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# Nanopore MinION long read sequencer: an overview of its error landscape

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# Nanopore MinION long read sequencer: an overview of its error landscape

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IRISA - Team Genscale



# Introduction

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How does Nanopore sequencing works ?

Constraints linked to the basecaller

Our working basis

# Nanopore: how does it work ?



DNA passing through nanopore alters voltage.

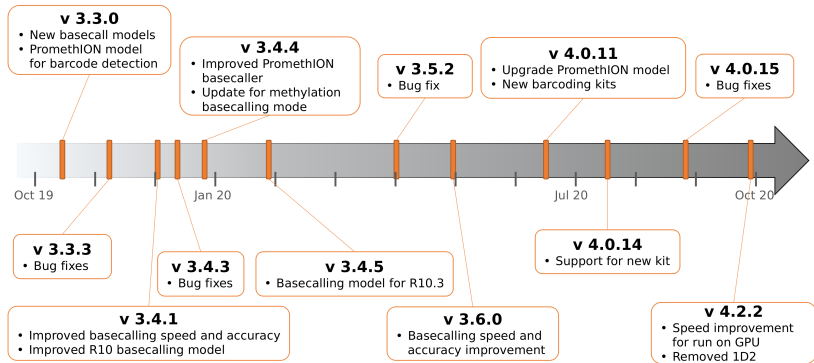
The **basecaller** exploits these variations to retrieve DNA sequence.

**Limitation:** bunch of same bases → no variation.

Basecaller: a core element.

# Basecaller: a core element. That is updated almost monthly

## Guppy basecaller releases



(+ Many other basecallers prior to Guppy [1] and to come.)

# What we have been working on

- Raw reads from various sources, sequenced with the MinION
  - Our sequencing experiments <sup>1</sup>
  - Wick *et al.* 2019 [1]
  - Shafin *et al.* 2019 [2]

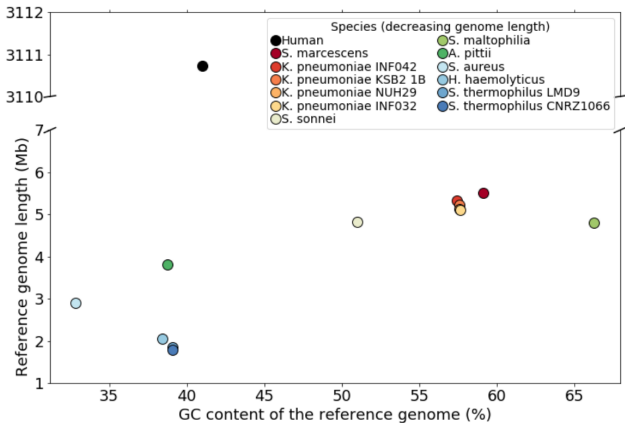


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<sup>1</sup>Thanks E. Roux, STLO INRA/Agrocampus and INRIA

# What we have been working on

- Raw reads from various sources, sequenced with the MinION
- 12 bacterial species with various GC content + 2 human datasets





# What we have been working on

- Raw reads from various sources, sequenced with the MinION
- 12 bacterial species with various GC content + 2 human datasets
- **Guppy basecaller** (latest v4.2.2)
  - HAC mode: High ACcuracy
  - FAST mode: faster but higher error rate

# What we have been working on

- Raw reads from various sources, sequenced with the MinION
- 12 bacterial species with various GC content + 2 human datasets
- Guppy basecaller (latest v4.2.2)

**Goal: outline landscape of MinION long read sequencing errors**

+ 2 preliminary steps:

- estimating error rates of reads
- evaluate HAC and FAST basecalling modes

# Estimate error rate from raw reads

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How to estimate error rates of reads?

Phred quality score

Estimate Nanopore error rates from quality

How to get error rate of reads ?

- Align reads on reference genome
- Count differences treated as sequencing errors

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## Problems

- Reference genome of poor quality (or even absent)
- Different strain / variants
- Requires to compute alignments

# Estimate error rate

How to get error rate of reads ?

- Align reads on reference genome
- Count differences treated as sequencing errors

## Problems

- Reference genome of poor quality (or even absent)
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→ **Estimate error rates directly from raw reads ?**

# Filtering reads on quality score

**Phred quality score:** confidence score for each sequenced base  
Ranging from 0 to 93 (the higher the better)

Base	T	G	A	T	A	G	T	T	A	T	G
Score	32	40	41	35	29	23	26	32	36	32	14
ASCII	A	I	J	D	>	8	;	A	E	A	/

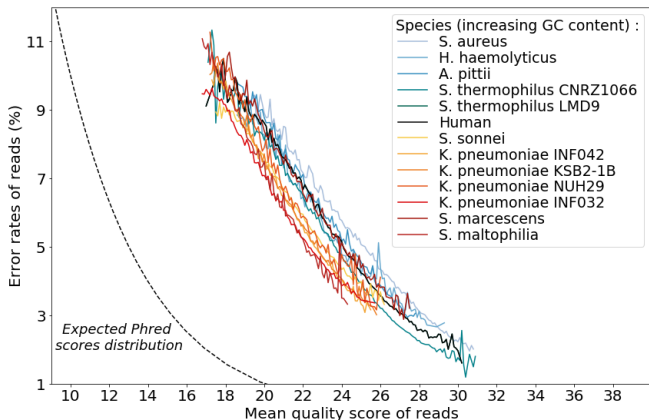
In FASTQ files scores are encoded in ASCII characters

Score indicates probability **P** of a wrong base:

$$P = 10^{\frac{-Q}{10}}$$

Phred score of 10  $\leftrightarrow$  10% error rate ; score of 20  $\leftrightarrow$  1% error rate

# Estimate error rate from quality score



Nanopore quality score (Q) does not follow Phred scores  
Yet enables to estimate error rate (E) (locally and at read level):  
 $E = 0.015Q^2 - 1.15Q + 24$



# Comparison between two main modes of the basecaller: HAC vs FAST

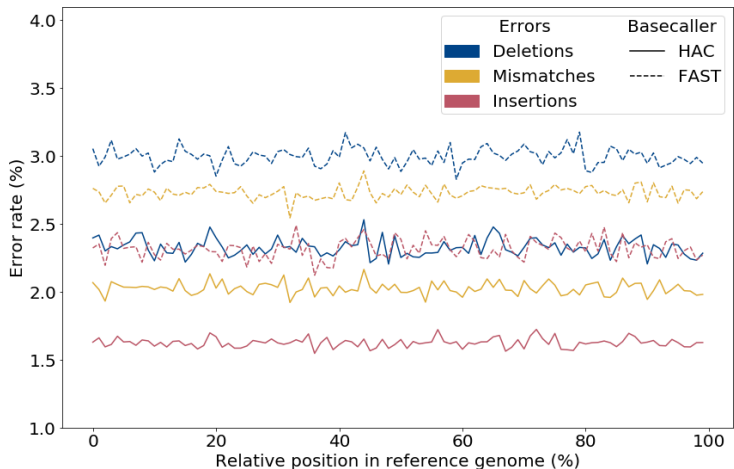
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Error rate

Calling homopolymers

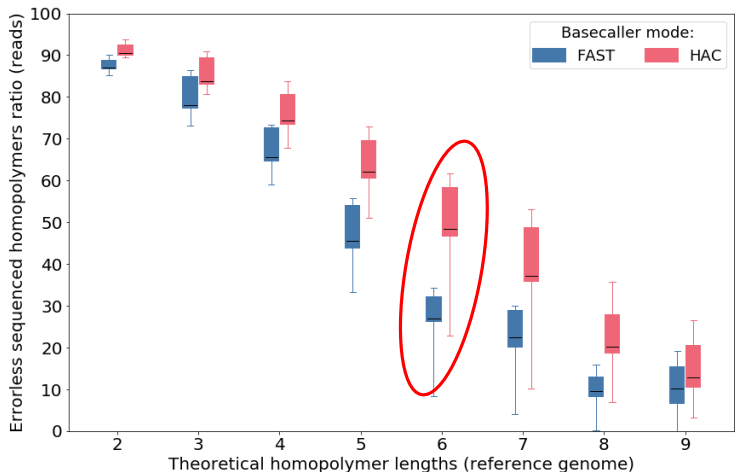
Running time

# HAC vs FAST - Error rates



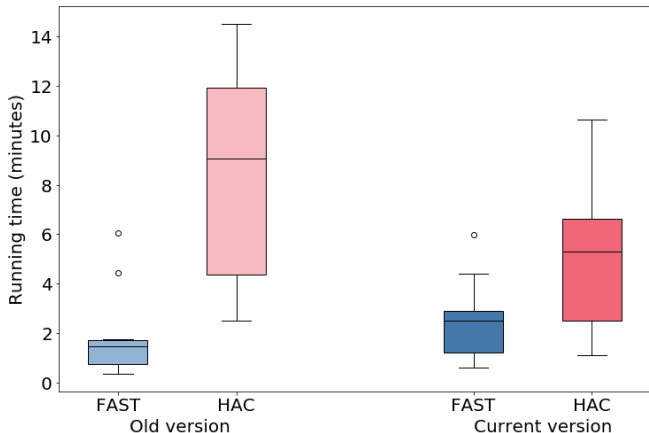
Similar error profile : deletions > mismatches > insertions  
HAC mode reduces error rate by 2%

# HAC vs FAST - Calling homopolymers



HAC mode basecalls homopolymers up to twice better than FAST

## HAC vs FAST - Running times (now and before)

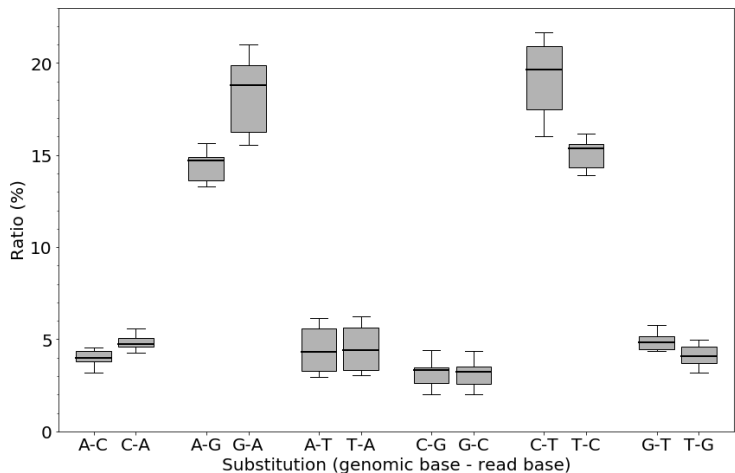


FAST mode is **only** about 2 times faster now  
FAST mode is no longer of practical interest

## Bias in substitution errors

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# Substitution errors bias



Transitions are three times more frequent than transversions

**Non-symmetrical bias:** decrease of reads' GC content

(-0.2% compared to reference genome)

# GC bias

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Depth of coverage

Error rate

Reads quality

## GC bias - Depth of coverage

NGS data are subject to GC-bias due to PCR amplification

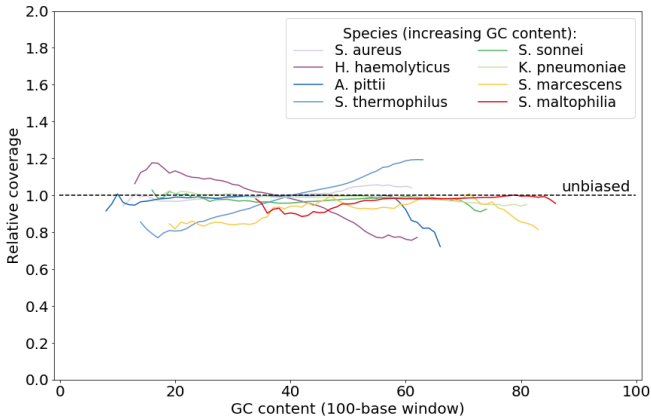
**What about Nanopore data (no PCR needed) ?**



# GC bias - Depth of coverage

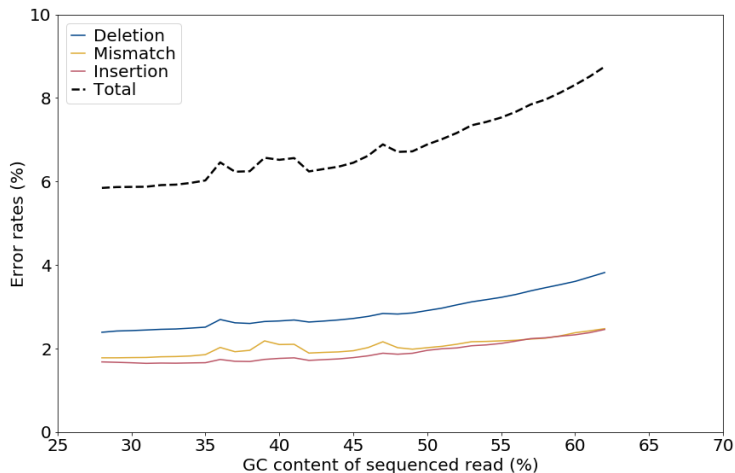
NGS data are subject to GC-bias due to PCR amplification

What about Nanopore data (no PCR needed) ?



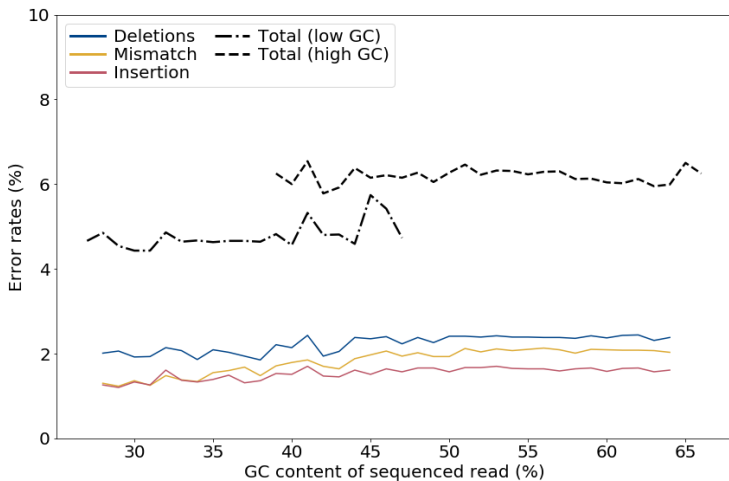
Roughly no bias, but drop for high GC + 2 species more impacted

## GC bias - Error rate (human)



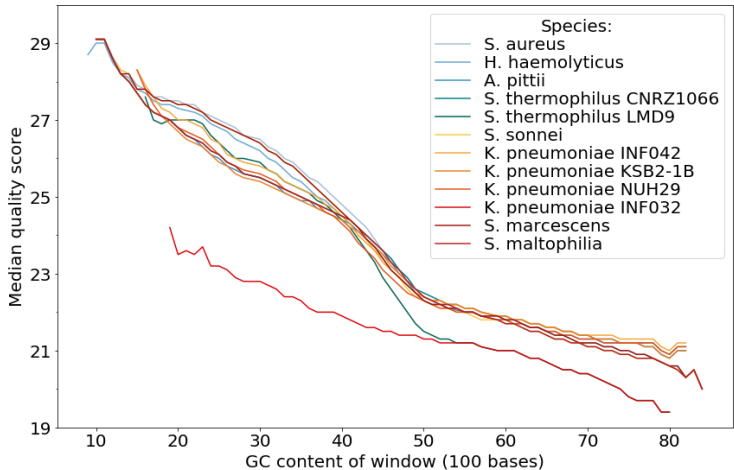
Low GC reads have about 1.5% lower error rates

## GC bias - Error rate (bacteria)



Low GC species have about 1.5% lower error rates

# GC bias - Reads quality



Quality decreases with GC content + break around 50%

# Sequencing low complexity regions

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Homopolymers  $(X)^n$

Heteropolymers  $(XY)^n$

Trinucleotides  $(XYZ)^n$

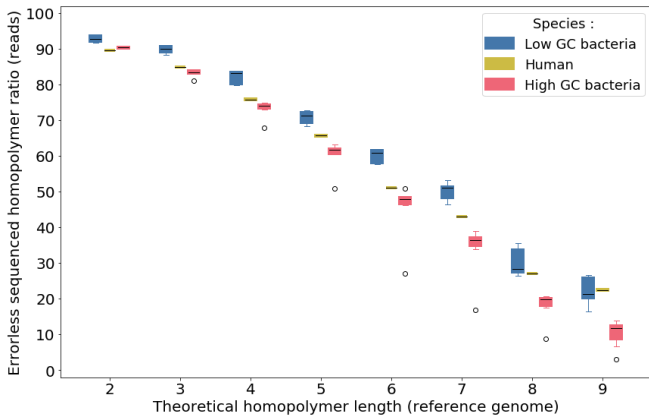
# Share of sequencing errors due to homopolymers

	Mismatches	Insertions	Deletions	Global
Bacteria	52.61	18.95	55.66	<b>44.59</b>
Human	56.57	25.07	60.51	<b>49.43</b>

**Table 1:** Ratio (%) of sequencing errors induced by homopolymers over all sequencing errors

**Near 50% of sequencing errors are linked to homopolymers**  
(mostly deletions and mismatches)

# Homopolymer sequencing accuracy

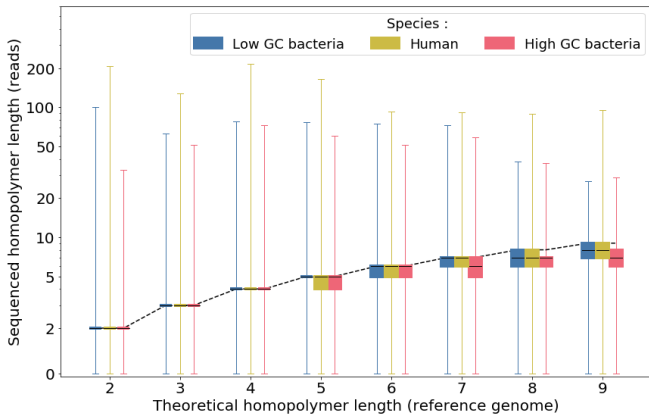


Rather good accuracy (>70%) for **short homopolymers** (< 4-5 bases)

Then **drop for higher lengths**: only 25% for length 8

Better results for **low GC**

# Estimating homopolymer lengths



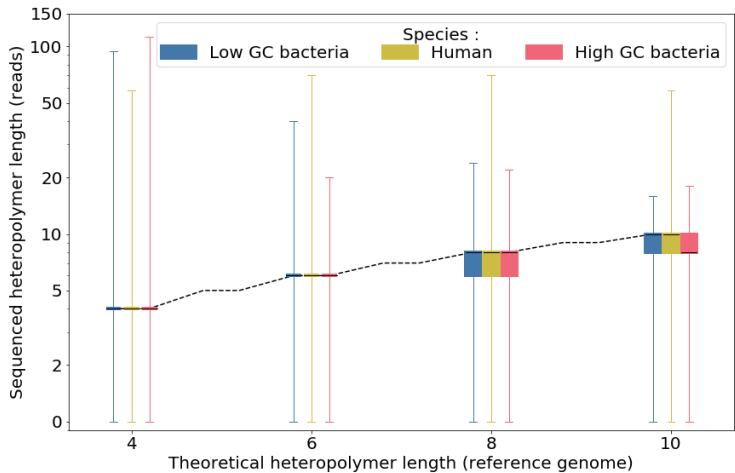
Rather good accuracy for **short homopolymers** (< 5 bases)

**Underestimated length** for longer homopolymers

**High variability:** overestimation of one order of magnitude

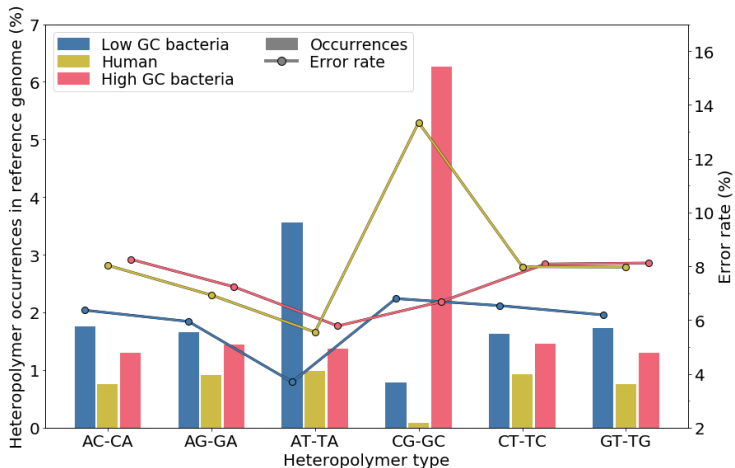


# Estimating heteropolymer lengths



Same trends as for homopolymers

# Heteropolymer sequencing accuracy



CpG islands are marginal, except for high GC species

Error rate proportional to species GC rate (inverted for CpG islands)

Minimal error rate for AT-TA heteropolymers

# Trinucleotides

Biological relevance: trinucleotide repeat expansion (mutation implied in several genetic diseases)

Notation:

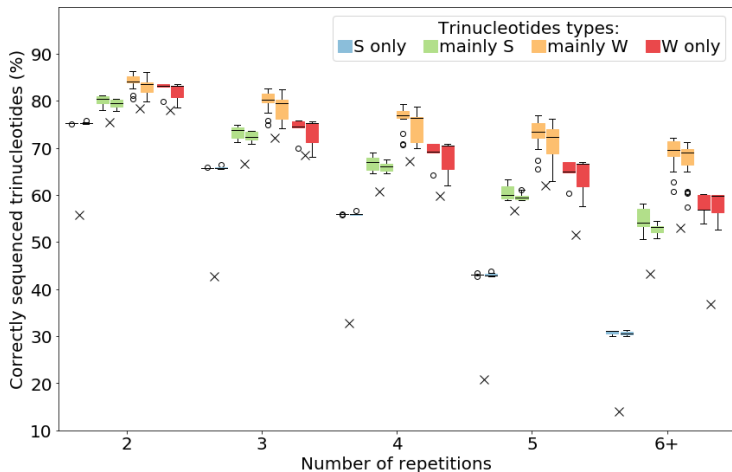
- S (Strong) for C and G bases
- W (Weak) for A and T bases

For example: (homopolymers removed as analysed in previous part)

- WWW = {AAT, ATA, TAA, ATT, TAT, TTA}
- WSW = {ACA, ACT, AGA, AGT, TCA, TCT, TGA, TGT}

→ **S only** (SSS), **mainly S** (SSW, SWS, WSS),  
**W only** (WWW) and **mainly W** (WWS, WSW, SWW)

# Trinucleotides sequencing accuracy



S-only are the worst (GC content) but W-only are not the best !  
Low GC trinucleotides are better sequenced  
Decrease of accuracy with increase of repetition number

## Conclusions and prospects



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# Conclusions and prospects

- estimate error rate from quality score
- use HAC basecalling mode rather than FAST one
- substitution bias
- GC bias: no depth bias but error bias
- low complexity regions

## **Few papers addressing error profile of Nanopore sequencing**

↔ Provide basis for future work on basecallers, read correction or assembly algorithms.

-  Wick, R. R., Judd, L. M., and Holt, K. E. (2019) Performance of neural network basecalling tools for Oxford Nanopore sequencing. *Genome Biology*, **20**(1), 129.
-  Shafin, K., Pesout, T., Lorig-Roach, R., Haukness, M., Olsen, H. E., Bosworth, C., Armstrong, J., Tigyi, K., Maurer, N., Koren, S., Sedlazeck, F. J., Marschall, T., Mayes, S., Costa, V., Zook, J. M., Liu, K. J., Kilburn, D., Sorensen, M., Munson, K. M., Vollger, M. R., Eichler, E. E., Salama, S., Haussler, D., Green, R. E., Akeson, M., Phillippy, A., Miga, K. H., Carnevali, P., Jain, M., and Paten, B. (2019) Efficient de novo assembly of eleven human genomes using PromethION sequencing and a novel nanopore toolkit. *bioRxiv*.