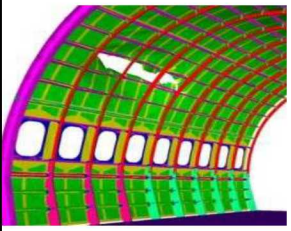


Nanopore Sequencing for Real-Time Pathogen Identification

SAND2017-2126C

PI : Michael Bartsch

Key Team Members:

Sara Bird, Steven Branda, Harrison Edwards, Hari Jayamohan, Raga Krishnakumar, Joe Schoeniger, Dan Throckmorton, and Anupama Sinha

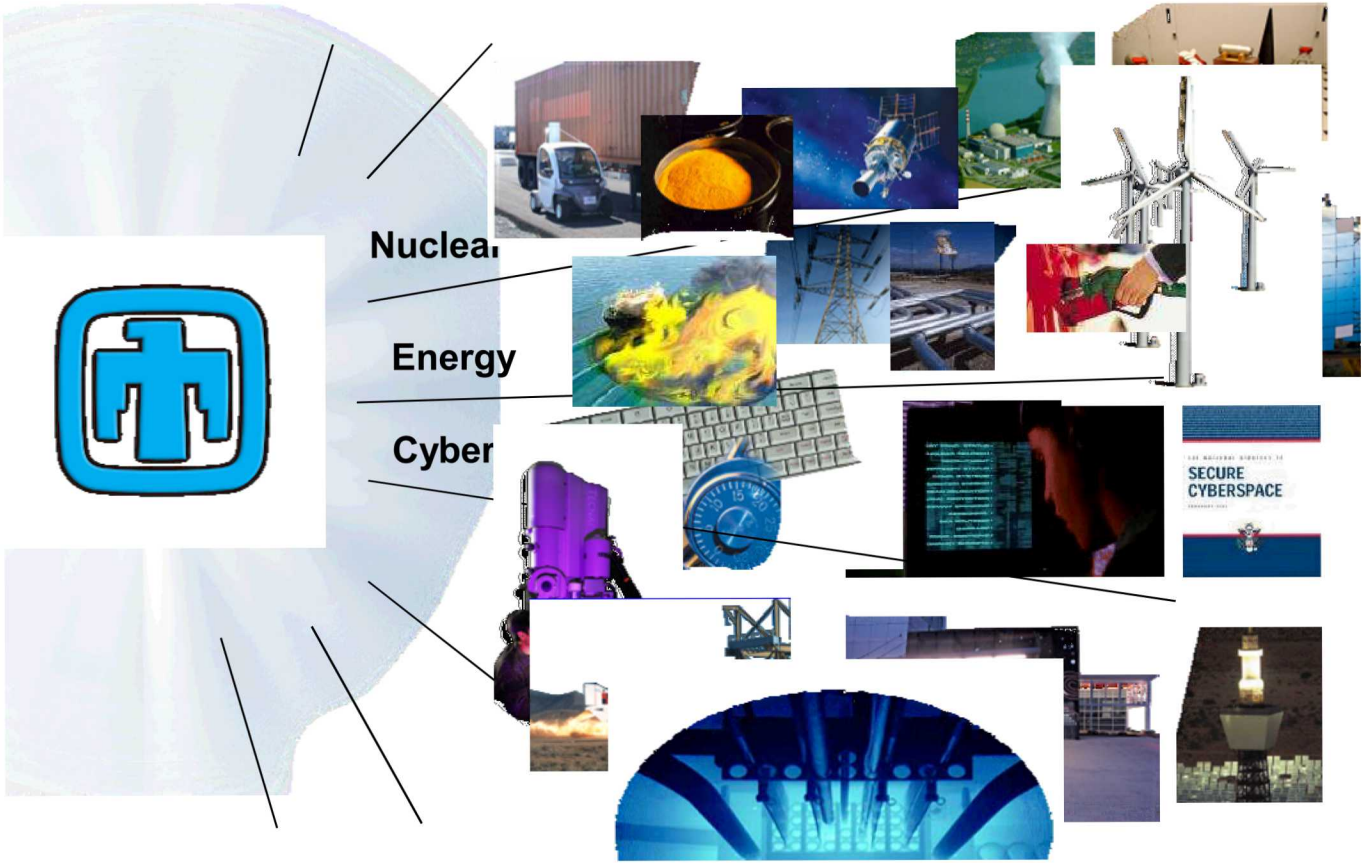
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Outline

- What is Nanopore sequencing?
- Applications
- Issues with sample & library preparation
- Solution – Automated library prep (ASPIRE)
- Future direction

2

Sandia National Labs- Focus Areas



Sandia engages in three broad areas of biological defense relevant to national security

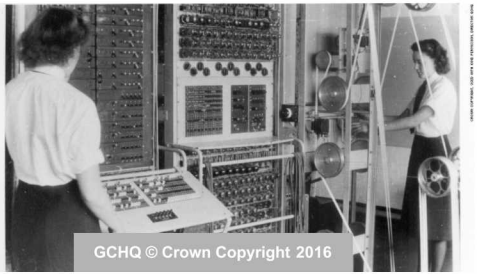
- Civilian population protection and homeland security
  - Biological threat assessment, detection, restoration, forensics and agricultural protection

- Military force protection
  - DoD seeks solutions in detection, protection, pretreatments, therapeutics, and diagnostics

- Human health and emerging infectious diseases
  - Early diagnosis and medical counter-measures against pandemic and emerging infectious diseases

Complete Sample-to-Answer systems for rapid actionable information

Evolution of sequencing



1st Gen/ *Sanger*

2nd Gen / NGS *Illumina,Roche…*

3rd Gen / *MinION…*

3rd Gen vs. 2nd Gen

	MinION	MiSeq
Size	80 cc	200,000 cc
Weight	0.103 kg	57.2 kg
Cost	$1k	$125k
Read Length	300 kilo bp	2 x 300 bp
Amplification Required	No	Yes
Selective Sequencing	Yes- *ReadUntil*	No
Accuracy	<90% (Q10)	>70% bases@99% (Q30)

Credits: Oxford Nanopore Technologies

“Democratization” of sequencing

3rd Gen / *MinION* …

Credits: Oxford Nanopore Technologies

- ✓ Personalized Diagnostics
- ✓ Water testing
- ✓ Food safety
- ✓ Environmental monitoring
- ✓ Forensics
- ✓ Biodefence ….

https://nanoporetech.com/applications

Significant interest in 3rd Gen Sequencing

Lasergen

Noblegen BIOSCIENCES

STRATOS genomics inc.

10X GENOMICS™

Complete genomics

GenapSys

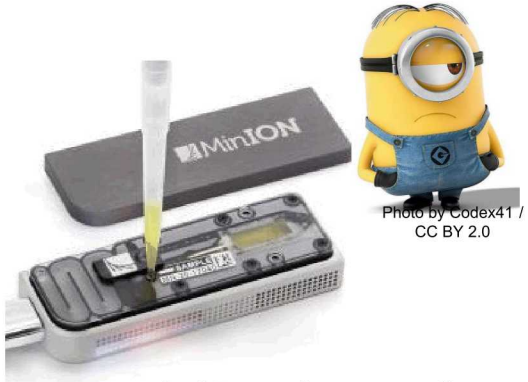


Photo by Codex41 / CC BY 2.0

QIAGEN

Nabsys

Only commercial product so far…

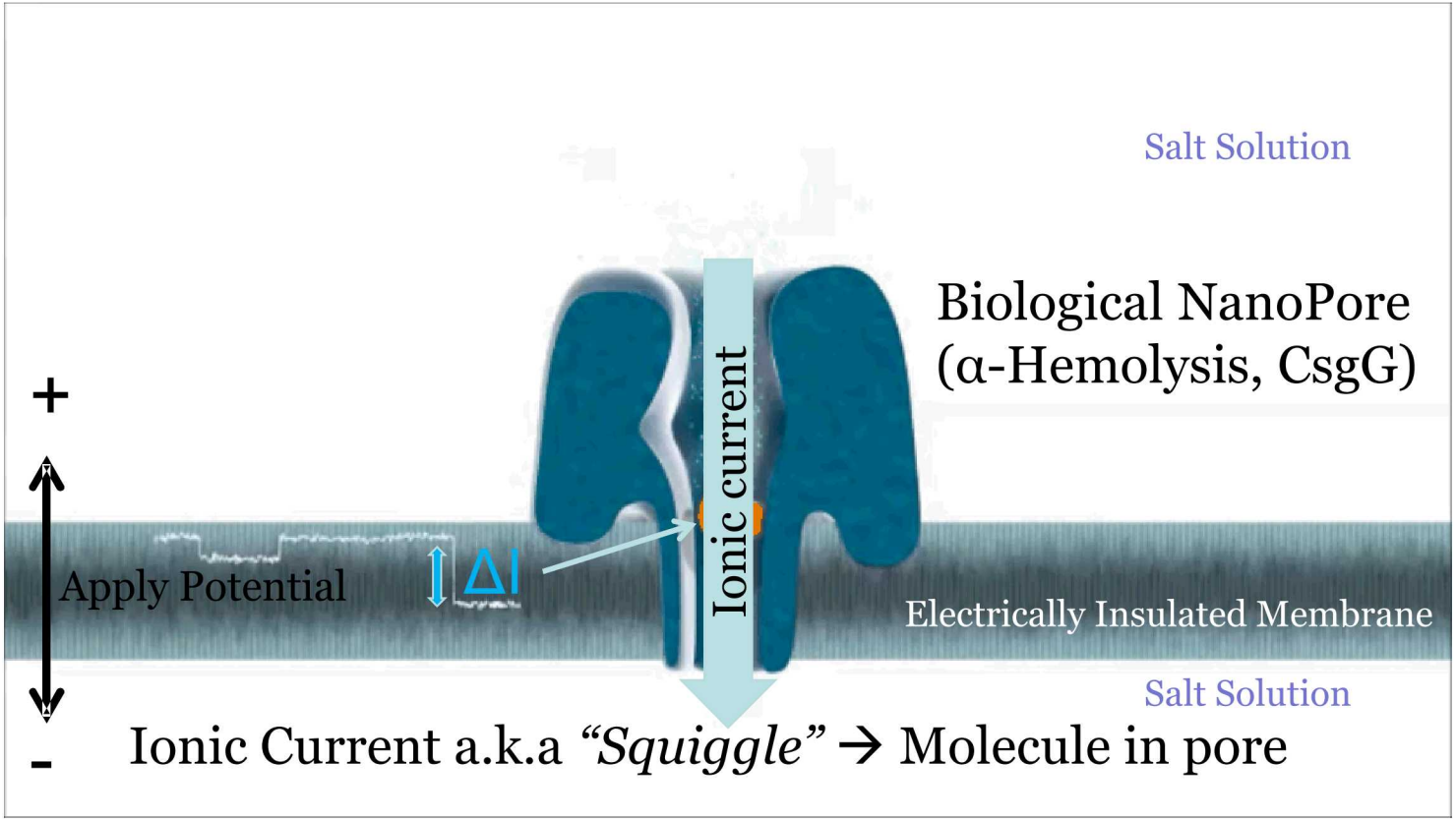
illumina®

gnubio

genia

ion torrent by life technologies™

MinION-Nanopore Sequencing Technology



Credit: https://nanoporetech.com/

MinION-Nanopore Sequencing Technology

Video credit: https://nanoporetech.com/

MinION Sequencer-In the Field…

- Ebola[1] & Zika virus surveillance[2]

Zika in Brazil Real-Time Analysis (ZiBRA) traveling laboratory (Berman, www.voanews.com, June 8, 2016)

1. Quick, Joshua, et al. *Nature* (2016)
2. Quick, Joshua, et al. bioRxiv (2017)

MinION Sequencer-In Extreme environments..

- Remote sequencing
  - Antarctica & Artic Glacier (78° N) [1]

Credits: Arwyn Edwards

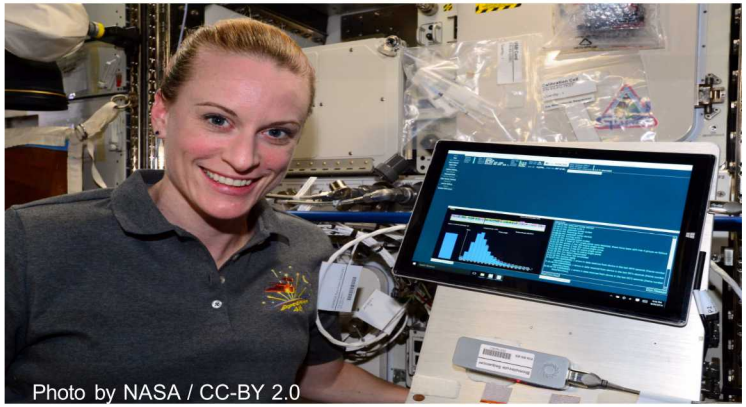
Credits: Sarah Johnson, Georgetown University

1. Edwards, Arwyn, et al. bioRxiv (2016)

MinION Sequencer-……and in Space!

- Remote sequencing
  - International Space Station
  - 62 feet below the Atlantic Ocean

ISS



NASA Aquarius Reef Base, Extreme Environment Mission Operations, Atlantic Ocean

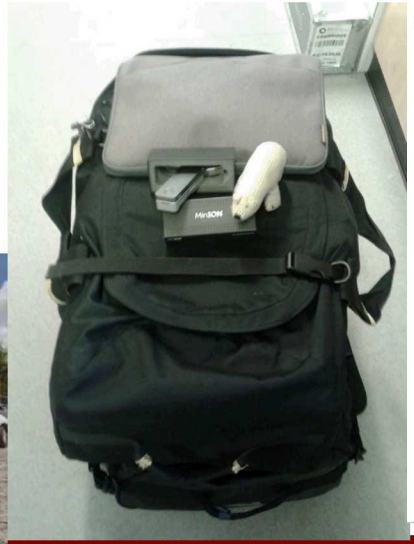
Sample prep– Still not portable

<100 grams (MinION)

Vs.

"50 kg of standard airline travel luggage "[1]

Copyright: Oxford Nanopore Technologies



Supplementary Table 1: Field Metagenomics Lab Equipment

Hardware	MinION mk1 device
	Laptop with minKNOW & Metrichor
	4TB Hard Drive
	microcentrifuge
	IKA Vortexor/beadbeater device & cardboard wedge
	Qubit microfluorimeter & protocol
	Pocket scales
	Multiplug
	UK EU electrical adapters
	Hybaid OmnE PCR cycler
	NEB foam rack for Eppendorfs
Pipettors	P2 pipettor
	P10 pipettor
	P20 pipettor
	P200 pipettor x2
	P1000 pipettor x2
Consumables	Gloves, L box
	Gloves, S box
	Duct tape
	parafilm
	LoBind Eppendorf tubes 2x bag

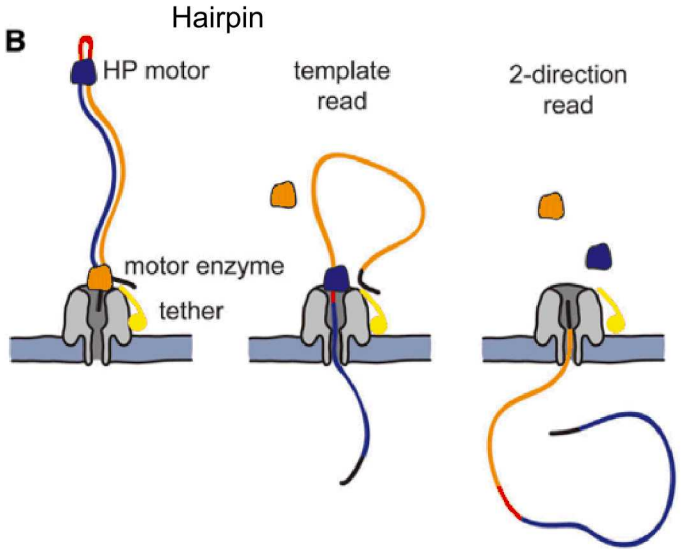
1. Quick, Joshua, et al. *Nature* (2016)

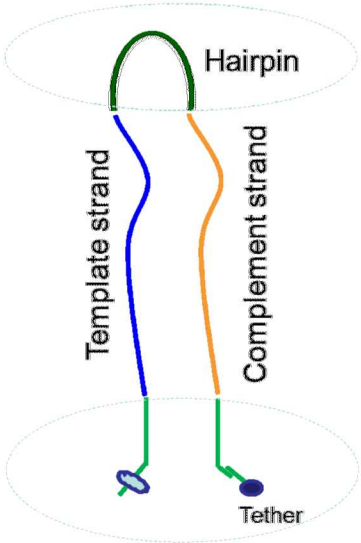
Portable Sample prep!

http://www.zerowait-state.com/blog/the-plm-state-the-cart-before-the-horse-using-plm-to-design-business-process/

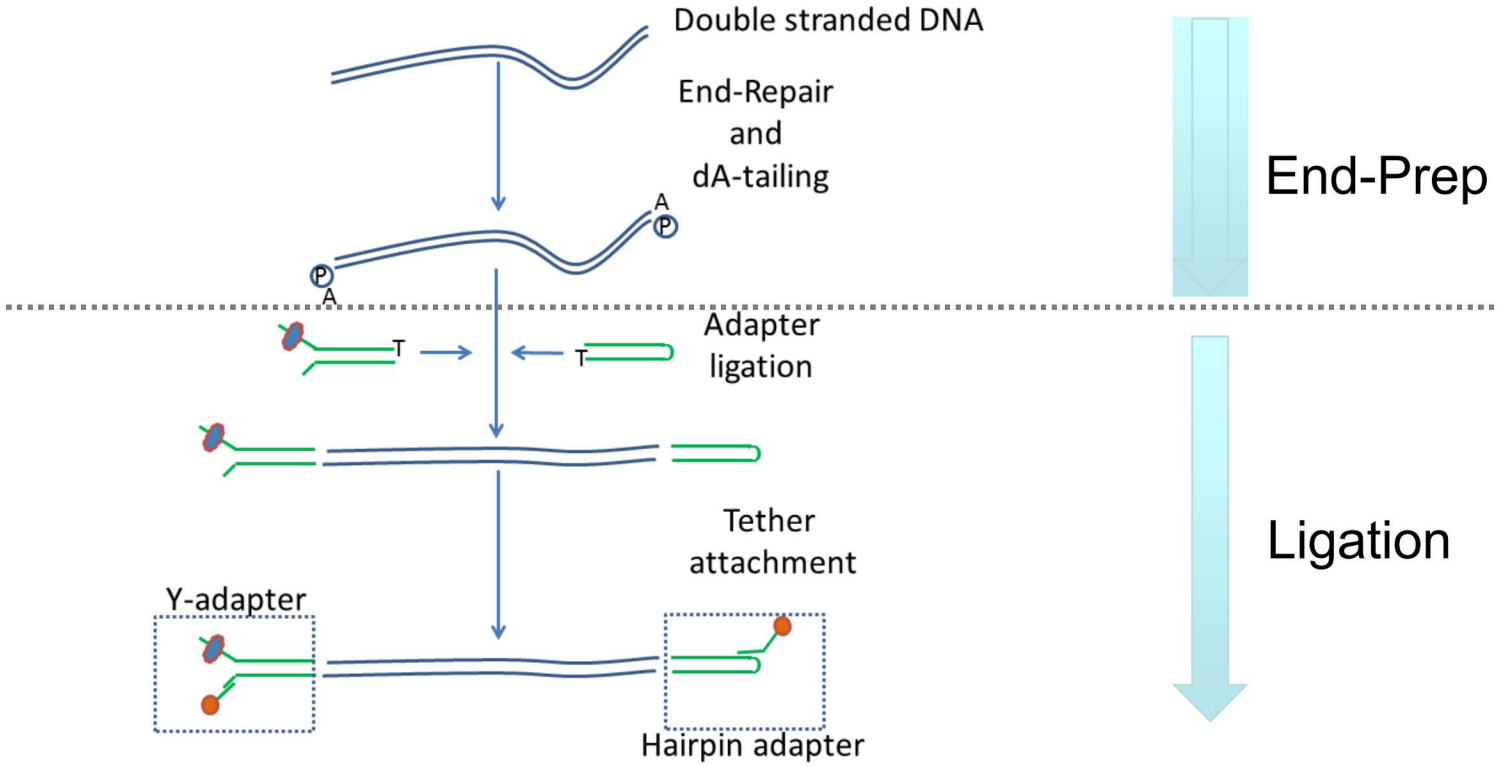
MinION Sequencer-Sample preparation

- Sample prep = DNA extraction + **Library prep**
- **Library prep** = DNA → *Sequencer compatible* DNA

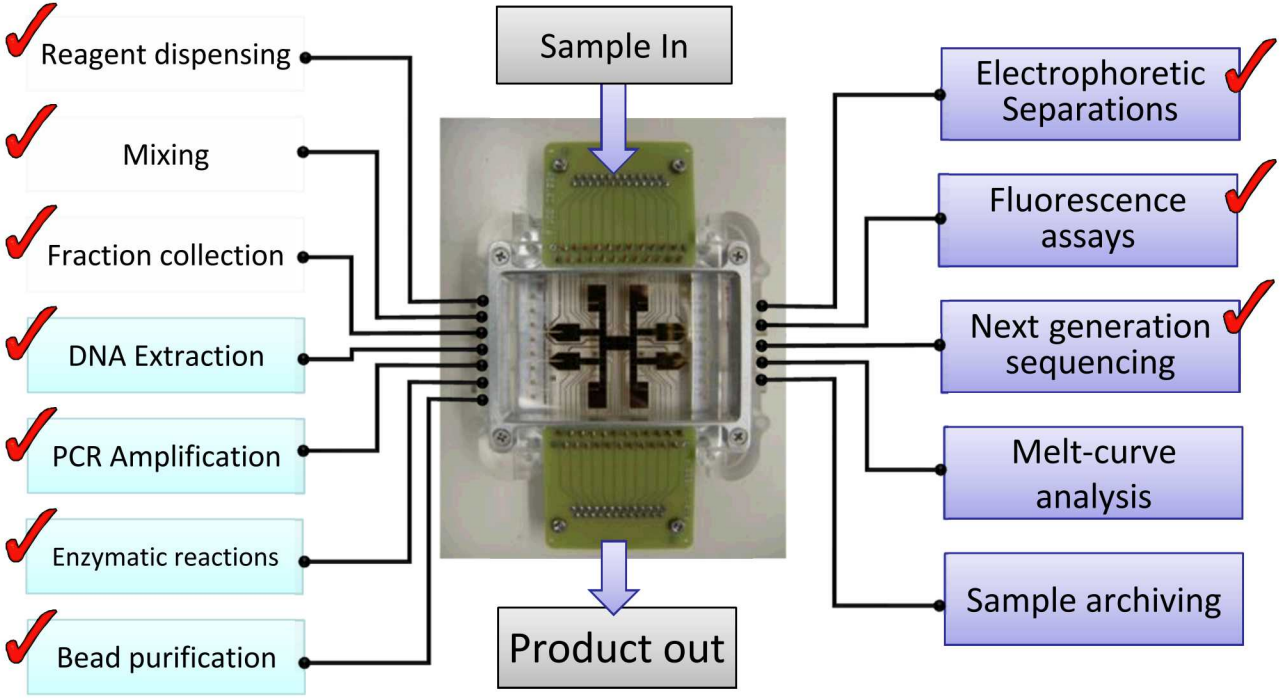




MinION-Library prep

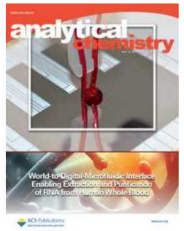


Sample prep Automation @ Sandia leveraging Digital Microfluidics (DMF)



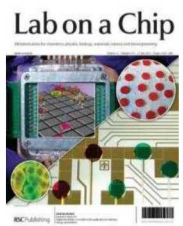
M. Bartsch et al. US8,940,147 **2015**

M. Jebrail, et al, A Chem *Lab Chip*, **2014**



Bartsch et al, R&D 100, **2012**

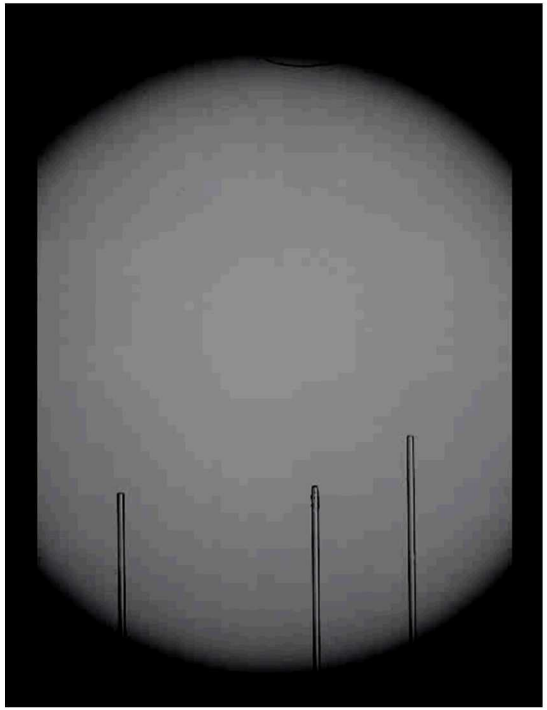
M. Jebrail, et al, Lab Chip, 2012



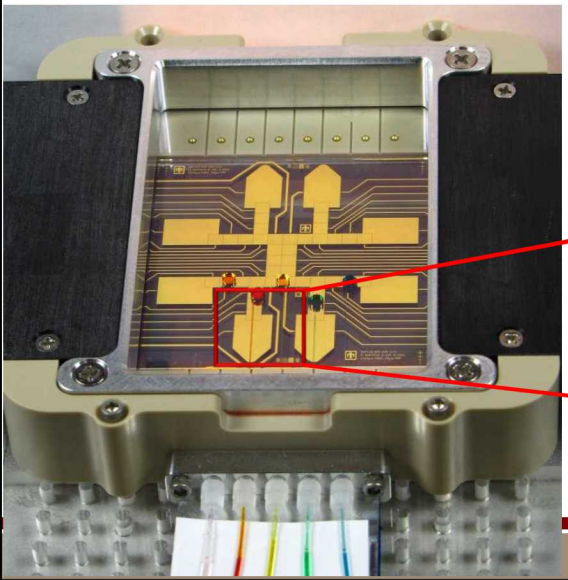
DMF allows configurability & connectivity for versatile operation

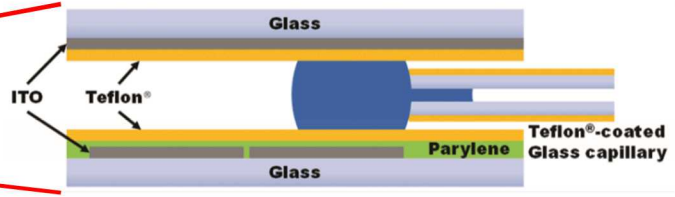
DMF is a central fluidic router for interfacing modules

- Capillary tubing offers a unified interface for all modules
- Programmable discrete manipulations of droplets
- Automation of *Illumina Nextera* library prep[1]



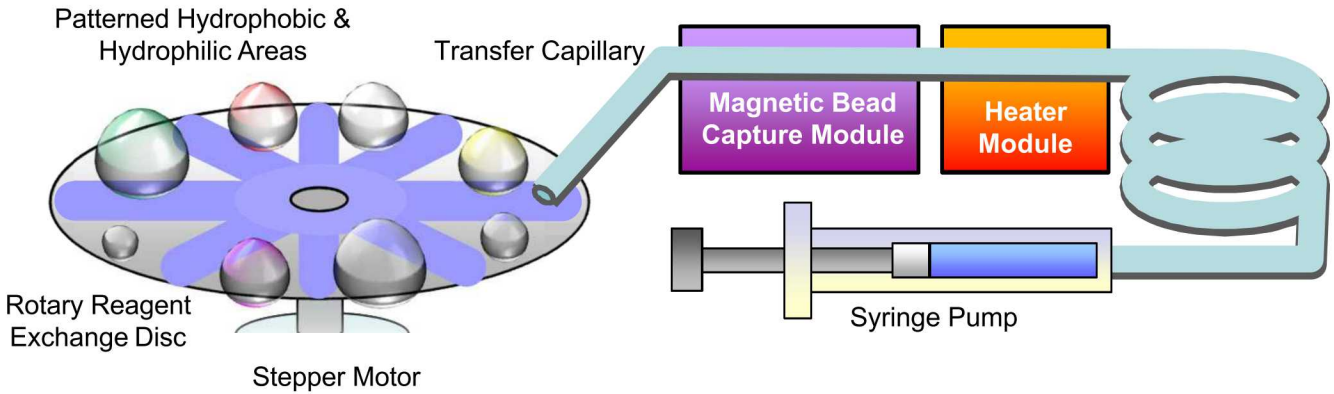
Sandia DMF Hub





1. Kim, Hanyoup, et al. *PLoSONE* (2013)

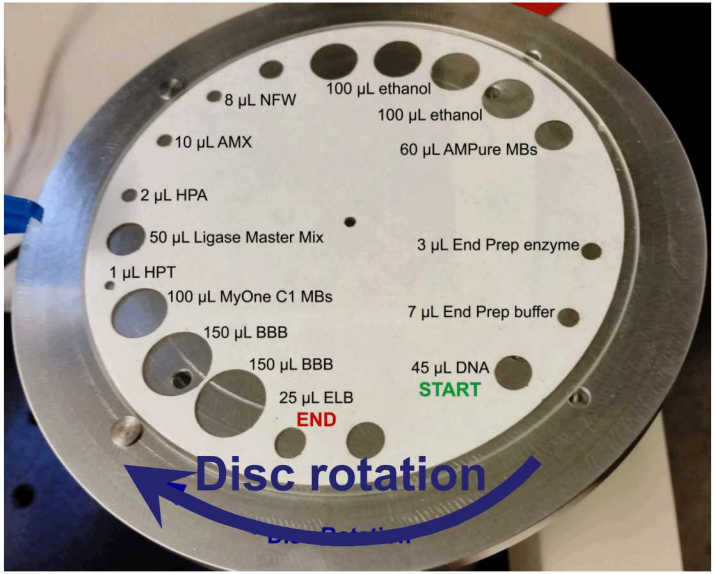
ASPIRE- central fluidic router for interfacing modules



ASPIRE -Automated Sample Preparation by Indexed Rotary Exchange

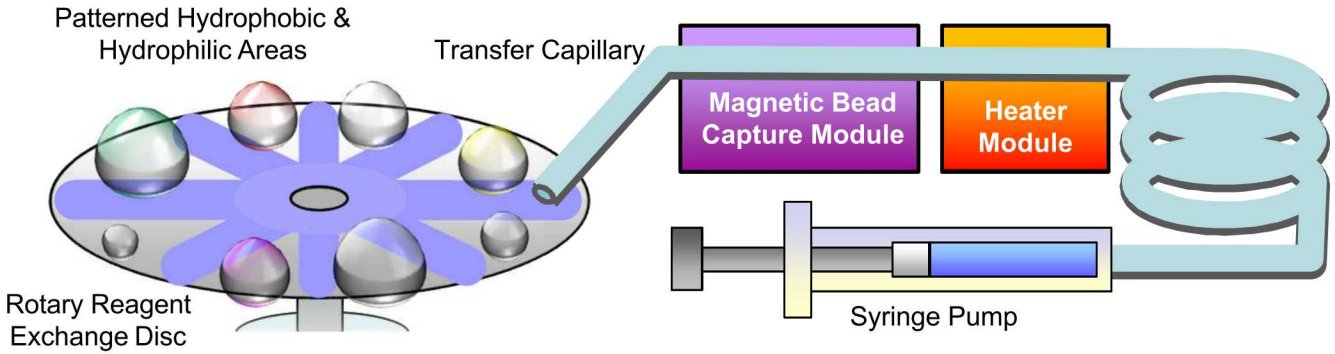
- ✓ Simplified design
  - No clean room fabrication or complex assembly
  - Disk & PFA tubing → Inexpensive, disposable components

ASPIRE- central fluidic router for interfacing modules



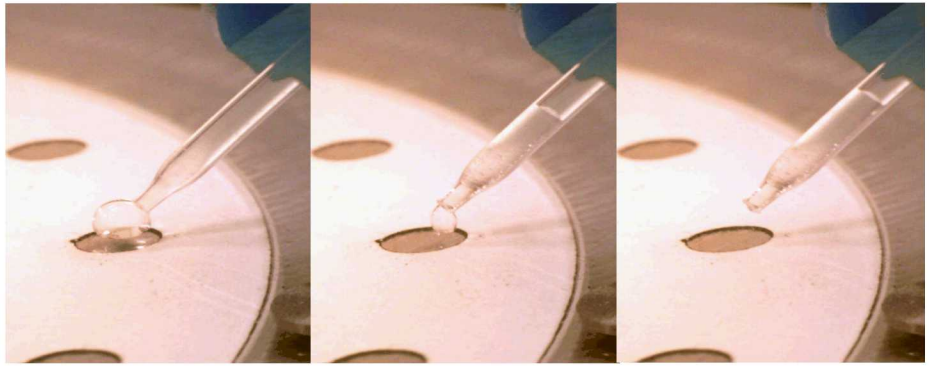
- ✓ Reagents pre-loaded at defined location on hydrophobic disk

ASPIRE- central fluidic router for interfacing modules



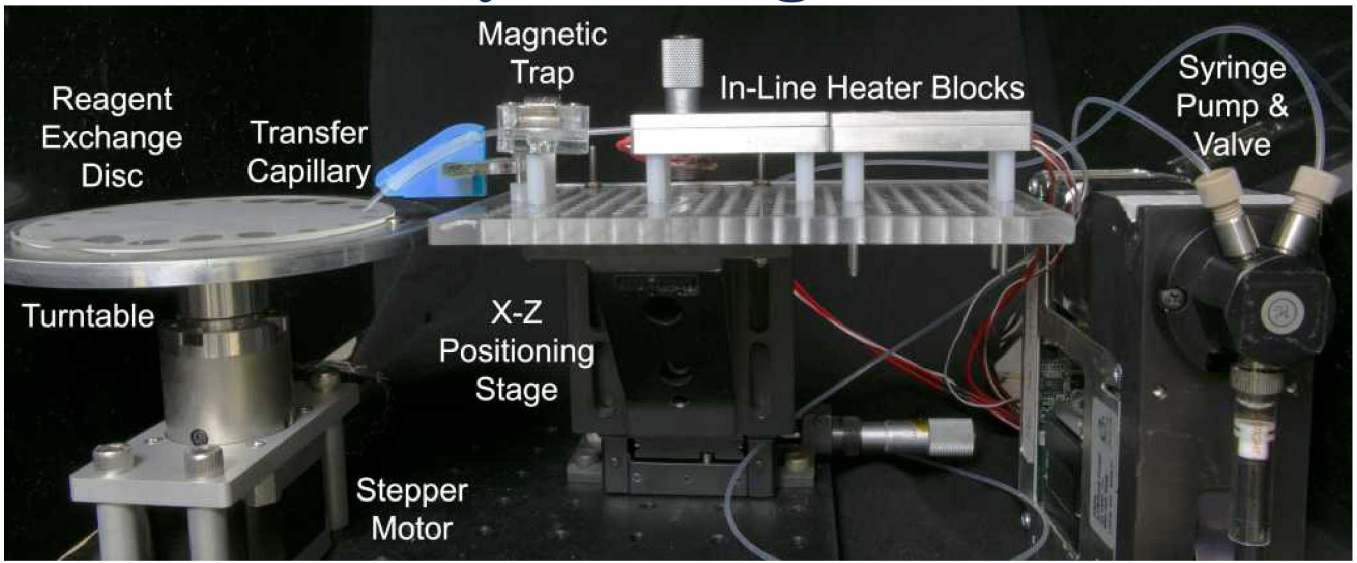
- ✓ Disc rotation brings droplets into contact sequentially with transfer capillary
- ✓ In-line magnetic bead trap, heater blocks, phase-change material for cooling
- ✓ Mixing → dispensing - aspirating from/to disk

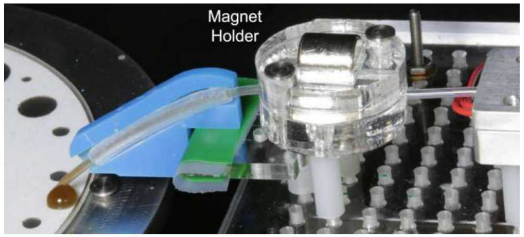
ASPIRE- Schematic

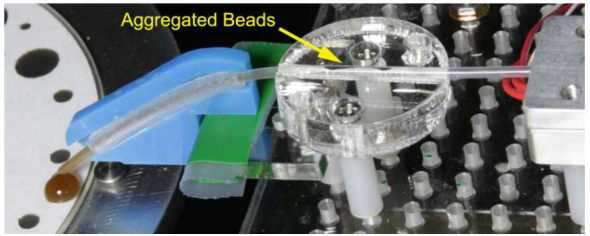


- ✓ Hydrophobic disc →Repeatable droplet pickup
- ✓ Hydrophilic zones →Waste dispensing
- ✓ Low Reynolds number laminar flow regime preserves long DNA strands
- ✓ Automate library prep operations
  - ✓ Mix, magnetic bead capture/wash/elution, sample heating, cooling

ASPIRE- Automated Sample Preparation by Indexed Rotary Exchange





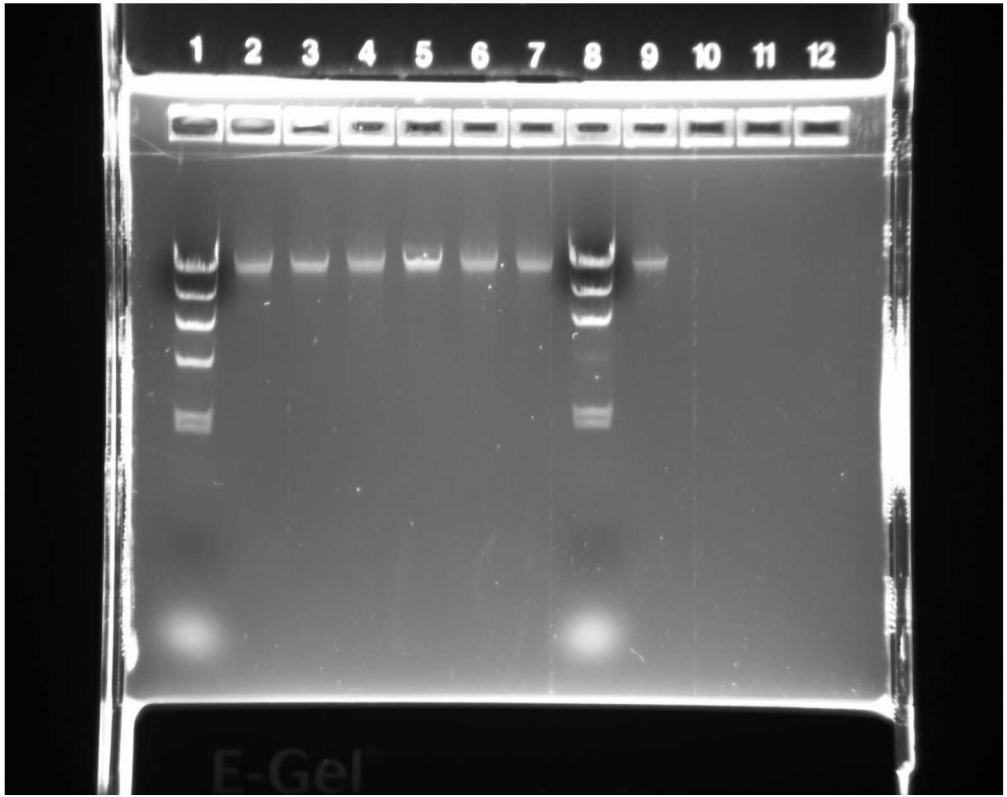


Magnetic bead capture/elution

ASPIRE-Working

2D Prep-Video.mp4

ASPIRE- Preserves integrity of High MW DNA



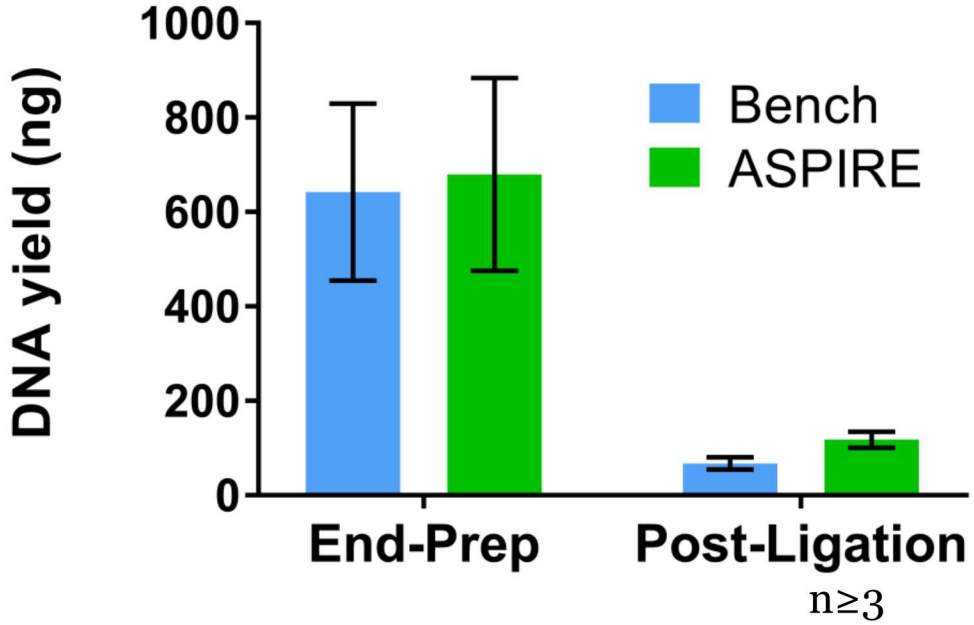
Lane 1 & 8: DNA ladder.

Lane 2 & 9: λ phage DNA (control)- 48,500 bp.

Lane 3-7: λ phage DNA subject to 10, 20, 40, 80 & 120 cycles.

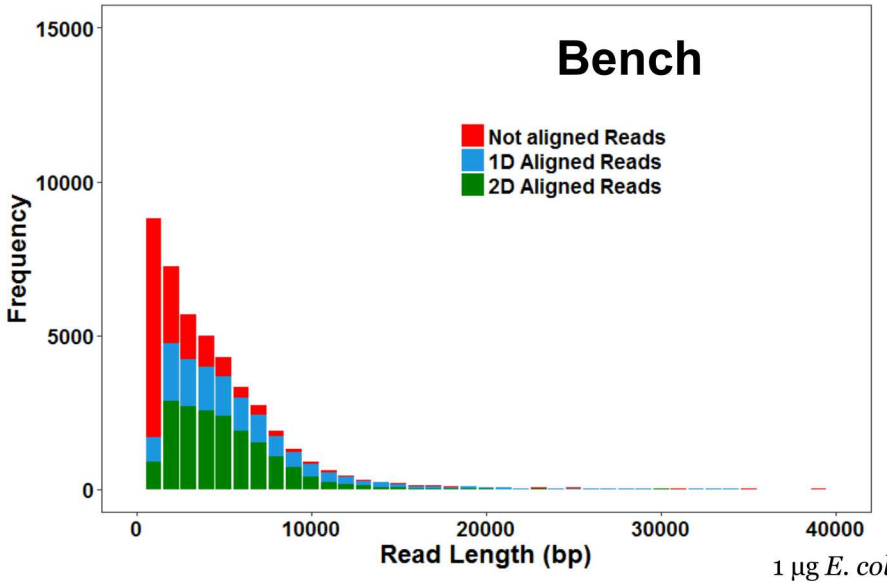
ASPIRE- Comparable DNA yield w.r.t Bench

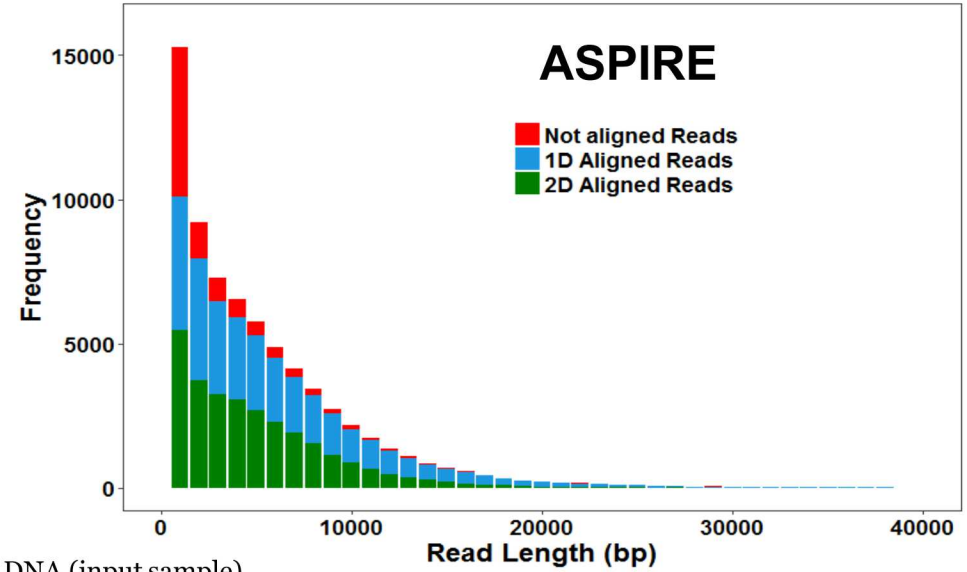
- ✓ ASPIRE platform as consistent as manual Benchtop methods



ASPIRE- Sequencing Results- *E. coli* DNA

✓ ASPIRE platform as consistent as manual Benchtop methods





1 µg *E. coli* DNA (input sample)

ASPIRE- Sequencing Results

	Sample	No. of 2D pass reads (Q-score>9)	Mean read length (bp)	Maximum read length (bp)	Percent Alignment
ASPIRE	λ phage	27,733	7055	35601	99.06%
ASPIRE	*E. coli*	27,467	4496	48520	98.43%
Bench	*E. Coli*	15,941	4520	56321	99.15%

ASPIRE- Conclusion & Future work

- Automate 2D prep for field-portable sequencers
- Preserves long DNA strands
- As consistent as bench operation
- 99% alignment (λ phage)
- Simple, disposable components
- Semi-automated operation
- Same volume of reagents as in bench protocol
  - No need to scale down volumes

Future Work

- Current prototype can be miniaturized (syringe pump & rotary actuator)
- Automate placement of magnet & phase change material
- Lyophilized reagents, disposable cartridge/tubing
- Integrate sample preparation

Thank you…

Mike Bartsch
Sara Bird
Steve Branda
Harrison Edwards
Raga Krishnakumar
Joe Schoeniger
Dan Throckmorton
Anupama Sinha

Sandia National Laboratories

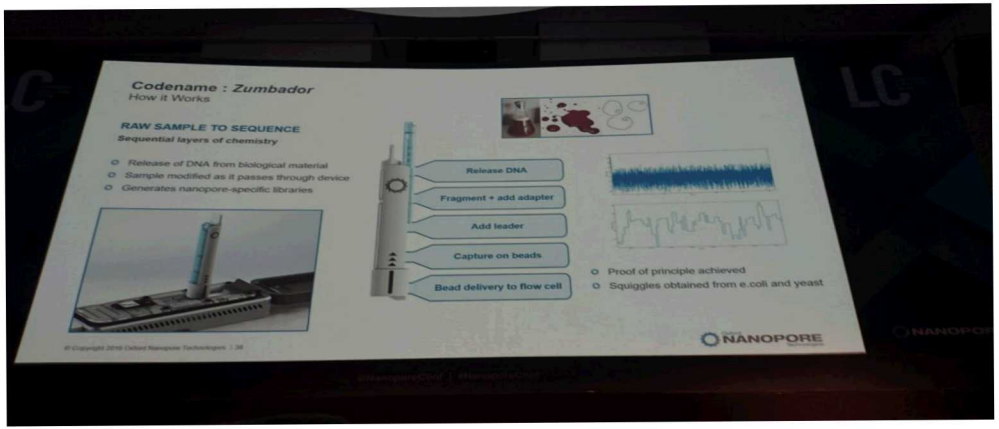
LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

: analysis of hair samples attributed to yeti, bigfoot and other
Soc. B. Vol. 281. No. 1789. The Royal Society, 2014.

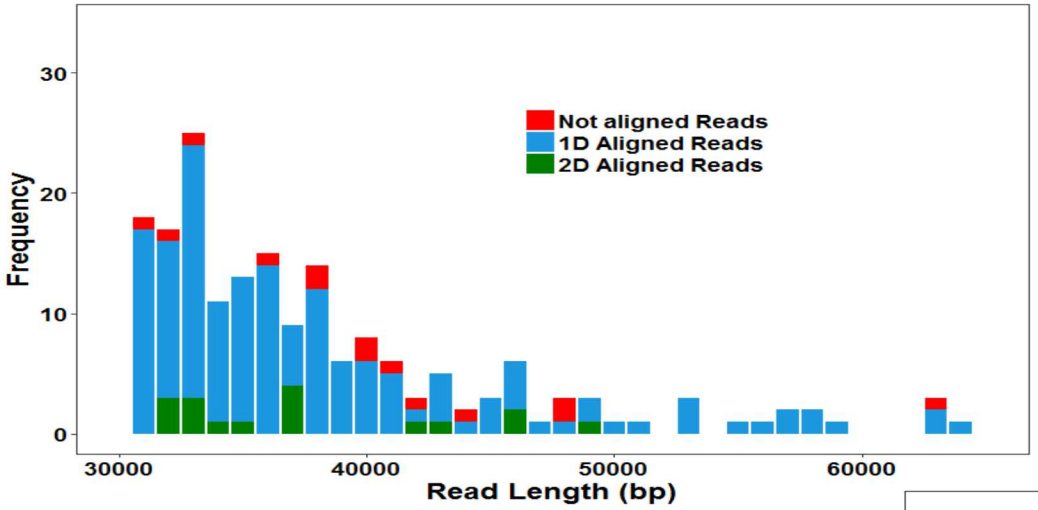
VolTRAX

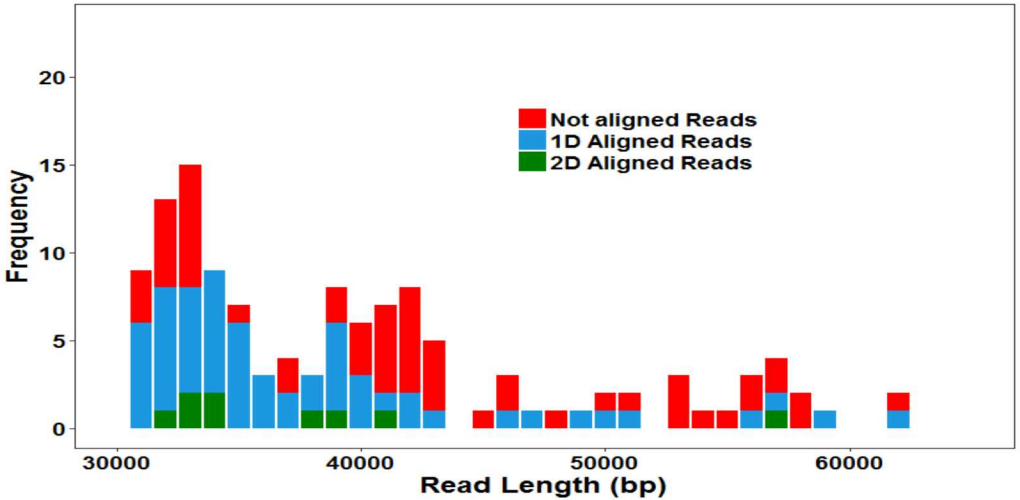
https://nanoporetech.com/products/voltrax

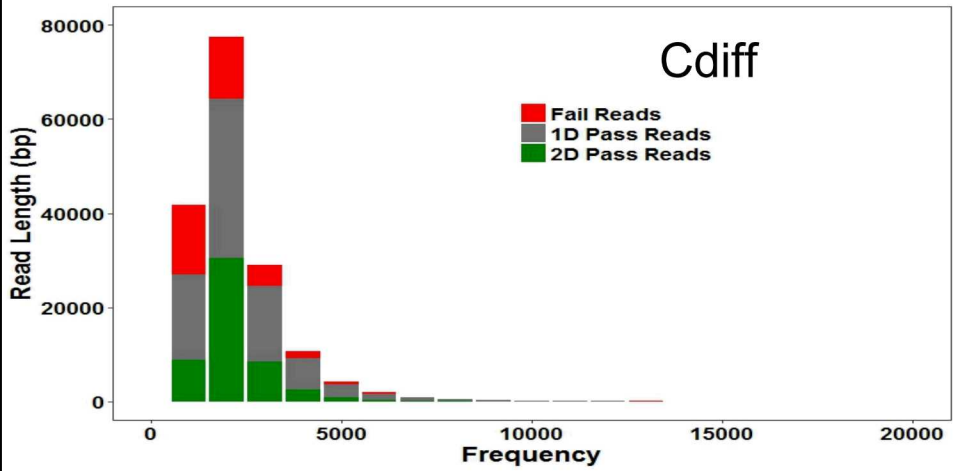
Sample prep

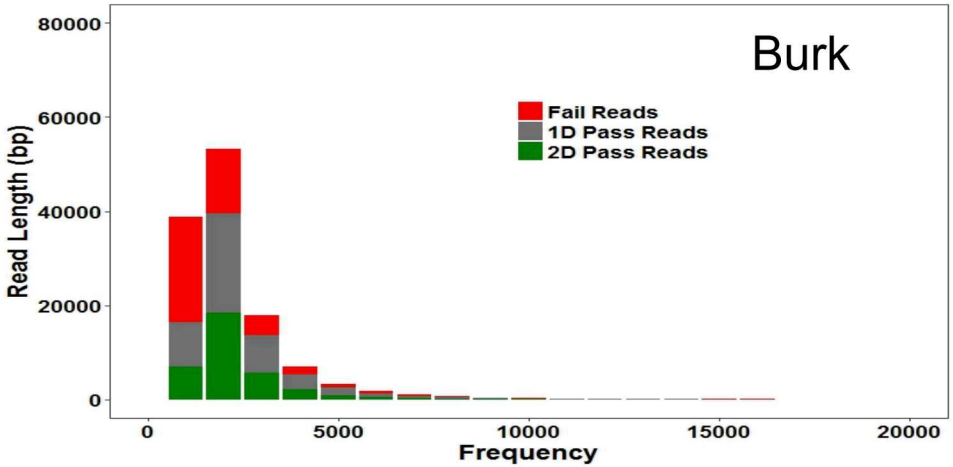


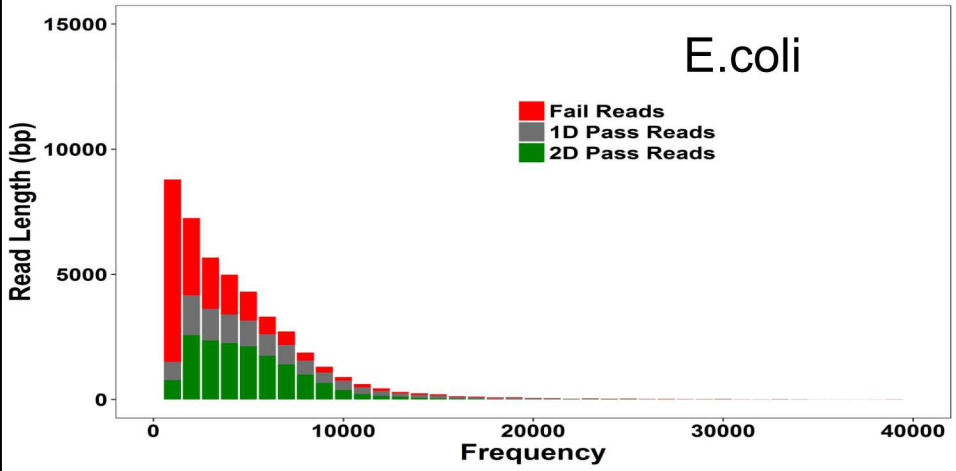
https://nanoporetech.com/

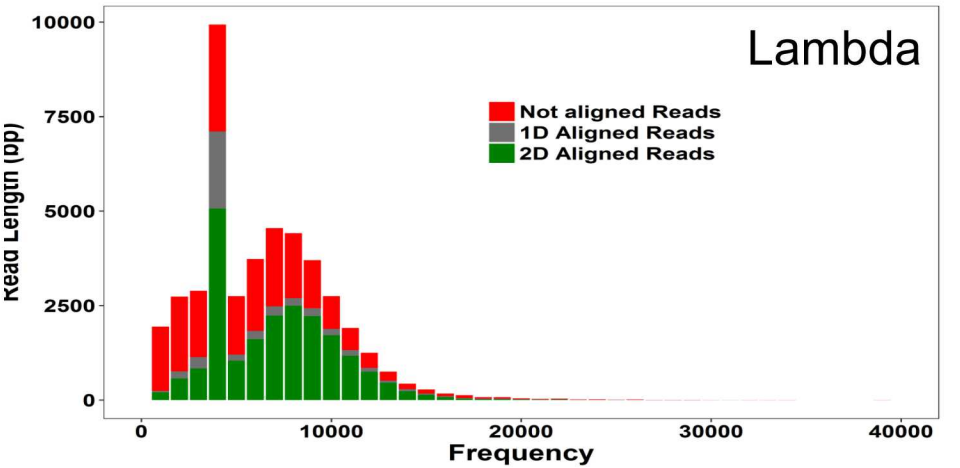

Not aligned Reads
1D Aligned Reads
2D Aligned Reads
Frequency
Read Length (bp)
0
10
20
30
30000
40000
50000
60000


Not aligned Reads
1D Aligned Reads
2D Aligned Reads
Frequency
Read Length (bp)
0
5
10
15
20
30000
40000
50000
60000


Cdiff
Fail Reads
1D Pass Reads
2D Pass Reads
Read Length (bp)
Frequency
0
20000
40000
60000
80000
0
5000
10000
15000
20000


Burk
Fail Reads
1D Pass Reads
2D Pass Reads
Read Length (bp)
Frequency
0
20000
40000
60000
80000
0
5000
10000
15000
20000


E.coli
Fail Reads
1D Pass Reads
2D Pass Reads
Read Length (bp)
Frequency
0
5000
10000
15000
0
10000
20000
30000
40000


Lambda
Not aligned Reads
1D Aligned Reads
2D Aligned Reads
Frequency
0
2500
5000
7500
10000
0
10000
20000
30000
40000