Pontificia Universidad Católica del Ecuador

Universidad de los Andes

ANÁLISIS DE DATOS DE SECUENCIACIÓN

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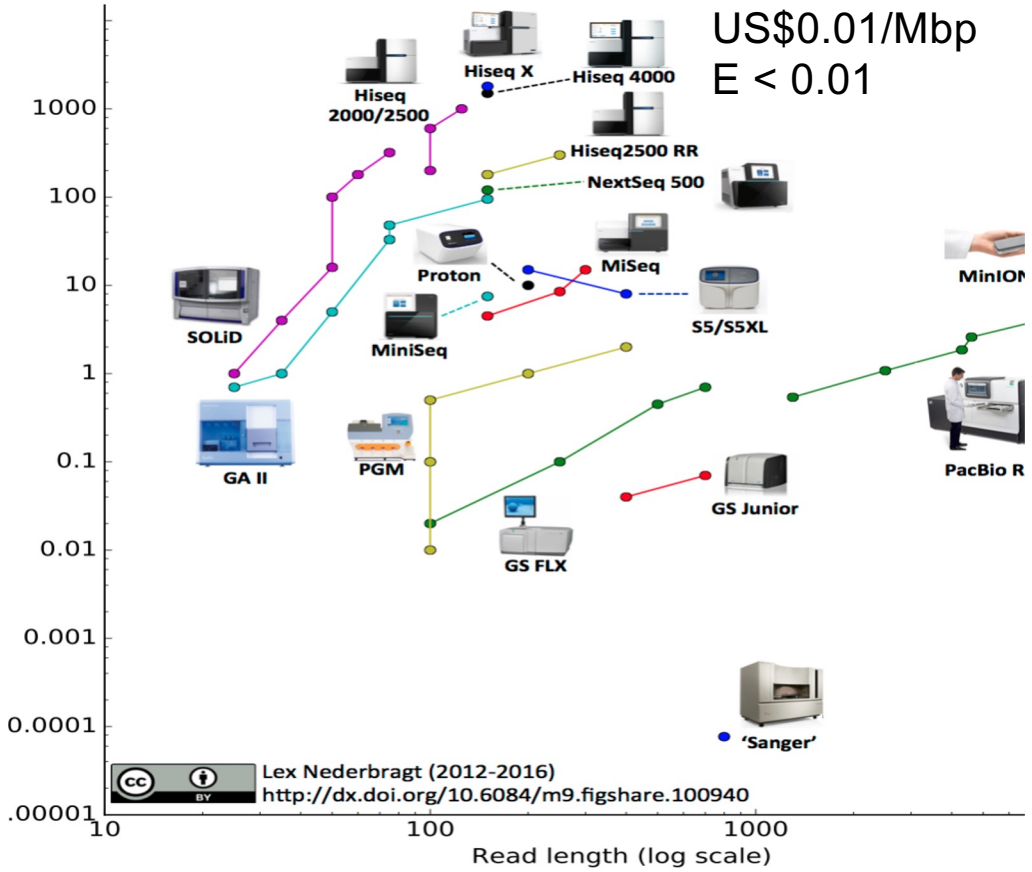
SGR
Sistema General de Regalías

TECNOLOGÍAS DE SECUENCIACIÓN

- Illumina puede producir hasta 1Tbp por corrida
- PacBio y Nanopore producen lecturas de más de 10Kbp

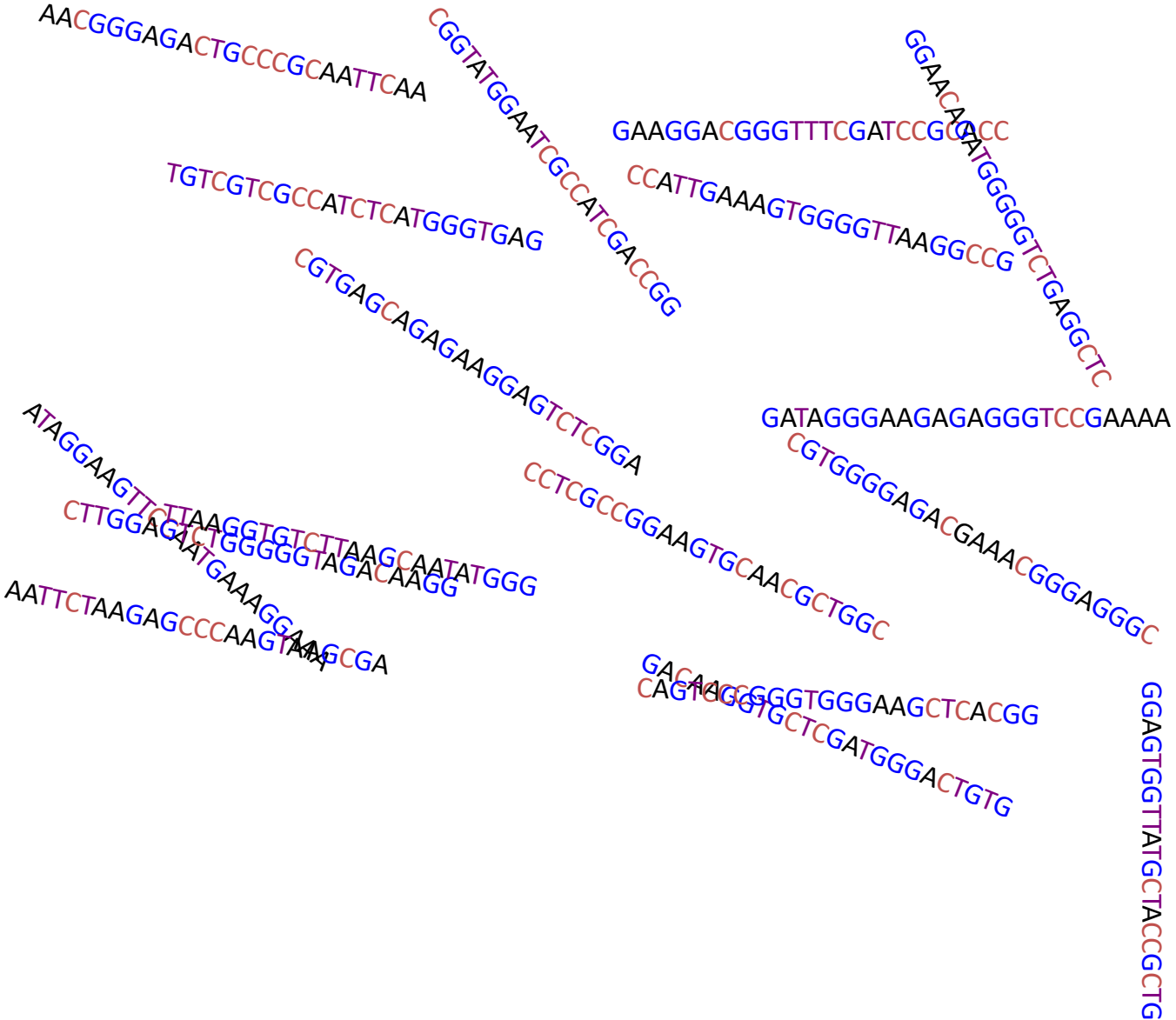


US$0.01/Mbp
E < 0.01

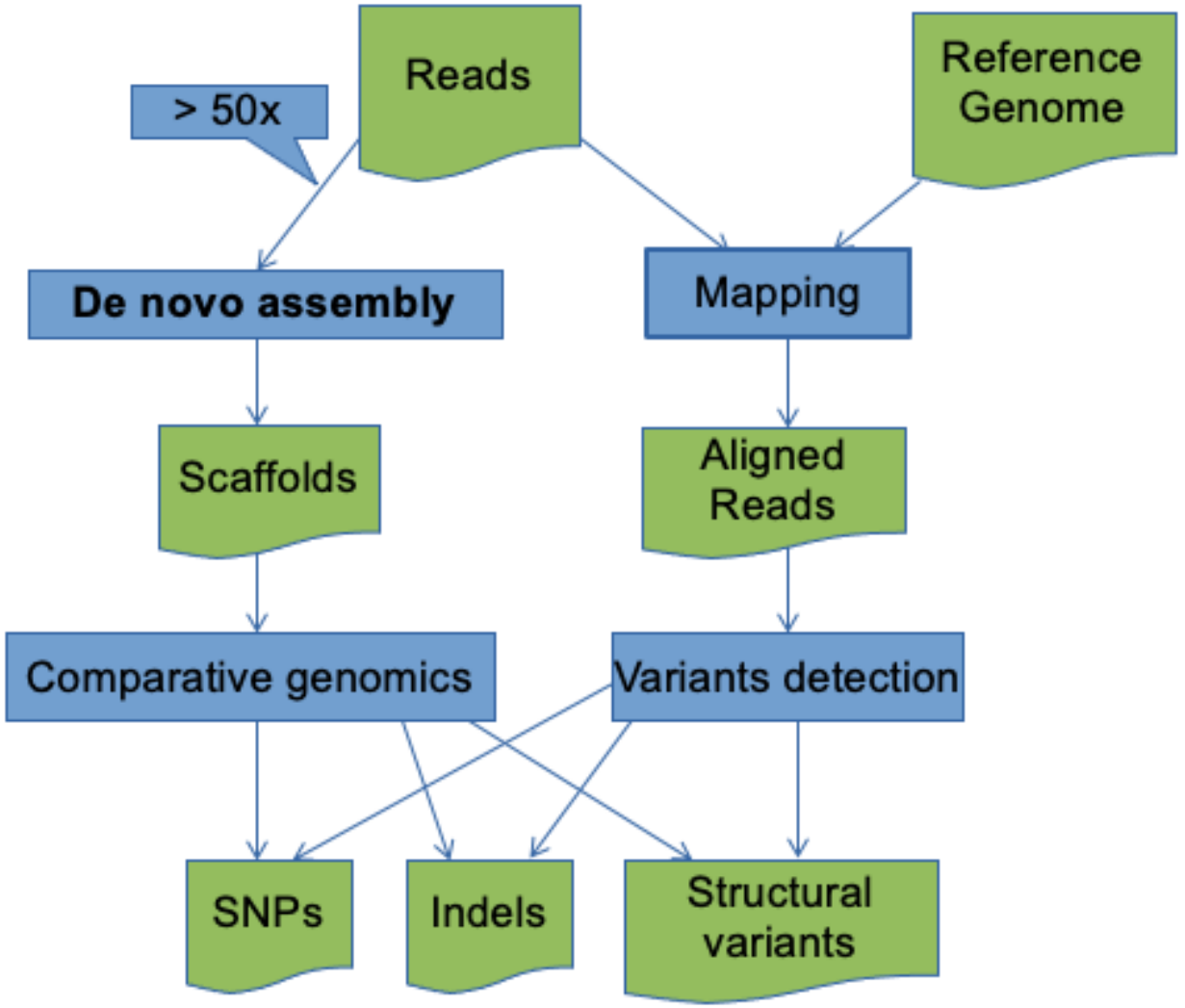
US$0.25/Mbp
E < 0.05

US$0.05/Mbp
E < 0.02

TECNOLOGÍAS DE SECUENCIACIÓN



BIOINFORMÁTICA PARA HTS



EJEMPLO CON TEXTO NATURAL

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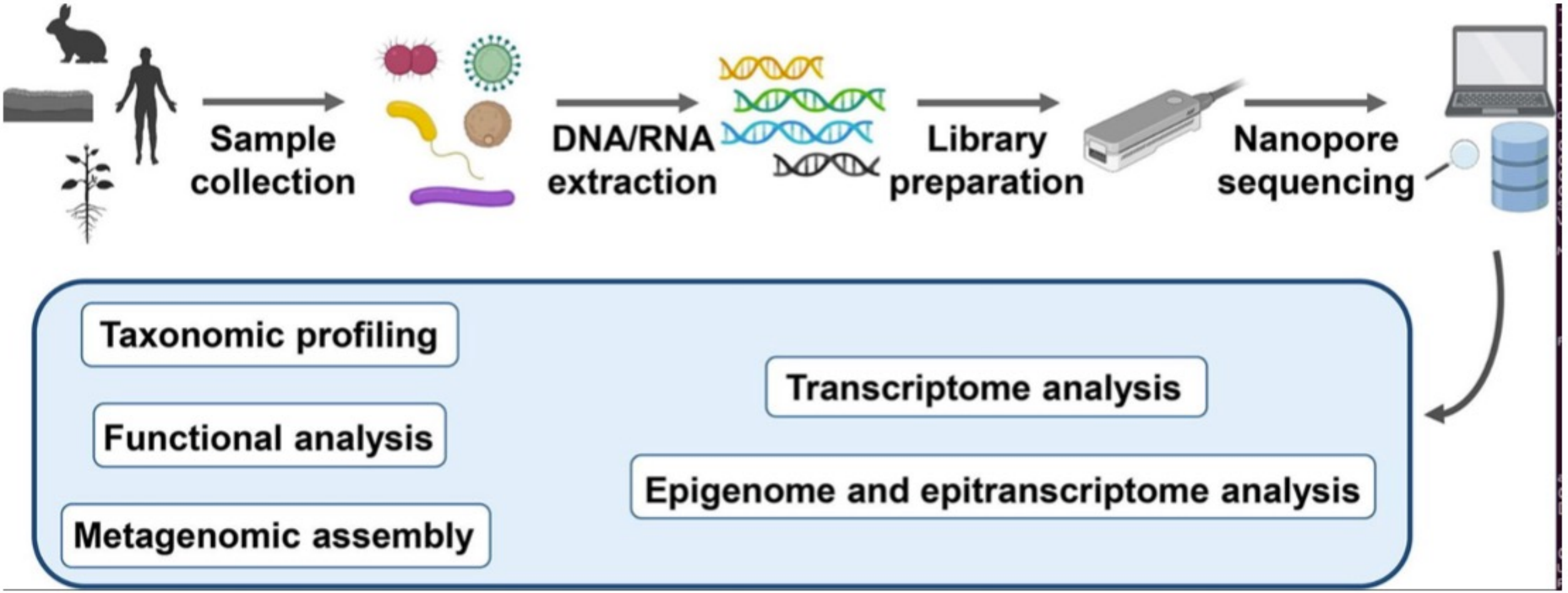
Tamaño estimado de secuencia: 50
Tamaño de lectura: 4
Número de lecturas: 50
Cubrimiento promedio: 4x
Texto: ?

What do you want to do with these long reads?

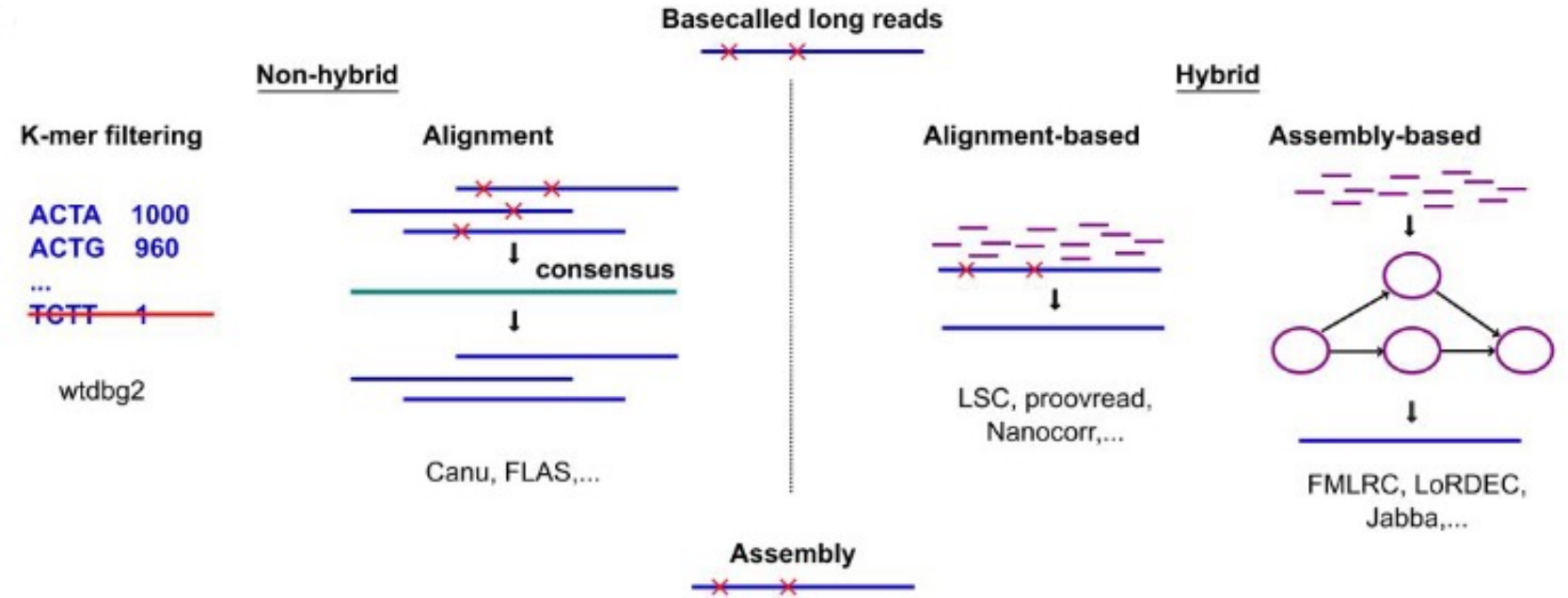


https://doi.org/10.1016/j.csbj.2021.02.020

Type	Reference	Application
Aligners/Alignment-based classifiers		
BLAST, MEGABLAST	[58,59]	Targeted; Shotgun
minimap2	[33]	Targeted; Shotgun
Alignment-free classifiers		
Kraken, Kraken2	[35,64]	Targeted; Shotgun
KrakenUniq	[65]	Shotgun
Bracken	[66]	Targeted; Shotgun
Metamaps	[69]	Shotgun
Centrifuge	[34]	Targeted; Shotgun
Mash	[72]	Targeted; Shotgun
Long-read assemblers		
Canu	[90]	Shotgun
miniasm	[73]	Shotgun
wtdbg2	[91]	Shotgun
OPERA-MS	[95]	Shotgun
MetaFlye	[96]	Shotgun
MetaSPAdes	[74]	Shotgun
Sequence correction and polishing tools		
Nanopolish	https://github.com/jts/nanopolish	Targeted; Shotgun
Medaka	https://github.com/nanoporetech/medaka	Targeted; Shotgun
Metagenomic analysis pipelines		
MEGAN-LR	[60]	Shotgun
NanoCLUST	[25]	Targeted
Reticulatus	https://github.com/SamStudio8/reticulatus	Shotgun
MUFFIN	[70]	Shotgun
NanoSPC	[71]	Shotgun
BusyBee	https://ccb-microbe.cs.uni-saarland.de/busybee/	Shotgun

https://doi.org/10.1016/j.csbj.2021.02.020

Reads Correction or not?



Reads Correction process

Correction strategies (*hybrid*)

– External reads : Illumina

– Internal reads : Only long reads or long reads corrected by short ones

Correction pipeline (*non-hybrid*)

– Read alignment

– Consensus calling

Canu module,

Racon can also be used as a read error-correction tool.

Assembly without reads correction

- Miniasm, Smartdenovo, Flye are members of this “new” family
- Improves speed
- Can work with less read depth.
- Can also assemble corrected reads

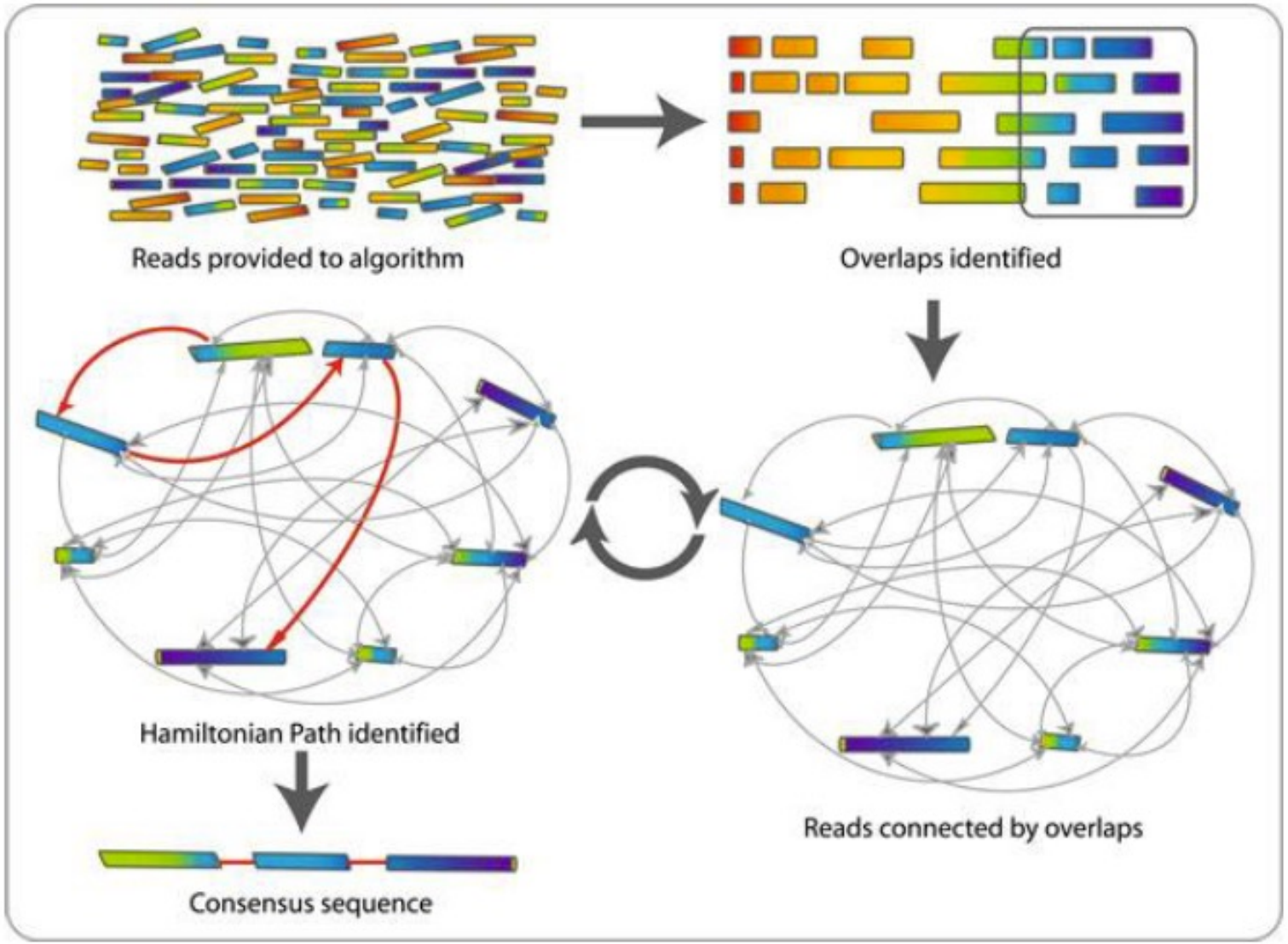
https://genomebiology.biomedcentral.com/track/pdf/10.1186/s13059-020-1935-5.pdf

What assembler to use over my favorite organism?

Long reads simplify genome assembly, with the ability *to* span repeat-rich sequences (characteristic of antimicrobial resistance genes) and structural variants. Nanopore sequencing also shows a lack of bias in GC-rich regions, in contrast to other sequencing platforms. To perform microbial genome assembly, we suggest using the third-party de novo assembly tool Flye. We also recommend one round of polishing with Medaka.
https://nanoporetech.com/sites/default/files/s3/literature/microbial-genome-assembly-workflow.pdf

For assembly, ONT recommend sequencing a human genome to a minimum depth of 30x of 25–35 kb reads. However, sequencing to a depth of 60x is advisable to obtain the best assembly metrics. We also recommend basecalling in high accuracy mode. Greatest contig N50 is usually obtained with Shasta and Flye. Polishing/Correction is also recommended (Racon and Medaka).
https://nanoporetech.com/sites/default/files/s3/literature/human-genome-assembly-workflow.pdf

Overlap–layout–consensus genome assembly algorithm (OLC)



Canu, Flye, Miniasm, Raven, Smartdenovo, Shasta

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3055744/

EJEMPLO CON TEXTO NATURAL

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Tamaño estimado de secuencia: 50
Tamaño de lectura: 4
Número de lecturas: 100
Cubrimiento promedio: 8x
Texto: ?

EJEMPLO CON TEXTO NATURAL

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Tamaño estimado de secuencia: 50
Tamaño de lectura: 8
Número de lecturas: 50
Cubrimiento promedio: 8x
Texto: ?

EJEMPLO CON ADN

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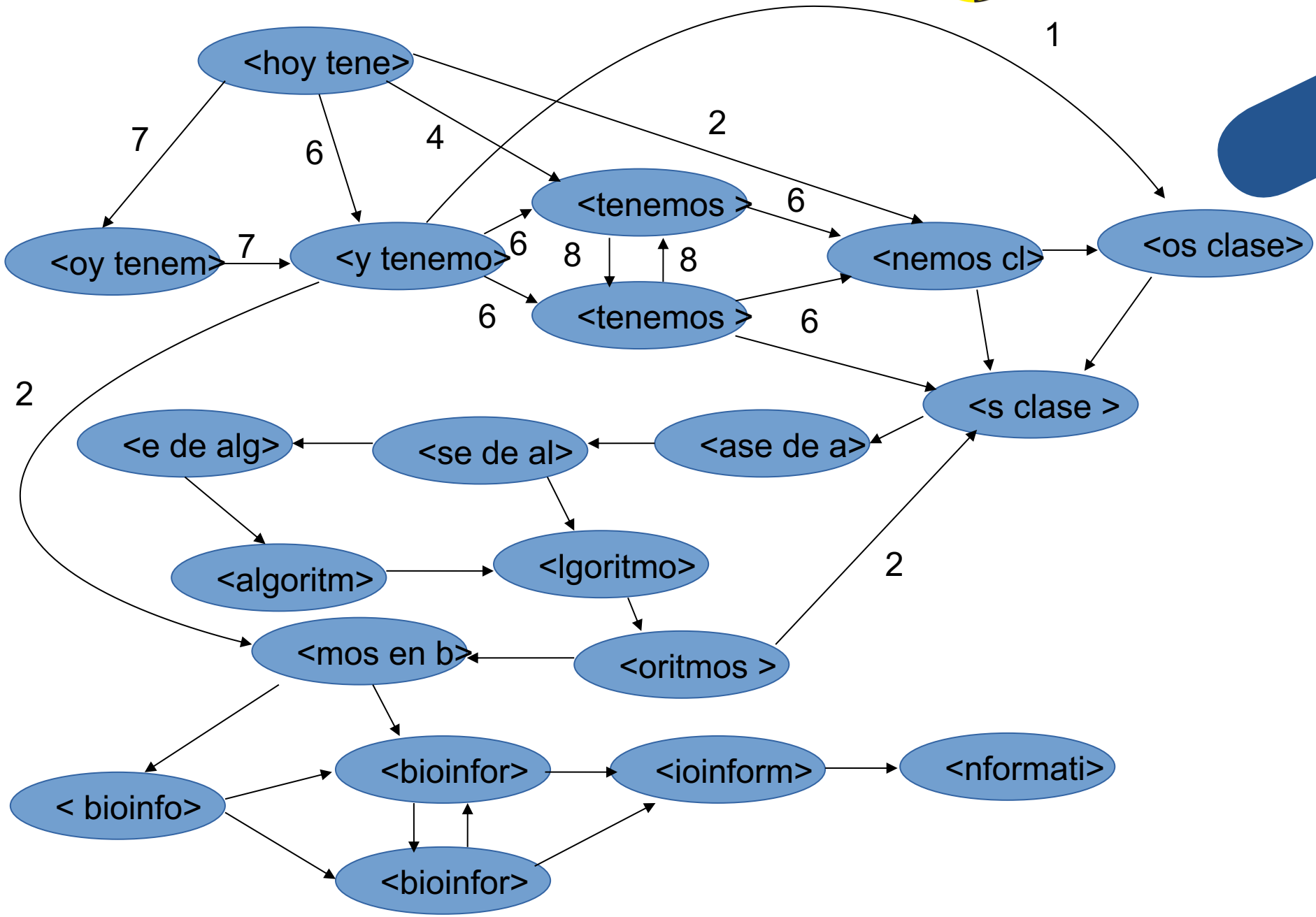
Tamaño estimado de secuencia: 50
Tamaño de lectura: 8
Número de lecturas: 50
Cubrimiento promedio: 8x

Secuencia: AGGCAGCGTCGTACGTACGTACCGCTAGCTAATAACAGCGTACTTGCGT

GRAFO DE ENSAMBLAJE

- Grafo dirigido
- Los vértices son las lecturas
- Hay un eje entre cada par de vértices si las lecturas se sobrelapan
- El número de bases en las que se sobrelapan es el peso del eje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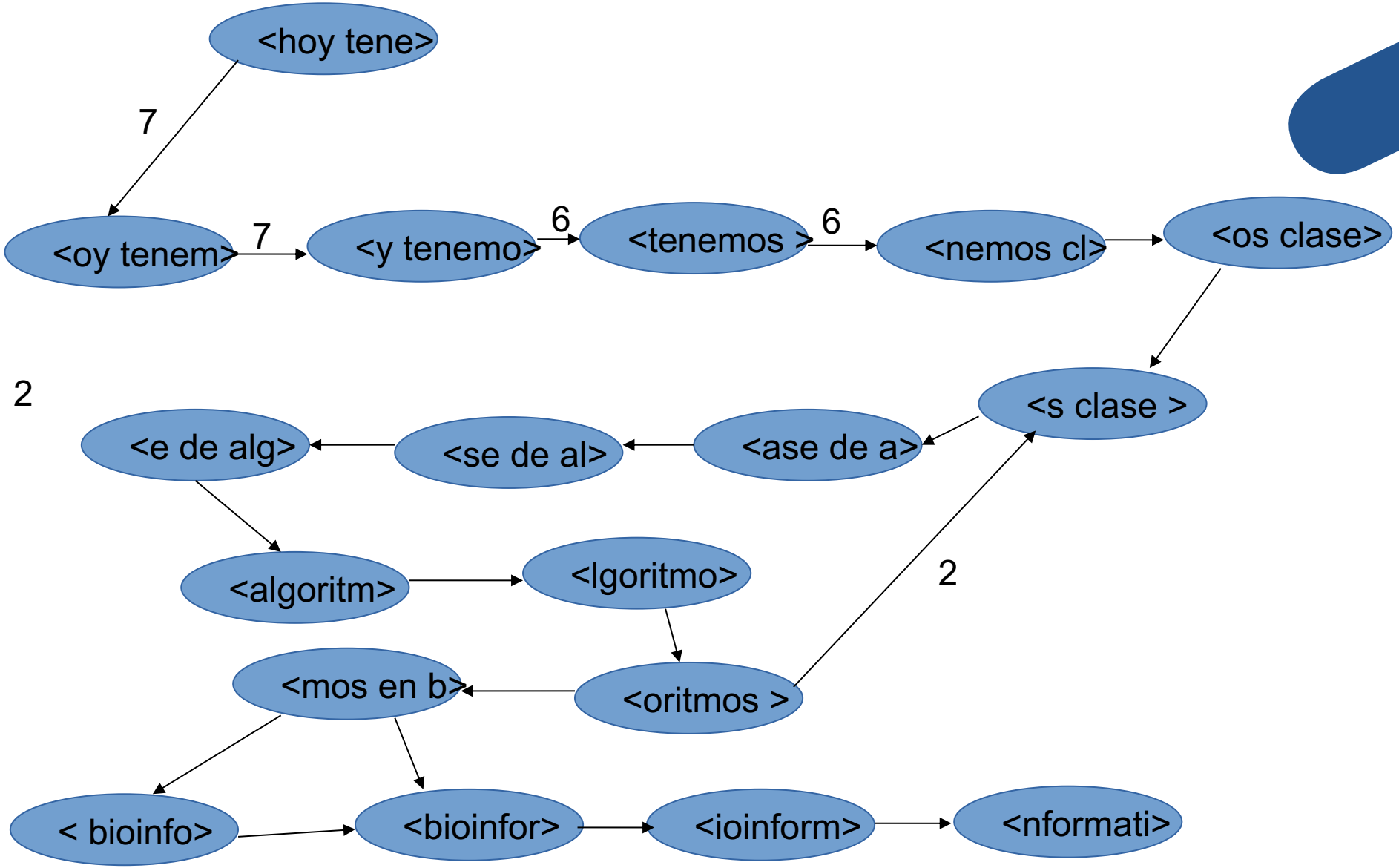
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EJERCICIO CON TEXTO

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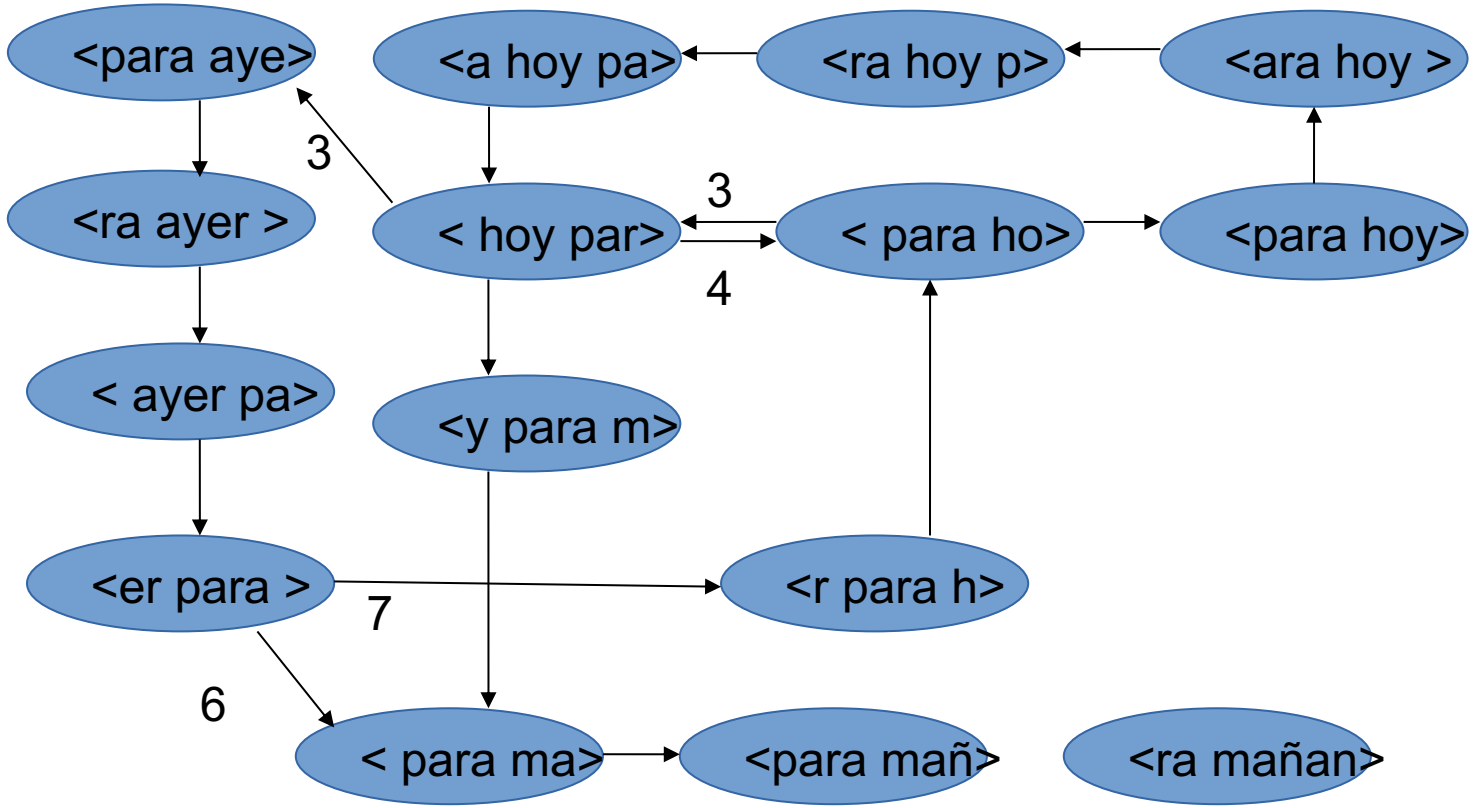
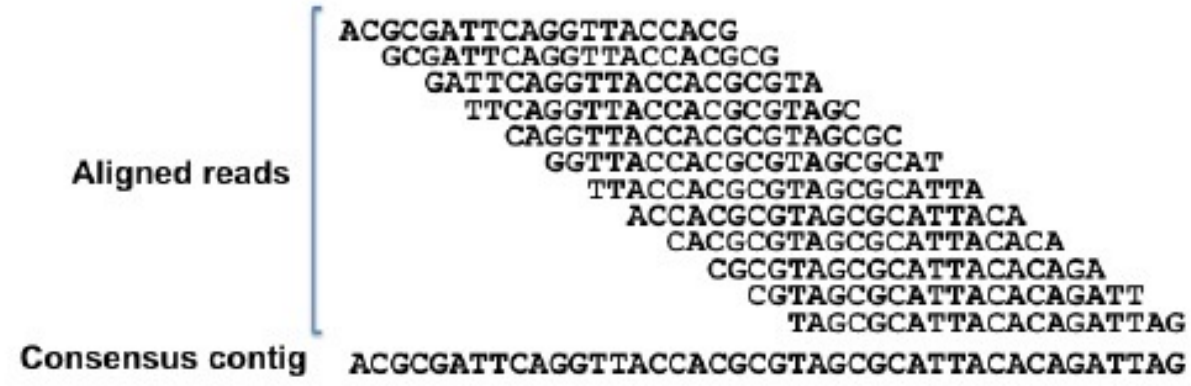


PROCESO DE ENSAMBLAJE

1. **Overlap**: Construir el grafo de sobrelapes (Overlap graph)
2. **Layout**: Encontrar el o los caminos en el grafo de sobrelapes que explican las lecturas
3. **Consensus**: Construir la secuencia de consenso a partir de las lecturas alineadas



http://image.slidesharecdn.com/110928clsioncodgenome-110929063927-phpapp01/95/how-to-sequence-a-large-eukaryotic-genome-15-728.jpg?cb=1357998263

Polishing / Correction

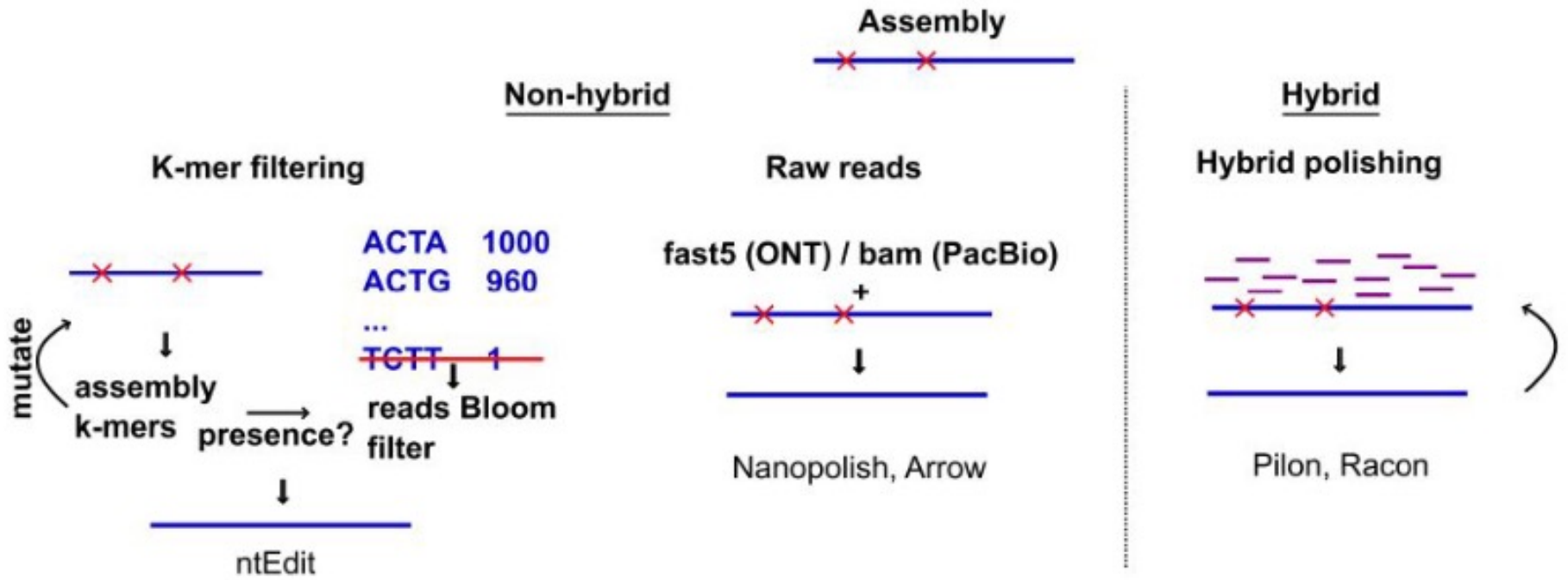
Racon correct raw contigs generated by rapid assembly methods which do not include a consensus step. It can polish with either Illumina data or data produced by third generation of sequencing. (recursive use)

Medaka and **Nanopolish** create a consensus sequence of nanopore sequencing data. (mapping + consensus)

+ Medaka uses neural networks where Nanopolish uses HMMs.
+ Medaka uses basecalled reads, not the raw signal.
+ Medaka propose the ability to train one's own basecalling model

Pilon correct assemblies using illumina reads. (recursive use)

Autres : NeuralPolish , ntEdit



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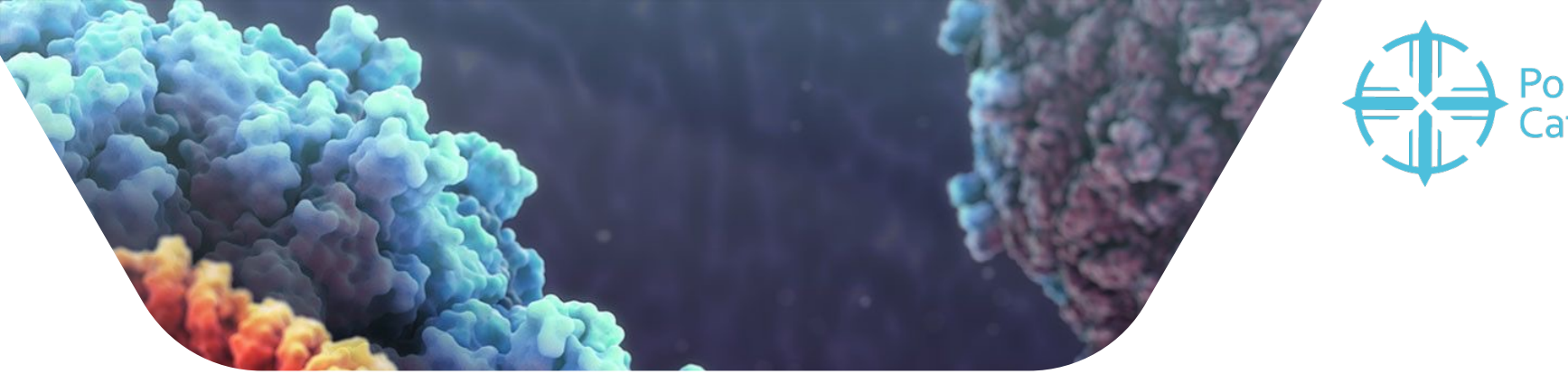

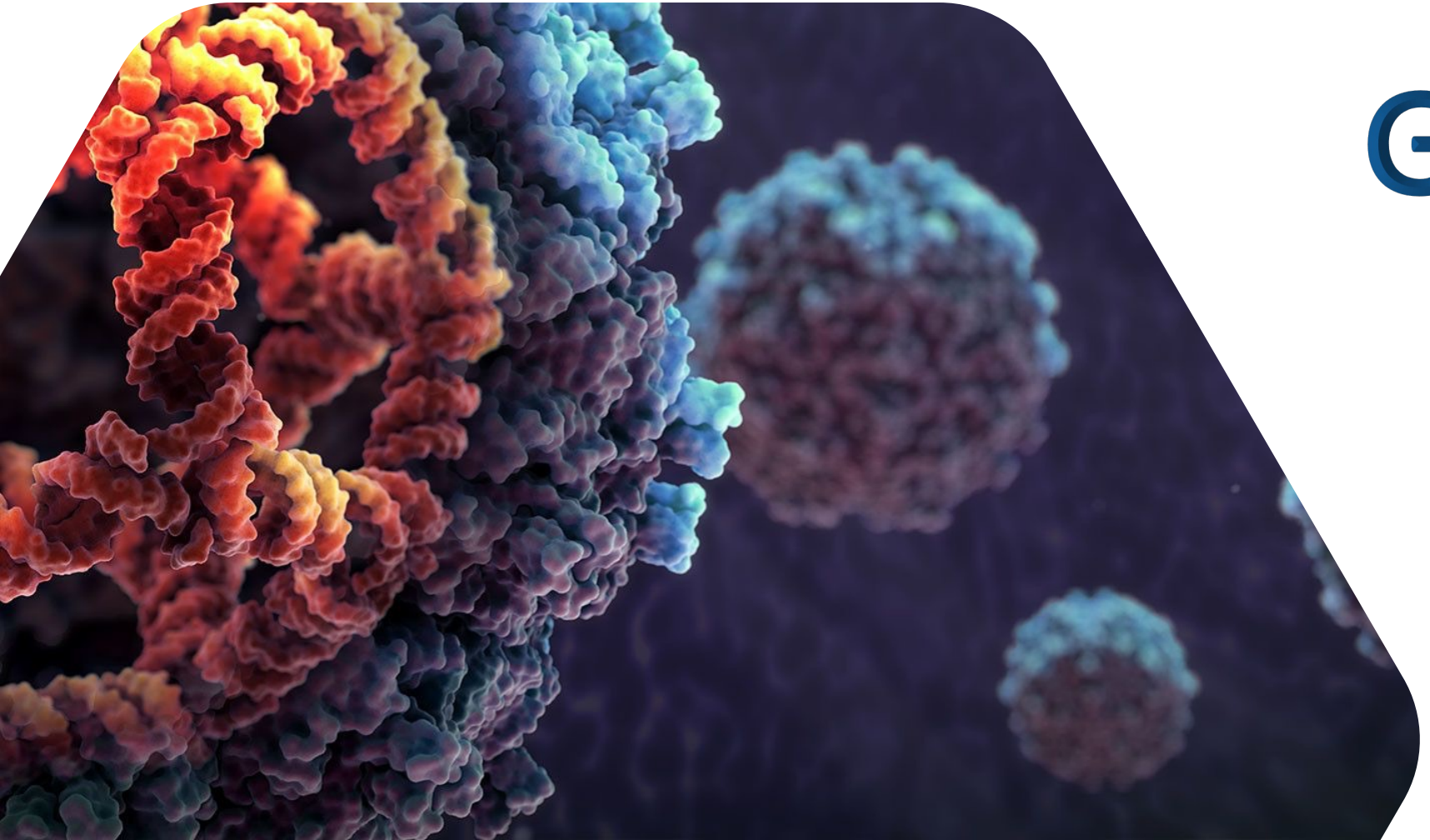

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GRACIAS

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