# Long-read metagenomics with MetaMaps

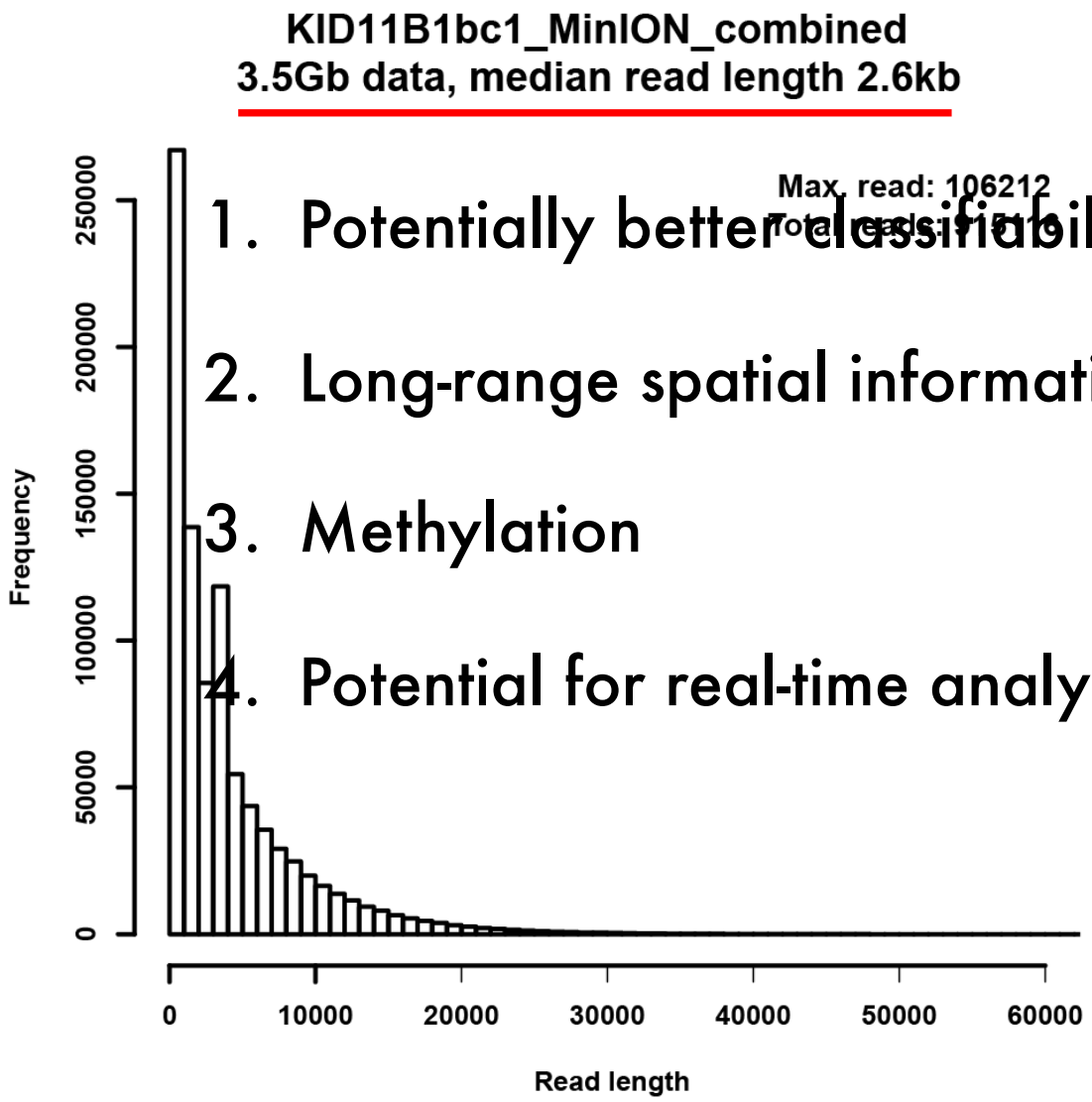
Alexander Dilthey (DPhil)

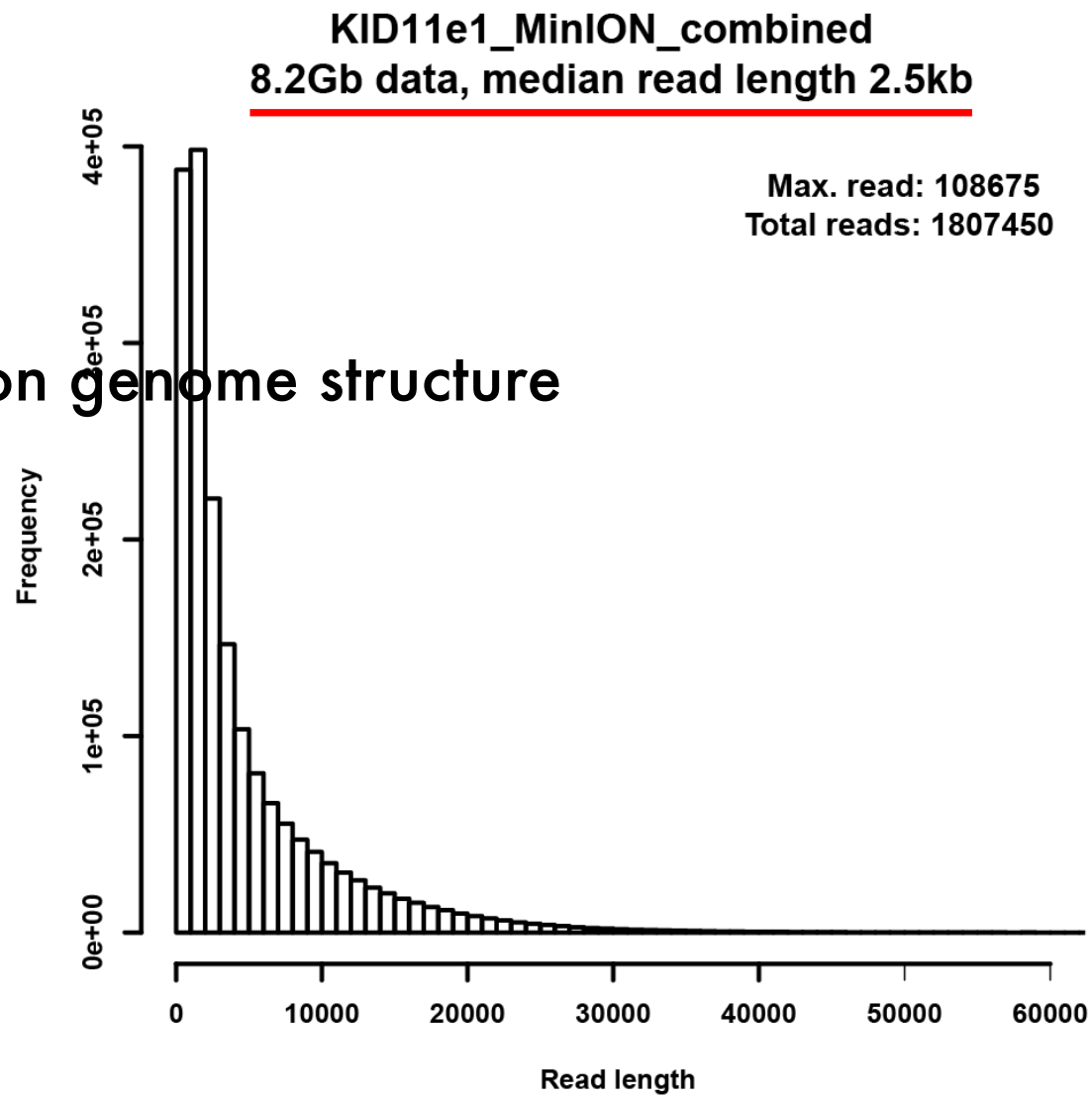
Heinrich Heine University Düsseldorf

UKD Universitätsklinikum Düsseldorf

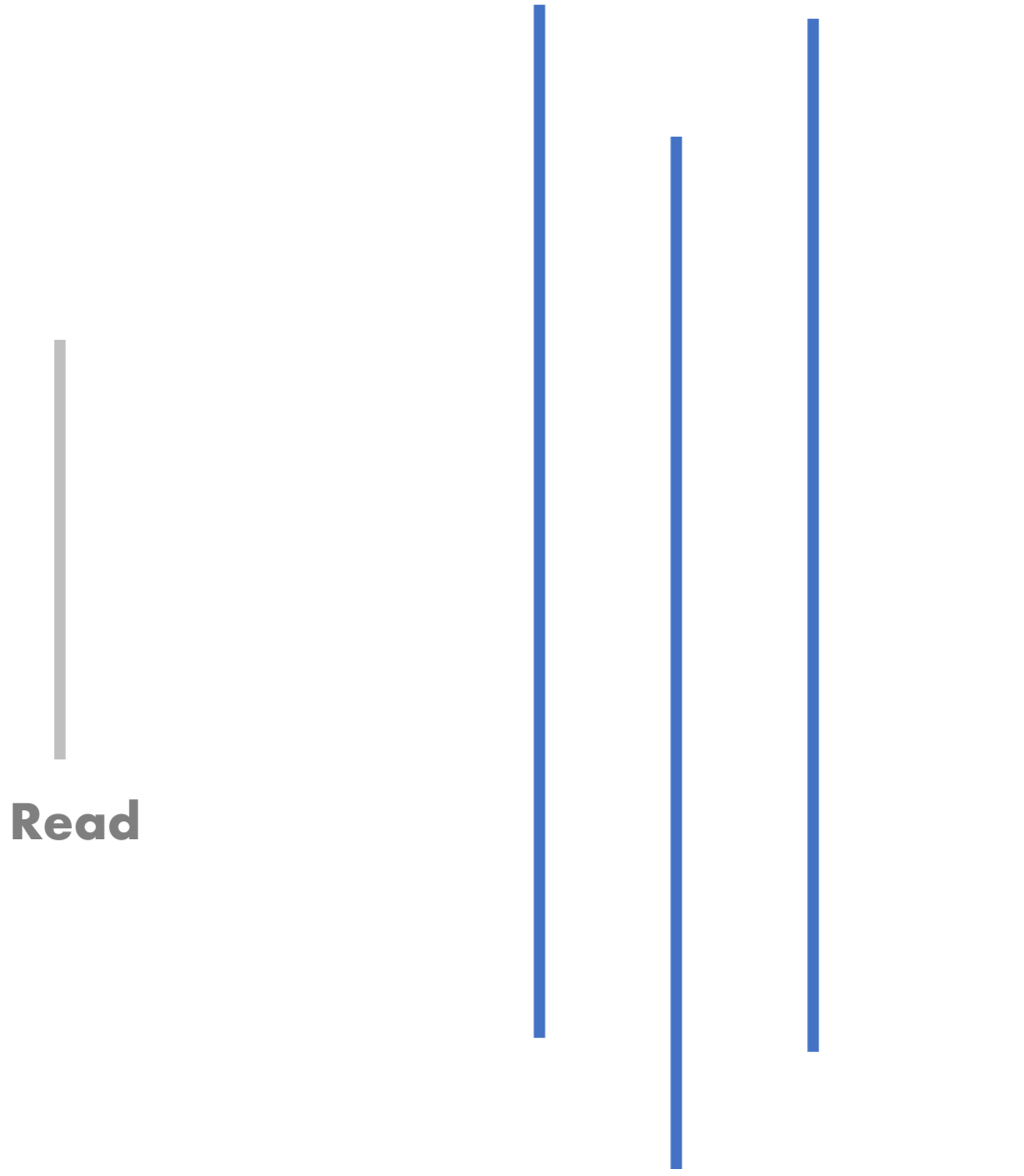
hhu.

# Long-read metagenomics

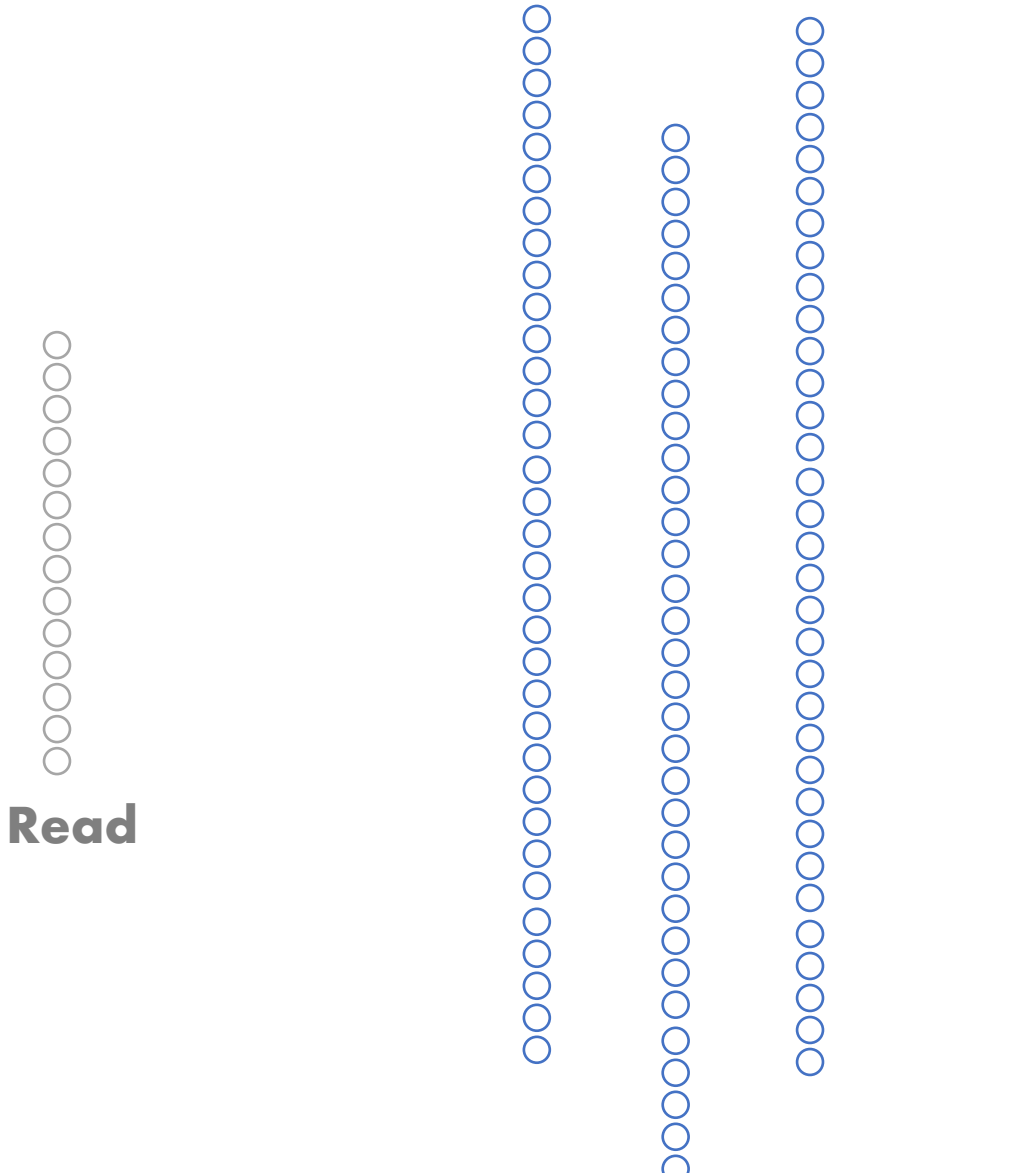



1. Potentially better classifiability
2. Long-range spatial information on genome structure
3. Methylation
4. Potential for real-time analysis

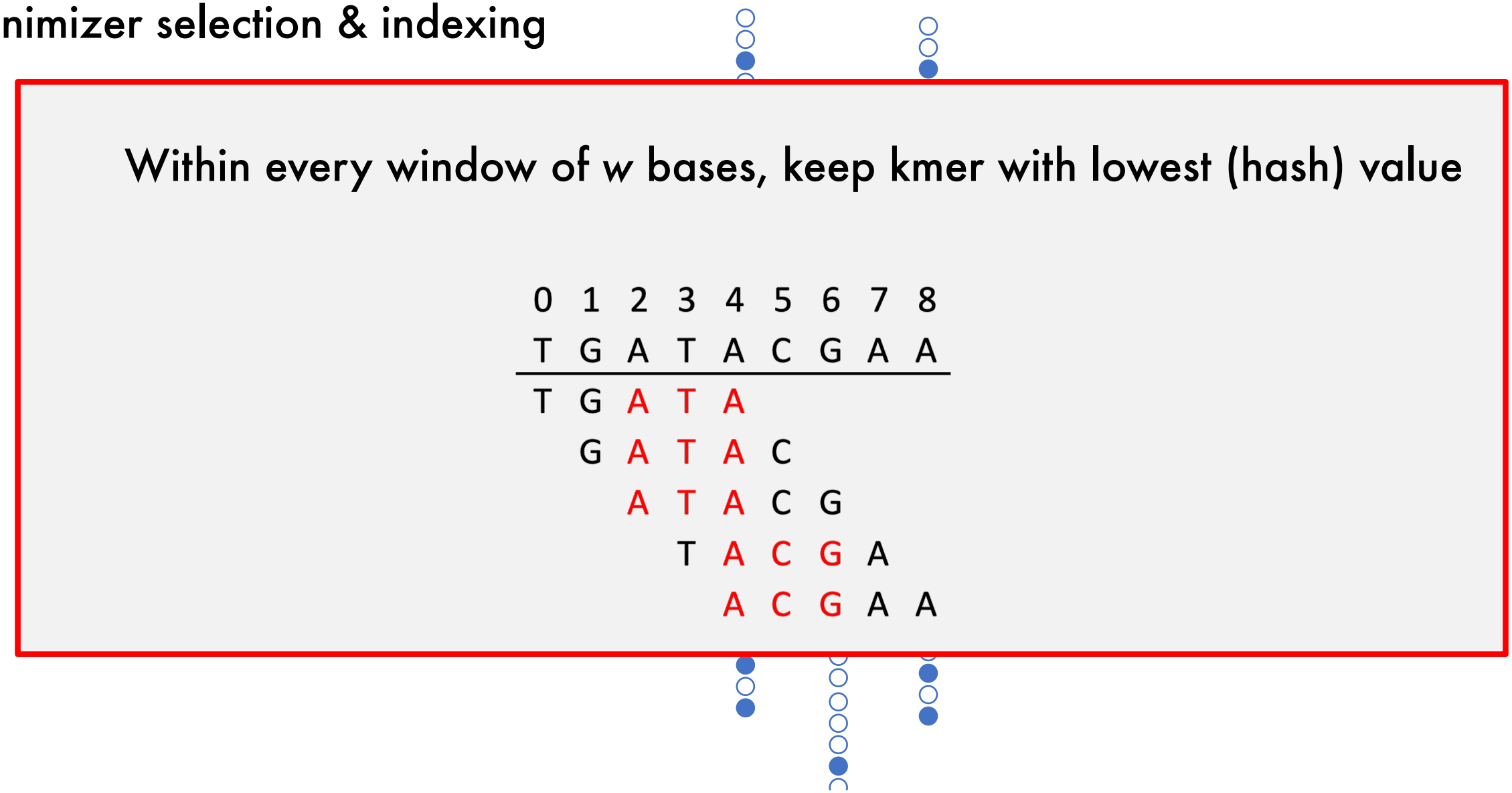

Read
Reference genomes

# kMer transformation



kMer transformation

Minimizer selection & indexing

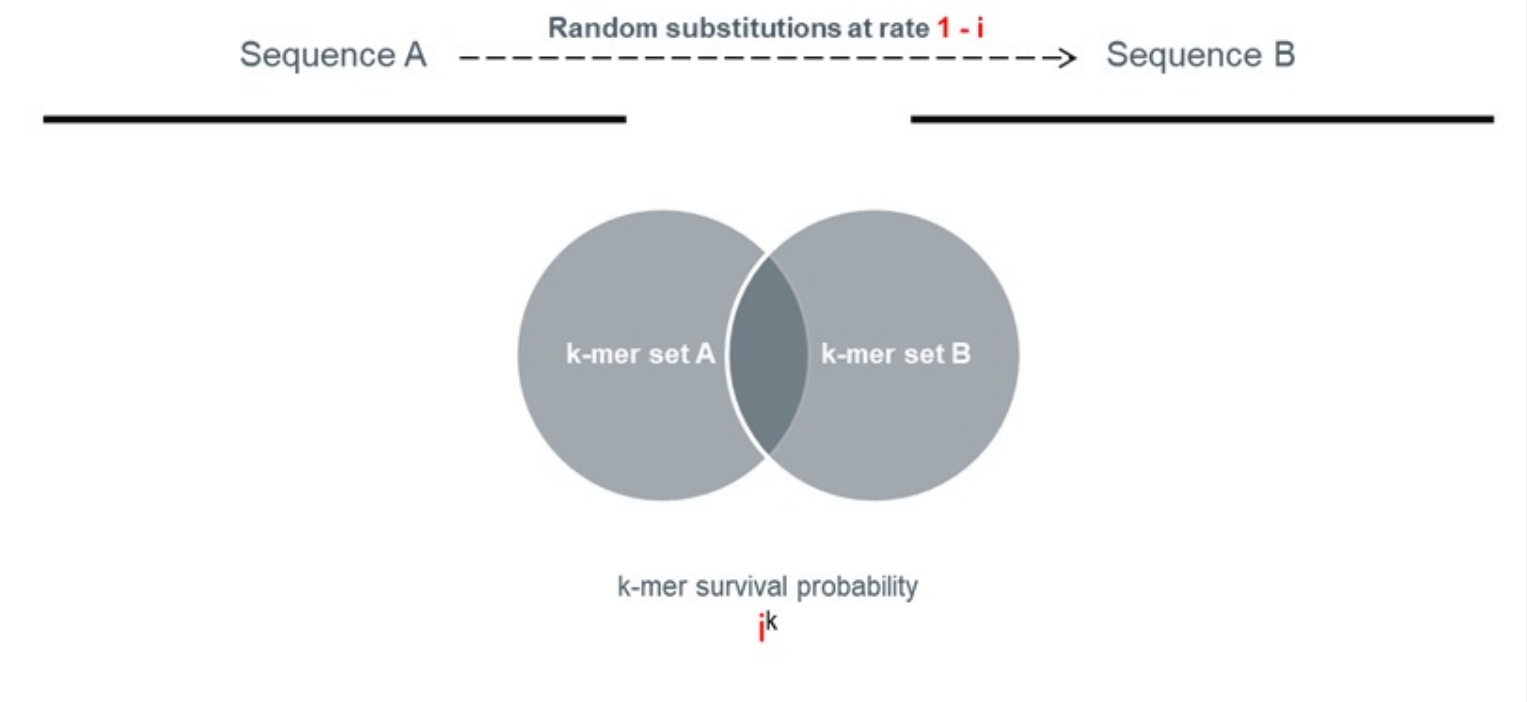


kMer transformation

Minimizer selection & indexing

Car

Sco

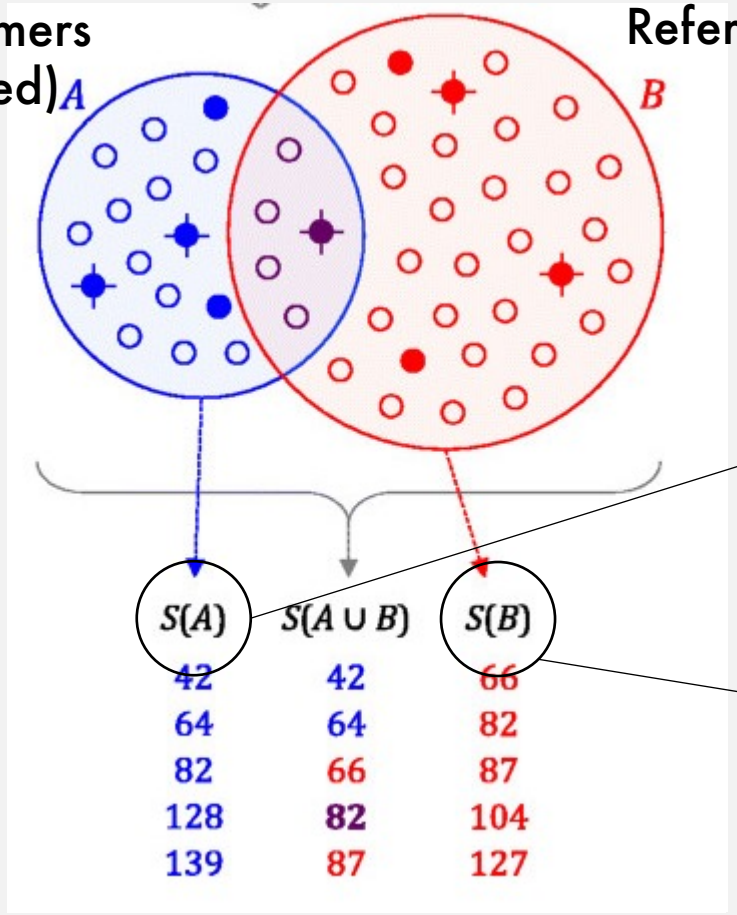



$$E[J(A,B)] := \frac{E[|A \cap B|]}{E[|A \cup B|]} = \frac{n_{kmers} \times i^k}{2 \times n_{kmers} - (n_{kmers} \times i^k)}$$

**Reference genomes**

Read kmers (hashed)

Reference window kmers (hashed)



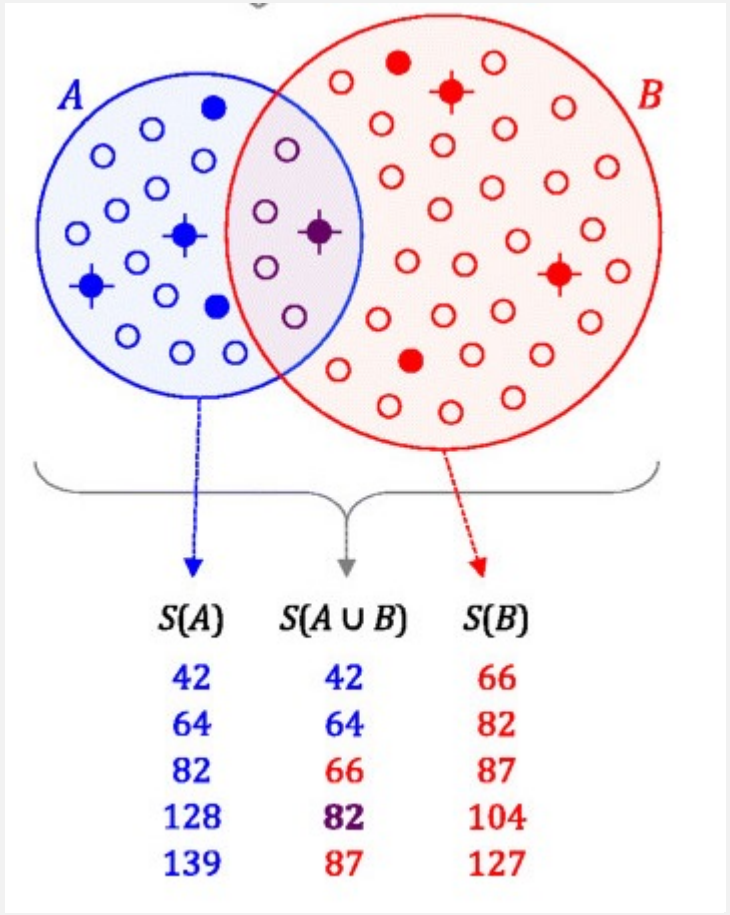

Read minimizers

Reference window minimizers

Ondov et al.

$x \in S(\mathrm{A} \cup \mathrm{B})$: $\mathrm{P}(x \in A \cap B) = Jaccard(\mathrm{Read}, \mathrm{Ref})$

$x \in A \cap B \iff x \in S(A) \wedge x \in S(B).$



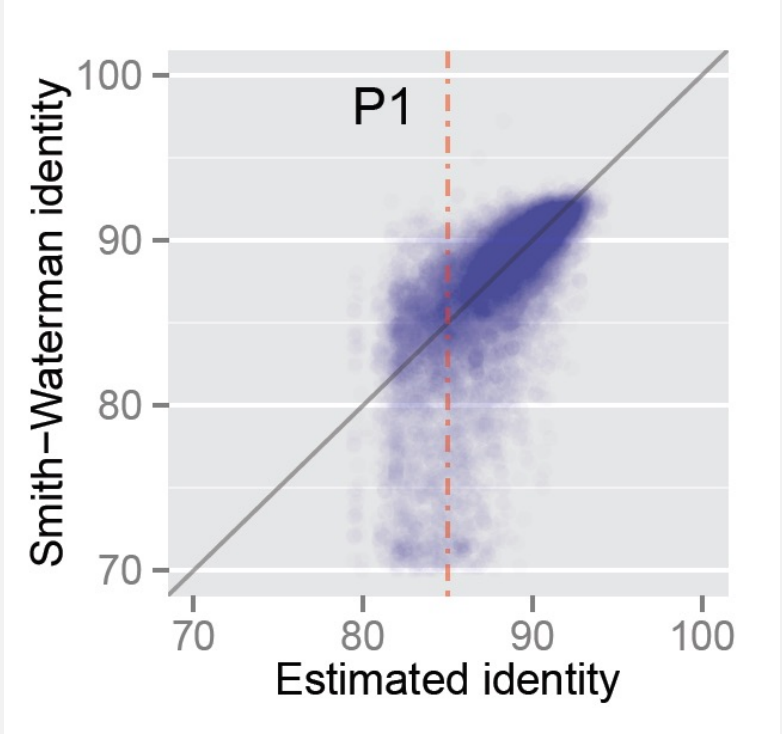

Ondov et al.

$x \in S(\mathrm{A} \cup \mathrm{B})$: $\mathrm{P}(x \in A \cap B) = Jaccard(\mathrm{Read}, \mathrm{Ref})$

$x \in A \cap B \iff x \in S(A) \wedge x \in S(B).$

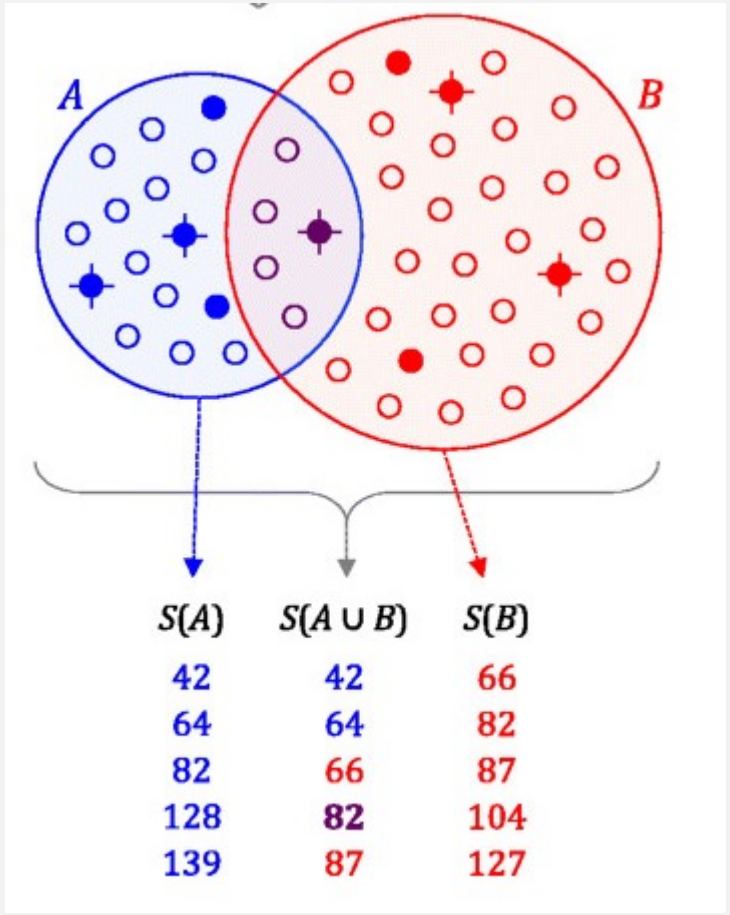
## Estimated alignment identities

MashMap, Mash




Jain et al.

**Reference genomes**




Ondov et al.

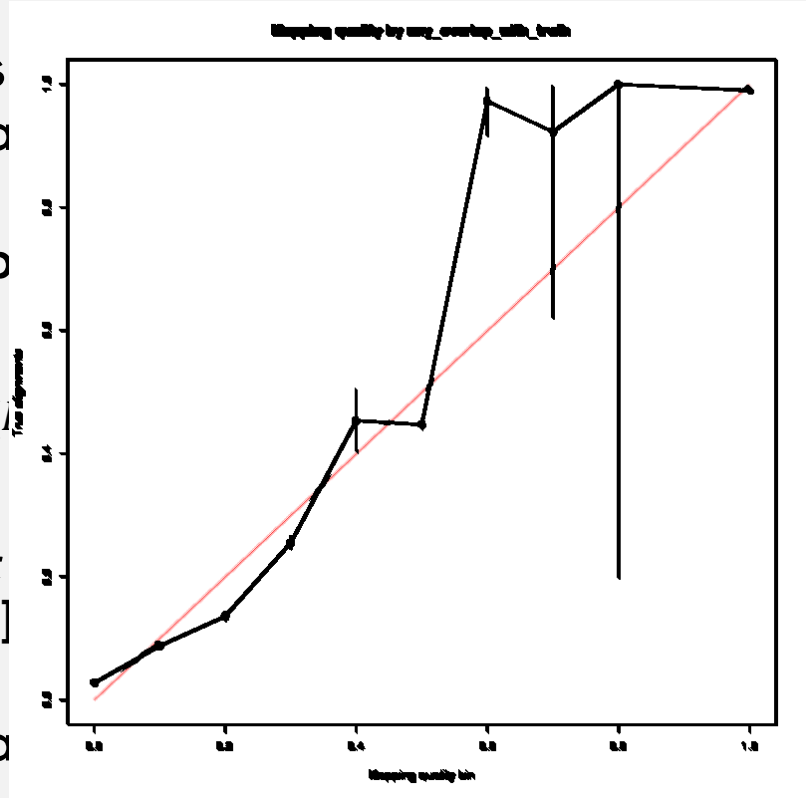
$x \in S(\mathrm{A} \cup \mathrm{B})$: $\mathrm{P}(x \in A \cap B) = Jaccard(\mathrm{Read}, \mathrm{Ref})$

$x \in A \cap B \iff x \in S(A) \wedge x \in S(B).$

## Mapping qualities

Probability distribution over mapping locations

- Ass
  Rea
  ce.
- Exp
  $E[J($
  $\frac{{}_{s} \times i^{k}}{n_{kmers} \times i^{k})}$
- P(x
  Ref)]
  d(Read,
- Rea
  t identity
- Normalize over mapping locations.



**Reference genomes**

kMer transformation

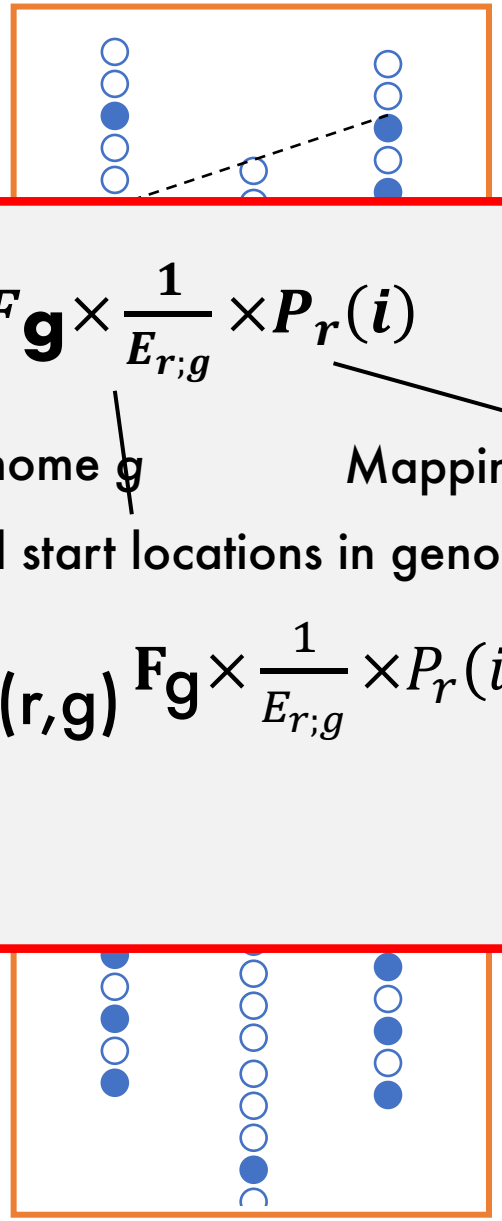
Minimizer selection & indexing

Candidate mapping regions

Sco

Sar

Read mapping locations (cond. on composition)




1. Likelihood of a mapping: $F_{\mathbf{g}} \times \frac{1}{E_{r;g}} \times P_r(i)$

Probability that read emanates from genome g

Possible read start locations in genome g

Mapping quality of location i

2. $L_G(r;\mathbf{F}) := \sum_{g \in G} \sum_{i \in map(r,g)} \mathbf{F}_g \times \frac{1}{E_{r;g}} \times P_r(i)$

3. Optimization via EM.

kMer transformation

Minimizer selection & indexing

Candidate mapping regions

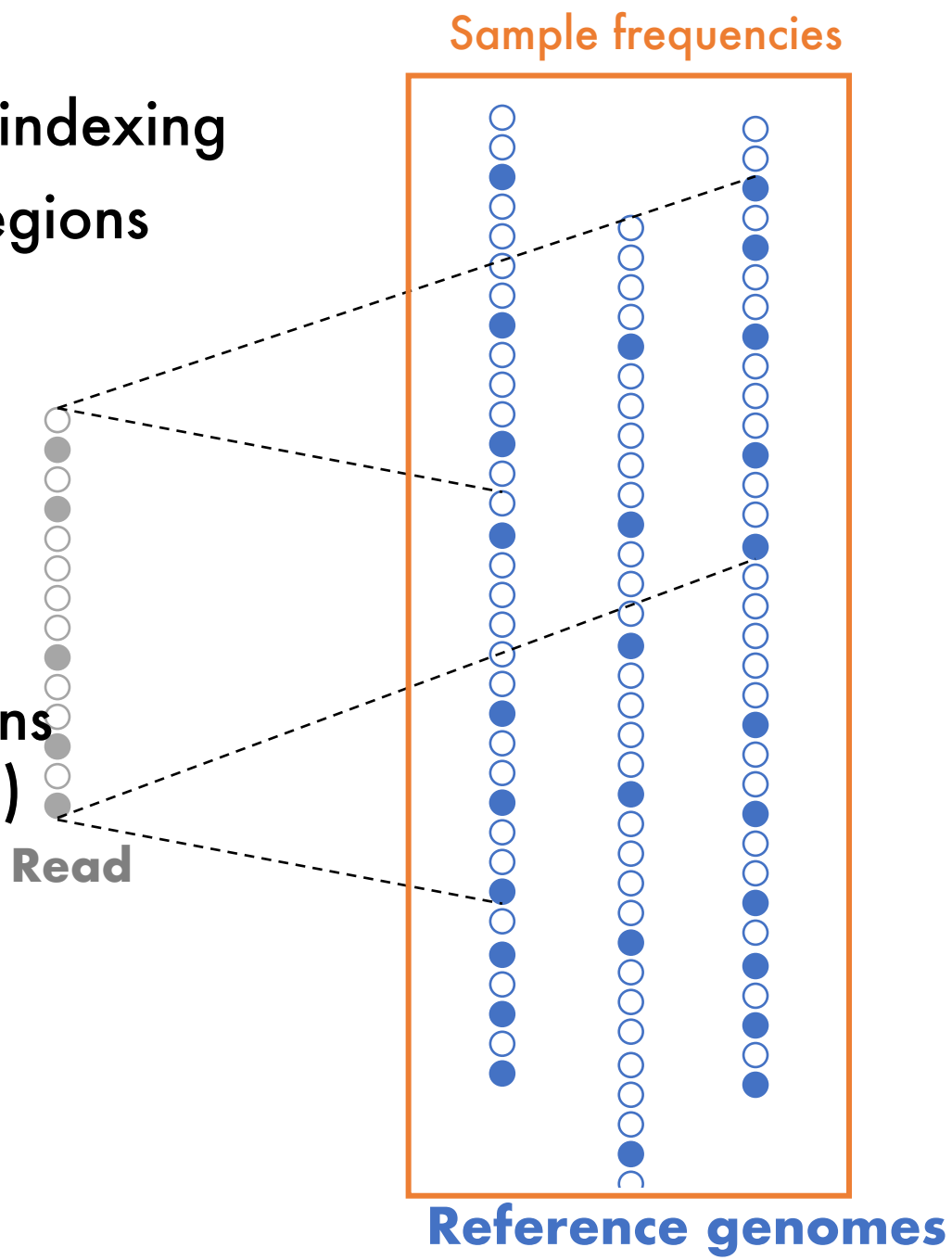
Scoring (MinHash)

Alignment idt.

Mapping quality

Sample composition

Read mapping locations (cond. on composition)



kMer transformation

Minimizer selection & indexing

Candidate mapping regions

Sco

Sar

Rec
(co

1. MetaMaps is an integrated metagenomic classification system.
2. MashMap [Jain et al. 2018], Mash [Ondov et al. 2016].
3. Auto-tuning: minimum read length & identity.
4. EM also used in Kallisto, Pathoscope, Centrifuge…

# Standard DB & evaluation

## Standard database – 25 gigabases

| | |
|---|---|
| Bacteria | 5774 |
| Viruses/viroids | 6059 |
| Archaea | 215 |
| Fungi | 7 |
| Human | 1 |
| Other eukaryotes | 2 |
| Total | 12058 |

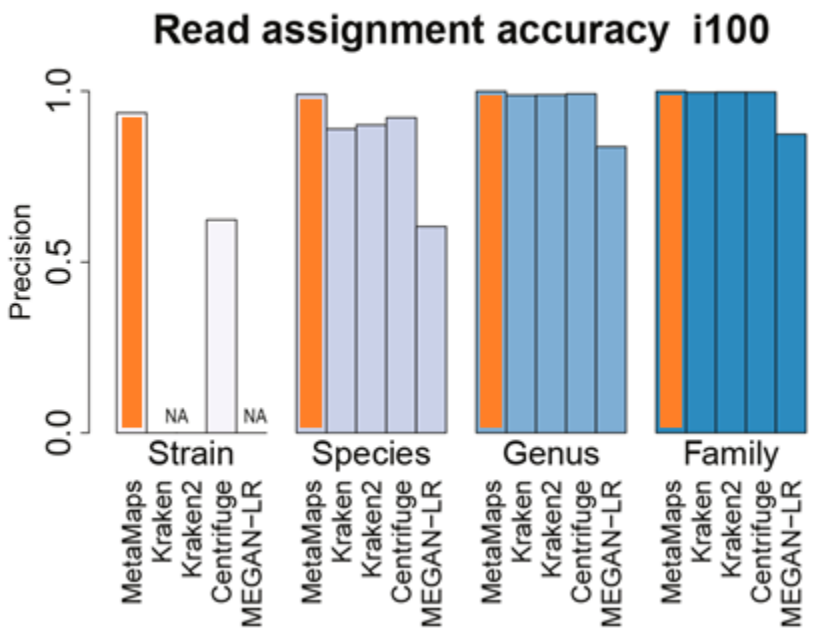
- 2 simulation experiments
  - i100: 100 different species
  - p25: 25 pathogens with 3 x 5 closely related strains
- 2 real-data experiments
  - PacBio HMP data
  - Zymo Mock Community by Loman et al
- Contamination experiments and simulated CAMI mouse gut data

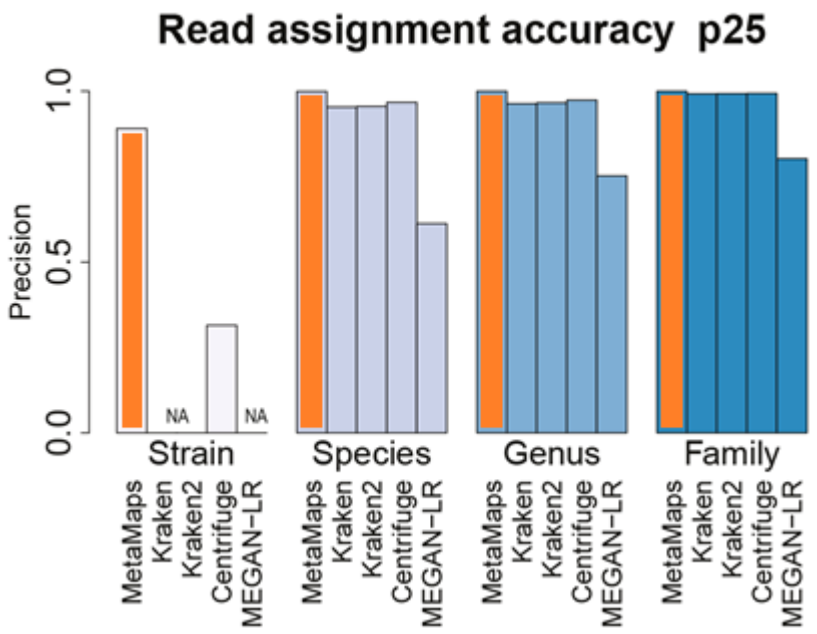
ARTICLE
https://doi.org/10.1038/s41467-019-10934-2 OPEN

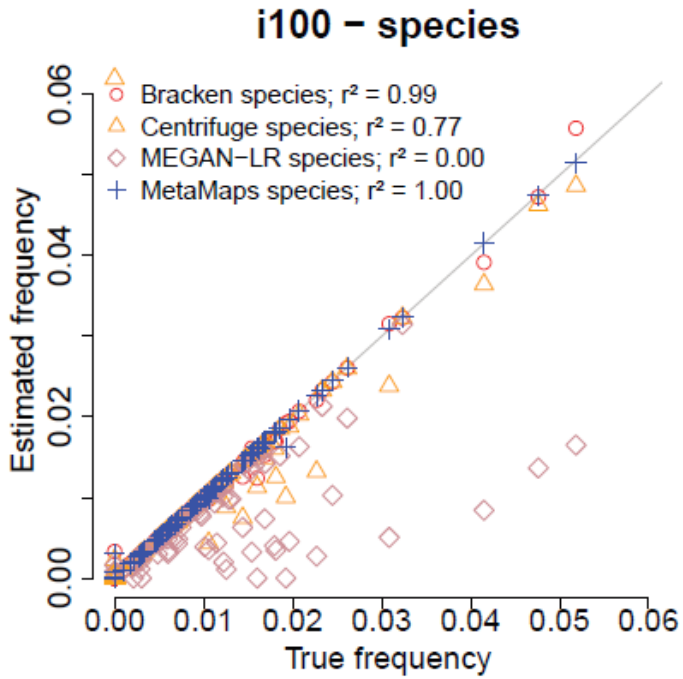
Strain-level metagenomic assignment and compositional estimation for long reads with MetaMaps

Alexander T. Dilthey[1,2], Chirag Jain[2,3], Sergey Koren[2] & Adam M. Phillippy[2]

# High accuracy on most simulated datasets



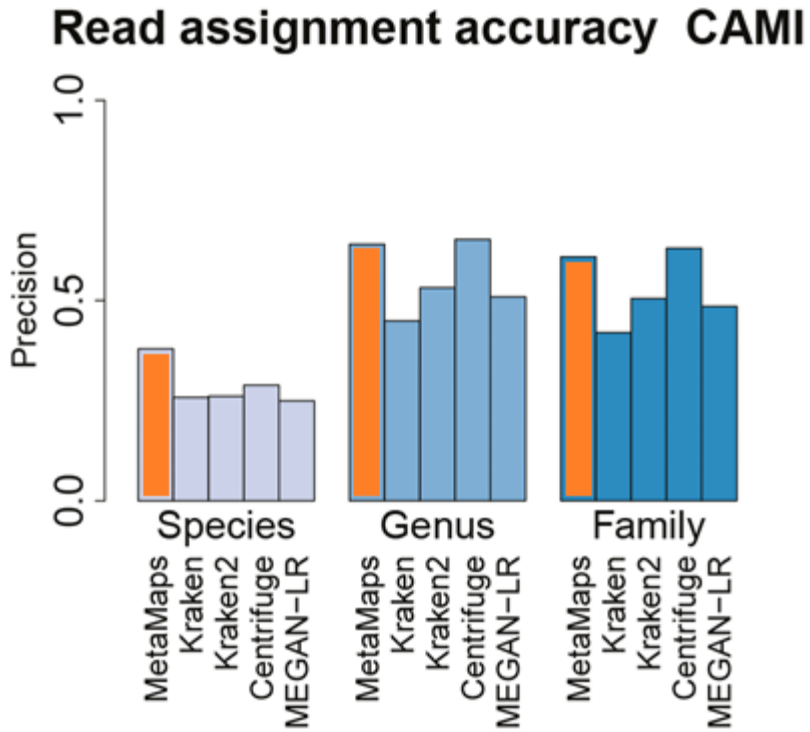



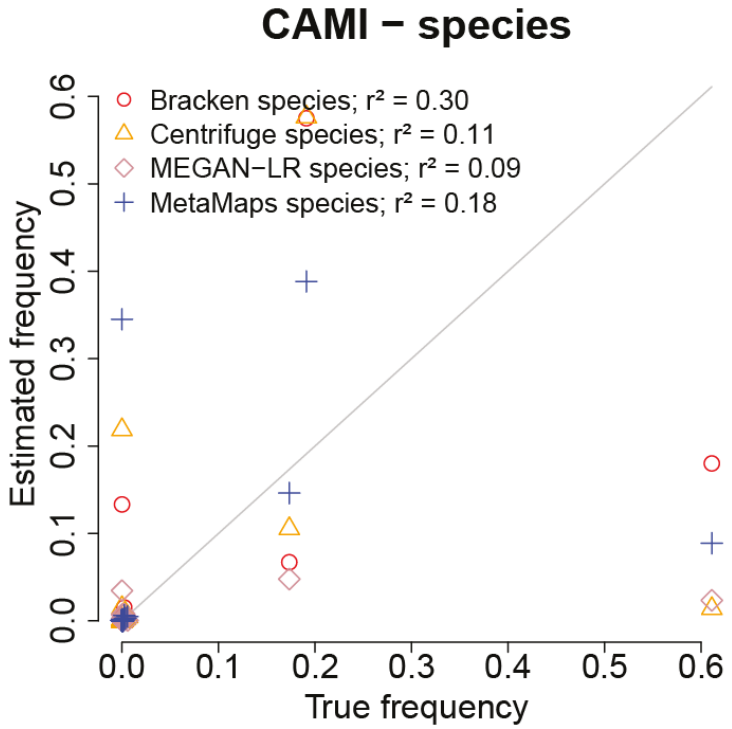

only 28% of species and 63% of genera present in the sample are represented in the database

First, the median estimated MetaMaps alignment identity in the CAMI experiment is around 82%; this is

# Very high degrees of divergence are problematic (CAMI simulated data)

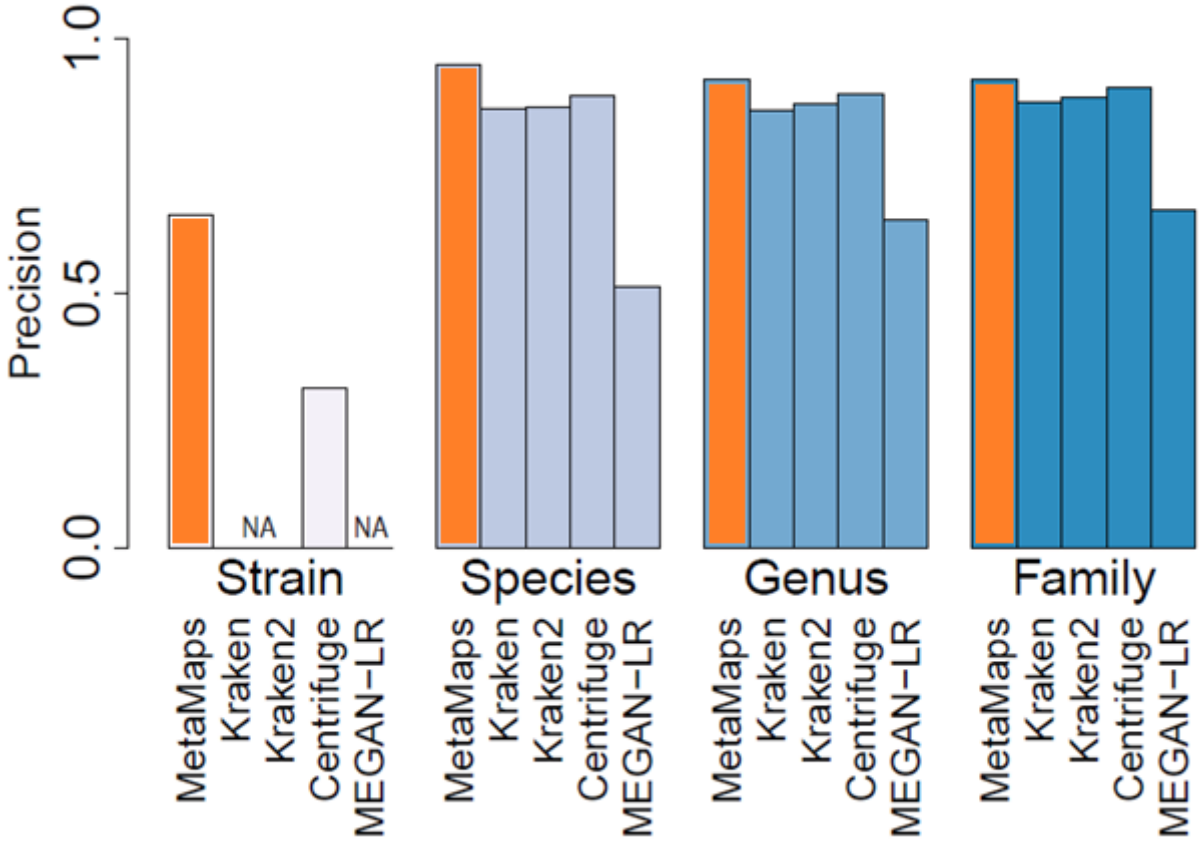
- 28% of species and 63% of genera present in the MetaMaps database
- Median estimated alignment identity ~82%.



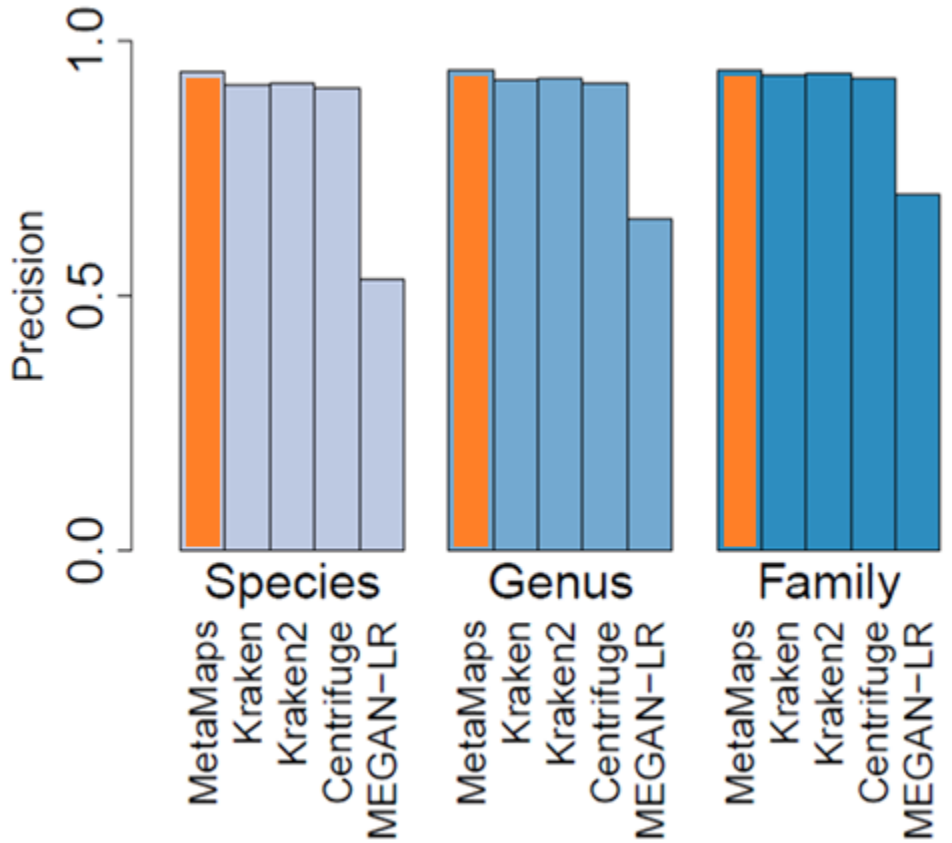



# Real data: High precision

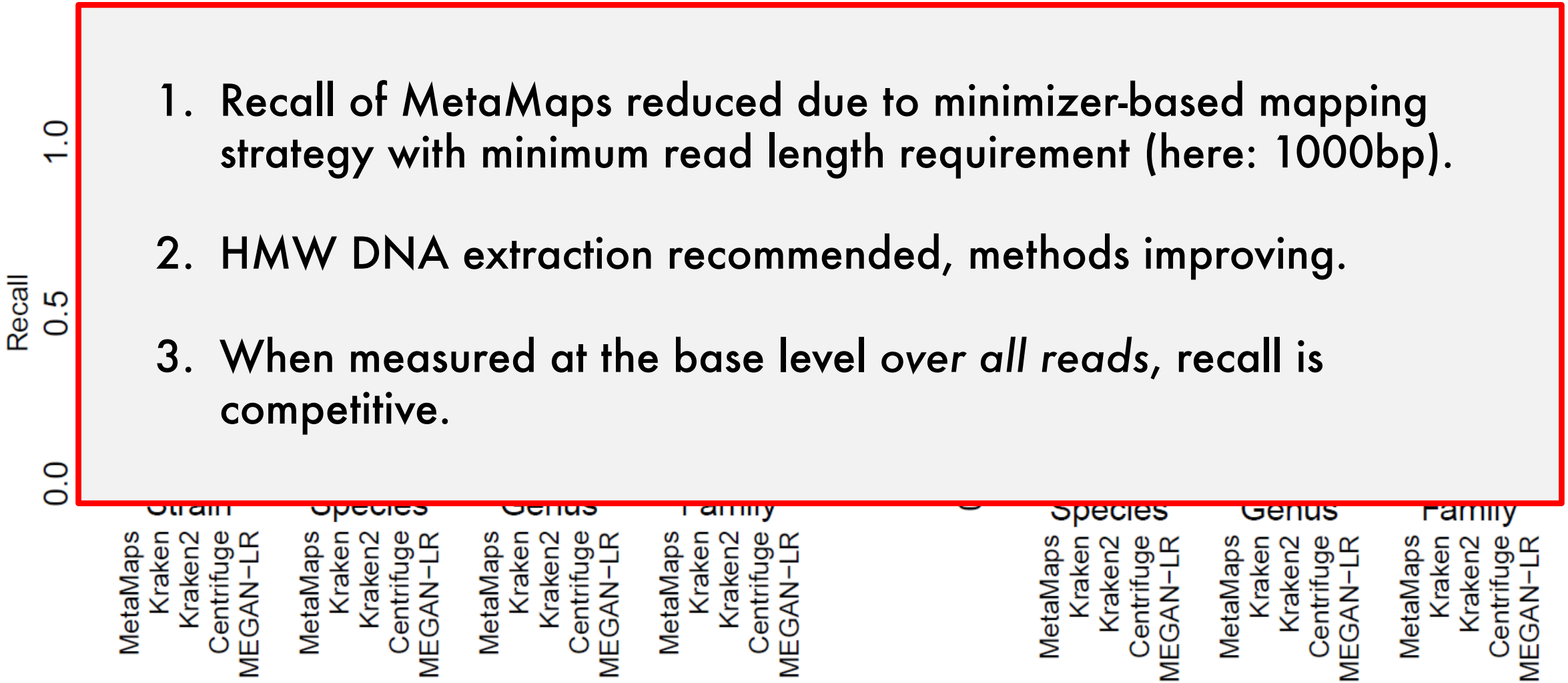
**Read assignment accuracy HMP**




**Read assignment accuracy Zymo**

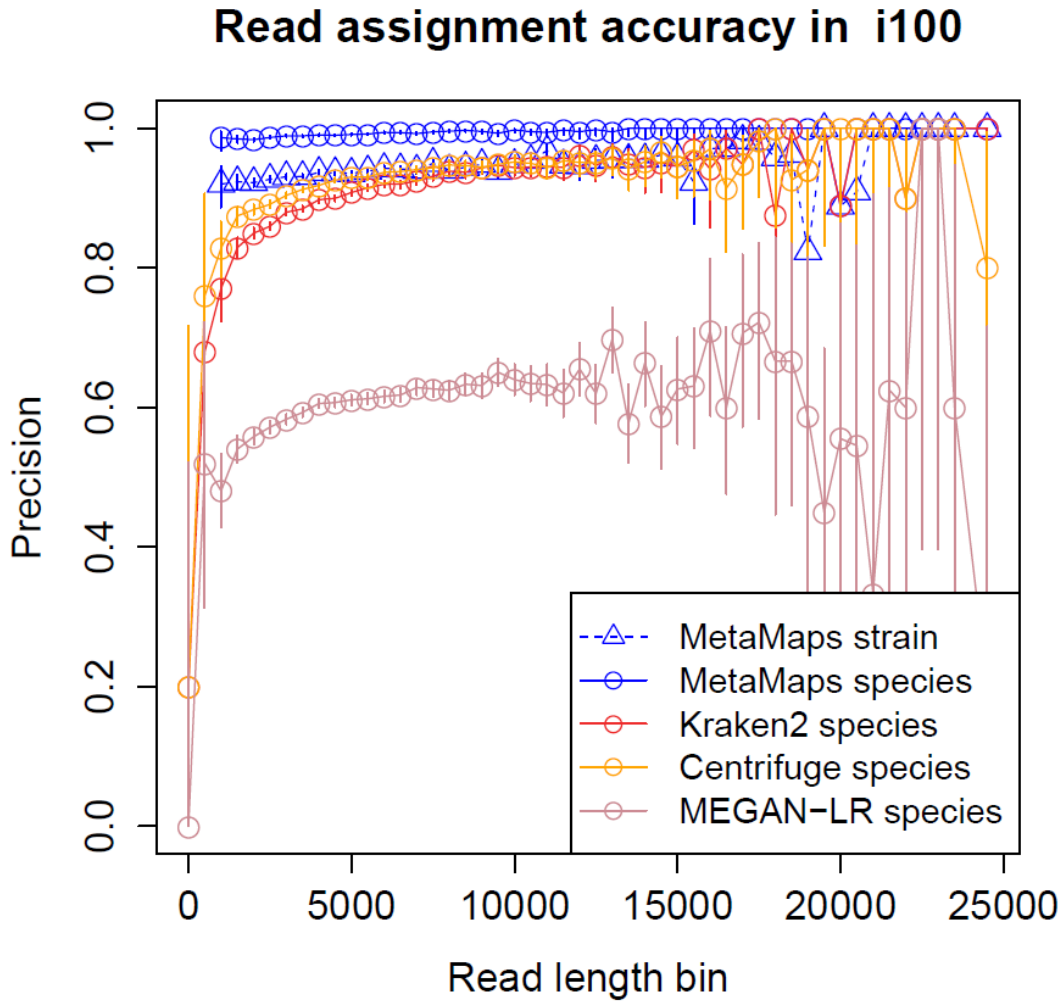


# Recall reduced due to minimum read length



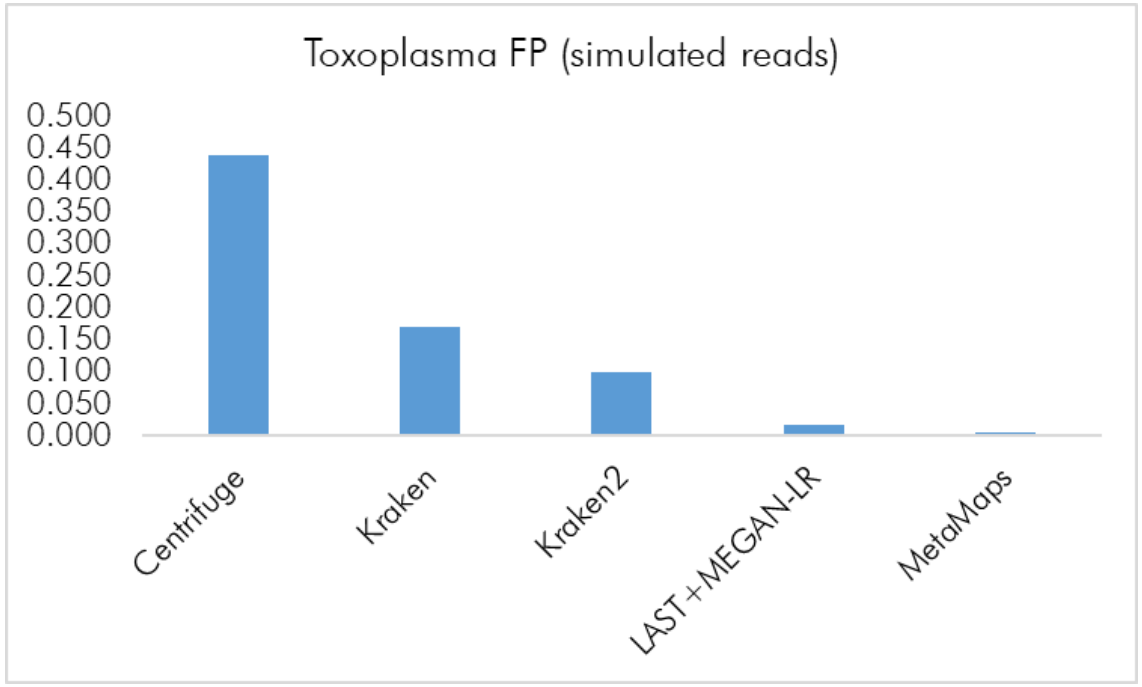

1. Recall of MetaMaps reduced due to minimizer-based mapping strategy with minimum read length requirement (here: 1000bp).
2. HMW DNA extraction recommended, methods improving.
3. When measured at the base level *over all reads*, recall is competitive.

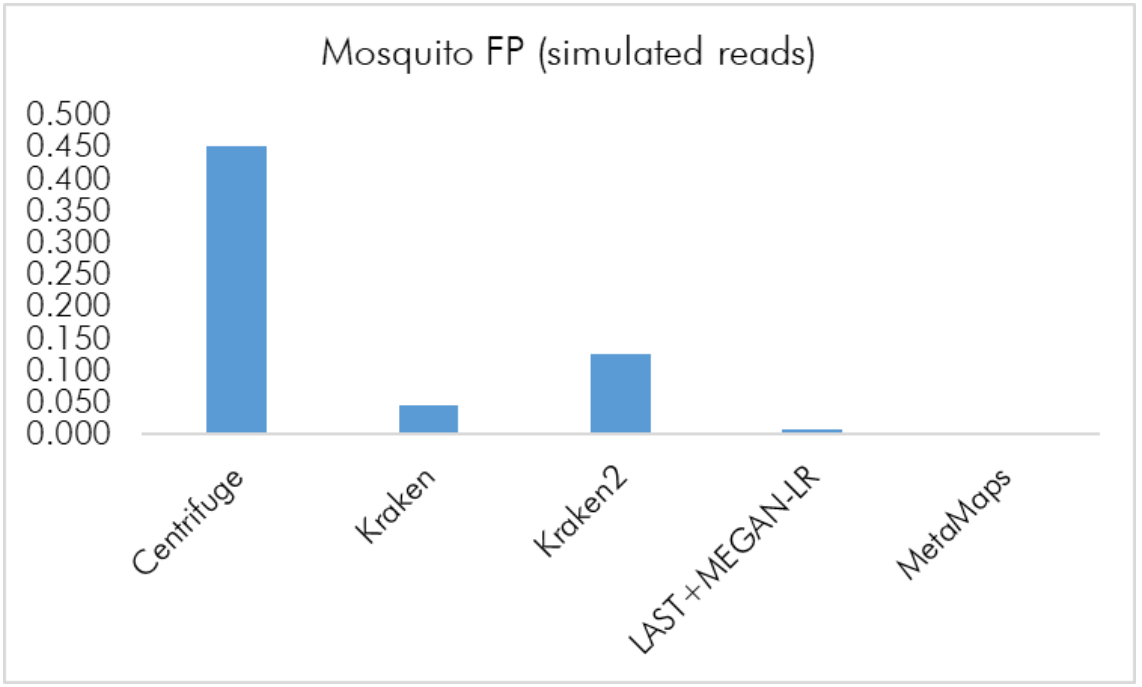
# High accuracy for „medium-length“ reads



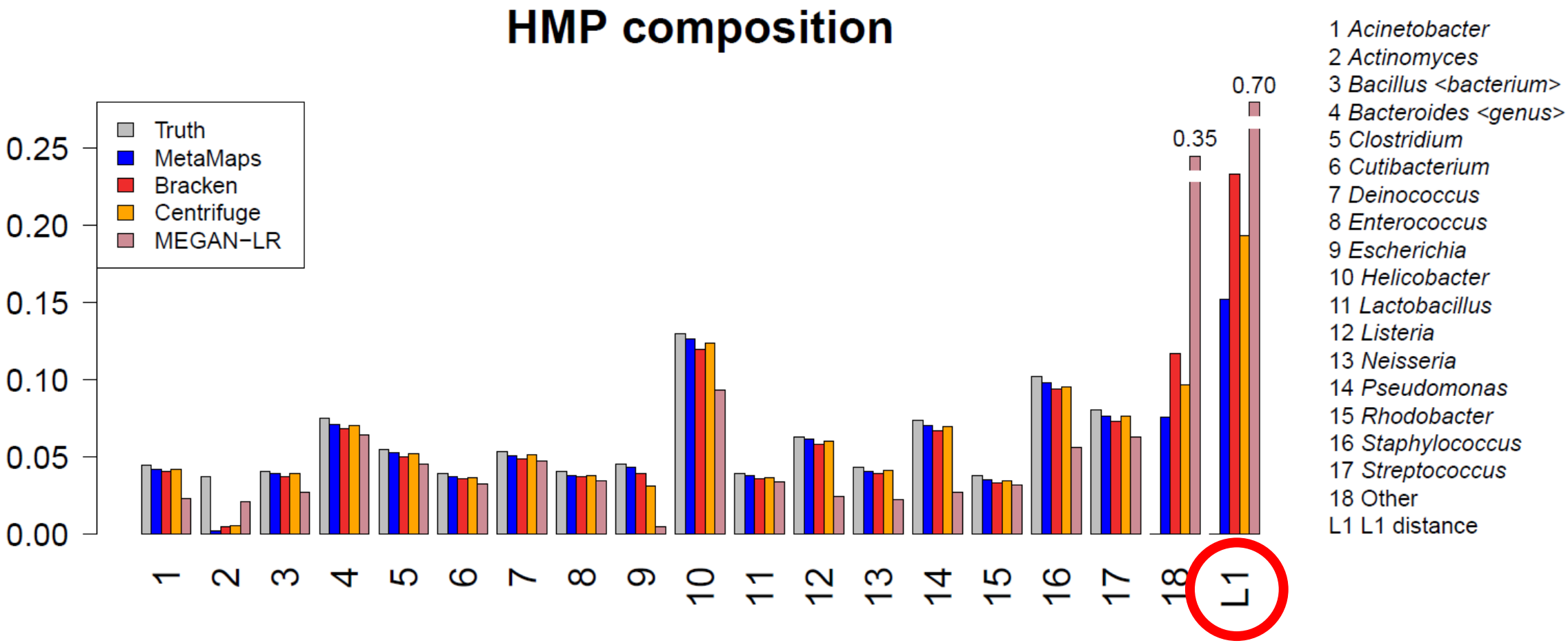

- In general: longer reads are easier to classify.
- MetaMaps achieves high accuracy from the minimum read length onwards.
- Likely cause: EM.

# Alignment-based method is more robust against large-genome contaminants

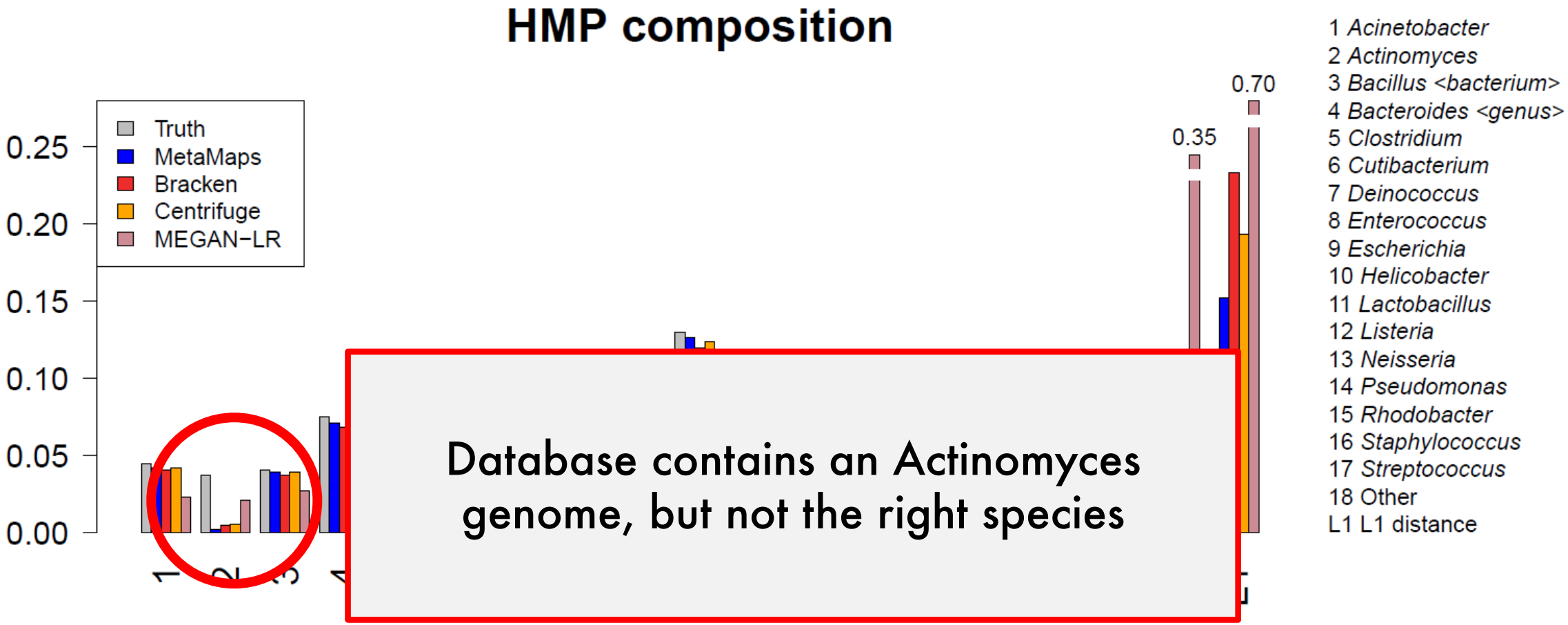


# High accuracy of compositional estimation



# Database-sample mismatches become apparent




Database contains an Actinomyces genome, but not the right species

# Database-sample mismatches become apparent




| Reads | Reads % | | COG group |
|---|---|---|---|
| 390 | 0.14% | A | RNA processing and modification |
| 399 | 0.15% | B | Chromatin structure and dynamics |
| 47263 | 17.31% | C | Energy production and conversion |
| 12889 | 4.72% | D | Cell cycle control, cell division, chromosome partitioning |
| 68289 | 25.02% | E | Amino acid transport and metabolism |
| 29636 | 10.86% | F | Nucleotide transport and metabolism |
| 58230 | 21.33% | G | Carbohydrate transport and metabolism |
| 31347 | 11.48% | H | Coenzyme transport and metabolism |
| 26591 | 9.74% | I | Lipid transport and metabolism |
| 49020 | 17.96% | J | Translation, ribosomal structure and biogenesis |
| 70430 | 25.80% | K | Transcription |
| 61699 | 22.60% | L | Replication, recombination and repair |
| 59690 | 21.87% | M | Cell wall/membrane/envelope biogenesis |
| 12514 | 4.58% | N | Cell motility |
| 37550 | 13.76% | O | Post-translational modification, protein turnover, and chaperones |
| 55657 | 20.39% | P | Inorganic ion transport and metabolism |
| 13808 | 5.06% | Q | Secondary metabolites biosynthesis, transport, and catabolism |
| 154932 | 56.76% | S | Function unknown |
| 33782 | 12.38% | T | Signal transduction mechanisms |
| 18251 | 6.69% | U | Intracellular trafficking, secretion, and vesicular transport |
| 24103 | 8.83% | V | Defense mechanisms |
| 408 | 0.15% | W | Extracellular structures |
| 116 | 0.04% | Y | Nuclear structure |
| 131 | 0.05% | Z | Cytoskeleton |

# Long-read benchmarking

- Difference between „reads correct“ and „bases correct“
- Even with no DB/sample mismatches, simulated accuracy > empirical accuracy
  [sensitivity and recall, >5%].
- Rapid development: Good simulators for current Nanopore and PacBio CCS?
- More complex empirical datasets with known truth urgently needed (ultra-deep PromethION + *de novo* assembly?)

# Conclusion

- Integrated solution: Assignment, composition, mapping locations.
- Fast minimzer-based mapping plus probabilistic mapping qualities.
  Enables statistical modeling of sample composition via EM.
  Unique / high-confidence alignments serve as „attractors“ for ambiguous reads.
- Auto-tuning: Will improve with longer, higher-quality reads (e.g. PacBio CCS).
- Future work: speed; prediction of novel species.

# Acknowledgements and funding

https://github.com/DiltheyLab/MetaMaps


NHGRI-NIH:

- **Chirag Jain**
- **Sergey Koren**
- **Adam Phillippy**
- **Brian Ondov**
- Arang Rhie
- Brian Walenz

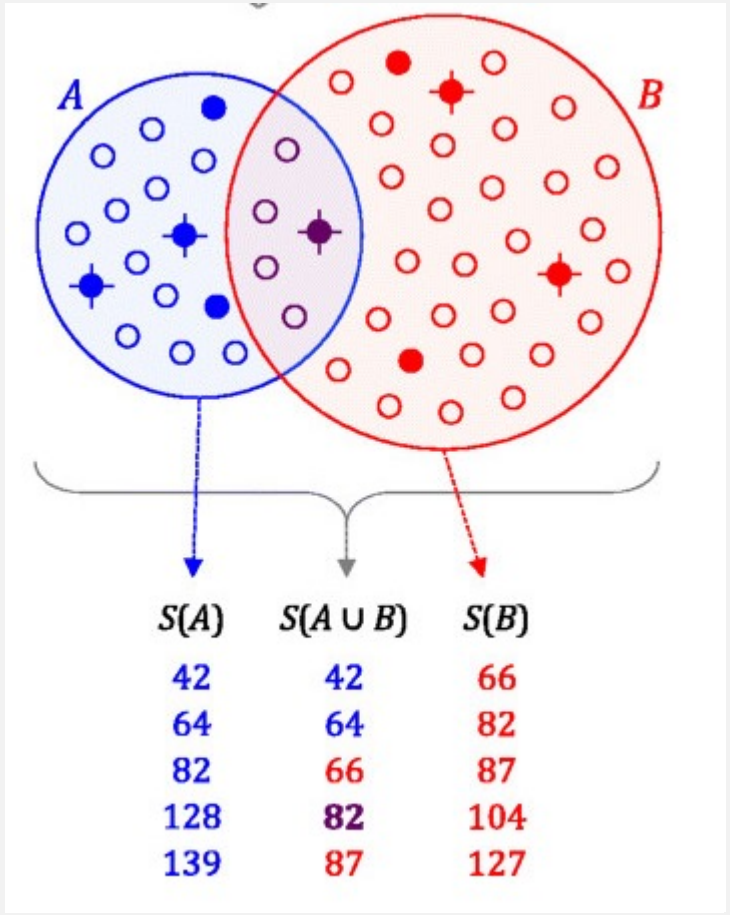
HHU Düsseldorf:

- Sebastian Scharf
- Daniel Strelow
- Alona Tyshaieva
- Birgit Henrich

Georgia Institute of Technology:

- **Chirag Jain**
- **Srinivas Aluru**



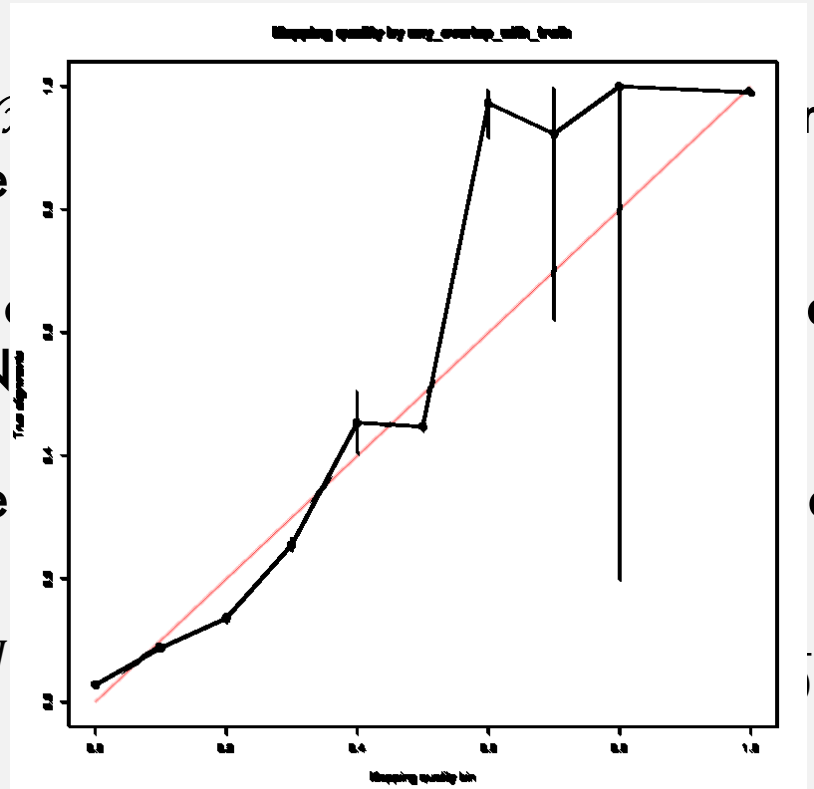

Ondov et al.

$x \in S(\mathrm{A} \cup \mathrm{B})$: $\mathrm{P}(x \in A \cap B) = Jaccard(\mathrm{Read}, \mathrm{Ref})$

$x \in A \cap B \iff x \in S(A) \wedge x \in S(B).$

## Mapping qualities

Probability distribution over mapping locations



- P(
- Ja
- Re
- $E[J$
- Normalize over mapping locations.

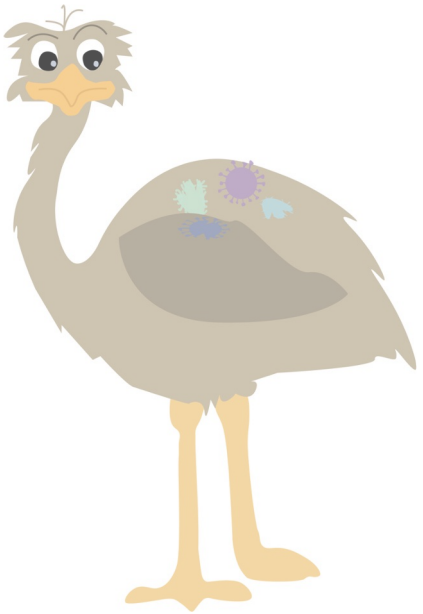
rd(Read, Re

ate (N

dentity

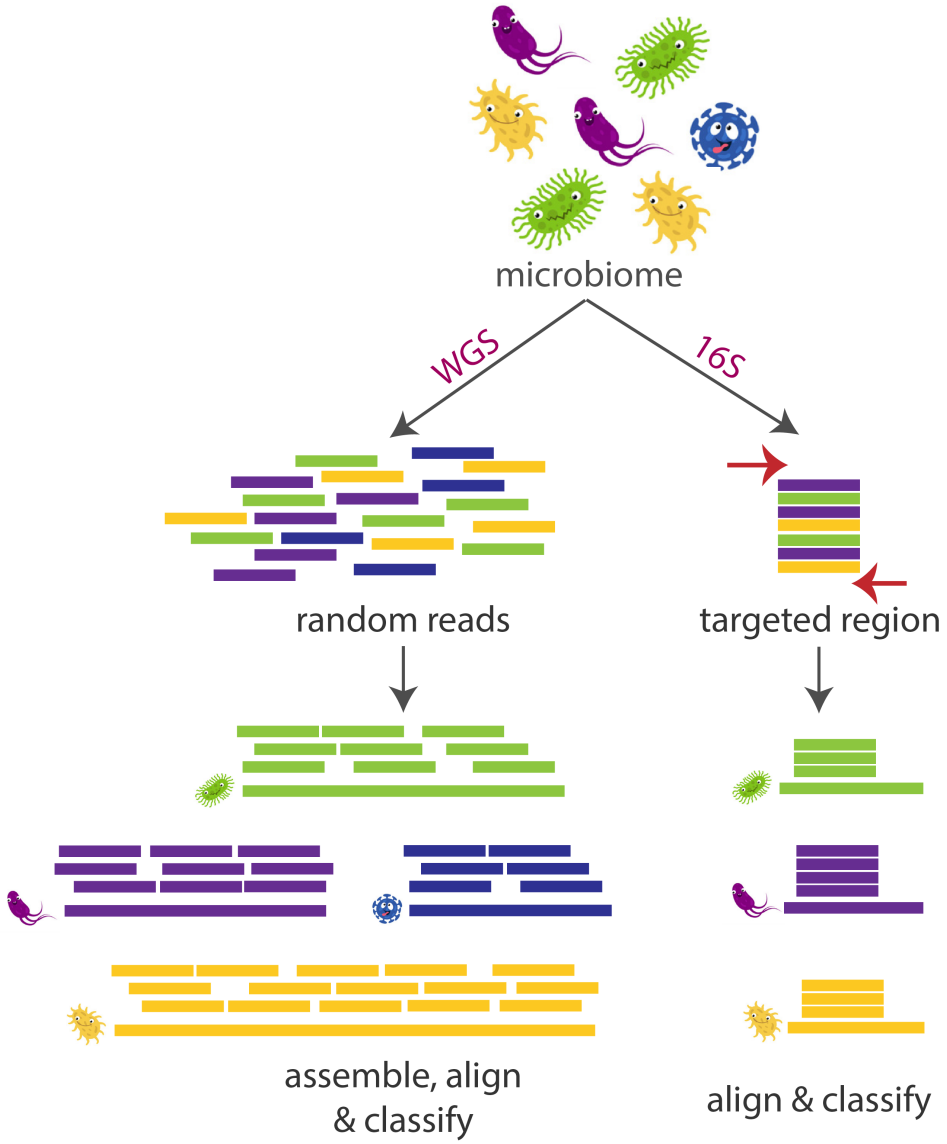
**Reference genomes**

# **Emu:** species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data



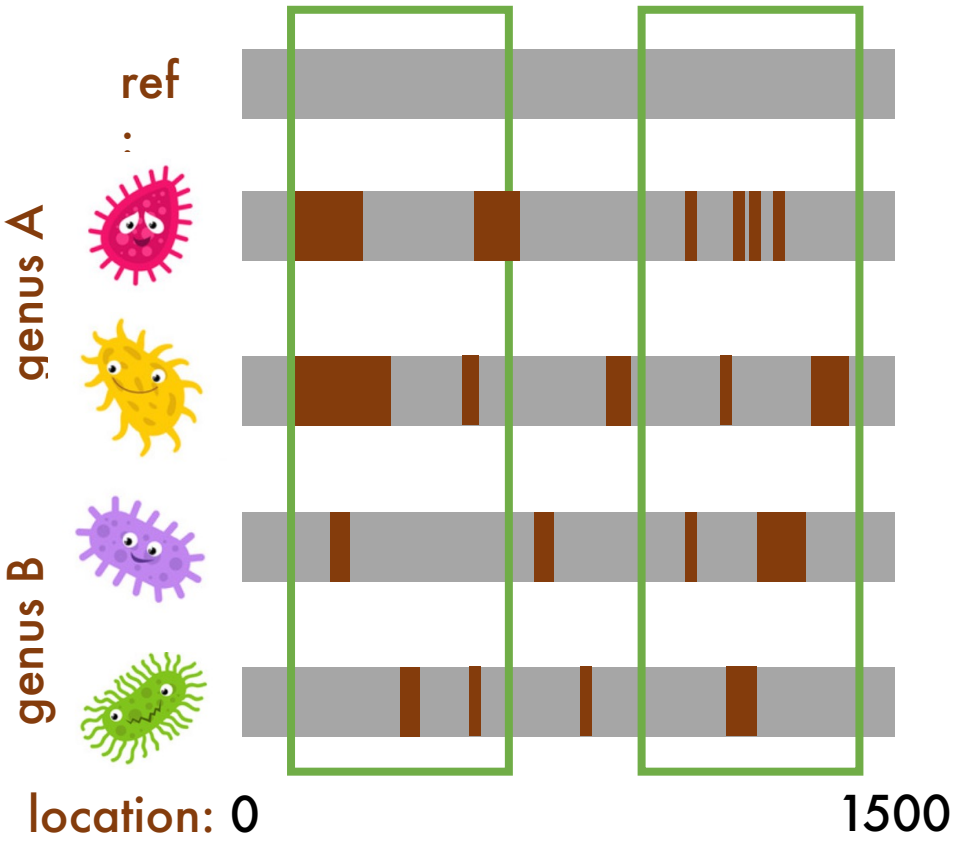
Kristen Curry, Rice University PhD student
*Treangen Lab; Dept. Computer Science*
Dilthey Lab; Institute of Medical Microbiology,
University Hospital of Düsseldorf Germany

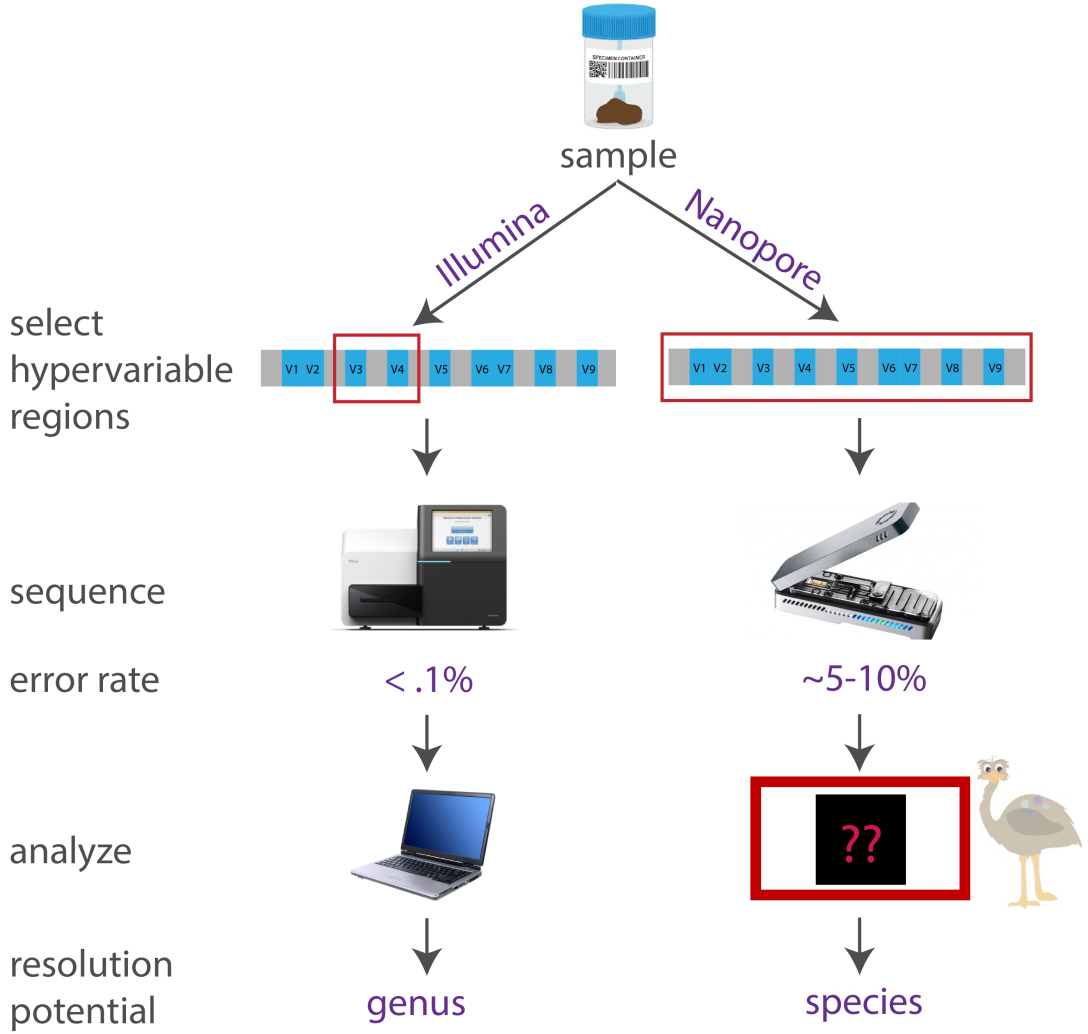
# Shotgun [WGS] vs. 16S Sequencing




| | Shotgun | 16S |
|---|---|---|
| kingdoms | all | bacteria & archea |
| composition | definite | relative |
| sequencing depth | high | shallow |
| computation | complex | direct |
| cost | $$$ | $ |
| accessbility | outsource | in-house |

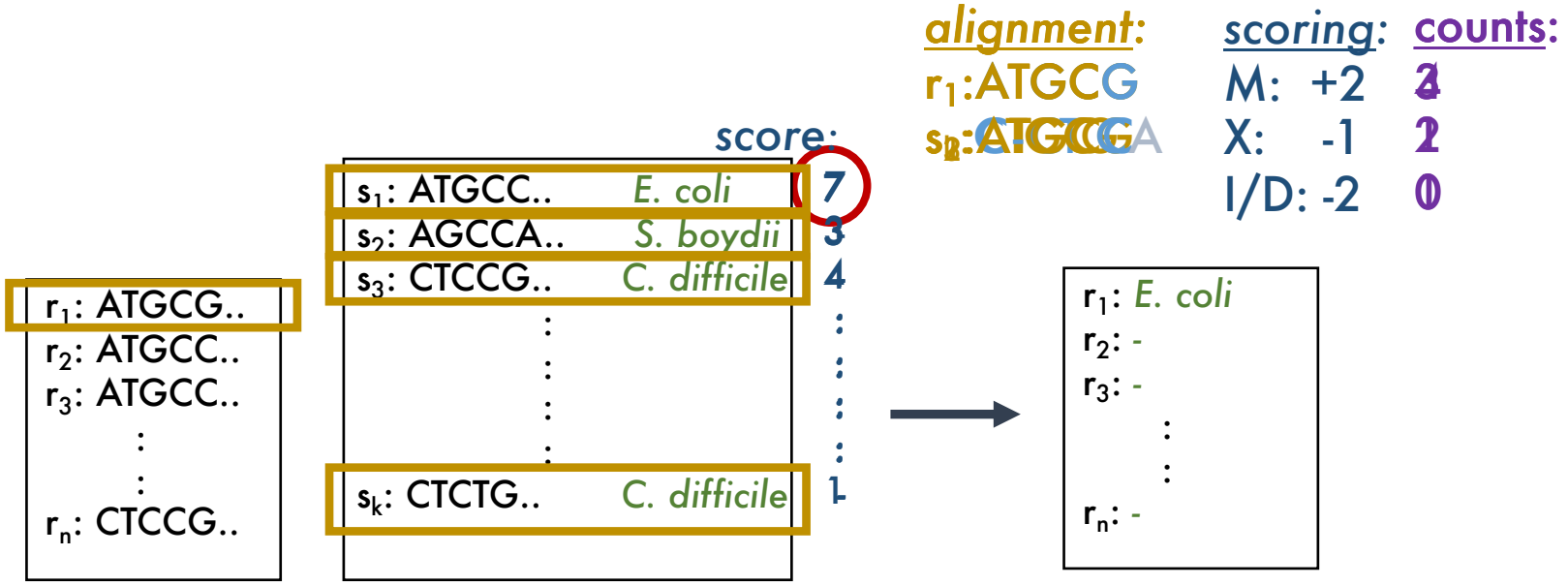
# Sequencing technologies



$r_1$: ATGCT..
$r_2$: ATTGG..
:
:
$r_n$: AGTGC..

# Reference-based classification



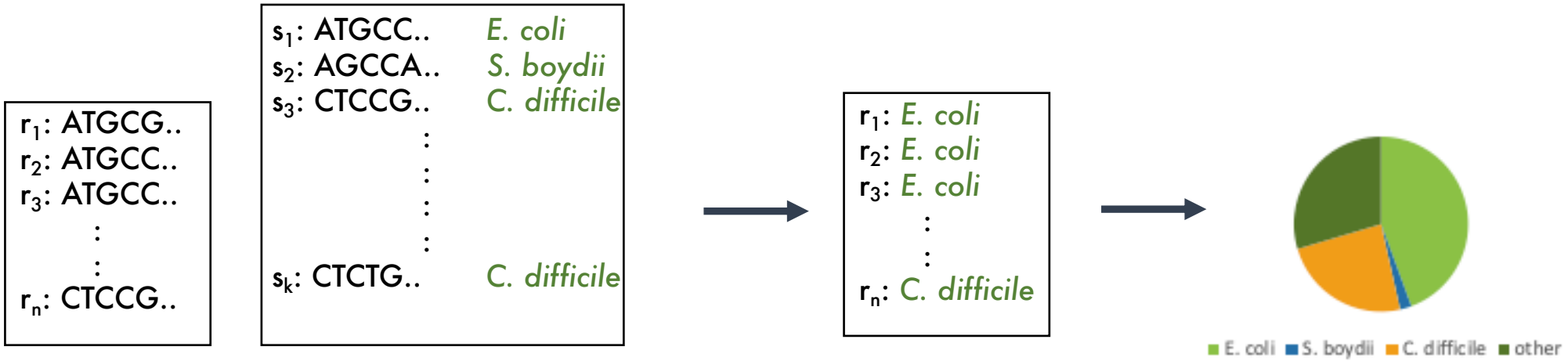

reads

reference database

classification

$r_1$: ATGCT..
$r_2$: ATTGG..
:
:
$r_n$: AGTGC..

# Reference-based classification




reads | reference database | classification | community profile


# Reference-based classification with errors

*Oxford Nanopore Technologies MinION**

"anywhere, anyone, anytime"

- portable
- real-time
- cost efficient
- **high error**

Emu

$r_1$: ATGCTG..
$r_2$: ATGCC..
$r_3$: ATGCG..
:
:
$r_n$: CTCCG..

reads

| | |
|---|---|
| $s_1$: ATGCC.. | *E. coli* |
| $s_2$: AGCCA.. | *S. boydii* |
| $s_3$: CTCCG.. | *C. difficile* |
| : | |
| $s_k$: CTCTG.. | *C. difficile* |

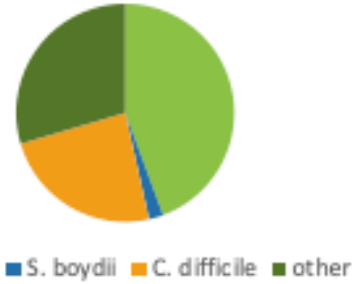
reference database

*E. coli*

*expectation-maximization error-correction*

$r_1$: *E. coli*
$r_2$: *S. boydii*
$r_3$: *E. coli*
:
:
$r_n$: *C. difficile*

classification

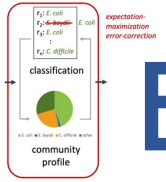




community profile

"An unknown read is **MORE** likely to be a bacteria that is already in the sample"

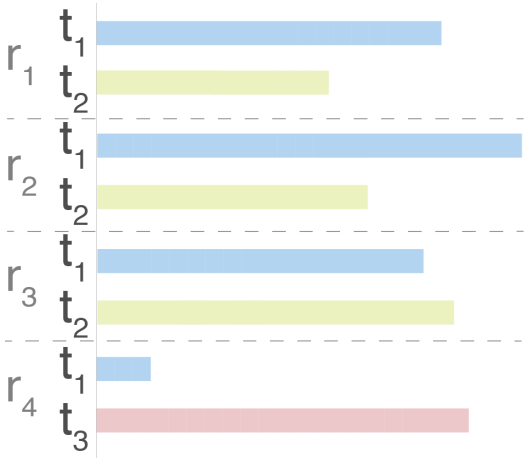


*Andrea D. Tyler et al., "Evaluation of Oxford Nanopore's MinION Sequencing Device for Microbial Whole Genome Sequencing Applications," *Scientific Reports* 8, no. 1 (July 19, 2018): 10931

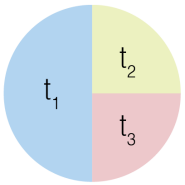



# EM in Emu

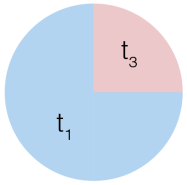
## Alignment scores




best hit

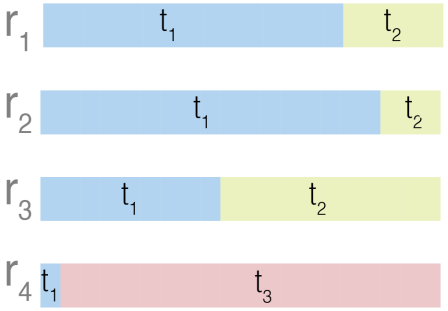



truth

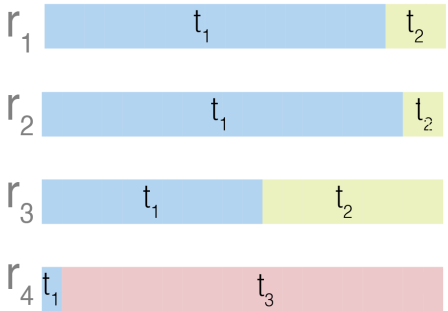



iteration 1

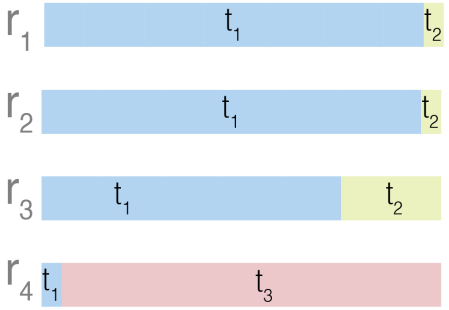
classification likelihood




iteration 2

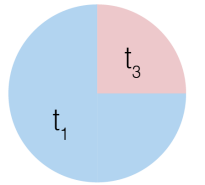



iteration 3




"A read is **MORE** likely to be a bacteria that is in the sample in **HIGHER** abundance"

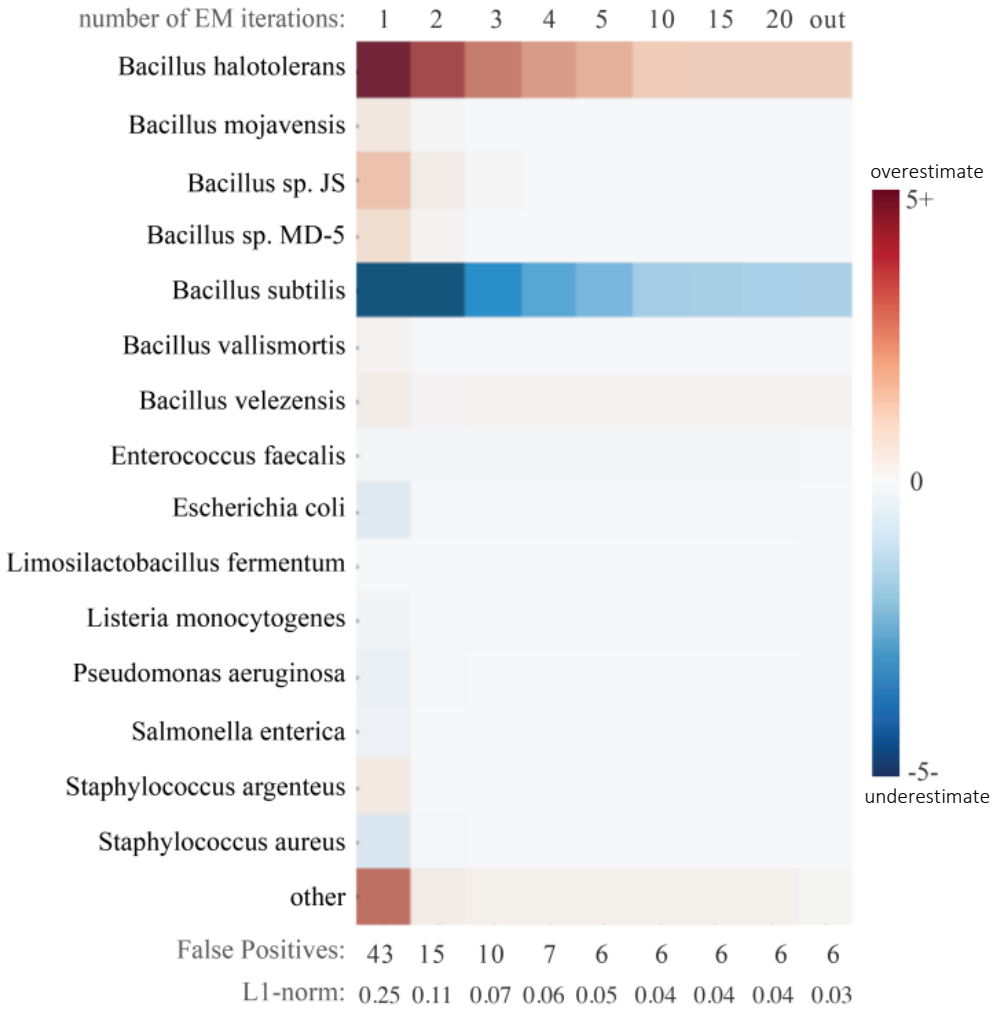
trim noise



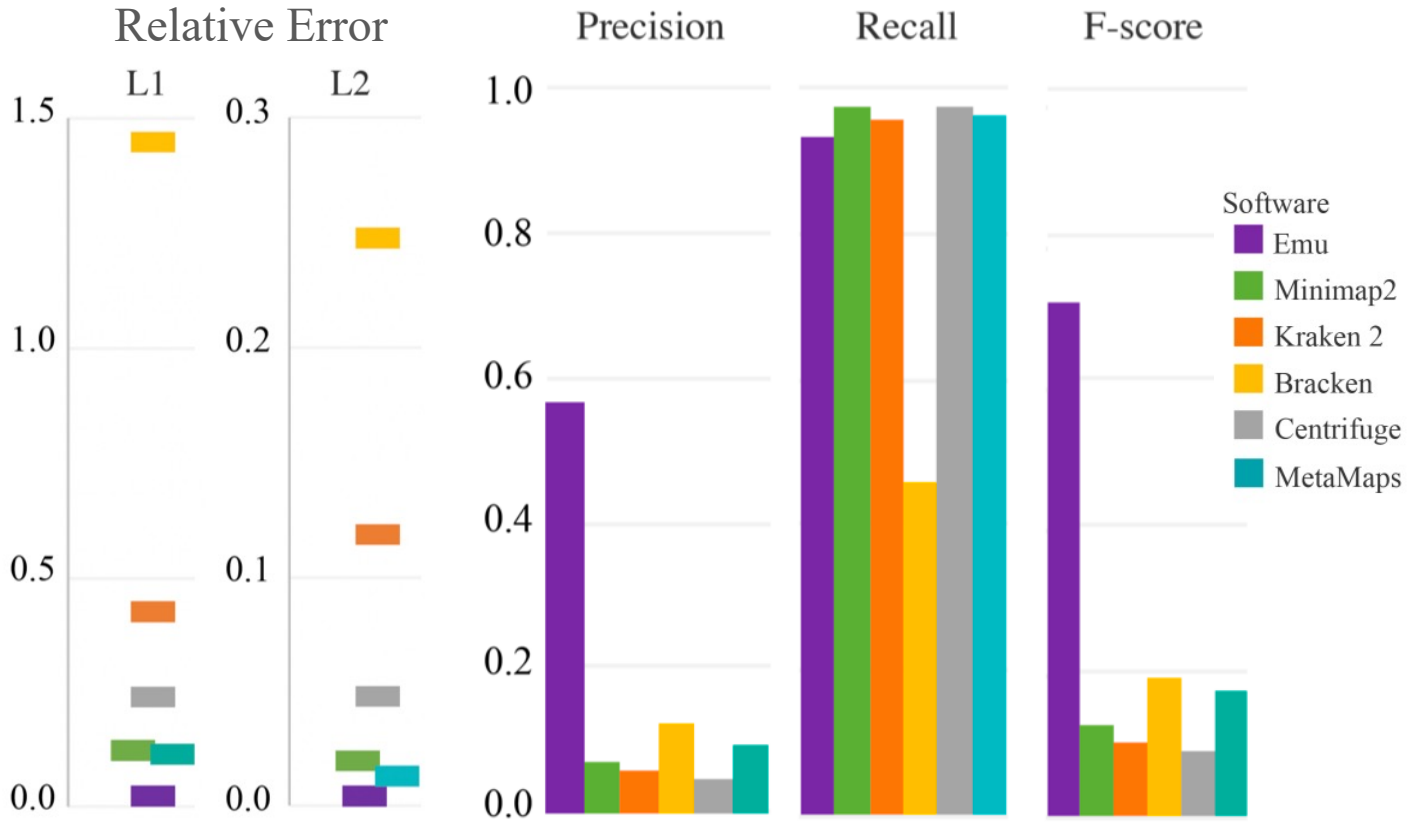

Emu output

# Test data results

## Error throughout EM iterations in Emu



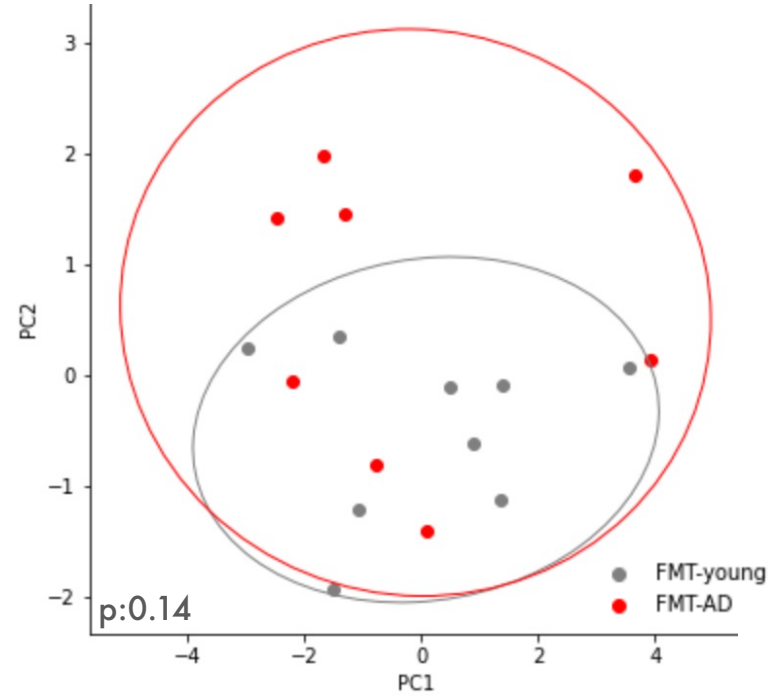

## Classification software comparison

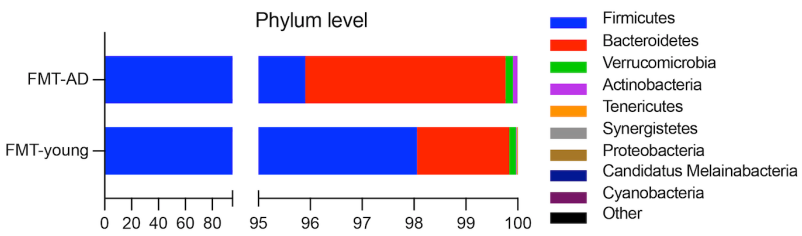



*Critical Assessment of Metagenome Interpretation (CAMI) 2 Mouse Gut*

# Emu in gut microbiome studies

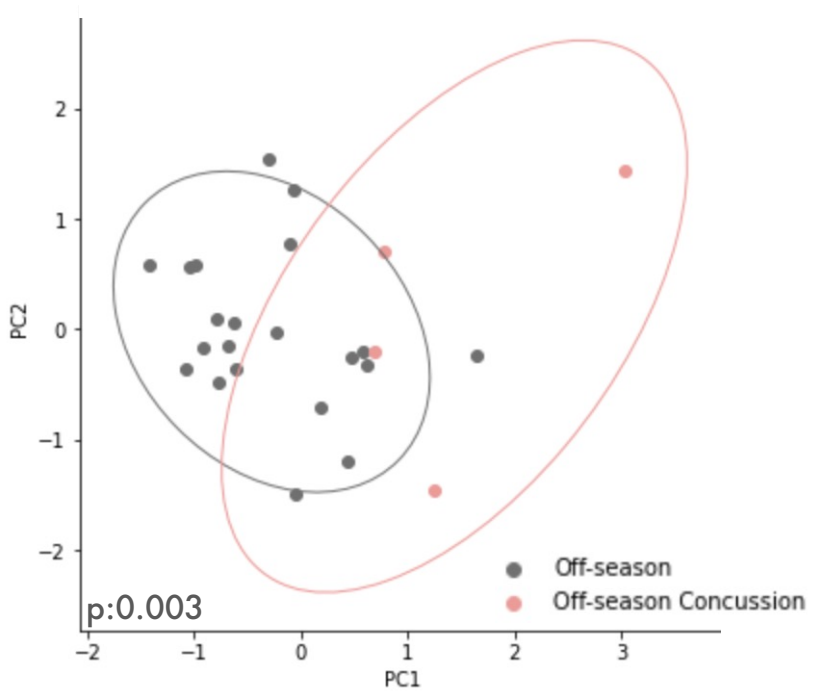
## *Alzheimer's Disease*

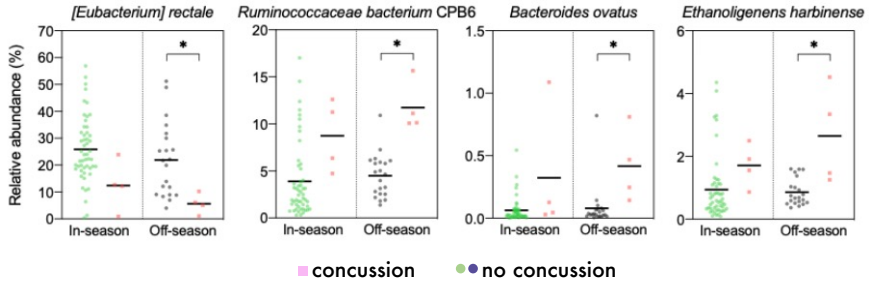



Soriano, Sirena et al. "Fecal Microbiota Transplantation Derived from Alzheimer's Disease Mice Worsens Brain Trauma Outcomes in Young C57BL/6 Mice," *medRxiv*, November 23, 2021.

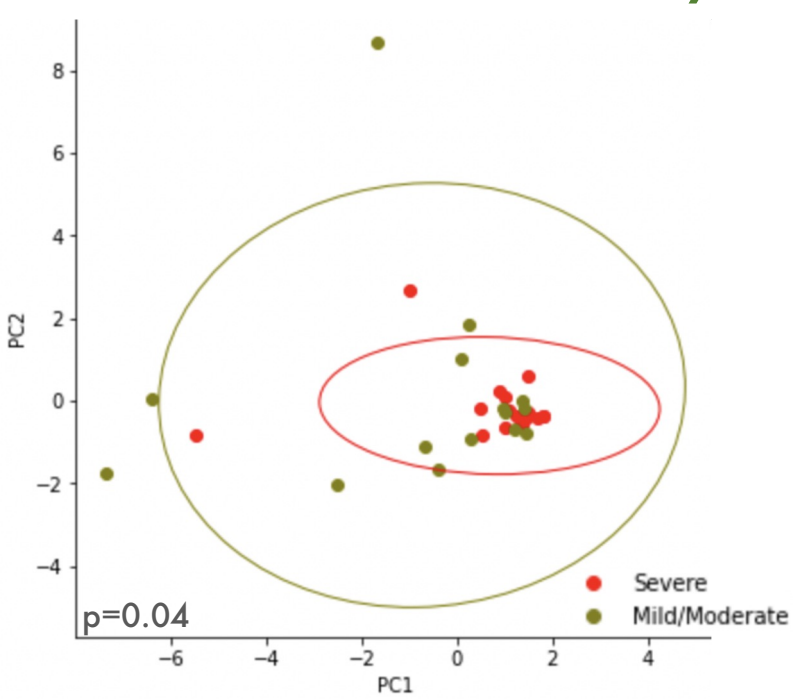
## *Concussion*

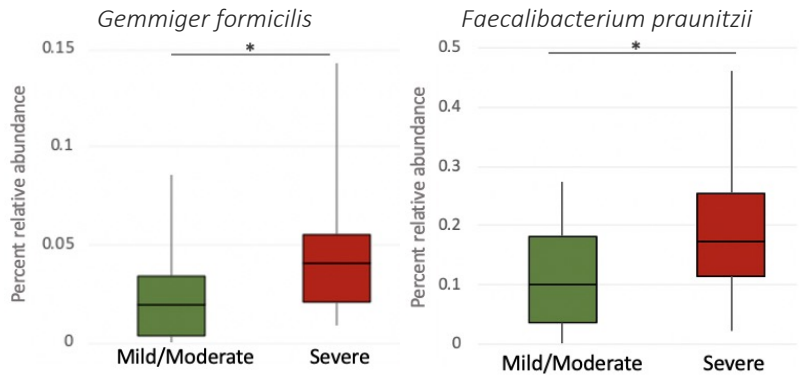



Soriano, Sirena, et al. "Alterations to the Gut Microbiome after Sport-Related Concussion and Subconcussive Impacts in a Collegiate Football Players Cohort," *medRxiv*, July 14, 2021.

## *COVID-19 severity*

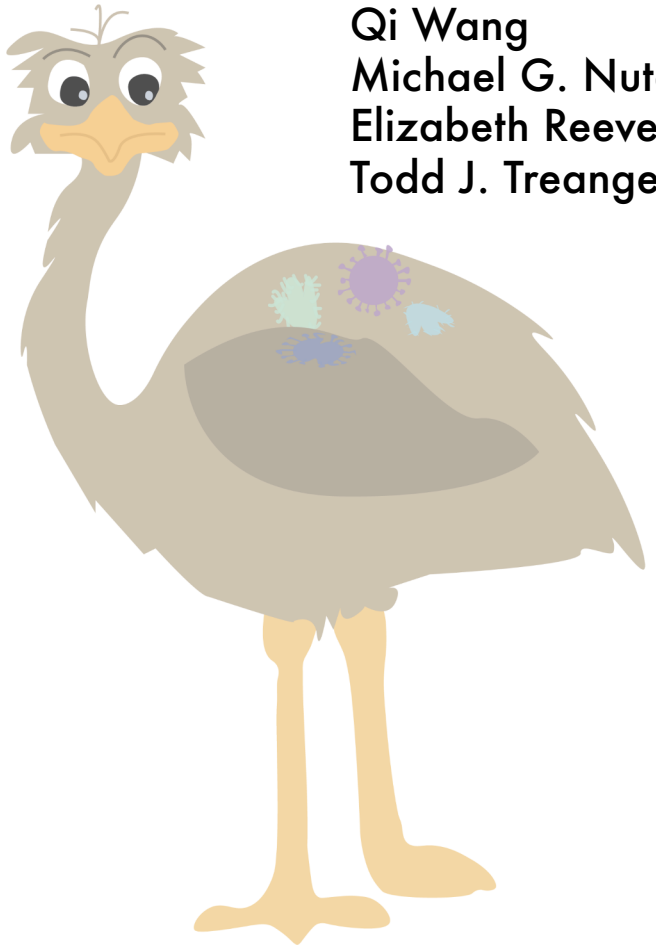



Villapol Lab, Houston Methodist Research Institute

# Acknowledgements

## Emu – profile microbial communities with error-prone 16S reads




[Treangen Lab - Rice University](#)
Qi Wang
Michael G. Nute
Elizabeth Reeves
Todd J. Treangen

[Heinrich Heine University Düsseldorf](#)
Alexander Dilthey
Alona Tyshaieva
Enid Graeber
Patrick Finzer

[Helios University Clinic Wuppertal](#)
Werner Mendling

[Villapol Lab - Houston Methodist Research Institute](#)
Sirena Soriano
Sonia Villapol

[Savidge Lab - Baylor College of Medicine](#)
Qinglong Wu
Tor Savidge



Article | Published: 30 June 2022


**Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data**


Kristen D. Curry, Qi Wang, Michael G. Nute, Alona Tyshaieva, Elizabeth Reeves, Sirena Soriano, Qinglong Wu, Enid Graeber, Patrick Finzer, Werner Mendling, Tor Savidge, Sonia Villapol, Alexander Dilthey & Todd J. Treangen



*Nature Methods* **19**, 845–853 (2022) | Cite this article




https://gitlab.com/treangenlab/emu