

# The Long and Short of it: PacBio Amplicon Sequencing Applications

Dave Corney, PhD

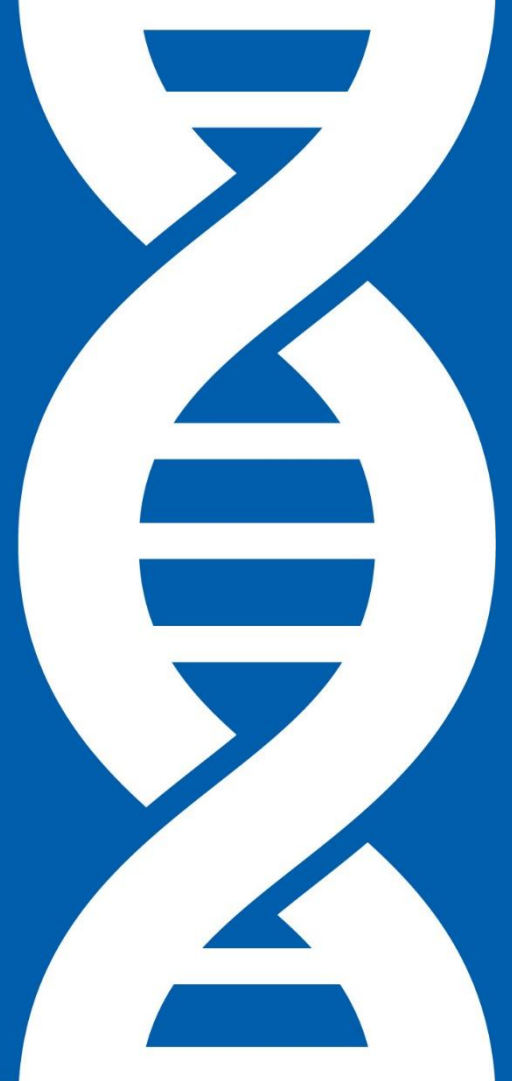
Senior Scientist

Next Generation Sequencing, GENEWIZ



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# GENEWIZ Offers Multiple NGS Services

## DNA-Seq

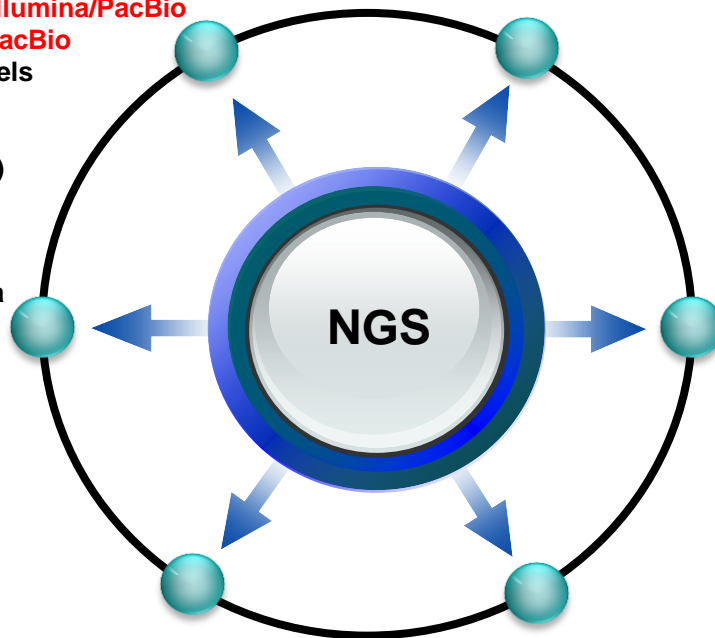
- **Whole Genome Sequencing by Illumina/PacBio**
- **Structural variant detection by PacBio**
- Exome, cancer, and custom panels
- 10x Genomics DNA phasing
- High-throughput genotyping
- Epigenomics (Methylation, ChIP)

## Amplicon Seq

- <500 bp amplicons with Illumina
- **>500 bp amplicons with PacBio**
- Amplicon-EZ

## Cell Line Development

- GenoTYPER-NEXT
- Insertion Site Identification



## RNA-Seq

- Strand-specific RNA-Seq
- Low input RNA-Seq
- Small RNA-Seq
- Targeted RNA-Seq
- 10X Genomics single cell RNA-seq
- **PacBio Iso-Seq Analysis**

## Microbial Communities

- 16S MetaVx™ sequencing
- Full Metagenomics
- **Full Length 16S by PacBio**

## Antibody Discovery

- Immunoseq
- Single chain seq on MiSeq
- **Paired chain seq on PacBio**
- 10X Genomics V(D)J

# PacBio Sequel System Sequencing at GENEWIZ



WGS

Iso-Seq

Amplicon

## Overwhelming Diversity of Amplicon Sequencing Applications

**Full-length 16S**

**STR repeat analysis**

**Full-length ITS1/2**

**Phasing**

**Antibody sequencing & diversity**

**Synthetic library screening**

**Viral genome library screening**

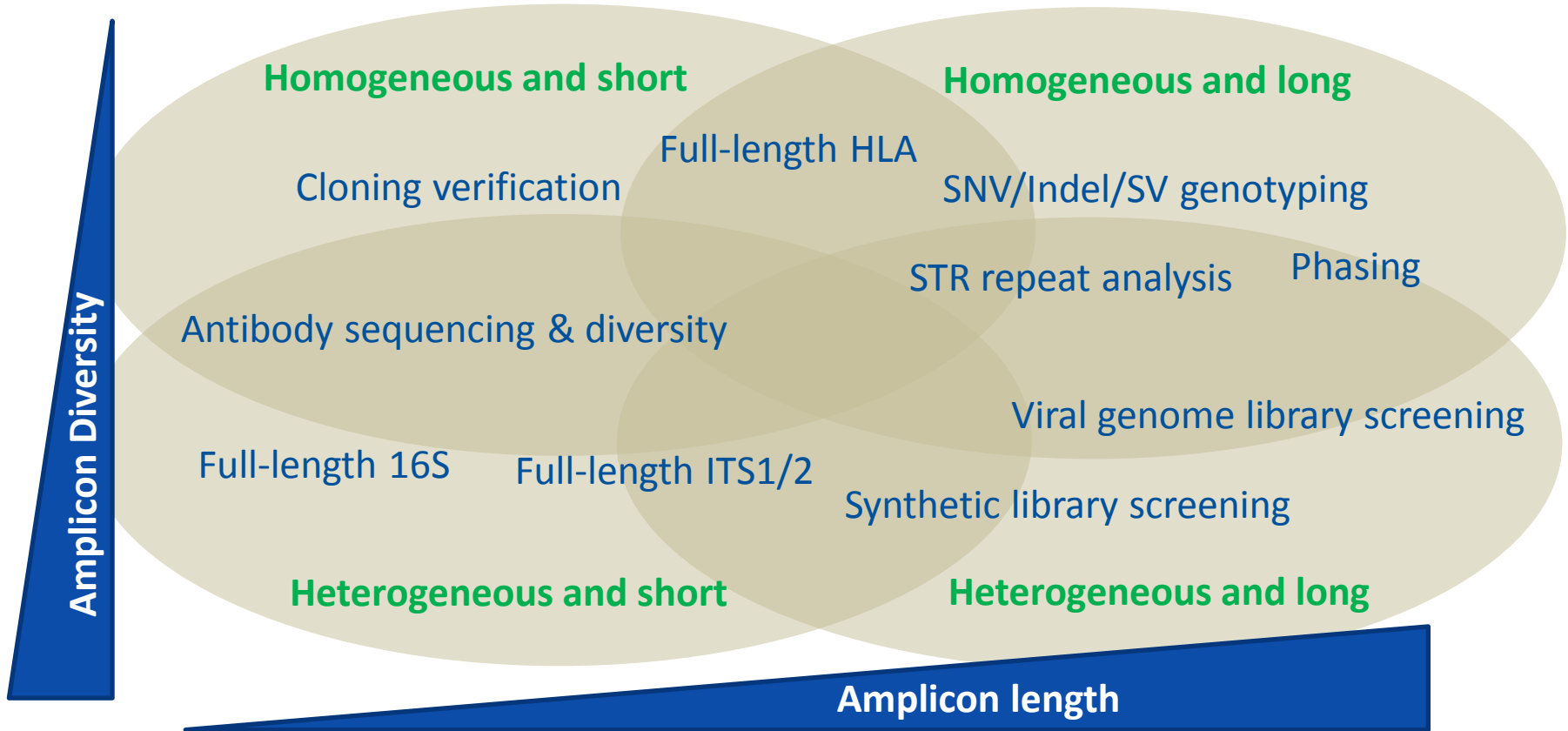
**Full-length HLA**

**Cloning verification**

**SNV/Indel/SV genotyping**

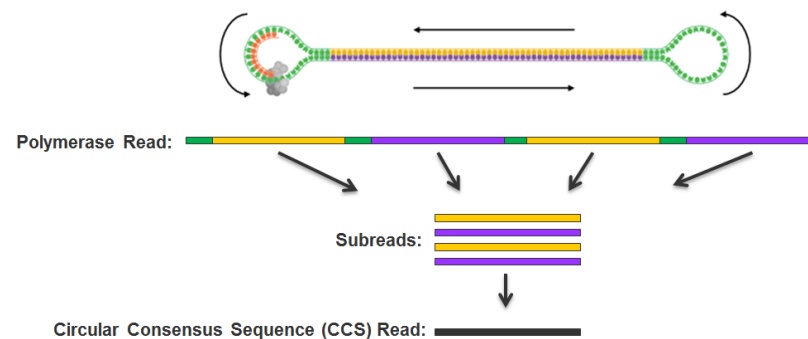
**PolyA tail length analysis**

## Diverse Amplicon Sequencing Applications



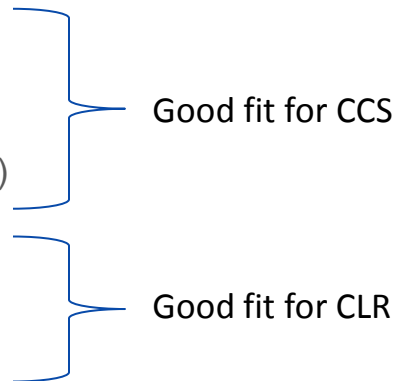
## Importance of Polymerase Read Length

- Longer polymerase read lengths are *really helpful*
  - Sequence longer amplicons and with greater single-molecule accuracy
  - Increase opportunities for multiplexing
- Major factors affecting polymerase RL
  - Amplicon length
  - Loading conditions (pre-extension, on-plate molarity)
  - Movie time



## Impact of Amplicon Length

- Short amplicon (>500bp, < 3 kb)
  - Can obtain highly accurate reads (many passes of template)
  - Can be highly multiplexed
  - Samples can be highly heterogeneous (given sufficient coverage)
- Longer amplicons (> 3 kb, up to ~10 kb)
  - Can obtain full-length but less accurate reads
  - Some reads may not have barcode



- Shorter amplicon
- Easier to sequence barcodes
- Higher single-molecule accuracy

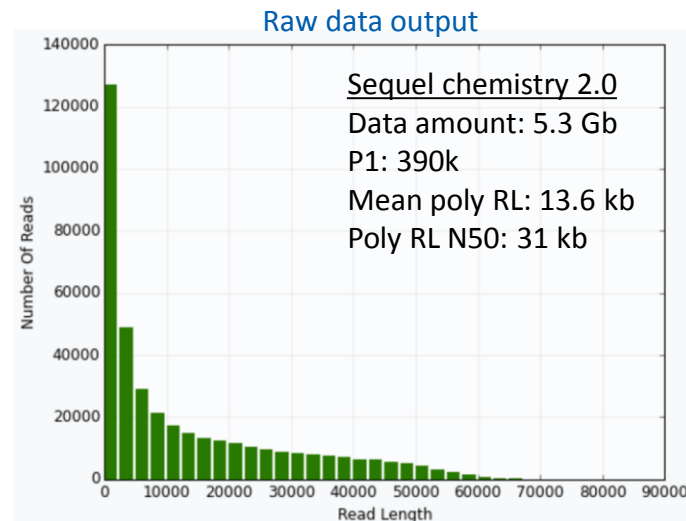
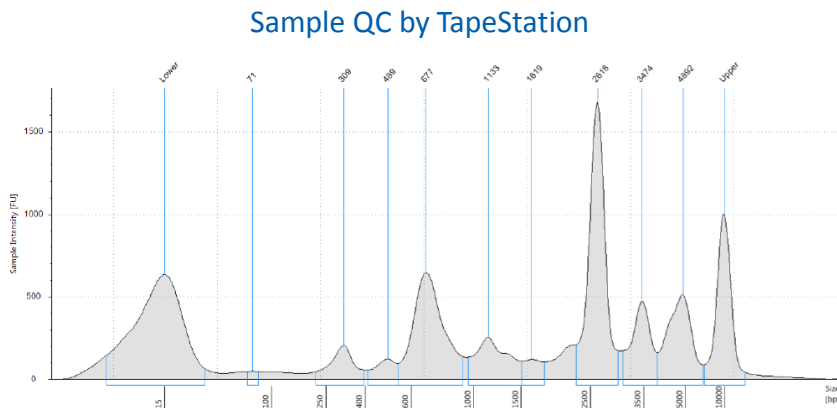
- Longer amplicon
- Reads without bc/low quality bc
- Lower single-molecule accuracy



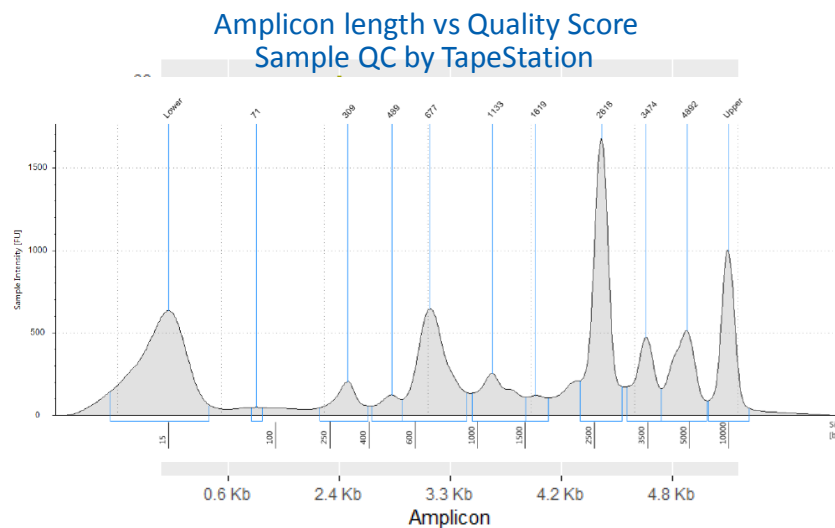
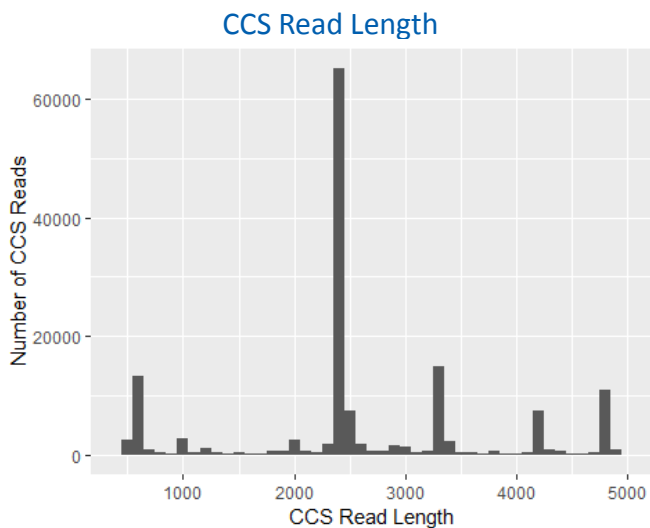


## Sequencing of Diverse Amplicon Sizes

- Proof-of-principle to determine ability to sequence wide distribution of amplicon sizes on one SMRT cell
- Amplicon sizes ranging from 300 bp to ~5000 bp
- Iso-seq (MagBead) loading



# Single-Molecule Accuracy vs Amplicon Size

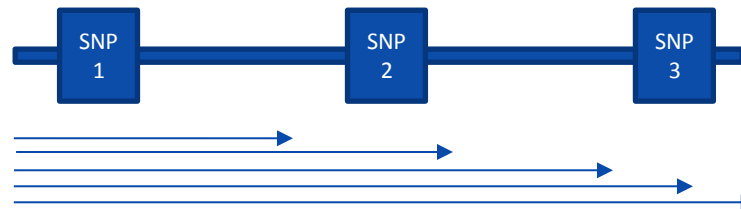


- CCS read accuracy drops off with longer amplicon size
- Short amplicons are not preferentially sequenced
  - Limiting pooling amplicons  $\pm 10\%$  is not necessary with adequate loading but application-dependent
  - Allows scientists to pool more amplicons for cost-effective experimental design

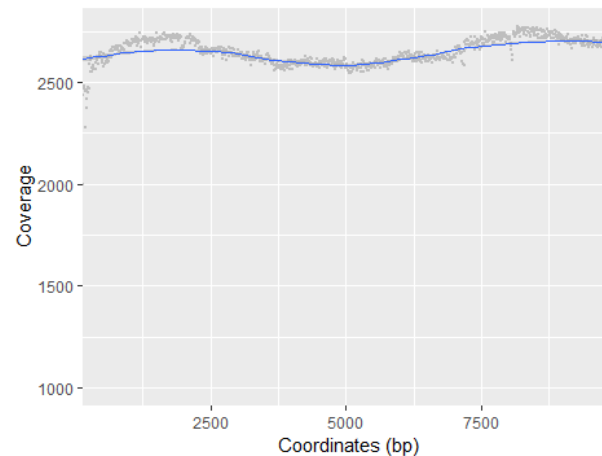
## Long amplicon sequencing

- Generated a 9.7 kb amplicon from genomic DNA
- Sequenced on one SMRT cell

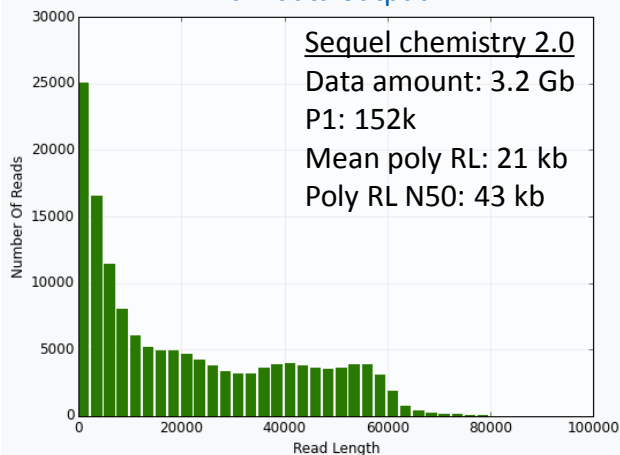
Continuous long reads to span entire amplicon



Coverage is even across length of amplicon



Raw data output



## Looking to the future

- The ability to amplify an amplicon, not sequence it, will become the bottleneck
- Improvements in polymerase RL offer potential for multiplexed long amplicon sequencing with higher single-molecule fidelity
- These advancements pave the way for novel applications and clinical diagnostics

## Contact Information

Dave Corney, Ph.D.

Senior Scientist, Next-Generation Sequencing

[david.corney@genewiz.com](mailto:david.corney@genewiz.com)

GENEWIZ Next-Generation Sequencing

[NGS@genewiz.com](mailto:NGS@genewiz.com)

877-GENEWIZ (436-3949) x1

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