Sample QC Workshop – Outline

- **2:30-2:45p – Tootie Tatum**
  - Project Management Office (PMO) & User Project Data Submission
- **2:45-3:00p – Mansi Chovatia**
  - Sample Management & QC Overview
- **3:00-3:05p – Kathleen Lail**
  - New Feature Support – DNAstable
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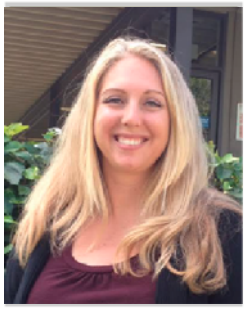
JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

U.S. DEPARTMENT OF ENERGY
Office of Science

Project Initiation at the JGI

Project Management Office

Project Management Office (PMO) Team



Danielle Graham
Workflow Management

Kerrie Barry, Plant and Fungal Genomics Programs

Natasha Brown, Supply Chain Manager

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Miranda Harmon-Smith, DNA Synthesis and Single-Cell Genomics

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Christa Pennacchio, Special Projects

Nicole Shapiro, Microbial Genomics Program

Randy Girsang
Workflow Management

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UNITED STATES DEPARTMENT OF ENERGY

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Our Science Our Projects Data & Tools User Program Info News & Publications

A DOE OFFICE OF SCIENCE NATIONAL USER FACILITY

APPLY NOW: Letters of Intent Due April 7 for Community Science Program

News

Email SUBSCRIBE JGI NEWS

MARCH 10, 2016

JGI Director to Step Down to Assume Scientific Helm of Startup

FEBRUARY 18, 2016

Biofuel Tech Straight from the Farm

LATEST JGI NEWS RELEASES

User Meeting is March 21-24

Apply Now: sequencing and proteomics in one

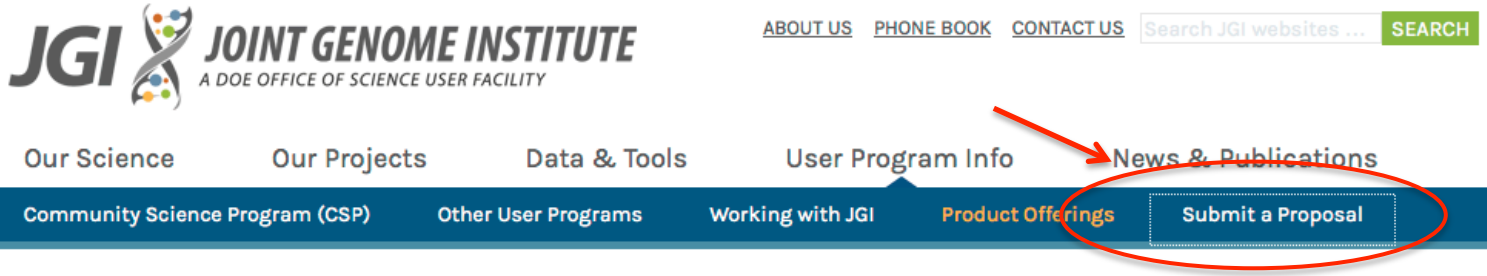
Apply Now: large-scale CSP proposals

Apply Now: Microbial and Plant Systems Modulated by Secondary Metabolites Meeting

User Program Info

Product Offerings

Scientific Program	Product	Brief Description	Deliverables	FY15 target cycle time (median), days	FY15 target cycle time (75th %), days
Fungal	Minimal Draft	Low coverage whole genome shotgun sequencing for evaluation. May turn into a standard draft or improved standard draft.	Assembly. Annotation optional (JGI portal); raw data submitted to SRA	250	400
Fungal	Resequencing	SNP and short indel calls, rearrangement detection, population analysis.	Text file of SNPs (incl location in genome, coding/vs non, syn vs non-syn aa change etc) and structural rearrangements, alignment files, tracks for upload to genome browser and fastq files; raw data submitted to SRA	140	200
Fungal	Standard Draft	Whole genome shotgun sequencing. Exact scope items and quality of finished product depend on genome. Selected genomes will be improved based on feasibility and scientific merit.	Assembly, annotation (JGI Portal + Genbank); raw data submitted to SRA	250	400



User Program Info

Log in

Submit a sequencing or synthesis proposal to the JGI:

The **Work Initiation Process (WIP)** application provides a web-based interface that can be used to request sequencing or synthesis from the JGI (view current product list). The current release supports submissions of all proposal types below. Please login to WIP to view specific instructions and templates for proposal submission.

JGI Director's Science R&D Long Read Proposal Call:

This call is open to everyone. About R&D Long Read Call

DOE JGI Emerging Technologies Opportunity Program (ETOP):

This user program is open to everyone during the annual call. The ETOP 2015 Call for Letters of Intent is now closed for new Letters of Intent. About ETOP 2015

JGI-EMSL Collaborative Science Initiative:

This user program is open to everyone during the annual call. The JGI-EMSL 2017 call is now open for new Letters of Intent. LOIs should be submitted via the EMSL portal.

About JGI-EMSL joint call.

Community Science Program (CSP) annual call:

This user program is open to everyone during the annual call. The CSP 2017 call for Letters of Intent is now open until April 7, 2016 23:59 PDT. About CSP

Community Science Program (CSP) small-scale microbial and metagenome:

This program is open to anyone. Small-scale proposals for microbial and metagenome projects may be submitted at any time as brief white papers and will be reviewed every six months. Proposals may include microbial isolates, RNA, resequencing and epigenomics projects, metagenome and metatranscriptome projects, and single cells. Deadline for submission is 60 days prior to the review. See proposal schedule for specific dates. About CSP

Community Science Program (CSP) synthetic biology:

This program is open to anyone. Proposals for synthesis projects may be submitted at any time as white papers and will be reviewed every six months. Deadline for submission is 60 days prior to the review. See proposal schedule for specific dates. About CSP

Bioenergy Research Centers (BRCs):

This program is open to PIs associated with DOE Bioenergy Centers only. Proposals may be submitted at any time. About BRCs

→ All users must create a "JGI Single Sign-On Account"

JGI **Proposal Submission**

JGI HOME CONTACT US

PROPOSALS | FD PROJECTS | SEQUENCING PROJECTS | ANALYSIS PROJECTS | METADATA | CONTACTS | SCIENTIFIC REVIEWS

MY PROPOSALS | ALL PROPOSALS

Start a new proposal...

Director's Science R&D Long Read Call for Proposals

Begin

CSP 2017 Annual Letter of Intent

Begin

CSP small-scale microbial and metagenome

You can see an example of such proposal here.

Begin

CSP synthetic biology

Begin

Bioenergy Research Center

New proposal: select a focus area | Begin

All other proposal types (Director's Science/R&D/WFO)

New proposal: select a focus area | Begin

JGI Proposal Submission

JGI HOME CONTACT US

PROPOSALS FD PROJECTS SEQUENCING PROJECTS ANALYSIS PROJECTS METADATA CONTACTS SCIENTIFIC REVIEWS

MY PROPOSALS ALL PROPOSALS

CSP2017 call

New CSP 2017 Letter of Intent

Required fields are marked with an *.

Title * 255 characters

Please select the area(s) of DOE mission relevance appropriate to your proposal

- [ ] bioenergy
- [ ] biogeochemistry
- [ ] carbon cycling
- [ ] phylogenetic diversity

Relevant categories

Please select one or more categories that are relevant to your proposal.

- [ ] Extreme Environments
- [ ] Plant flagship genomics
- [ ] Plant microbiomes
- [ ] Algal genomics
- [ ] Fungal genomics
- [ ] Microbial function
- [ ] Metagenomics
- [ ] Chromatin analysis
- [ ] FACS
- [ ] Synthesis
- [ ] Metabolomics
- [ ] Raman-based cell sorting
- [ ] Other

Proposal summary

Description * 4000 characters

Please briefly describe sequencing or synthesis needs for your proposal. Include estimated genome size for each whole genome shotgun organism, or estimated total resource needs for other types of projects. Please refer to sequencing and synthesis limits at http://jgi.doe.gov/collaborate-with-jgi/community-science-program/how-to-propose-a-csp-project/csp-annual-call/.

Justification * 4000 characters

Please briefly describe the scientific rationale for performing this work.

Utilization * 4000 characters

Please briefly describe how the resource produced will be used.

User Program Info

Institutional User Agreements
Project Materials Submission Overview
Approval To Ship Samples
Project Management FAQ
Policies
Protocols

Working with JGI

The DOE JGI Project Management Office (PMO) carries out a number of activities to facilitate world-class science. DOE JGI Project Managers collaborate with users to develop project requirements, define the scope of genomic work and outline roles and responsibilities resulting in a formal Statement of Work (SOW).

All work performed at the DOE JGI is initiated via the Work Initiation Process (WIP) interface. To submit a proposal, users must first log in (Note: new users of the DOE JGI will need to register and create a login at https://signon.jgi.doe.gov).

Once users are notified that their proposal has been accepted, the PMO will assist with initiation of work through:

- Coordinating execution of Institutional User Agreements
- Coordinating with DOE JGI technical experts
- Project design with Principal Investigator
- Providing a General Orientation to DOE JGI systems to Investigators
- Preparation of formal Statement of Work (project roadmap)

DOE JOINT GENOME INSTITUTE

Statement of Work for JGI DNA Sequencing Projects

1. General Information

Title:
Functional genomics of moss-cyanobacteria interactions in boreal forest ecosystems

Abstract:
We propose coupled genomics, transcriptomics, and proteomics to characterize the feather moss-cyanobacteria association that covers the understory in boreal forests worldwide and serves as the main form of biological N input to the mature boreal forests. The two predominant and widespread Pleurocarpous feather mosses, i.e., Pleurozium schreberi and Hylocomium splendens, support a wide diversity of filamentous cyanobacteria dominated by the genera Nostoc and Stigonema. Single gene based surveys suggest host specificity and high genetic diversity in this community of filamentous cyanobacteria. Genome sequences of the associated cyanobacteria and the moss host are not available, although genome sequences exist for the distantly-related association-competent cyanobacterium Nostoc punctiforme ATCC 29133/PCC 73102, originally isolated from the cycad Macrozamia.

Our approach targets all levels of the central dogma, as well as organismal and ecosystem scales. Genome sequencing of cyanobacterial isolates collected in natural boreal ecosystems will provide an understanding of the functional potential and diversity of this crucial partner of the associations and provide fundamental knowledge on how the two partners communicate prior to and during colonization. Our first set of genome sequencing samples will consist of ten cyanobacteria associated with feather mosses that have been isolated in pure culture by Co-PI Rasmussen. Our second set of genome sequencing samples will be from cyanobacterial filaments unable to be cultivated in isolation (i.e. Stigonema). The filaments will be removed from the moss by micro-manipulation and genomic DNA will be amplified. For both sets of cyanobacterial genome samples, we assume a genome size of approximately 8-10 Mb for each isolate or environmental colony, based on the genome size of the related N. punctiforme. This estimate includes plasmids that may be important repositories of genetic diversity for these species.

Signaling pathways involved in forming successful associations will be studied using transcriptomics and proteomics of both partners. We will use an experimental setup developed by Co-PI Rasmussen that allows for communication between the partners without colonization. This novel setup allows the initial signaling phase to be differentiated from the subsequent establishment phase of the interaction. P. schreberi will be used as the moss partner and three different cyanobacteria strains will be used: 1) a Nostoc sp. isolate from the moss P. schreberi as a successful interaction, 2) a free-living Nostoc sp. that does not form associations, and 3) the model cyanobacterium N. punctiforme ATCC 29133 because of its well-established genetic system. Samples will be collected for each cyanobacteria and the P. schreberi moss partner under three conditions: 1) separated (in culture/growing separately), 2) with chemical but not physical contact, and 3) during/after colonization. The use of these strains will enable differential transcriptional patterns to be deduced and will identify candidate cyanobacterial genes to test by genetic manipulation. If time allows, altered physical conditions, such as high CO2 and high temperature, will be tested to understand how changing climate may affect these critical ecosystem interactions. The large transcriptomic data set will facilitate global and targeted proteomic analysis at various states of the interaction.

Finally, the metatranscriptome and metaproteome of several distinct natural moss-cyanobacterial communities will be examined. These will be the same communities from which samples used in this proposal have been collected (see Sample Preparation) and will allow for comparisons of gene expression patterns with the laboratory-based association studies. It will also permit correlation of transcriptional profiles from other organisms in the community leading to the formation of successful associations.

Scope of Work:
ISOALTES
TOTAL: 6 organisms
Improved drafts, PacBio – 10kb library.

SAGs
TOTAL: 8
Send JGI the MDA product
Will be a mixed culture (pools of cells) 10-20 cells per MDA reaction

TRANSCRIPTOME
TOTAL: 30
3 cyanobacteria strains, each with 3 conditions, and 3 replicates (=27 samples), plus 3 moss only samples. For the cyanobacterial only samples (18), rRNA depletion will be used. For the samples that have both cyanobacteria and moss together (9), these will get both rRNA and poly-A library prep methods to allow both the cyanobacterial and moss samples to be represented in the transcriptome. For the three moss-only samples (3), these will get only poly-A library prep.

JGI will pilot 2 transcriptomes for feasibility: a moss-only transcriptome and a moss-cyanobacteria transcriptome.

Institutional User Agreements
Sample Material Submission Overview
Sequence Submission Requirements - DNA Synthesis Program
Approval To Ship Samples
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Sample Material Submission Overview

Please follow the steps outlined below to submit samples for sequencing or synthesis to the JGI. If you have any questions, please contact your Project Manager or pmo_jgi_project_management@lists.jgi-psf.org.

DNA Sequence (Synthetic Biology) Submission

DNA sequence files for all constructs should be emailed to Miranda Harmon-Smith, Synthetic Biology Project Manager. Please use the format referenced here.

DNA and RNA Sample Submission

1) Review JGI sample QC requirements and protocols

Obtaining DNA & RNA of suitable quantity and quality has been the rate-limiting step for many projects at the DOE JGI. The quality of the starting material is one of the greatest predictors of a successful sequencing project. It is imperative that users utilize the Qubit system for assessment of DNA or RNA mass prior to shipment to the DOE JGI. These documents (posted with permission) demonstrate the importance of utilizing this platform for nucleic acid quantification in our Illumina and PacBio sequencing workflows and provide guidance as to how to perform sample QC prior to shipment:

- Importance of Sample QC
- Sample Quality and Contamination
- Genomic DNA Sample QC Protocol
- Total RNA Sample QC Protocol
- iTag Sample Amplification QC v1.1 – before submitting samples for iTag sequencing, you must first confirm the quality of material through amplification. We strongly suggest that you use this protocol to confirm the ability of *all* samples to be used for library construction and sequencing of 16s, 18s or ITS.

User Program Info

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2) Review sample preparation guidelines detailing quantity and quality requirements

Please review the appropriate guidelines detailing the quantity & quality requirements for samples submitted to the DOE JGI.

- Plate-based Sample Requirements
- DNA Preparation Requirements
- RNA Preparation Requirements

3) Complete the sample metadata form

Complete metadata must be submitted for all samples being prepared for sequencing at the DOE JGI. Your Project Manager will assist you in preparation of this information. Once complete, your Project Manager will initiate the sample shipping approval process. Please do not ship your samples until you receive shipping approval from the JGI.

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Approval To Ship Samples

Please follow the steps outlined below to submit samples for sequencing at the DOE JGI. If you have any questions, please contact your Project Manager or pmo_jgi_project_management@lists.jgi-psf.org.

Obtain Approval for Shipping

PLEASE NOTE: The JGI will not accept any materials classified above NIH Biosafety Level 2. Before approval to ship is granted, you must have completed submission of all project metadata for all project materials.

Review Shipping Instructions

After receiving approval, please refer to the emailed shipping instructions for shipping your materials to the DOE JGI. The Shipping Checklist below will assist you in verifying that all required information is included. Please note that shipment of materials to the JGI without prior approval or complete documentation may result in destruction of the shipment. Please retain your carrier's tracking information.

- JGI Shipping Checklist
- International Shipments (For shipping packages internationally (including Canada!) please use these instructions.)
- TCSA Form for International Shipments

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Protocols

Protocols provided by JGI and the JGI user community.

JGI sample QC protocols:

- Genomic DNA sample QC protocol
- Total RNA sample QC protocol
- iTag Sample Amplification QC protocol

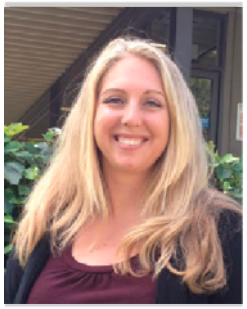
DNA preparation protocols:

- JGI Bacterial DNA isolation CTAB-2012
- Isolation_of_Genomic_DNA_from_Phytophthora
- Isolation_of_Melampsora_Nuclear_DNA
- DNA_Isolation_of_Plant Nuclear_DNA
- Populus nuclear DNA purification with Qiagen
- DNA_extraction_from_Activated_Sludge
- Cloning of High Molecular Weight eDNA (soil)
- RNase_I_DNA_Clean_up_protocol
- eDNA _purification (removal of humics)
- High Molecular Weight DNA Extraction from Soil
- http://1000.fungalgenomes.org/home/protocols/

RNA preparation protocols:

- DNase Treatment of Total RNA

Project Management Office (PMO) Team



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JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Overview of Sample Management

Mansi Chovatia
March 22, 2016

Sample Management Team

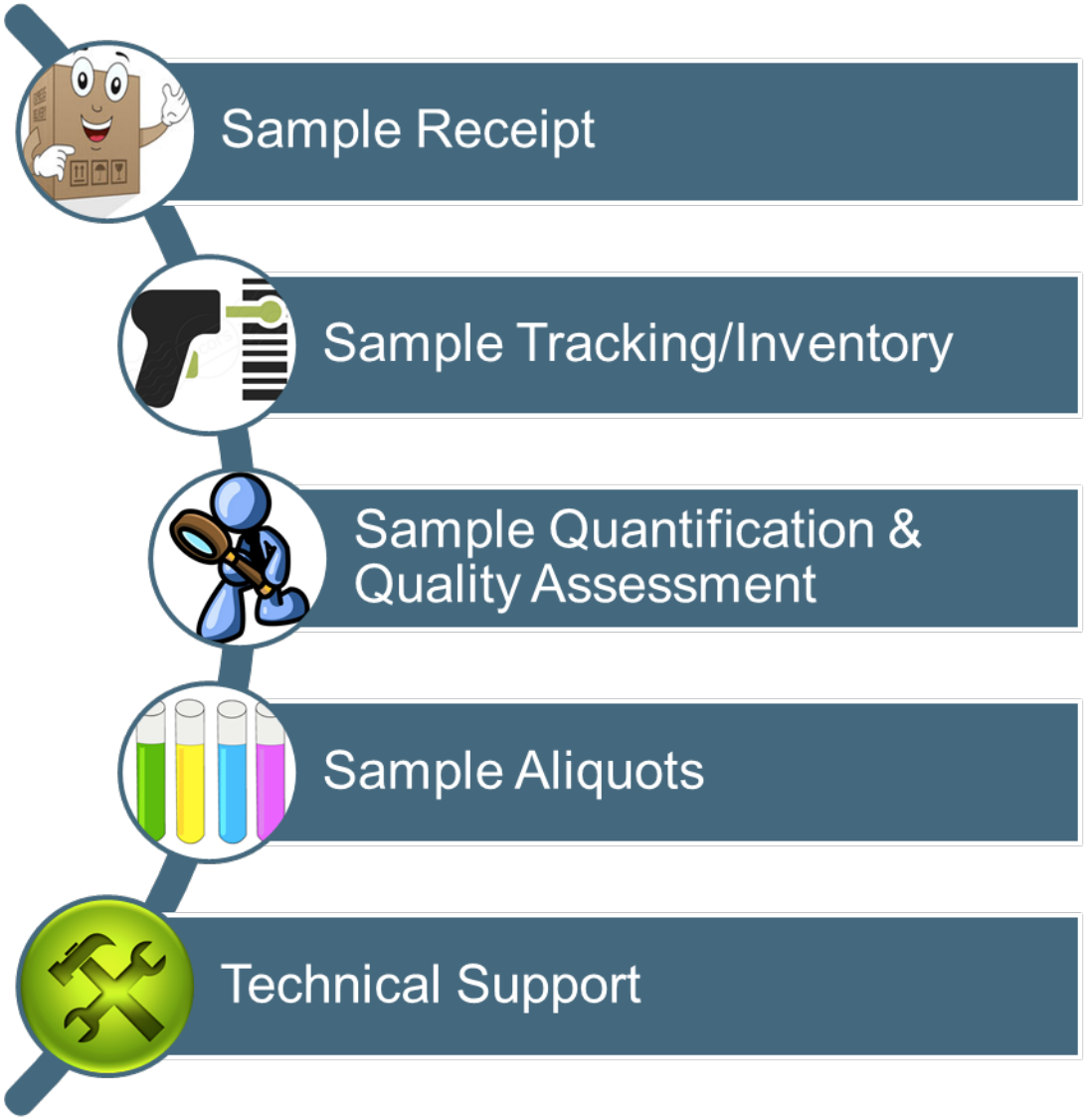

Kathleen Lail


Juying Yan


Yuko Yoshinaga


Aditi Sharma

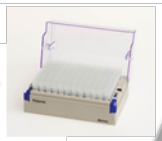

David Dilworth



Sample Storage

-Coordinates shipping schedule
-Ships out labeled containers

Supply Chain Manager



Sample Receipt
-Receive packages
-Barcode association
-Log sample into LIMS

Sample Management

Sample Return & Disposal
-Completed projects returned to users or discarded

JGI Users

Project Manager

-User Agreements
-Project initiation
-Sample metadata
-Project tracking

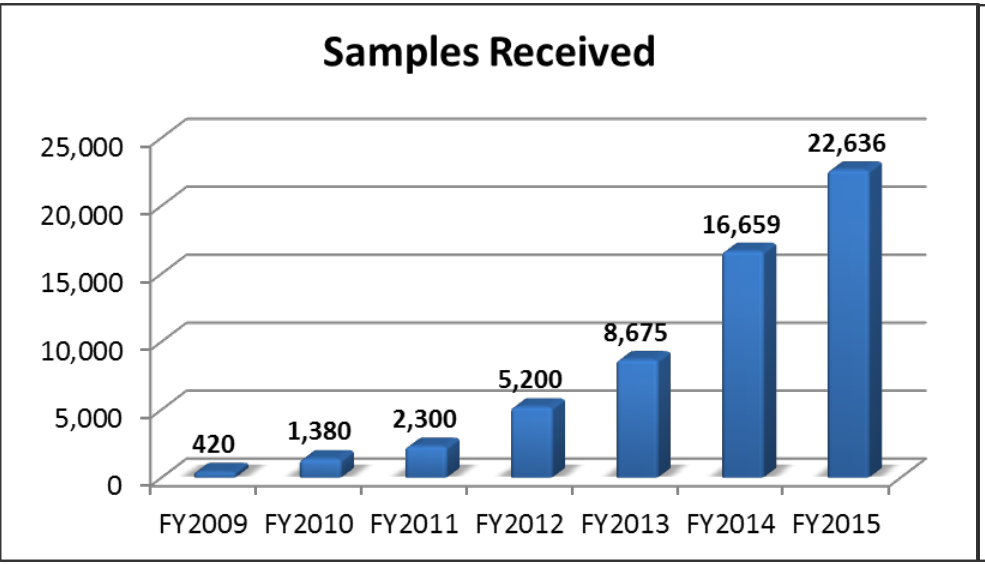
Sample QC

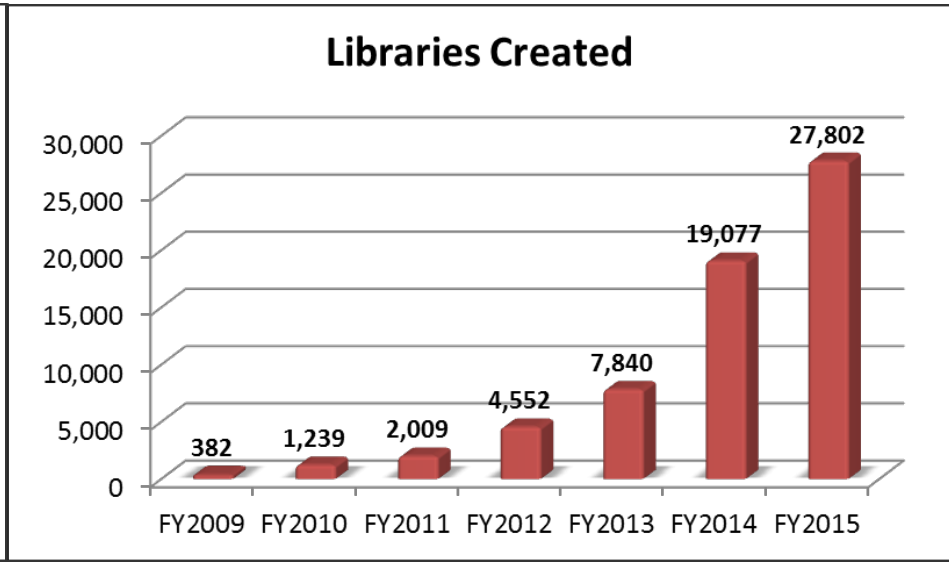
- Measure sample volume
- Top-off low volume samples
- Quantify samples using fluorometric dyes
- Assess sample quality based on scope of work (SOW)
- Record measurements and analysis in LIMS

Sample Aliquot

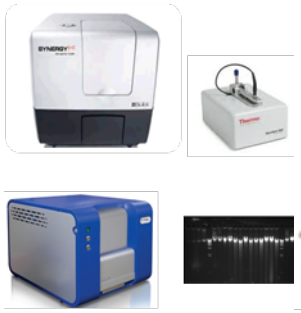
- Transfer required amount for library construction
- Top-off low volumes to required amount
- Create intermediate dilution for highly concentrated samples
- Re-array plates and tubes into a plate for library construction
- Record sample transfer information in LIMS

Throughput


Samples Received
25,000
20,000
15,000
10,000
5,000
0
420
1,380
2,300
5,200
8,675
16,659
22,636
FY2009
FY2010
FY2011
FY2012
FY2013
FY2014
FY2015


Libraries Created
30,000
25,000
20,000
15,000
10,000
5,000
0
382
1,239
2,009
4,552
7,840
19,077
27,802
FY2009
FY2010
FY2011
FY2012
FY2013
FY2014
FY2015

Automation



Storage	Volume Check	QC	Aliquot
• Sample archival • Manage in-progress containers • Houses a total of 532 plates and 11,320 tubes • Barcode association • Retrieves upon command • Improved tracking • Provides security • Eliminates the human error	• Sonic pulses • Non-contact measurement • Compatible with plates and tubes	• Microplate reader for quantification • NanoDrop for the purity • Agarose gels for DNA quality • 2 LabChips for RNA sample quality	• 2 STARlets in production • Main workforce for both sample QC and aliquots • Integrated tube decapper • Re-array capability • High consistency • Reduces ergonomic issues

Sample Receipt

Common concerns:

- Packaging
- Dry ice
- Secondary containers
- Container seals
- Labels/documents

JGI Shipping Checklist

Your shipment to the JGI must include the following items:

- ☐ Hard copy of "Approval to Ship" email from the JGI
- ☐ JGI USDA Import Permit (soil and soil-derived materials only)
- ☐ Completed shipping checklist (this page)

Investigator Name	Date

Enter Total Number of Sample Tubes or Plates in this Shipment:

Total Tubes:
Total Plates:

Fill out below ONLY if shipping at room temperature with RNAstable or DNAstable Plus:

☐ Check this box if you have included RNAstable treated sample/s and list the total amount of tubes.

_____# of tubes of RNAstable treated samples

☐ Check this box if you have included DNAstable Plus treated sample/s and list the total amount of tubes.

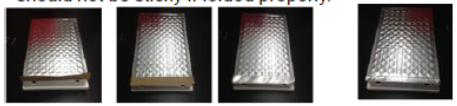
_____# of tubes of DNAstable Plus treated samples

Correct Packaging

✓ Example of tube **WITH** secondary containment (15mL conical tube) Please ship on crushed dry ice.
Multiple sample tubes can be placed in a larger conical tube or secondary containment.

Example of a plate **WITH** secondary containment, and properly labeled and tightly sealed with a **FOIL** seal. Make sure label is on **A12** side. Do not cover the label. (Please ship in cardboard box on crushed dry ice).

Ensure that the tab of the foil seal is NOT folded down on the side of the plate. Fold the tab over itself on top of the plate as shown in the 4 steps below. The tab should not be sticky if folded properly.



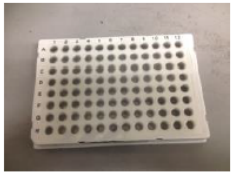
1. **Apply label with tab to A1 side of the plate.**
2. **Fold tab on top of plate surface.**
3. **Remove the paper backing from the tab.**
4. **Fold the tab over itself so that the sticky sides are together and sealed on top of the plate, and not over the side of the plate.**

Incorrect Packaging

✖

Example of tube arriving **WITHOUT** secondary containment (placed directly on dry ice).

Example of a plate arriving with tab sealed down and covering the plate label.

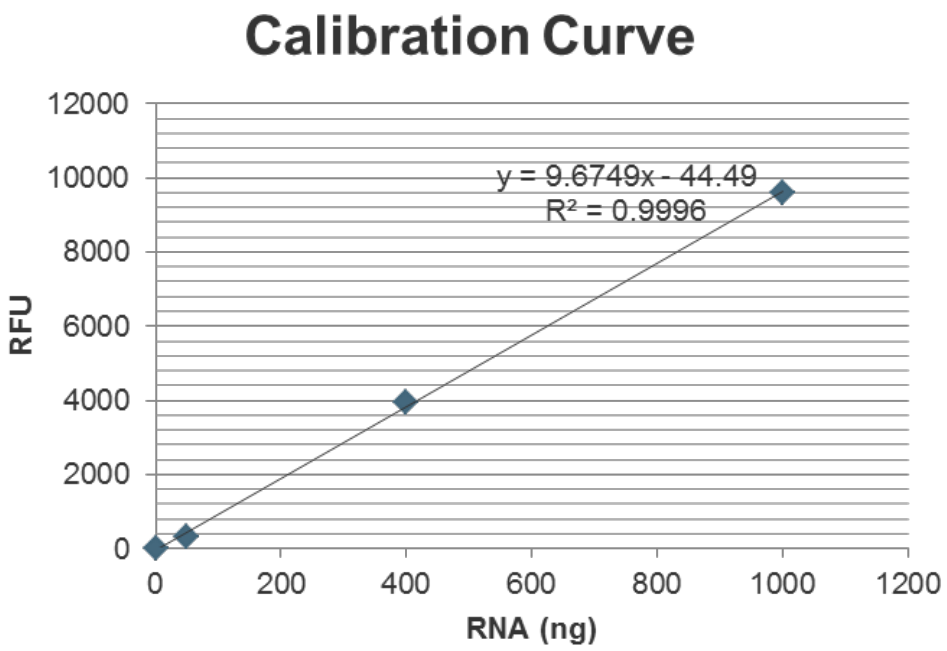


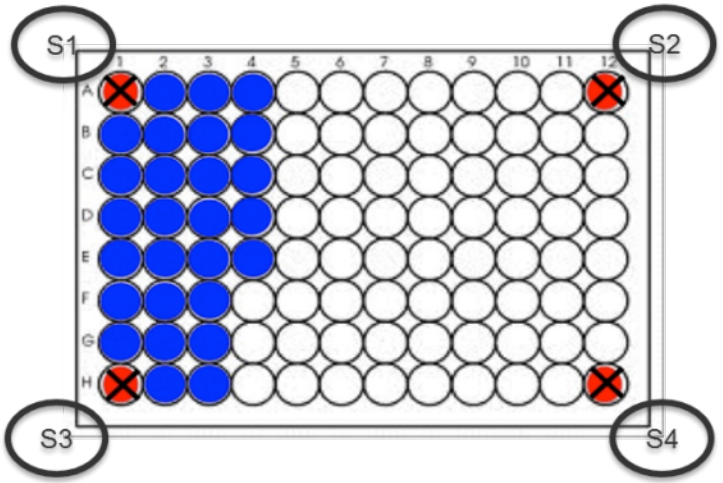
DO NOT use clear plate seals.

Example of a plate arriving **WITHOUT** secondary containment - plate seal has been damaged.

Sample Quantity

DNA assays	RNA assays
Quantifluor dsDNA system (0.4-20ng, 2-200ng)	Quant-iT RNA BR kit (50-1000ng) Quantifluor RNA system (1.5-80ng)

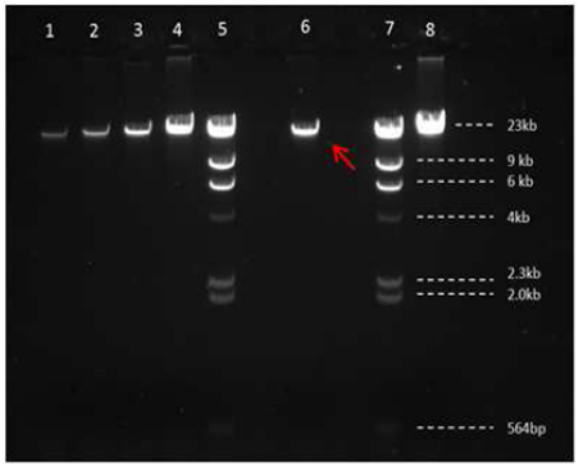




Common cause for discrepancy:

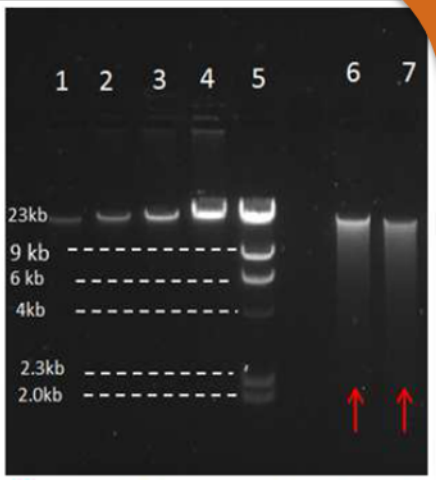
- Pipetting errors
- Calibration curve
- Contaminants
- Assays/kits
- Standards

Sample Quality



A. Lane 6 – High molecular weight DNA at 23kb

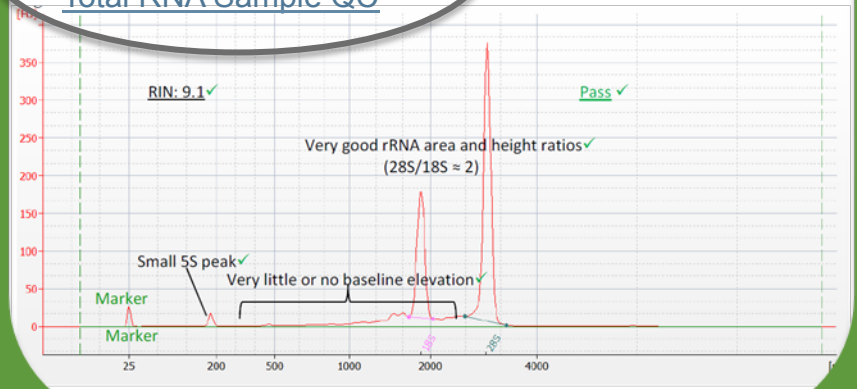
B. Lane 6 & 7 – Partially degraded DNA (smearing visible)

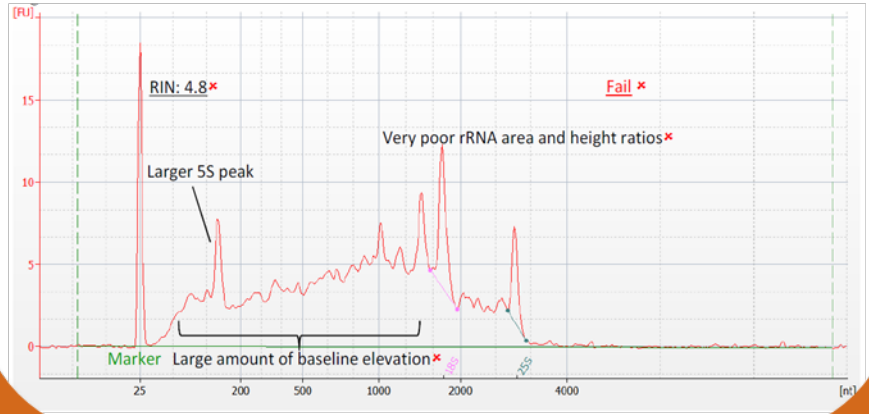


C. Lane 6 & 7 – Completely degraded DNA

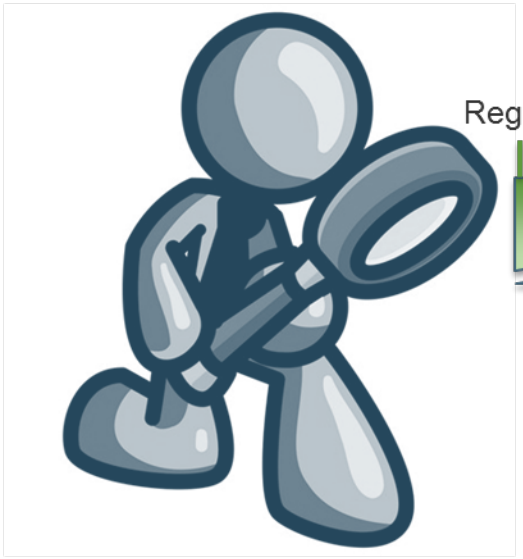
Genomic DNA Sample QC

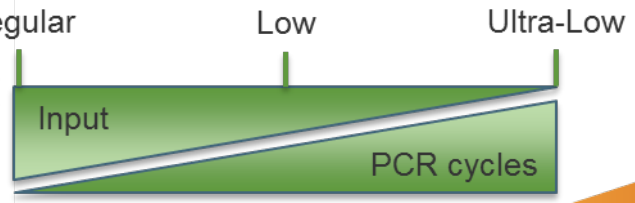
Total RNA Sample QC





Sample QC Requirements per Library





Relaxed

Stringent

Volume

All library types

Quantity

All library types except:

- Ribosomal iTags

Quality

- Nextera LMP
- PacBio Long reads
- Stranded RNA-seq
- smRNA
- Iso-seq

Purity

- PacBio Long reads

New Technology

Instrument & Method Evaluation

- Fragment Analyzer
- Agilent Tape Station
- Biotek Synergy
- STARlet method validation
- SAM validation
- Pippin Pulse

Optimization

- Chlamy and Microbial DNA/RNA sample prep
- Biomatrica RNAstable & DNAstable
- QC for ssDNA/dsDNA ratio in Metagenome samples
- Miniaturization of QC assay

Technical Guidance

- Genomic DNA QC protocol
- Total RNA QC protocol
- Chlamy RNA extraction
- Etc…

Protocols


thank you!

Sample QC Workshop – Outline

- **2:30-2:45p – Tootie Tatum**
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UNITED STATES DEPARTMENT OF ENERGY

DNAstable Plus® Testing on JGI in-house prepped Microbial gDNA

3/22/2016
Sample Management
Kathleen Lail

DNAstable Plus® Testing

JGI has tested an alternative shipping format for DNA:

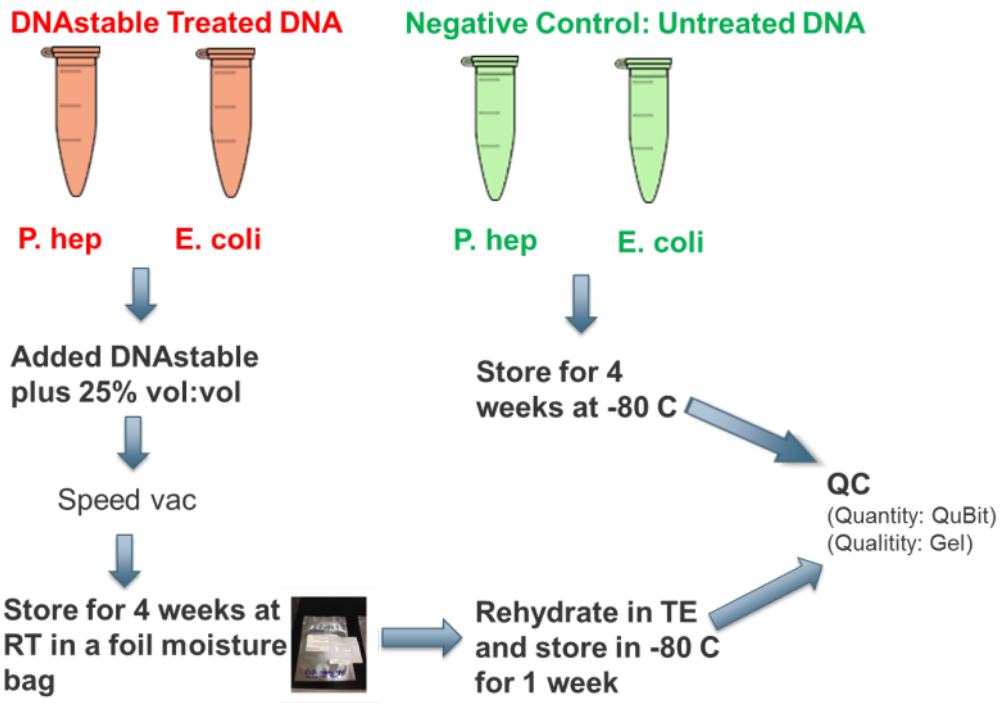
Background

- Tested a commercial product from Biomatrica to enable collaborators to ship DNA as dry samples not requiring the use of dry ice (For use only where there are restrictions on dry ice or shipping liquids.)

DNAstable Plus®

- Synthetic polymer that forms a protective seal around DNA to prevent degradation during storage at room temperature.

Test conditions for DNAstable Plus using microbial gDNA were set-up in duplicate:



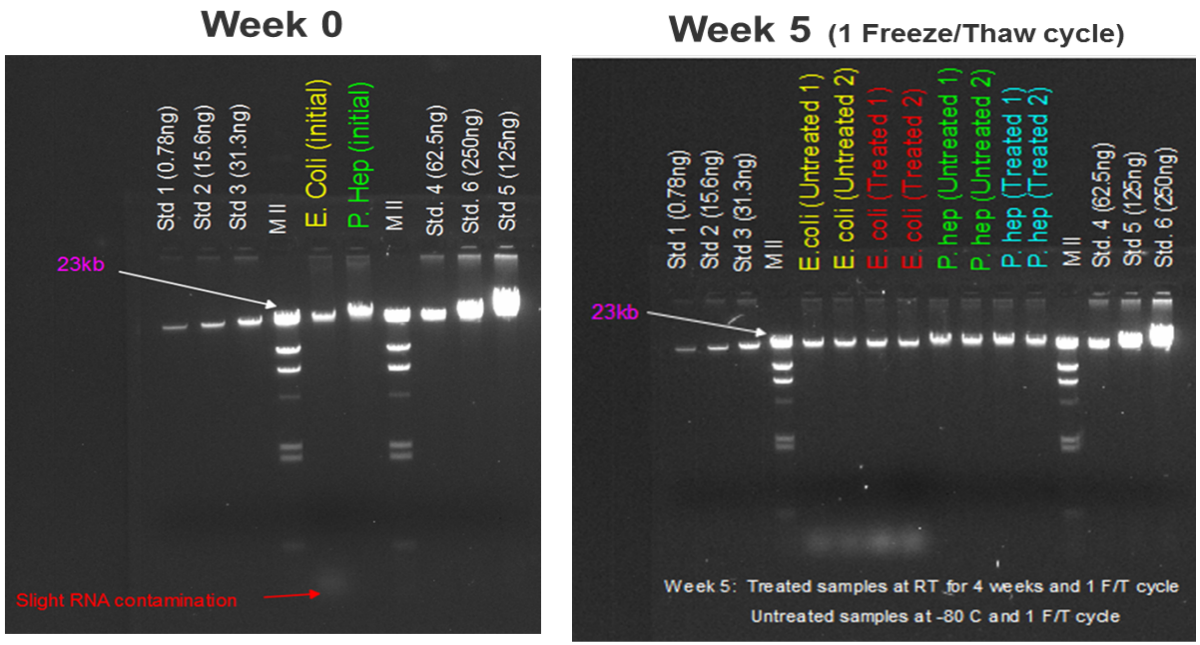
1) Compared DNAstable Plus® treated DNA in **dry** format with the same DNA stored at -80C

2) Checked if DNAstable Plus® affected
- Recovery
- Quantification
- Quality of the DNA

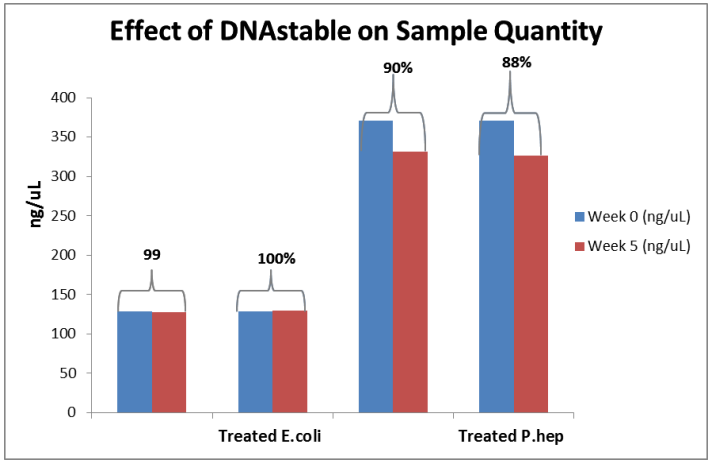
3) Checked if DNAstable Plus® affected
- Library quality
- Library yield
- Sequencing results
- Assembly

*In earlier experiments, compared DNAstable Plus® treated DNA in both **dry** and **liquid** formats, using in-house prepped Chlamy DNA. In addition, tested controls of the same DNA stored at Room Temp untreated. Also, tested blank samples of DNAstable Plus® to check for background.*

Effect of DNAstable Plus® on Quality and Quantity



There was no effect on quantity or real drop in quality from the untreated controls, as seen on the gel.
Even the presence of RNA in the E.coli did not have an effect when comparing the untreated with the treated.

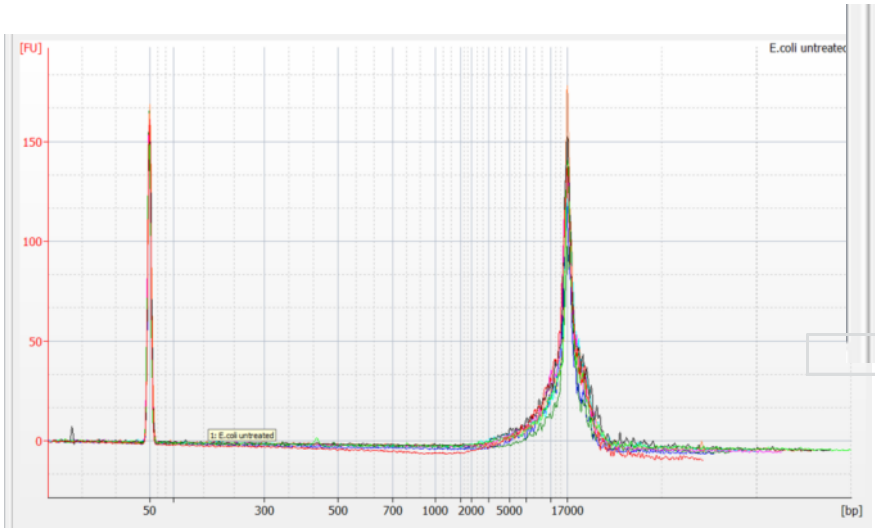


	Week 0 (ng/uL)	Week 5 (ng/uL)
Untreated E. coli	129	128
Treated E. coli	129	130
Untreated P. hep	370.5	332
Treated P. hep	370.5	326

Pedobacter heparinus (P. hep) Samples were viscous; both control and treated showed similar concentrations at week 0 and week 5.

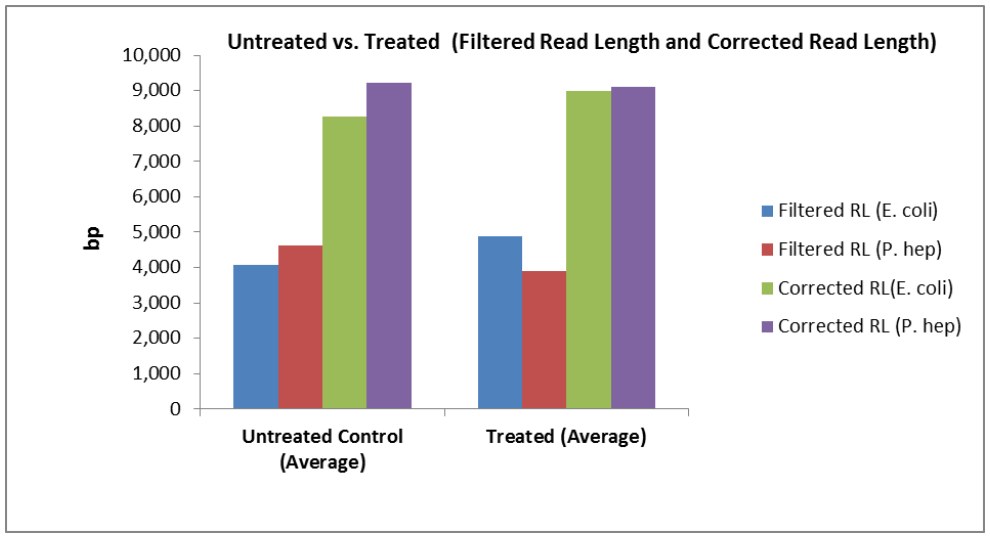
Effect of DNAstable Plus® on PacBio >10kb Library Construction and Sequencing QC

Overlay of all Treated and Untreated >10kb PacBio libraries



Library Construction:
DNAstable compound had no effect on 300bp Illumina fragment or >10kb PacBio libraries made from microbial DNA.

Sequencing:
DNAstable compound had no effect on sequencing QC for both 300bp fragment libraries and >10kb Pacbio Libraries made from microbial DNA.



Pacbio >10kb libraries						
	N SNPs (E. coli)	N SNPs (P. hep)	Filtered RL (E. coli)	Filtered RL (P. hep)	Corrected RL(E. coli)	Corrected RL (P. hep)
Control (Average)	30 ± 4	66 ± 15	4,083 ± 3,019	4,611 ± 3,397	8,255 ± 4,172	9,224 ± 4,928
Treated (Average)	52 ± 30	41 ± 1	4,891 ± 3,488	3910 ± 3,007	8,980± 4,778	9,105 ± 4,563

Rolling QC: When comparing N SNPs/library insert sizes DNAstable Plus did not affect assembly quality, within the precision of quality assessment. Pipeline noise was significantly higher then individual differences.

JGI Shipping Checklist

Shipping Checklist

JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Shipping Checklist

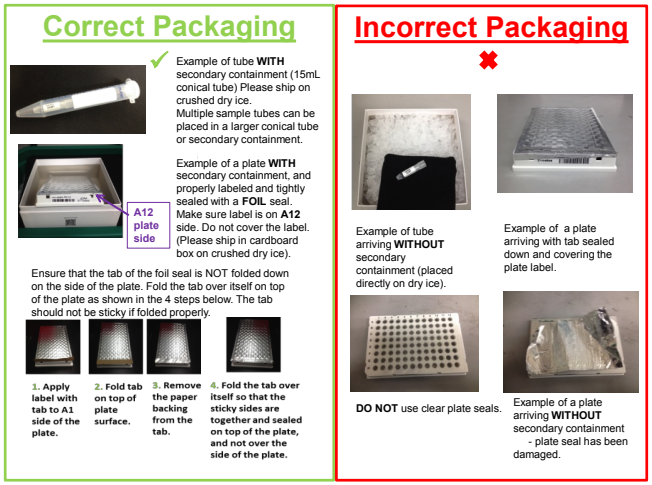
Please include this completed checklist in your shipment to the JGI. Shipments containing incorrect or improperly packaged materials or incomplete/inaccurate documentation will not be accepted. Contact your Project Manager prior to shipment if you have any questions.

Preparing your shipment:
It is important to follow these instructions for preparation of your shipment to ensure that all materials arrive in good condition.

All samples must be placed in the barcoded tubes or plates contained in this package.

1. Place barcoded samples in sturdy secondary containment (examples include15 or 50 ml Falcon tubes, plastic freezer box, etc. for tubes). Plates should also be placed in sturdy secondary containment (cardboard or plastic box). Do not use Parafilm or clear seals to wrap your individual sample tubes or plates.

2. Add sufficient crushed dry ice to the box to cover all samples.



Last updated 3/10/2016

2800 Mitchell Drive, Walnut Creek, CA 94598
www.jgi.doe.gov

3. Place the pre-printed shipping label (included) on the outside of your package.

4. Once you have shipped your package, email shipping_to_jgi@lists.jgi-psf.org with tracking information for the shipment.

Your shipment to the JGI must include the following items:

☐ Hard copy of "Approval to Ship" email from the JGI

☐ JGI USDA Import Permit (soil and soil-derived materials only)

☐ Completed shipping checklist (this page)

Investigator Name	Date

Enter Total Number of Sample Tubes or Plates in this Shipment:

Total Tubes:	
Total Plates:	

Fill out below ONLY if shipping at room temperature with RNAstable or DNAstable Plus:

☐ Check this box if you have included **RNAstable treated sample/s** and list the total amount of tubes.

_____**# of tubes of RNAstable treated samples**

☐ Check this box if you have included **DNAstable Plus treated sample/s** and list the total amount of tubes.

_____**# of tubes of DNAstable Plus treated samples**

Requirements of gDNA storage in DNAstable Plus ® (Dry format)

Storage Requirements for Collborators

- **Important: sample purity is critical for DNAstable.**
  - Free from DNase activity to prevent degradation.
- **Store in dry format up to 50ug.**
- **Store dried tubes in heat sealed moisture-barrier bag with desiccant pack.**
- **Sample must be completely dry to prevent degradation of DNA.**

Supplies:

(**Please note JGI does not support plate format at this time**)

Supplies can be purchased from **http://www.biomatrica.com/dnastable.php**

Example:

DNAstable® Plus: 2mL: 53091-016 10mL: 52091-026

Moisture barrier foil bags: **14001-007**

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UNITED STATES DEPARTMENT OF ENERGY

Sample Quantification at the JGI

David Dilworth 3/22/2016

Sample Quantification at the JGI

- Describe importance of nucleic acid-specific dyes
- Creating a standard curve & selecting inputs
- Implementation on Hamilton Starlet instruments
- Next steps and new testing

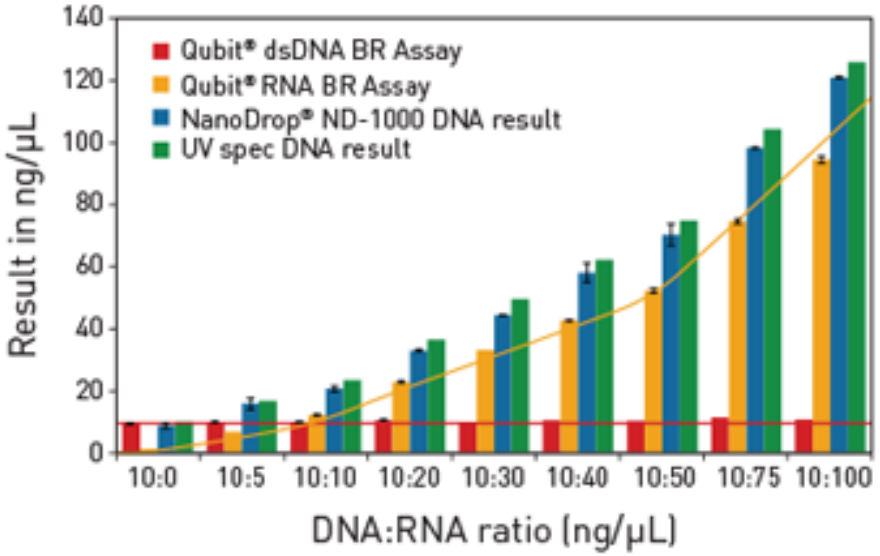
Importance of Nucleic Acid-specific Dyes

- **Why are nucleic acid-specific dyes necessary?**

	Fluorescence-based Kits	NanoDrop® ND-1000
Quantification method	Fluorescence-based dyes that bind specifically to DNA or RNA	UV absorbance measurements
Selectivity for DNA or RNA	Accurately measure both DNA and RNA in the same sample	Results for samples containing both DNA and RNA are nondiscriminatory
Can indicate contamination	No	Gives peaks revealing the presence of contaminants

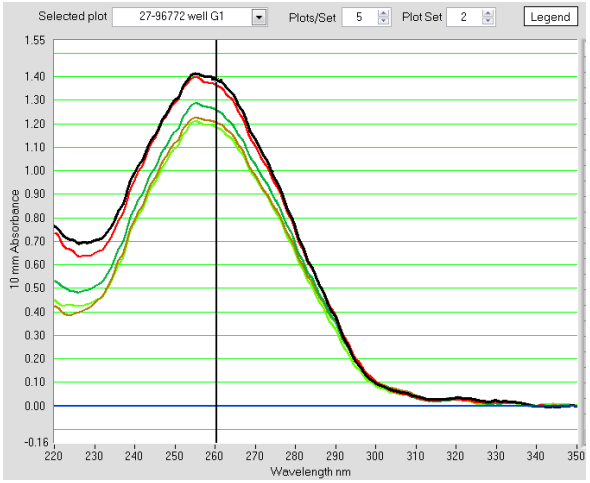
Importance of Nucleic Acid-specific Dyes

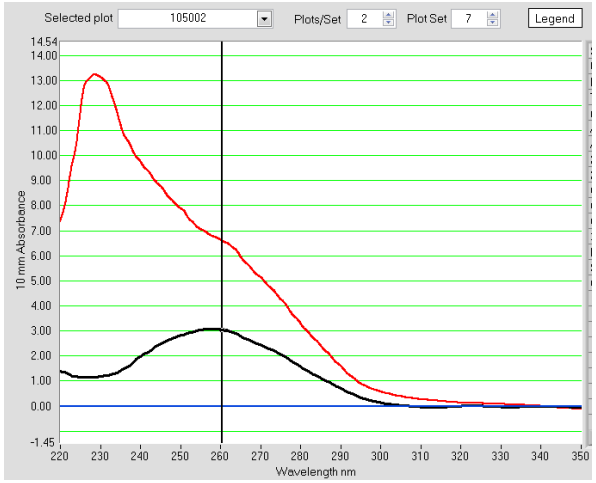
- **Why are nucleic acid-specific dyes necessary?**

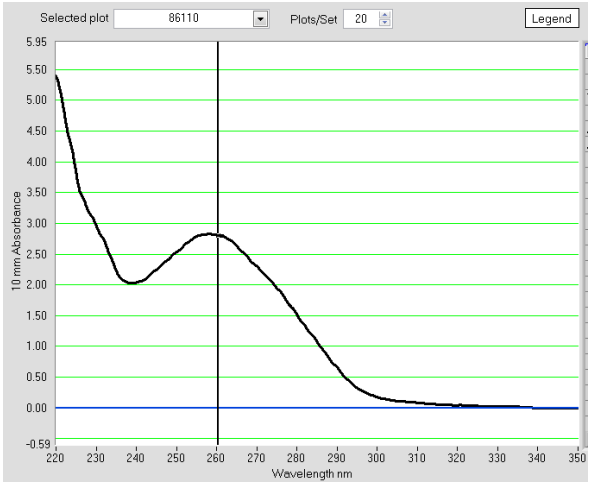


Importance of Nucleic Acid-specific Dyes

- **Why are nucleic acid-specific dyes necessary?**

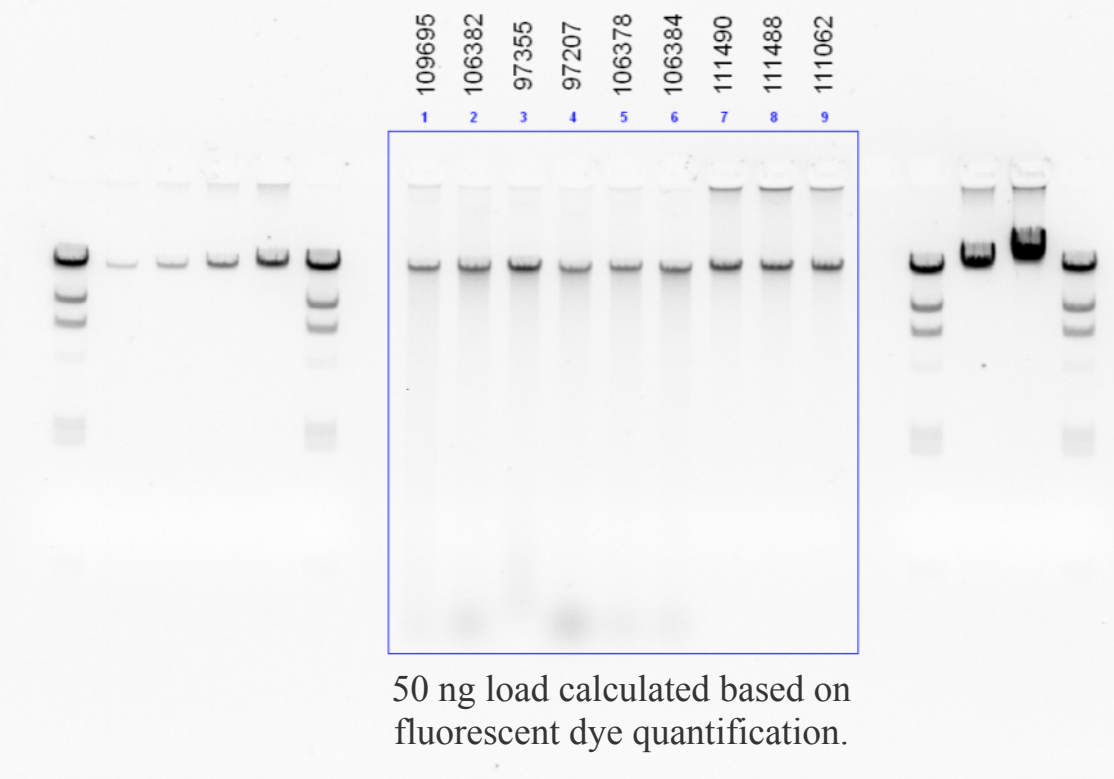






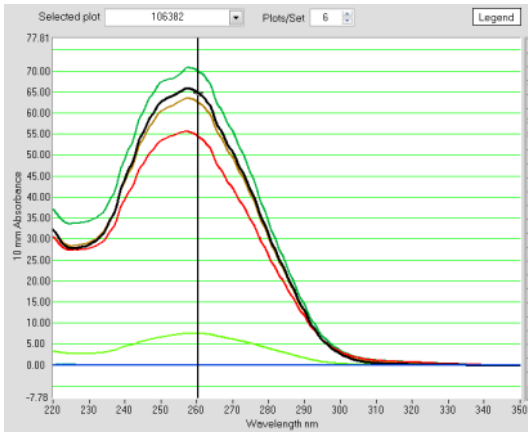
Importance of Nucleic Acid-specific Dyes

- **Why are nucleic acid-specific dyes necessary?**



50 ng load calculated based on fluorescent dye quantification.

	[DNA] ng/µL	
Sample	Qubit	NanoDrop
106382	187	3254
97207	182	2735
106378	329	3510
106384	295	3139
111062	352	375



Importance of Nucleic Acid-specific Dyes

- **The JGI uses fluorescent dyes for quantification:**
  - **DNA: broad range or high sensitivity assay for high throughput; another (BR or HS) for repeats or low throughput.**
  - **RNA: broad range or high sensitivity assay for high throughput; another (BR or HS) for repeats and low throughput.**

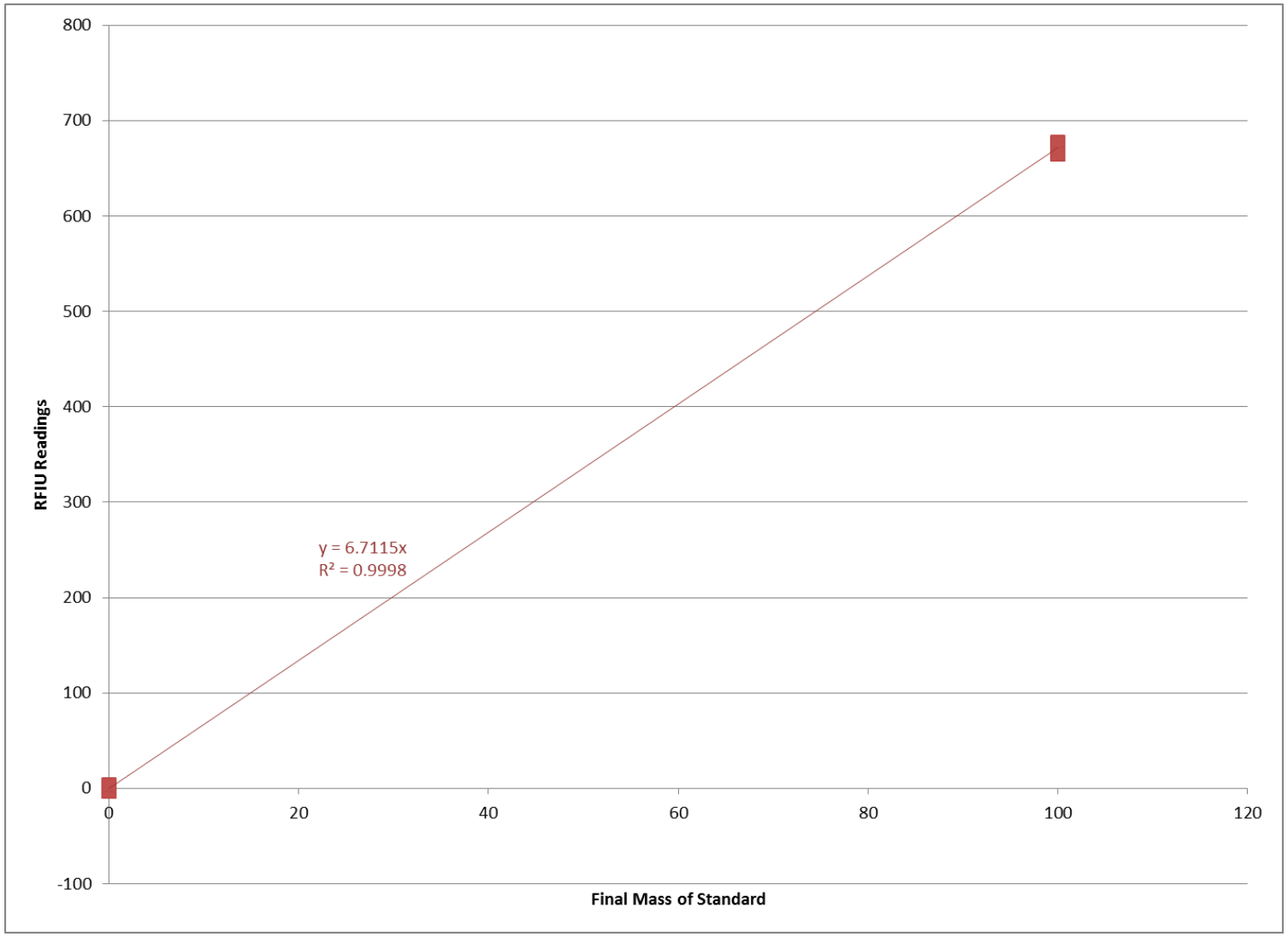
- **The JGI uses NanoDrop® ND-1000 to read purity**

Creating a Standard Curve & Selecting Inputs

- **Why is a multi-point standard curve necessary?**
  - This allows the operator to be able (with each run) to correlate the RFU readings of standards to their concentrations
  - Multi-point standard curves enable accuracy for a range of sample concentrations
  - When a single standard is used, the curve will be skewed if that one sample is off in concentration or pipetting)

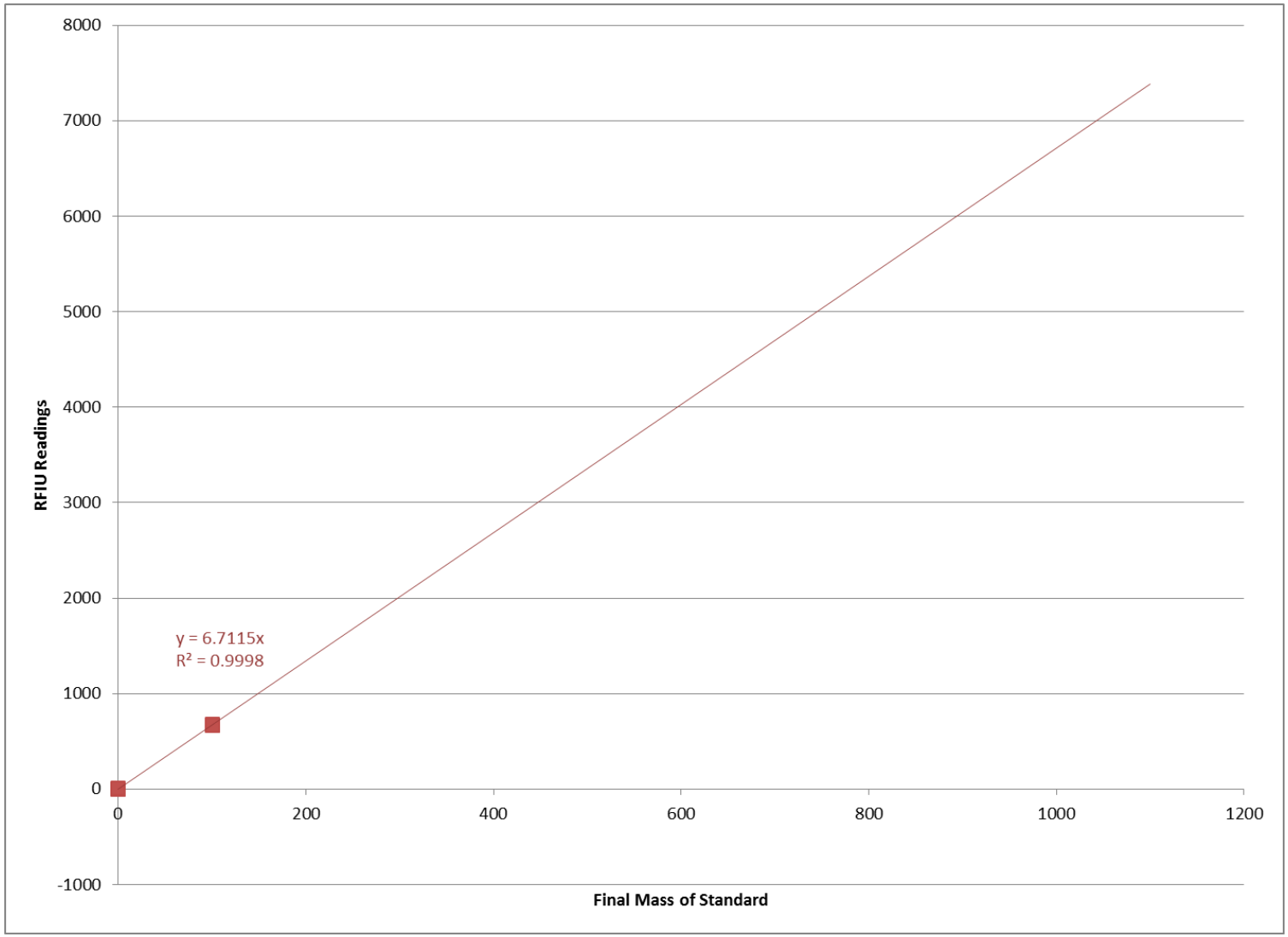
Creating a Standard Curve & Selecting Inputs

- **Why is a multi-point standard curve necessary?**



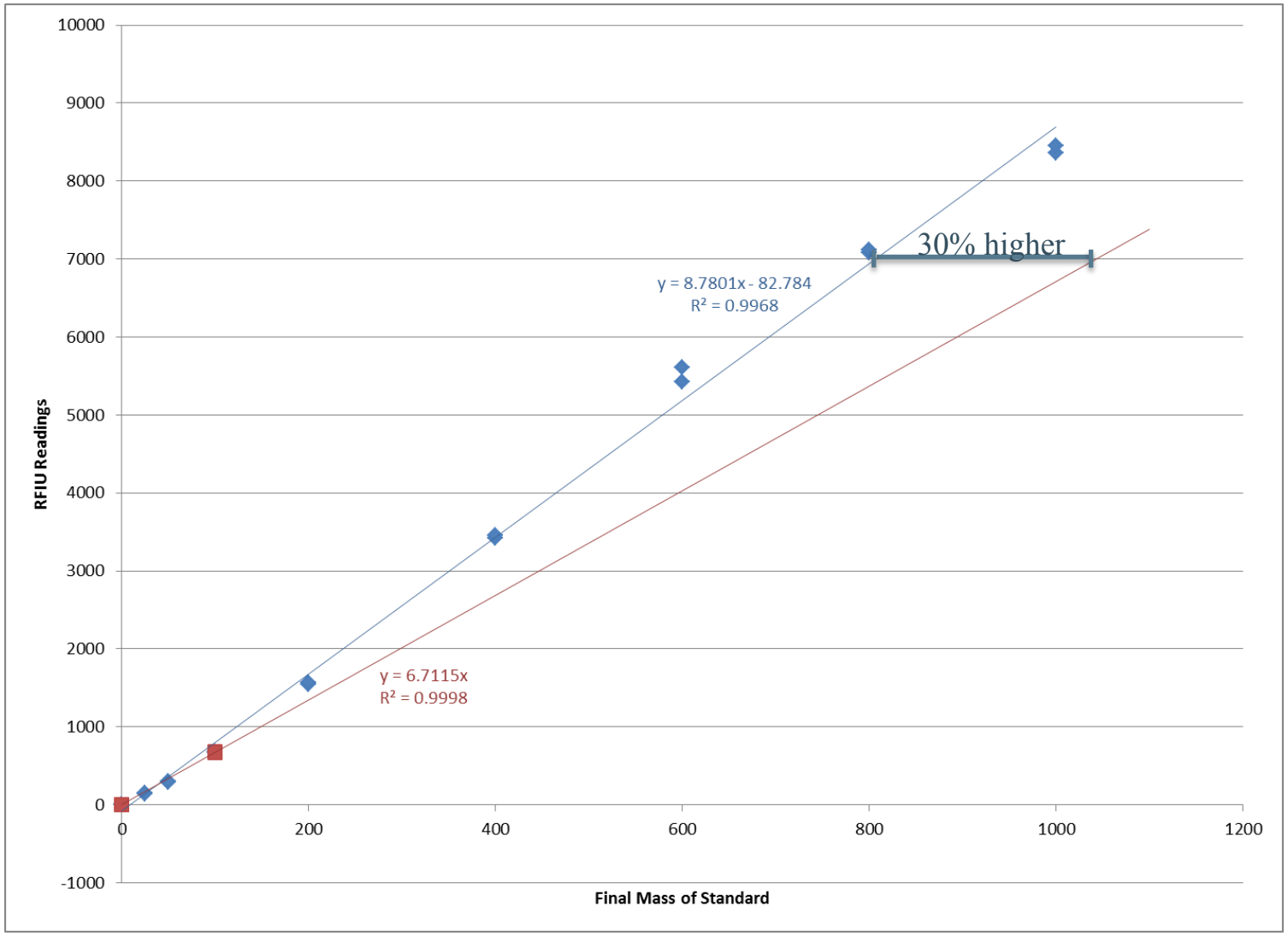
Creating a Standard Curve & Selecting Inputs

- **Why is a multi-point standard curve necessary?**

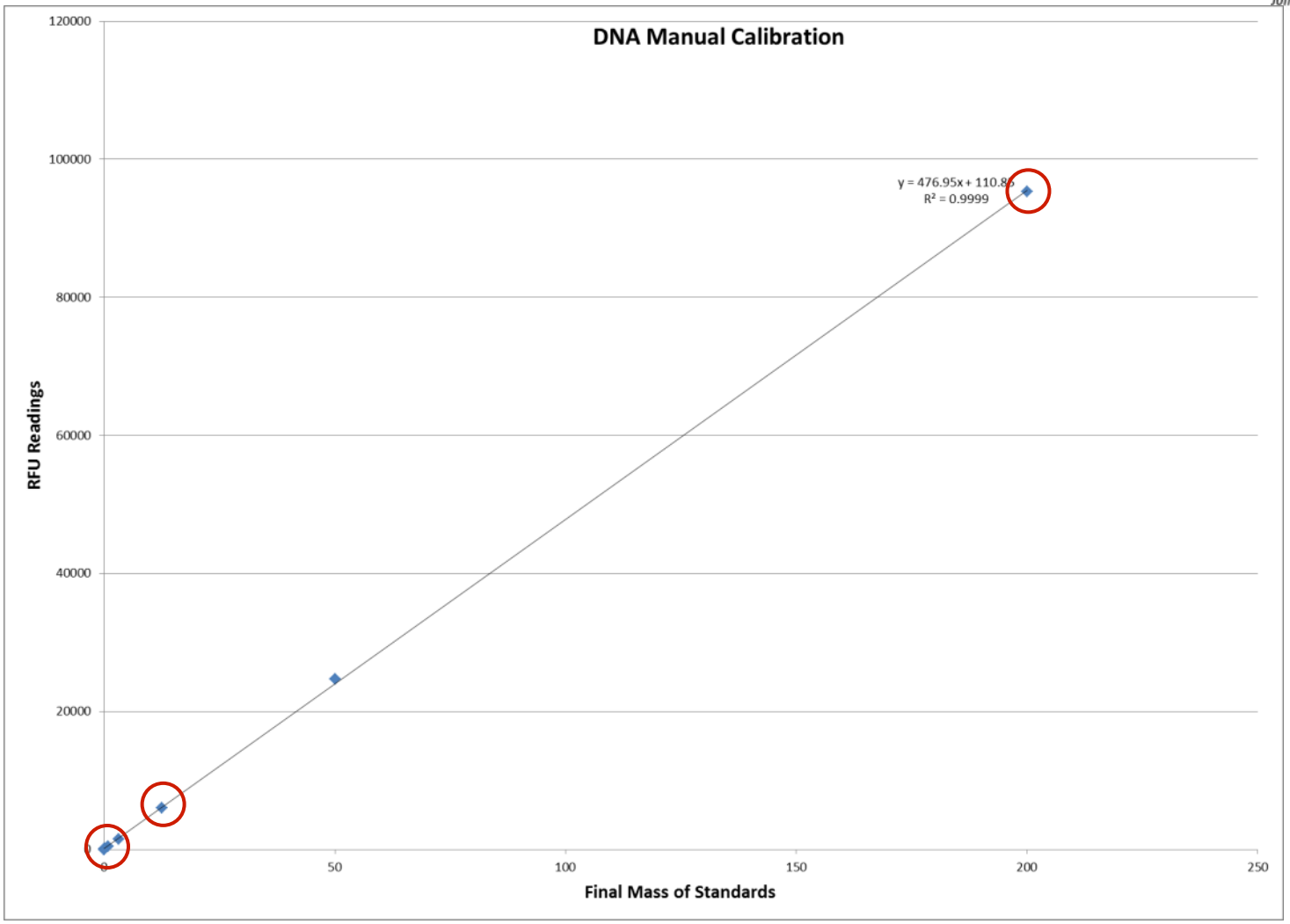


Creating a Standard Curve & Selecting Inputs

- **Why is a multi-point standard curve necessary?**



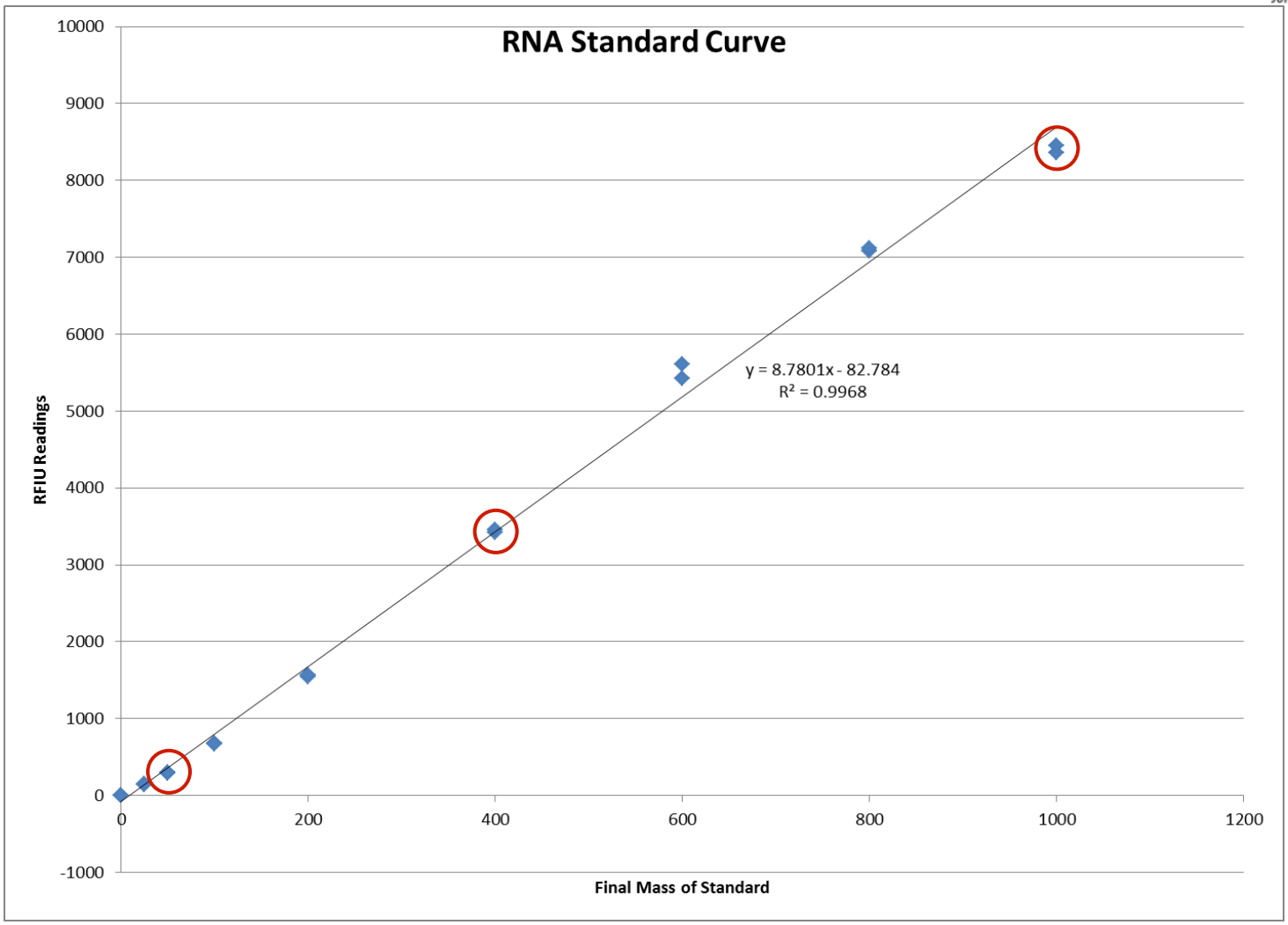
Creating a Standard Curve & Selecting Inputs - DNA



Selected 2ng, 10ng & 200ng (final mass) for DNA Broad Range.

Also implemented 0.4ng, 2ng & 20ng (final mass) for DNA High Sensitivity.

Creating a Standard Curve & Selecting Inputs - RNA

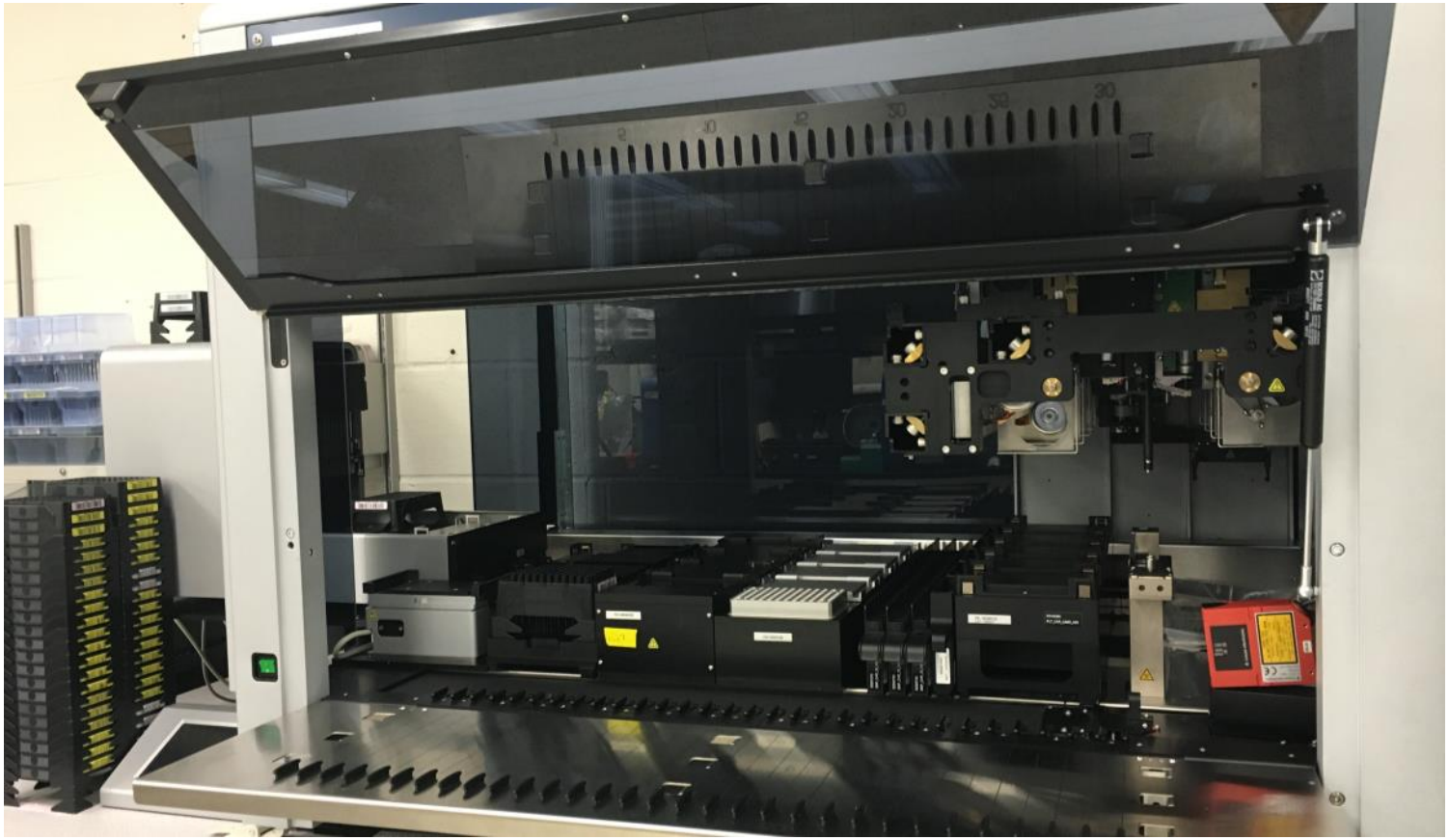


Selected 50ng, 400ng & 1000ng (final mass) for RNA Broad Range.

Also implemented 1.5ng, 10ng & 80ng (final mass) for RNA High Sensitivity.

Implementation on Hamilton Starlet

Implementation on Hamilton Starlet

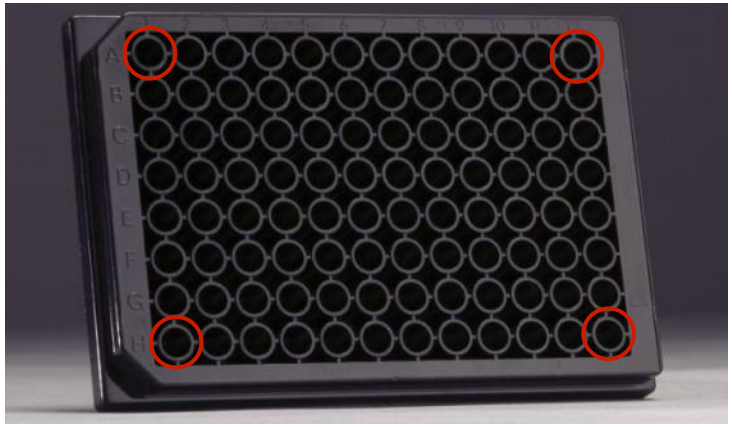


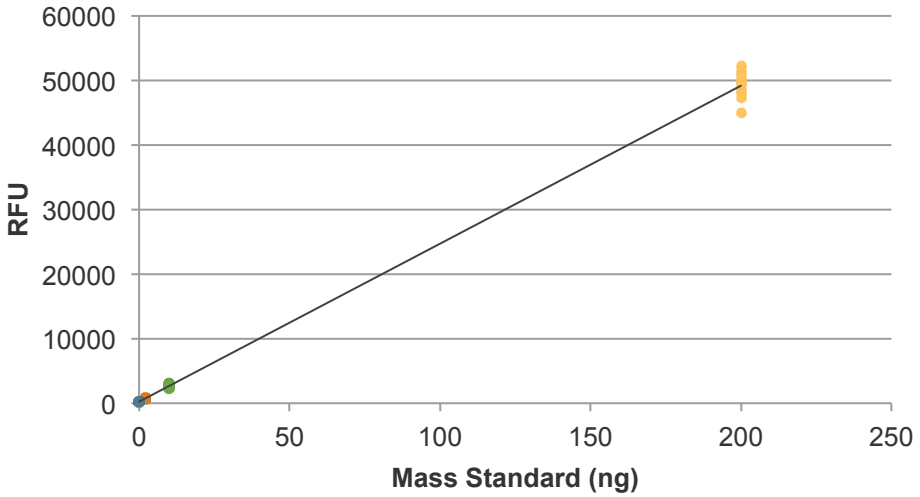
Implementation on Hamilton Starlet

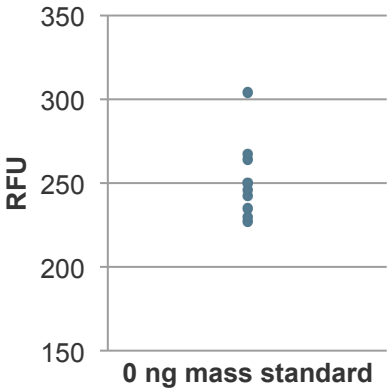
BioTek Synergy H1 microplate reader

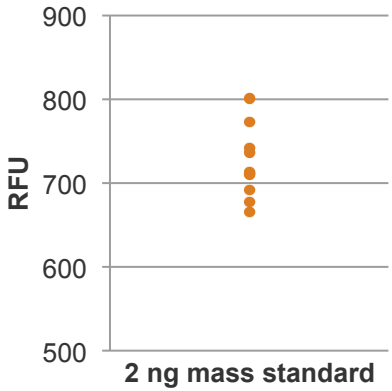
Implementation on Hamilton Starlet

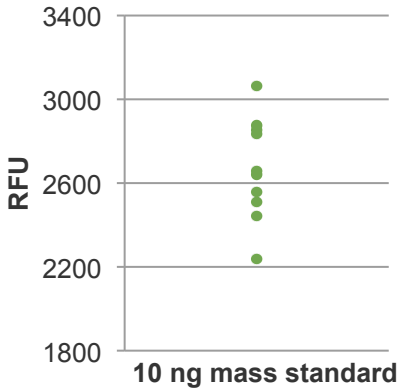
Tracking RFU Readings for Starlet Quantification

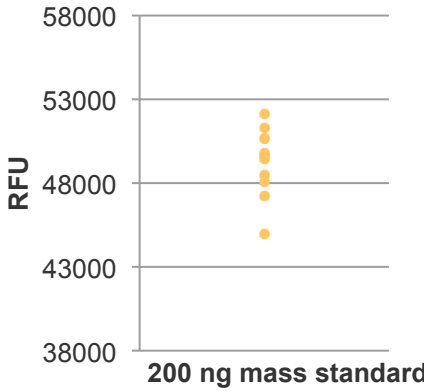




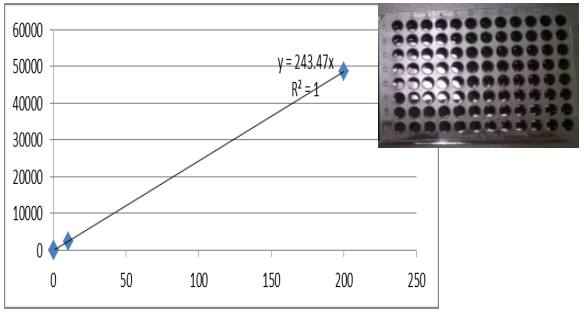


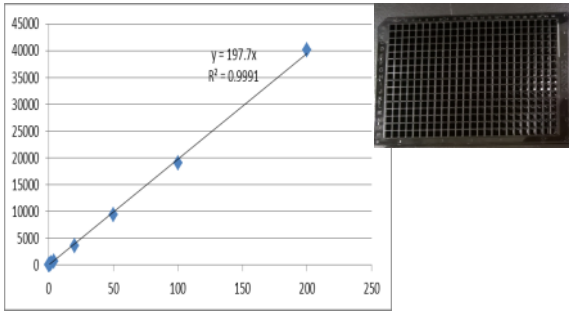






Miniaturization of QC assay
Improve sample management sample QC workflow

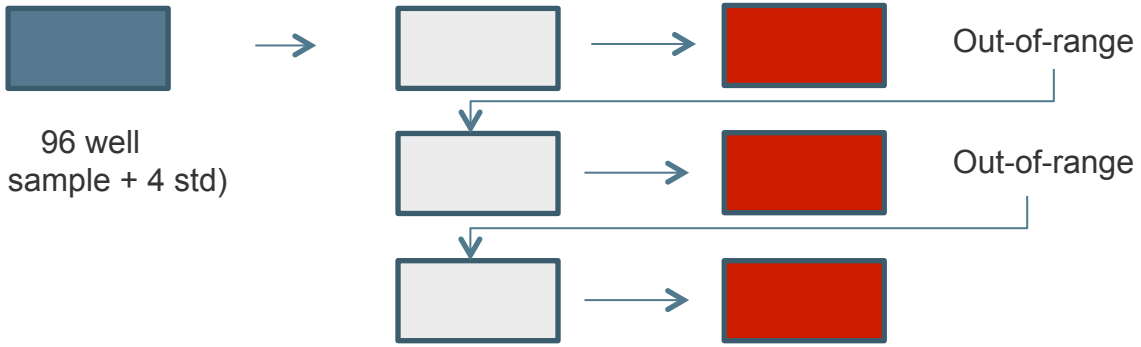




Comparable linear detection range

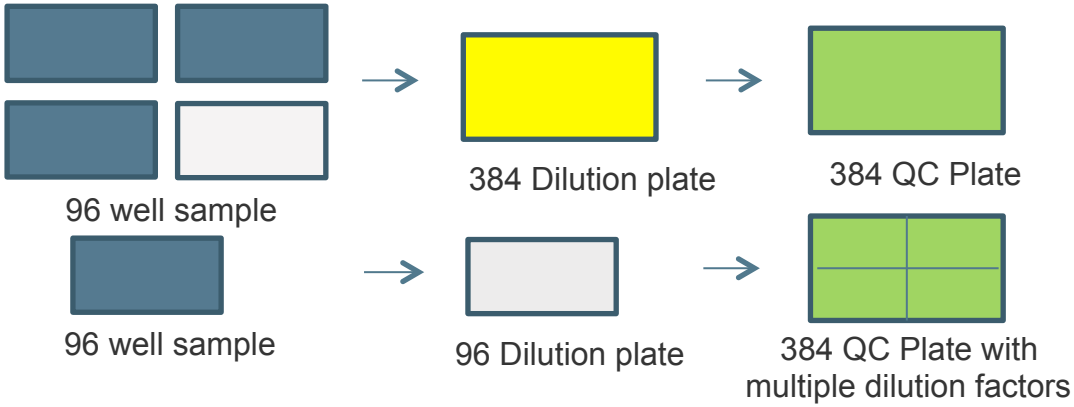
Current workflow (2-4hr)

- Repetitive if samples are in a different detection range.
- 3-point calibration limited by space on plate



Improved workflow (~2hr)

- Shorter processing time
- > 3 point calibration curve to improve accuracy
- Efficient workflow



Summary and Next Steps

- High-throughput DNA and RNA BR and HS assays have been developed and are currently employed in production QC
  - Current Starlet methods allow for quantification within various ranges; however,
  - The within sample set variability for QC samples often requires repeat runs to cover multiple ranges.

- The major limitation, the number wells available for use in multipoint calibration, is being overcome through conversion of current Starlet methods to 384-well format.

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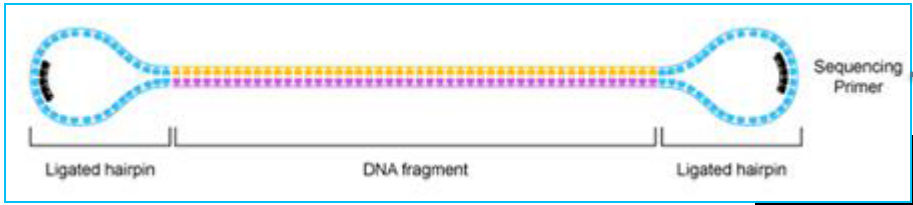
JGI JOINT GENOME INSTITUTE
A DOE OFFICE OF SCIENCE USER FACILITY

Sample Quality for PacBio Sequencing

Yuko Yoshinaga
Sample Management JGI

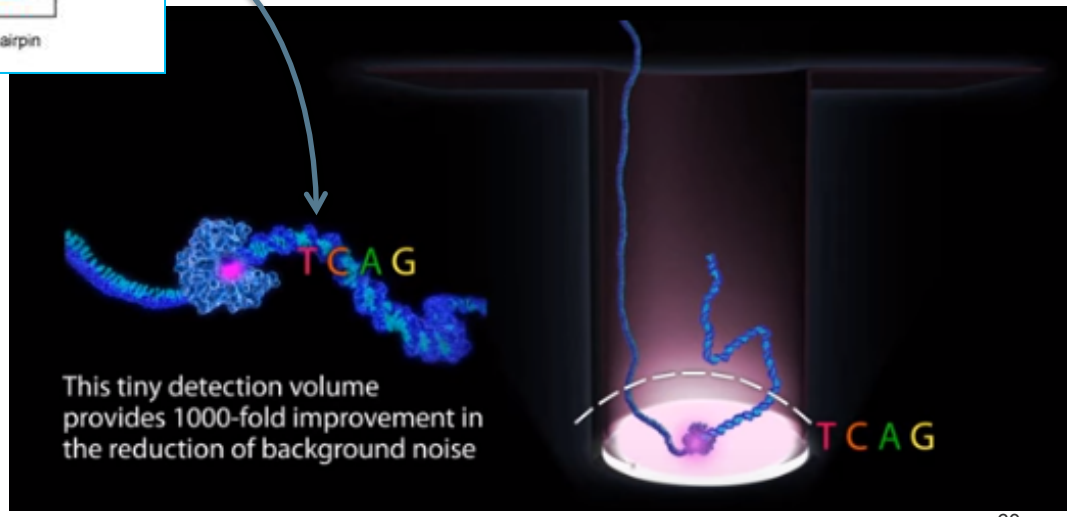
PacBio: SMRT

- Single Molecule, Real-time sequencing Technology: SMRT
- No amplification & long reads
- Quality of the DNA directly reflected in the sequencing results
- DNA damage (e.g, inter-strand crosslinks), chemical contamination and short fragment contamination will result in impaired performance in the system
- **High-quality, high-molecular-weight** genomic DNA is required



Key Advantages:

- Structurally linear
- Topologically circular
- Structural homogeneity of templates
- Provides sequences of both forward and reverse strands in the same trace



Quantity-Quality-Purity

1. **High Quality**: High Molecular Weight DNA
   – Degraded DNA causes shorter shearing size, less library yield and short fragment contamination

2. **Large Quantity and High Concentration**:
   – Less input causes less library yield
   – Lower concentration causes shorter shearing size and short fragment contamination

3. **High Purity**:
   – Contaminants of DNA binders or polymerase inhibitors cause low library yield and low quality sequencing

General recommendations

HMW DNA-Treat DNA as a crystal vase: it is fragile when in solution

- **No overdry: air dry or no heat in a SpeedVac**
- **Elute in neutral buffered solution (Tris/TE, pH 8.0)**
- **Never vortex: invert or tap the tubes gently**
- **Use wide-bore tips, limit pipetting**
- **No >65°C heating**
- **DNA storage:**
  - 4°C (short-term) or –20°C / –80°C (long-term)
- **Minimize freeze/thaw**

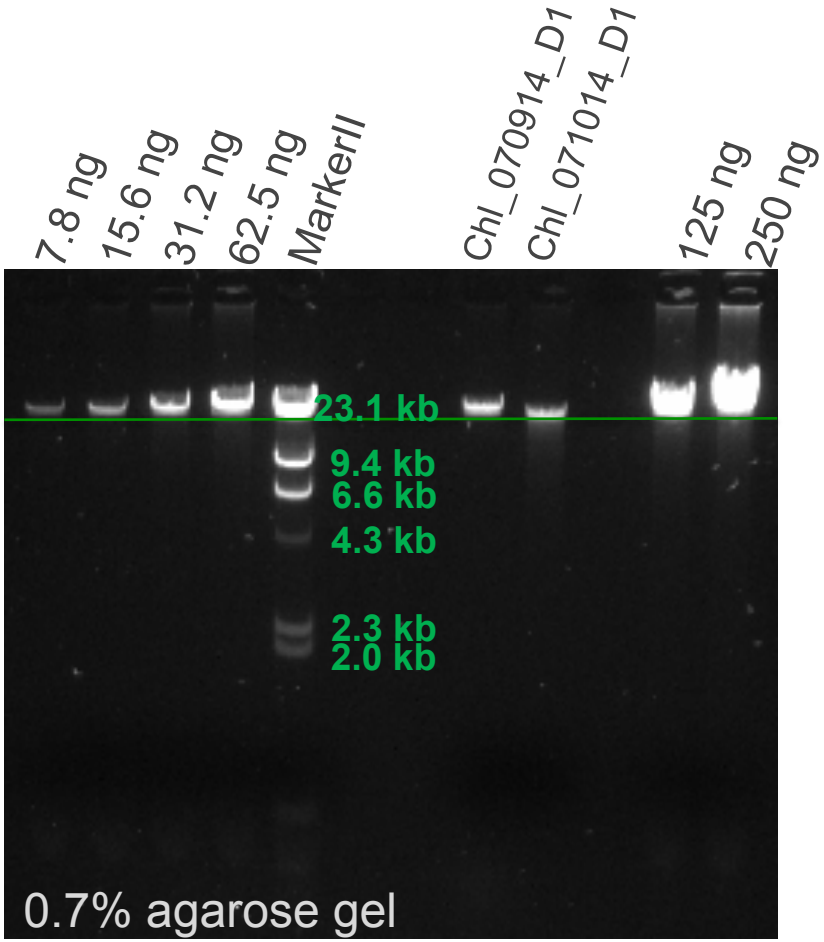
Amplicons:

- **Clean amplicons, with no non-specific products**
- **No ethidium bromide/UV for gel purification**
  - Use SYBR® Safe (Invitrogen) and blue light

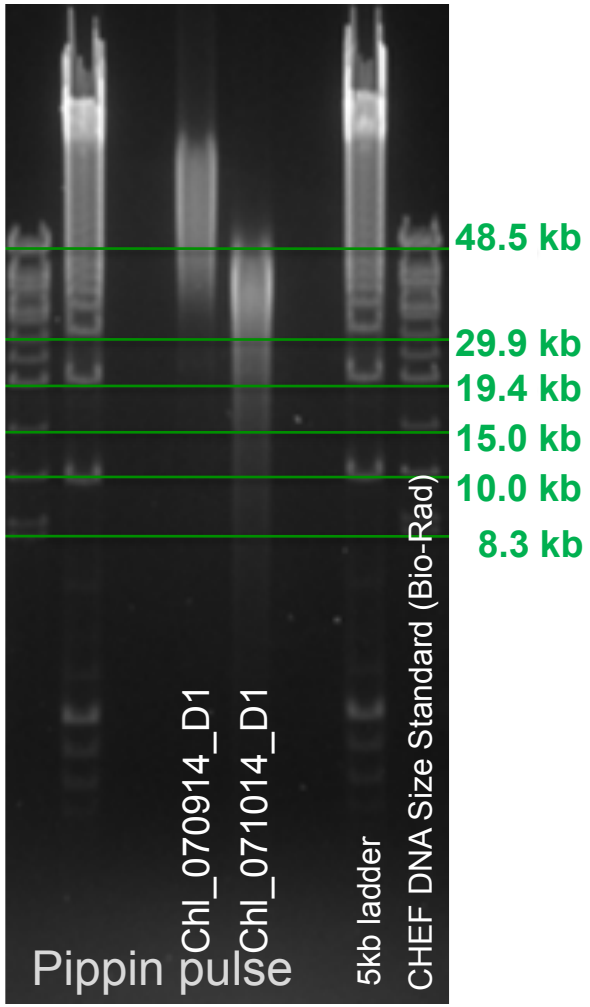
JGI catalog - PacBio

Library Types	Lib Status	Container	Required QC Type	Mass per attempt	Max Vol per attempt	Quality criteria
2kb	Active	Tube	Quantity	500 ng	10 uL	Amplicon only
3kb	Active	Tube	Quantity	2 ug	200 uL	No quality check
Low Input 10kb	Active	Tube	Quantity Quality Purity	1 ug	100 uL	Marginal DNA quality
>10kb	Active	Tube	Quantity Quality Purity	5 ug	30 uL	Marginal DNA quality
>10kb	Active	Plate	Quantity Quality Purity	875 ng	28 uL	Marginal DNA quality acceptable
>10kb Blue Pippin	Active	Tube	Quantity Quality Purity	10 ug	70 uL	High MW DNA
20kb Blue Pippin	Active	Tube	Quantity Quality Purity	40 ug	200 uL	Very high MW DNA
30kb Blue Pippin	RnD	Tube	Quantity Quality Purity	40 ug or more	TBA	Very high MW DNA

High quality: gel assessment

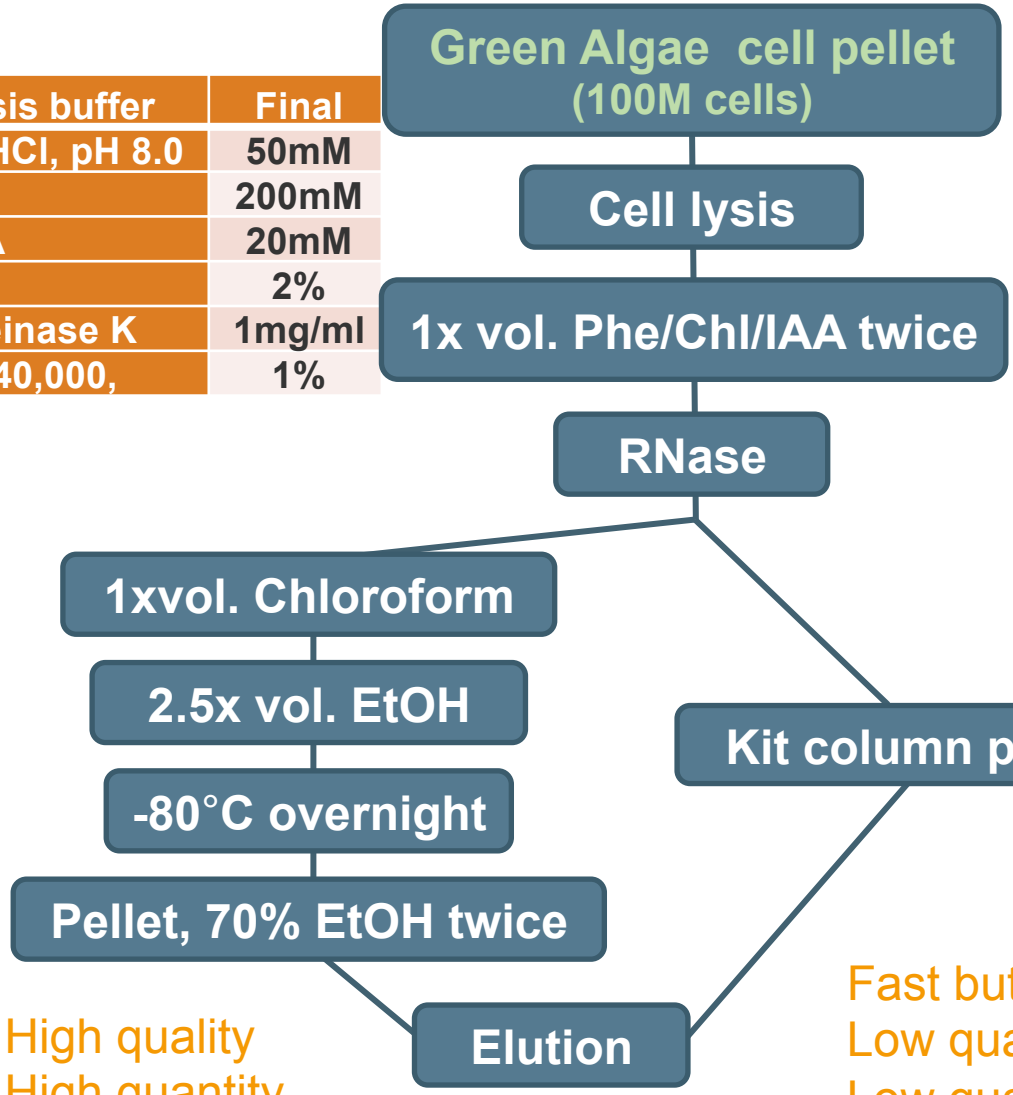


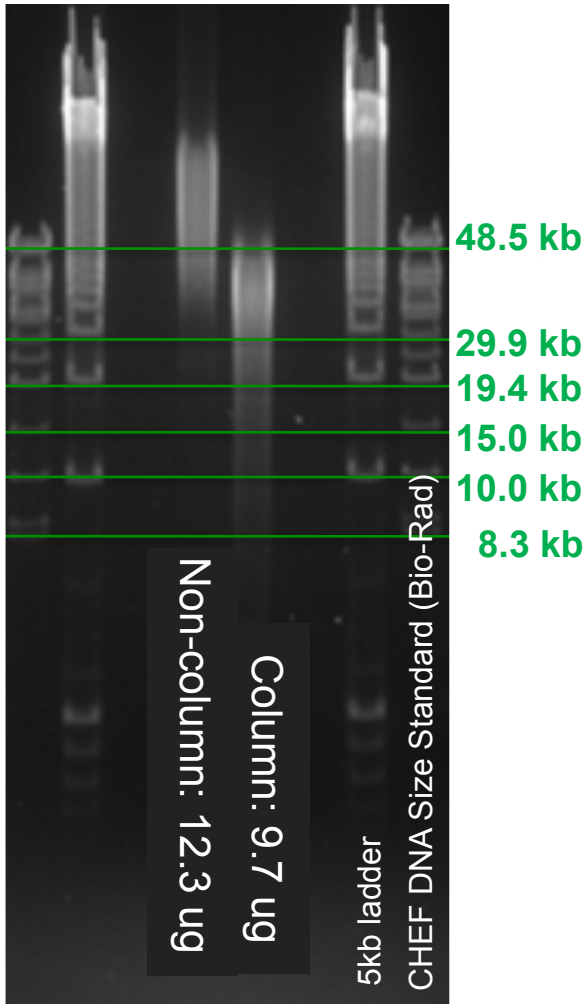
50ng of *Chlamydomas* gDNA extracted in two methods: Aditi Sharma



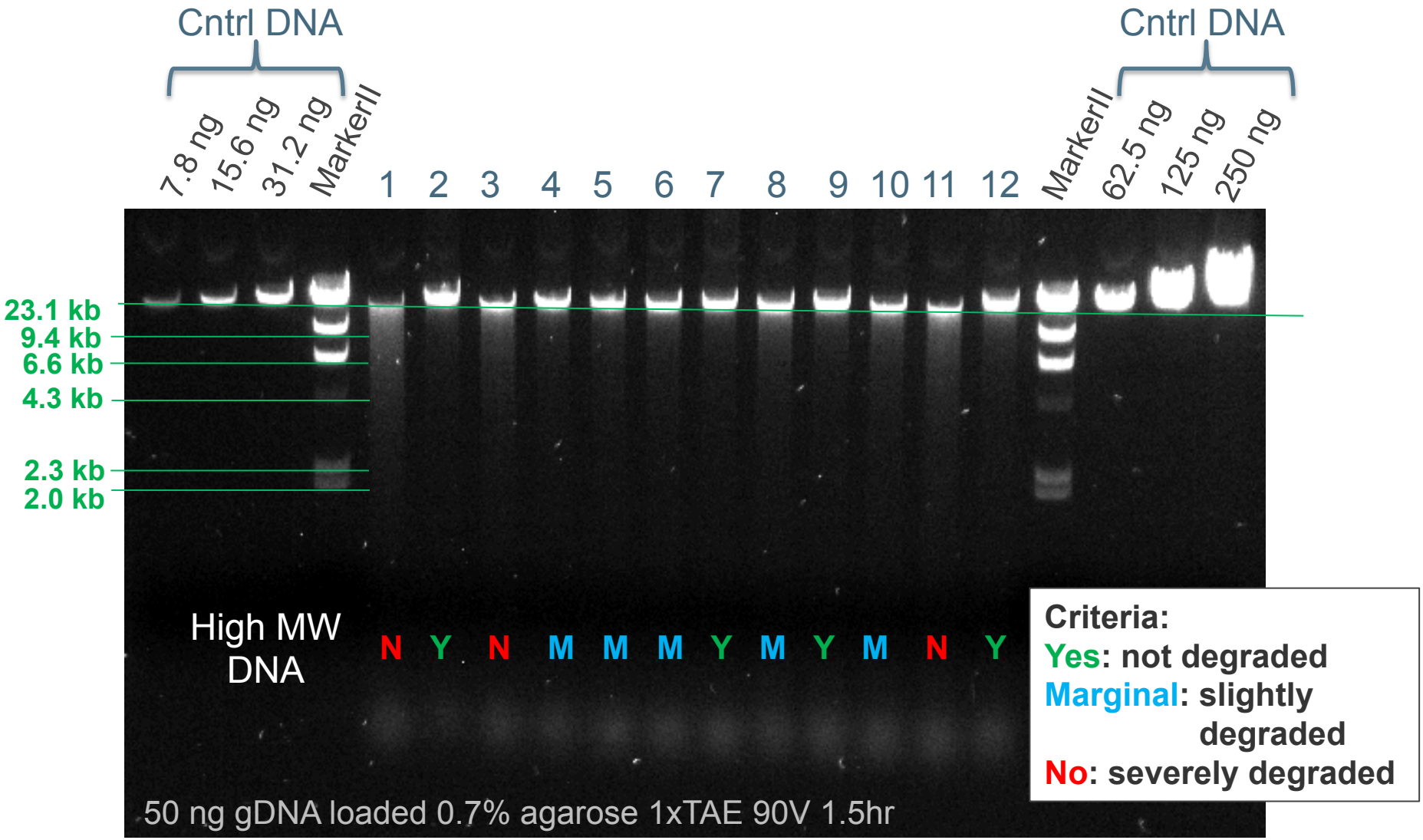
Column vs non-column DNA purification

Lysis buffer	Final
Tris/HCl, pH 8.0	50mM
NaCl	200mM
EDTA	20mM
SDS	2%
Proteinase K	1mg/ml
PVP 40,000,	1%





HMW DNA-pass/fail



*The samples have RNA contamination. It will be noted but not be failed.

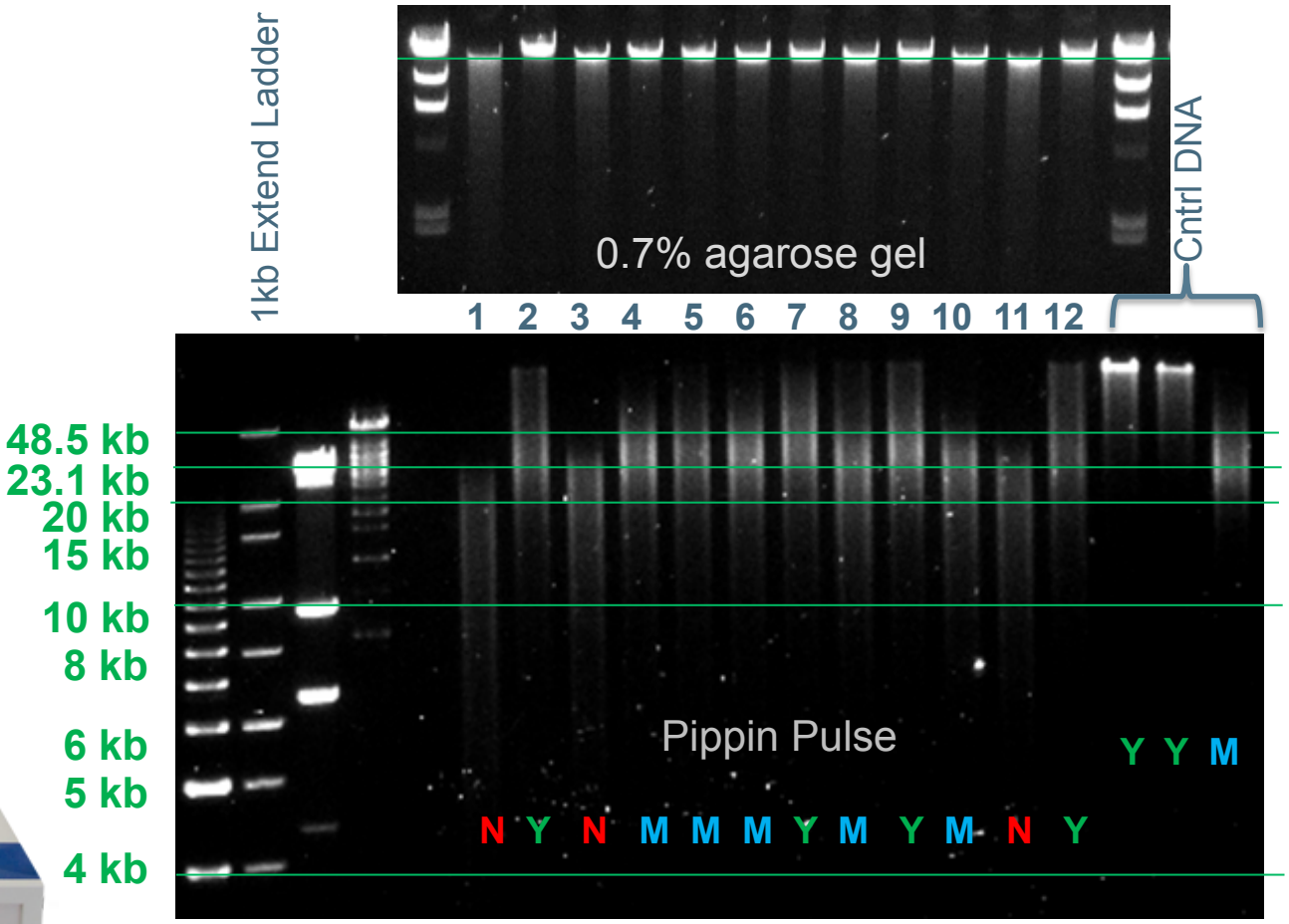
Pippin Pulse electrophoresis power supply (Sage Science)

Pulse-field gel QC:
Aditi Sharma

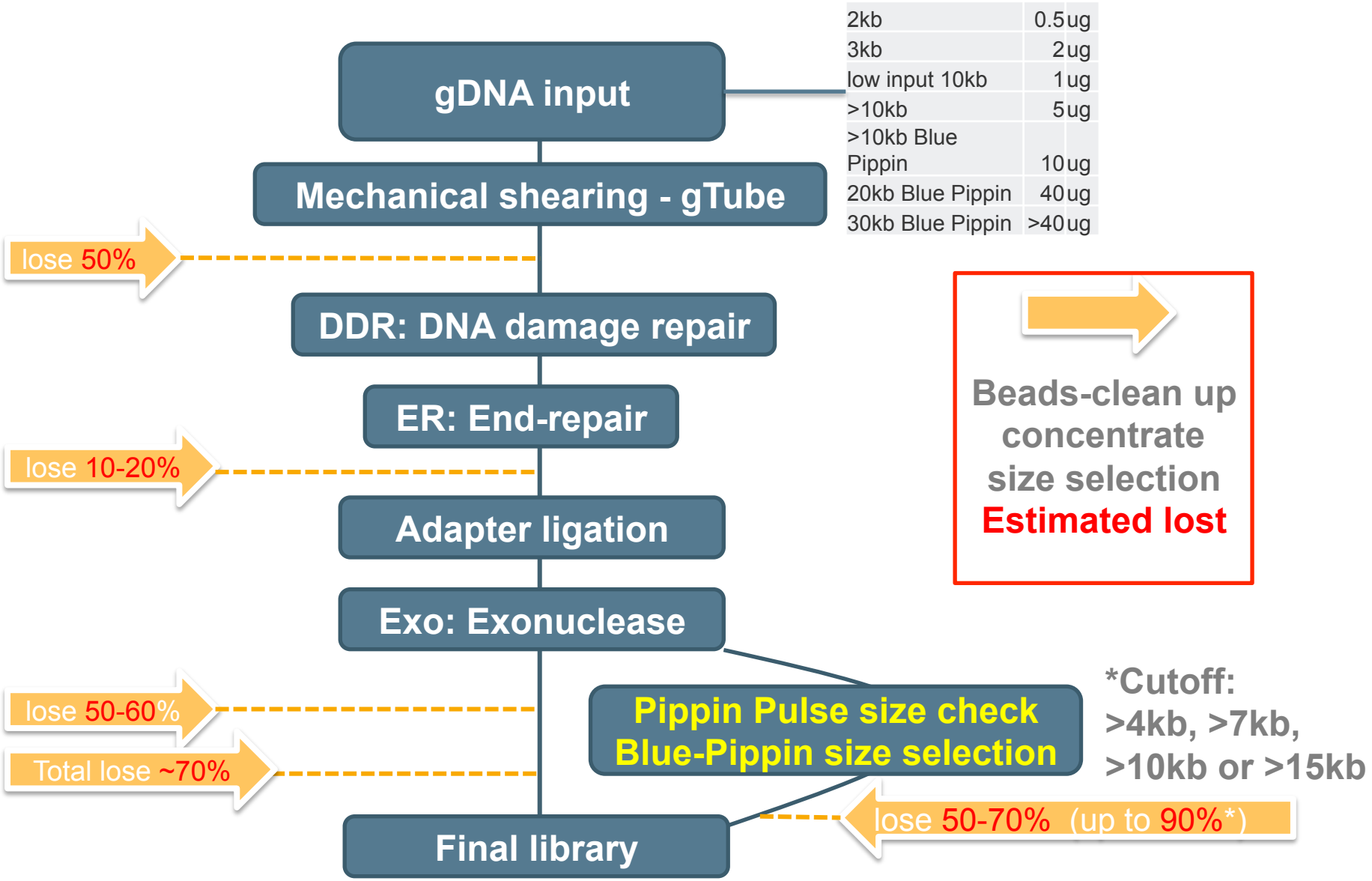
Resolution of Pippin Pulse is >100 kb

Criteria:

Yes: **> 48.5kb**
Marginal: 20-48.5kb
No: **< 23.1kb**

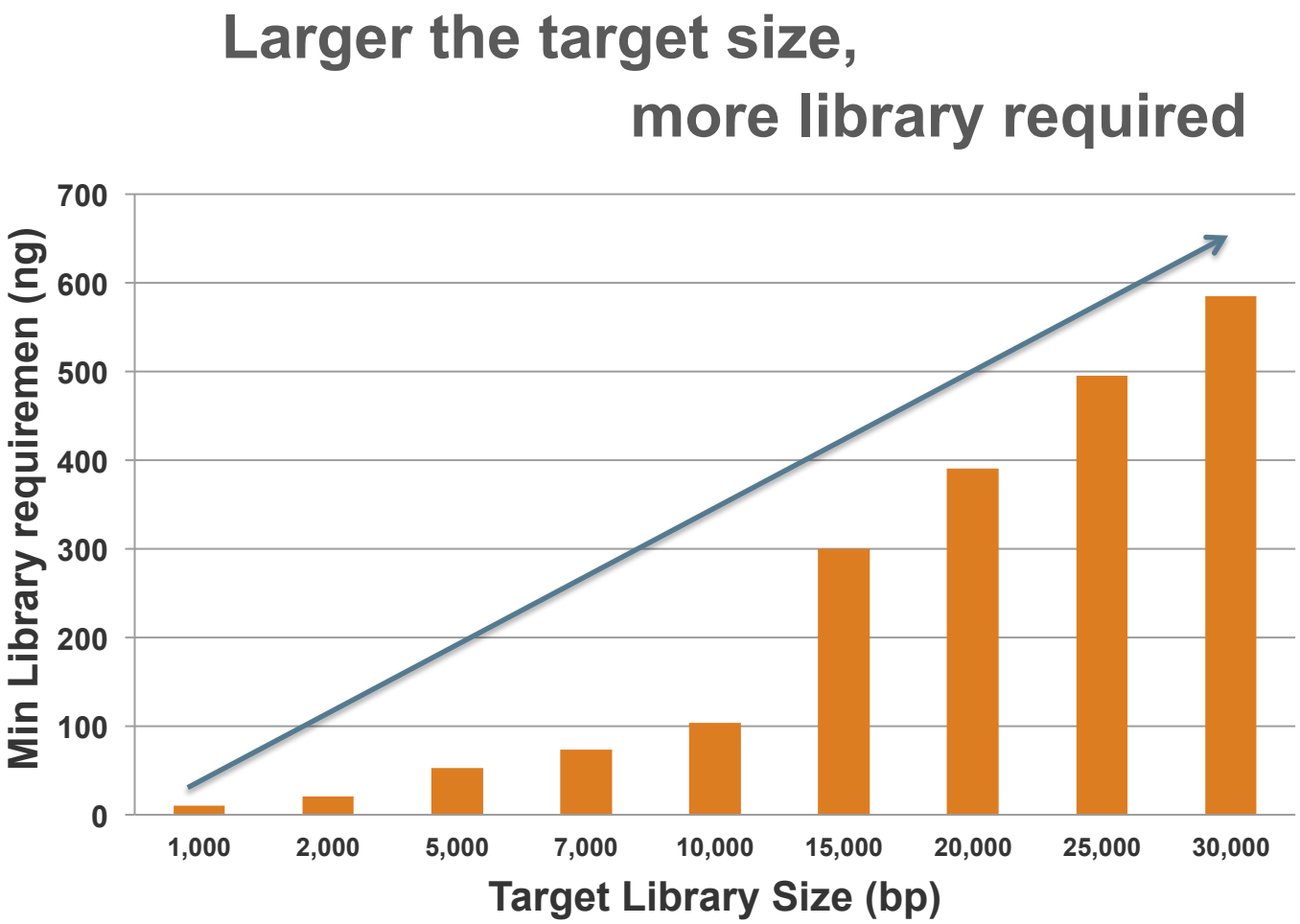


PacBio procedure



2kb	0.5ug
3kb	2ug
low input 10kb	1ug
>10kb	5ug
>10kb Blue Pippin	10ug
20kb Blue Pippin	40ug
30kb Blue Pippin	>40ug

Large quantity

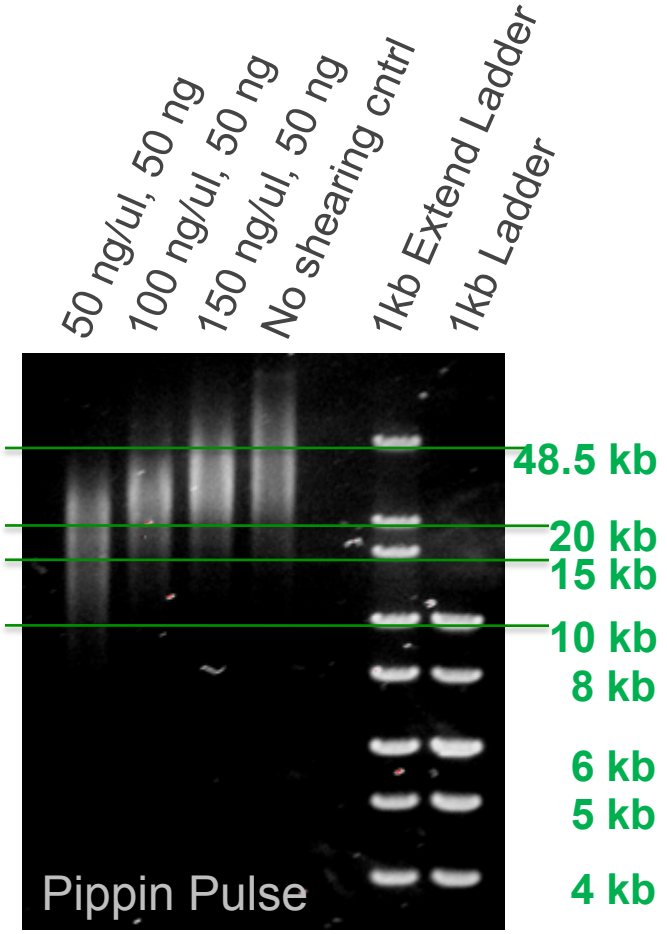


Lower concentration – lower shearing size

20kb Blue pippin: shearing optimization:
Guifen He

- **10-20kb shearing:**
  - **Covaris g-Tube**
  - **5,500 rpm x 60 sec**
- **Same DNA but lower concentration shears lower**

- **10kb: 50 ng/ul**
- **20kb: >100 ng/ul**
- **Depends on starting material quality also**



High purity: absorbance

- DNA-binders: Proteins, polyphenols, secondary metabolites (e.g. toxins), pigments, polysaccharides
- Polymerase inhibitors: Salts/Phenol/Alcohols
- Physical inhibiting factors – debris

Photometrically active contaminants: phenol, polyphenols, EDTA, thiocyanate, protein, RNA, nucleotides (fragments below 5 bp)

Pure DNA 260/280: 1.8 – 2.0

< 1.8: presence of organic contaminants: proteins and phenol; glycogen

- **absorb at 280 nm**

> 2.0: High share of RNA

Pure DNA 260/230: 2.0 – 2.2

<2.0: Salt, humic acids, peptides, aromatic compounds, polyphenols, urea, guanidine, thiocyanates (latter three are common kit components) – **absorb at 230 nm**

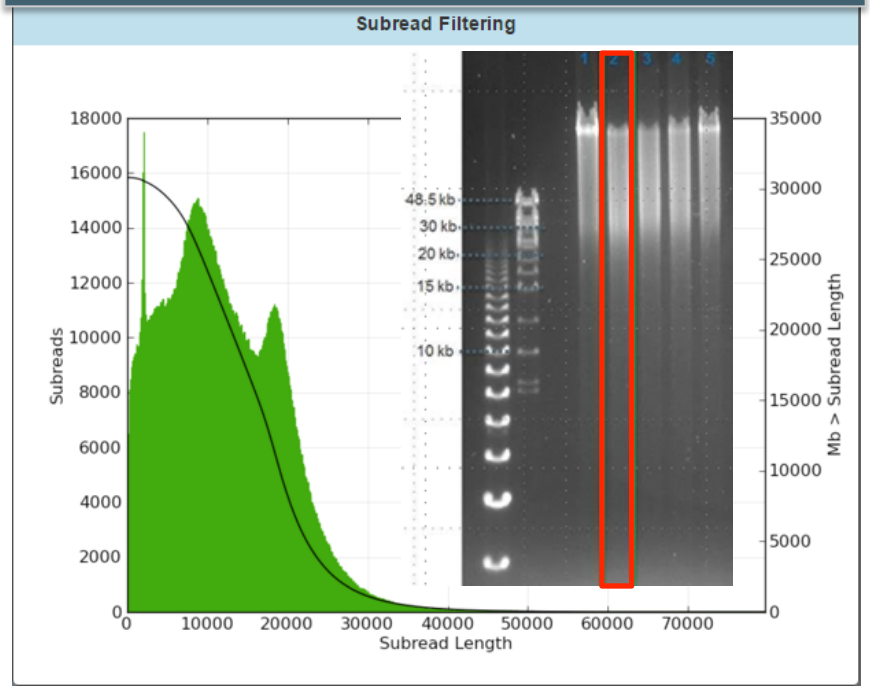
>2.2: High share of RNA, very high share of phenol, high turbidity, dirty instrument, wrong blank

☺ High quality (20kb)

AXHYY – 30 cells – 11,773 bp

BAR26844A3

Mean Subread length	11,773	N50	16,194
Total Number of Bases	30,916,677,709	Number of Reads	2,625,957



Observation

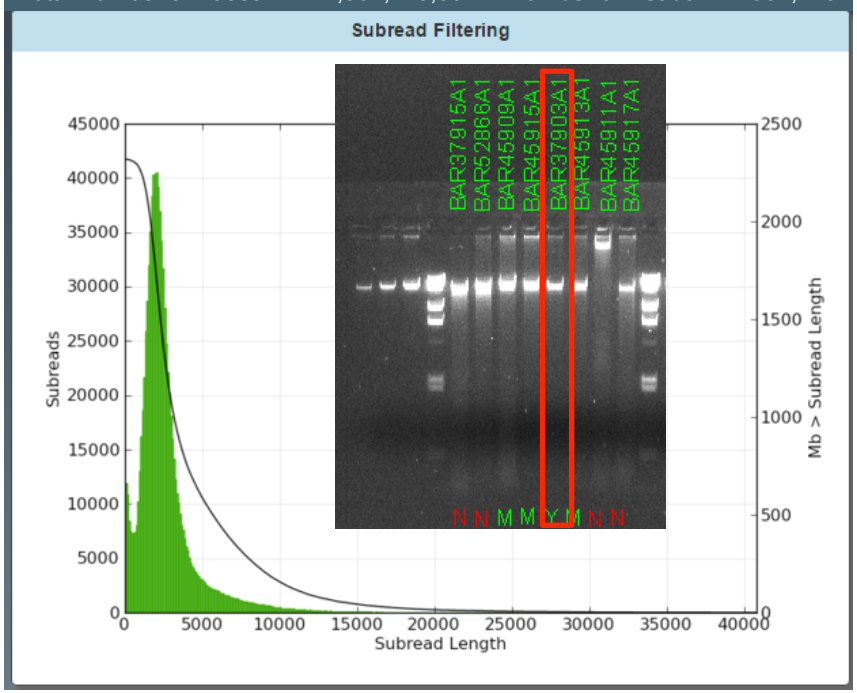
- **High quality, pass purity**
  - 260/230=1.72
  - 260/280=1.88
- **15kb cutoff used**
- **Long insert size**

☹ Low purity (low input 10kb)

AUBZB - 4 cells - **2,614bp**

BAR37903A1

Mean Subread length	2,614	N50	2,903
Total Number of Bases	2,364,213,004	Number of Reads	904,223



Observation

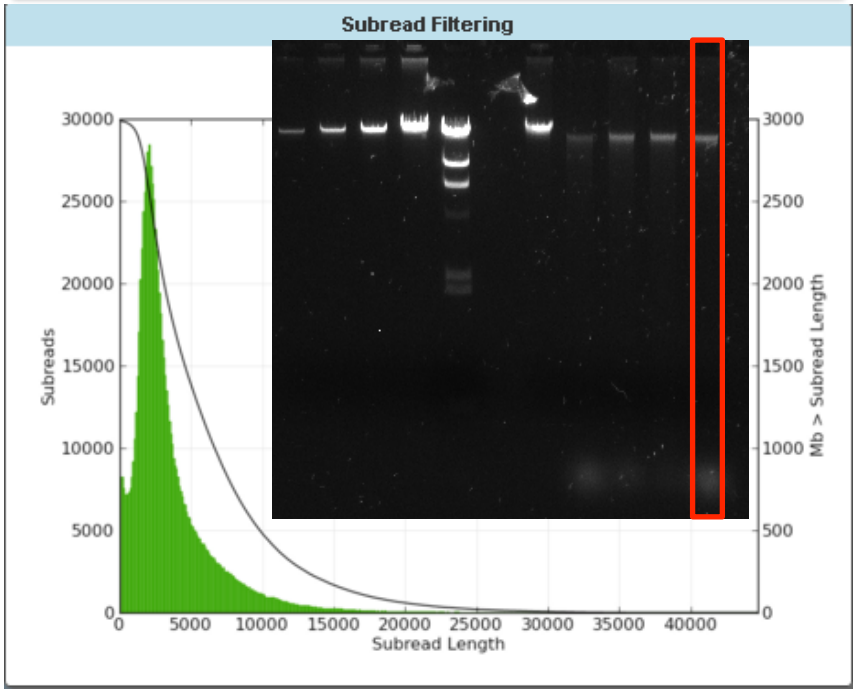
- **High quality**
- **Low purity:**
  - 260/230=1.28
  - 260/280=1.69
- **Low library yield:**
  - 1 ng/ul (require >5ng/ul)
  - **Inhibited library construction enzymes**
- **Low data yield: <1Mb**
  - **Inhibited sequencing enzymes**
- **Short insert size**
- **Remade library: AXWUT**
- **Low data yield:**
  - **Request new sample**

☹ Low quality (>10kb)

AXCAU – 3 cells – 3,534 bp

BAR57312A1

Mean Subread length	3,534	N50	4,648
Total Number of Bases	3,030,418,955	Number of Reads	857,453



Observation

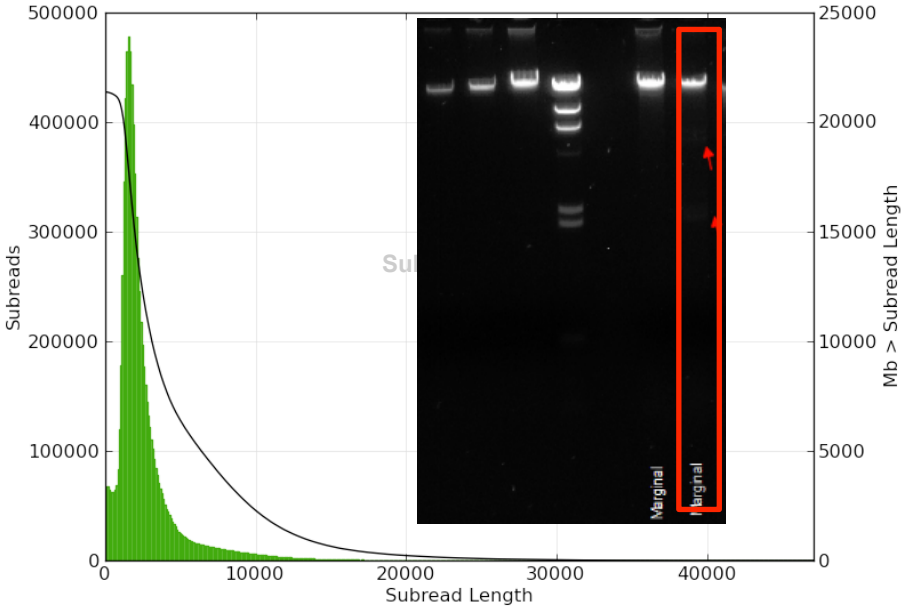
- **Low quality**
  - Degraded sample
- **High purity**
  - 260/230=2.3
  - 260/280=2.12
- **Short insert size**

☹ Low quality & purity (>10kb)

AWSBX – 16 cells – 2,597 bp

BAR25711A2

Mean Subread length	2,597	N50	2,959
Total Number of Bases	21,793,272,159	Number of Reads	8,389,749



Observation

- **Low quality**
  - Unknown short bands and degradation
- **Low purity**
  - A260/A230= -4.52
  - A260/A280= 1.96
- **Primary QC failed but library was made**
- **Short insert size**

Sample QC Workshop – Outline

- **2:30-2:45p – Tootie Tatum**
  - Project Management Office (PMO) & User Project Data Submission
- **2:45-3:00p – Mansi Chovatia**
  - Sample Management & QC Overview
- **3:00-3:05p – Kathleen Lail**
  - New Feature Support – DNAstable
- **3:05-3:20p – David Dilworth**
  - Sample Quantification & QC Developments
- **3:20-3:45p – Yuko Yoshinaga**
  - Sample Quality for PacBio Sequencing
- **3:45-4:00p – Q&A**