

Experience from the Geneva University Hospitals (HUG) laboratory

Vladimir Lazarevic

Genomic Research Laboratory, HUG–CMU, Geneva, Switzerland



**Fourth**  
International  
Conference on  
**Clinical**  
**Metagenomics**

4th International Conference on Clinical Metagenomics  
Geneva | 17–18 October 2019



Article

# Detection of Bacterial Pathogens from Broncho-Alveolar Lavage by Next-Generation Sequencing

Stefano Leo <sup>1,\*</sup>, Nadia Gaïa <sup>1</sup>, Etienne Ruppé <sup>1</sup>, Stephane Emonet <sup>2,3</sup>, Myriam Girard <sup>1</sup>, Vladimir Lazarevic <sup>1</sup> and Jacques Schrenzel <sup>1,2,3</sup>

case#1

38-year-old man

Allogenic human stem cell transplantation (HSCT) for lymphoblastic leukemia.

Severe graft-versus-host disease (GvHD)

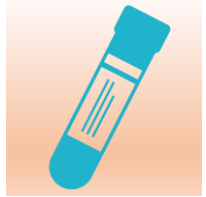
Major immunosuppressive treatment

Left lobar pneumonia



Bronchoalveolar lavage fluid

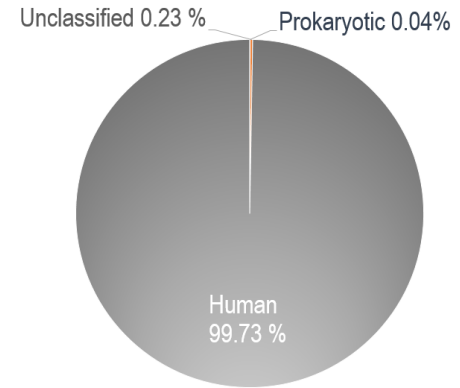
Bronchoalveolar lavage fluid (600  $\mu$ L)



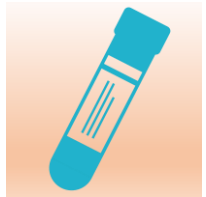
NucleoSpin Soil (MN)



NGS (HiSeq 2  $\times$  100)



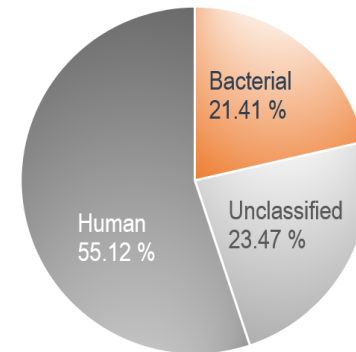
Bronchoalveolar lavage fluid (600  $\mu$ L)



Ultra-Deep Microbiome Prep (Molzym)  
*Bacterial DNA enrichment*

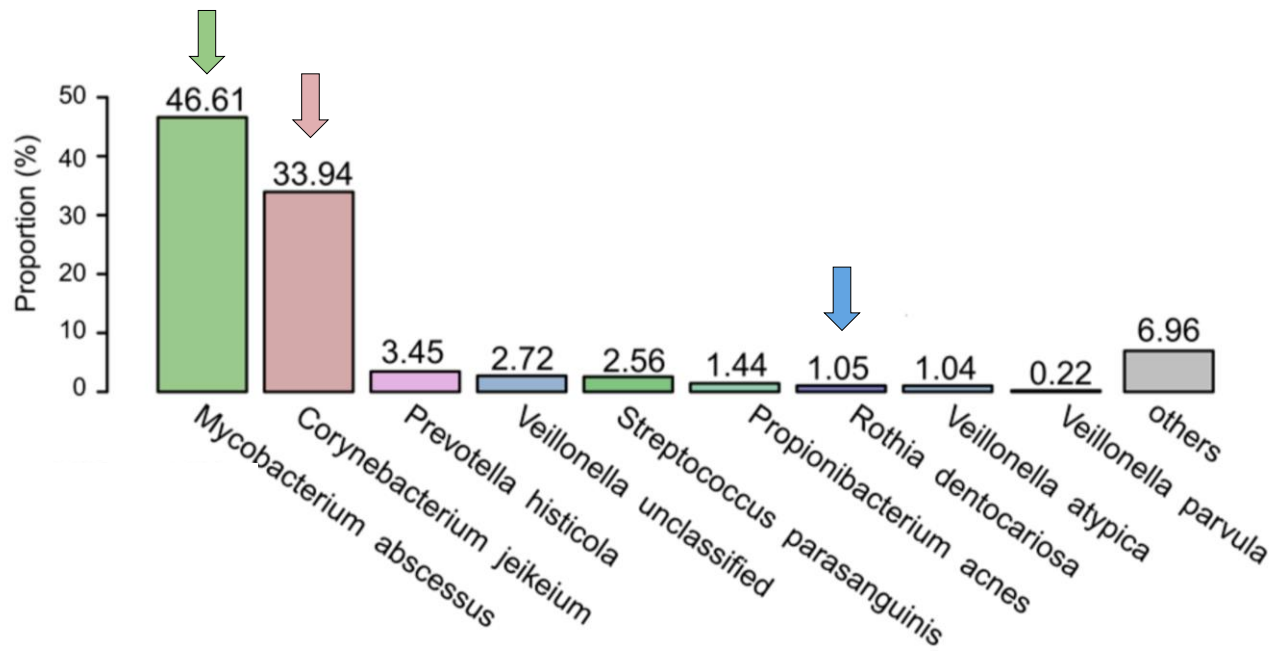


NGS (MiSeq 2  $\times$  100)



975 $\times$  increased bacterial-to-human signal

### NGS



### Routine clinical tests

Bacteria	Detection
<i>Corynebacterium jeikeium</i>	$>1.0 \times 10^5$ CFU/mL
<i>Rothia dentocariosa</i>	$1.0 \times 10^2$ CFU/mL
<i>Mycobacterium abscessus</i>	Present

## Rare Case of Community-Acquired Endocarditis Caused by *Neisseria meningitidis* Assessed by Clinical Metagenomics

Vassili Choutko<sup>1†</sup>, Vladimir Lazarevic<sup>2†</sup>, Nadia Gaia<sup>2</sup>, Myriam Girard<sup>2</sup>, Gesuele Renzi<sup>3,4</sup>, Stefano Leo<sup>2</sup>, Peter M. Keller<sup>5</sup>, Christoph Huber<sup>6</sup> and Jacques Schrenzel<sup>2,3,4\*</sup>

case#2

A 54-year old woman

Initially diagnosed (by GP) community-onset pneumonia

Dyspnoea, cough and fever, 39.2°C

Th: Co-amoxicillin and clarithromycin

TEE and TTE: Signs suggestive of **infective endocarditis**

Semi-urgent aortic valve replacement (day 2)

Negative blood and valve cultures



Fresh valve tissue





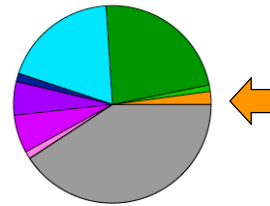
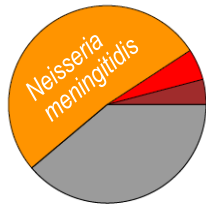
Valve



NC

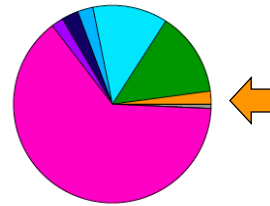
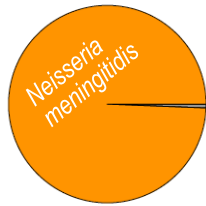
### CLARK

Ounit et al, BMC Genomics 16:236, 2015



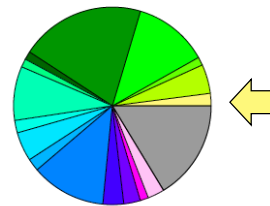
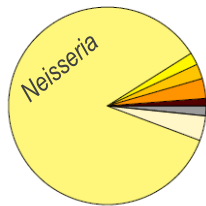
### MetaPhlan2

Segata et al, Nature Methods 8:811, 2012



### Mothur

Schloss et al, Appl Environ Microbiol 75:7537, 2009



- *Neisseria elongata*
- *Neisseria gonorrhoeae*
- *Neisseria lactamica*
- *Neisseria meningitidis*
- *Neisseria polysaccharea*
- *Neisseria skkuensis*
- *Neisseria unclassified*
- *Neisseria FJ193127*
- *Hathewayia histolytica*
- *Hathewayia limosa*
- *Hathewayia unclassified*
- *Serratia fonticola*
- *Serratia marcescens*
- *Serratia unclassified*
- Actinobacteria c unclassified
- Clostridiaceae unclassified
- *Corynebacterium unclassified*
- *Cutibacterium (Propionibacterium) acnes*
- *Enhydrobacter aerosaccus*
- Enterobacteriaceae unclassified
- *Haemophilus influenzae*
- *Halomonas unclassified*
- *Klebsiella pneumoniae*
- Micrococcales unclassified
- *Micrococcus luteus*
- *Moraxella osloensis*
- Moraxellaceae unclassified
- *Propionibacterium phage P100D*
- *Streptococcus mutans*
- *Streptococcus unclassified*
- Others

*de novo*  
assembly of  
*Neisseria* reads



362 contigs  
2,041,449 Mb



ResFinder  
ARD analysis

### PorB Asp-121

Partial resistance to penicillins,  
tetracyclines, and  
cephalosporin



PubMLST analysis

Allele	% Identity
--------	------------

abcZ_3	100%
--------	------

adk_6	100%
-------	------

aroE_34	100%
---------	------

fumC_5	100%
--------	------

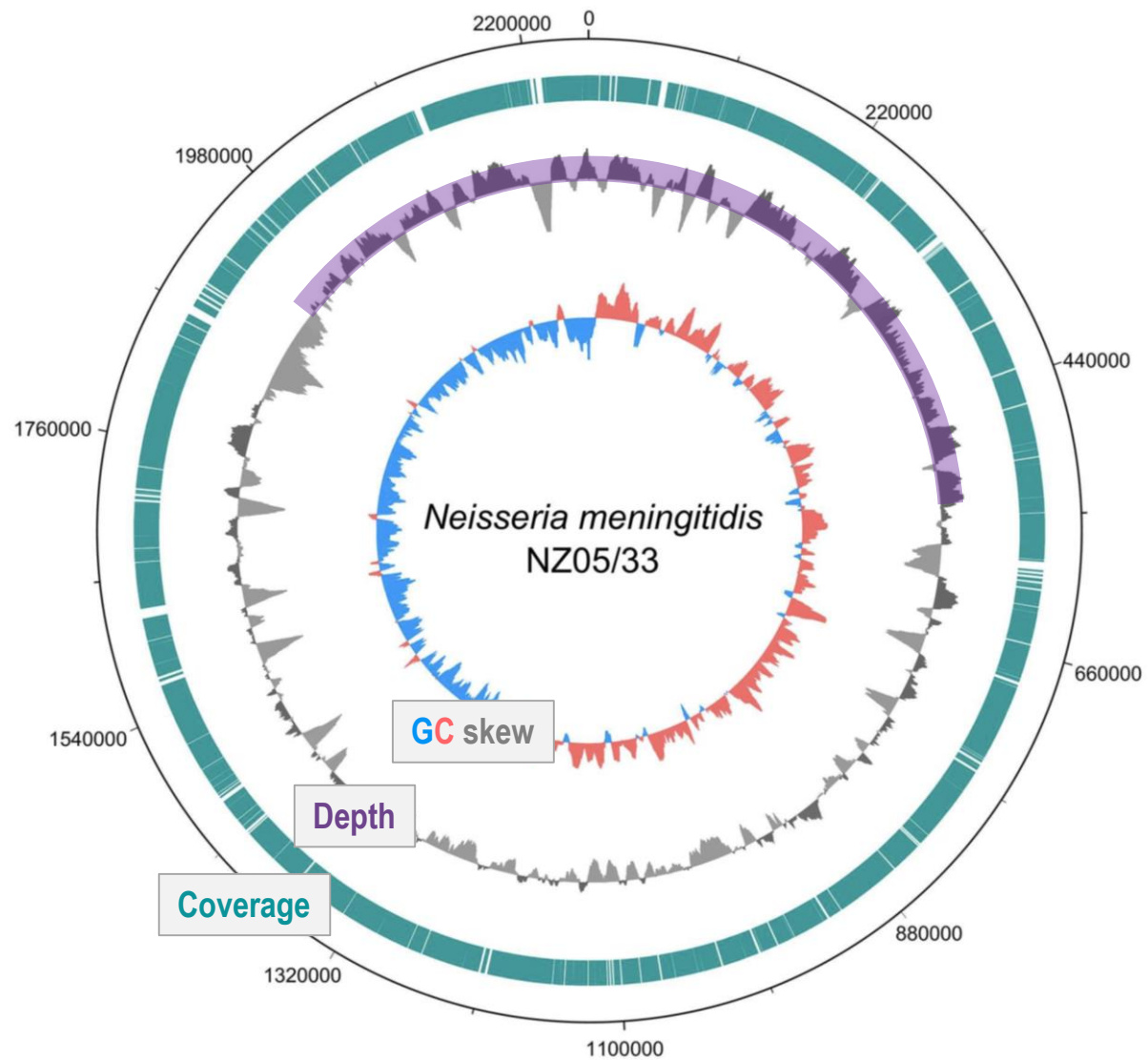
gdh_22	100%
--------	------

pdhC_6	100%
--------	------

pgm_9	100%
-------	------

### ST-41/44 complex





## When Bacterial Culture Fails, Metagenomics Can Help: A Case of Chronic Hepatic Brucellosis Assessed by Next-Generation Sequencing

Vladimir Lazarevic<sup>1\*</sup>, Nadia Gaia<sup>1</sup>, Myriam Girard<sup>1</sup>, Stefano Leo<sup>1</sup>,  
Abdessalam Cherkaoui<sup>2</sup>, Gesuele Renzi<sup>2</sup>, Stéphane Emonet<sup>2</sup>, Sharon Jamme<sup>3</sup>,  
Etienne Ruppé<sup>1</sup>, Sandrine Vijgen<sup>4</sup>, Laura Rubbia-Brandt<sup>4</sup>, Christian Toso<sup>5</sup> and  
Jacques Schrenzel<sup>1,2</sup>

case#3

35-year old woman

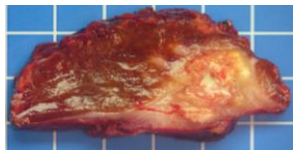
Fever, pain in the right abdomen and shoulder

Abdominal echography & CT scan:

**relapsed (6-y) liver abscess (brucellosis)**

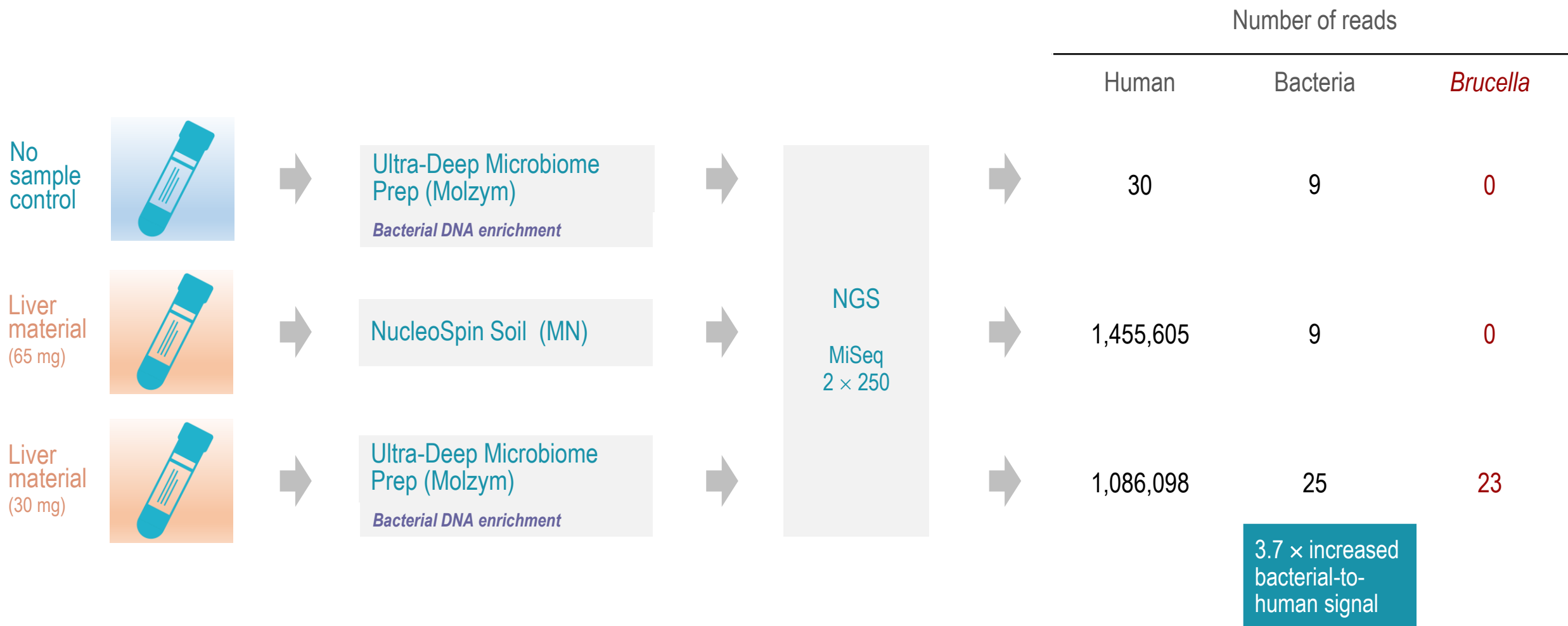
Negative blood and abscess draining cultures

Hepatectomy segments 6 & 7



Hepatic surgical  
material

→ -80 °C



## Next-Generation Sequencing for the Diagnosis of Challenging Culture-Negative Endocarditis

Manon Kolb<sup>1†</sup>, Vladimir Lazarevic<sup>2†</sup>, Stéphane Emonet<sup>3,4</sup>, Alexandra Calmy<sup>4</sup>, Myriam Girard<sup>2</sup>, Nadia Gaïa<sup>2</sup>, Yannick Charretier<sup>2</sup>, Abdessalam Cherkaoui<sup>3</sup>, Peter Keller<sup>5</sup>, Christoph Huber<sup>6</sup> and Jacques Schrenzel<sup>2,3,4</sup> \*

case#4

58-year old man

Breathlessness, chest tightness and chill

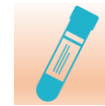
Physical examination, laboratory tests, TEE and TTE:  
Signs suggestive of **infective endocarditis**

ABT: Co-amoxicillin, gentamicin (day 0)

Aortic valve replacement (day 3)

Negative blood and valve cultures

Negative valve BR 16S PCR



Valve tissue



-80 °C





Fragment 1



Fragment 2

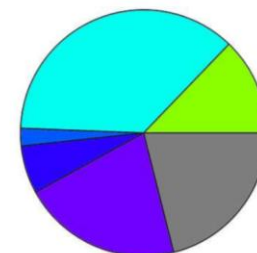
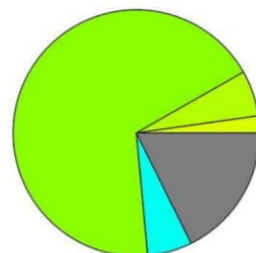
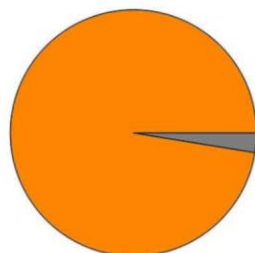
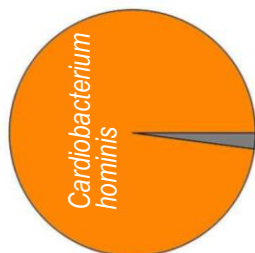


NC1

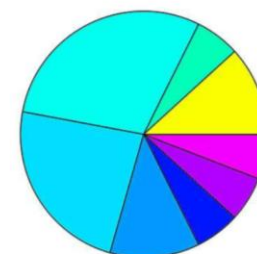
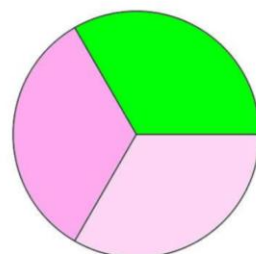
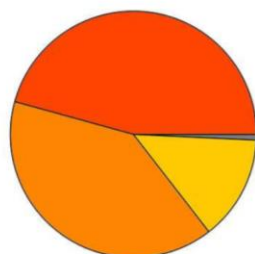
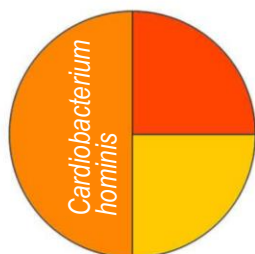


NC2

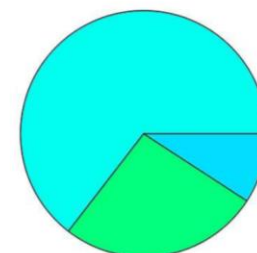
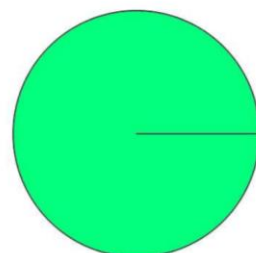
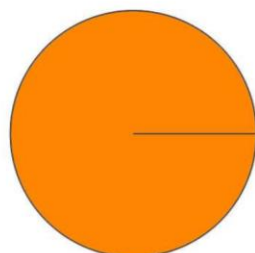
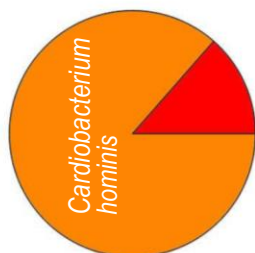
CLARK



mothur/Wang



MetaPhlAn2



- Cardiobacteriaceae unclassified
- *Cardiobacterium* - JQ451561
- *Cardiobacterium hominis*
- *Cardiobacterium* unclassified
- *Acinetobacter* unclassified
- *Ralstonia insidiosa*
- *Ralstonia mannitolilytica*
- *Ralstonia pickettii*
- *Ralstonia syzygii*
- *Ralstonia* unclassified
- Core eudicotyledons unclassified
- *Cutibacterium acnes*
- *Enhydrobacter aerosaccus*
- *Kocuria arsenatis*
- *Kocuria rhizophila*
- *Kocuria* unclassified
- *Micrococcus luteus*
- *Moraxella osloensis*
- Moraxellaceae - HQ143323
- Moraxellaceae unclassified
- *Mycosphaerella mycopappi*
- *Staphylococcus capitis*
- Others

Sample type	Number of samples analysed
Heart valve	28
Cerebrospinal fluid	6
Aorta	3
Bronchoalveolar lavage fluid	3
Endocardium	2
Bone	1
Skin	1
Hepatic biopsy	1
Joint fluid	1
<b>Total</b>	<b>46</b>

Valve culture		Valve NGS		Blood culture	Days between blood culture positivity and surgery
<i>Cardiobacterium hominis</i>	=	<i>Cardiobacterium hominis</i>	<i>mecA</i>	= <i>Cardiobacterium hominis</i>	2
<i>Staphylococcus aureus</i> MRSA	=	<i>Staphylococcus aureus</i> MRSA		= <i>Staphylococcus aureus</i> MRSA	2
<i>Streptococcus constellatus</i>	=	<i>Streptococcus anginosus</i> group	<i>fusB</i>	= <i>Streptococcus constellatus</i>	0
<i>Aggregatibacter aphrophilus</i>	=	<i>Aggregatibacter aphrophilus</i>		= <i>Aggregatibacter aphrophilus</i>	
<i>Prevotella oralis</i>	=	<i>Prevotella oralis</i>		. / .	
. / .		<i>Tannerella forsythia</i>		. / .	
<i>Staphylococcus epidermidis</i>	=	<i>Staphylococcus epidermidis</i>		= <i>Staphylococcus epidermidis</i>	21
<i>Streptococcus gallolyticus</i>	=	<i>Streptococcus gallolyticus</i>		= <i>Streptococcus gallolyticus</i>	2
negative		<i>Abiotrophia defectiva</i>	<i>IsaA</i>	= <i>Abiotrophia defectiva</i>	27
NA		<i>Enterococcus faecalis</i>		= <i>Enterococcus faecalis</i>	NA
negative		<i>Streptococcus mitis</i> group		= <i>Streptococcus mitis</i>	11
negative		<i>Kingella kingae</i>		= <i>Kingella kingae</i>	2
negative		<i>Staphylococcus aureus</i>	<i>norA</i>	= <i>Staphylococcus aureus</i> MSSA	5
NA		<i>Staphylococcus aureus</i>		= <i>Staphylococcus aureus</i>	NA
negative		<i>Streptococcus agalactiae</i>	<i>tetL</i>	= <i>Streptococcus agalactiae</i>	6
NA		<i>Streptococcus gallolyticus</i>		= <i>Streptococcus gallolyticus</i>	NA
negative		<i>Streptococcus pneumoniae</i>		= <i>Streptococcus pneumoniae</i>	12
negative		<i>Neisseria meningitidis</i>		negative	NA
negative		<i>Staphylococcus aureus</i>		negative	NA
negative	=	negative		= negative	NA
negative	=	negative		= negative	NA
negative	=	negative		= negative	NA
negative	=	negative		<i>Aggregatibacter aphrophilus</i>	18
negative	=	negative		<i>Staphylococcus aureus</i> MSSA	44
negative	=	negative		<i>Staphylococcus aureus</i> MSSA	17



- Identification of reagent contaminants
- Removal of host DNA to increase pathogen-to-human signal ratio
- Pathogen quantification
- Validation of the whole CMg workflow (including the bioinformatics pipeline)

Pig muscle tissue  
100 mg

+

Bacterial cells ( $\sim 3.5 \times 10^5$  cells each):

Bs

*Bacillus subtilis* (Firmicutes)

Ec

*Escherichia coli* (Proteobacteria)

Rd

*Rothia dentocariosa* (Actinobacteria)

Sm

*Sphingobacterium multivorum* (Bacteroidetes)



Homogenisation

- Glycerol

+ Glycerol



DNA Extraction

MagCore

Molzym Tissue

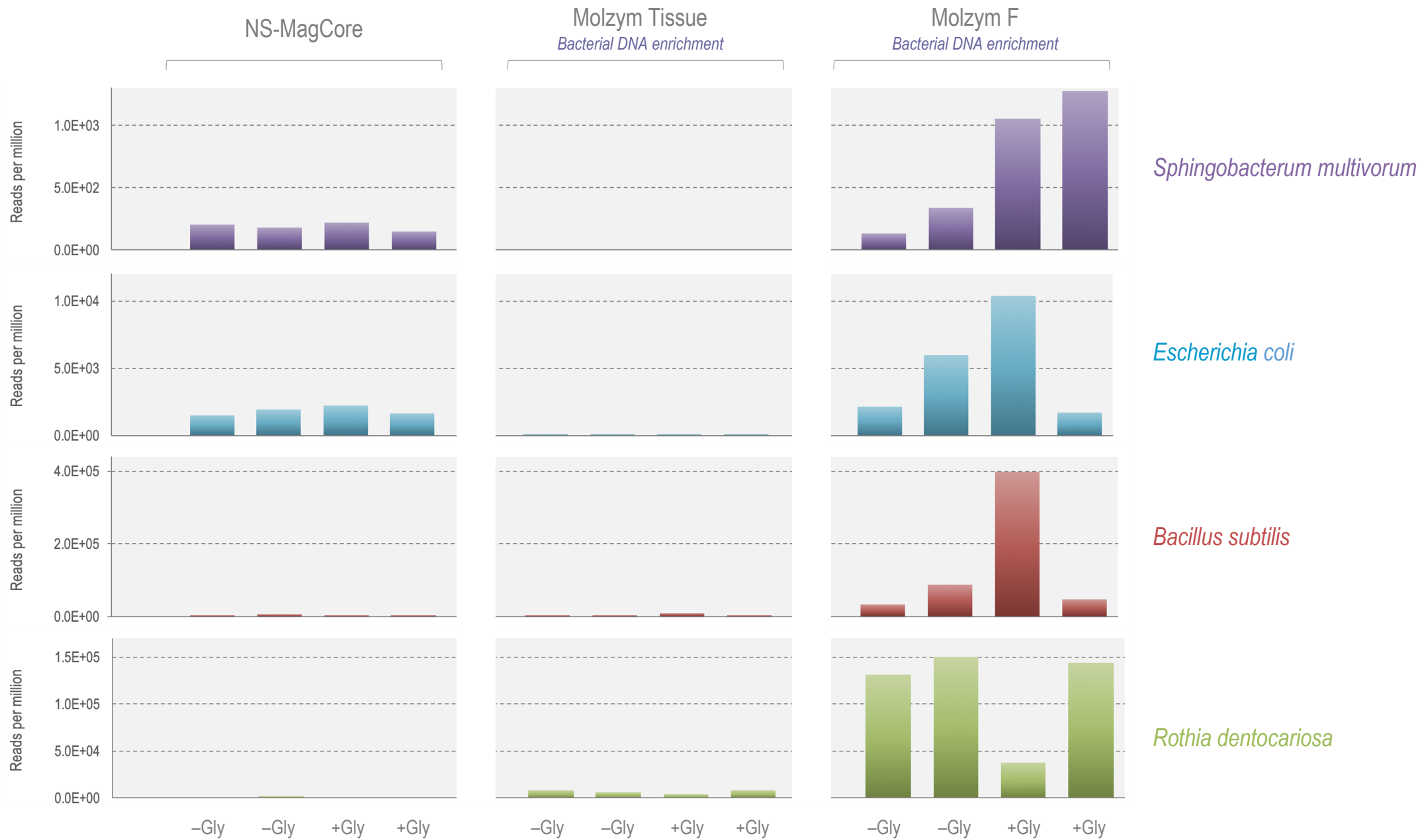
Molzym F

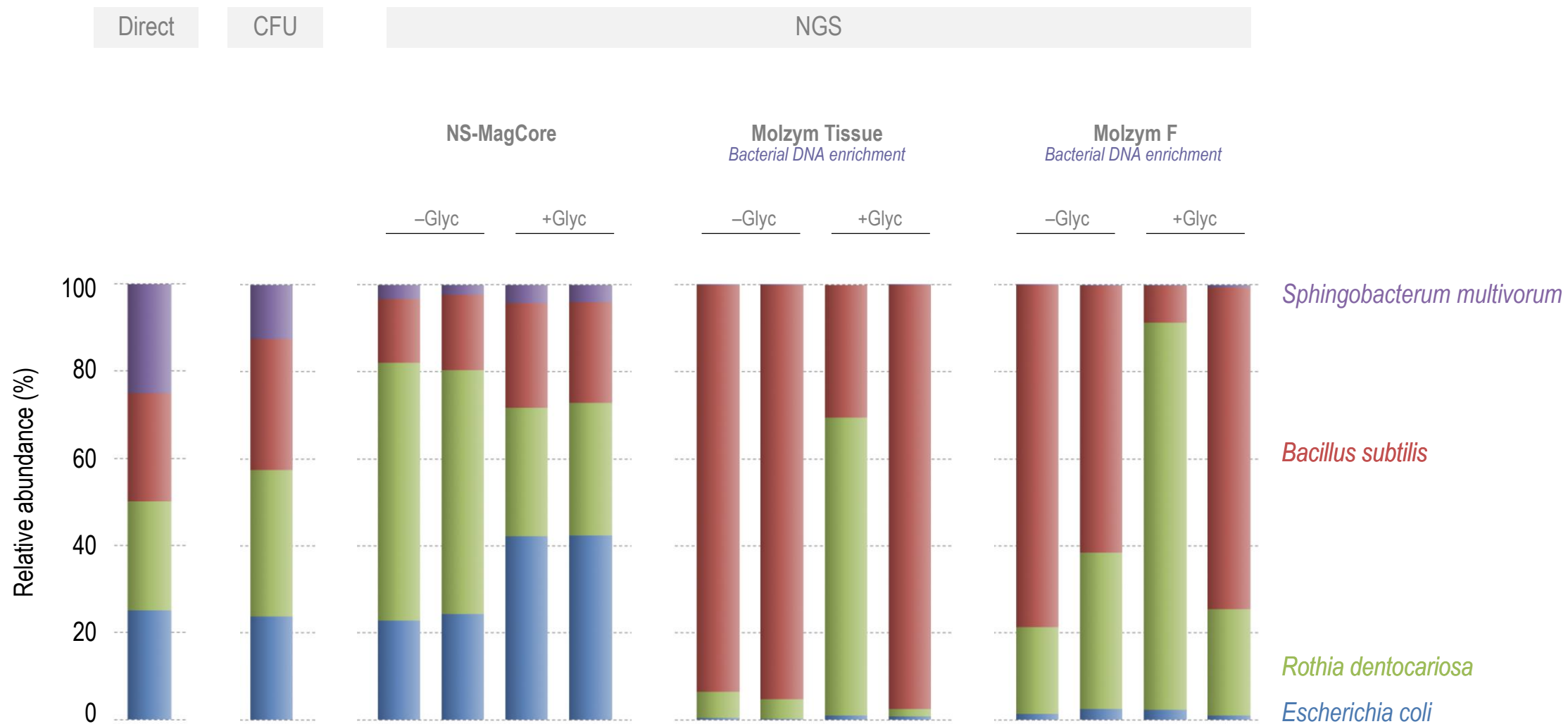


qPCR (bacterial & human)  
iSeq



Huge-Map Pipeline



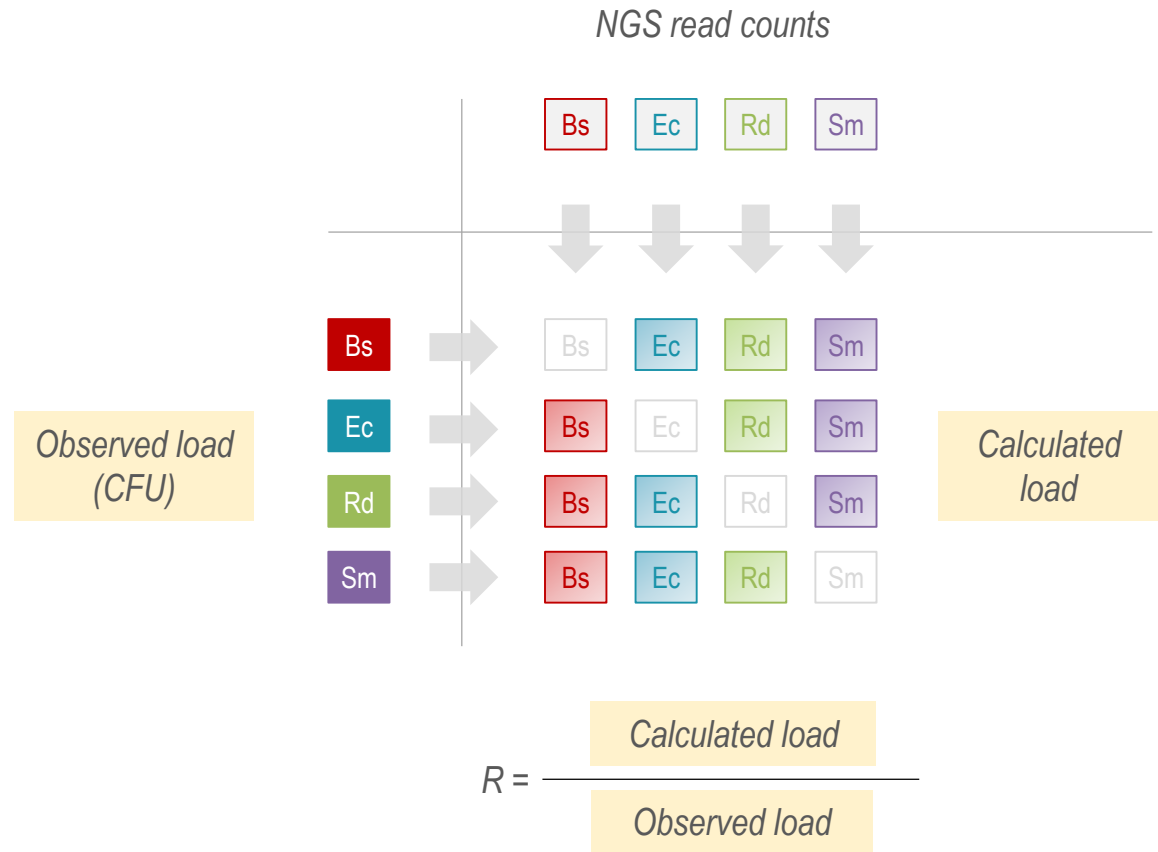
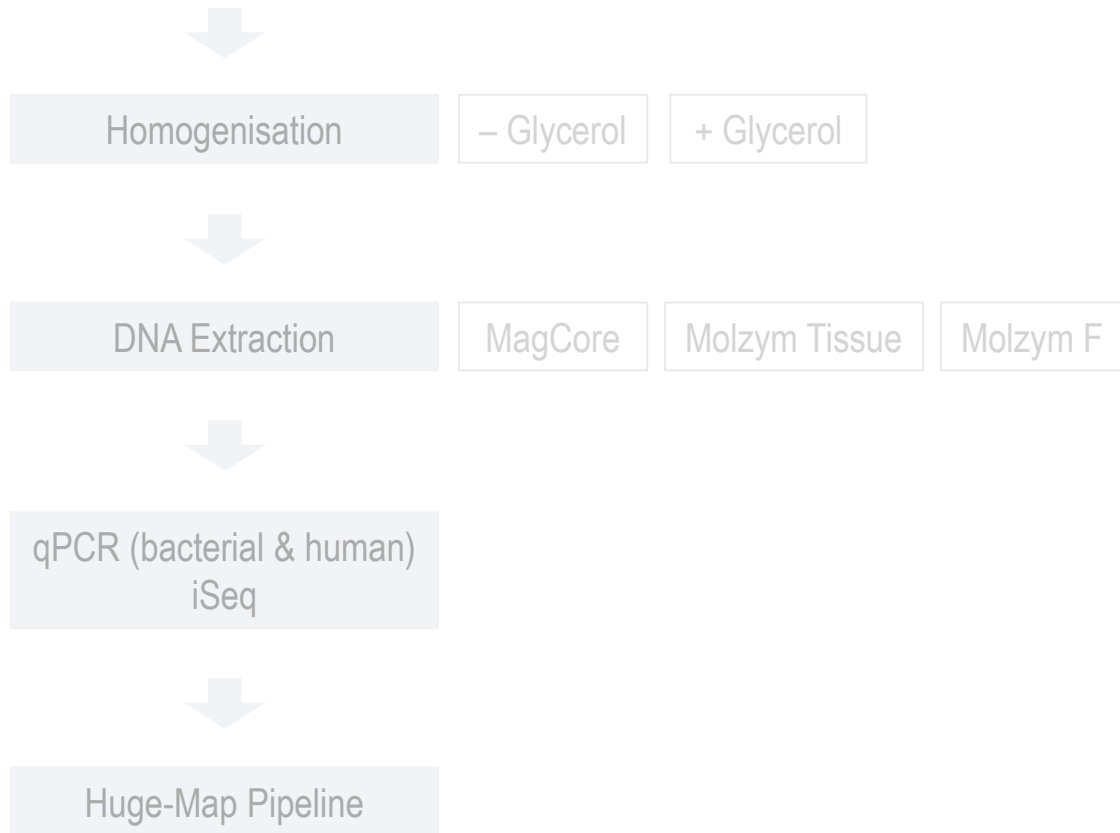


Pig muscle tissue

+

Bacterial cells ( $\sim 3.5 \times 10^5$  cells each):

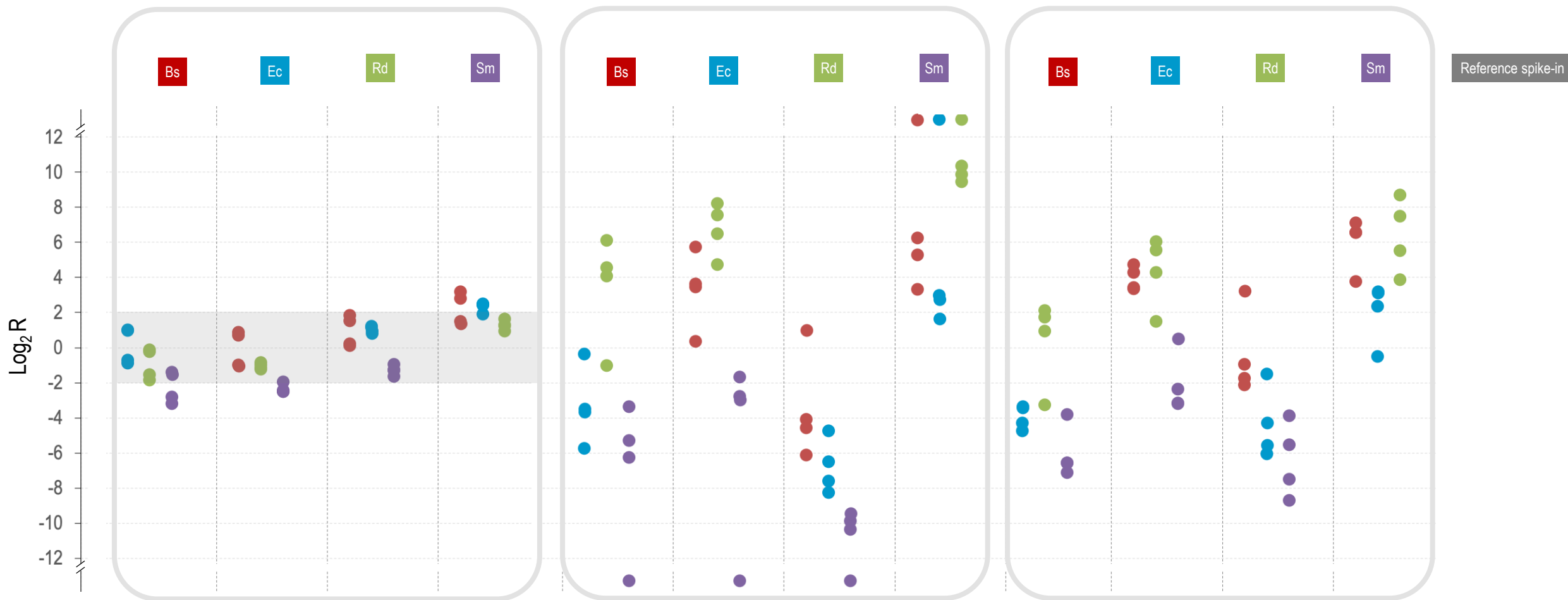
- Bs *Bacillus subtilis* (Firmicutes)
- Ec *Escherichia coli* (Proteobacteria)
- Rd *Rothia dentocariosa* (Actinobacteria)
- Sm *Sphingobacterium multivorum* (Bacteroidetes)



### NS-MagCore

### Molzym Tissue (bacterial DNA enrichment)

### Molzym F (bacterial DNA enrichment)



Reference spike-in

$$R = \frac{\text{Calculated load}}{\text{Observed load}}$$

- Universal DNA extraction protocol may be used for different sample types
- No-sample controls are required for identification of reagent contaminants
- Removal of host DNA improves identification of pathogens but distorts the proportion of different community members

# Acknowledgments

**Genomic Research Laboratory (GRL)  
Geneva University Hospitals (HUG)  
Geneva, Switzerland**

Myriam Girard

Nadia Gaïa

Stefano Leo

Dr Yannick Charretier

Miguel De Albuquerque

Prof Jacques Schrenzel

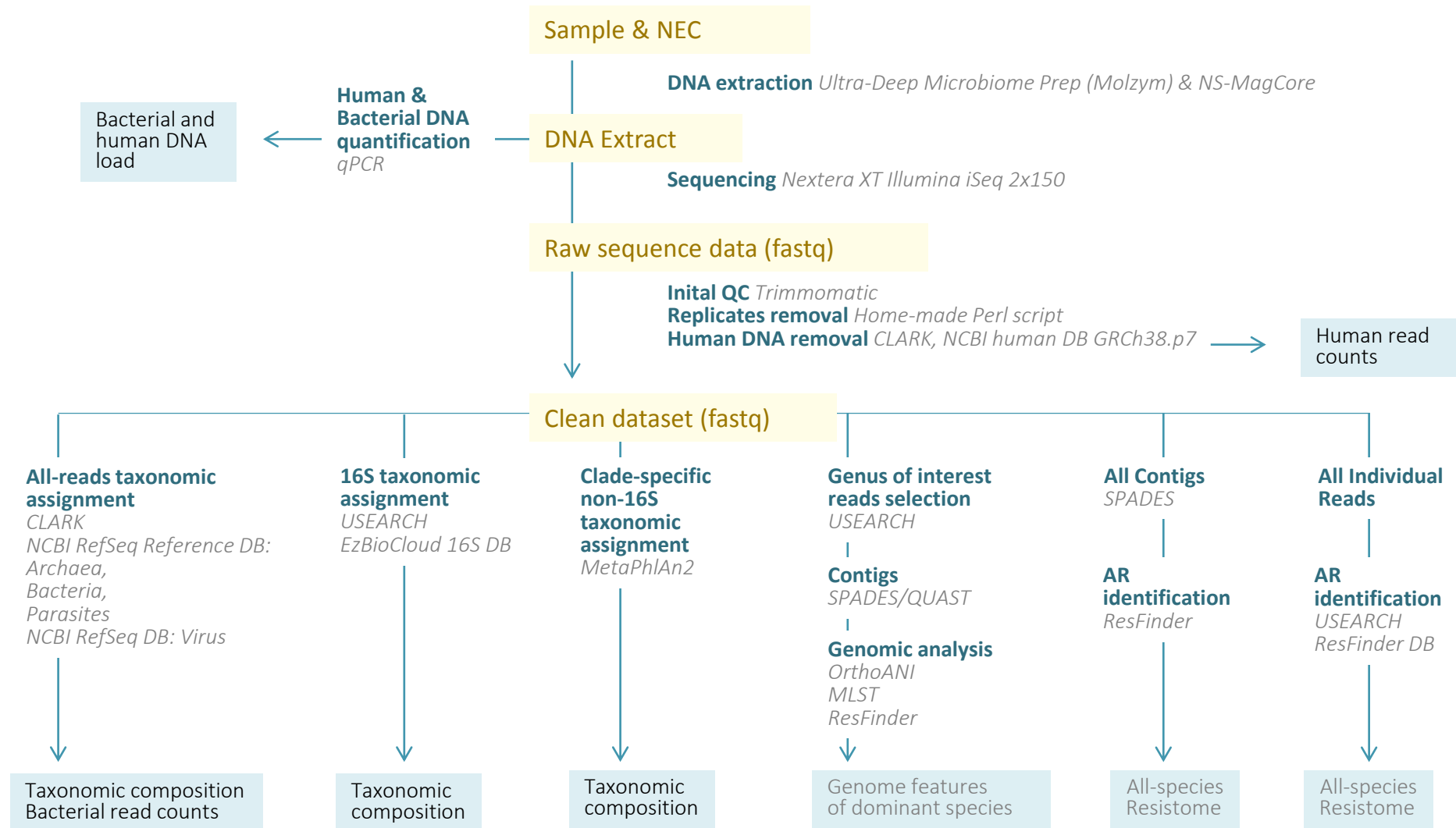
**Paris Diderot University & Paris Hospitals  
Paris, France**

Prof Etienne Ruppé

The Paris-Geneva clinical metagenomics group



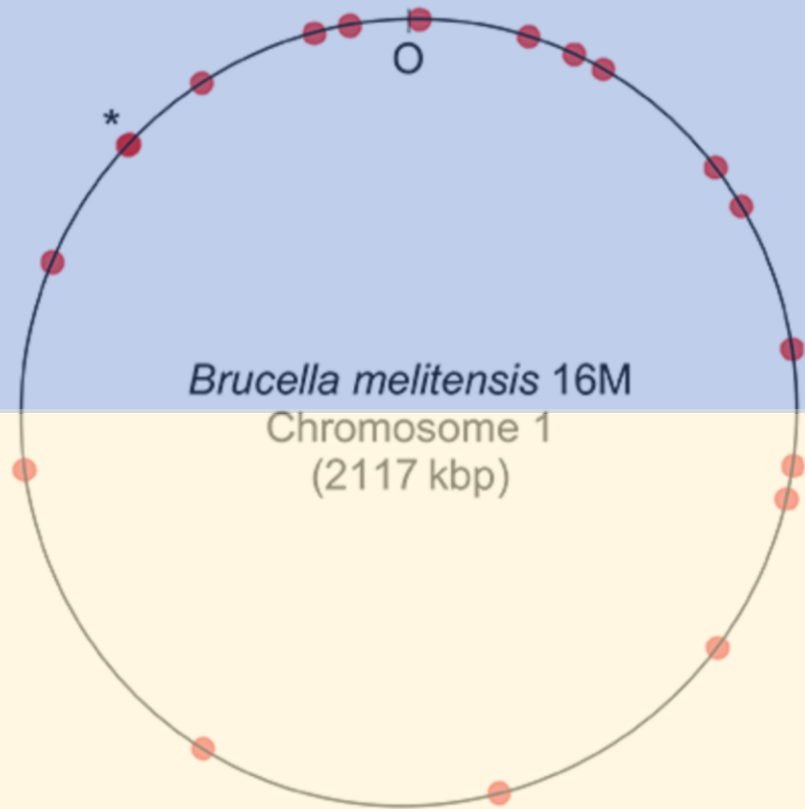




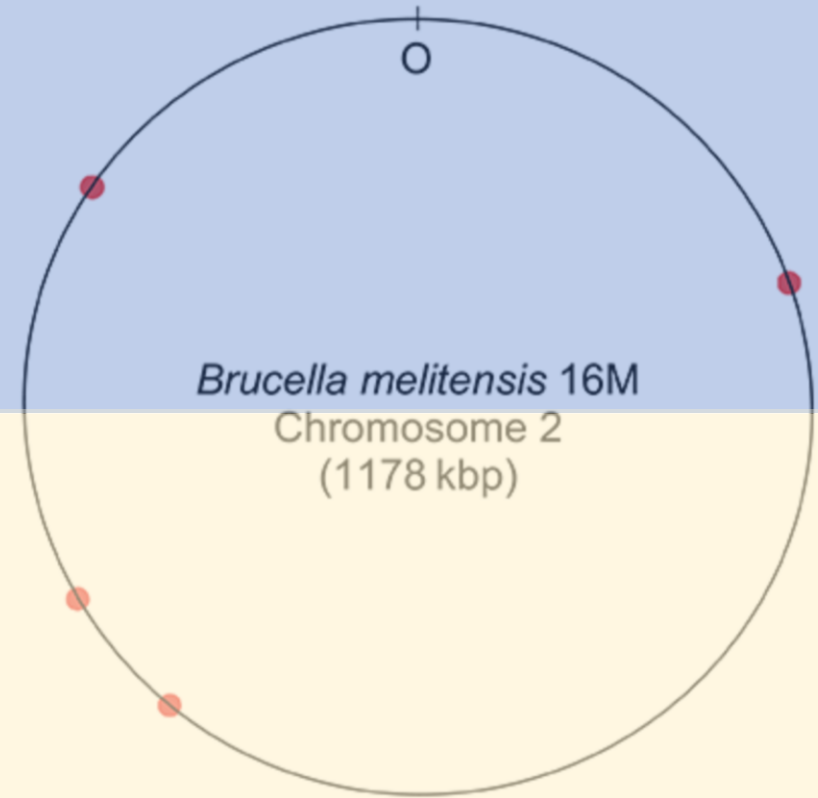
REPORT FOR CLINICIANS

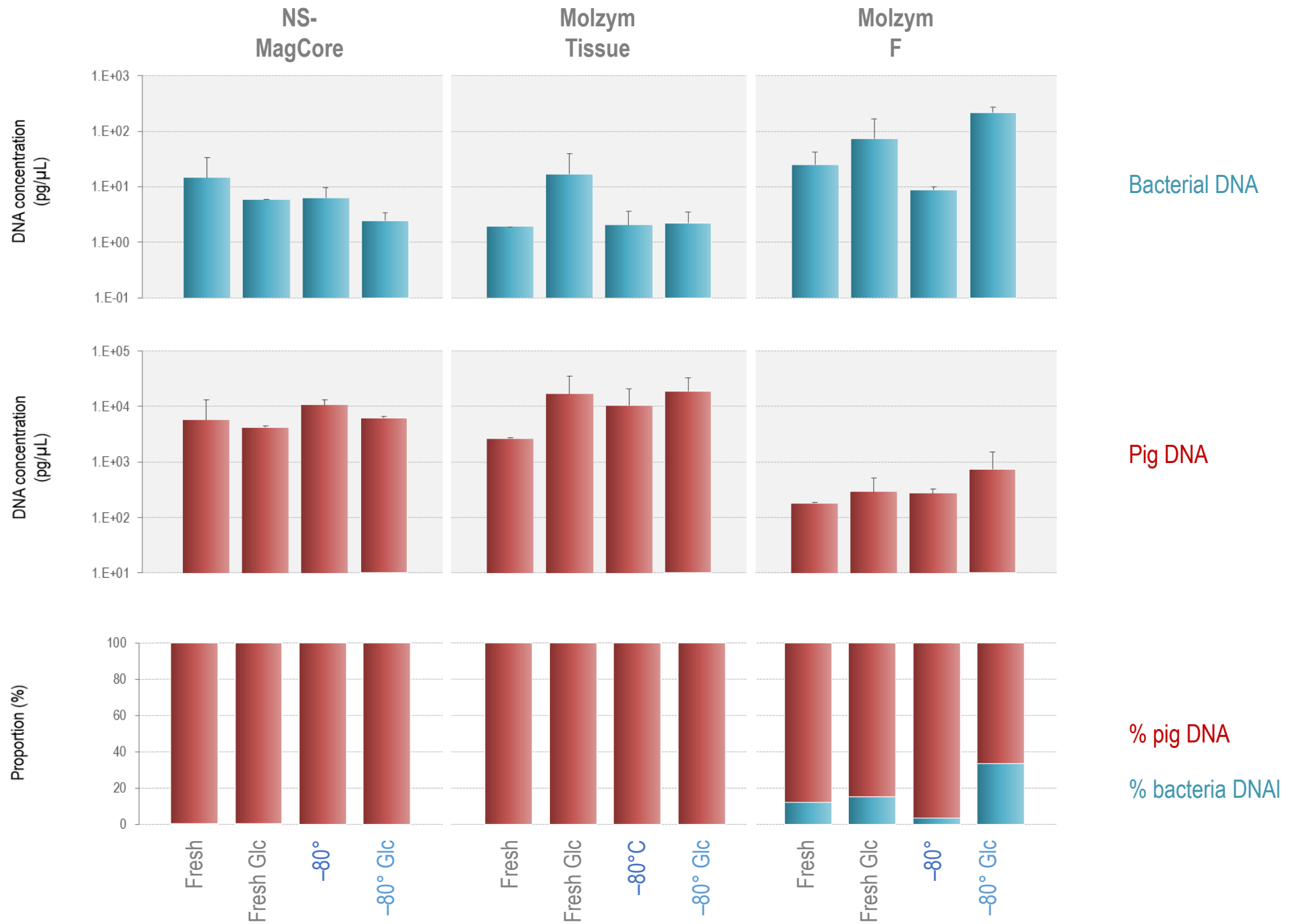


65%



35%





Pig muscle tissue  
100 mg

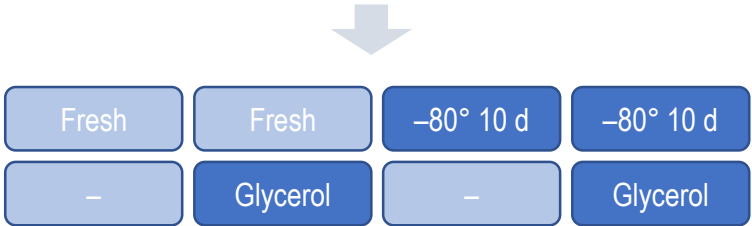
*Escherichia coli* (Proteobacteria)  
*Bacillus subtilis* (Firmicutes)  
*Rothia dentocariosa* (Actinobacteria)  
*Sphingobacterium multivorum* (Bacteroidetes)  
~  $3.5 \times 10^5$  cells each (by microscopic examination)



CFU (20 h, 37°C)



Direct microscopic counts



Homogenization

DNA Extraction

MagCore

Molzym Tissue

Molzym F

qPCR (bacterial & human)  
iSeq

Huge-Map Pipeline

### Summary of QUAST results

Nr of contigs >0 bp	362
Nr of contigs >500 bp	292
Largest contig	49,572
Total length	2,041,449
GC (%)	52.49
N50	12,645
L50	49

# Identification of reagent contaminants

## CLARK

*Ounit et al, BMC Genomics 16:236, 2015*

## MetaPhlAn2

184 ± 45 clade markers per genome  
*Segata et al, Nature Methods 8:811, 2012*

## Mothur

1–15 16S rRNA markers  
*Schloss et al, Appl Environ Microbiol 75:7537, 2009*

Unclassified 23.47%

Prokaryotes 0.04%

Unclassified 0.23%

Prokaryotes 21.41%

CLARK

(*Ounit et al, BMC Genomics 16:236, 2015*)

MetaPhlAn2

184 ± 45 clade markers per genome  
(*Segata et al, Nature Methods 8:811, 2012*)

mothur

1–15 16S rRNA markers  
(*Schloss et al, Appl Environ Microbiol 75:7537, 2009*)