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:

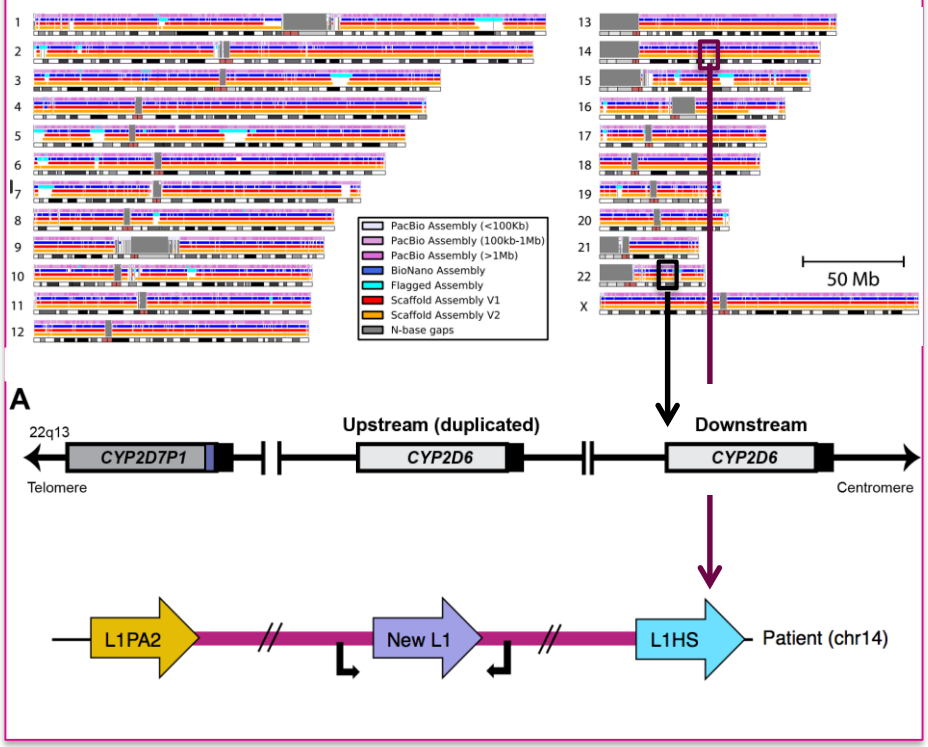
- 1 10X Genomics Chromium
- 1 BLI Beacon
- 1 BioRad/Illumina ddSeq

Using long read sequencing as a translational research tool

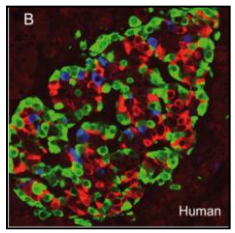
Technology Toolbox and Expertise:

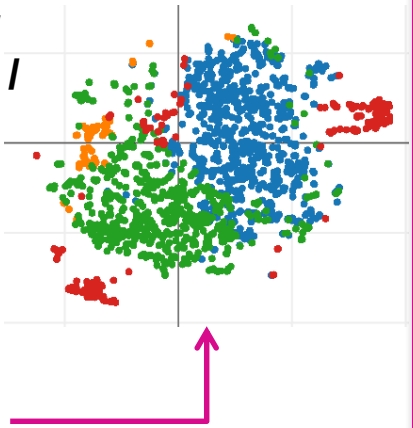
- 2 Sequel & 3 RSII sequencing systems with >3000 RSII & >250 Sequel SMRTcells in 2016
- 10X Chromium and BLI Beacon for unique single cell genomics
- BioNano Genomics optical mapping and scaffold generation expertise
- Novel data integration, spanning all technologies to inform R&D and novel diagnostics

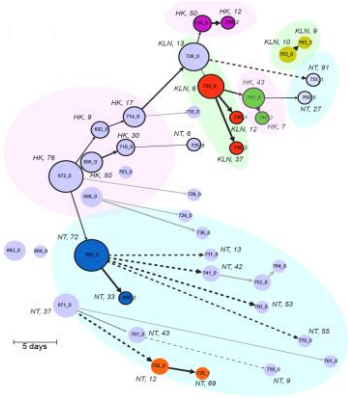
Addressing complex structure & targeting comprehensive diagnostics



Rapid validation of low freq variants in somatic / heterogeneous tissue







Infectious Disease & Immune Repertoire Profiling and Surveillance

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

Progress in Human SMRT-WGS Across 7 Genomes

(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

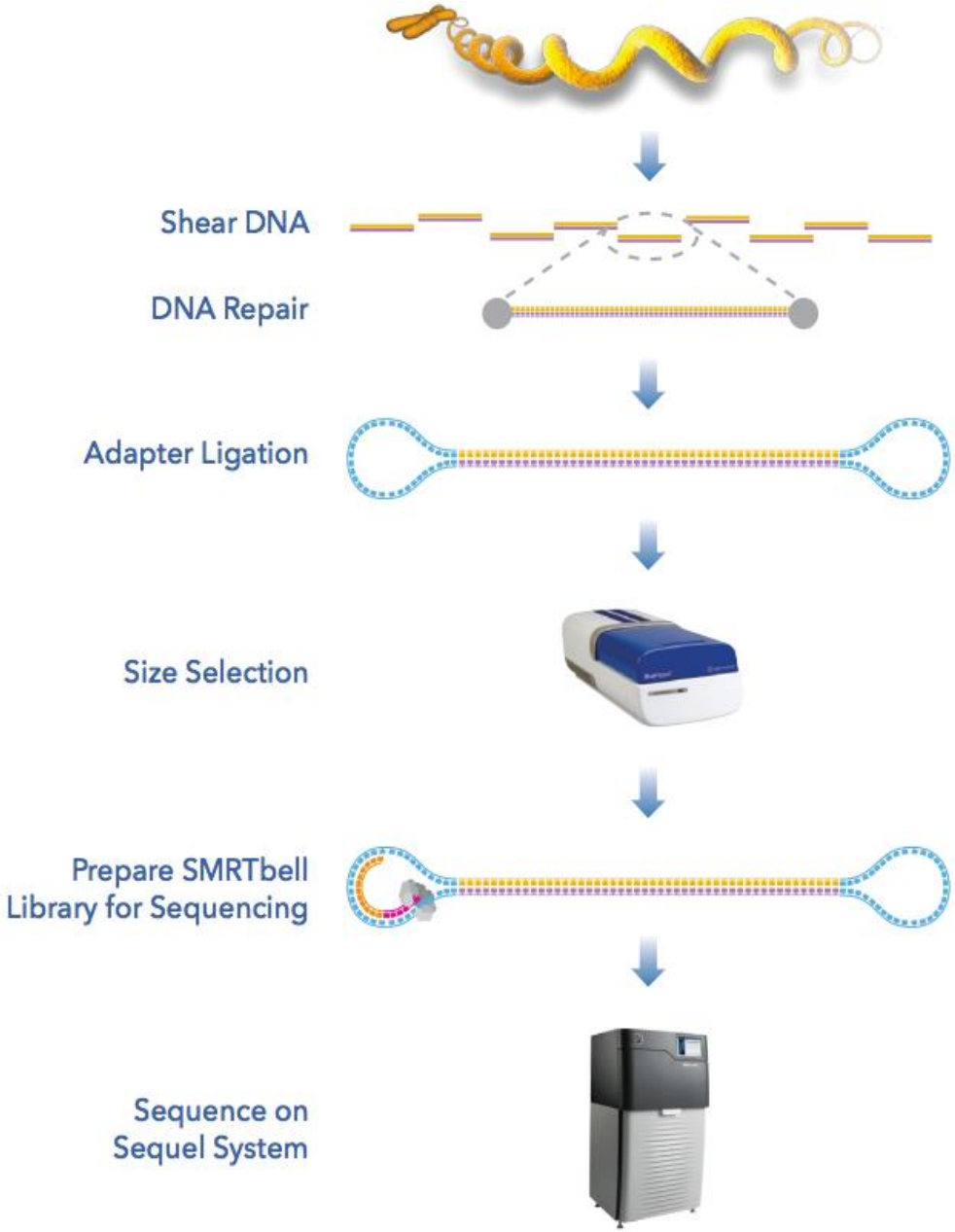
(6) Cau-3 haplotype (SQL-6hr)
~1 Mb* de novo PB-FLCN
~50 SMRTcells
~ weeks
~ 13 kb subRL N50

(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)



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)

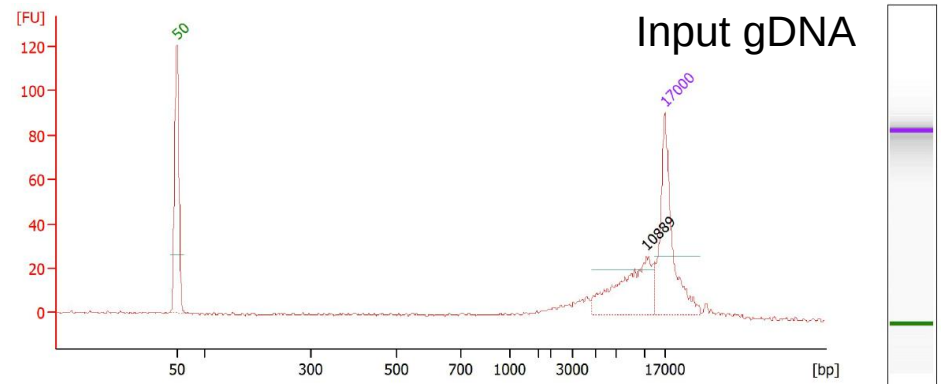
Annealing/Binding: Primer annealing time (30min); Pol binding time (4h)

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

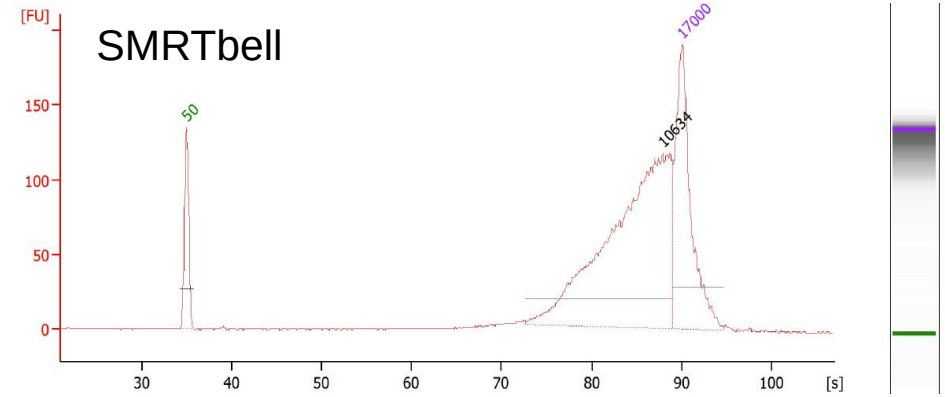
Sample Data Sets: Input, Library Prep and Size Selection

“Just Fine” sample

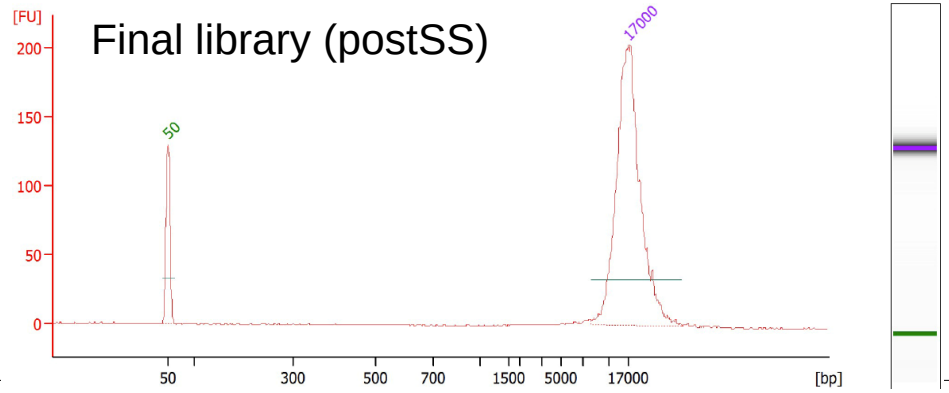
Input gDNA



SMRTbell

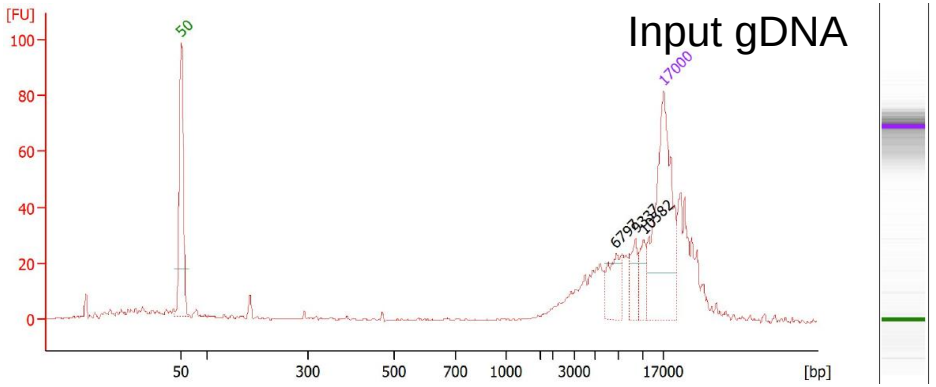


Final library (postSS)

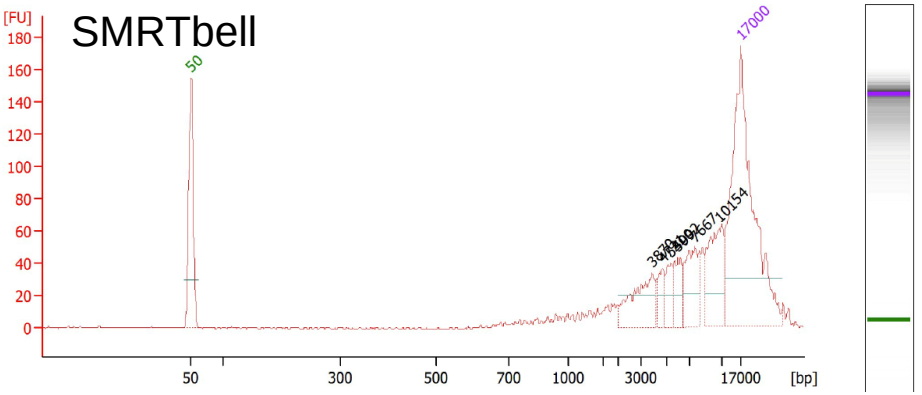


“Great” sample

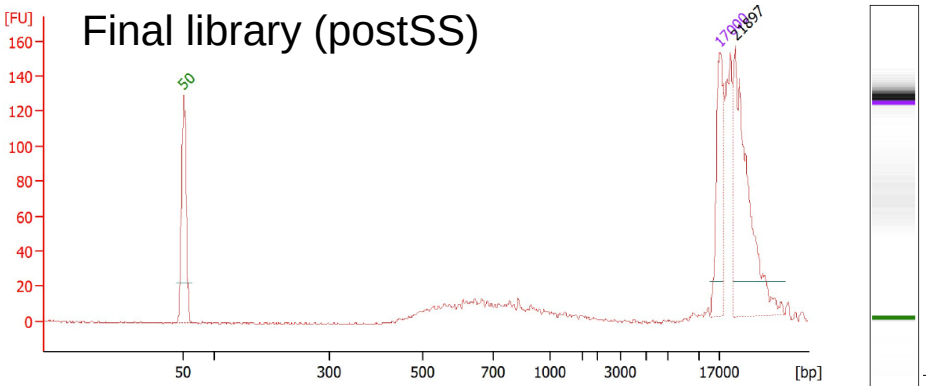
Input gDNA



SMRTbell



Final library (postSS)

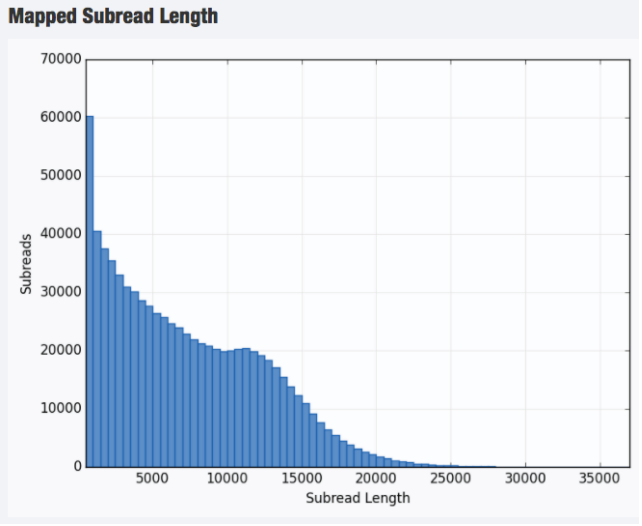


Sample Data Sets : Sequel Stats

"Just Fine (2 cell pilot)"

Mapping Report

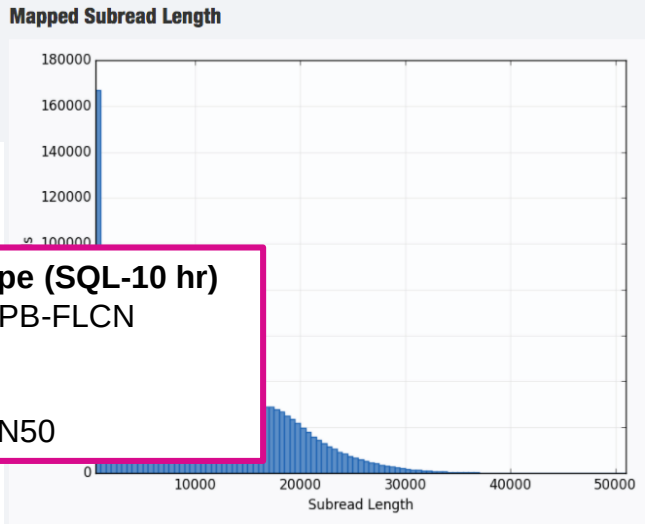
Value	Analysis Metric
83.52%	Mean Concordance (mapped)
794,400	Number of Subreads (mapped)
5,394,918,161	Number of Subread Bases (mapped)
6,791	Subread Length Mean (mapped)
10,718	Subread Length N50 (mapped)
15,840	Subread Length 95% (mapped)
36,431	Subread Length Max (mapped)
642,617	Number of Polymerase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



"Great (4 cell pilot)"

Mapping Report

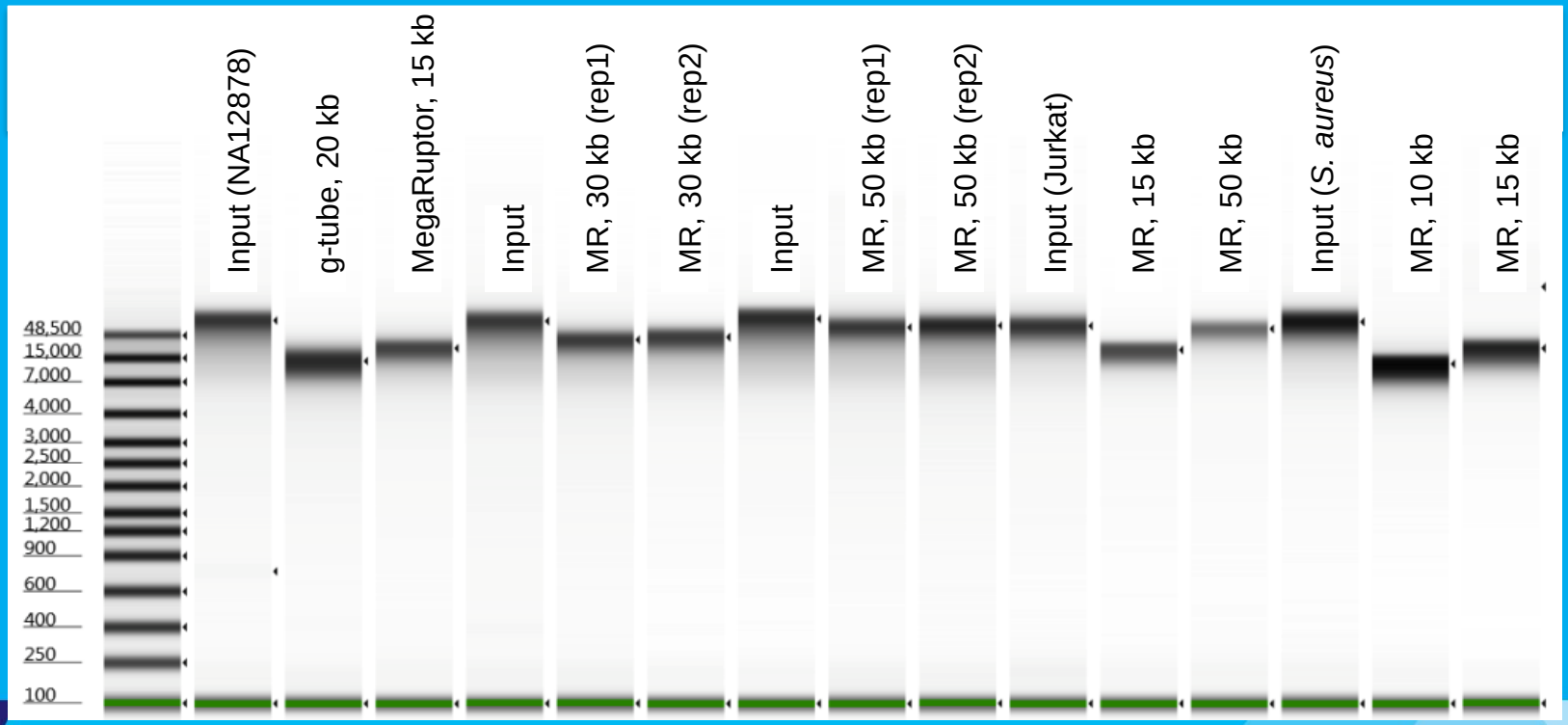
Value	Analysis Metric
84.04%	Mean Concordance (mapped)
2,172,791	Number of Subreads (mapped)
18,590,421,740	Number of Subread Bases (mapped)
8,556	Subread Length Mean (mapped)
13,904	Subread Length N50 (mapped)
21,620	Subread Length 95% (mapped)
50,116	Subread Length Max (mapped)
1,758,655	Number of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



(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!

Tech Dev Team

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Open to all the Q&A!

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