Best Practices for Large Insert Libraries and Sequel System Sequencing

PacBio UGM
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# Our Technology Development team & capabilities

Mission: To enable investigators to perform translational and interdisciplinary biomedical research using cutting edge genomics tools

Multi-Platform DNA Sequencing:
- 3 PacBio RS IIs
- 2 PacBio Sequels
- 8 Illumina HiSeq 2500/4000
- 2 MiSeq
- 8 Ion Protons
- 7 Ion S5XL
- 2 Ion PGM
- 11 Ion Chefs
- 1 10X Genomics Chromium
- 1 BioNano Genomics Irys
- 1 Applied Biosystems 3730xl
- ONT MinION

Single Cell Technology:
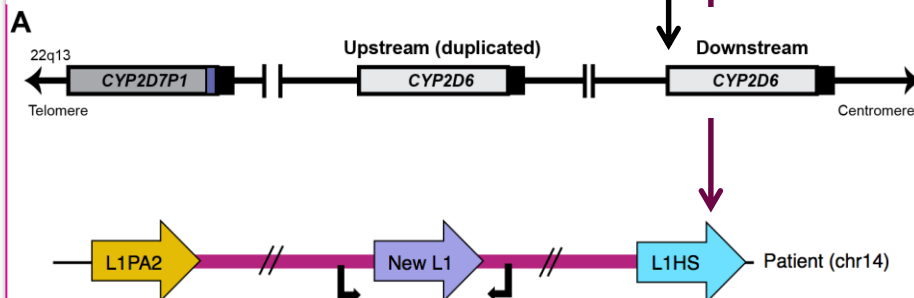- 1 10X Genomics Chromium
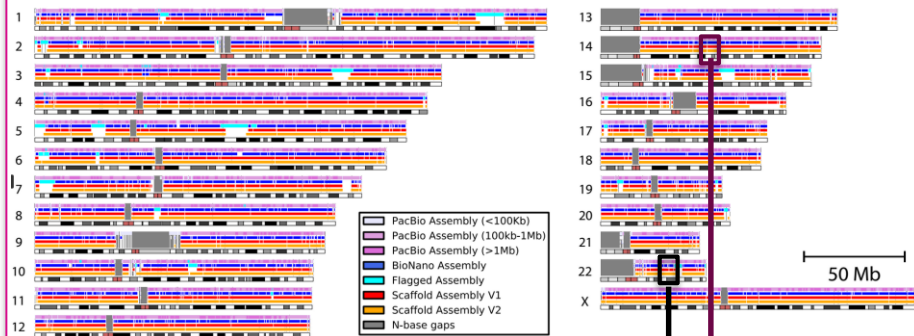- 1 BLI Beacon
- 1 BioRad/Illumina ddSeq
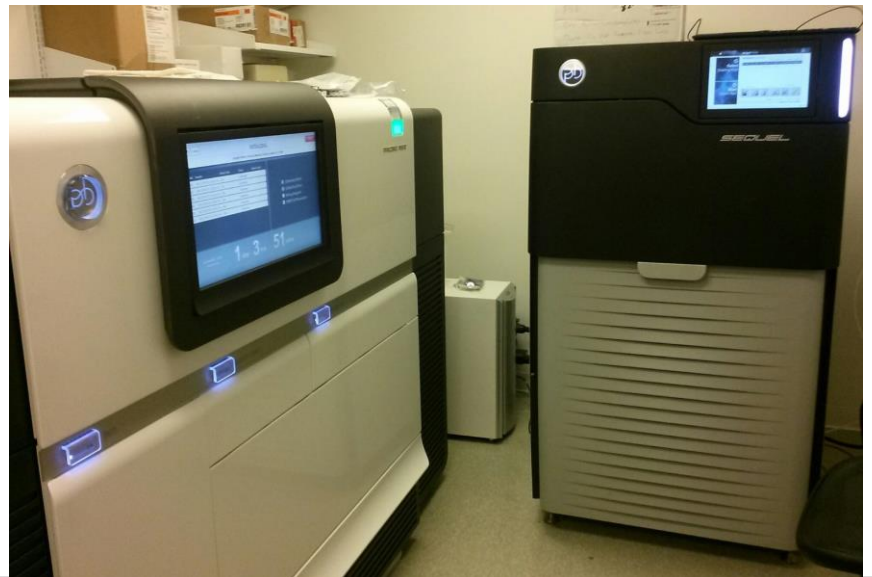
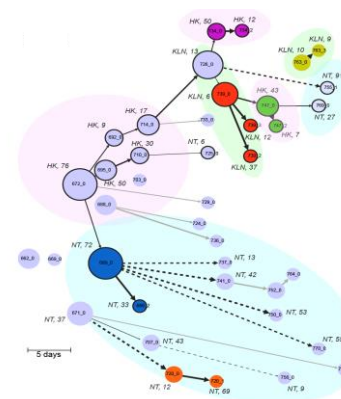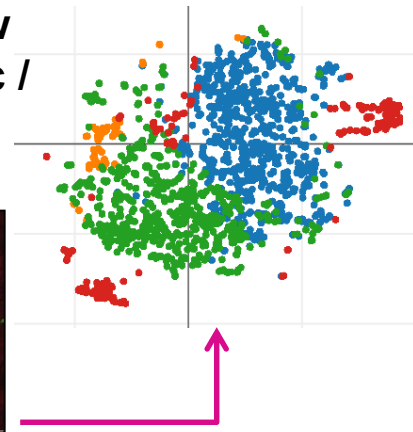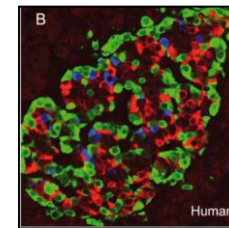# Using long read sequencing as a translational research tool

Technology Toolbox and Expertise:
- 2 Sequel & 3 RSII sequencing systems with >3000 RSII & >250 Sequel SMRTcells in 2016
- 10X Chromium and BLI Beacon for unique single cell genomics
- BioNano Genomics optical mapping and scaffold generation expertise
- Novel data integration, spanning all technologies to inform R&D and novel diagnostics

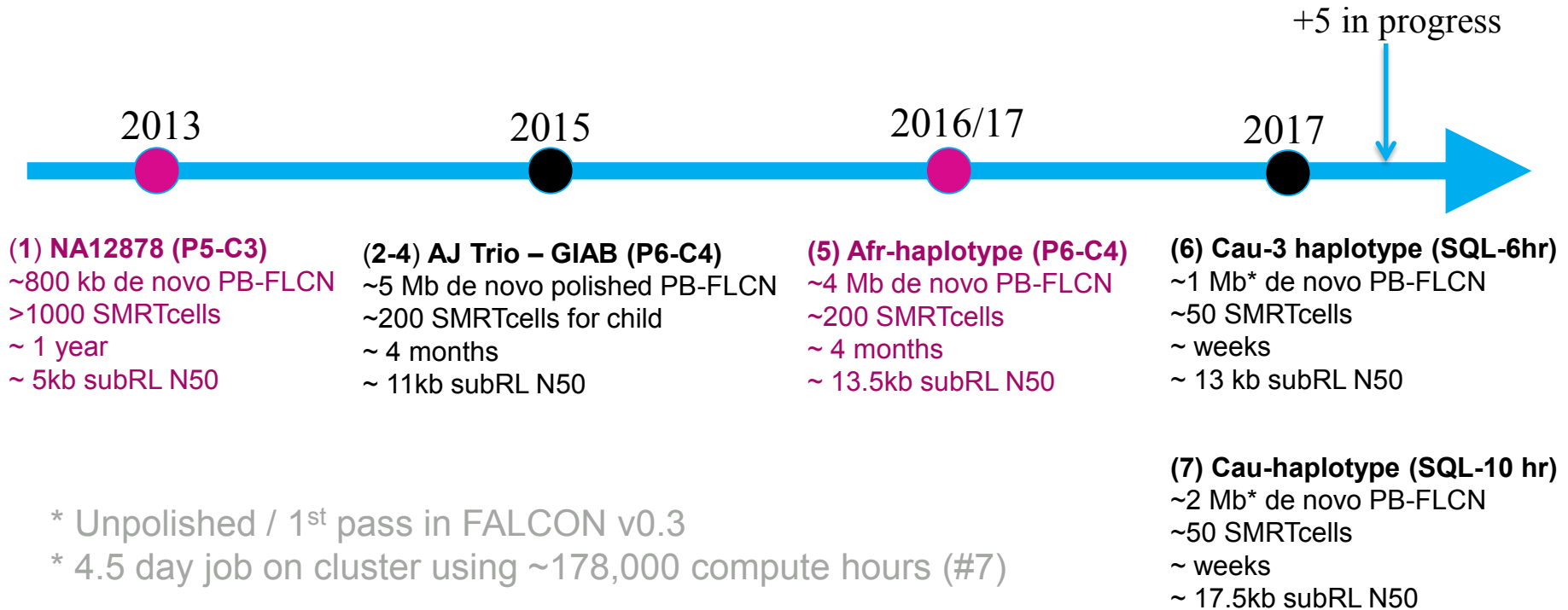## Addressing complex structure & targeting comprehensive diagnostics

Legend:
- PacBio Assembly (<100Kb)
- PacBio Assembly (100kb-1Mb)
- PacBio Assembly (>1Mb)
- BioNano Assembly
- Flagged Assembly
- Scaffold Assembly V1
- Scaffold Assembly V2
- N-base gaps

50 Mb

A

22q13

CYP2D7P1 — Telomere

Upstream (duplicated) CYP2D6

Downstream CYP2D6 — Centromere

L1PA2 → New L1 → L1HS → Patient (chr14)

Rapid validation of low freq variants in somatic / heterogeneous tissue

B — Human

Infectious Disease & Immune Repertoire Profiling and Surveillence

5 days

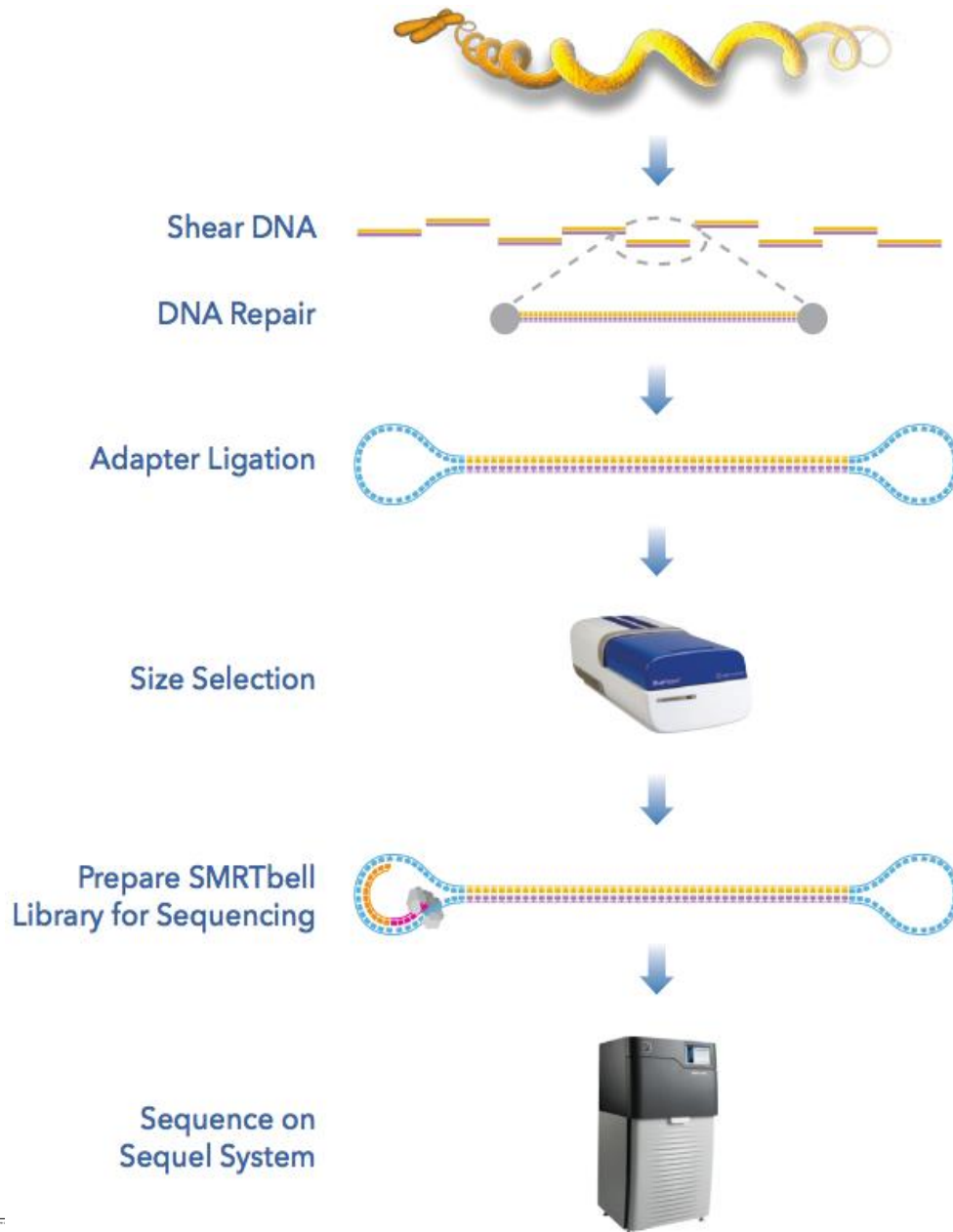**Our Goal:** To Address Structural Complexity by WGS with Comprehensive Long Read Sequencing Methods

Progress in Human SMRT-WGS Across 7 Genomes

+5 in progress

2013　　　　　2015　　　　　2016/17　　　　　2017

(1) NA12878 (P5-C3)
~800 kb de novo PB-FLCN
>1000 SMRTcells
~ 1 year
~ 5kb subRL N50

(**2-4**) **AJ Trio – GIAB (P6-C4)**
~5 Mb de novo polished PB-FLCN
~200 SMRTcells for child
~ 4 months
~ 11kb subRL N50

(5) Afr-haplotype (P6-C4)
~4 Mb de novo PB-FLCN
~200 SMRTcells
~ 4 months
~ 13.5kb subRL N50

(6) Cau-3 haplotype (SQL-6hr)
~1 Mb* de novo PB-FLCN
~50 SMRTcells
~ weeks
~ 13 kb subRL N50

(7) Cau-haplotype (SQL-10 hr)
~2 Mb* de novo PB-FLCN
~50 SMRTcells
~ weeks
~ 17.5kb subRL N50

* Unpolished / 1st pass in FALCON v0.3
* 4.5 day job on cluster using ~178,000 compute hours (#7)

- From previous experience with BNG, can lift scaffold N50 to 22-32 Mb
- Current max contig sizes, even on pass #1 FALCON 0.3 with Sequel are ~10 Mb
- Polished max contigs up to ~20 Mb on RSII completed assemblies
- Speed and cost on Sequel greatly reduced for generating more haplotypes faster
- Additional large genomes prepped for Sequel – mammalian, plants & insects

Overview of Large Insert Library Prep Process (w/Notes)

Shear DNA

DNA Repair

Adapter Ligation

Size Selection

Prepare SMRTbell Library for Sequencing

Sequence on Sequel System

Upfront QC: request at least 20 μg; clean (1X AMPure), BA, Qubit

Shear: Covaris g-tubes (20 kb shear)

Pre-library repair: DR, ER + exoVII always!
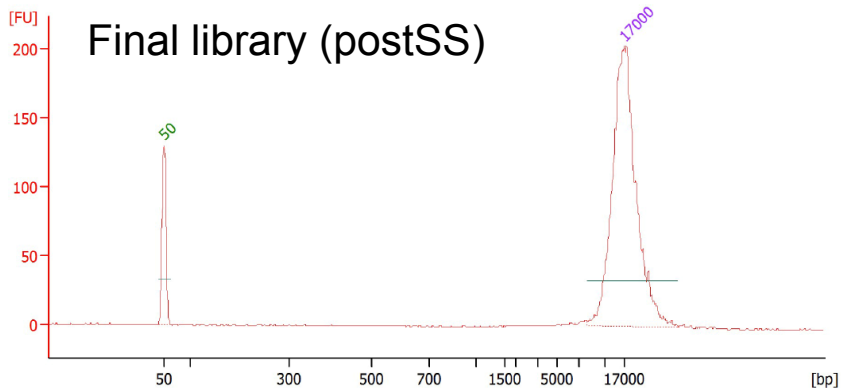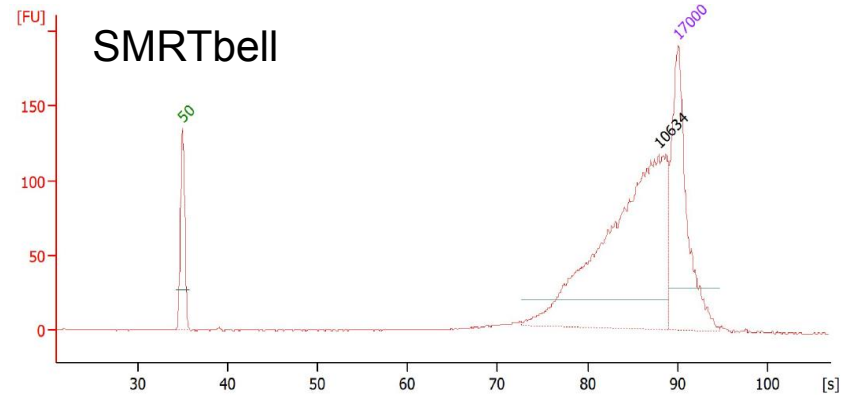
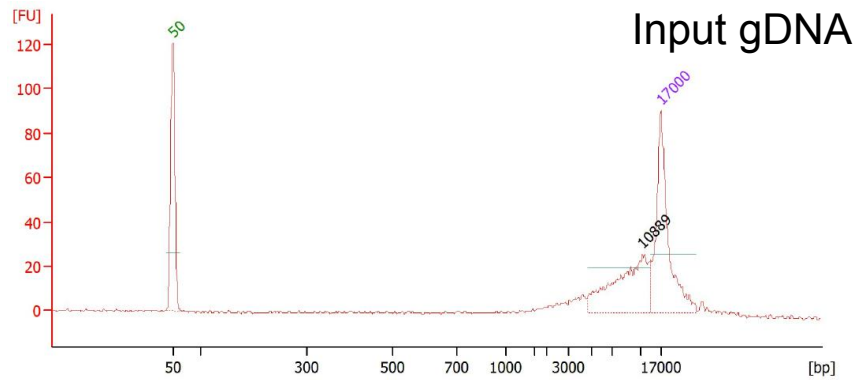**Library Prep:** standard protocols

Size Selection: Based on quality & quantity of library, select 10-50 kb or 20-50 kb (BluePippin and/or PippinHT)

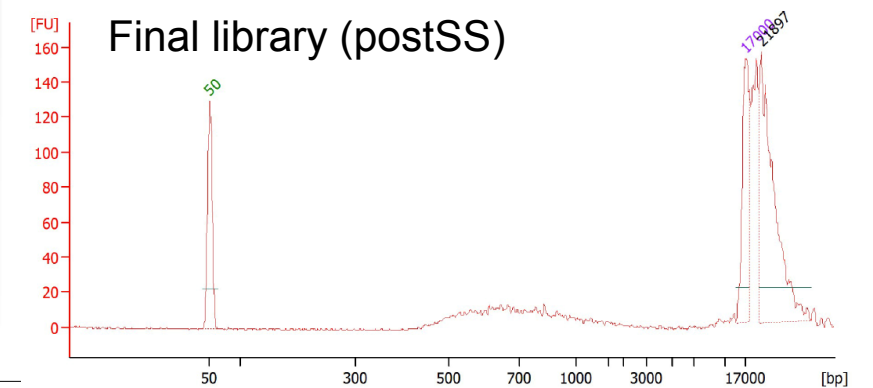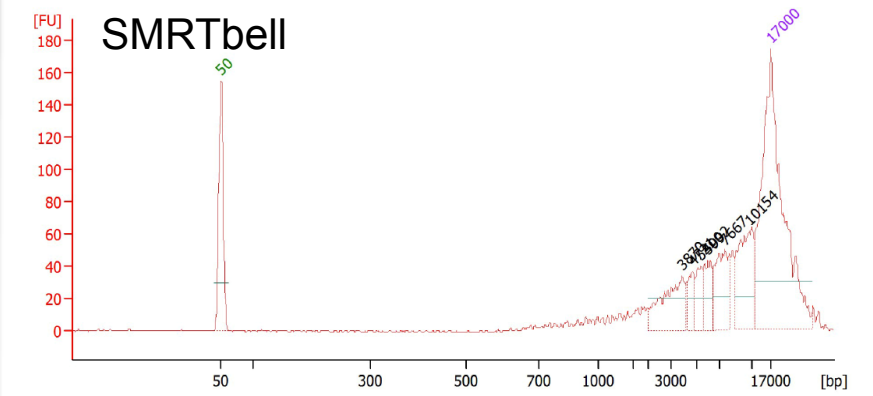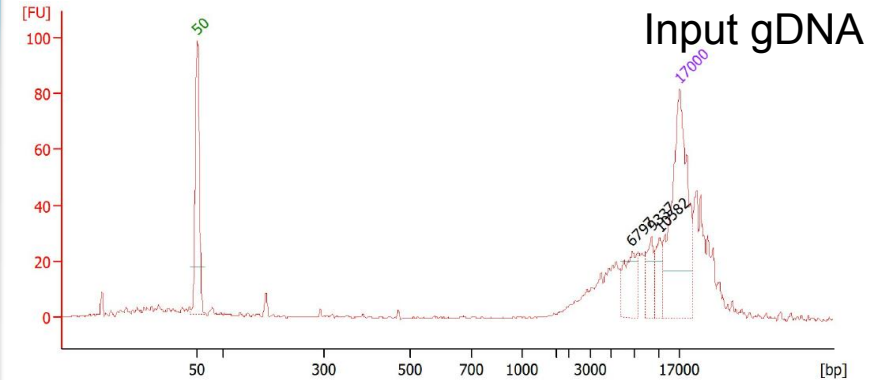**Annealing/Binding:** Primer annealing time (30min); Pol binding time (4h)

Sequel: *Titrate* for large projects; 10h movies; 2.0 chemistry

# Sample Data Sets: Input, Library Prep and Size Selection

Sample Data Sets : Sequel Stats

"Just Fine (2 cell pilot)"

Mapping Report

Value	Analysis Metric
83.52%	Mean Concordance (mapped)
794,400	Number of Subreads (mapped)
5,394,918,161	Number of Subread Bases (mapped)
6,791	Subread Length Mean (mapped)
10,718	Subread Length N50 (mapped)
15,840	Subread Length 95% (mapped)
36,431	Subread Length Max (mapped)
642,617	Number of Polymerase Reads (mapped)
8,555	Polymerase Read Length Mean (mapped)
13,008	Polymerase Read N50 (mapped)
24,010	
77,546	

Mapped Subread Length
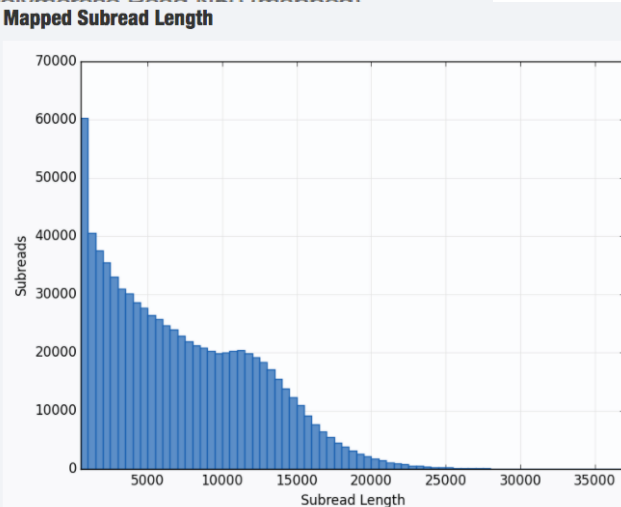
"Great (4 cell pilot)"

Mapping Report

Value	Analysis Metric
84.04%	Mean Concordance (mapped)
2,172,791	Number of Subreads (mapped)
18,590,421,740	Number of Subread Bases (mapped)
8,556	Subread Length Mean (mapped)
13,904	Subread Length N50 (mapped)
21,620	Subread Length 95% (mapped)
50,116	Subread Length Max (mapped)
1,758,655	Number of Polymerase Reads (mapped)
10,732	Polymerase Read Length Mean (mapped)
17,498	Polymerase Read N50 (mapped)
32,120	
75,133	

Mapped Subread Length
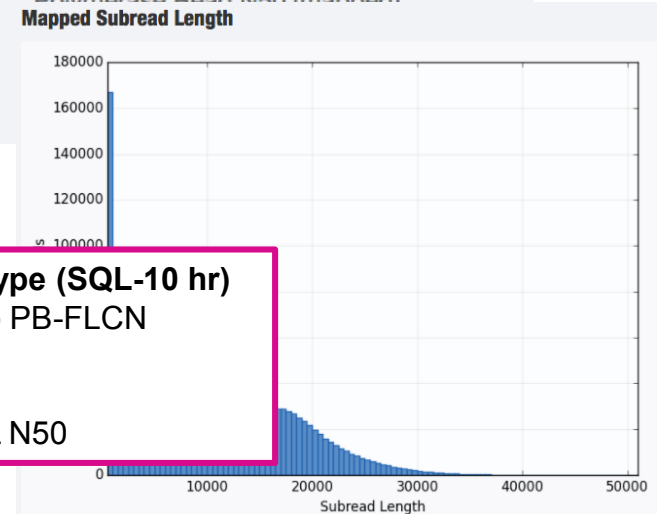
(7) Cau-haplotype (SQL-10 hr)
~2 Mb* de novo PB-FLCN
~50 SMRTcells
~ weeks
~ 17.5kb subRL N50

Looking Forward

- Awaiting SMRTLink 5.0 and IPS upgrades soon
- Currently:
  - 5 whole human genomes in progress/in queue
  - Multiple additional large genomes, including mammalian, insect and plants
- Expanding to MegaRuptor (≥ 30 kb)-derived libraries
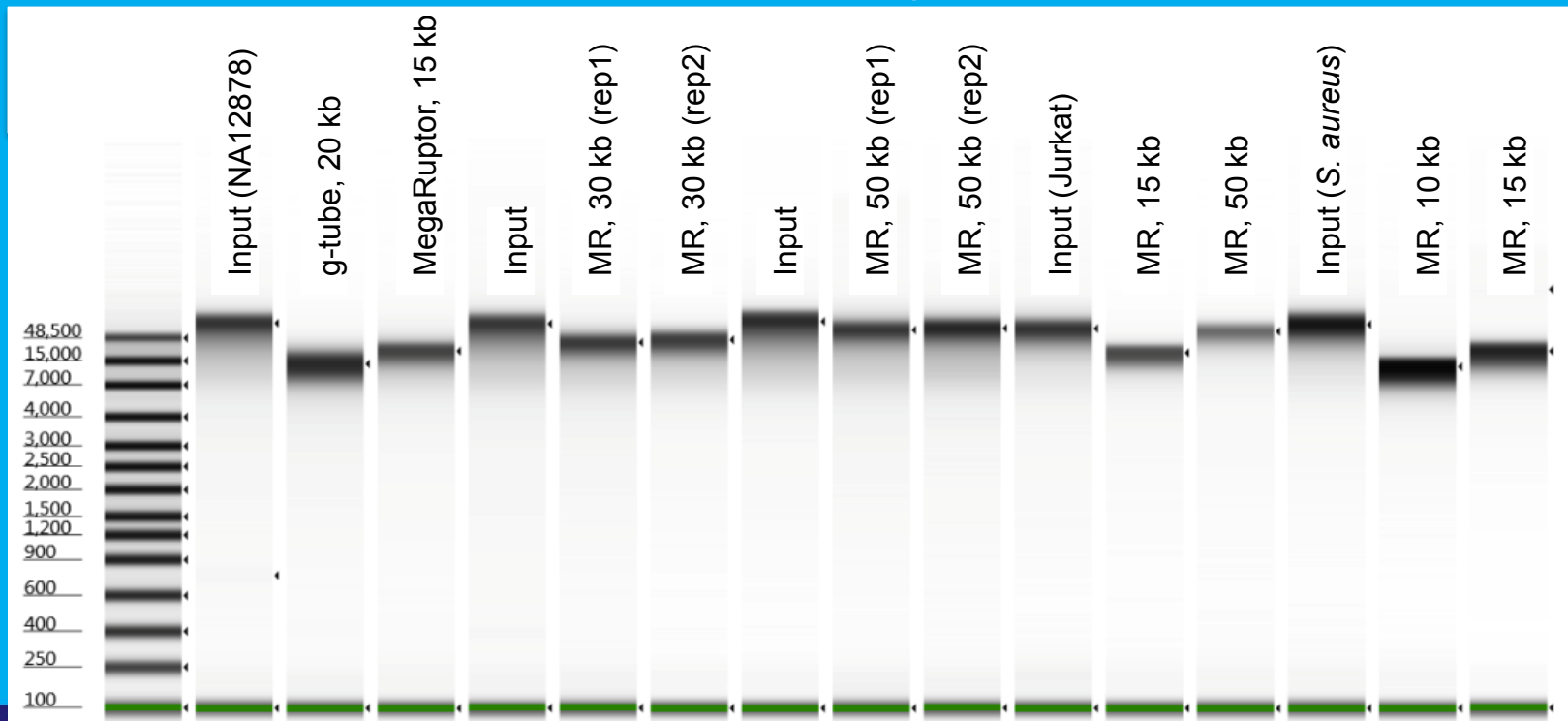
Many Thanks!

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Open to all the Q&A!

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