Nanopore Community Meeting 2016
New York

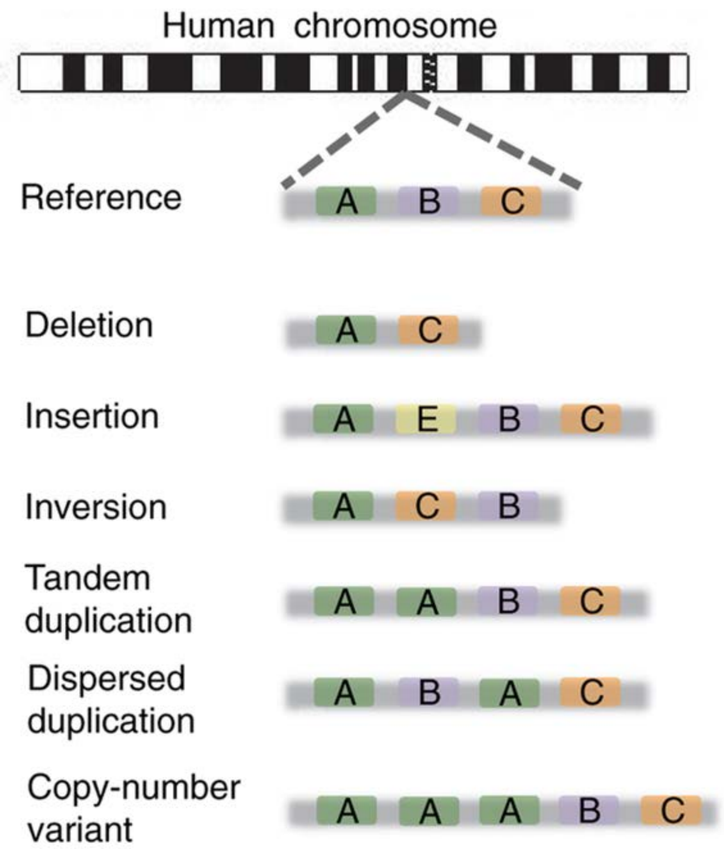
Structural variation detection on human DNA using targeted sequencing

Isac Lee
Department of Biomedical Engineering, Johns Hopkins School of Medicine

December 2, 2016

Structural Variation

- Abnormality in large region (50b-3mb) of a chromosome
- Pervasive in cancer – 50% of pancreatic ductal adenocarcinoma (PDAC)
- Common in tumor suppressor genes such as *CDKN2A* and *SMAD4*
- Nanopore sequencing can resolve SVs
  - *But* High coverage desired for heterogeneous samples
  - and size of human genome : 3 Gbps
- We know where SVs tends to occur

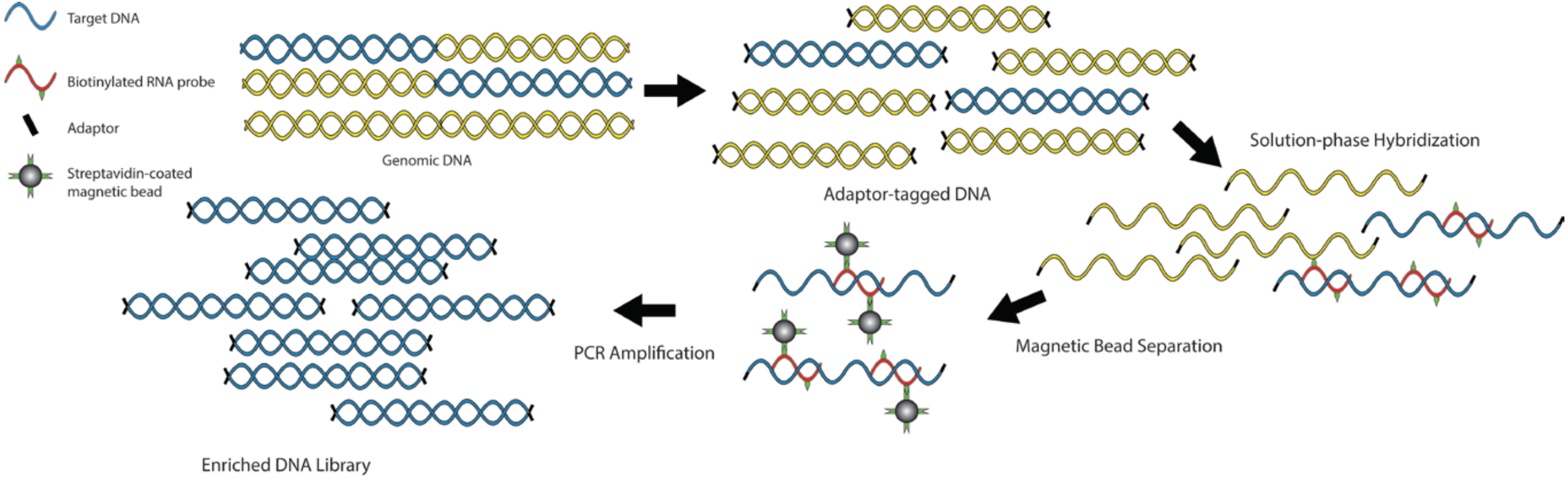


Baker, Monya. 2012. Nature Methods

© Copyright 2016 Oxford Nanopore Technologies

JOHNS HOPKINS BIOMEDICAL ENGINEERING Oxford NANOPORE Technologies

Solution-phase Hybridization Capture



Agilent SureSelectXT Targeted Sequencing System

- ~90 bps biotinylated RNA probes complementary to target sequence
- Biotin-streptavidin interaction to enrich for the targeted region
- Optimization for long-reads : > 2 kb

© Copyright 2016 Oxford Nanopore Technologies

Targeted Capture Optimization

Collaboration with Josh Wang from Agilent

- Trial 1
- Probe tiling, No empty spaces between probes
- Target region
  - *CDKN2A :* 1.5 Mbps
- Low stringency to allow mismatches
- Result: 2.28 % on-target

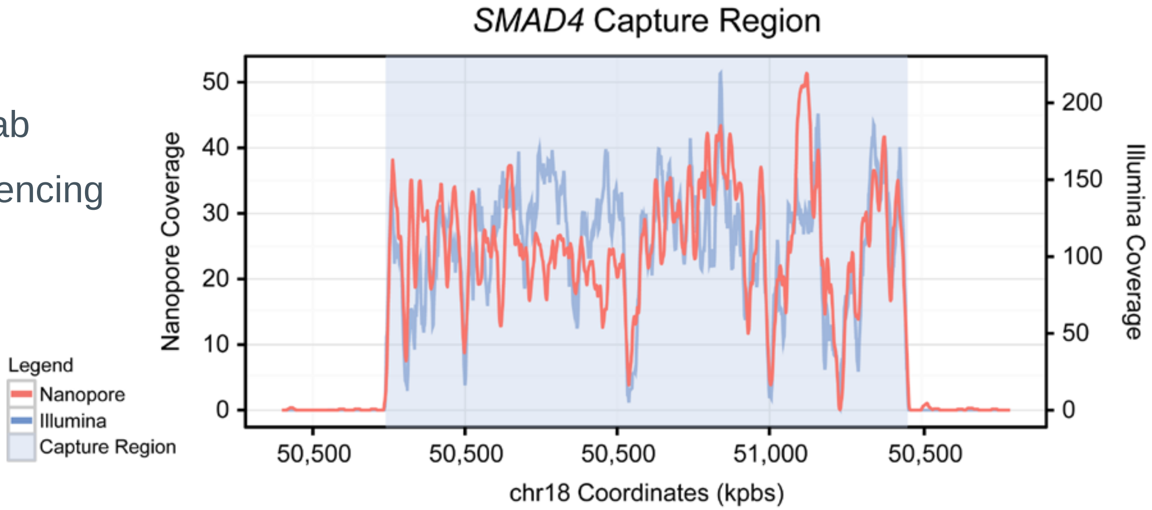
- Trial 2
- No tiling, average 400 bp space between probes
- Target regions
  - *CDKN2A* : 1.5 Mbps
  - *SMAD4* : 850 Kbps
- High stringency to limit off-target capture
- Consideration of known SV breakpoints
  - PDAC SVs from James Eshleman lab
- Result: 30 % on-target

© Copyright 2016 Oxford Nanopore Technologies

JOHNS HOPKINS BIOMEDICAL ENGINEERING
Oxford NANOPORE Technologies

Targeted Sequencing Performance

- Control : NA12878 lymphoblast
- Sample : PDAC from Eshleman lab
- Illumina short-read targeted sequencing for comparison
- > 300-fold enrichment
- > 20X average coverage



	Total yield (reads)	On-target	On-target percentage	Fold enrichment	Coverage
Illumina NA12878	4.4m	3.7m	85%	641X	113X
Nanopore NA12878	107k	32k	30%	353X	27X
Nanopore PDAC	56k	20k	26%	332X	20X

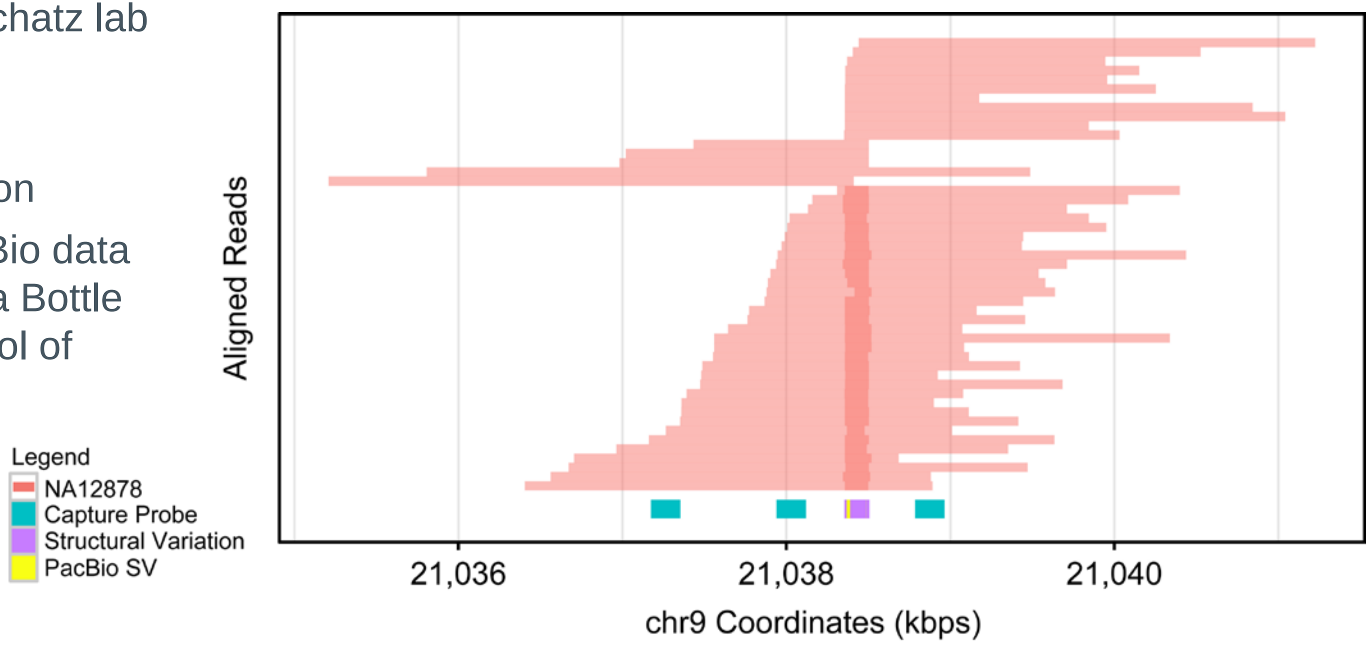
JOHNS HOPKINS BIOMEDICAL ENGINEERING
Oxford NANOPORE Technologies

Nanopore Structural Variation Detection

NA12878 SVs

Sniffles by Michael Schatz lab

- chr9:21,038,354 - 21,038,506
- 152 bps duplication
- Validated with PacBio data from Genome in a Bottle (Mt. Sinai School of Medicine)

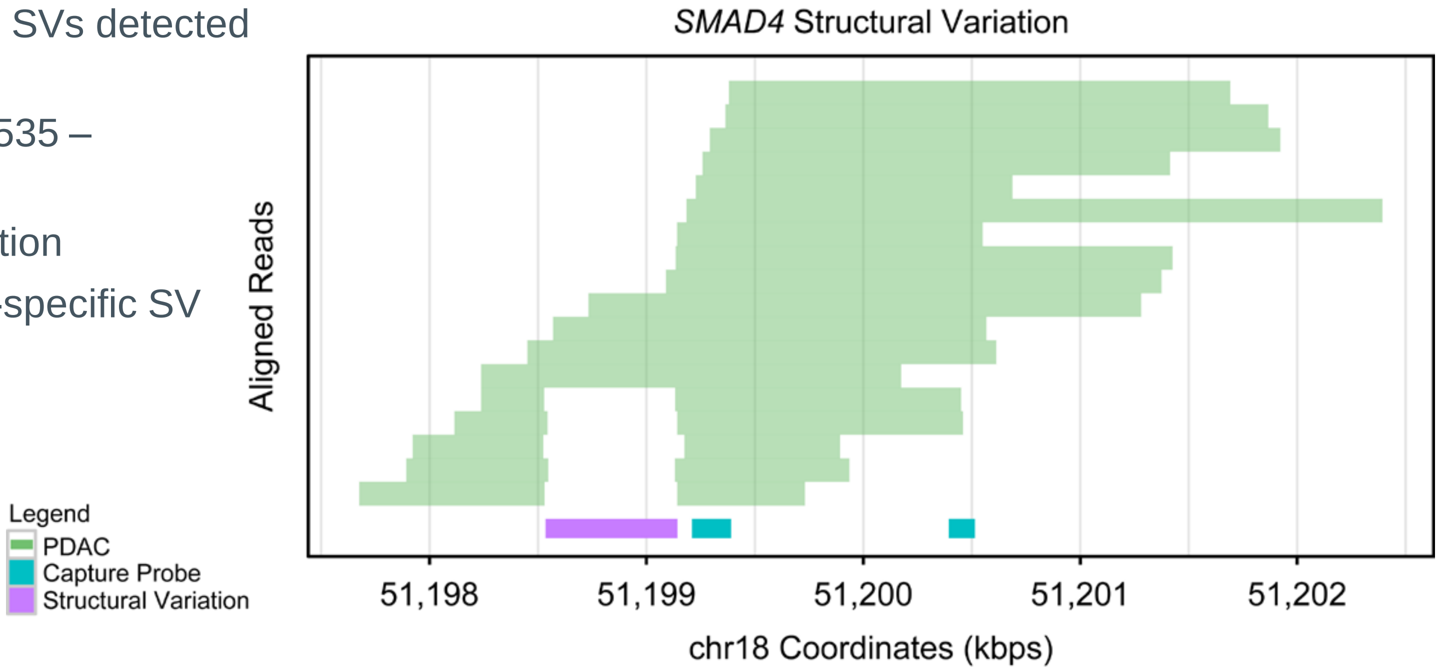


© Copyright 2016 Oxford Nanopore Technologies

Nanopore Structural Variation Detection

PDAC SVs

- Novel, putative SVs detected from PDAC
- chr18: 51,198,535 – 51,199,143
- 600 bps deletion
- Possibly allele-specific SV



Nanopore Structural Variation Detection

PDAC SVs

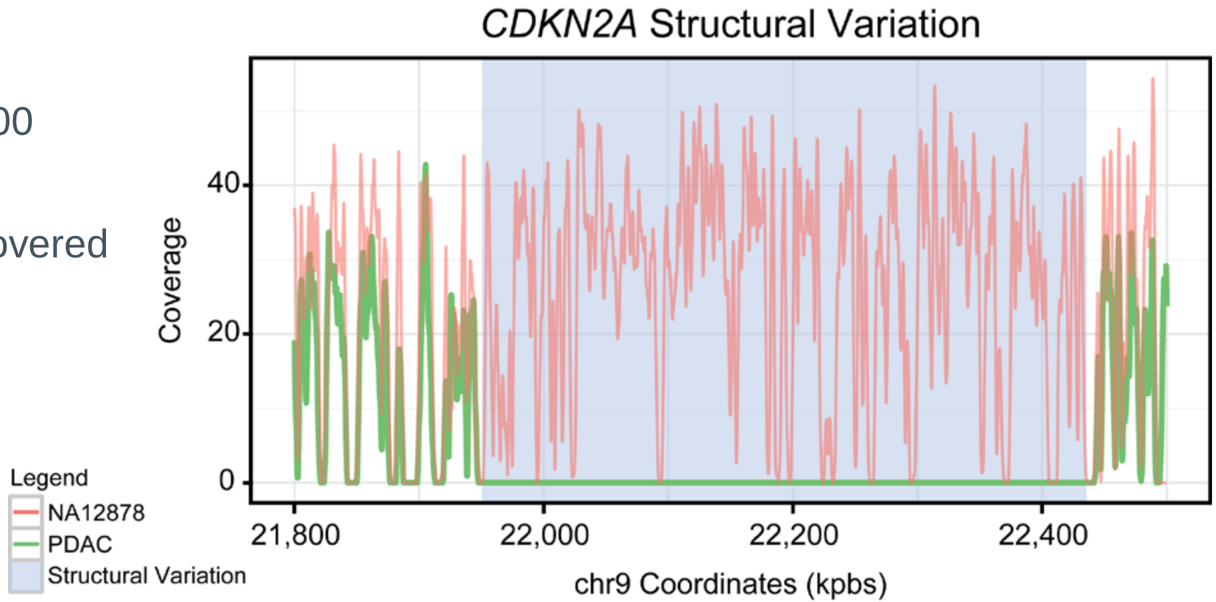
Large window of coverage

Absence in *CDKN2A* region

- chr9:21,950,000 - 22,436,000
- 486 kbps SV
- Homozygous Deletion discovered previously

Sniffles did not detect this SV

- No reads covering either breakpoint



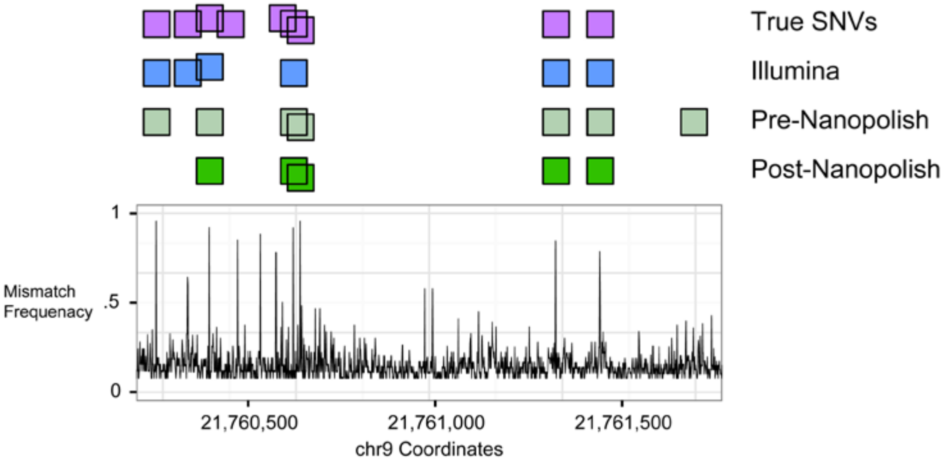
Single Nucleotide Variation Detection
NA12878 SNVs

- Nanopolish by Jared Simpson et al to improve SNV calling

SNV comparisons

	Illumina	Pre-polish	Post-polish
Avg. Coverage	113	27	27
Correct	1133	2485	947
Total	1211	4138	1017
Precision	**94%**	60%	**93%**
Sensitivity	**32%**	69%	**26%**

Number of True SNVs: 3587(Eberle,et al. bioRxiv, 2016)



- Nanopore SNV detection after nanopolish is comparable to Illumina
- Phased SNV analysis is possible with coverage from targeted sequencing

JOHNS HOPKINS BIOMEDICAL ENGINEERING Oxford NANOPORE Technologies

Conclusions and Future Works

Solution-phase Hybridization-Capture

- Achieves sufficient coverage for SV and SNV detection
- Longer reads and higher on-target percentage : recent run 40% on-target, 3kb avg. length

Structural Variation Detection

- SVs can be detected with a single flowcell
- Allele-specific SVs may also be resolved

Single Nucleotide Variation Detection

- SNV detection is comparable to Illumina sequencing
- Nanopolish for haplotyping and phased SNV analysis

© Copyright 2016 Oxford Nanopore Technologies

JOHNS HOPKINS BIOMEDICAL ENGINEERING

Oxford NANOPORE Technologies

Acknowledgments

Timp Lab – Johns Hopkins University
Winston Timp, PhD
Rachael Workman, MS

Agilent Technologies
Josh Wang, PhD
Jonathan Levine, PhD

Eshleman Lab – Johns Hopkins School of Medicine
James Eshleman, MD, PhD
Alexis Norris, PhD

© Copyright 2016 Oxford Nanopore Technologies