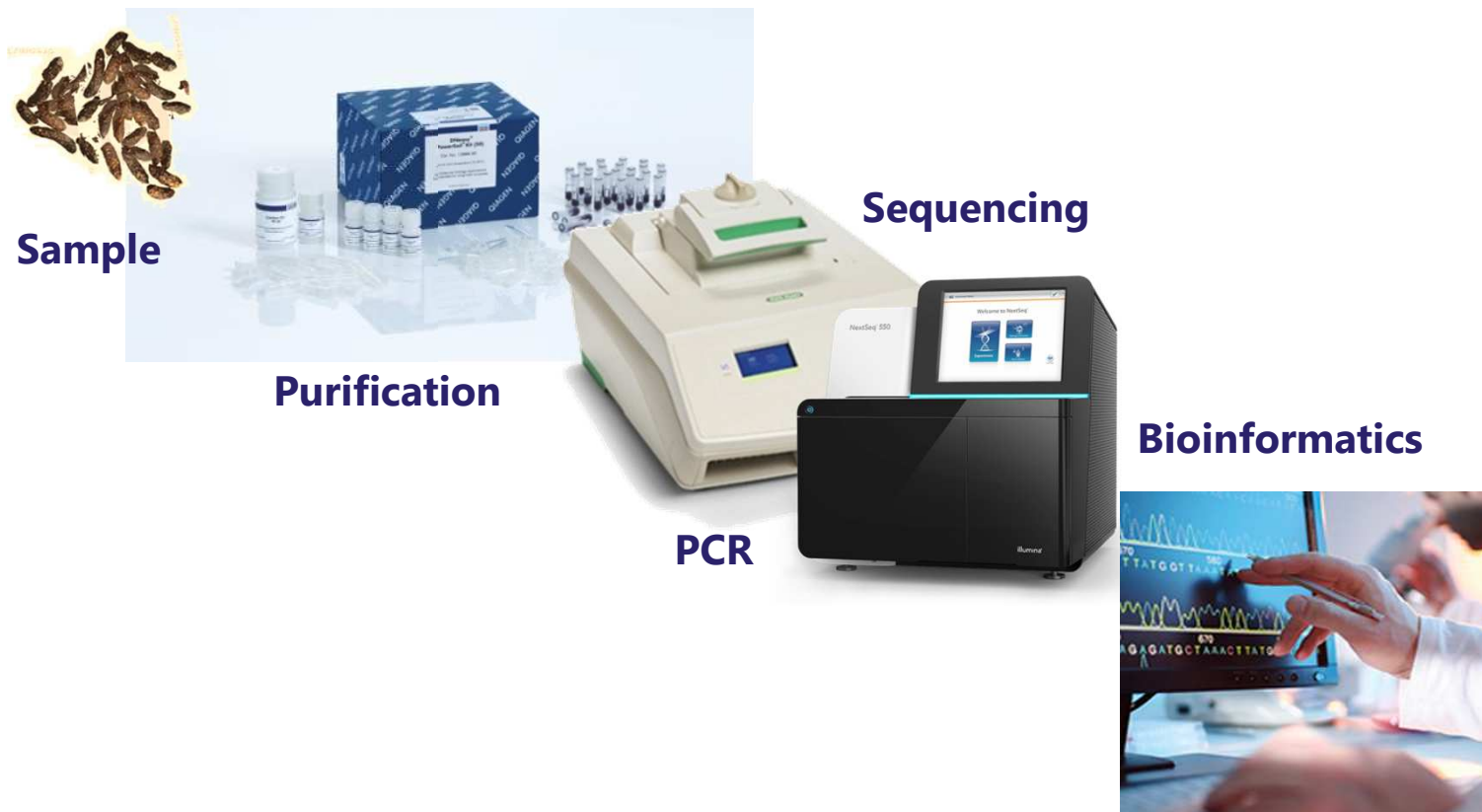
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C15

1 mouse + 1000 different germs



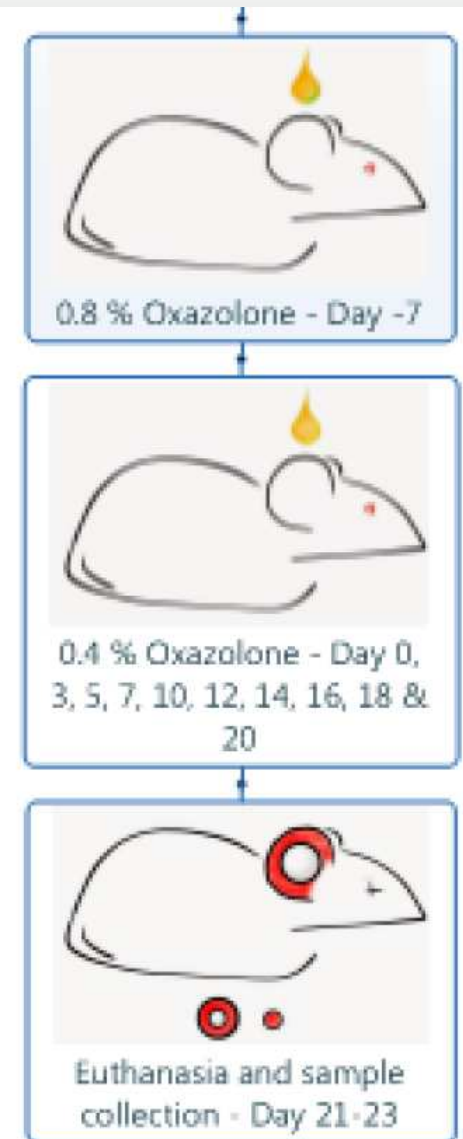
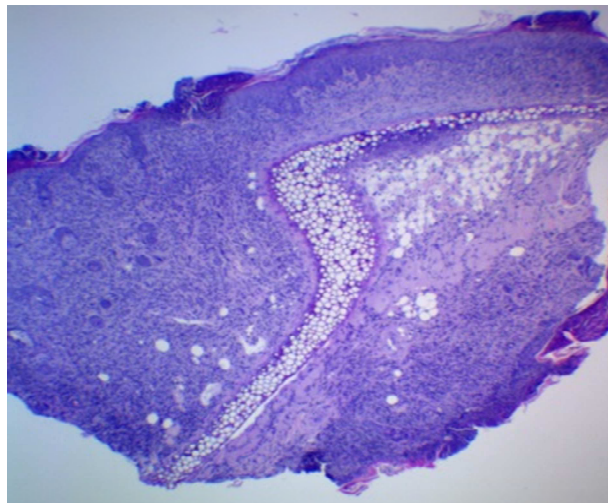
16S sequencing



The atopic dermatitis hapten model

Sensitisation with a Th2 inducing hapten

Oxazolone





Comparative Medicine
 Copyright 2012
 by the American Association for Laboratory Animal Science

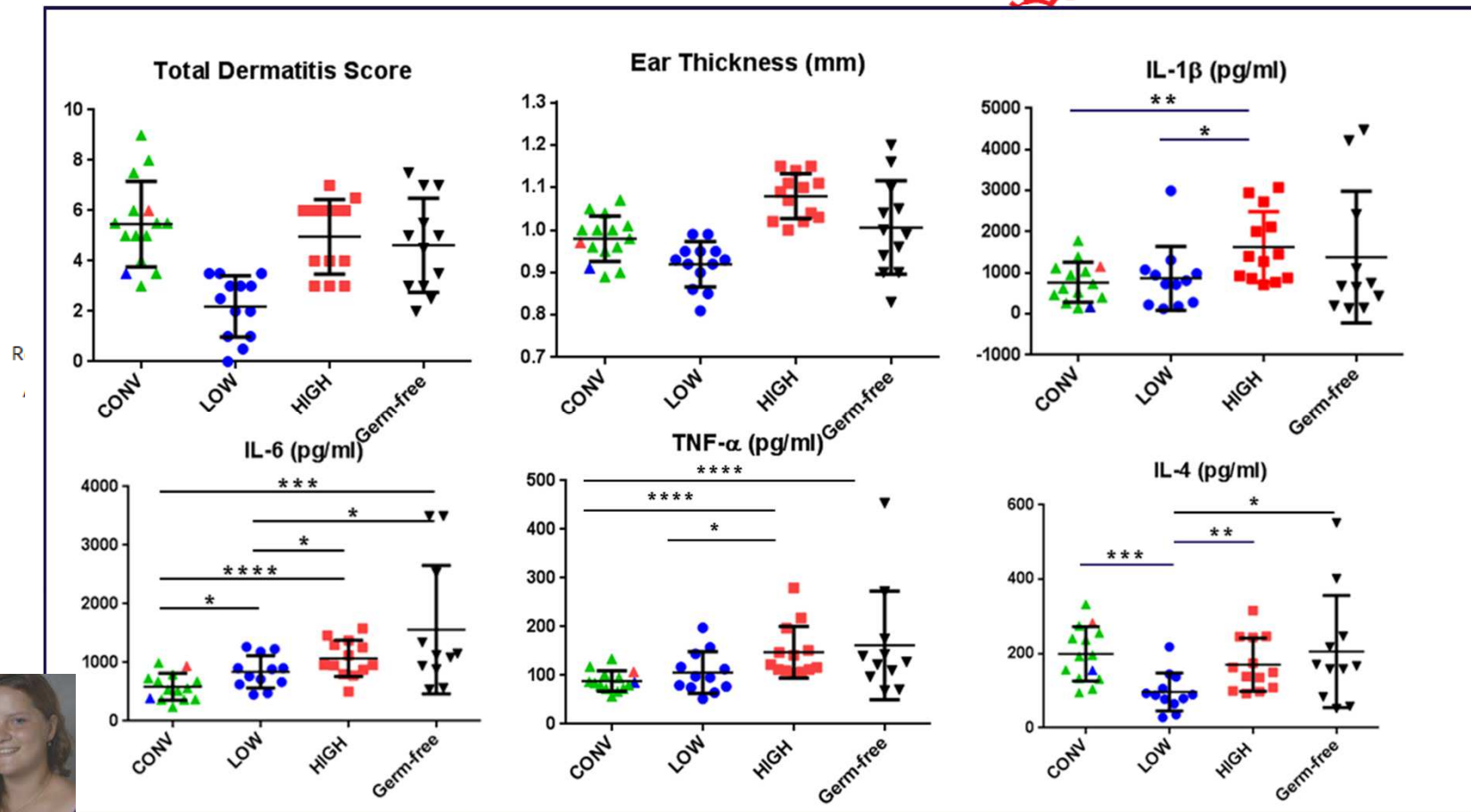
Original
Gastric and Intestinal Microbiota
 during Oxazolone-in
 BALB/c



Randi Lundberg,^{1*} Susanne K Clausen,³ Wanyong Pang,¹ Dennis S Nielsen,² Kristian Möller,³
 Knud E Josefsen,⁴ and Axel K Hansen¹

Correlation between start gut microbiota and final ear cytokines						
	IFN- γ	IL-1 β	IL-8	IL-10	IL-12	TNF- α
r²	0.93	0.83	0.88	0.86	0.71	0.87
P <	0.001	0.01	0.01	0.01	0.05	0.01

SCIENTIFIC REPORTS



The reproducibility crisis

ANNOUNCEMENT

Reducing our irreproducibility

Over the past year, *Nature* has published a string of articles that highlight failures in the reliability and reproducibility of published research (collected and freely available at go.nature.com/hubbyr). The problems arise in laboratories, but journals such as this one compound them when they fail to exert sufficient scrutiny over the results that they publish, and when they do not publish enough information for other researchers to assess results properly.

nature

Tools to increase power and reduce group sizes

- Standardization
- Standardization
- Standardization

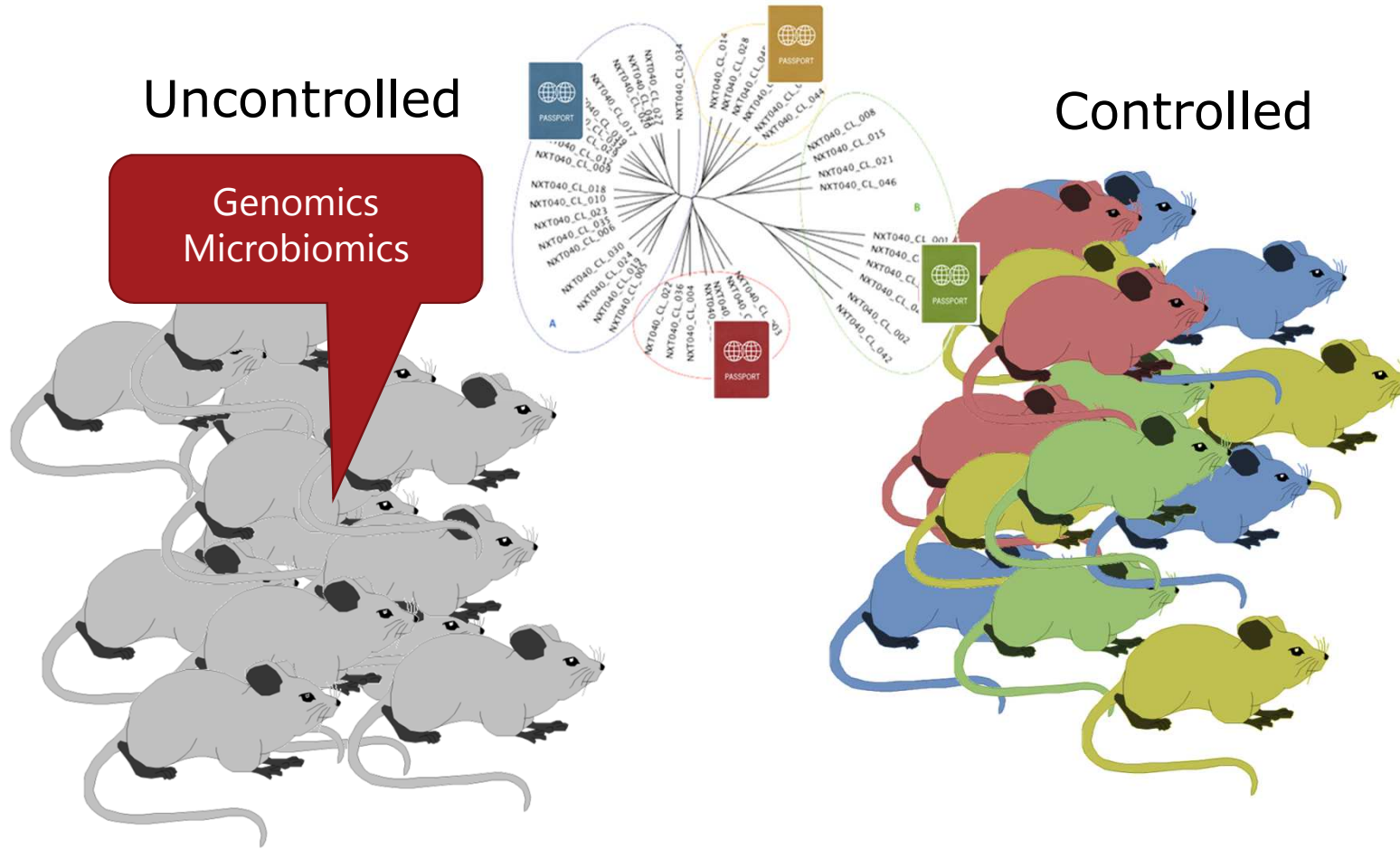
But is there an alternative ?

Yes, new 'omics technology gives us a new opportunity



An alternative to standardization

Describe the variation





Contents lists available at ScienceDirect

Research in Veterinary Science

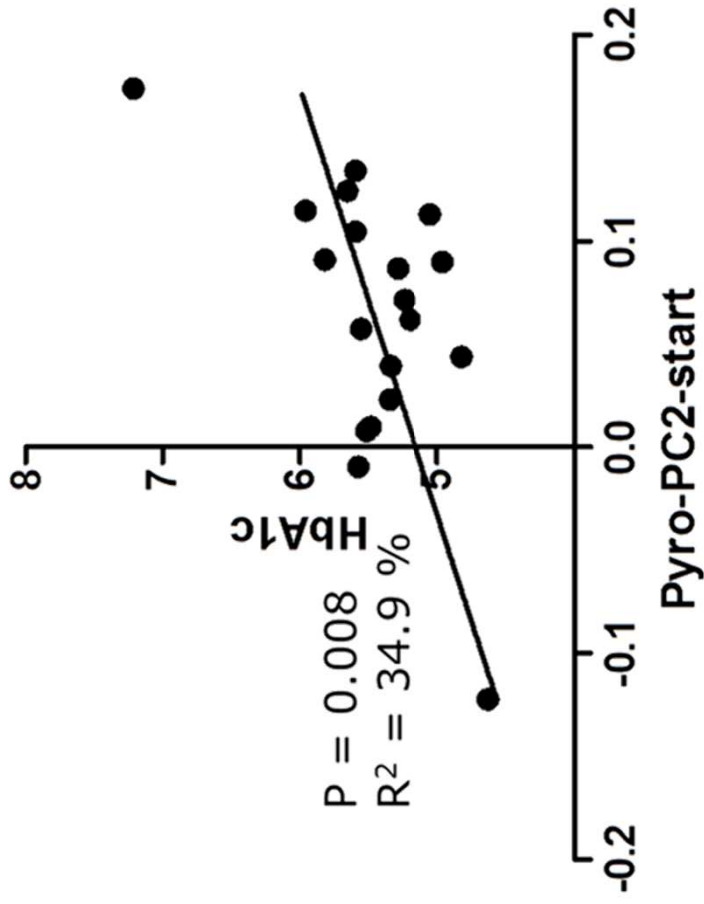
Research in Veterinary Science 96 (2014) 241–250

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



Characterization of the gut microbiota in leptin deficient obese mice – Correlation to inflammatory and diabetic parameters

M. Ellekilde^{a,*}, L. Krych^b, C.H.F. Hansen^a, M.R. Hufeldt^{a,b,c}, K. Dahl^d, L.H. Hansen^e, S.J. Sørensen^e,
F.K. Vogensen^b, D.S. Nielsen^b, A.K. Hansen^a



The mouse passport project

Taconic



Group size ?



Janvier



Group size ?



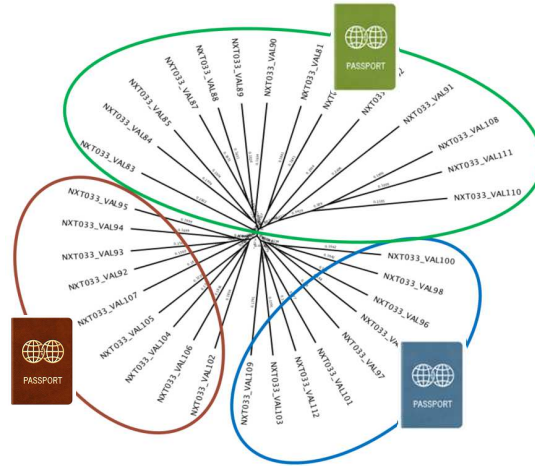
Clusters of early life microbiomics for B6 mice

Time 1

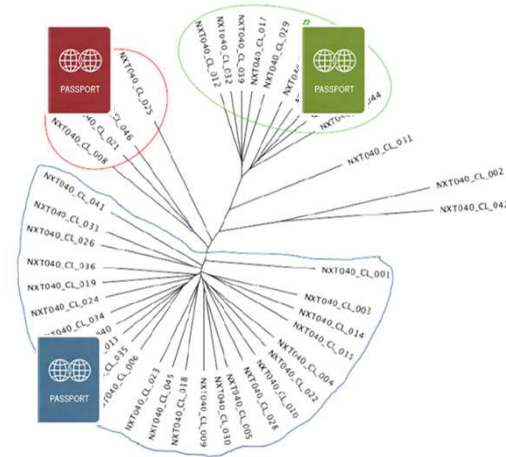
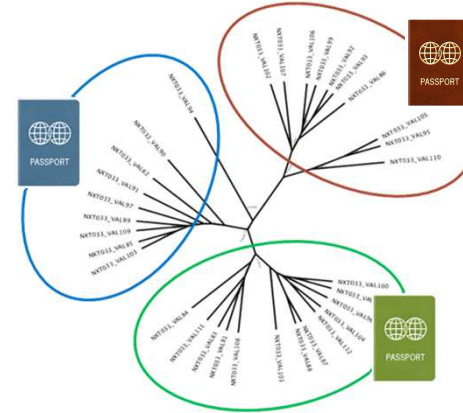
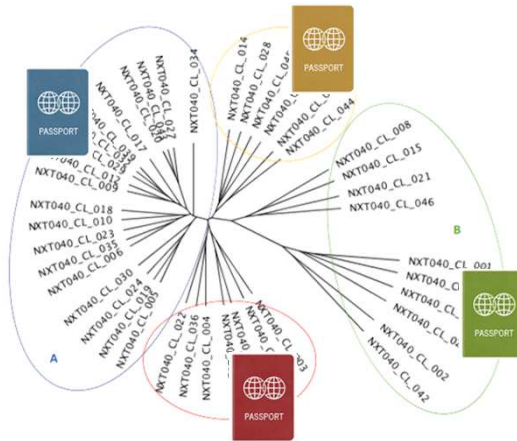
Time 2



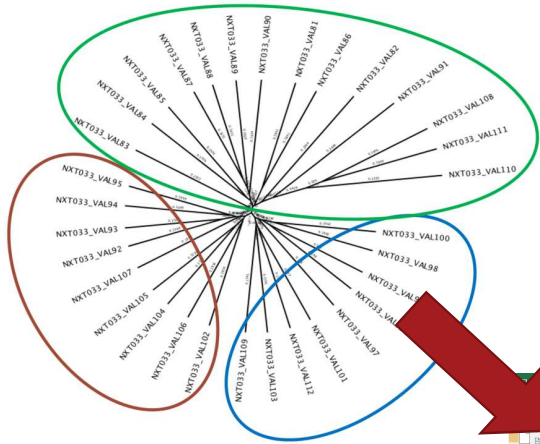
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Jackknifing of clusters



Calculate pooled s.d. and weighted means

B6NTac - Excel

Variable	Microbiot:Total Cour	Mean	StDev	dff.	Mear weighted diff.	s pooled
Δ AUC OGA	3	-3,87	16,64	-36,51	-292,08	553,7792 7246,71
B	4	-22,65	16,92	-9,85	-68,95	858,8592 26
C	9	-14,27	20,2	-28,94	-491,98	3264,32

Variable	Microbiot:Total Cour	Mean	StDev	dff.	Mear weighted diff.	s pooled
Δ AUC OGA	5	-40,38	16,99			1154,64
B	3	-32,5	10,07			202,8098
C	8	-43,21	13,16			1212,299
	32				-26,6566	16,6949

Calculate group size for a full factorial design

Minitab - B6NTac.mpx

Test for Equal Variances: leptin versus Treatment; Microbiota 2

Method

Null hypothesis: $\sigma^2_1 = \sigma^2_2 = \dots = \sigma^2_k$

Alternative hypothesis: $\sigma^2_1 \neq \sigma^2_2 = \dots = \sigma^2_k$

Significance level: 0,05

Specify values for any two of the following:

Replicates: 2,3

Values of the maximum difference between main effect means: 1,23

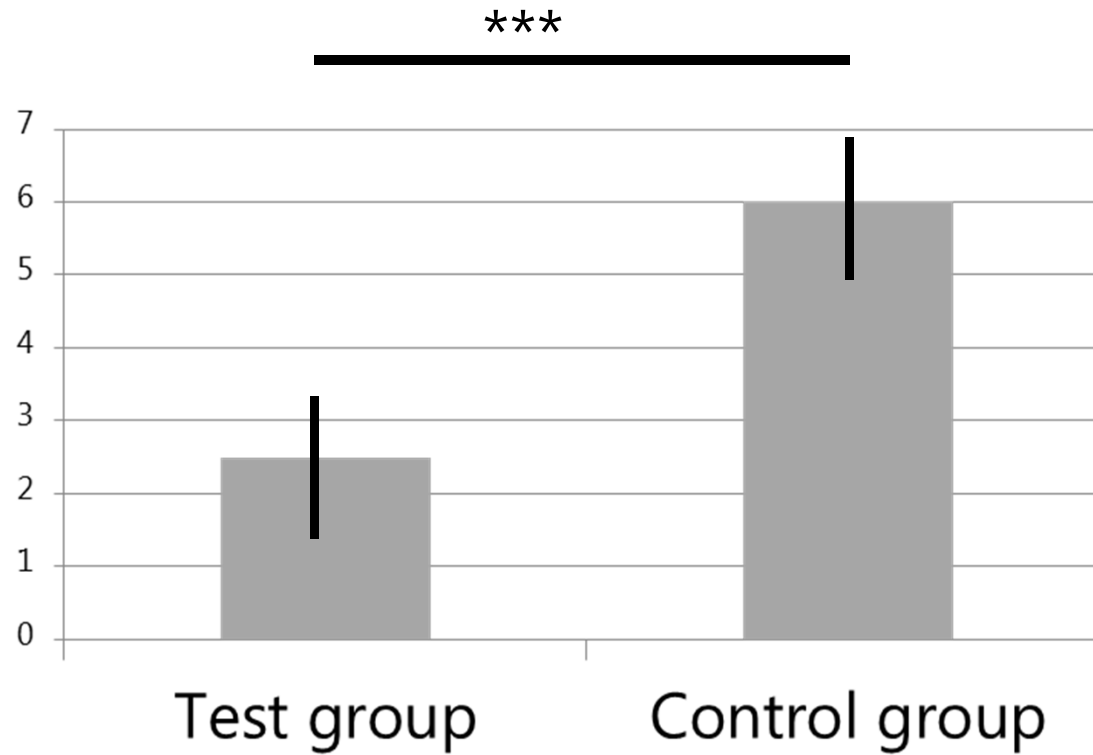
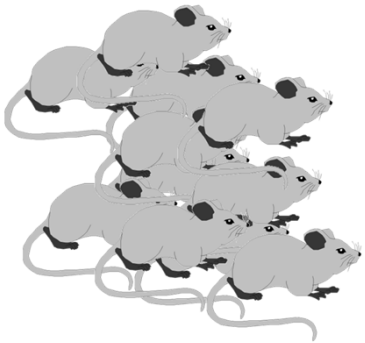
Power values: 0,9

Standard deviation: 1,1

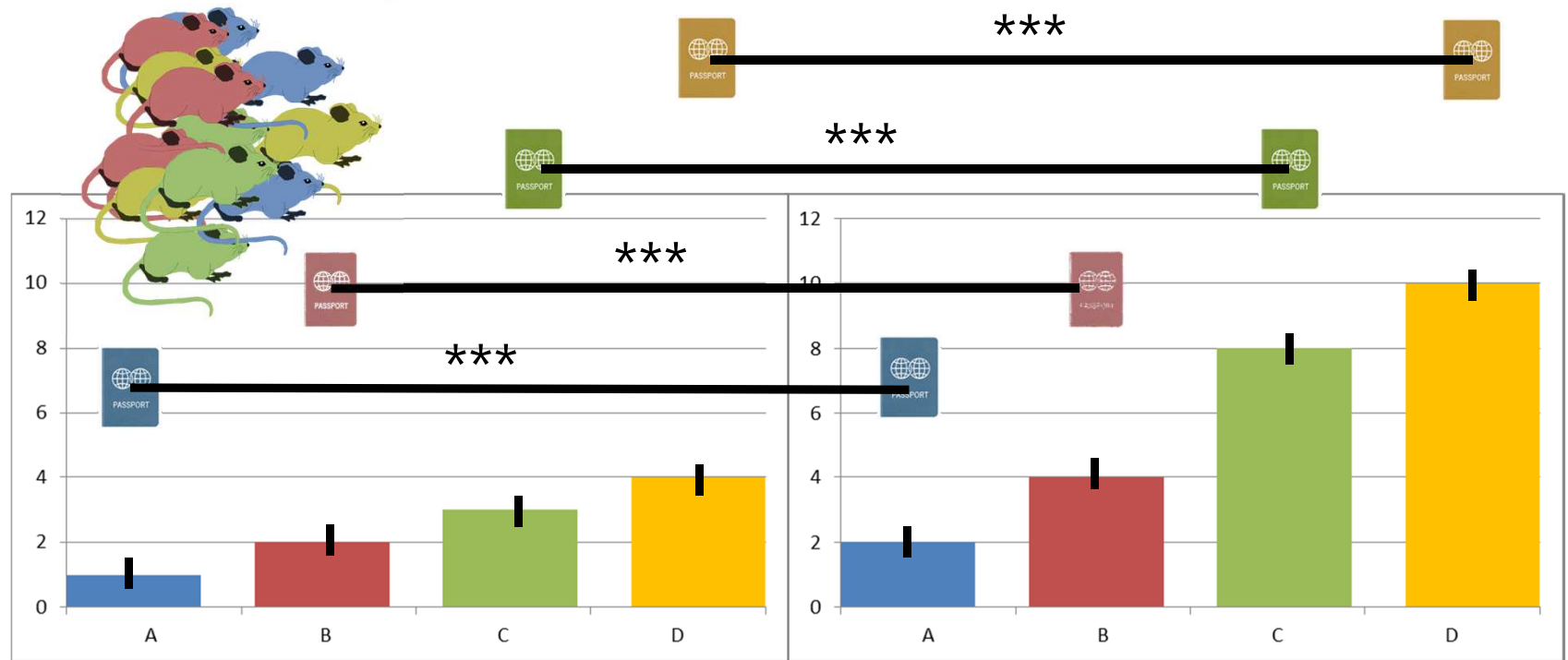
Design... Options... Graph... Help

rank	FBG OGT	Microbiota 1	Microbiota 2	D11S112	D12S206
1	5,4 D	*	cc	dd	
10	4,2 *	*	bd	bd	
30	4,6 A	C	bb	cd	
24	4,7 B	C	ab	cd	
43	6,2 C	D	cc	cc	
41	4,9 D	*	cd	cc	
45	5,1 A	A	bc	cd	
40	6,2 A	D	cd	cd	
23	4,2 C	B	bc	cc	
2	6,4 D	B	cd	cc	
20	4,3 D	B	cc	cd	
15	4,6 D	B	bc	cd	
27	5,2 D	*	bc	cc	
26	4,4 D	*	bb	cd	

Standard comparisons (One-way ANOVA)



Comparison with passport (Multi-factorial ANOVA)



Test group

Control group

Taconic mice

Janvier mice

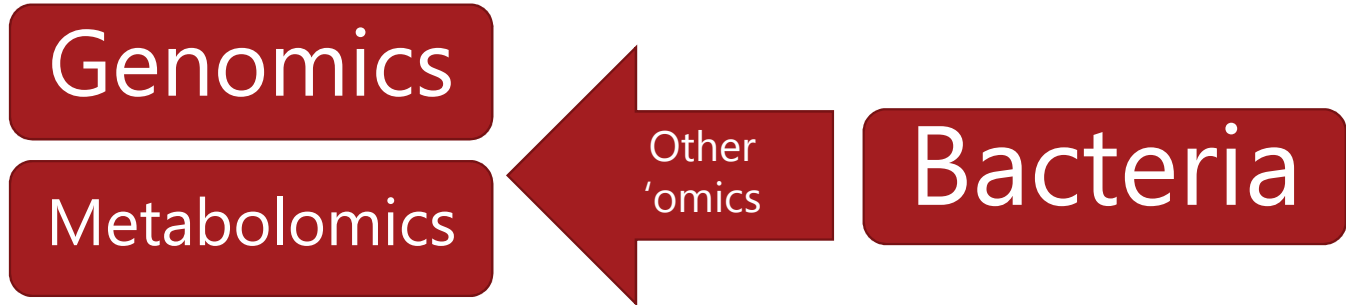
	ΔOGTT AUC%	Δ Body weight %	Primary readout ΔHbA1c%	Insulin pg/ml	Leptin pg/ml*	Fasting glucose mmol/l
Group size in study N	16	16	16	16	16	16
Liraglutide (Mean±s.d.)	-40.32±13.71	-11.94±4.12	-11.60±16.29	1235±780	7490±5304	5.81±0.93
Control ((Mean±s.d.)	-14.41±18.77	0.09±3.55	0.09±16.27	1568±967	23688±12103	7.09±1.25
One-way ANOVA						
Effect	-25.91	-12.03	-11.69	-335	-35737	-1.28
Pooled s.d.	16.44	3.84	16.28	878	9344	1.1
Significance for difference between treatments p	0.000	0.000	0.051	0.292	0.000#	0.003
Power	0.99	0.97	0.50	0.18	1.00#	0.97
Group size needed for p< 0.05 and power>0.9	10	4	42	146	8#	17
Two-way ANOVA						
<i>Microbiome time 1</i>						
Efficient group size	16	16	16	16	16	16
Weighted effect	-26.66	-12.28	-13.00	-457	-16472	-1.23
Pooled s.d.	16.69	3.92	16.19	803	9422	1.10
Significance for difference between treatments p	0.000	0.000	0.033	0.133	0.000#	0.001
Significance for difference between clusters p	0.747	0.307	0.164	0.040	0.230#	0.270
Power at N _{one-way} animals	1.00	1.00	0.80	0.50	1.00#	0.98
Group size needed for p< 0.05 and power>0.9	6	3	21	40	5#	11
<i>Microbiome time 2</i>						
Efficient group size	15	15	15	15	15	15
Weighted effect	-28.54	-12.48	-17.20	-407	-16610	-1.35
Pooled s.d.	17.23	3.71	16.95	863	10038	1.12
Significance for difference between treatments p	0.000	0.000	0.038	0.154	0.000#	0.013
Significance for difference between clusters p	0.361	0.905	0.376	0.217	0.926#	0.614
Power	0.99	1.00	0.96	0.34	1.00#	0.98
Group size needed for p< 0.05 and power>0.9	6	2	12	58	4#	10

	ΔOGTT AUC%	Δ Body weight %	Primary readout ΔHbA1c%	Insulin pg/ml	Leptin pg/ml*	Fasting blood glucose mmol/l
Group size in study N _{one-way}	23	23	23	23	23	23
Liraglutide (Mean±s.d.)	-1.47±22.25	-10.52±4.05	-2.27±18.56	1769±2247	12865±11078	4.82±0.70
Control (Mean±s.d.)	-16.51±19.90	3.48±5.21	-2.93±12.02	3731±3265	43131±24610	6.00±1.35
One-way ANOVA						
Effect	-15.05	-14.00	-1.34	-1962	-30266	-1.18
Pooled s.d.	21.11	4.67	15.64	2803	19083	1.08
Significance for difference between treatments p	0.020	0.000	0.772	0.022	0.000#	0.001
Power	0.66	1.00	0.06	0.64	1.00#	0.95
Group size needed for p< 0.05 and power>0.9	43	4	2864	44	7*	19
Two-way ANOVA						
<i>Microbiome time 1</i>						
Efficient group size	15	15	15	15	15	15
Weighted effect	-17.85	-13.38	-1.99	-1762.99	-24451.45	-1.36
Pooled s.d.	20.11	4.80	16.11	3241.32	18567.93	1.26
Significance for difference between treatments p	0.034	0.000	0.681	0.194	0.000#	0.007
Significance for difference between clusters p	0.968	0.415	0.435	0.547	0.143#	0.881
Power	0.87	1.00	0.07	0.44	1.00#	0.97
Group size needed for p< 0.05 and power>0.9	17	3	830	44	6#	12
<i>Microbiome time 2</i>						
Efficient group size	12	12	12	12	12	12
Weighted effect	-27.30	-14.67	-0.97	-2248.36	-31657.00	-1.18
Pooled s.d.	17.46	4.84	13.68	3481.48	19561.78	1.37
Significance for difference between treatments p	0.004	0.000	0.805#	0.169	0.000#	0.042
Significance for difference between clusters p	0.970	0.337	0.155#	0.697	0.274#	0.948
Power	0.99	1.00	0.14#	0.49	1.00#	0.68
Group size needed for p< 0.05 and power>0.9	7	2	126#	30	6#	20

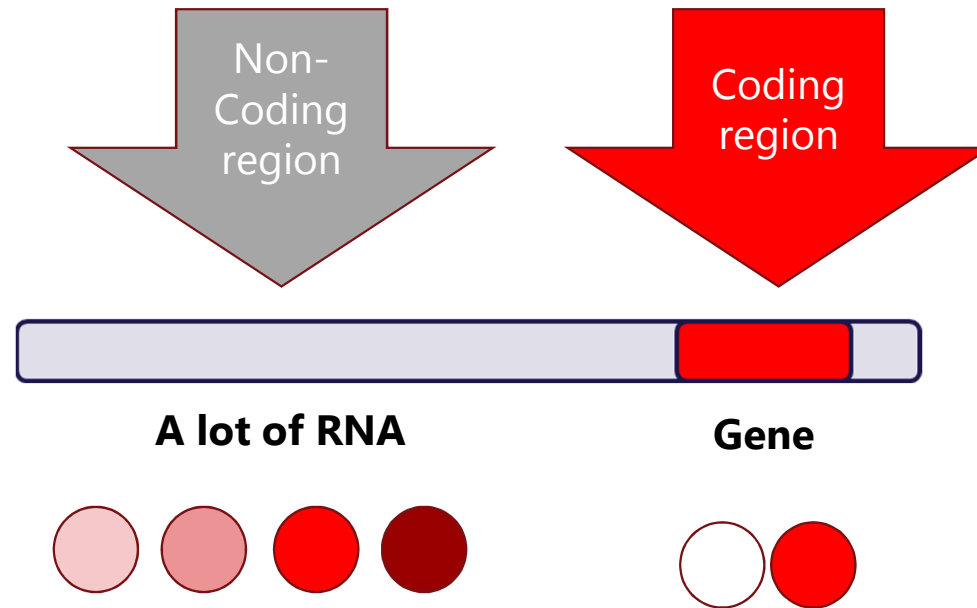
Tested on ranked data due failure in normality and/or equal variances

Group size	31-40	21-30	10-20	<10
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Perspectives



The genome (The simple version)



Standard tandem repeats STPs



- **STR** Short tandem repeats
 - Multiple repeats of a double nucleotide
 - Often in non-coding regions
 - May show inter-individual variation between inbred mice of the same strain



Taconic



● ●
Low genomic variability

Janvier

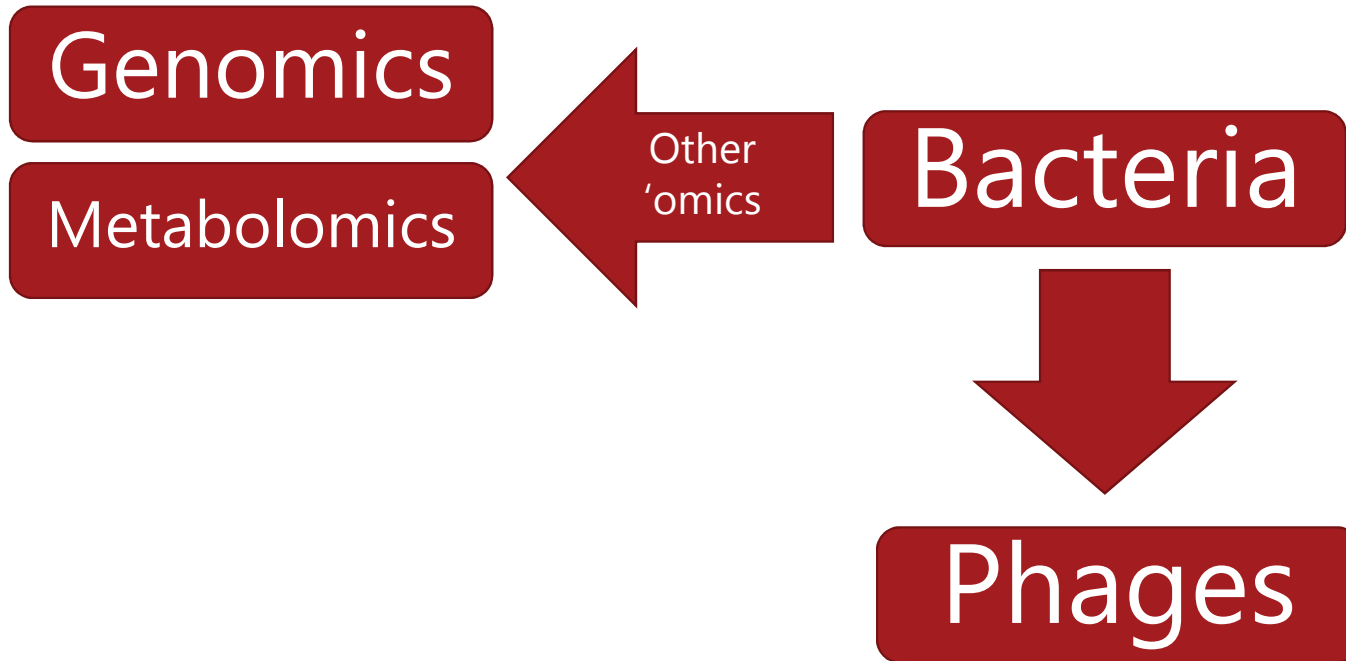


● ● ● ●
High genomic variability

Taconic Mice

	Δ OGTT AUC%	Δ Body weight %	Primary readout Δ HbA1c%*	Insulin pg/ml	Leptin pg/ml*	Fasting glucose mmol/l
Group size _y	16	16	16	16	16	16
Liraglutide (Mean \pm s.d.)	-40.32 \pm 13.71	-11.94 \pm 4.12	-11.60 \pm 16.29	1235 \pm 780	7490 \pm 5304	5.81 \pm 0.93
Control (Mean \pm s.d.)	-14.41 \pm 18.77	0.09 \pm 3.55	0.09 \pm 16.27	1568 \pm 967	23688 \pm 12103	7.09 \pm 1.25
One-way ANOVA						
Effect	-25.91	-12.03	-11.69	-335	-16198	-1.28
Pooled s.d.	16.44	3.84	16.28	878.44	6.84	1.1
Significance for difference between treatments p	0.000	0.000	0.051	0.292	0.000	0.003
Power μ	0.99	1.00	0.50	0.18	1.00	0.89
Group size needed for p < 0.05 and power > 0.9	10	4	42	146	8	17
Two-way ANOVA						
			<i>Genome (STRs with most even gene frequencies)</i>			
			D9S218			
Weighted effect	-22.78	-11.72	-5.83	-433	-14507	-1.27
Pooled s.d.	17.24	3.68	14.58	823.69	9599.22	1.08
Significance for difference between treatments p	0.001	0.000	0.254	0.129	0.000	0.002
Significance for difference alleles p	0.877	0.232	0.013	0.044	0.482	0.267
Power	0.99	1.00	0.27	0.44	0.99	0.99
Group size needed for p < 0.05 and power > 0.9	8	2	80	47	6	10

Perspectives



bioRxiv preprint first posted online Mar. 25, 2019; doi: <http://dx.doi.org/10.1101/588160>. The copyright holder for this preprint (which was not peer-reviewed) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity.

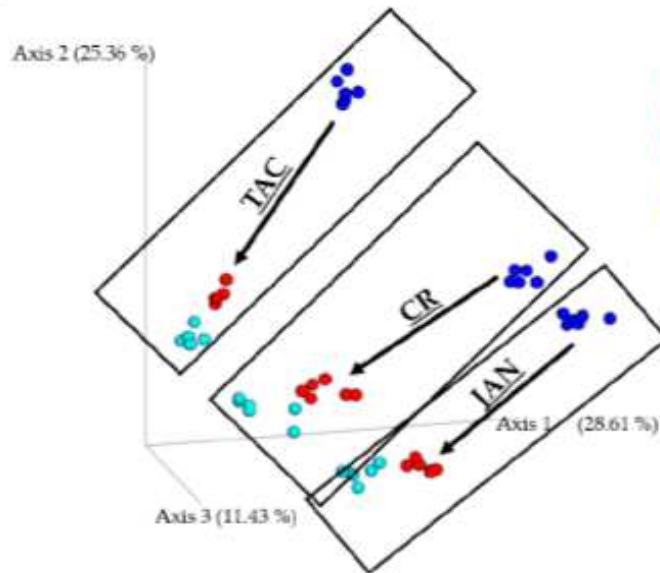
1 Article

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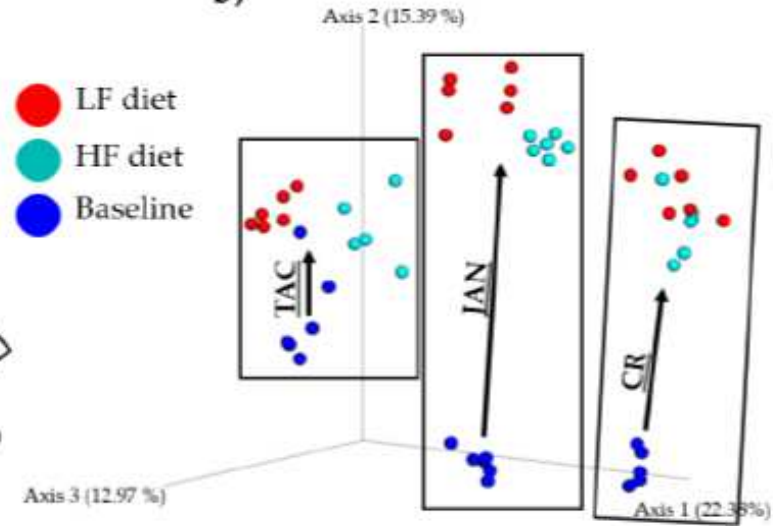


2 **Mouse vendor influence on the bacterial and viral gut**
 3 **composition exceeds the effect of diet**

4 a)



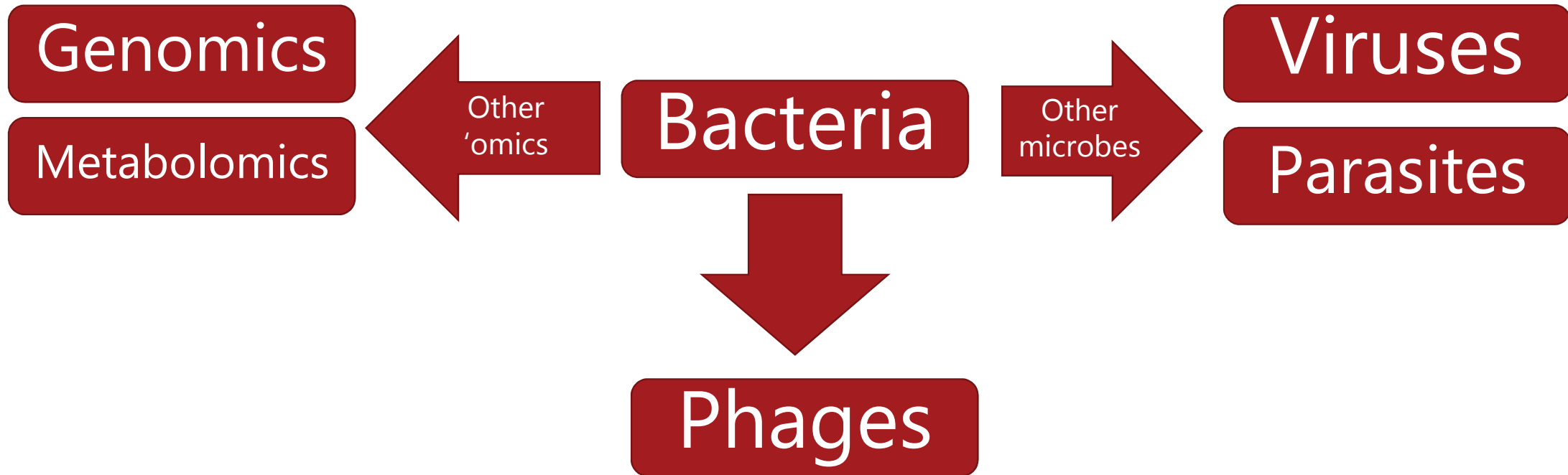
b)



c)

Effect	R-value	p-value	Sample size	Sample type	Community
Vendor - Endpoint	0.988	0.001	35	Cecum	Bacterial
Vendor - Baseline	1.000	0.001	18	Cecum	Bacterial
Diet	0.207	0.006	35	Cecum	Bacterial
Vendor - Endpoint	0.989	0.001	35	Cecum	Viral
Vendor - Baseline	1.000	0.001	18	Cecum	Viral
Diet	0.104	0.028	35	Cecum	Viral

Perspectives





Contents lists available at ScienceDirect

Research in Veterinary Science

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T.G. Rasmussen et al.

Inter-vendor variance of enteric eukaryotic DNA viruses in specific pathogen free C57BL/6N mice

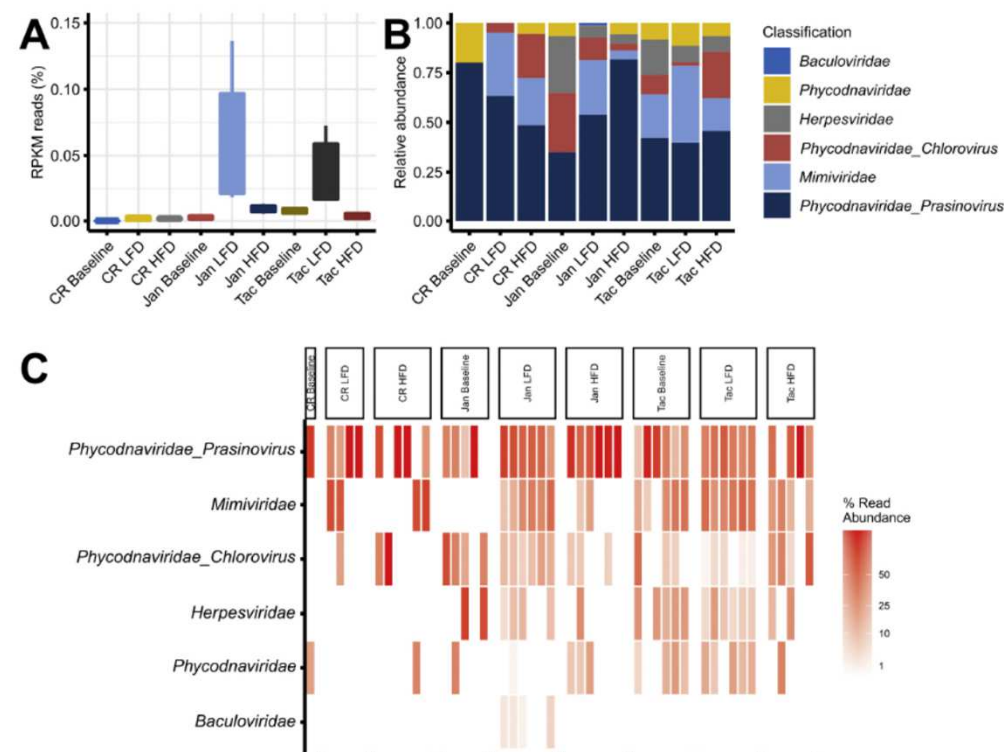
Torben Sølbeck Rasmussen^a, Rasmus Riemer Jakobsen^a, Josué L. Castro-Mejía^a, Witold Kot^b, Allan Randrup Thomsen^c, Finn Kvist Vogensen^a, Dennis Sandris Nielsen^a, Axel Kornerup Hansen^{d,*}

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^c Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

^d Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark



Take home messages

- Variation can be described
 - included in group size/power calculations
 - by characterization of the microbiota
- This will for some - but not all - models
 - decrease group sizes
 - or increase power
- More knowledge on the microbiota background from each study
 - is likely to increase reproducibility in the long run
- This may also be extended to other types of 'omics

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Randi Lundberg, Dept. Vet. & Anim. Sci., Univ. Copenhagen & Taconic

Line Sidsel Zachariassen, Dept. Vet. & Anim. Sci., Univ. Copenhagen

Peter Dobrowolski, GVG Genetic Monitoring



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