Basecalling – How Good is Good Enough?

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About ATCC®

- ATCC® was founded in 1925
- 501(c)(3) not-for-profit organization
- World's largest, most diverse biorepository
- Quality Accreditation by multiple industry standards
  - ISO 9001 Certified
  - ISO 13485 Certified
  - ISO/IEC 17025 Accredited
  - ISO 17034 Accredited
- Standards development partner with multiple industry working groups
  - ANSI Standards Working Groups
  - AOAC International Working Group
  - IMMSA/NIST Microbiome Standards

- Global supplier of authenticated cell lines, microorganisms, and molecular standards
- Sales and distribution to 150+ countries
- Talented team of 600+ employees

Thousands of authenticated biomaterials

- 5000+ cell lines & primary tissue
- 2900+ viruses
- 17000+ bacteria & archaea
- 53000+ fungi
- 2000+ protists

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Background

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Basecalling Need to Know

Nanopore basecalling is, by default, performed via the high accuracy (HAC) model

- Fast and super accurate (SUP) models are also available
- Dorado is designed to produce identical results at higher speeds compared to the Guppy basecaller
  - Also offering additional models for methylation analysis

ATCC®'s Role

High-throughput (>1000/year) ONT® sequencing

- Using standard recommended software and settings (HAC)
- Sequences wide array of microbes across all taxa
- GridION® platform
- Hybrid assembly workflow with Illumina® data

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Benchmarking Goals

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Set expectations and recommendations for sequencing labs of variable:

- Throughput demand
- Organism diversity
- Upgrade capacity

Evaluate the analysis of dorado and guppy FAST/HAC/SUP basecalling models in terms of:

- Output read quality scores
- Basecalling speed
- Read length and count
- Illumina®-hybrid and ONT®-only *de novo* assembly

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Study

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More benchmarking than done before

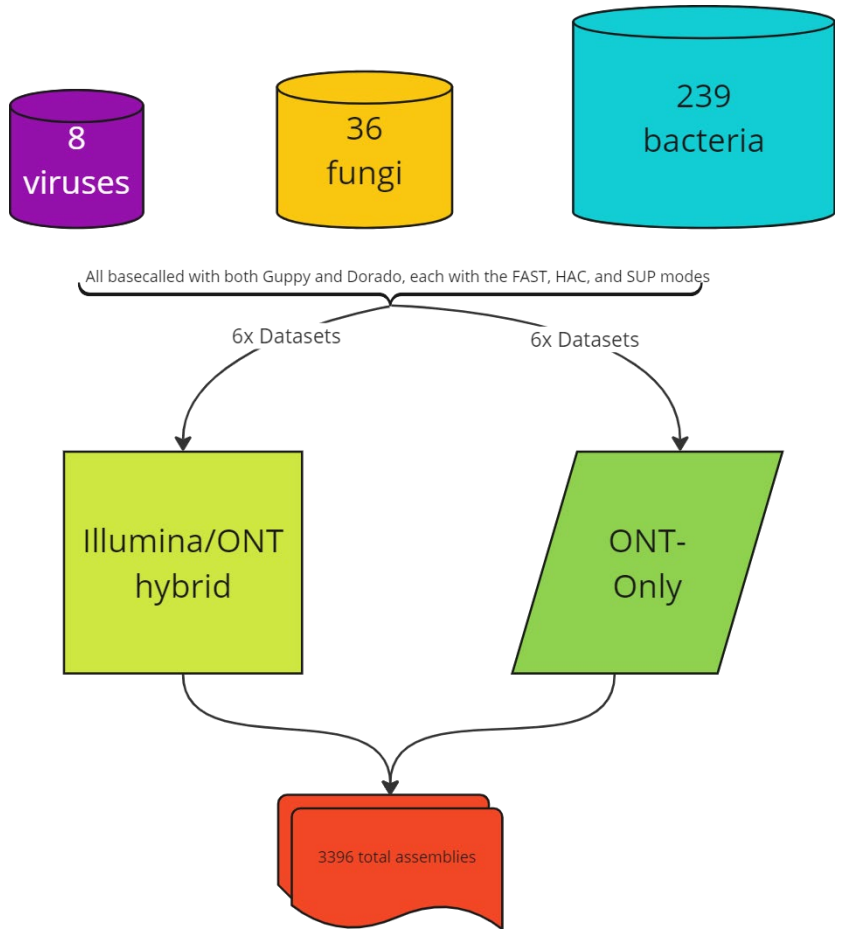
Taking a mix of samples we had available:

- Compare the performance of both guppy and dorado in terms of speed and quality at the read level
  - Across basecalling models fast, HAC, and SUP
- Compare the impact of these different basecallers on the downstream assembly quality
  - ONT®-only assembly with flye
  - ONT®-first assembly with flye, polished with Illumina® data
  - Illumina®-first assembly with unicycler, scaffolded with ONT® data
  - Measured by N50, assembly completion score, gene annotation content

- Establish sense of value vs sacrifice of each mode

Minimal experimental variability across 283 different organisms

- Sequenced by the same user
- Same handling and preparation (as the organisms allow)
- Same flow cell
- Same physical instrument
- Same basecalling hardware



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Basecalling Speeds

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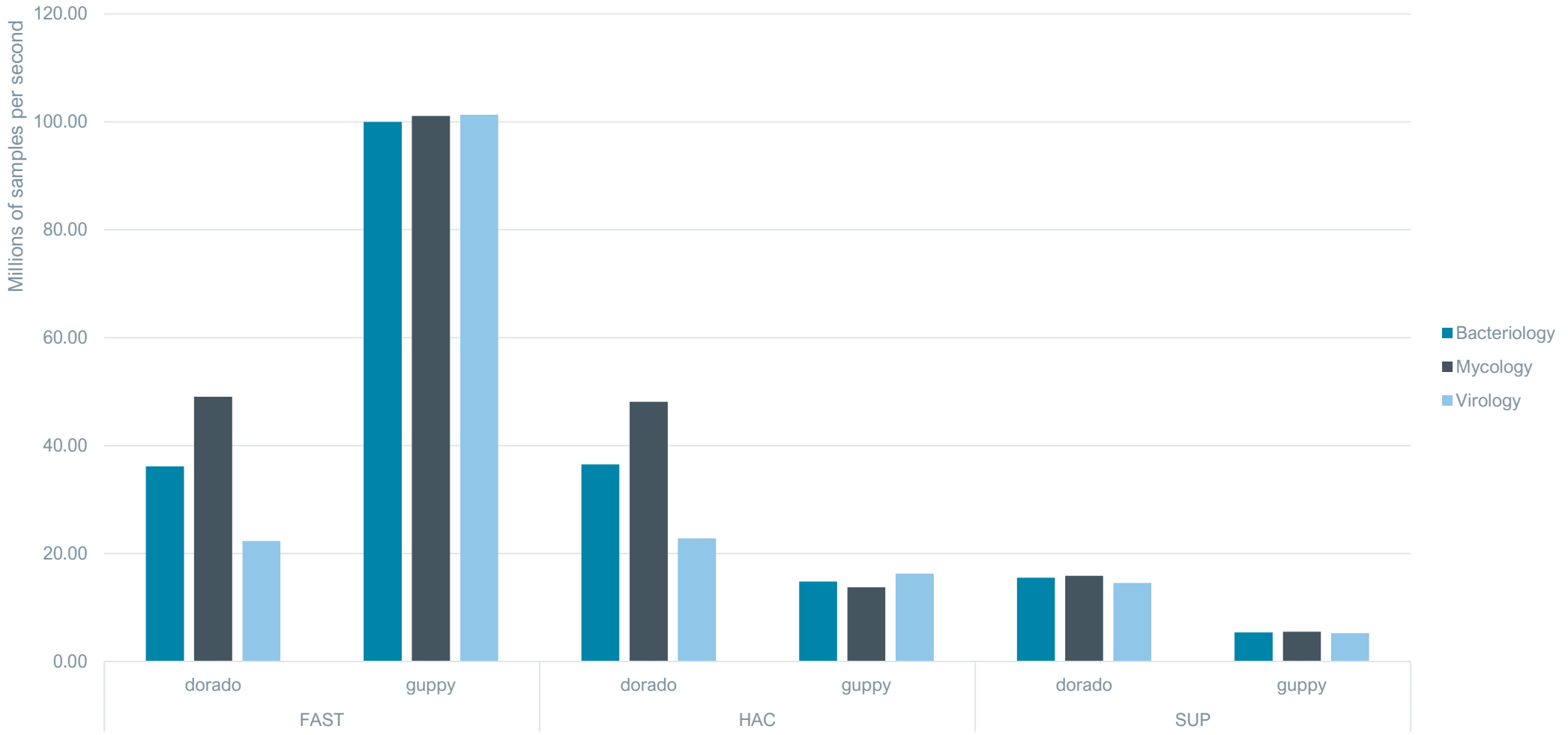

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Assembly Statistics

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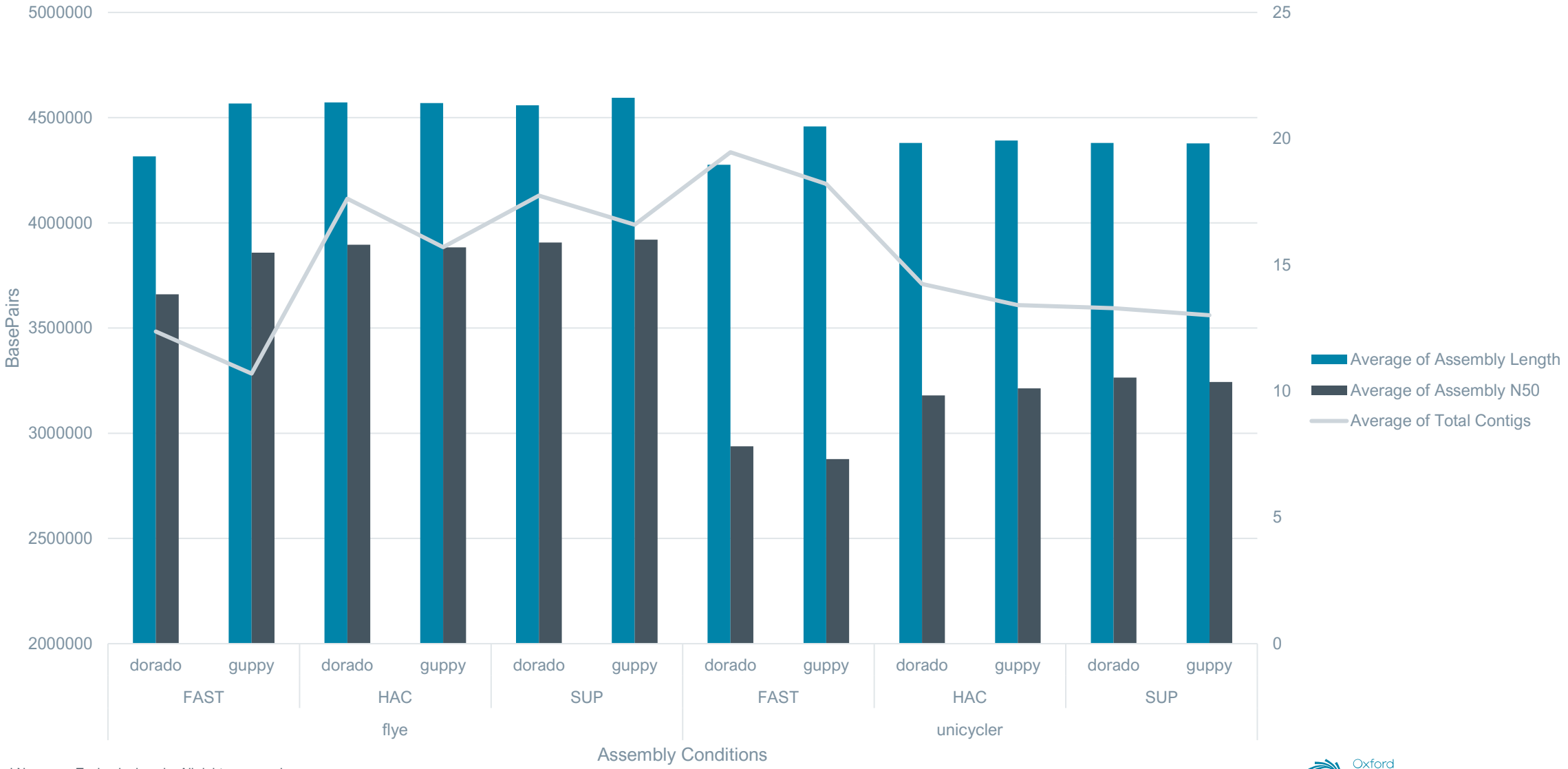

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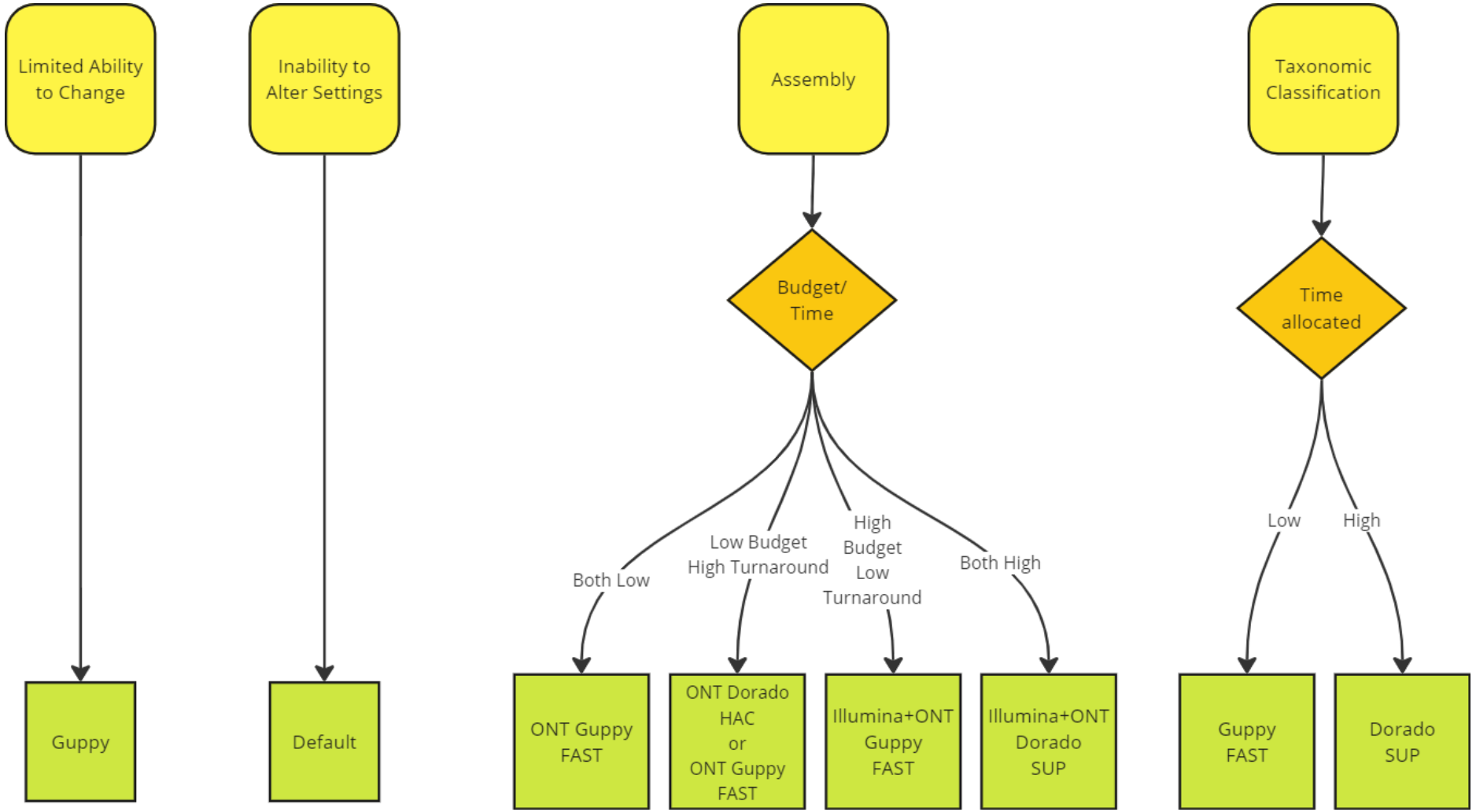
Application (preliminary)



Recap

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ATCC's Collection offers unparalleled insight into Nanopore sequencing efficacy

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- With the goal of optimizing our usage of the GridION® platform, ATCC® has found that for our usage, Dorado run with its SUP mode best serves our needs:
  - It is substantially faster than the Guppy equivalent
  - It allows for one GridION run per week, compared to two if in HAC or FAST modes
  - Assembly accuracy is slightly superior to HAC and greatly superior to FAST

- Future work includes:
  - Describing virology and mycology collections in more detail
  - Tuning parameters beyond default settings
  - Methylation analysis

Other Considerations

- Other use cases may find other requirements:
  - Guppy's FAST mode remains substantially faster (nearly 3x faster than Dorado) at the cost of higher bacterial contamination
    - Dorado is otherwise nearly 3X faster than Guppy

- Hybrid assembly is relatively unaffected by basecaller or basecalling speed

- ONT®-only *de novo* assembly is still outperformed by hybrid assembly methods
  - Using simple default parameters
    - Tuning parameters for trimming and assembly to be performed in future work

- Dorado offers superior methylation analysis capability

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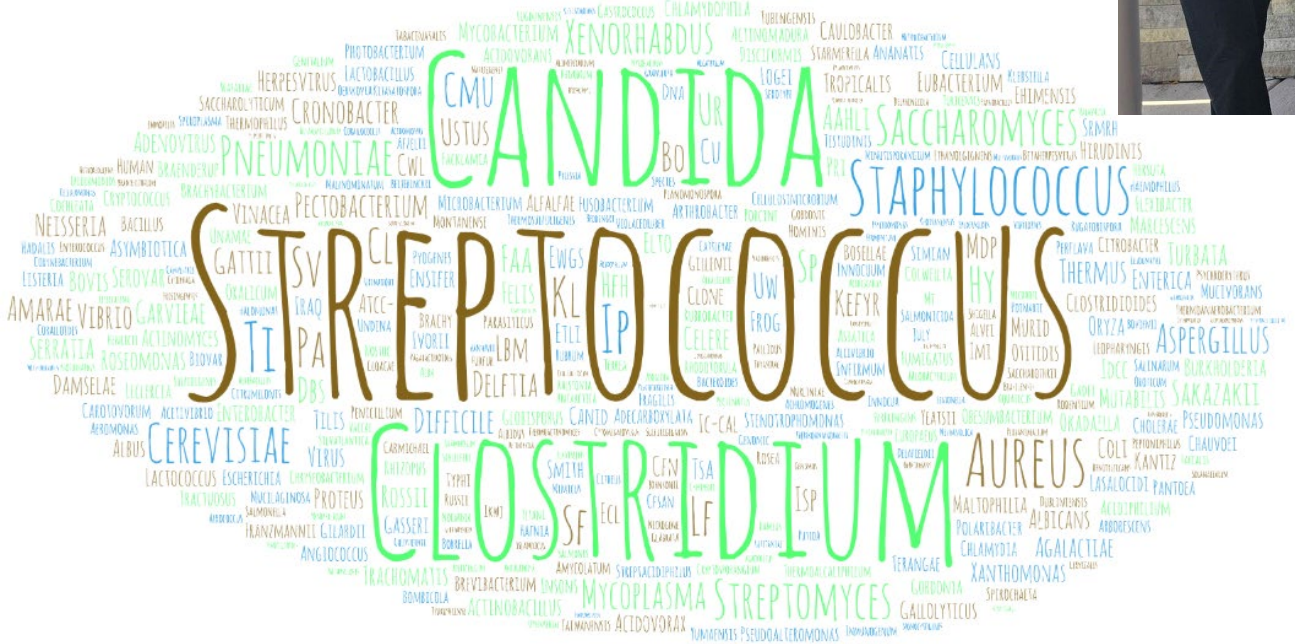
Acknowledgements

- ATCC's Sequencing and Bioinformatics Center
- Corina Tabron



Thank you