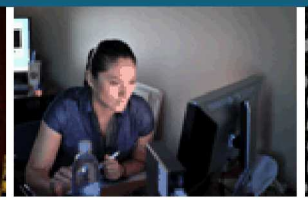
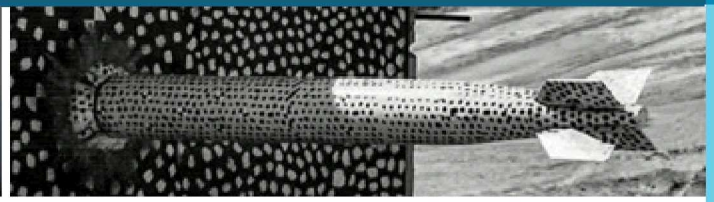
SAND2018-10347PE

LDRD Ending Project Review
Real-time Automated Pathogen Identification by Enhanced Ribotyping (RAPIER) - 191175





PI - Michael Bartsch (8645)
PM – James Carney (8631)

Team Members – Sara Bird, Steven Branda, Harrison Edwards, Harikrishnan Jayamohan, Raga Krishnakumar, Kamlesh Patel, Joseph Schoeniger, Anupama Sinha

FY16-18, $530k/yr

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology and Engineering Solutions of Sandia LLC, a wholly owned subsidiary of Honeywell International Inc. for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525.

PROBLEM STATEMENT

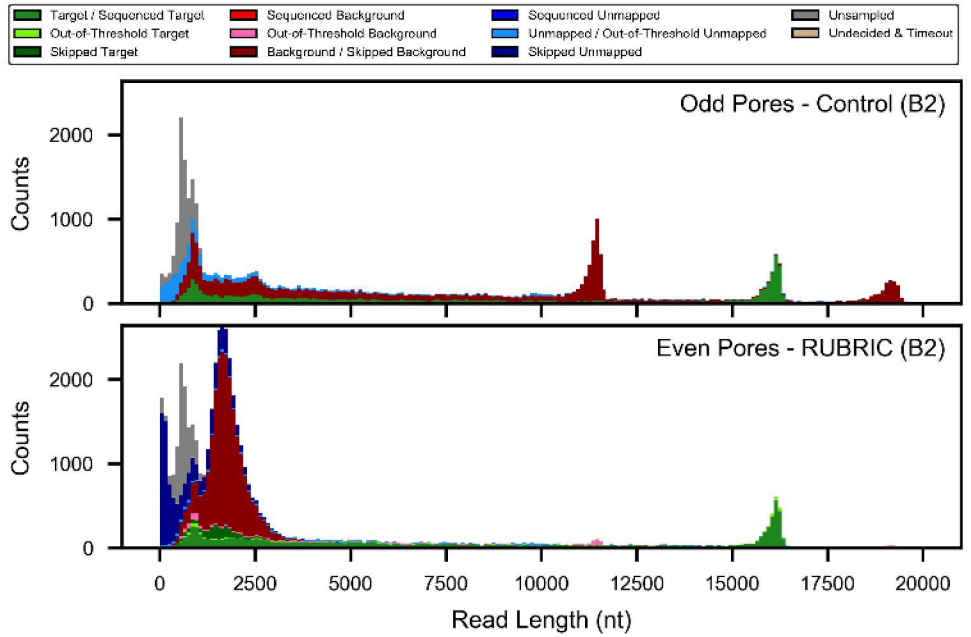
- Rapid, accurate diagnosis of infectious disease is critical to
  - Provide timely and appropriate treatment
  - Identify new/emerging pathogens before they can spread
  - Enable biosurveillance networks for public health & national security
- Conventional clinical diagnostics
  - Bacterial culture: time-consuming, highly pathogen-dependent, potential biosecurity risk
  - PCR panels: require *a priori* knowledge of the target(s), problematic for new/emerging pathogens
  - Short read sequencing: unsuitable for rapid or portable diagnostics, large instruments, 12+ hours for batch sequencing & analysis

Illumina MiSeq vs. Oxford MinION

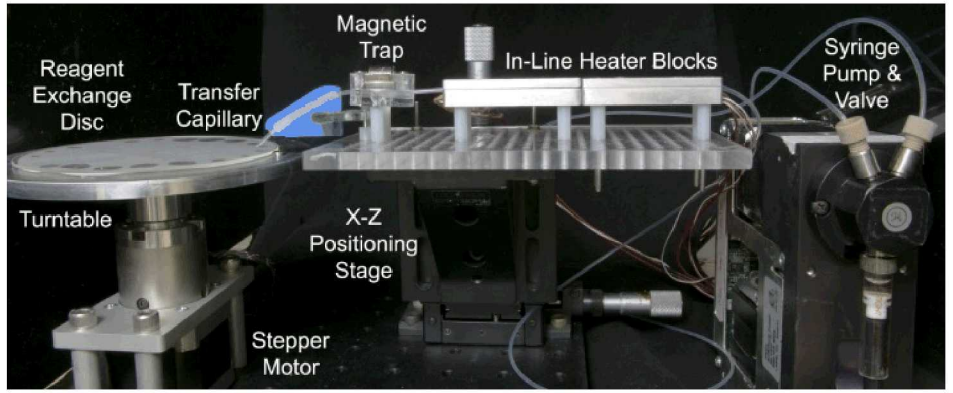
- RAPIER LDRD Goal: To develop a rapid, universal, amplification-free, pathogen ID & characterization capability suitable for clinical, field-forward, & low-resource settings
  - Assess the Oxford MinION nanopore sequencer performance for relevant sample types
  - Develop sample/library prep automation facilitating its use in the field
  - Explore sample & library prep methods enabling pathogen-independent diagnostics
  - Leverage MinION real-time data output to enable new diagnostic approaches

PI's PROJECT LEGACY

- Oxford MinION nanopore sequencing at Sandia
  - Our team has developed deep expertise with the MinION and unique insights into its performance, advantages, and shortcomings (Krishnakumar, Scientific Reports, 2018)
  - These hard-won insights have enabled us to develop a number of related concepts applicable to future applied nanopore work and R&D
- RUBRIC selective sequencing with the Oxford MinION
  - Thanks to a fantastic cross-disciplinary team effort and a great deal of tenacity in the face of adversity, this work has singlehandedly made a name for Sandia and our team in the nanopore sequencing space
  - We are now one of ~3-5 teams in the world working on nanopore selective sequencing, and we anticipate that our upcoming publication of the RUBRIC method could be one of the seminal works on the topic
  - Our team has built relationships & credibility with ONT staff through participation in their developer community, providing us with early access to software/features
  - When sponsor agencies finally recognize the potential of nanopore sequencing, we will be uniquely positioned to respond…assuming any of us are still available
- ASPIRE microfluidic library prep automation
  - Overtaken by events and hampered by staffing problems
  - Release of ONT (10 min) rapid library prep and Voltrax digital microfluidic automated library prep system significantly undercut this activity
  - Still, our upcoming ASPIRE paper may nevertheless be one of the first published examples of automating MinION library prep, albeit for an obsolete kit



RUBRIC Selective Sequencing



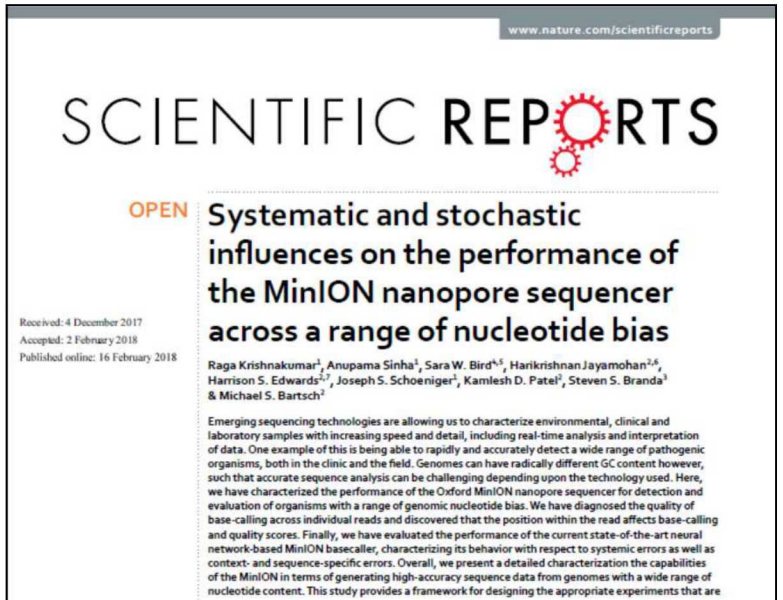
ASPIRE Automated Library Prep

PI's PROJECT LEGACY

- Potential mission-relevant Impacts and timeframe
  - Nanopore sequencing is poised to revolutionize DNA/RNA sequencing and epigenetics
    - 5 years from now, *everyone who does sequencing* will be using nanopore sequencers in some capacity
    - 10 years from now, nanopore-sequencing based diagnostics will be in the mainstream
    - 15 years from now, kids will wonder why anyone ever did short read Illumina-style sequencing…
  - Real-time selective sequencing like RUBRIC will in turn revolutionize nanopore sequencing (in some applications)
    - The insights developed through our RUBRIC work will significantly accelerate the adoption and broaden the appeal/accessibility of real-time selective sequencing, to the benefit of Sandia, our collaborators, and the field generally
    - 1-3 years from now, real-time selection will be a standard "drop-down box" option when running a nanopore sequencer
  - Our RAPIER work uniquely position Sandia to capitalize on the novel capabilities of nanopore sequencing
    - Portable, rapid DNA/RNA sequence-based diagnostics for pathogen detection/identification in clinical, military, and biosurveillance applications
    - Detection of genetic and epigenetic (e.g., methylation) changes/mutations enabled by long reads and/or targeted selection
    - Ultralong read-enabled insights into the complex genomics of algae/plants for bioenergy applications
- Lessons learned
  - Building a project around an immature technology is risky but offers tremendous potential for innovation/impact
  - Building a project around *someone else's* immature technology is *doubly* risky, but can still offer worthy opportunities
  - Staff projects to address your team's lack of specific expertise, not to duplicate existing expertise inexpensively
  - 3-year LDRDs must obtain key results by the 1.5 year mark to maintain continuity through outside follow-on funding
  - Genuinely novel, high-risk research is unlikely to become self-sustaining after an initial 1.5 years of activity

PROJECT OUTPUTS

- Publications (accepted or submitted)
  - R. Krishnakumar, A. Sinha, S.W. Bird, H. Jayamohan, H.S. Edwards, J.S. Schoeniger, K.D. Patel, S.S. Branda, M.S. Bartsch, "Systematic and Stochastic Influences on the Performance of the MinION Nanopore Sequencer Across a Range of Nucleotide Bias," Scientific Reports, 8:3159, 16 Feb., 2018.
  - H.S. Edwards, R. Krishnakumar, A. Sinha, S.W. Bird, K.D. Patel, M.S. Bartsch, "Real-Time Selective Sequencing with RUBRIC: Read Until with Basecall- and Reference-Informed Criteria," Nature Methods/BioRxiv, currently in final R&A, submission imminent.
  - H. Jayamohan, A. Sinha, H.S. Edwards, R. Krishnakumar, M.S. Bartsch, "Preparing Nanopore Sequencing Libraries with ASPIRE: Automated Sample Preparation by Indexed Rotary Exchange," Lab on a Chip, in preparation, submission 2-3 weeks out.


www.nature.com/scientificreports

SCIENTIFIC REPORTS

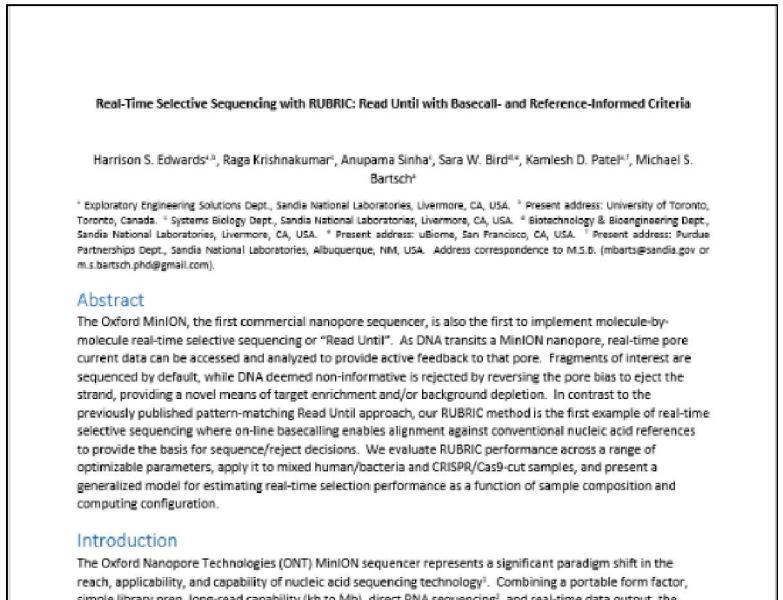
OPEN

Systematic and stochastic influences on the performance of the MinION nanopore sequencer across a range of nucleotide bias

Received: 4 December 2017
Accepted: 2 February 2018
Published online: 16 February 2018

Raga Krishnakumar[1], Anupama Sinha[1], Sara W. Bird[4,5], Harikrishnan Jayamohan[2,6], Harrison S. Edwards[2,7], Joseph S. Schoeniger[1], Kamlesh D. Patel[3], Steven S. Branda[3] & Michael S. Bartsch[2]

Emerging sequencing technologies are allowing us to characterize environmental, clinical and laboratory samples with increasing speed and detail, including real-time analysis and interpretation of data. One example of this is being able to rapidly and accurately detect a wide range of pathogenic organisms, both in the clinic and the field. Genomes can have radically different GC content however, such that accurate sequence analysis can be challenging depending upon the technology used. Here, we have characterized the performance of the Oxford MinION nanopore sequencer for detection and evaluation of organisms with a range of genomic nucleotide bias. We have diagnosed the quality of base-calling across individual reads and discovered that the position within the read affects base-calling and quality scores. Finally, we have evaluated the performance of the current state-of-the-art neural network-based MinION basecaller, characterizing its behavior with respect to systemic errors as well as context- and sequence-specific errors. Overall, we present a detailed characterization the capabilities of the MinION in terms of generating high-accuracy sequence data from genomes with a wide range of nucleotide content. This study provides a framework for designing the appropriate experiments that are


Real-Time Selective Sequencing with RUBRIC: Read Until with Basecall- and Reference-Informed Criteria

Harrison S. Edwards[1,a], Raga Krishnakumar[2], Anupama Sinha[2], Sara W. Bird[3,a], Kamlesh D. Patel[3,f], Michael S. Bartsch[4]

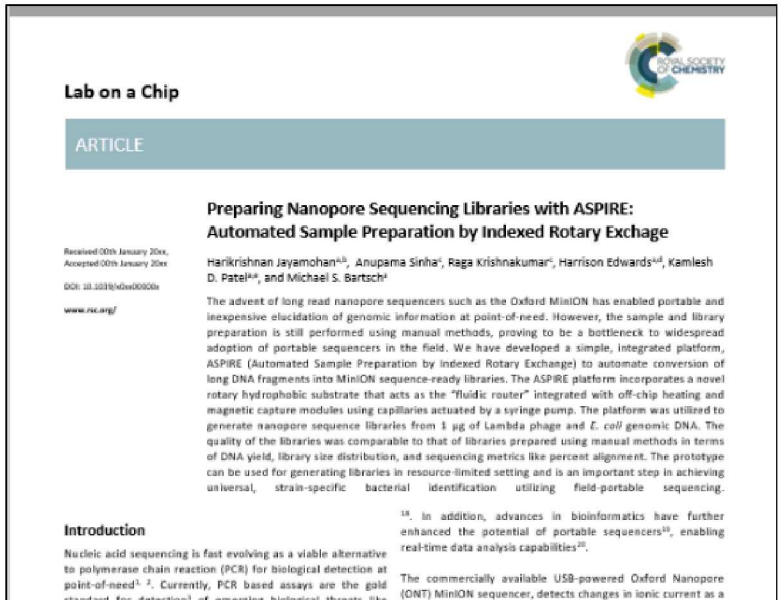
[1] Exploratory Engineering Solutions Dept., Sandia National Laboratories, Livermore, CA, USA. [a] Present address: University of Toronto, Toronto, Canada. [2] Systems Biology Dept., Sandia National Laboratories, Livermore, CA, USA. [3] Biotechnology & Bioengineering Dept., Sandia National Laboratories, Livermore, CA, USA. [f] Present address: uBiome, San Francisco, CA, USA. [f] Present address: Purdue Partnerships Dept., Sandia National Laboratories, Albuquerque, NM, USA. Address correspondence to M.S.B. (mbartsch@sandia.gov or m.s.bartsch.phd@gmail.com).

Abstract

The Oxford MinION, the first commercial nanopore sequencer, is also the first to implement molecule-by-molecule real-time selective sequencing or "Read Until". As DNA transits a MinION nanopore, real-time pore current data can be accessed and analyzed to provide active feedback to that pore. Fragments of interest are sequenced by default, while DNA deemed non-informative is rejected by reversing the pore bias to eject the strand, providing a novel means of target enrichment and/or background depletion. In contrast to the previously published pattern-matching Read Until approach, our RUBRIC method is the first example of real-time selective sequencing where on-line basecalling enables alignment against conventional nucleic acid references to provide the basis for sequence/reject decisions. We evaluate RUBRIC performance across a range of optimizable parameters, apply it to mixed human/bacteria and CRISPR/Cas9-cut samples, and present a generalized model for estimating real-time selection performance as a function of sample composition and computing configuration.

Introduction

The Oxford Nanopore Technologies (ONT) MinION sequencer represents a significant paradigm shift in the reach, applicability, and capability of nucleic acid sequencing technology[1]. Combining a portable form factor,


Lab on a Chip

ARTICLE

Preparing Nanopore Sequencing Libraries with ASPIRE: Automated Sample Preparation by Indexed Rotary Exchage

Harikrishnan Jayamohan[a,b], Anupama Sinha[c], Raga Krishnakumar[c], Harrison Edwards[a,d], Kamlesh D. Patel[a,e], and Michael S. Bartsch[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

The advent of long read nanopore sequencers such as the Oxford MinION has enabled portable and inexpensive elucidation of genomic information at point-of-need. However, the sample and library preparation is still performed using manual methods, proving to be a bottleneck to widespread adoption of portable sequencers in the field. We have developed a simple, integrated platform, ASPIRE (Automated Sample Preparation by Indexed Rotary Exchange) to automate conversion of long DNA fragments into MinION sequence-ready libraries. The ASPIRE platform incorporates a novel rotary hydrophobic substrate that acts as the "fluidic router" integrated with off-chip heating and magnetic capture modules using capillaries actuated by a syringe pump. The platform was utilized to generate nanopore sequence libraries from 1 µg of Lambda phage and *E. coli* genomic DNA. The quality of the libraries was comparable to that of libraries prepared using manual methods in terms of DNA yield, library size distribution, and sequencing metrics like percent alignment. The prototype can be used for generating libraries in resource-limited setting and is an important step in achieving universal, strain-specific bacterial identification utilizing field-portable sequencing.

Introduction

Nucleic acid sequencing is fast evolving as a viable alternative to polymerase chain reaction (PCR) for biological detection at point-of-need[1, 2]. Currently, PCR based assays are the gold

[18]. In addition, advances in bioinformatics have further enhanced the potential of portable sequencers[19], enabling real-time data analysis capabilities[20].

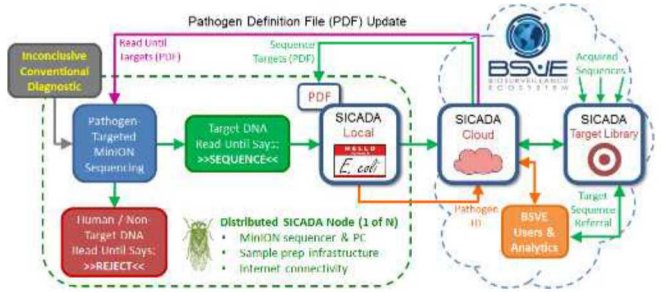
The commercially available USB-powered Oxford Nanopore (ONT) MinION sequencer, detects changes in ionic current as a

PROJECT OUTPUTS

- Presentations (invited)
  - M.S. Bartsch, "Real-time selective sequencing with RUBRIC (Read Until with Basecall- and Reference-Informed Criteria)", invited talk, London Calling 2018, London, 25 May, 2018.
  - M.S. Bartsch, "Read Until with Basecall- and Reference-Informed Criteria (RUBRIC)," invited talk, Nanopore Day at UCSF, 13 March, 2018.
  - R. Krishnakumar, "Characterizing the performance of the MinION for real-time detection," invited talk, Nanopore Day at UCSF, 13 March, 2018.
  - M.S. Bartsch, "The evolution of MinION selective sequencing: Read Until with Basecall- and Reference-Informed Criteria (RUBRIC)," Nanopore Community Mtg., NYC, 30 Nov., 2017. (poster + lightning talk)
  - R. Krishnakumar, "Selective Long-Read Nanopore Sequencing for Real-time Point-of-need Pathogen Identification", DTRA CBD S&T Conference, Long Beach, 28 Nov., 2017.
  - R. Krishnakumar, "Real-time diagnostics using nanopore sequencing," Molecular Tricon, San Francisco, CA, 24 Feb., 2017.
  - H. Jayamohan, "Nanopore sequencing for real-time pathogen identification," Molecular Tricon, San Francisco, CA, 24 Feb., 2017.
  - R. Krishnakumar, "Real-Time Auttomated Pathogen Identification by Enhanced Ribiotyping (RAPIER)", Oxford Nanopore Community Mtg., NYC, 1-2 Dec., 2016. (poster)
- Proposals submitted/awarded based on this LDRD
  - Submitted: Sandia National Laboratories Perspective and Response to IARPA RaDNAS RFI 17-06 for Nanopore-Based DNA Sequencing for ID and Characterization
  - Submitted: Sandia National Laboratories Response to BARDA ENACT RFI #HHS-18-BARDA-RFI-00019
  - Submitted, not funded: NIH R21 "Rapid diagnostics of disease states in blood cells using DNA accessibility and real-time nanopore sequencing"
  - Submitted, not funded: "Sequence-Informed Context-Adaptive Detection Architecture (SICADA)", DTRA JSTO FY17 Service Call, CBA-03
  - Submitted, not funded: LDRD idea 19-0680 "Detecting Genome and Epigenome Editing with Methylation-Aware 'Hand Grenade' Selective Sequencing"
  - Submitted, not funded: LDRD idea 19-0468 "Syndromic Surveillance Nanopore Pilot for Rapid Detection of Plague and Tularemia"

https://nanoporetech.com/lc18/whats-on#modal=Real-timeselectivesequencingwithRUBRIC

https://nanoporetech.com/resource-centre/evolution-minion-selective-sequencing-read-until-basecall-and-reference-informed



SICADA selective sequencing-enabled cloud-integrated biosurveillance architecture (DTRA proposal)

CAPABILITIES DEVELOPMENT

- Establishment of Capabilities expected to impact future work
  - The knowledge base developed as a result of RAPIER positions us uniquely to respond to future sponsor interests related to fieldable, rapid sequencing-based biodiagnostics in a host of applications
  - RUBRIC selective sequencing software and the associated data analysis pipeline represent a world-leading foundation upon which to build future programs
- Career Development
  - Junior staff funded and mentored as team members
    - Harrison Edwards – RAPIER involvement contributed to transition into doctoral program (fully funded)
    - Raga Krishnakumar – RAPIER involvement contributed to conversion from postdoc to staff
  - ST&E staff and post doc hires
    - Raga Krishnakumar – hired as postdoc, now staff
    - Sara Bird – hired as postdoc, now at uBiome
    - Harikrishnan Jayamohan – hired as postdoc, now at Roche Sequencing Solutions
  - Retention and career development of mid-career PI

TEAM BUILDING AND PARTNERSHIPS

- Internal collaboration discussions with Carrie McNeil, pending with Ben Brodsky
- External collaboration and joint proposal discussions underway:
  - Fred Hutch Cancer Research Center
    - LLS translational research program proposal (10/2018)
  - University of New Mexico Medical School
    - Possible NIH proposal (10/2018)
  - Ohio State University Medical School

IA/PM PROJECT LEGACY

How did this project contribute to IA strategic goals and objectives?

- This project secured Sandia a position of recognized leadership in an emerging technology with significant potential benefits to the biodefense and biosecurity portions of the IA's CBRN-D mission
- Substantial staff development benefits were realized by fostering new capabilities and expertise among existing Sandians and facilitating the hiring and conversion to staff of new Sandians with key subject matter expertise
- In addition to building staff expertise and capabilities, software tools and data processing pipelines developed through this work position Sandia uniquely to be responsive to future sponsor calls in this and related topic areas
- The high visibility and benefits of the unique selective sequencing (RUBRIC) work developed through this project have already produced a number of collaboration and co-proposal discussions, and the impending publication of key results on this topic will likely result in many more

What are the key results from this research that will be useful to other current and future projects?

- Our team now has deep expertise in the application of nanopore sequencing generally and selective sequencing in particular, offering significant benefits to future projects where rapid/real-time diagnostics, fieldable/portable bioanalysis, or rare target detection from complex samples ("needle in a haystack") is required
- Development of the RUBRIC real-time selective sequencing software and data analysis pipeline provides Sandia with a (for now) unique capability to enable purely software-based enrichment of rare targets in diagnostic workflows, with potential application to genomic (DNA), transcriptomic (RNA), and epigenetic (e.g., DNA methylation) targets using the same instrument

Technology insertion and follow-on funding for potential and realized ROI

- Related proposals submitted to Sandia LDRD, DTRA, and NIH. RFI responses based on this work submitted to IARPA and BARDA
- Discussions underway for collaboration / co-proposal with UNM, Fred Hutch CRC, OSU