

VIRAL METAGENOMICS

FROM SEA TO SHORE



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& Global viral forecasting initiative, USA



14:00	Biotechnologies and modern Microbiology	Giorgio Palù
14:30	Is classical microbiology dead? An introduction to 'culturomics'	Didier Raoult
15:00	Carbohydrate-active enzymes and the latent power of glycogenomics	Bernard Henrissat

NO

Culture

vs.

16S rRNA sequencing

Many viruses cannot be cultured
Viruses lack a common gene

VIRUSES DURING THE 'OMICR' ERA

(UNCULTURABLE, LACK A GENE COMMON TO ALL VIRUSES)



- How many are they?
- How diverse are they?
- ~10 viruses for every bacteria
- 10^{10} virus like particles/ liter of sea water

Viruses are the most abundant biological entity on earth

How to characterize viruses?

Traditional methods

- Culture
 - Virus isolation
- Microscopy
 - Electron microscopy
- Immunological assays
 - Complement fixation test
 - Haemagglutination inhibition test
 - Enzyme linked immunosorbent assay (ELISA)
 - Single radial Haemolysis
 - Immunofluorescence
 - Neutralization
 - IgG antibody avidity
- Molecular methods
 - Amplification methods: PCR
 - Hybridization methods: Southern blot, microarrays etc.

Traditional methods for virus detection

- Culture
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~ 1% of culturable viruses

Known target

HOW DIVERSE ARE THEY?

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VIRAL METAGENOMICS

CULTURE-INDEPENDENT AND SEQUENCE-INDEPENDENT APPROACH FOR DETECTION OF VIRUSES

San Diego Scripps Pier



San Diego Mission Bay



Viral metagenomics

- Culture-independent
- Target-independent

~ 1% of culturable viruses

Known target

Procedure

Samples preparation

High-throughput sequencing

Bioinformatics

Viral metagenomics

- Culture-independent
- Target-independent

~ 1% of culturable viruses

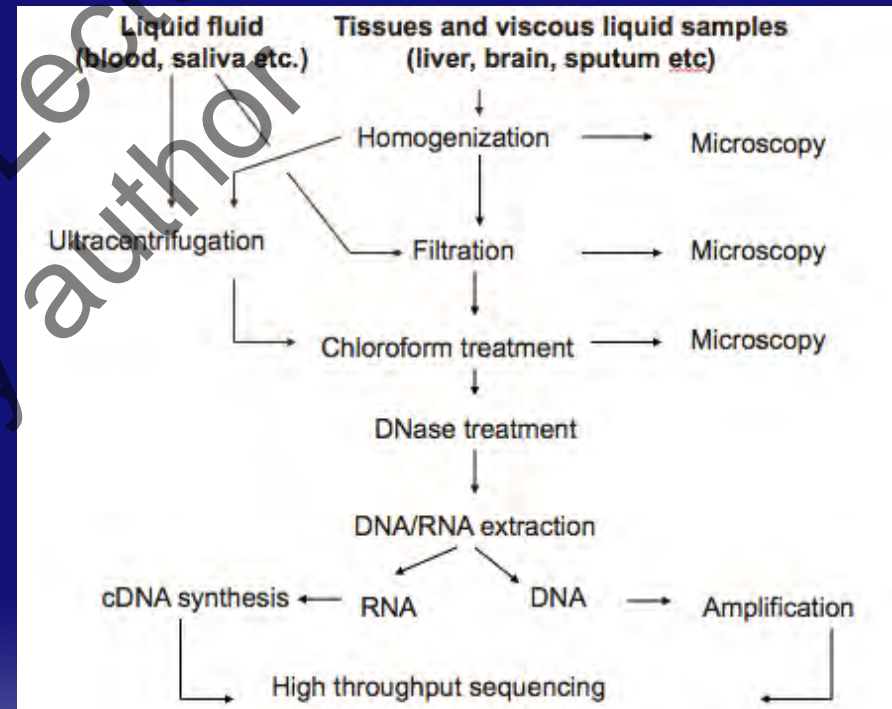
Known target

Procedure

Samples preparation

High-throughput sequencing

Bioinformatics



Examples

Study	Sample
Pig-to-baboon <u>xenotransplantation</u>	Plasma
Flu-like illnesses of unknown origin	Nasopharynx swabs
Liver failure of unknown etiology	Liver tissue
Normal viromes in <u>nonhuman</u> primates at San Diego Zoo	Plasma
Biomarkers in brain tissues of patients with <u>Chronic encephalopathy of unknown origin</u> (former football players- NFL)	Brain tissue
Diffuse large B-cell lymphoma of unknown origin (Cameroon Africa)	Plasma

Example 1

Risk assessment of pig-to-baboon xenotransplantation

Objectives:

Comparative analysis of viromes of donors and recipients of xenografts

Study population:

4 pigs (donors)
5 baboons (recipients)

Xenograft

Kidneys



Pre-transplantation

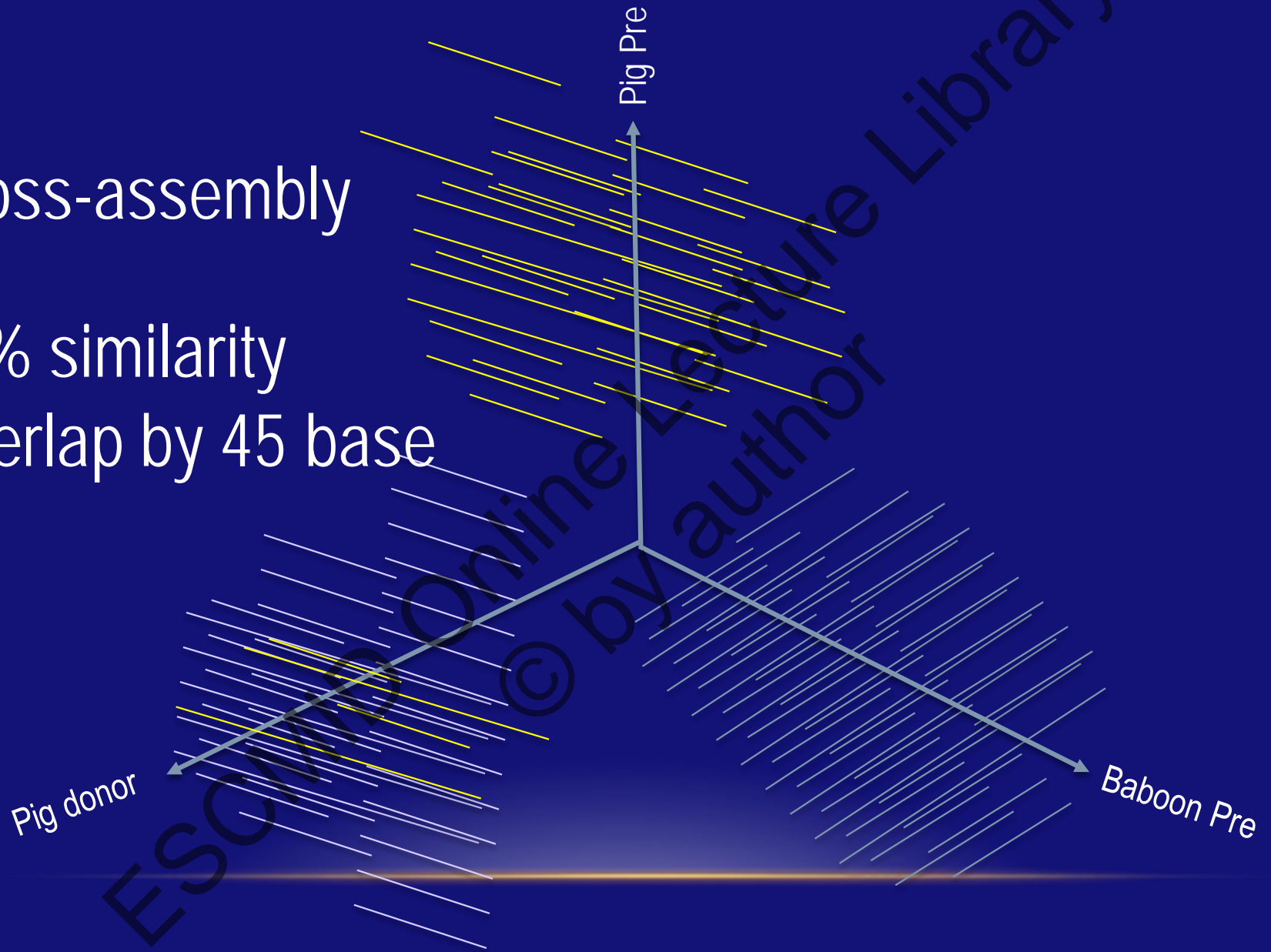
Pre-transplantation

Post-transplantation

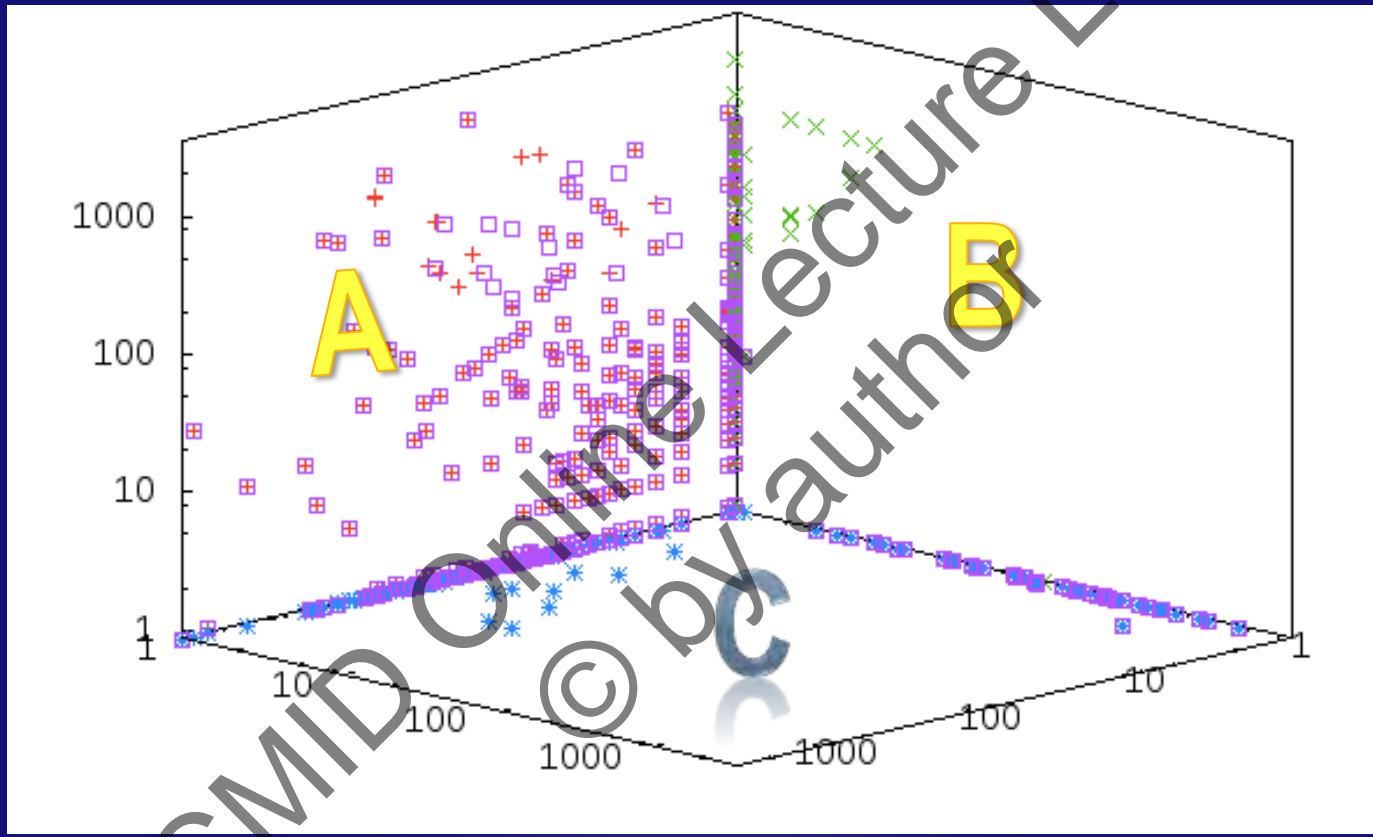
Cross-assembly

98% similarity

Overlap by 45 base



Baboon Post



Pig Pre

Baboon Pre

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Example 1: FLU LIKE ILLNESS OF UNKNOWN ORIGIN

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Objectives:

Human virome

Study population:
n=95

Military recruits n=35

Dependents of military personnel n= 40

Civilian from San Diego n=10

Civilian from Mexicali, Mexico n=10

All 95 patients tested (-) by PCR, culture and ELISA for influenza and common cause of ILI



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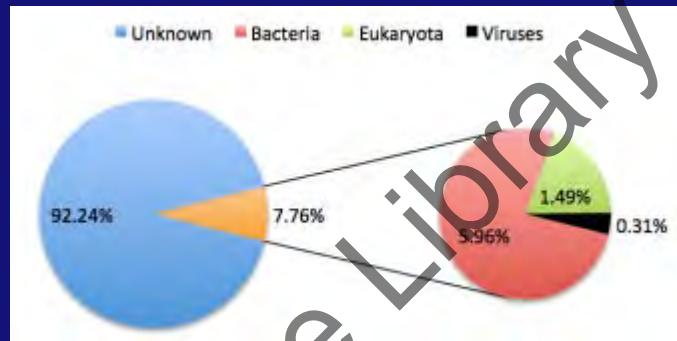
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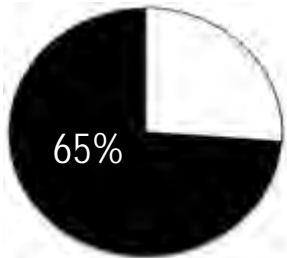
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The unknowns

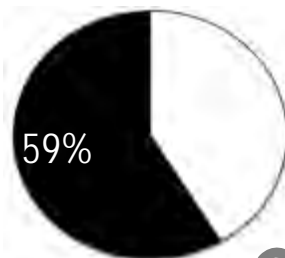


Seawater



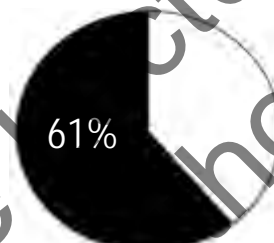
Breitbart et al, 2002

Gut



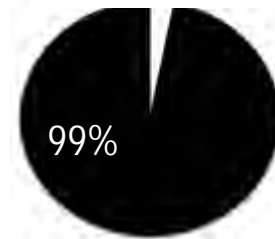
Breitbart et al, 2003

Virioplankton



Benchet al, 2007

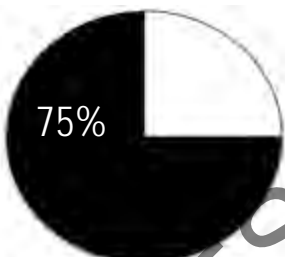
Stromatolite



Desnues et al, 2008

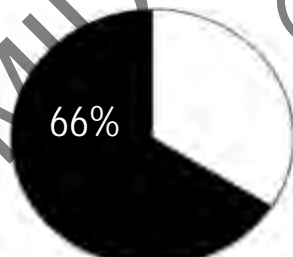
Water

Marine sediment



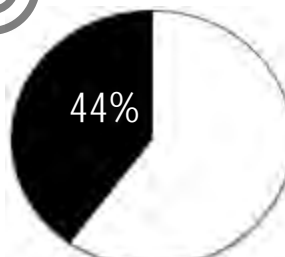
Breitbart et al, 2004

Infant gut (<3 months)



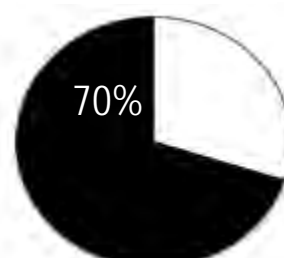
Breitbart et al, 2008

Potable

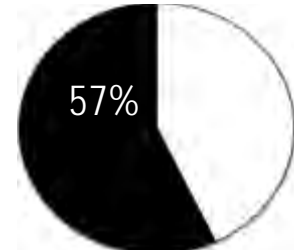


Rosario et al, 2009

Reclaimed DNA



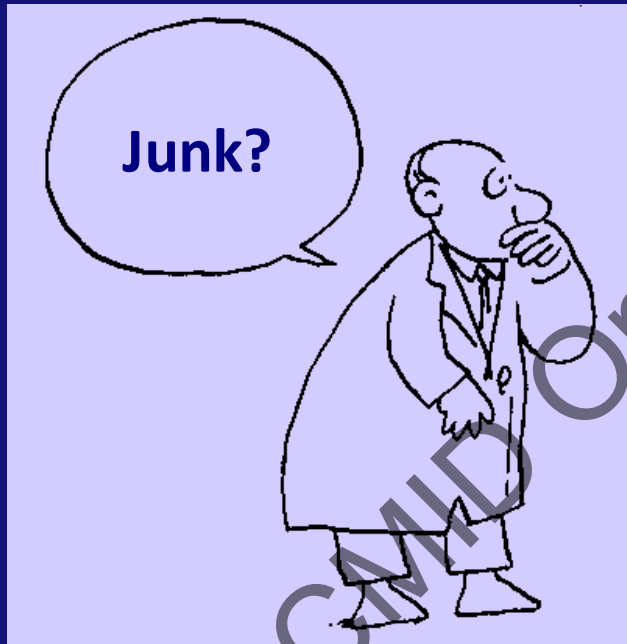
Reclaimed RNA



Unknowns

Knowns (Hits to Genbank)

How do we deal with the unknowns?



Just because you don't understand us, you can't just call us junk

How to deal with the unknowns

1. Junk-----→ do nothing

2. Not Junk

- Assembled
- Open reading frames
- Viral genes: reverse transcriptase etc.
- GC contents
- Linear or circular



? Blueprint of novel viruses



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Conclusion

1. **Multiple known viruses:** rhinoviruses, enteroviruses, novel human papilloma virus, Torque teno virus
2. **Multiple unknowns** including potential novel circular structure (?viral)
3. **Bacteria** sequences from phages

Acknowledgments

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