JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Oxford Nanopore Sequencing

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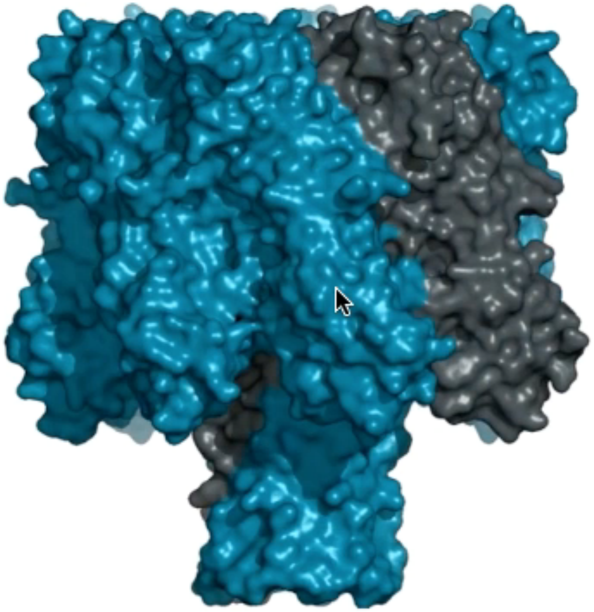
Outline

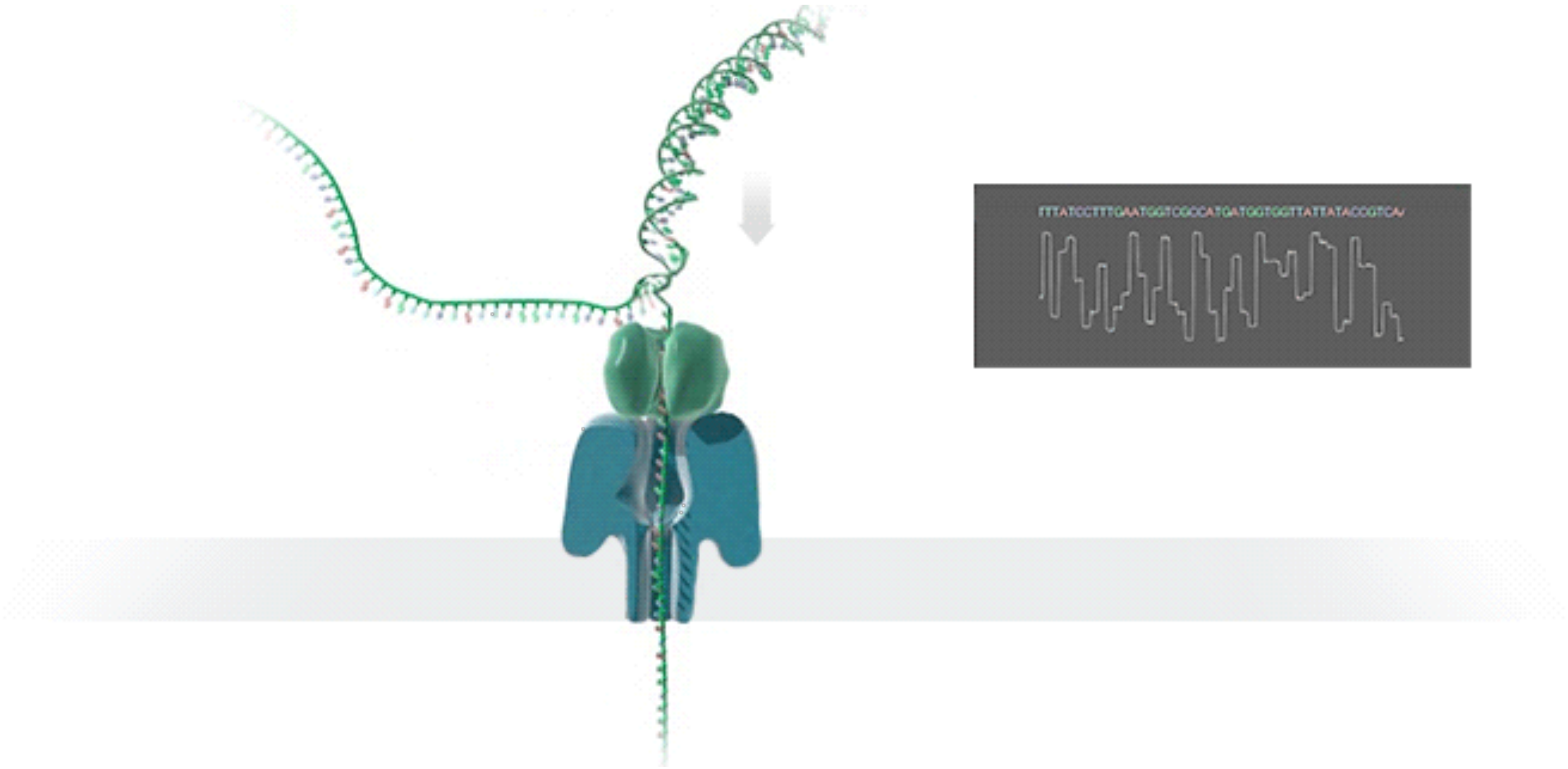
- **Technology Introduction**
- **Current Experience:**
    - Overview of ONT library types
    - Sequencing Run Performance
    - Application: Mini Mock Metagenome
- **Future Directions**

Nanopore Sequencing



Source: Oxford Nanopore Technologies

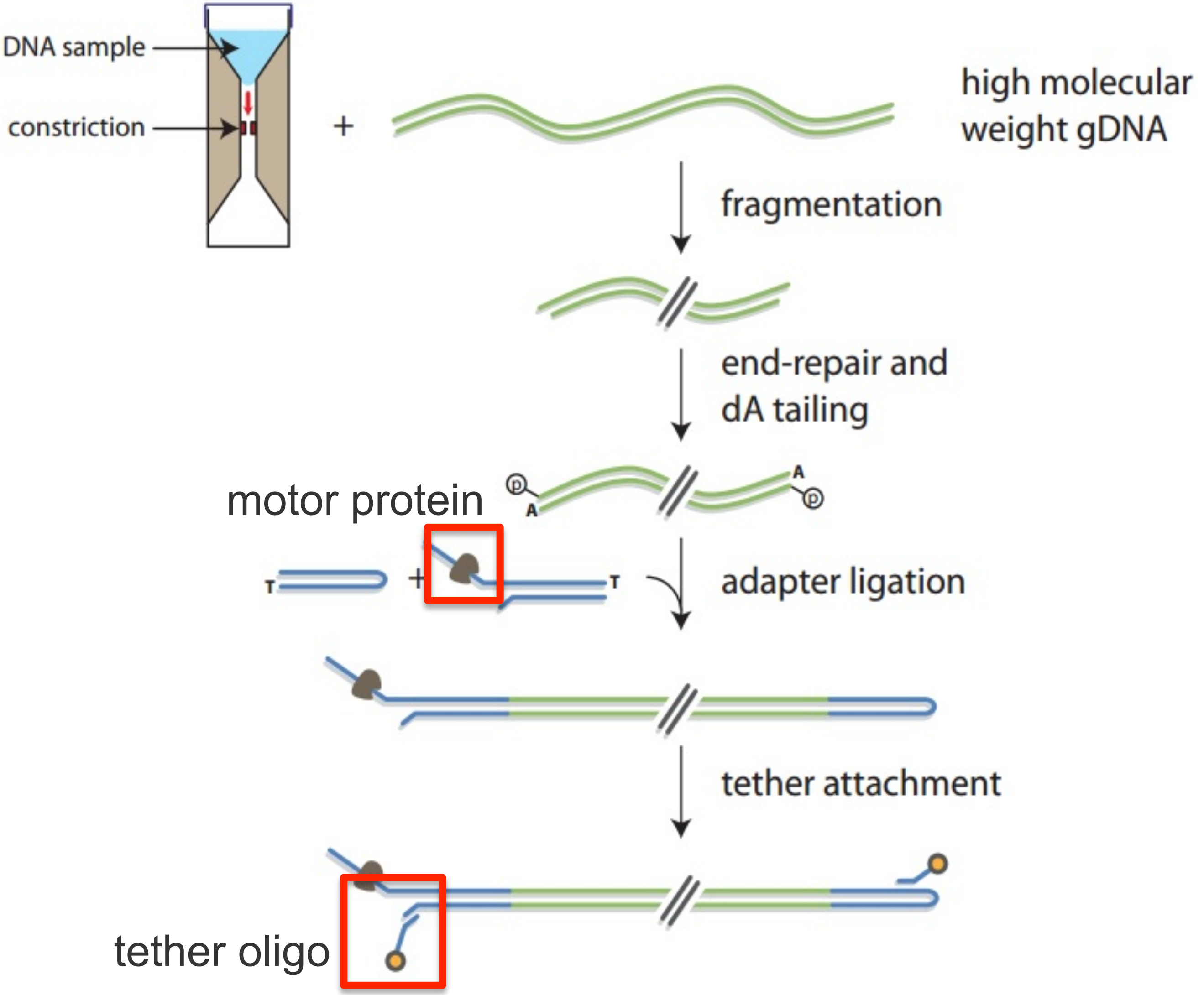
ONT Sequencing



No polymerase or amplification needed → as long as DNA intact, long reads possible

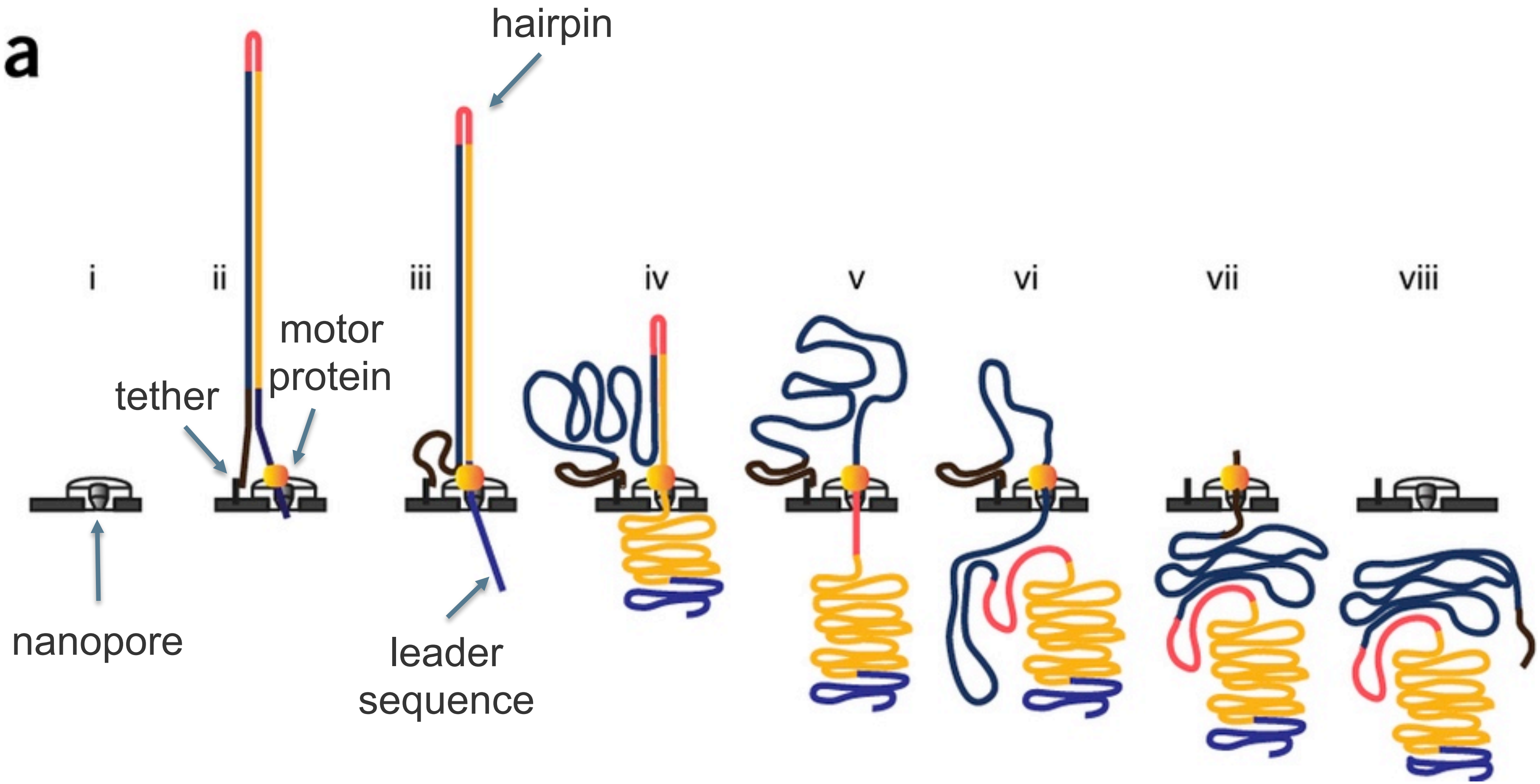
Source: Oxford Nanopore Technologies

Simple Library Preparation



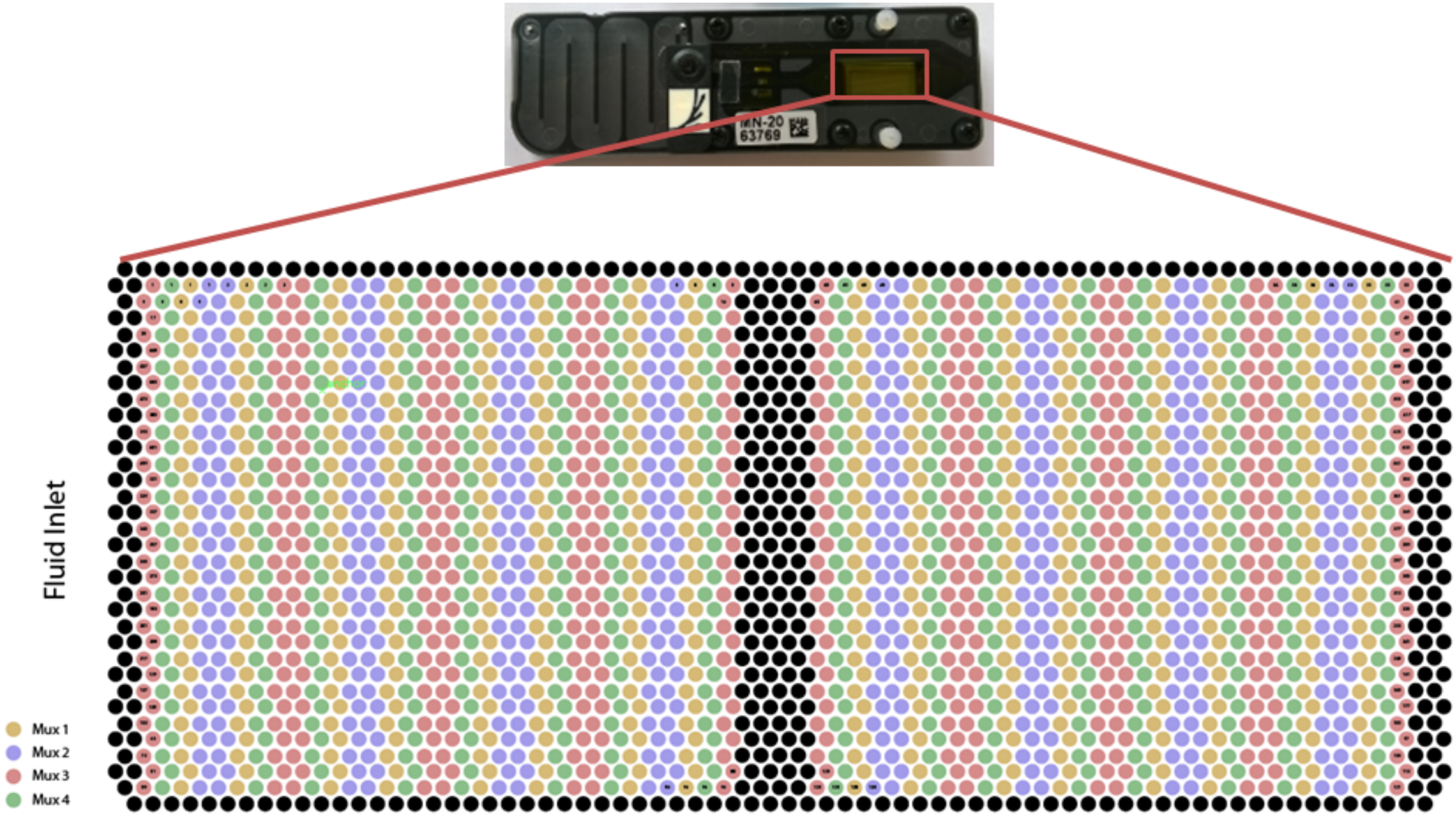
Source: Oxford Nanopore Technologies

Nanopore Sequencing



Source: Jain *et al.*, *Nature Methods* 2015

Flow Cell Layout



512 sequencing channels → each channel sequences multiple molecules

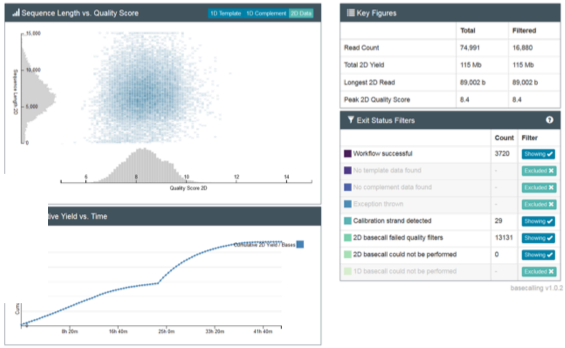
Source: Oxford Nanopore Technologies

Cloud-Based Data Processing

METRICHOR

MinKNOW

MinION



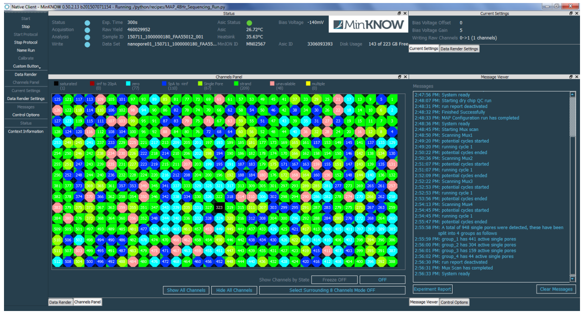
Key Figures

	Total	Filtered
Read Count	74,991	16,880
Total 2D Yield	115 Mb	115 Mb
Longest 2D Read	89,002 b	89,002 b
Peak 2D Quality Score	8.4	8.4

Exit Status Filters

	Count	Filter
Workflow successful	3720	Showing ✓
No template data found	-	Excluded ✗
No complement data found	-	Excluded ✗
Exception thrown	-	Excluded ✗
Calibration strand detected	29	Showing ✓
2D basecall failed quality filters	13131	Showing ✓
2D basecall could not be performed	0	Showing ✓
1D basecall could not be performed	-	Excluded ✗

basecalling v1.0.2



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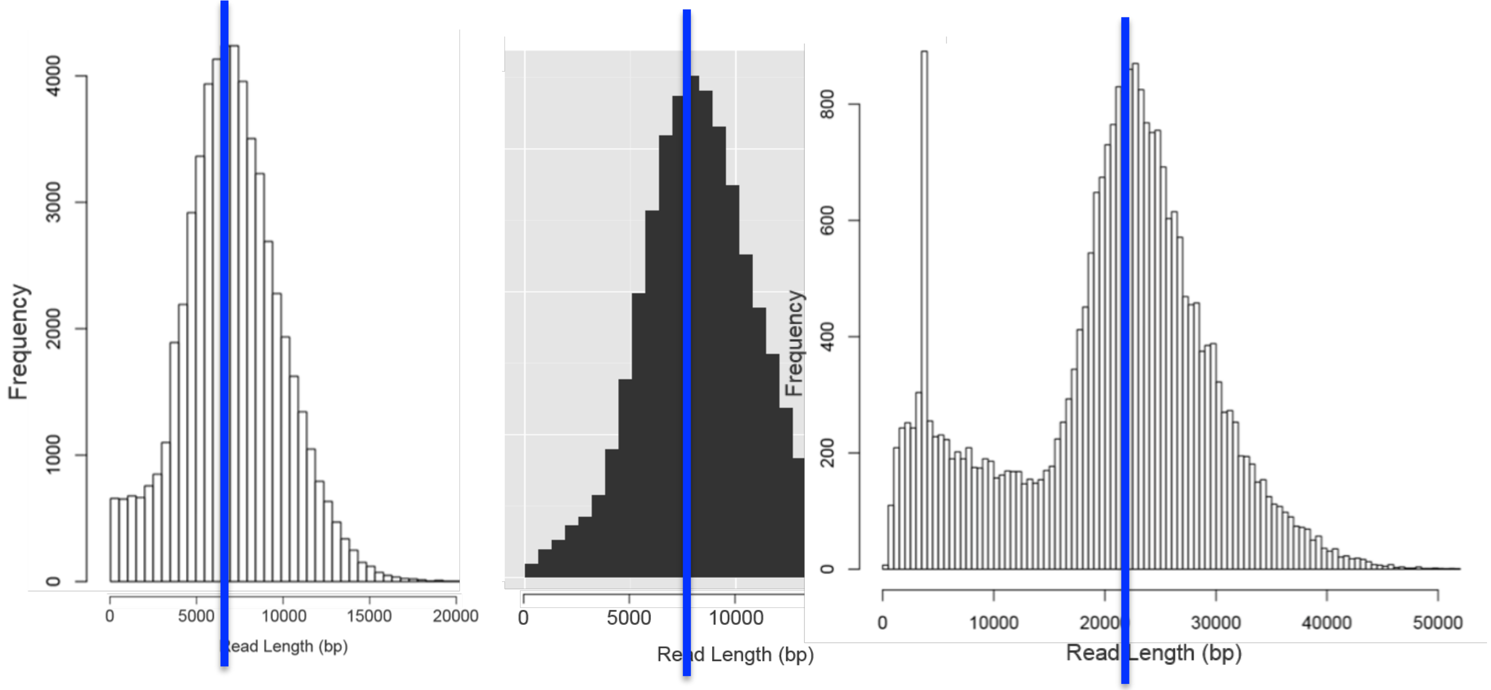
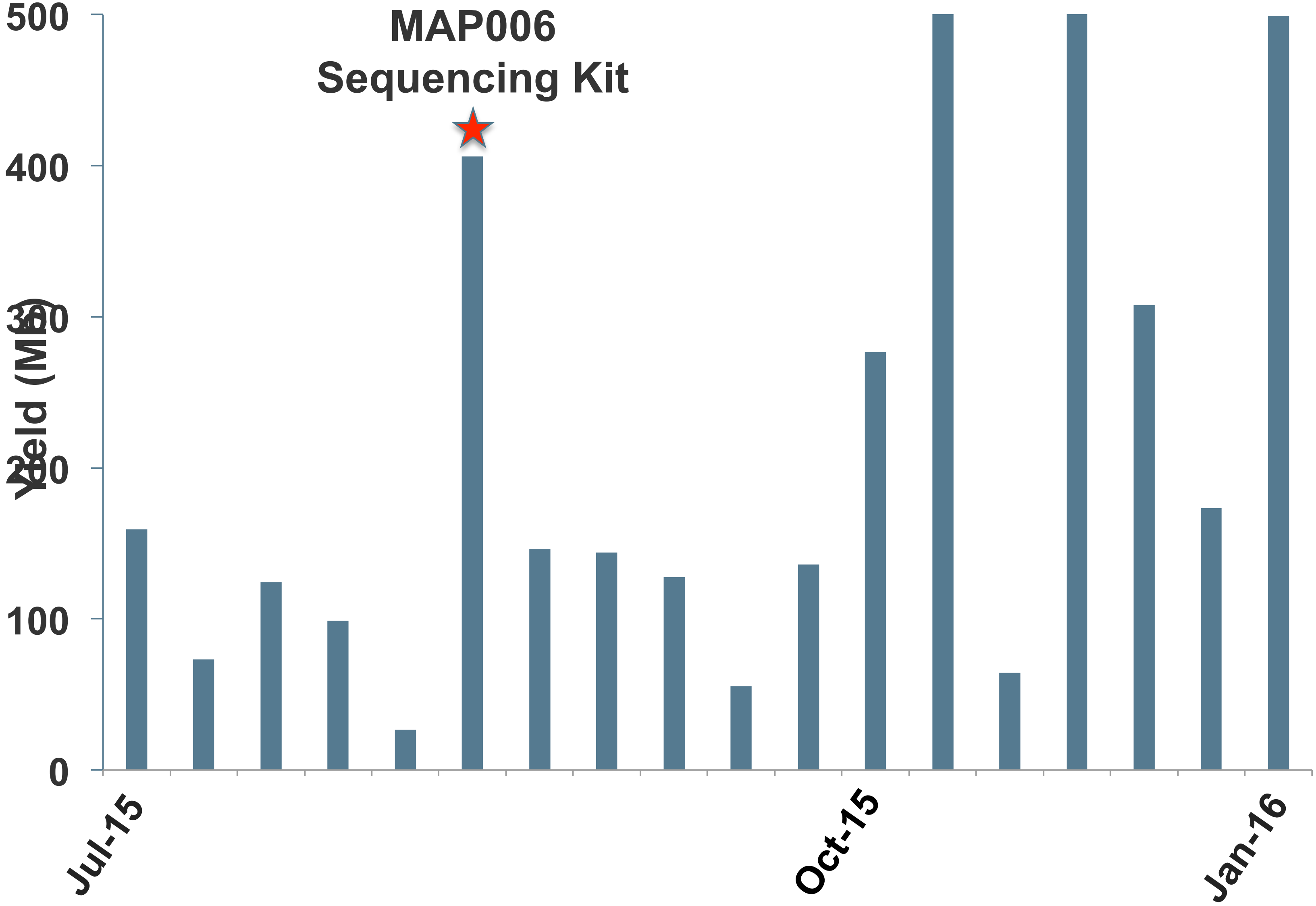
Library Types



Run Statistic	8kb Standard	8kb Low Input	20kb
Input	1µg	100ng	15µg
Yield (Mb)	406 Mb	303 Mb	537 Mb
# Reads	57K	37K	26K
Mean Size	7263 bp	8524 bp	21310 bp

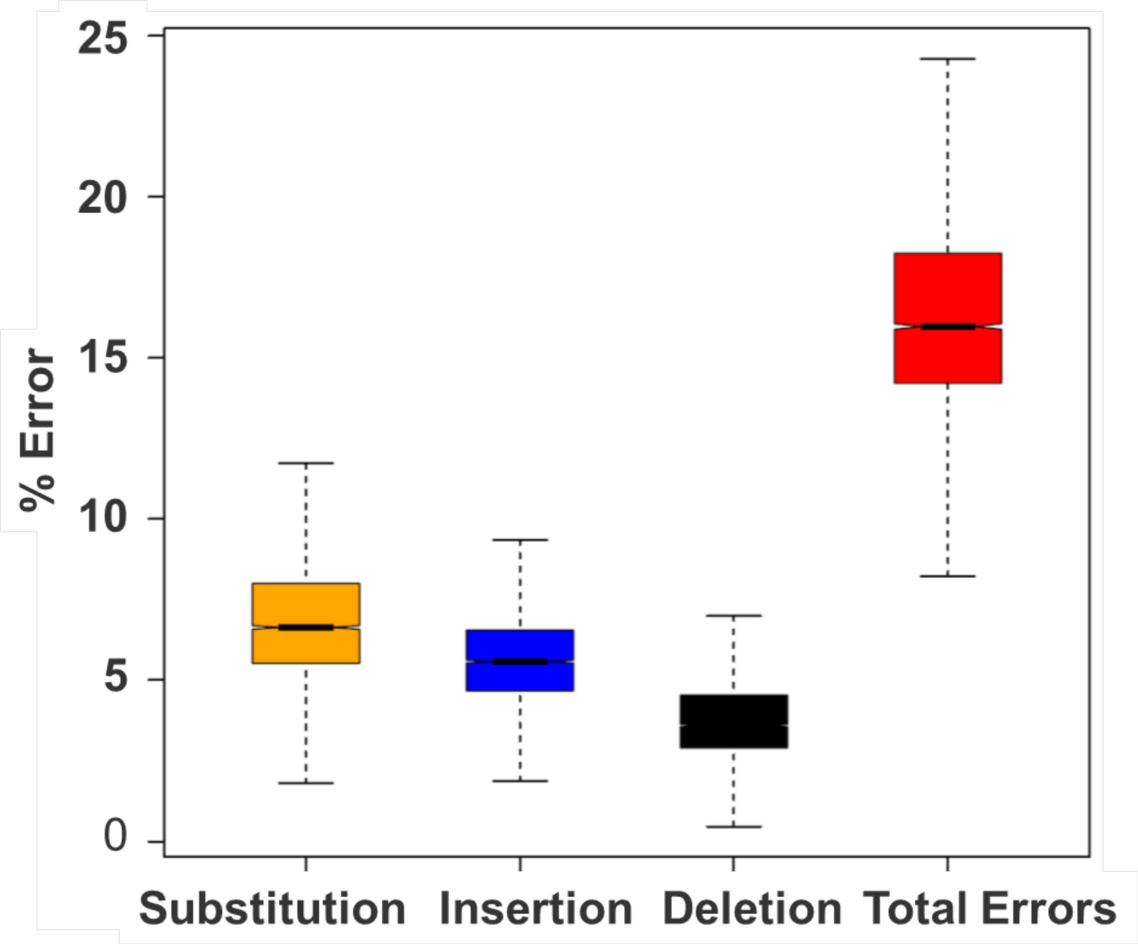
Review of Runs – Yield (Mb)



For Ref: PacBio 1-10Gb; Illumina 10-1000Gb

High Error Rate

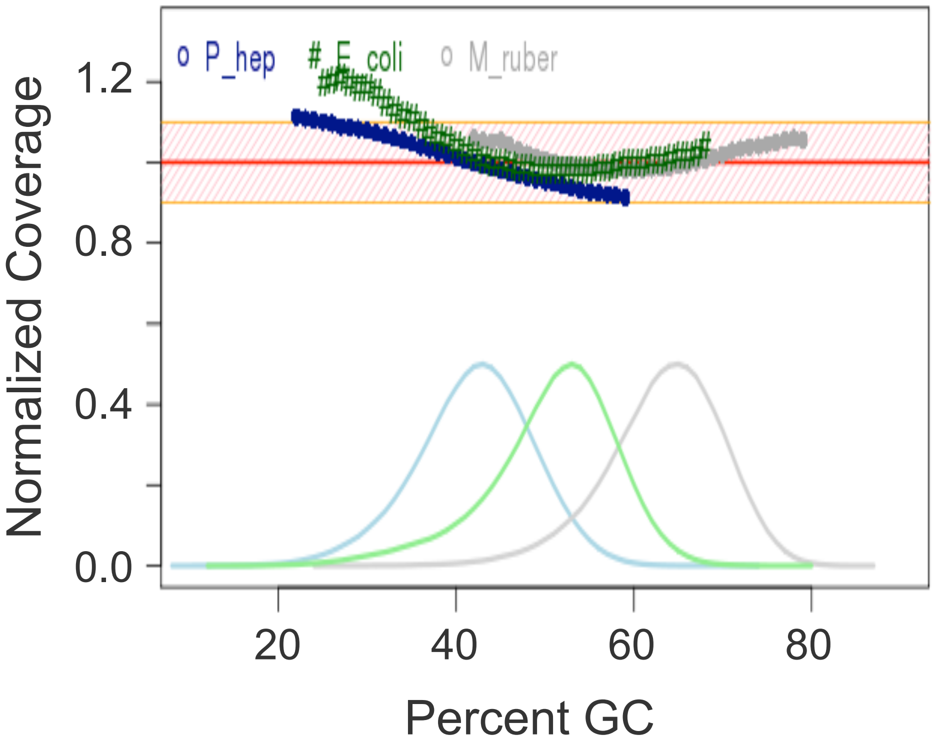
- **Percent ID = ~ 83%**
  - Note: Illumina >99%, PacBio ~ 85%
- **Substitutions > Insertions > Deletions**



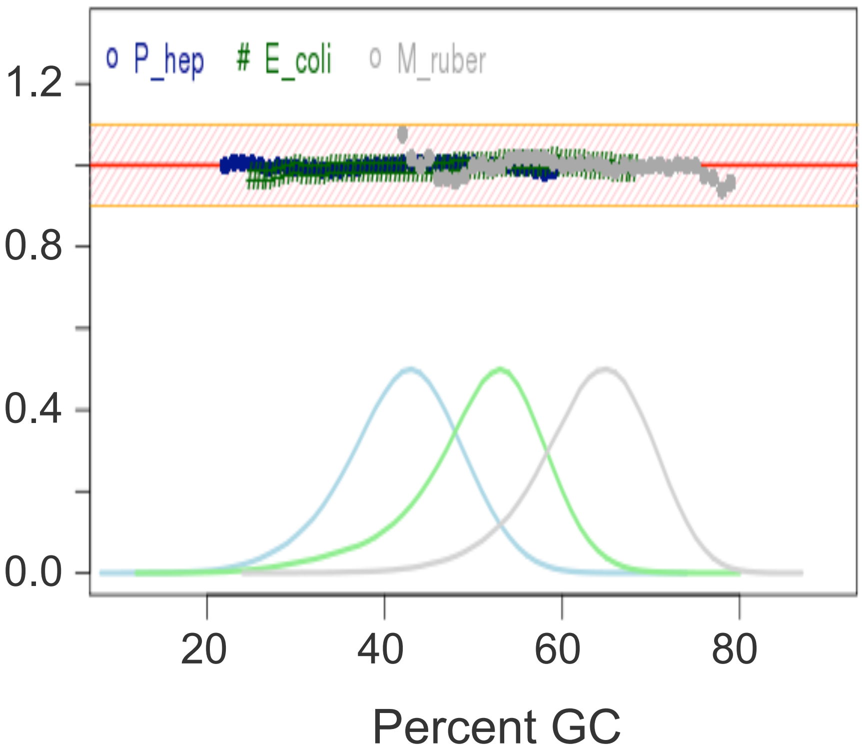
ONT Minimal GC Coverage Bias

Species	P. heparinus	E. coli	M. ruber
GC content	42%	51%	63%

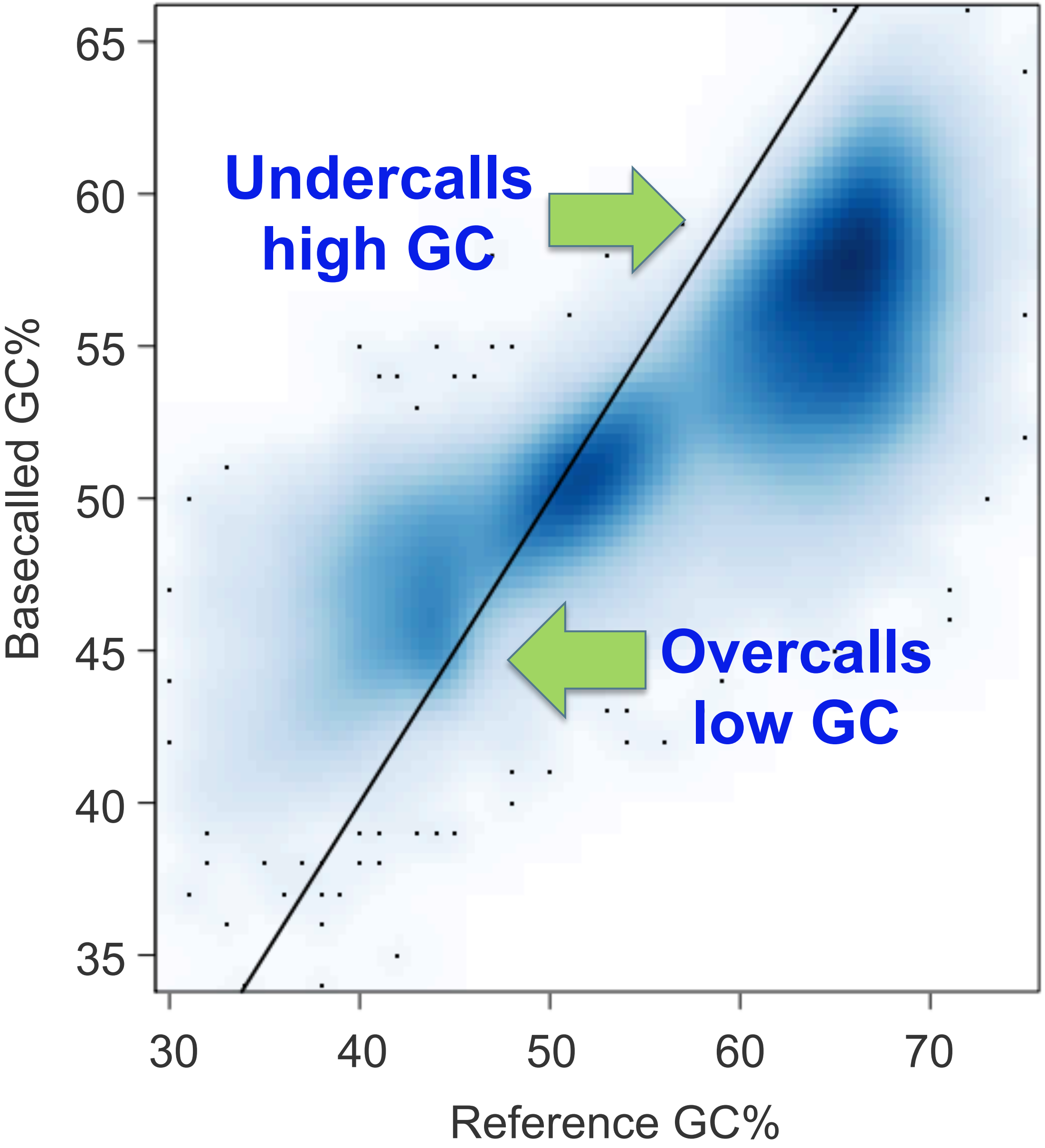
Illumina



ONT

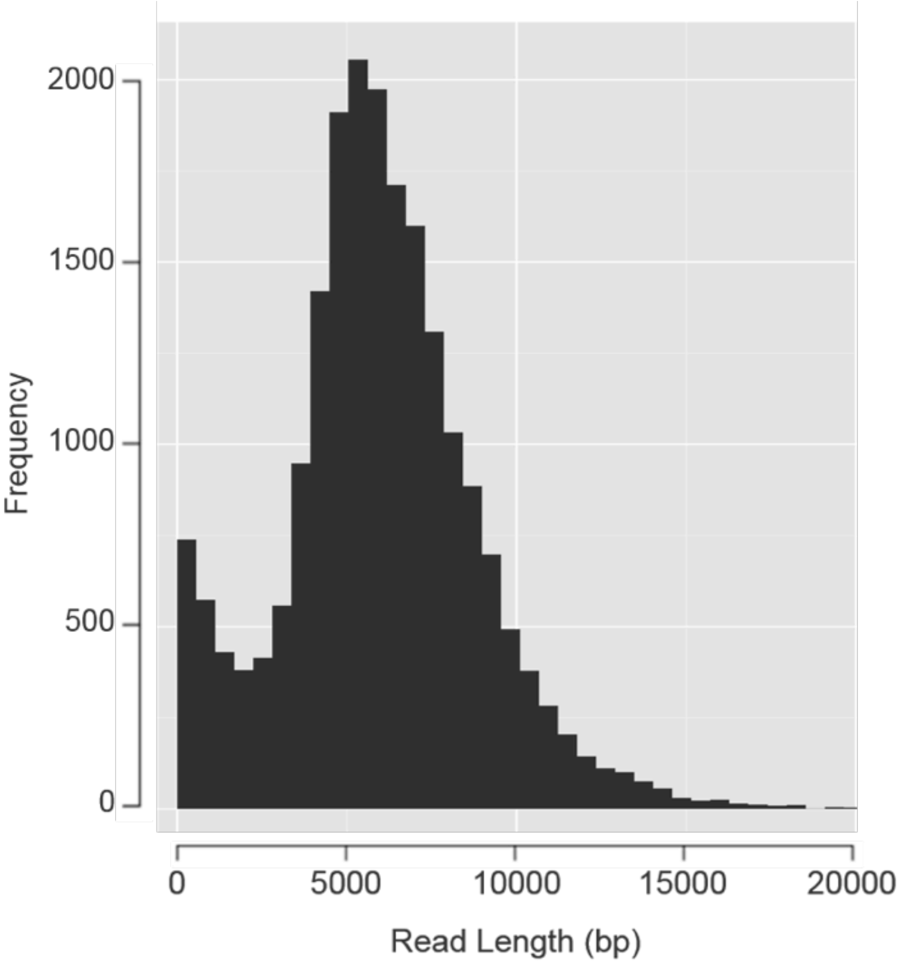


Incorrect GC Calls at Extremes



Application: Mini Mock Metagenome

- **4 organisms pooled in equimolar amounts**
  - GC content from 40% to 70% -- 2 organisms with GC content > 60%
- **8 kb library constructed and sequenced**



Run Statistic	Amount
Yield (Mb)	458 Mb
# Reads	77K
Mean Size	7723 bp
Median Size	7674 bp

ONT: Conclusions and Considerations

- **8kb Protocol Modifications Yield 20kb+ Libraries**
  - Consistent and robust protocol performance

- **100ng Low-Input Protocol Provides Similar Yields**

- **Yields and Read Lengths from Mini Mock Metagenome Similar to Single Organisms**

- **Currently Higher Error Rate in Extreme GC Content**

- **Yet, Minimal GC Coverage Bias Observed**

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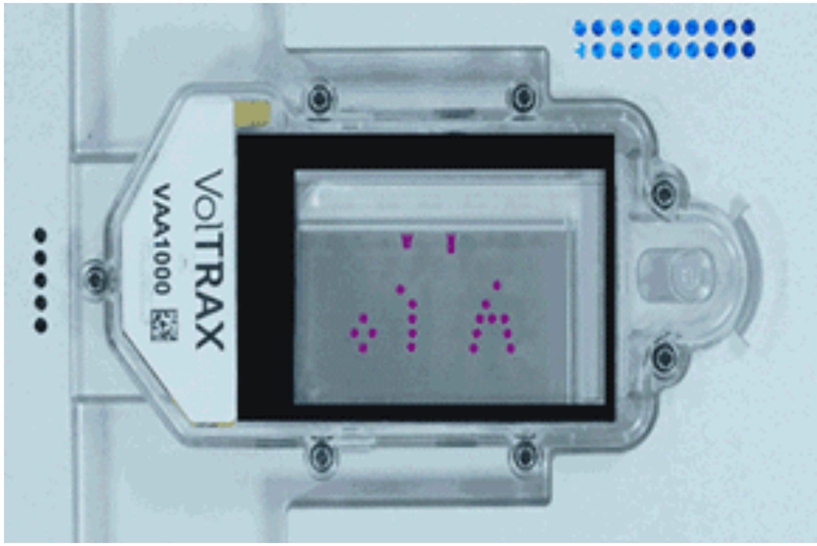
Future Directions

- **Protocol development and applications:**
  - Ex. longer read lengths
  - Ex. complex metagenome communities

- **New ONT protocols:**
  - New sequencing chemistry (R9) – faster speed, more accurate basecaller
  - Sequencing of native RNA

- **Software development:**
  - minoTour analysis platform
    - Monitor reads in each pore in real-time and reject reads that are unacceptable

ONT Updates -- Hardware



VOLTRAX

- Automated sample preparation
- 2016

PROMETHION

- 300X greater throughput than MinION
- JGI early access site
- 2016

Director's Call for Proposals

- Use ONT's long-read sequencing capability for novel applications

- http://jgi.doe.gov/user-program-info/other-programs/

Thanks to the JGI Nanopore Group

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