Optimization of fecal sample processing benefits metagenomic studies of human gut microbiota

Joint Graduate Student Seminar

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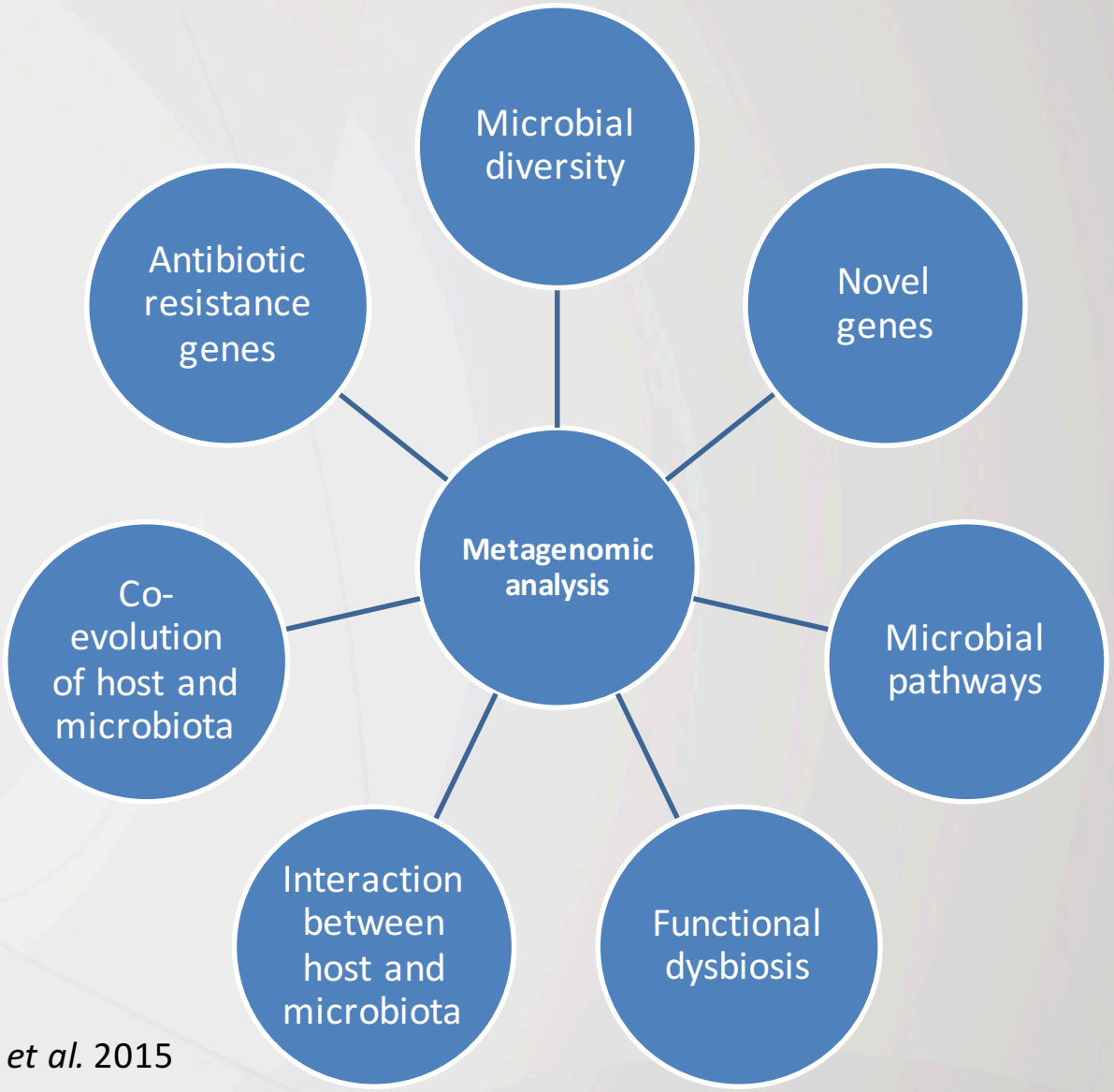
Today's content

- Background
- Study Design & Methods
- Results
- Conclusion

BACKGROUND

Why study the gut microbiome?

- ‘Dysbiosis of it is closely associated with some human diseases (e.g. diabetes & inflammatory bowel disease)
- Core microbial community in host could facilitate the immune networks to combat against many pathogenic species (e.g. *Citrobacter rodentium & Shigella flexneri*)
- Advancement of next-generation sequencing (NGS) technologies



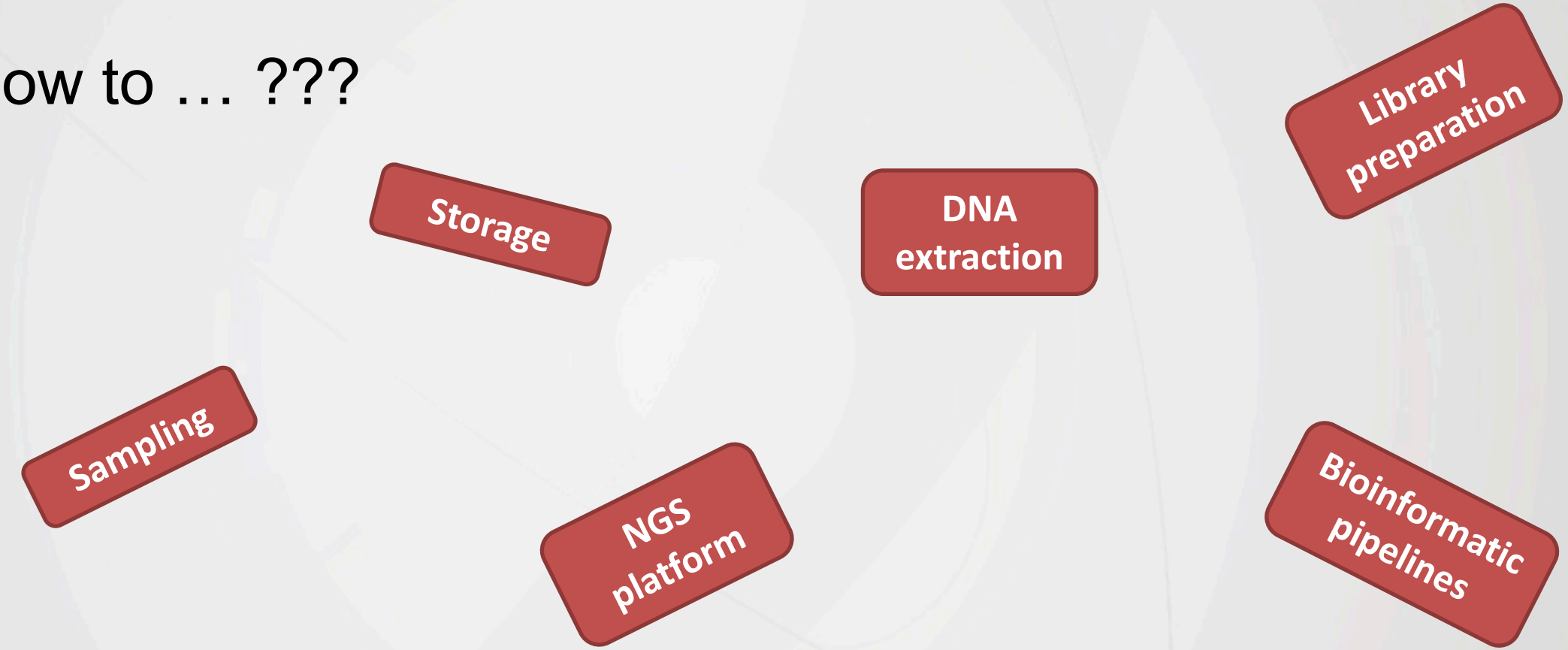
Wang *et al.* 2015

Why doing metagenomics?

- Application of metagenomics in the human gut microbiome
  - Diversity
  - Functional implications (e.g. genes and pathways of interest)
- New insights for disease examination and subsequent treatment

Too diverse !

How to … ???



nature biotechnology

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Towards standards for human fecal sample processing in metagenomic studies

Paul I Costea[1], Georg Zeller[1], Shinichi Sunagawa[1,2], Eric Pelletier[3–5], Adriana Alberti[3], Florence Levenez[6], Melanie Tramontano[1], Marja Driessen[1], Rajna Hercog[1], Ferris-Elias Jung[1], Jens Roat Kultima[1], Matthew R Hayward[1], Luis Pedro Coelho[1], Emma Allen-Vercoe[7], Laurie Bertrand[3], Michael Blaut[8], Jillian R M Brown[9], Thomas Carton[10], Stéphanie Cools-Portier[11], Michelle Daigneault[6], Muriel Derrien[11], Anne Druesne[11], Willem M de Vos[12,13], B Brett Finlay[14], Harry J Flint[15], Francisco Guarner[16], Masahira Hattori[17,18], Hans Heilig[12], Ruth Ann Luna[19], Johan van Hylckama Vlieg[11], Jana Junick[8], Ingeborg Klymiuk[20], Philippe Langella[6], Emmanuelle Le Chatelier[6], Volker Mai[21], Chaysavanh Manichanh[16], Jennifer C Martin[15], Clémentine Mery[10], Hidetoshi Morita[22], Paul W O'Toole[9], Céline Orvain[3], Kiran Raosaheb Patil[1], John Penders[23], Søren Persson[24], Nicolas Pons[6], Milena Popova[10], Anne Salonen[13], Delphine Saulnier[8], Karen P Scott[15], Bhagirath Singh[25], Kathleen Slezak[8], Patrick Veiga[11], James Versalovic[19], Liping Zhao[26], Erwin G Zoetendal[12], S Dusko Ehrlich[6,27], Joel Dore[6] & Peer Bork[1,28–30]

STUDY DESIGN AND METHODS

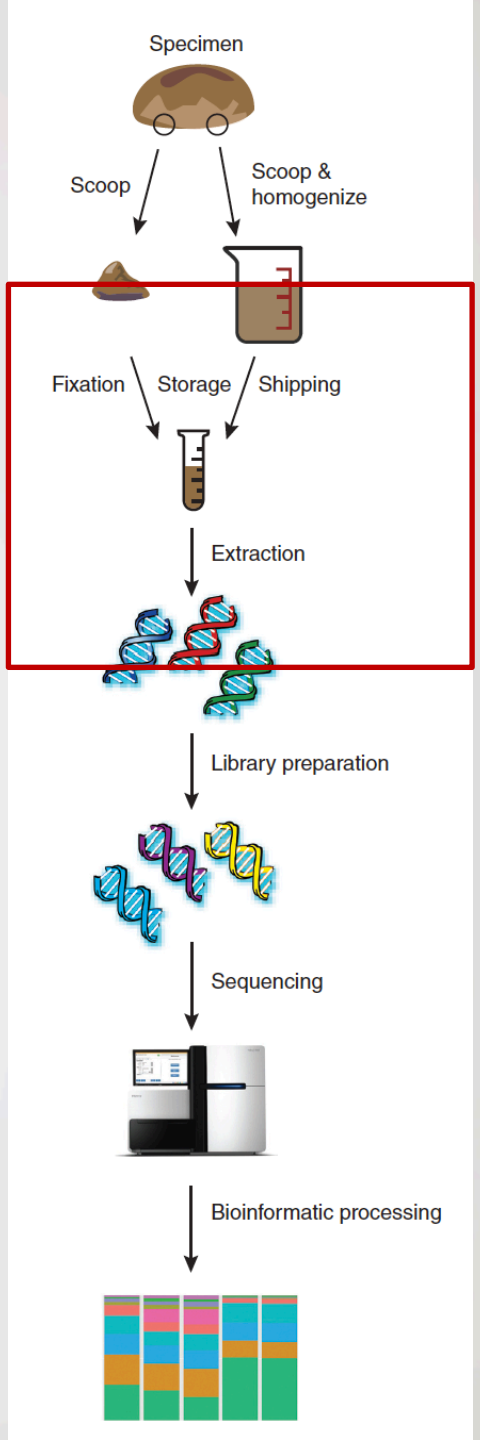
Human fecal samples processing

Three study phases:

I. To assess the variability introduced by different DNA extraction methods
   - Comparison of DNA extraction derived technical variation to other possible biological and technical effects

II. Comparative analysis of the ‘best-performing’ protocols

III. To quantify the extraction accuracy by a mock community with known bacterial species
   - Estimation of the recovery of relative species abundances in samples



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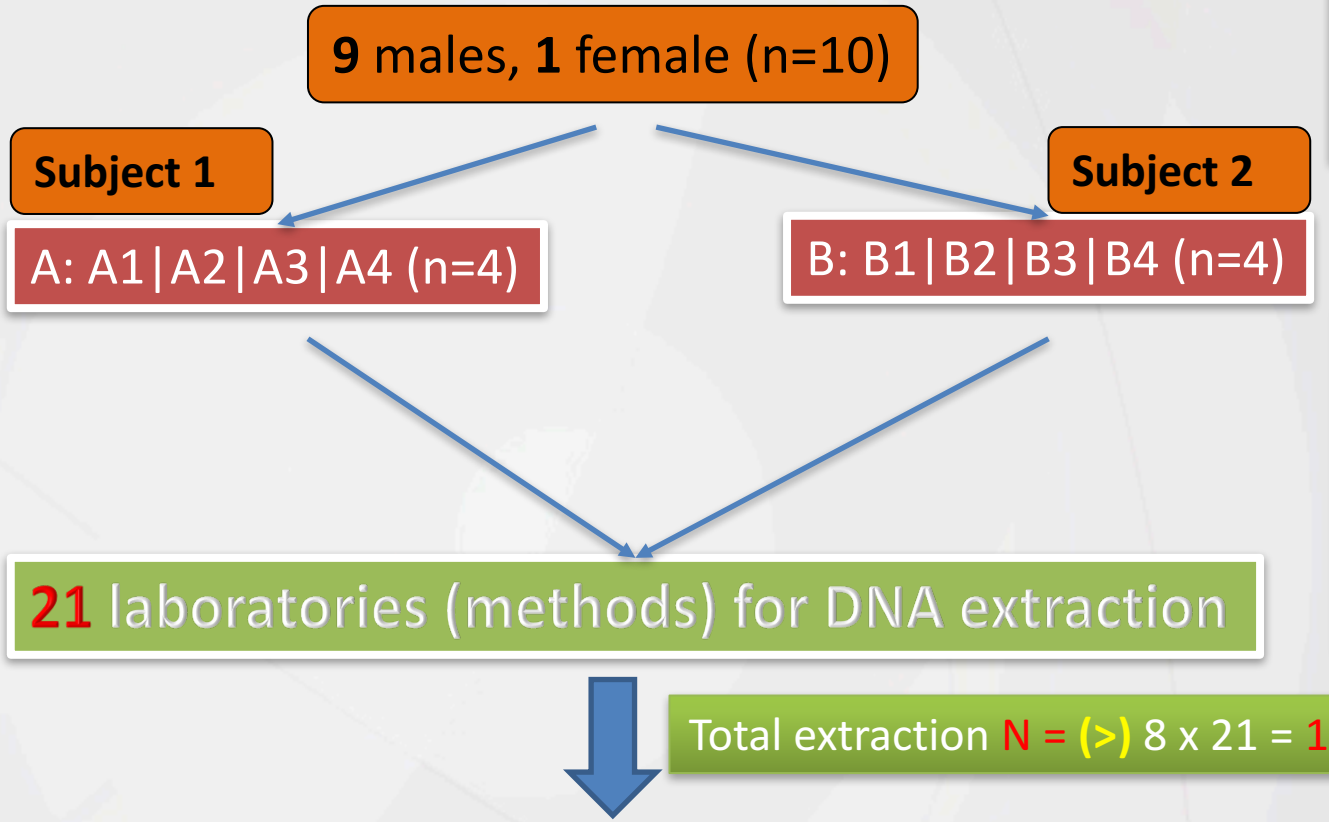
Costea *et al.* 2017

Outline of Phase 1



- Homologized with 2hrs
- Aliquots for 200mg each
- -80℃ within 4hrs

- Ship with dry ice
- 11 countries in 3 continents
- > 4 extraction of each sample

One single NGS facility for library preparation, NGS, and bioinformatics

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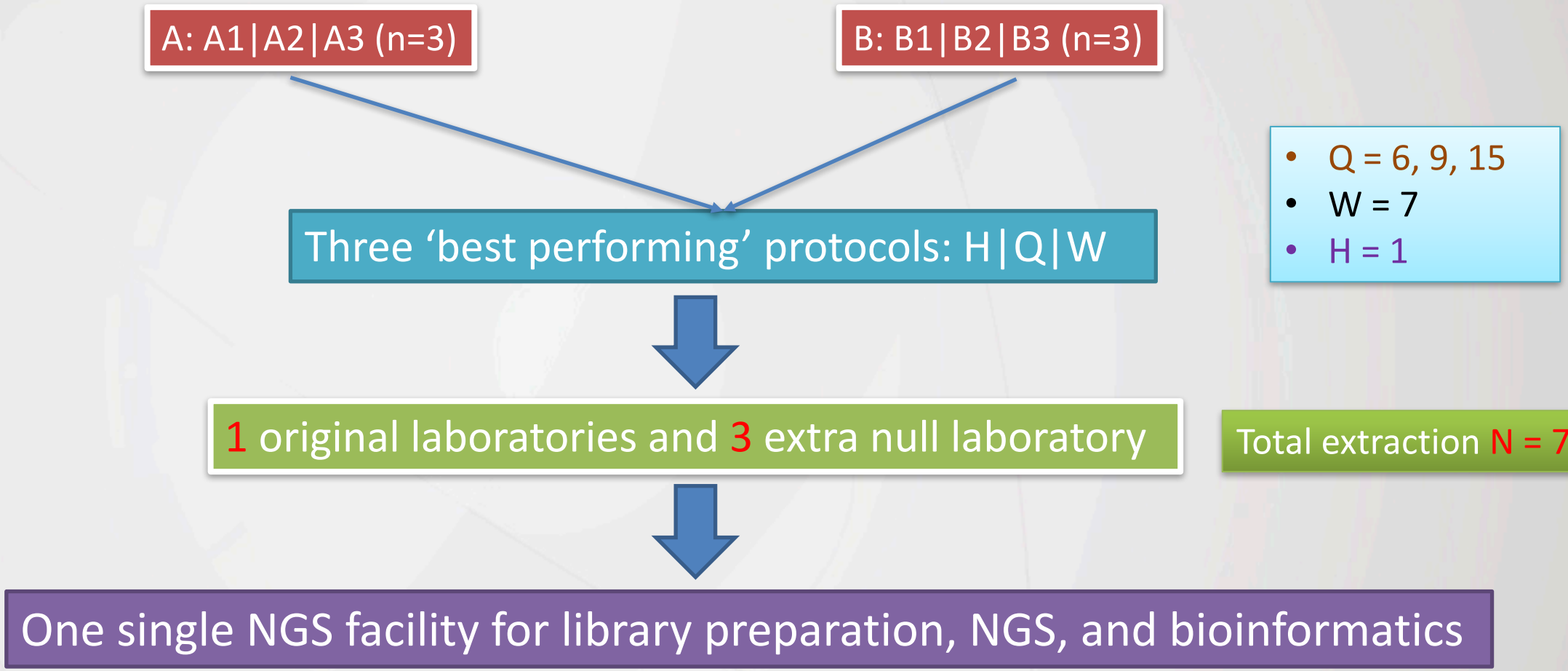
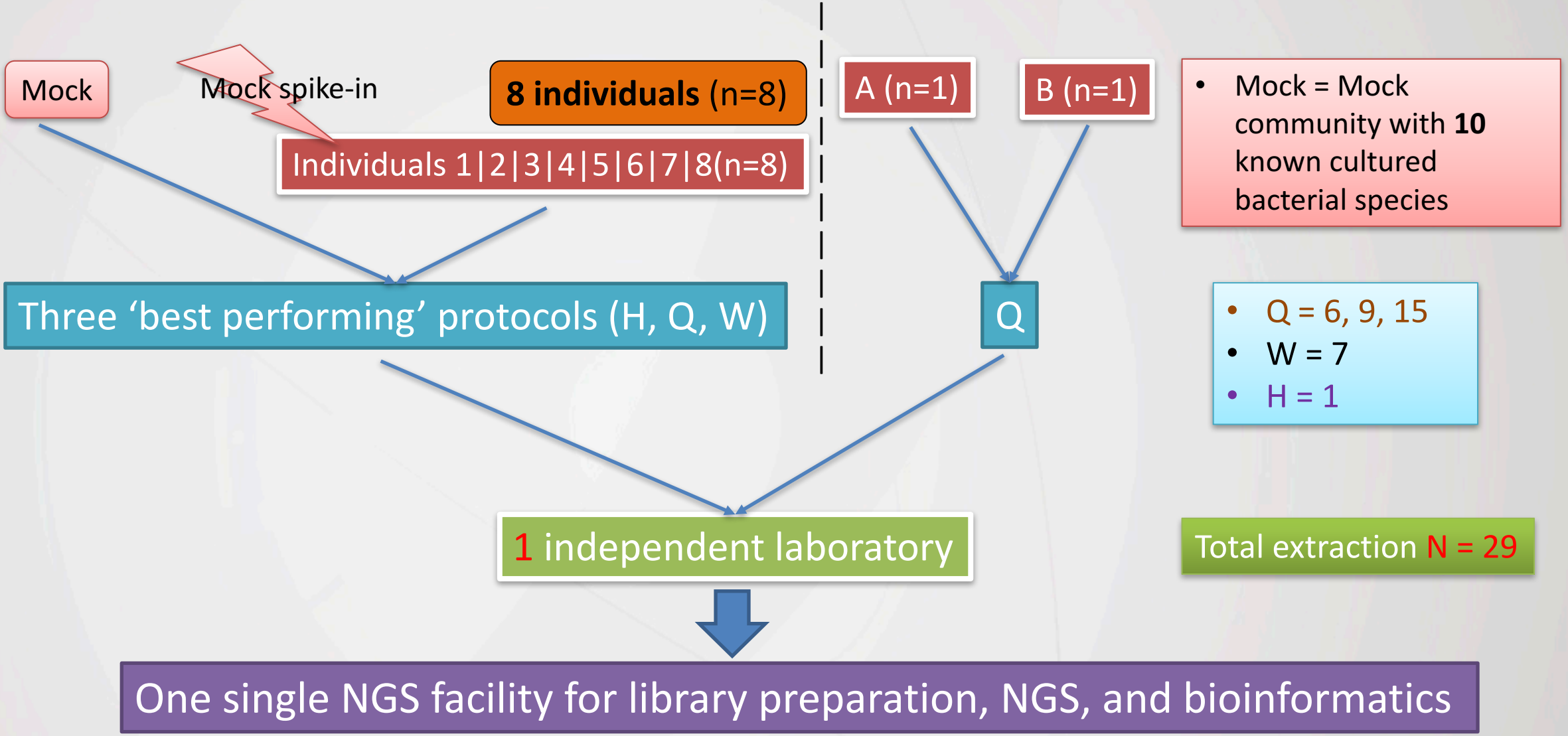
Outline of Phase 2



Outline of Phase 3



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RESULTS

Brief introduction of 21 methods

Invitek_PSPStool
Mobio_PowerSoil
Omega_Bio_Tek_EZNAstool
Promega_Maxwell
Qiagen_QiIAampStoolMinikit
Bio101_G'Nome
MP-Biomedicals_FastDNAspinSoil
Roche_MagNAPureIII
No-Kit_GodonMethod
No-Kit_OtherMethod

Use_of_crude_feaces
Treatment_before_lysis
Use_for_extraction
Chemical lysing agent_buffer
Mechanical_lysis
Shaking_aparatus
Protectant_versus_lysis
Protein_precipitant
DNA's_precipitation
DNA's_wash
DNA's_dry
Suspension_solution

Specific combinations of the use of protocol descriptors – 21 methods in total

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Method	**Use_of_Kit**			■	■	■	■		■	■		■		■	■	■	■	■		■	■	■
Method	Invitek_PSPStool																■					■
Method	Mobio_PowerSoil			■																		
Method	Omega_Bio_Tek_EZNAstool				■																	
Method	Promega_Maxwell				■																	
Method	Qiagen_QilAampStoolMinikit					■	■		■	■		■		■		■					■	
Method	Bio101_G'Nome																	■				
Method	MP-Biomedicals_FastDNAspinSoil														■							
Method	Roche_MagNAPureIII																			■		
Method	No-Kit_GodonMethod	■	■																			
Method	No-Kit_OtherMethod							■			■		■						■			
Treatment_before_lysis	pretreatment_before_lysis			■				■						■					■	■		
Chemical lysing agent_buffer	SDS			■		■	■	■	■	■	■	■			■	■			■			
Lysis_Incubation	shaking					■	■			■						■	■	■		■	■	■
Lysis_Incubation	mechanical_lysis	■	■		■	■	■	■	■	■	■		■	■	■	■	■	■		■	■	■
Lysis_Incubation	glass_beads_0,1mm	■	■					■										■			■	
Lysis_Incubation	glass_beads_0,5mm																				■	
Lysis_Incubation	glass_beads_>1mm					■				■												■
Lysis_Incubation	zirconia_beads_0,1mm				■	■	■		■	■	■			■		■						
Lysis_Incubation	zirconia_beads_0,5mm						■															■
Lysis_Incubation	silica_beads_0,1mm									■					■	■						
Shaking_aparatus	MM200_MM400	■							■													
Shaking_aparatus	Bead_Beater		■		■		■				■			■				■				
Shaking_aparatus	Vortex			■										■			■					
Shaking_aparatus	Bath_dry_waving																		■			
Shaking_aparatus	break_during_shking		■		■	■				■											■	■
Shaking_aparatus	Guanidine_thiocyanate	■	■	■		■	■		■	■		■	■		■	■	■			■	■	■
Shaking_aparatus	InhibitEX_Tablet								■			■		■		■	■				■	
Protectant_versus_lysis	Tris_EDTA_NaCl_SDS					■	■															

- Q = 6, 9, 15
- W = 7
- H = 1

■ Yes
□ No

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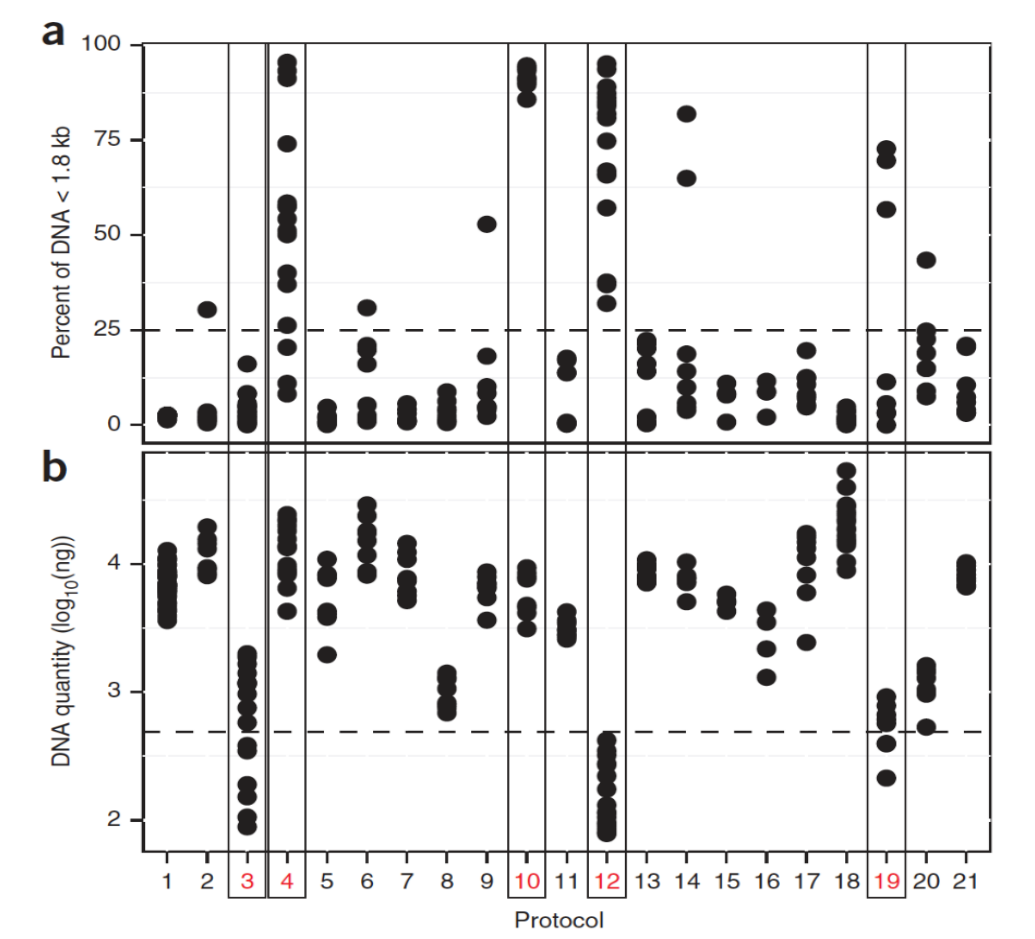
DNA extraction and Fragmentation



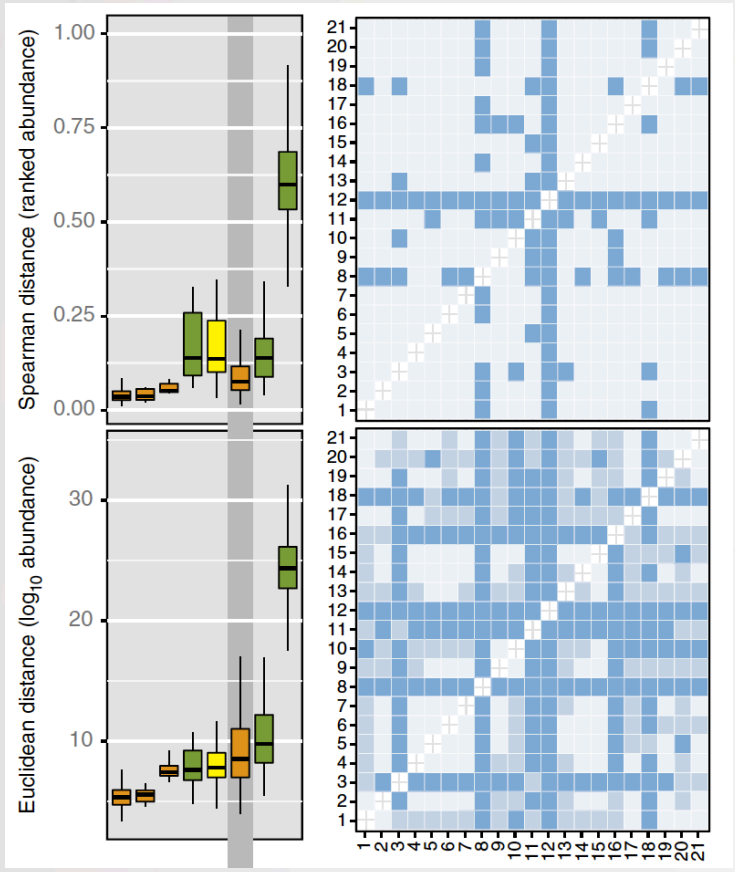
❖Minimizing small fragmentation

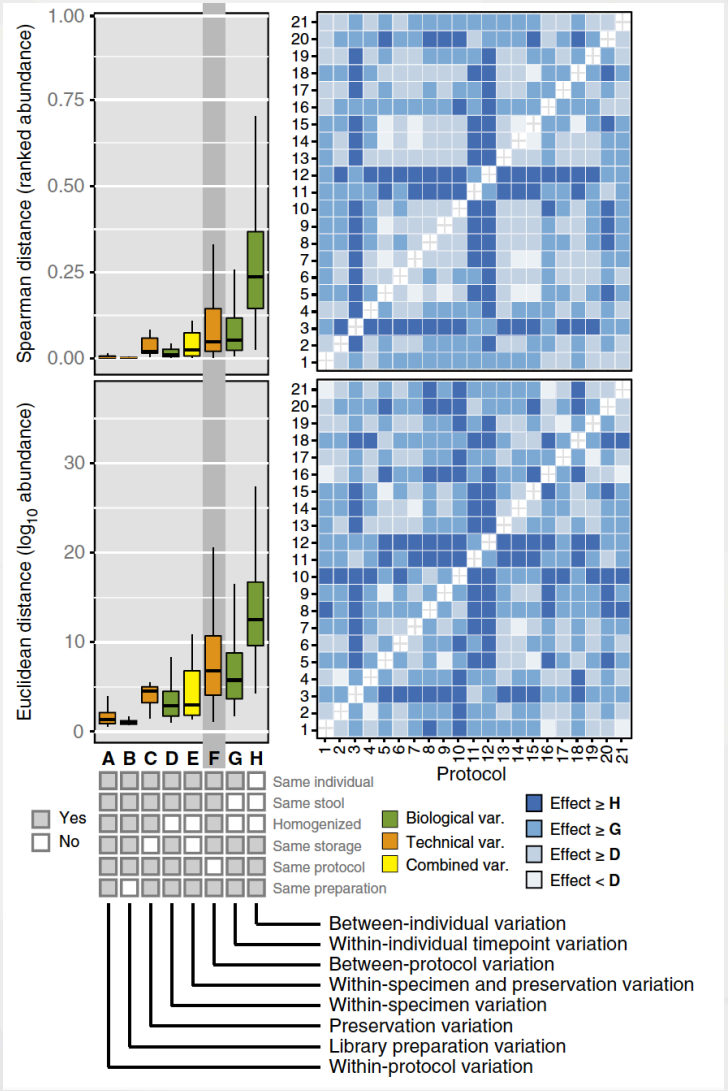
- While using protocols 4, 10,12,19 lead to high yield of fragmented DNA, protocol **1** produces nearly no observable fragmentation

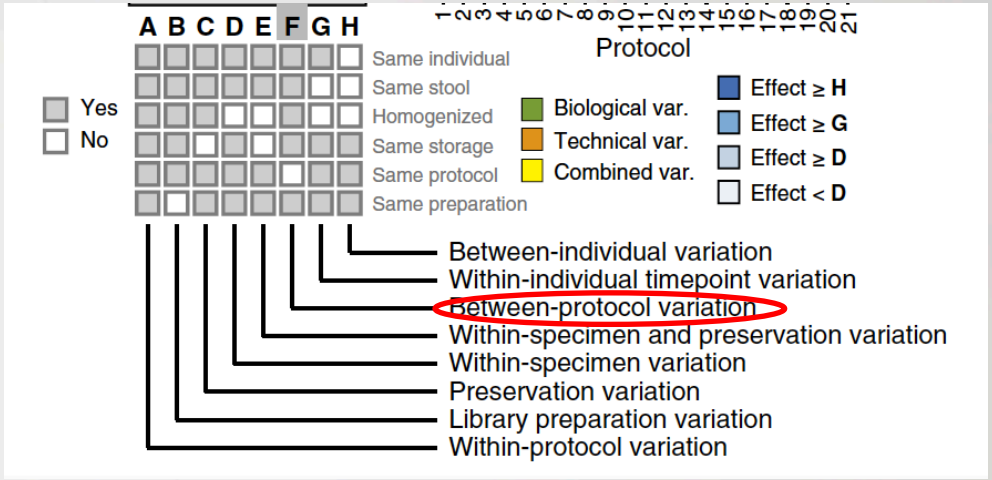
❖Maximizing DNA quantity

- Protocol **18** reproduced 100 times more DNA than protocols 3 and 12, respectively

Variability in microbial composition







- Library preparation and within-protocol variation have the smallest effects
- Between-protocol variation may be greater than some biological effects

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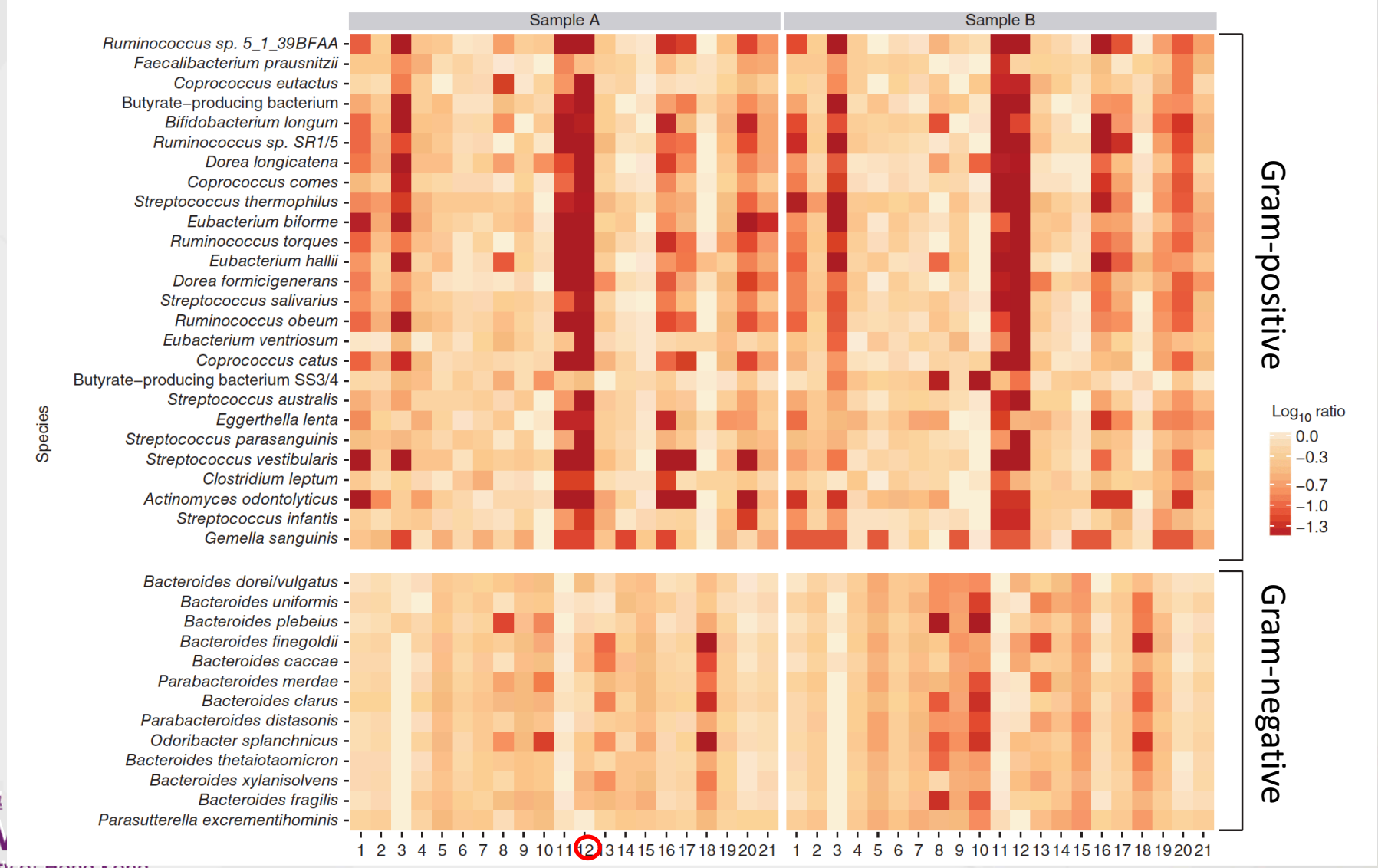
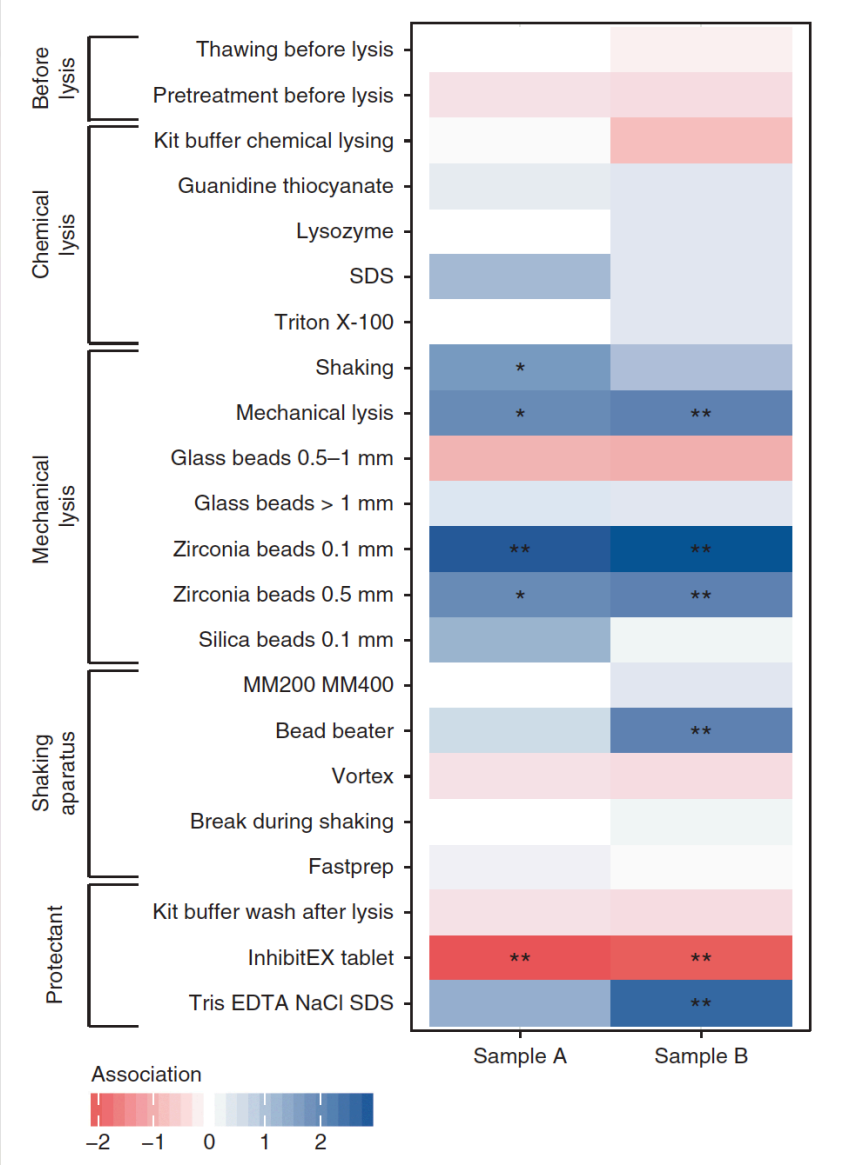
Species-specific abundance variation



Effects of protocol manipulations on sample composition



- Among ***22*** protocol descriptors that vary between the **Qiagen-based** methods, ***7*** were significantly associated with diversity outcomes

  ❖ **Qiagen-based** kits, # 5, 6, 8, 9, 11, 13, 15 and 20

- **Mechanical lysis**, **zirconia beads** and **shaking** were positively associated with diversity

- The only significant negative association was with the **InhibitEX tablet**

Associations are
coded as negative (red) or positive (blue)

Potential methods…

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Method	**Use_of_Kit**			■	■	■	■		■	■		■		■	■	■	■	■		■	■	■
Method	Invitek_PSPStool																■					■
Method	Mobio_PowerSoil			■																		
Method	Omega_Bio_Tek_EZNAstool				■																	
Method	Promega_Maxwell				■																	
Method	Qiagen_QilAampStoolMinikit					■	■		■	■		■		■		■					■	
Method	Bio101_G'Nome																	■				
Method	MP-Biomedicals_FastDNAspinSoil														■							
Method	Roche_MagNAPureIII																			■		
Method	No-Kit_GodonMethod	■	■																			
Method	No-Kit_OtherMethod							■			■		■						■			
Treatment_before_lysis	pretreatment_before_lysis			■				■						■					■	■		
Chemical lysing agent_buffer	SDS			■		■	■	■	■	■	■	■			■	■			■			
Lysis_Incubation	shaking			■		■	■			■						■	■	■		■	■	■
Lysis_Incubation	mechanical_lysis	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■
Lysis_Incubation	glass_beads_0,1mm	■	■	■				■										■			■	
Lysis_Incubation	glass_beads_0,5mm			■																	■	
Lysis_Incubation	glass_beads_>1mm			■		■				■												■
Lysis_Incubation	zirconia_beads_0,1mm			■	■	■	■		■	■	■			■		■						
Lysis_Incubation	zirconia_beads_0,5mm			■			■															■
Lysis_Incubation	silica_beads_0,1mm			■						■					■	■						
Shaking_aparatus	MM200_MM400	■							■													
Shaking_aparatus	Bead_Beater		■		■		■				■			■				■				
Shaking_aparatus	Vortex			■										■			■					
Shaking_aparatus	Bath_dry_waving																		■			
Shaking_aparatus	break_during_shking		■		■	■				■											■	■
Shaking_aparatus	Guanidine_thiocyanate	■	■	■		■	■		■	■		■	■		■	■	■			■	■	■
Shaking_aparatus	InhibitEX_Tablet								■			■		■		■	■				■	
Protectant_versus_lysis	Tris_EDTA_NaCl_SDS					■	■															

- Q = 6, 9, 15
- W = 7
- H = 1

■ Yes
□ No

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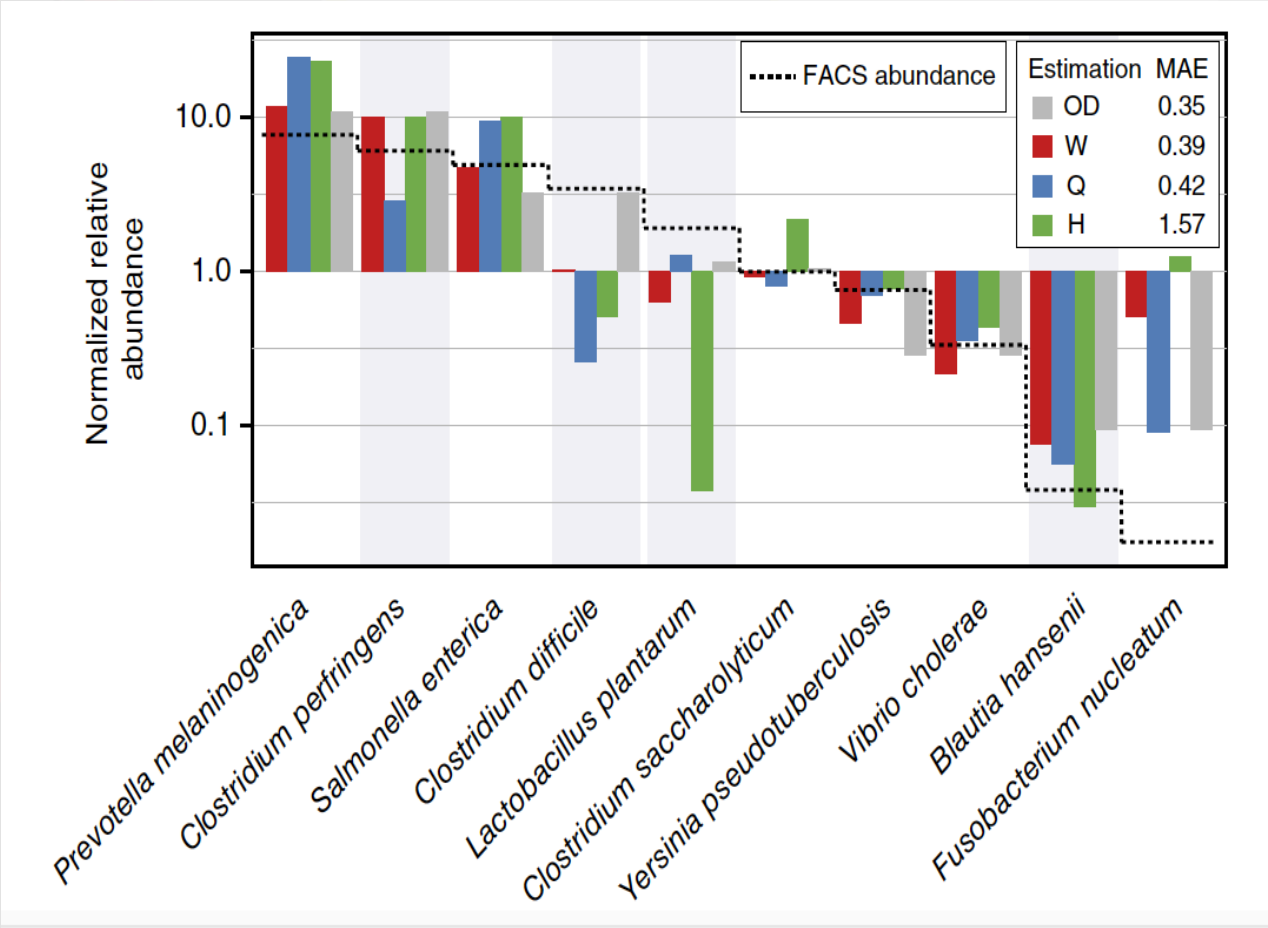
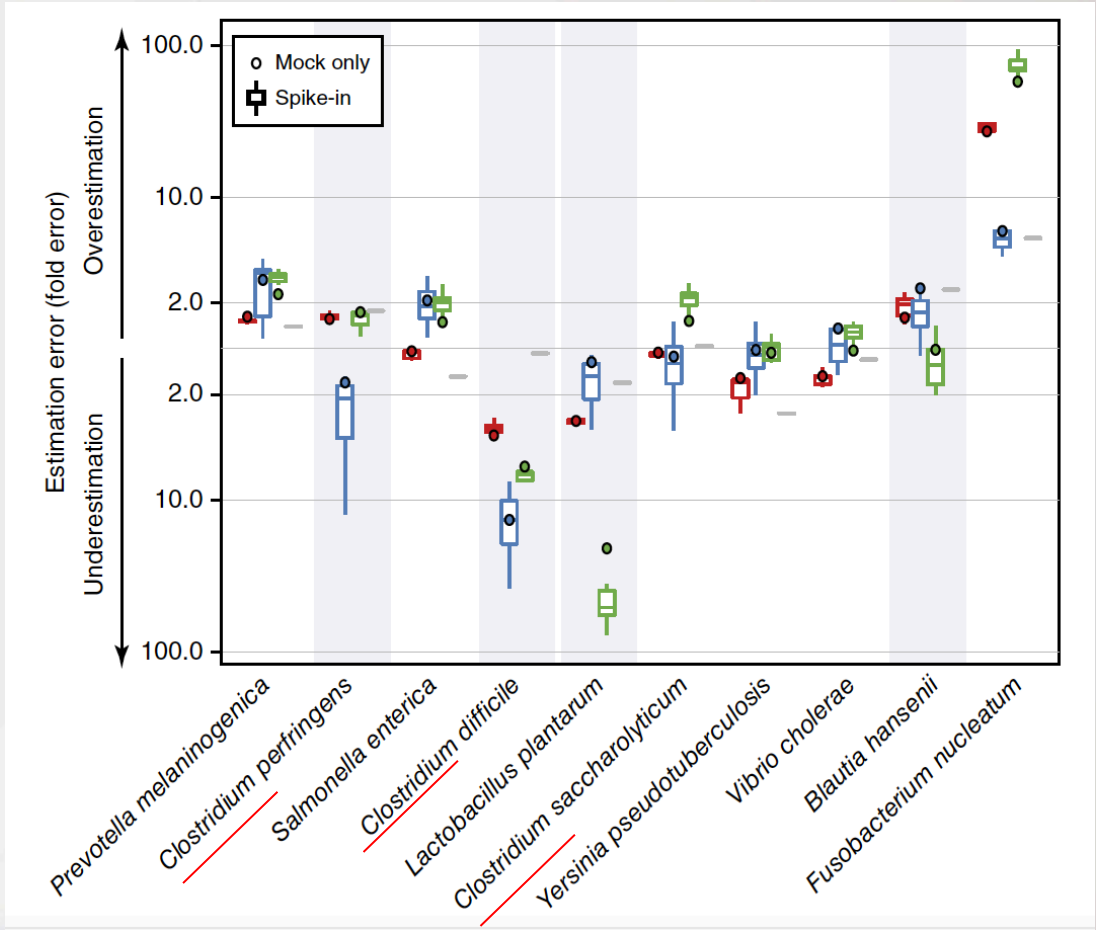
Mock community extraction quality





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Less is better

Conclusion

- A Bead-beating step significantly influences the composition
- *Combined* protocol **Q (6,9,15)** seemed to be the best overall and is predicted to suit most applications
  - With a median absolute quantification error of **≤ 0.5x**
  - Potential benchmark for new DNA extraction methods
- Protocol #3 (Mobio PowerSoil) was expected to improve its performance by introducing a bead beating step.
- Remarks: Potential impact of kit contamination on samples with low biomass

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THANK YOU!

Q&A

Bibliography

- Wang W-L, Xu S-Y, Ren Z-G, Tao L, Jiang J-W, Zheng S-S. Application of metagenomics in the human gut microbiome. *World J Gastroenterol.* (2015) **21:**803–14
- Costea, P. I., *et al.* Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol.* (2017) **35**(11)**:** 1069-1076