Breakout: "SMRT Sequencing Project Success: Start to Finish"

- Carmen Guarco: **Using Bioinformatic Resources to Plan Your Project**
- Greg Young: **SMRT Sequencing Project Success: Start to Finish**
- Nick Sisneros: **What Happened to My Sequencing Run?**
- Carmen Guarco: **Best Practices in Bioinformatics Data QC**

Using Bioinformatic Resources to Plan Your Project

Carmen Guarco

For Research Use Only. Not for use in diagnostics procedures. © Copyright 2017 by Pacific Biosciences of California, Inc. All rights reserved.

STEPS FOR SUCCESS

Design your project

Sample, DNA, Library Prep, Loading

- Ensure the high quality of your sample and DNA to generate the optimal library.

Sequencing

- Scale coverage based on your project needs and achieve up to 10 Gb of sequence data per SMRT Cell.

Data Analysis

- In-house bioinformatic tools with SMRT Link package (GUI and Command line) and guides.

STEPS FOR SUCCESS

We will explore the resources for **tips**, **tricks**, and **best practices** at each step in the workflow for generating and analyzing high-quality Single Molecule Real-Time (SMRT) Sequencing Data.

STEPS FOR SUCCESS

- Key Questions to Ask Yourself
  - Which Analysis Application fits my goals?
  - What is the coverage I need to utilize this application?
    - How many SMRTCells do I need to hit this coverage?

Which Analysis Application fits my goals?

ANALYSIS APPLICATIONS & THEIR PRIMARY OUTPUTS

Whole Genome Sequencing

De novo Assembly	Assembly (HGAP4)	Polished Assemblies (FASTA/FASTQ)
Structural Variant Calling	Structural Variant Calling	VCF, BED
Resequencing	Resequencing	VCF, GFF, FASTA/FASTQ, BED

Targeted Sequencing

Single Molecule Consensus Sequences	CCS	FASTA/FASTQ
Long Amplicon Analysis	LAA	FASTA/FASTQ
Minor Variant Detection	Minor Variant Detection	JSON / HTML report

RNA Sequencing

Iso-Seq	Iso-Seq, Iso-Seq3	FASTA/FASTQ

Epigenetics

Base Modification Detection	Base modification detection	GFF, BAM

PACBIO®

How much coverage do I need?

WHOLE GENOME SEQUENCING

Target	Coverage
Human	>50 fold (**inbred / diploid / human**)
Plants & Animals	~100 fold (***de novo* outbred & highly heterozygous genomes**)
Microbial	~70-100 fold
Structural Variation	5 to 10 fold: **population genetics** 10 fold: **rare undiagnosed disease** 10 to >20 fold: **genetic disease**
Resequencing (all application areas)	**For QV30+ average consensus accuracy***: >30 fold coverage

*Currently Arrow does NOT support diploid whole genome variant calling, only haploid genomes

RNA SEQUENCING

Goal	Coverage
Human Whole Transcriptome Characterization	~20 Gb overall -or- 250-350K full length reads per SMRT Cell >2 SMRT cells for better genome characterization
Plants & Animals Whole Transcriptome Characterization	

EPIGENETICS

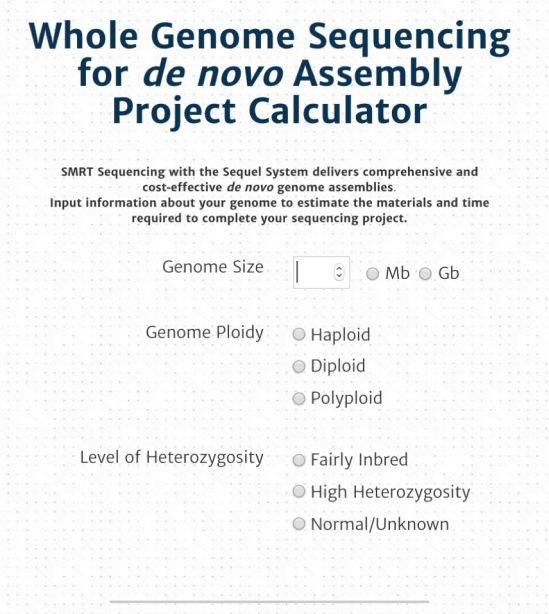
Goal	Coverage
Characterize Microbial DNA Modification Motifs	25x coverage (per strand)- 4mC 40x coverage (per strand)- 6mA

AMPLICON PROJECTS

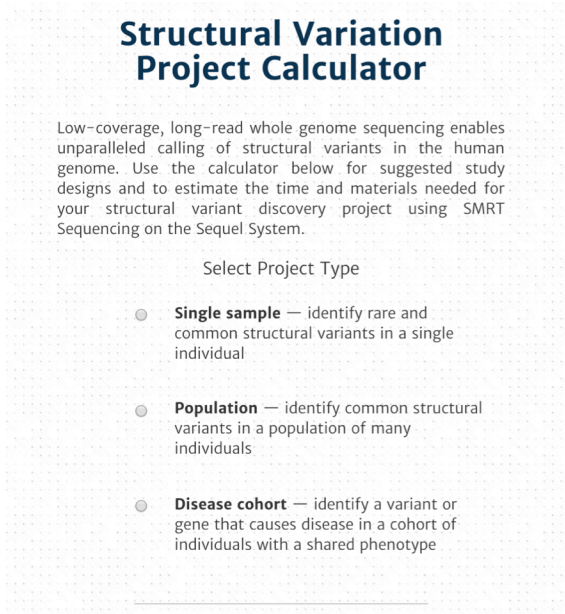
Goal	Coverage
Full length consensus sequences (Amplicons > ~10kb with 3.0 Sequencing Kit)	> 30 fold ***subread*** coverage per expected allele
Minor Variant Detection	~600 fold ***CCS*** coverage for variants at 5% frequency ~6000 fold ***CCS*** coverage for variants at 1% frequency
SNV Validation (Amplicons <= 10kb with 3.0 Sequencing Kit)	~100 fold ***CCS*** coverage per amplicon

How Many SMRTCells Do I Need?

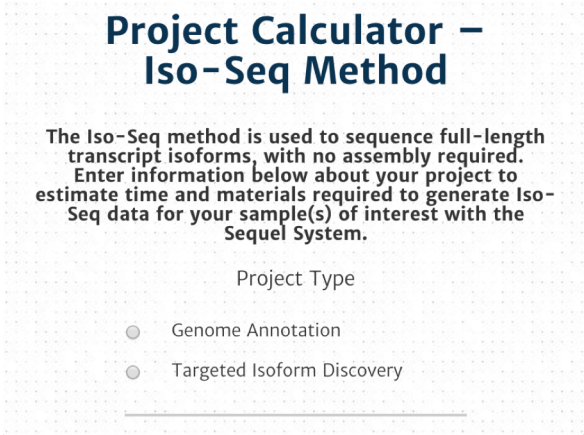
USE OUR ONLINE PROJECT CALCULATORS



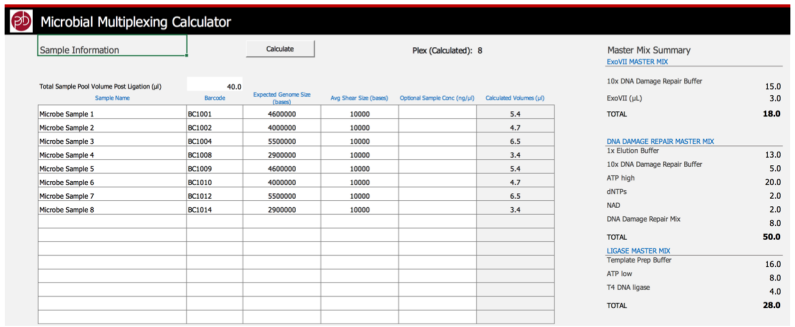
https://www.pacb.com/calculator-whole-genome-sequencing/



https://www.pacb.com/calculator-structural-variation/



https://www.pacb.com/calculator-iso-seq/



[Microbial Multiplex calculator]

WHEN IN DOUBT, ASK US!

Feel free to send any project inquiries to your local account team- FAS or FBX

AND CC

Support@pacb.com

This ensures your question will be seen and addressed by the next available appropriate person within 24-48 business hours.

We HIGHLY RECOMMEND you do this BEFORE sequencing when possible to ensure you have the best information for designing your SMRT Sequencing Project!

Best Practices in Bioinformatics Data QC

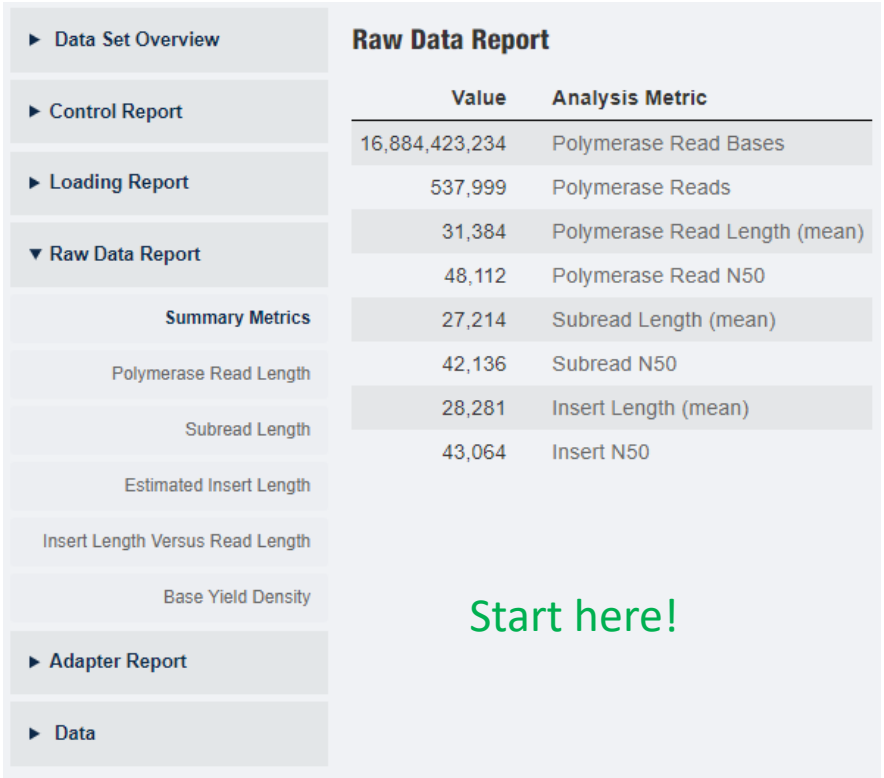
For Research Use Only. Not for use in diagnostics procedures. © Copyright 2017 by Pacific Biosciences of California, Inc. All rights reserved.

3 KEY QUESTIONS TO ASK YOURSELF

1) Does the sequencing data properly reflect the expected library generated during sample prep?
2) (For multiplexed projects) Did I get adequate representation of every sample I pooled into this experiment?
3) Do I have enough data to expect reasonable results from [insert pipeline]?

1) DOES THE SEQUENCING DATA PROPERLY REFLECT THE EXPECTED LIBRARY GENERATED DURING SAMPLE PREP?

Use the Data Management information to determine how a library "looked" during sequencing.



Raw Data Report >> Summary Metrics

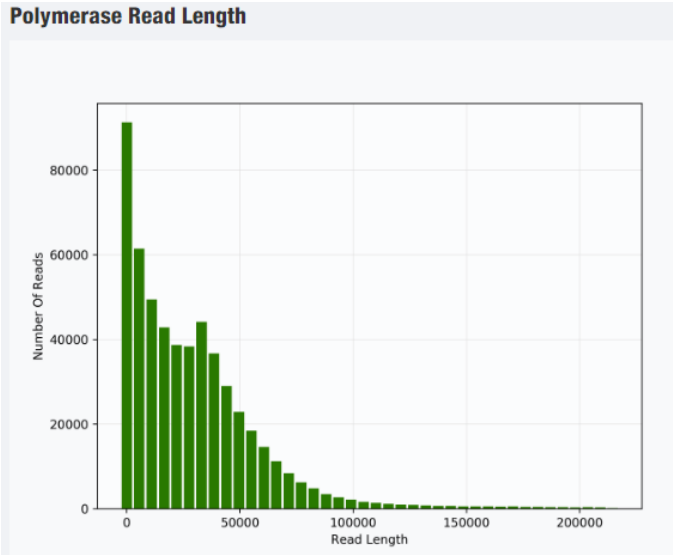
1) Do I have between 350k - ~700k polymerase reads? Yes
2) Is my polymerase read length mean > 10kb (2.1 Seq Chem)? Yes
3) Is my insert length mean close to my polymerase read length mean?

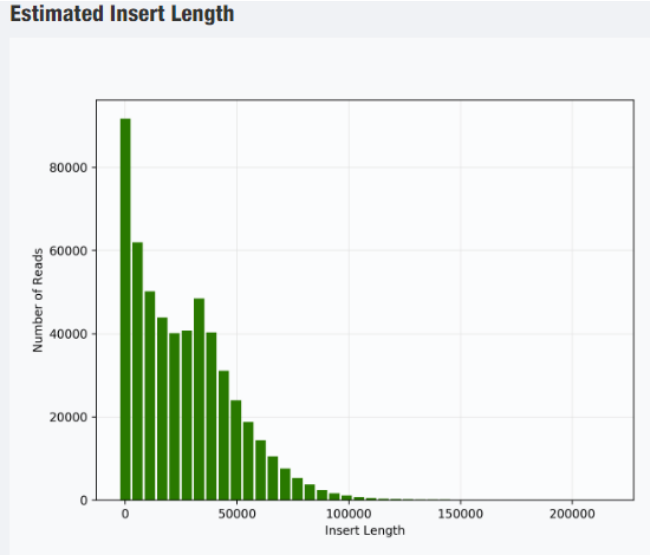
   Yes?- sequencing was from a large insert library OR damaged DNA if answer to (2) is NO

   No? – sequencing was from a fixed size library

1) DOES THE SEQUENCING DATA PROPERLY REFLECT THE EXPECTED LIBRARY GENERATED DURING SAMPLE PREP?

Use the Data Management information to determine how a library "looked" during sequencing.

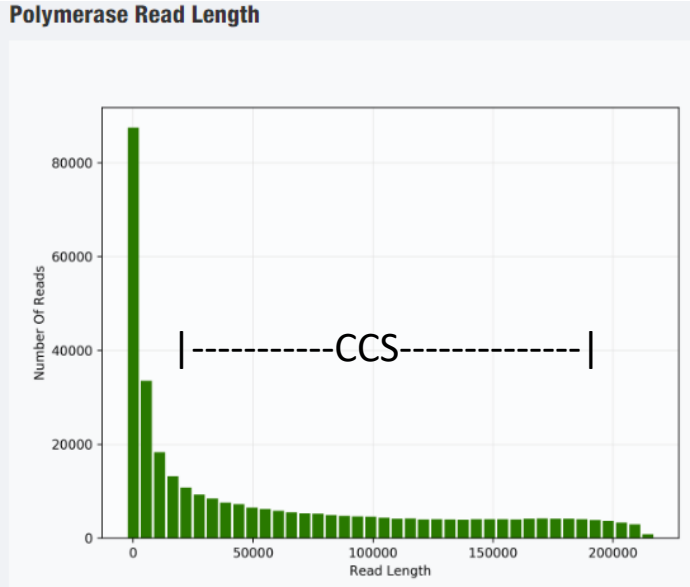


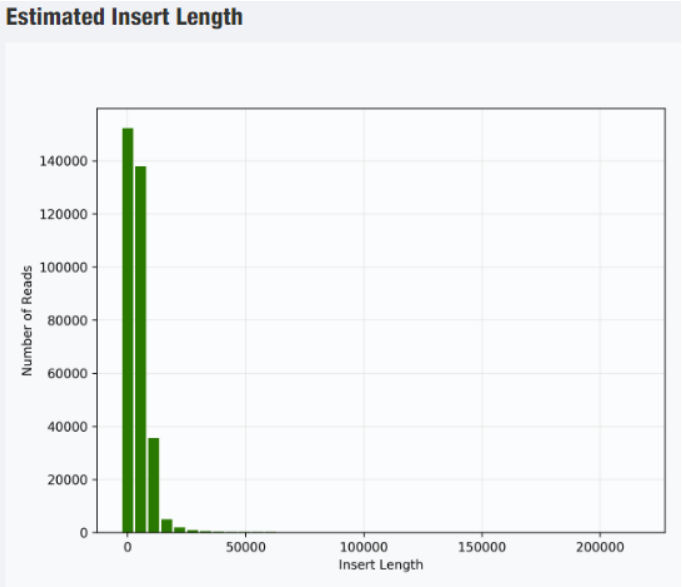


Compare these two graphs. For large insert libraries (20kb libraries and higher), these graphs should closely mimic one another!

1) DOES THE SEQUENCING DATA PROPERLY REFLECT THE EXPECTED LIBRARY GENERATED DURING SAMPLE PREP?

Use the Data Management information to determine how a library "looked" during sequencing.

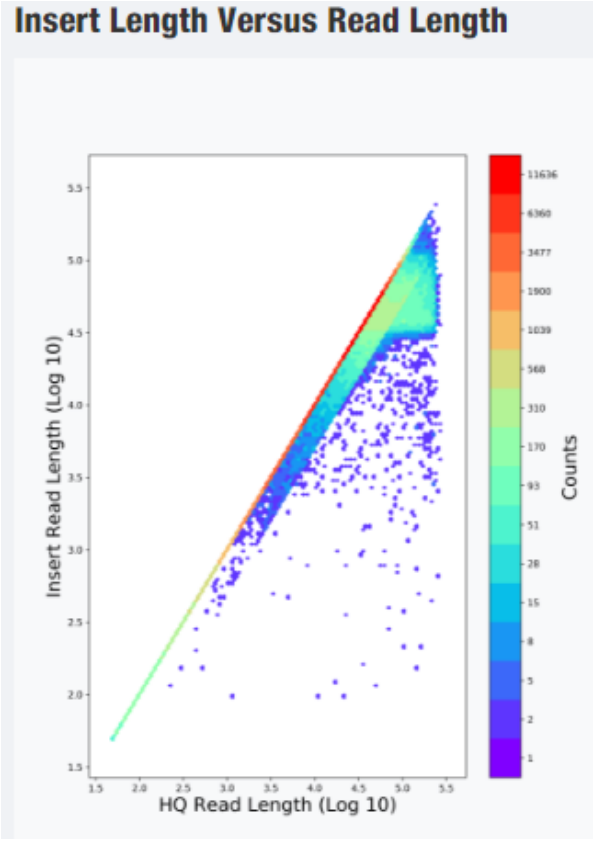




Compare these two graphs. For fixed length libraries (<=10kb libraries), these graphs should look very different from one another! Note the long, right handed rail in the polymerase read length graph. The estimated insert length graph shows a much more normally distributed size range centered around the expected size of the insert in the fixed length library.

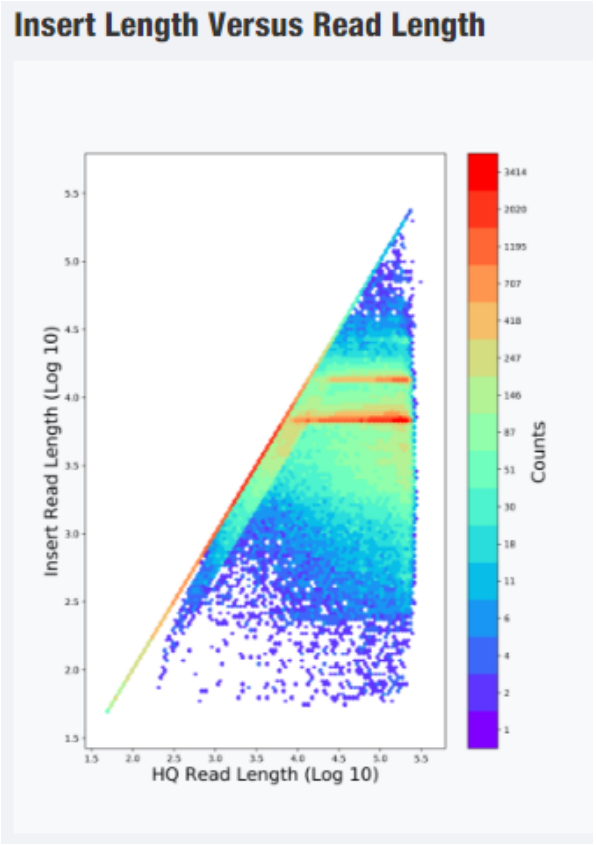
1) DOES THE SEQUENCING DATA PROPERLY REFLECT THE EXPECTED LIBRARY GENERATED DURING SAMPLE PREP?

New plots have been added to Data Management in SMRTLink 6.0 to assist in assessing library performance



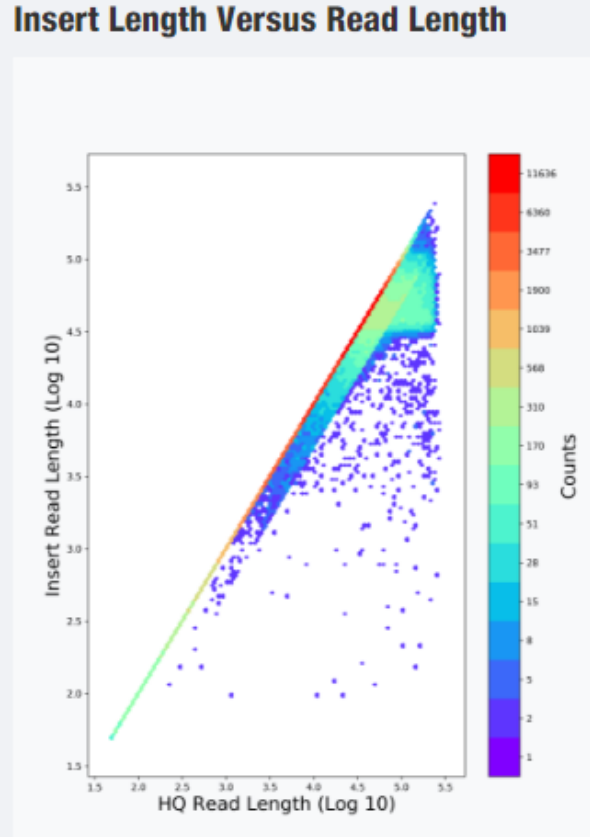
<<35kb whole genome size selected library

2.5kb PCR generated fixed insert size library>>



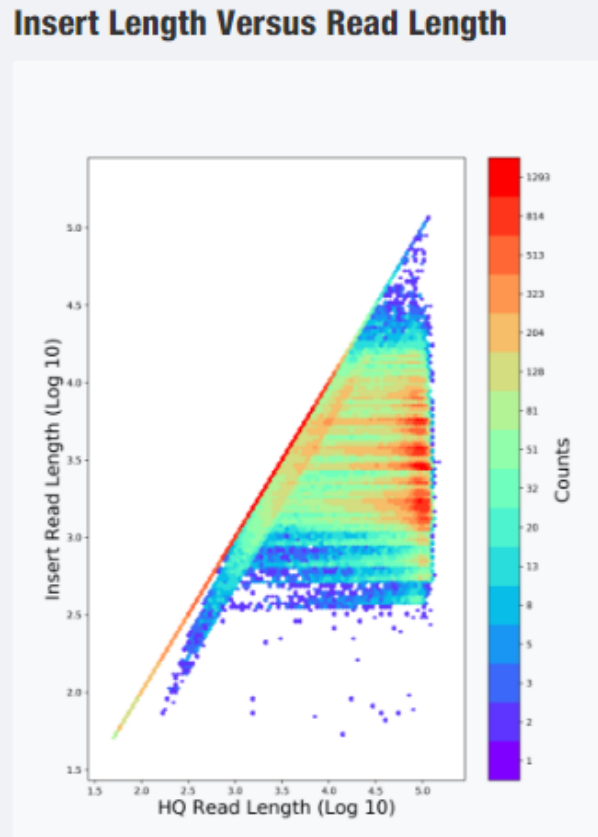
1) DOES THE SEQUENCING DATA PROPERLY REFLECT THE EXPECTED LIBRARY GENERATED DURING SAMPLE PREP?

New plots have been added to Data Management in SMRTLink 6.0 to assist in assessing library performance



<<35kb whole genome size selected library

10kb whole genome non-size selected library>>



2. (FOR MULTIPLEXED PROJECTS) DID I GET ADEQUATE REPRESENTATION OF EVERY SAMPLE I POOLED INTO THIS EXPERIMENT?

Examine the Demultiplexing Results in Data Management

Microbial Multiplexing

- >70 fold coverage per experiment? (based on expected assembled genome size per sample)
- Mean read length for each sample relatively consistent?
- Mean barcode quality > 60?

Fixed insert length libraries

- Do I have enough coverage per expected allele?
- Mean barcode quality > 60?
- <15% of total yield (in ***bases***) is unbarcoded?

*__For process QC__: did I get approximately the same number of binned reads and bases per barcode? If not- this suggests there may be problems with equimolar pooling of samples upstream

3) DO I HAVE ENOUGH DATA TO EXPECT REASONABLE RESULTS FROM [INSERT PIPELINE]?

Target	Coverage
Human	>50 fold coverage(**inbred / diploid / human**)
Plants & Animals	~100 fold coverage (***de novo* outbred & highly heterozygous genomes**)
Microbial	~70-100 fold coverage
Structural Variation	5 to 10 fold: **population genetics** 10 fold: **rare undiagnosed disease** 10 to >20 fold: **genetic disease**
Resequencing (all application areas)	**For QV30+ average consensus accuracy***: >30 fold coverage
Human Whole Transcriptome Characterization	~20 Gb overall -or- 250-350K full length reads per SMRT Cell >2 SMRT cells for better genome characterization
Plants & Animals Whole Transcriptome Characterization	
Characterize Microbial DNA Modification Motifs	25x coverage (per strand)- 4mC 40x coverage (per strand)- 6mA
Full length consensus sequences (Amplicons > ~10kb with 3.0 Sequencing Kit)	> 30 fold ***subread*** coverage per expected allele
Minor Variant Detection	~600 fold ***CCS*** coverage for variants at 5% frequency ~6000 fold ***CCS*** coverage for variants at 1% frequency
SNV Validation (Amplicons <= 10kb with 3.0 Sequencing Kit)	~100 fold ***CCS*** coverage per amplicon

SUMMARY

Utilize SMRTLink's Data Management module to quickly assess how your library performed during sequencing.

This checklist can quickly help you assess if you have sufficient and adequate data to successfully perform analyses.

>70% of troubleshooting bioinformatics cases lead back to one of the 3 questions in this checklist!

SMRT Sequencing Project Success: Start to Finish

UGM September 19, 2018

Gregory Young

For Research Use Only. Not for use in diagnostics procedures. © Copyright 2017 by Pacific Biosciences of California, Inc. All rights reserved.

AGENDA

- HMW Sample Preparation
- Internal Control
- Accurate Sample QC
- To Shear, or Not to Shear
- Loading
- Customer Support Resources
- Questions

HMW Sample Prep

PUSHING THE BOUNDARIES OF LONG-READ SEQUENCING

Good sample preparation is the key to attain multi-kilobase read sequencing and to maximizing throughput per SMRT Cell.

Components:

- **Purity**
  - A260/280 nm should be ~1.8
  - A260/230 nm should be >2.0

- **Molecular Weight (MW)**
  - Use fresh, or flash-frozen tissue/cells
  - Use gentle agitation, and wide-bore tips
  - Allow HMW DNA to resuspend (3-7 days)[1]

- [1] Olga Petterson's presentation from SMRT Leiden: Perfect Genome is a Treasure, DNA is the Key

HMW DNA EXTRACTION OPTIONS FOR PACBIO APPLICATIONS

Microbial and plant samples tend to be the most challenging

Microbial Solutions:

- Nanobind CBB Big DNA Kit (Circulomics)
- Master Pure Complete DNA and RNA Purification Kit (Lucigen)

Plant Solutions:

- Nanobind Plant Nuclei Big DNA Kit (Circulomics)

- Comparable sequencing results to agarose plug nuclei extraction methods

	Bases (Gb)	Poly. N50 (bp)	P0	P1
Circulomics	10.95	31750	41%	55%
Agarose-plug	9.92	31250	43%	53%

Sequel DNA Internal Control

SPIKE-IN INTERNAL CONTROL AS TROUBLESHOOTING TOOL

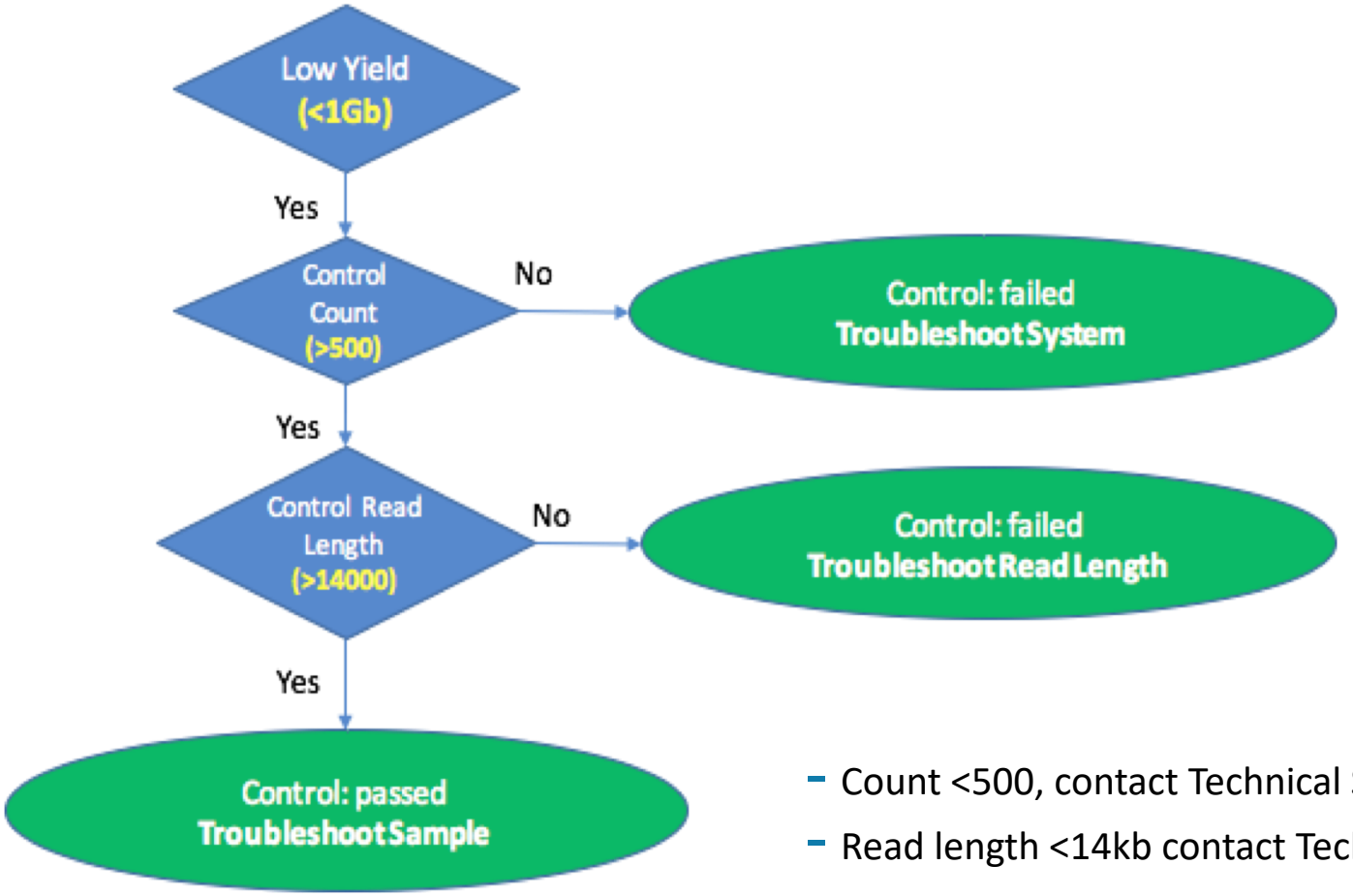
- Distinguish between sample and system issues
- Required for SMRT Cell and reagent reimbursement

Average Control Read Length for 600 min. movies on ICS v5.1:

- Sequel DNA Internal Control v2.1 – **27,348 bp**
- Sequel DNA Internal Control v2.0 – **19,136 bp**

Control count is sensitive to sample loading. In generally, there should be a few thousand reads (<1% of total P1).

SPIKE-IN INTERNAL CONTROL AS TROUBLESHOOTING TOOL



- Count <500, contact Technical Support
- Read length <14kb contact Technical Support

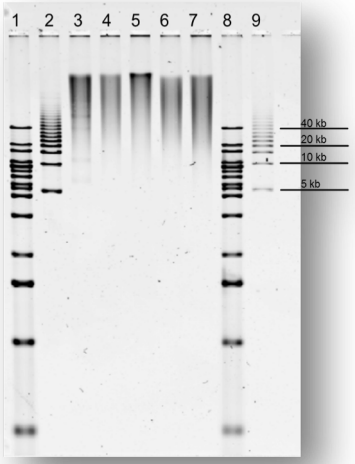
support@pacb.com

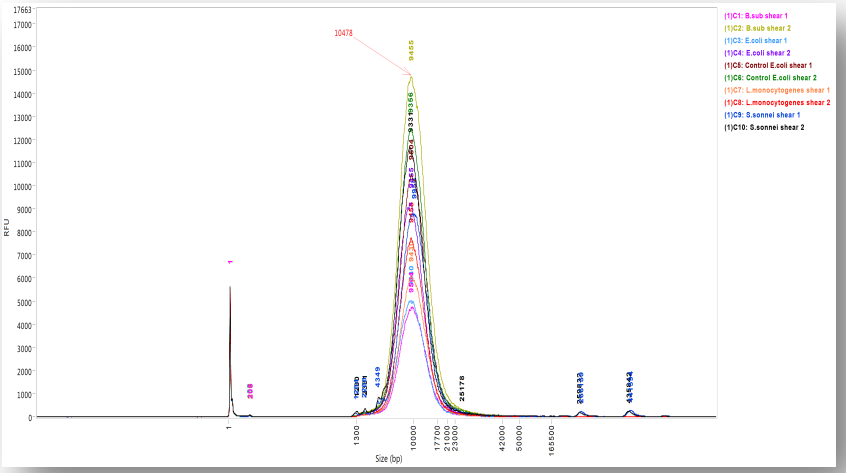
Accurate Sample QC

ACCURATELY QC HMW DNA

Solutions:

- CHEF Mapper XA Pulsed Field Electrophoresis (Bio-Rad)
- Pippin Pulse Electrophoresis Power Supply (Sage Science)
- FEMTO Pulse automated pulsed-field capillary electrophoresis (AATI / Agilent)





ACCURATE QC LEADS TO ACCURATE MOLARITY

Example: 20 kb size-selected library at 34 ng/uL; true mean size is 35 kb; On-Plate 8 pM.

- Molarity difference when insert size is inaccurately measured

Insert Size (bp)	Complex Conc (ng/µL)	Molarity (pM)	Actual On-Plate pM
20000	2.2	169	4.5
35000	2.2	96.7	8.0
45000	2.2	75.2	10

$$\frac{\text{Insert Concentration (ng/µL)}}{\text{Mean insert size (bp)}} * \frac{\text{1 mol basepairs}}{\text{650 g}} * \frac{\text{1 g}}{10^9 \text{ ng}} * \frac{10^{15} \text{ fmol}}{\text{1 mol base pairs}} = \frac{\text{fmol}}{\text{µL}} = \text{nM}$$

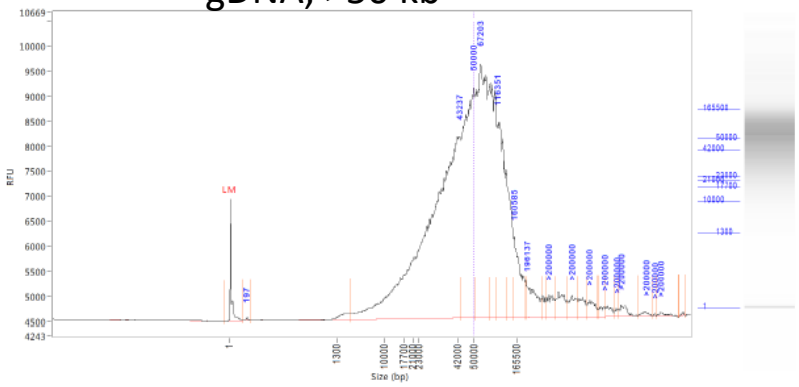
- **Inaccurate size estimations can lead to over or under loading**

To shear or not to shear

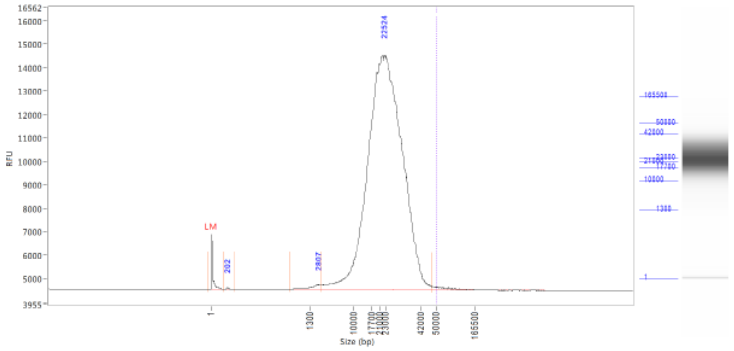
TO SHEAR OR NOT TO SHEAR?

- Two libraries made from same genomic DNA, SMRTbell Express Template Prep

gDNA, >50 kb

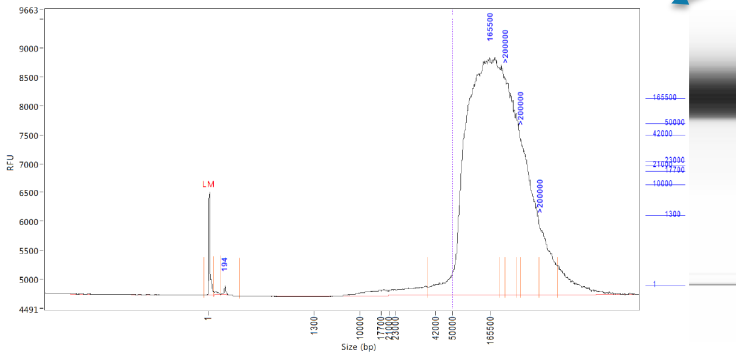


65 kb target size shear (Megarupter)

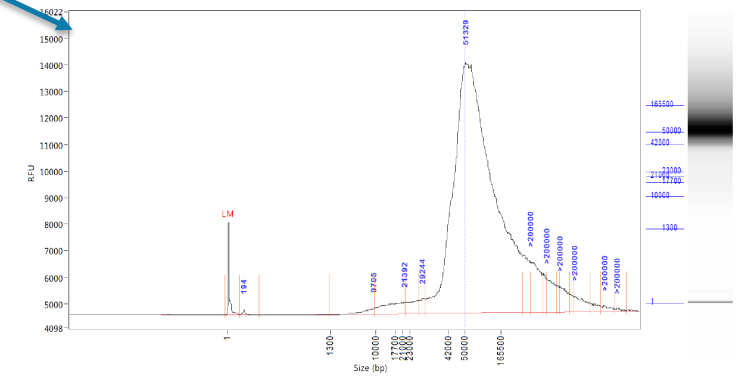


Library

- No shearing
- BP start 40 kb



- Sheared
- BP Size 20 kb



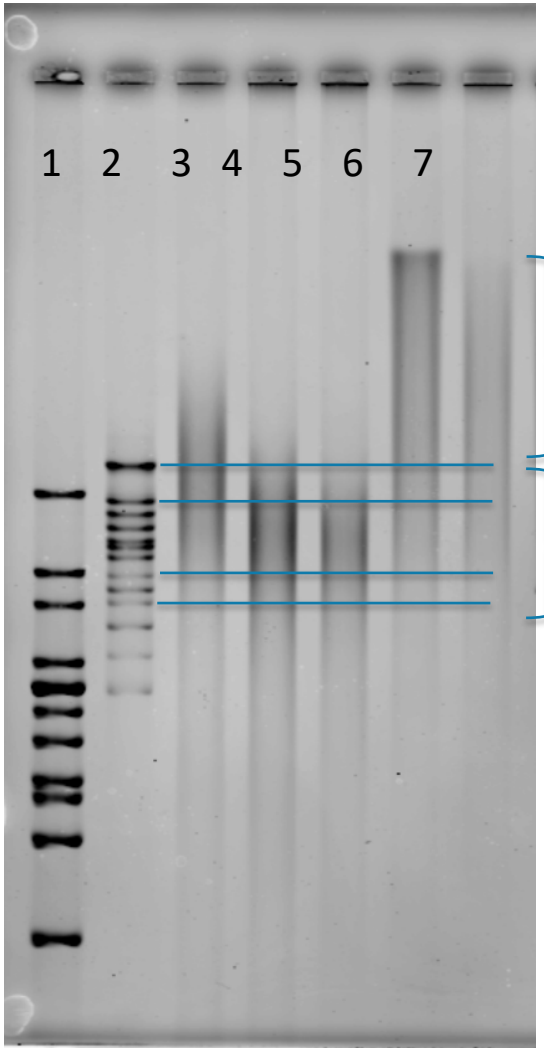
SMRTbell Express Template Prep Kit v1

sample	library		on-plate [pM]	Movie Length (mins)	Total Bases (GB)	Polymerase Reads (bp)	Subread (bp)	Productivity		
	size [bp]	Bp cut [Kb]						(P0)	(P1)	(P2)
Sheared	50000	20	8	1200	8.48	14976	14439	36.30%	56.20%	7.50%
Not Sheared	100000	40	8	1200	6.36	16340	15633	52.20%	39.20%	8.60%

- Un-sheared library had slightly greater read length (1,194 bp) than the sheared library
- Sheared library loaded better (17% gain) at same calculated on-plate molarity
- Sheared library produced ~2 Gb more of data

TO SHEAR OR NOT TO SHEAR?

- Quality of 5 human gDNA samples on PFGE



- High molecular weight gDNA
- (shearing is required)
- Degraded gDNA
- (shearing is **not** necessary)

1. 1 kb DNA Extension Ladder (Invitrogen; Cat #10511-012)
2. 8-48 kb CHEF DNA Size Standard (Bio-Rad; Cat #170-3707)
3. Sample 1 (no shear for prep)
4. Sample 2 (no shear for prep)
5. Sample 3 (no shear for prep)
6. Sample 4 (need to shear)
7. Sample 5 (need to shear)

- No shearing for samples under ~50 kb
- Shearing for samples >50 kb

TO SHEAR OR NOT TO SHEAR

- Shear samples with average molecular weight **greater than 50 kb** as measured on PFGE or FEMTO Pulse

- Shearing HMW DNA leads to better and more predictable loading

Exceptions:

- When bulk of DNA is between 20 and 50 kb

- Precious DNA or low input amount – cannot afford to lose DNA in the shearing process

PACBIO®

Loading

RECOMMENDATIONS FOR GOOD LOADING AND YIELD

Small, or single-cell projects:

- Purity
  - extra AMPure PB bead cleanups
  - Magbead cleanups
- Accurately measure both size and concentration
- Err on the side of higher loading molarity
  - pre-extension for smaller inserts (<15 kb)
- Use diffusion loading

RECOMMENDATIONS FOR GOOD LOADING AND YIELD

Multi-cell projects

Same recommendations as for small projects plus:

- Large polymerase binding reactions for multiple runs
- Titration and production runs on same SMRT Cell lot
- Same SMRTbell bound complex on titration and production runs
- Target P1 >50%; 20%≤ P0 ≥50%
- Run 6-8 cells at a time (10-hour movies)

AMPure PB cleaned SMRTbell complex is stable for at least 7 days at 4°C.

PITFALLS TO AVOID:

- Measuring large-insert libraries on the wrong technology
- Skipping size-selection for large-insert libraries (>15kb)
  - e.g. SMRTbell Express Template Prep Protocol.
- Micro-adjustments to on-plate concentration with different binding reactions
  - e.g. bind, run one cell, adjust, new binding reaction, run one cell …
- Long times in between runs without new titration
- Running across multiple SMRT cell lots without titrating

If ever in doubt, contact your local FAS, or PacBio Technical Support.

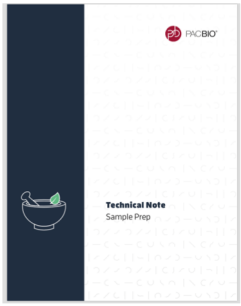
New Customer Support Resources

GENOME BUILDER WEBSITE AND TECHNICAL NOTES

New Genome builder https://www.pacb.com/genome-project-builder/

3 new Technical Notes on preparing for WGS:

- Samples
- DNA
- Libraries



Sample Best Practices

- Avoid tissue preservatives such as ethanol
- Fresh tissue/cells or flash frozen in liquid Nitrogen and stored at -80°C

Extraction Best Practices

- Wide-bore tips
- Minimize high heating steps (>65°C)
- Use gentle techniques such as inversion
- AMPure PB bead cleanups

Library Best Practices

- Blue Pippin size selection

LOW YIELD TROUBLESHOOTING GUIDE HAS 4 STEPS

1. Evaluation of the Internal Control to address concerns with the instrument and the consumables

2. Evaluation of the SMRT Link Sample Setup or Binding Calculator to investigate generic sample issues

3. Evaluation of the SMRT cell metrics for optimal loading

4. Application-specific troubleshooting: heavy-duty molecular biology
   - RNA for Iso-Seq Analysis
   - Target capture or PCR for targeted sequencing
   - DNA extraction for whole-genome sequencing

SUMMARY

- Sample preparation is #1 key to success
- Accurately QC HMW DNA
- Use the Internal Control
- Shear samples with average size >50 kb
- Perform titration and production runs on same SMRT Cell lot
- Perform large binding reactions for 6-8 cells runs

If in doubt, contact Technical Support.

support@pacb.com
1-877-920-PACB

QUESTIONS?

PACBIO®

What Happened to my Sequencing Run?

North America User Group Meeting Workshop

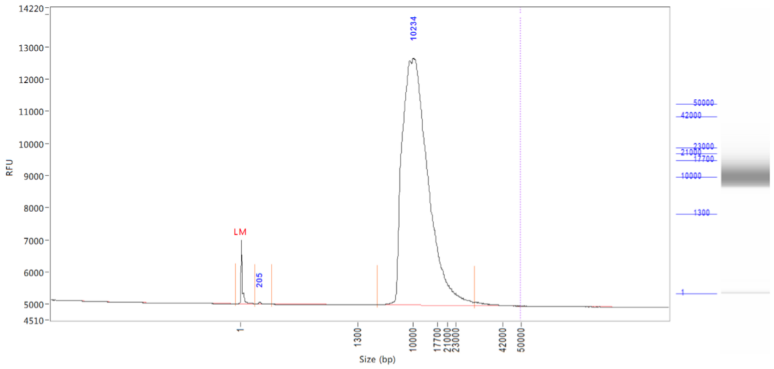
September 19th & 20th 2018

EXAMPLES TO BE COVERED

- **What does Pac Bio Tech Support and FAS look at when troubleshooting?**
  - Control reads- yield and read length
  - Yield
  - Sample Read length

- **Common troubleshooting issues:**
  - Contamination
  - Underloading
  - N2 issues
  - Express kit issues

CASE 1: 10 HOUR MOVIES, LOW YIELD



Sample 1 Femto pulse of final library

Fresh annealing/binding reaction run on Sequel #1

Well	Name	pM	Movie Time (min)	Total Bases (GB)	Read Length				Productivity			Control				Local Base Rate	Template	
					Polymerase		Longest Subread		P0	P1	P2	Total Reads	Poly RL Mean	Concordance			Adapter Dimer	Short Insert
					Mean	N50	Mean	N50						Mean	Mode			
C04	Sample 1	8	600	1.60	3445	7250	2787	5250	20.80%	45.80%	33.40%	2839	6008	0.85	0.85	1.72	0.00	0.01
D04	Sample 2	8	600	1.44	3206	7250	2646	5250	21.40%	44.40%	34.20%	3681	6163	0.85	0.85	1.59	0.00	0.01
E04	Sample 1	6	600	1.63	3899	8250	3075	5750	27.10%	41.40%	31.50%	4891	6825	0.85	0.85	1.65	0.00	0.01
F04	Sample 2	6	600	1.46	3381	7750	2769	5750	24.20%	42.70%	33.10%	3111	6493	0.85	0.85	1.65	0.00	0.01

Same annealing reaction, different binding run on Sequel #2

Well	Name	pM	Movie Time (min)	Total Bases (GB)	Read Length				Productivity			Control				Local Base Rate	Template	
					Polymerase		Longest Subread		P0	P1	P2	Total Reads	Poly RL Mean	Concordance			Adapter Dimer	Short Insert
					Mean	N50	Mean	N50						Mean	Mode			
C04	Sample 1	2	600	4.80	19294	43750	6490	9250	67.00%	26.40%	6.60%	20167	42906	0.85	0.85	1.82	0.00	0.01
D04	Sample 2	2	600	5.96	17890	41750	6373	9250	55.60%	34.70%	9.70%	20959	38697	0.85	0.85	1.76	0.00	0.01
E04	Sample 1	4	600	4.97	12044	36750	4823	8250	41.90%	41.50%	16.60%	9961	27613	0.85	0.85	1.64	0.00	0.01
F04	Sample 2	4	600	6.46	13976	36250	5588	8750	40.00%	40.00%	13.20%	14138	32995	0.85	0.85	1.64	0.00	0.01

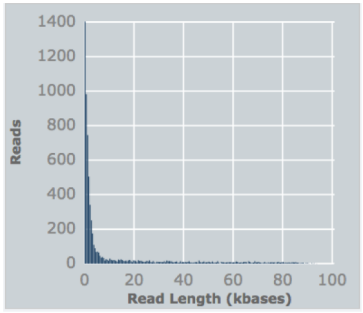
CASE 2: EXPRESS PROTOCOL- ONLY THE CONTROL SEQUENCED

A01: Genomic DNA was size-selected prior to library prep, but not after (8 pM)
B01: No size-selection pre or post-library prep. (8 pM)

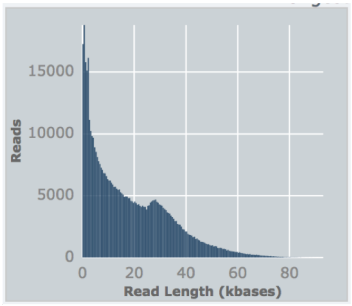
Movie Length (mins)	Total Bases (GB)	Polymerase Reads		Longest Subread		Control Reads			Template		Productivity		
		Length	Quality	Length	Quality	#	Length	Quality	Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)
600	0.08	11494	0.80	1525	0.00	19759	32042	0.86	0.00	0.00	984537 (97%)	26423 (3%)	8275 (1%)
600	0.05	8791	0.80	1576	0.00	17227	30348	0.86	0.00	0.00	983115 (96%)	22510 (2%)	13610 (1%)

Same library (A01) shown above was put through Blue Pippin size-selection (12 pM)

Movie Length (mins)	Total Bases (GB)	Polymerase Reads		Longest Subread		Control Reads			Template		Productivity		
		Length	Quality	Length	Quality	#	Length	Quality	Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)
600	9.96	19444	0.80	18087	0.00	18929	32476	0.85	0.00	0.01	432782 (42%)	531304 (52%)	55149 (5%)



A01 library not size-selected



A01 library size-selected

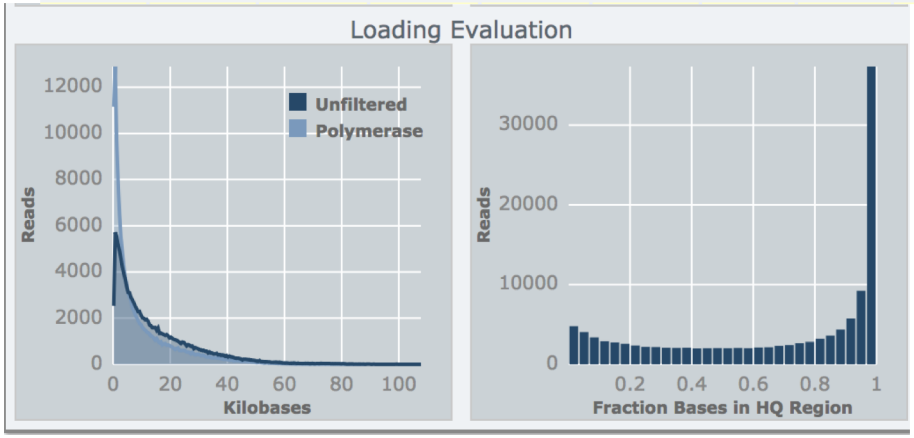
CASE 3: 10 HOUR MOVIES, SAME SAMPLE DIFFERENT LOADING CONCENTRATIONS

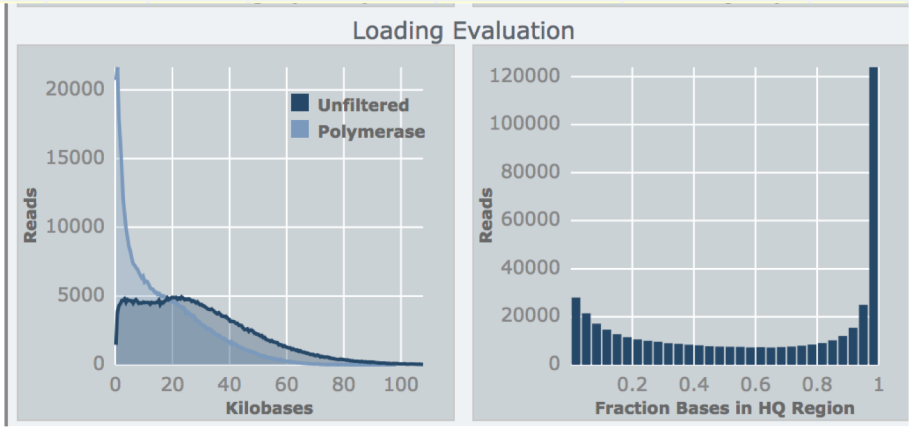
Library & Run Details

- 40kb size-selected Express library
- Same sample/binding reaction loaded at 2pM and 8pM
- No pre-extension
- Sequencing kit v2.1, primer v4

Movie Length (mins)	Total Bases (GB)	Polymerase Reads		Longest Subread		Control Reads			Template		Productivity			Local Base Rate
		Length	Quality	Length	Quality	#	Length	Quality	Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)	
600	1.32	9624	0.80	8862	0.00	20894	26088	0.86	0.00	0.00	829101 (81%)	158369 (16%)	31765 (3%)	1.90
600	7.64	15984	0.80	15179	0.00	15510	28582	0.86	0.00	0.01	447458 (44%)	493241 (48%)	78536 (8%)	2.07

Movie Length (mins)	Total Bases (GB)	Polymerase Reads		Longest Subread		Control Reads			Template		Productivity			Local Base Rate
		Length	Quality	Length	Quality	#	Length	Quality	Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)	
600	8.72	17829	0.80	16935	0.00	11746	29277	0.86	0.00	0.01	432466 (42%)	500952 (49%)	85817 (8%)	2.11



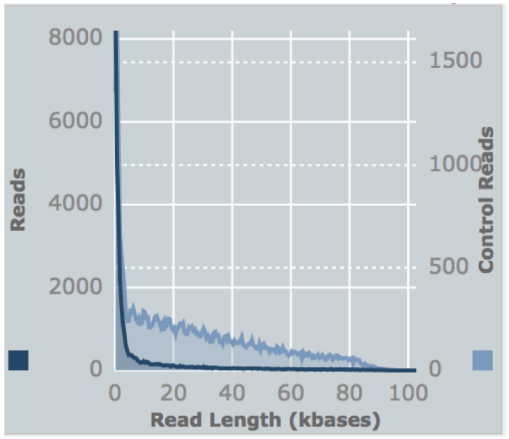


CASE 4: SHORT POLYMERASE RL, 10 HOUR MOVIE, VERY LOW YIELD

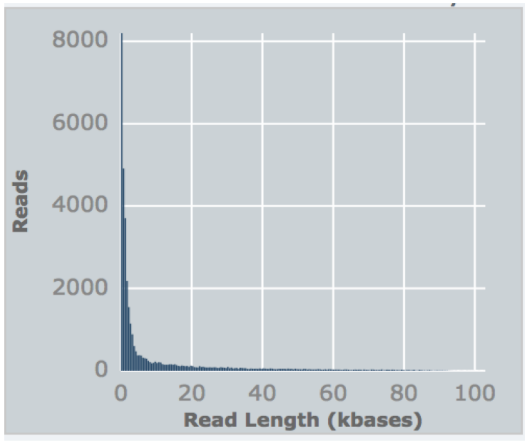
Movie Length (mins)	Total Bases (GB)	Polymerase Reads		Longest Subread		Control Reads			Template		Productivity			Local Base Rate
		Length	Quality	Length	Quality	#	Length	Quality	Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)	
600	0.34	9752	0.80	1568	0.00	29549	26574	0.85	0.00	0.00	792713 (78%)	64857 (6%)	161665 (16%)	1.79
600	0.30	9317	0.80	1570	0.00	21859	30147	0.85	0.00	0.00	666143 (65%)	54103 (5%)	298983 (29%)	1.80
600	0.35	10957	0.80	1668	0.00	9144	27014	0.84	0.00	0.00	651031 (64%)	41001 (4%)	327199 (32%)	1.86

"V phenotype": P0,P2>P1

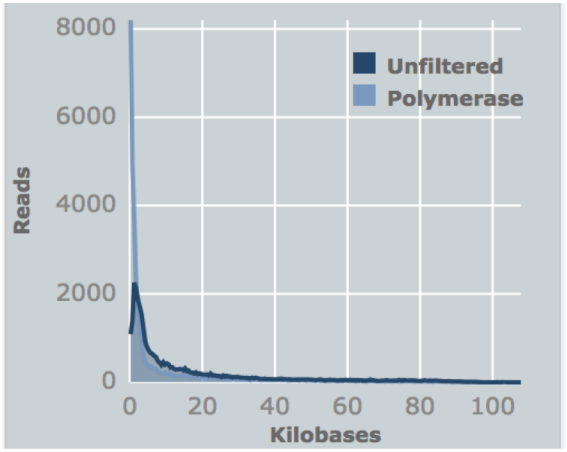
Control v Sample



Polymerase Read Lengths



Loading Evaluation

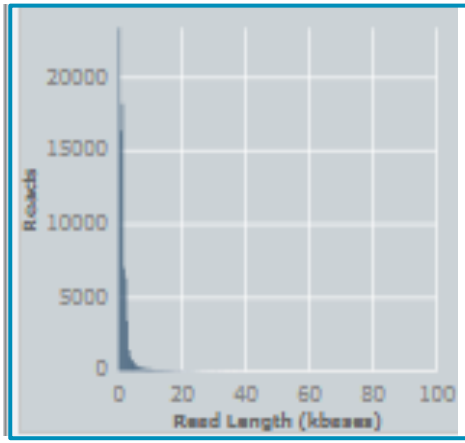


CASE 5: SHORT POLYMERASE READ LENGTH (10 HOUR MOVIE), VERY LOW YIELD FOR GEL-PURIFIED SAMPLE

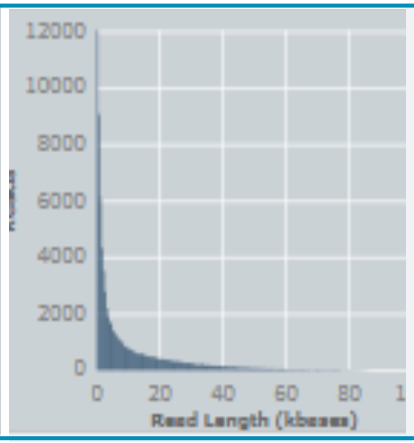
Movie Time (min)	Total Bases (GB)	Read Length AVG		Productivity			Local Base Rate	Template	
		Polymer-ase	Longest Subread	P0	P1	P2		Adapter Dimer	Short Insert
600	0.9	10817	1795	727180 (70%)	83531 (8%)	226089 (22%)	1.56	1.59	0.57

"V phenotype": P0,P2>P1

Subread Read Lengths

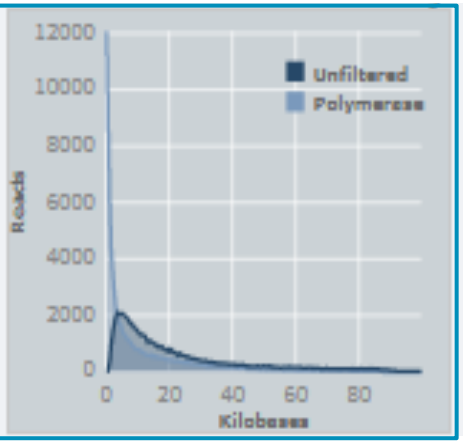


Polymerase Read Lengths



Loading Evaluation

Filtered vs unfiltered bases



CASE 6: LOW YIELD, NORMAL RL, NORMAL CONTROL

Sample Results

Show: ◉ Productivity ○ Loading

SMRT Cell	Well	Movie Context	Started @	Status	Movie Length (mins)	Total Bases (GB)
1	A01	m54198_171219_231231	12/19/17 23:12	Transferred	600	0.71

Polymerase Reads		Longest Subread		Control Reads			Productivity			Local Base Rate	SNR			
Length	Quality	Length	Quality	#	Length	Quality	Empty (P0)	Productive (P1)	Other (P2)		A	C	G	T
18887	0.80	16162	0.00	22078	24310	0.86	182916 (18%)	37792 (4%)	798527 (78%)	1.85	6.6	12.6	5.6	9.1

CASE 6: AFTER SIZE SELECTION

Sample Results

Show: (•) Productivity ( ) Loading

SMRT Cell	Well	Movie Context	Started @	Status	Movie Length (mins)	Total Bases (GB)
1	A01	m54198_180330_210858	03/30/18 21:08	Transferred	1200	8.96

Polymerase Reads		Longest Subread		Control Reads		
Length	Quality	Length	Quality	#	Length	Quality
16547	0.80	7197	0.00	6901	47500	0.85

Template		Productivity			Local Base Rate	SNR			
Adapter Dimer	Short Insert	Empty (P0)	Productive (P1)	Other (P2)		A	C	G	T
0.03	0.42	246280 (24%)	548480 (54%)	224474 (22%)	1.82	6.0	11.6	4.9	8.2

For Research Use Only. Not for use in diagnostics procedures. © Copyright 2015 by Pacific Biosciences of California, Inc. All rights reserved. Pacific Biosciences, the Pacific Biosciences logo, PacBio, SMRT, SMRTbell, Iso-Seq, and Sequel are trademarks of Pacific Biosciences. BluePippin and SageELF are trademarks of Sage Science. NGS-go and NGSengine are trademarks of GenDx.

All other trademarks are the sole property of their respective owners.