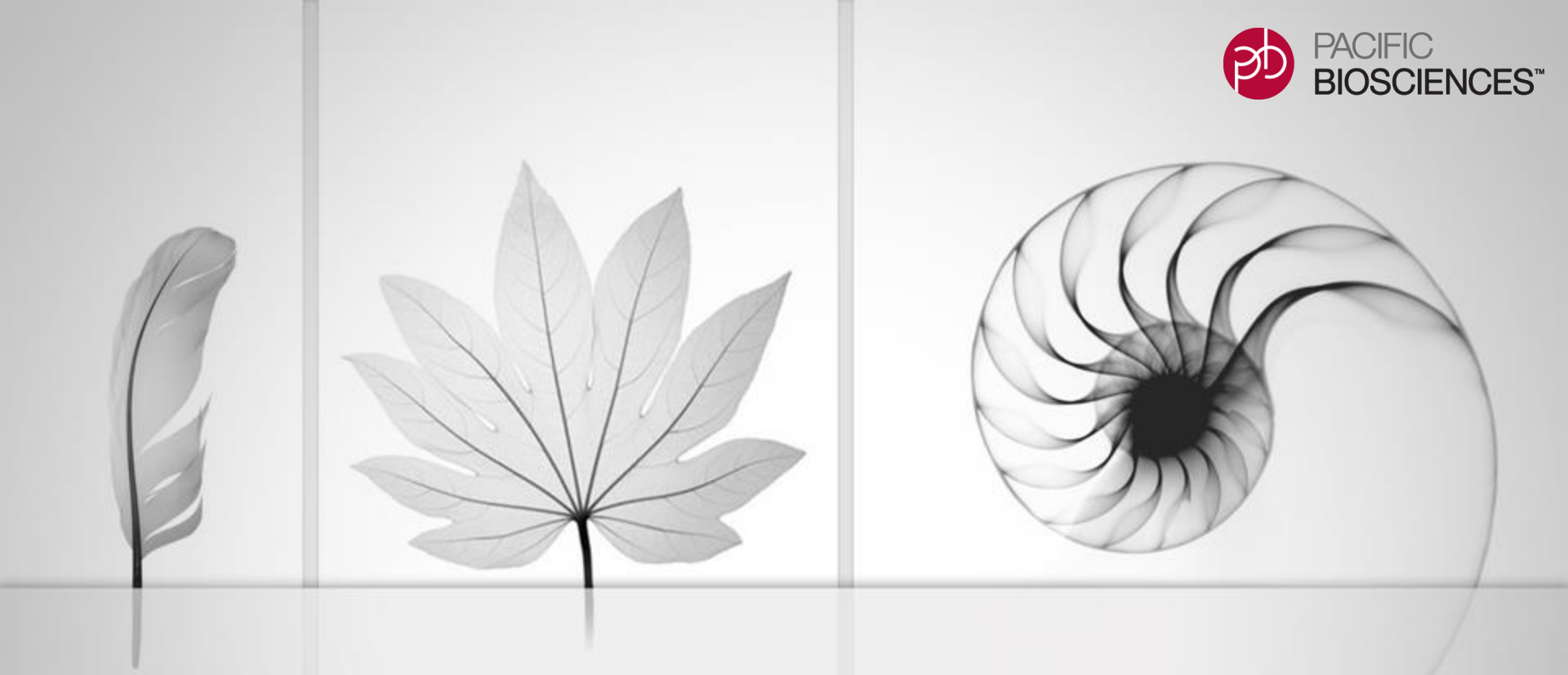


REVEALING SMRT™ BIOLOGY

On-site System Training Workflow

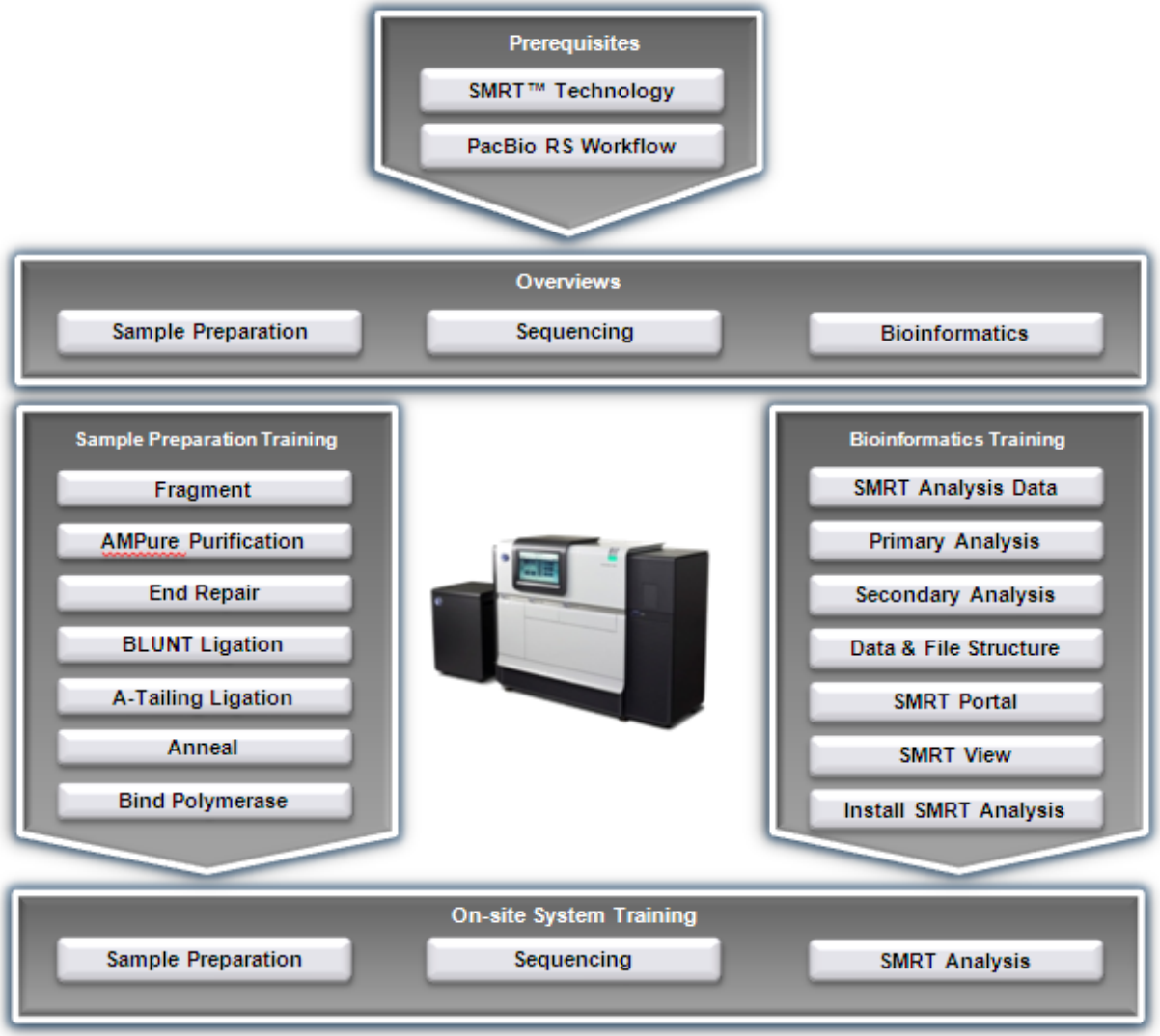
Pacbio RSII系统标准客户培训流程

FU Wei
Senior Application Specialist

Gene Company Limited
基因有限公司
A Gene Group Company

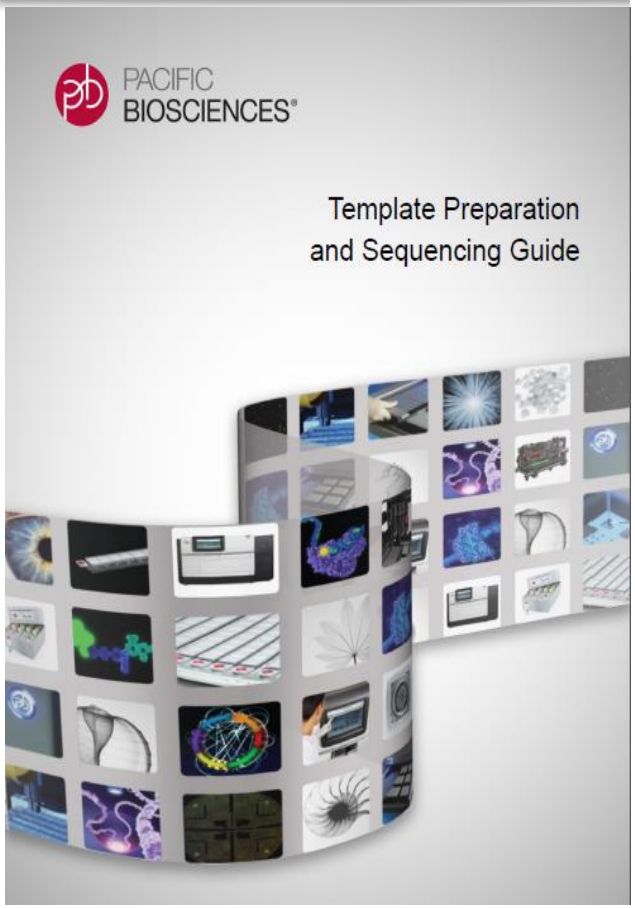
Login to the **PacBio Portal** and perform following tasks

http://www.pacificbiosciences.com/support/pubmap/training.html



PACIFIC BIOSCIENCES™

Download documents



PACIFIC BIOSCIENCES®

Procedure & Checklist - 2 kb Template Preparation and Sequencing

Before You Begin

To perform this procedure, you must have the Pacific Biosciences®:

- DNA Template Prep Kit (verify you have the correct kit for your insert size)
- DNA/Polymerase Binding Kit
- DNA Sequencing Kit
- DNA Control Complex
- SMRT® Cells for standard sequencing

This procedure can be used to prepare 1500 bp to 3 kb libraries from 500 ng up to 750 ng of sheared and concentrated DNA. If preparing larger amounts of DNA, scale all the reaction volumes proportionally (e.g., if the input amount of DNA is double the amount set forth in this procedure, then double all the reaction volumes listed in the tables).

Insert Size Target	Insert Size Range	Sheared and Concentrated DNA Amount	Ligation	DNA Damage Repair
2 kb	~ 1500 bp to 3			

For DNA sequencing, it is recommended

Fragment and Concentrate DN

Use a Covaris® System (with Adaptive Fo concentrate your DNA sample. Note that ple loss is to be expected as a result of th ficient amounts of starting DNA in order t (25 ng/µL) for the End-Repair reaction.

Target Shear Size	Tube	Shearing Volume	Temp Chille
2.0 kb	Mini-tube Clear	200.0	4°C

PACIFIC BIOSCIENCES®

Procedure & Checklist - 10 kb Template Preparation and Sequencing

Before You Begin

To perform this procedure, you must have the Pacific Biosciences®:

- DNA Template Prep Kit (verify you have the correct kit for your insert size)
- DNA/Polymerase Binding Kit
- DNA Sequencing Kit
- DNA Control Complex
- SMRT® Cells for standard sequencing.

This procedure can be used to prepare 10 kb libraries from 5 µg of sheared and concentrated DNA. For sheared DNA libraries and PCR products greater than 4 kb, any DNA damage (generated during DNA extraction and PCR amplifications) must be repaired using the DNA Damage Repair reagents provided by Pacific Biosciences and according to the instructions as set forth in this Procedure. Common types of damage may include abasic sites, cytosine deamination, and oxidation.

all the reaction volumes proportionally (e.g., if the input amount of procedure, then double all the reaction volumes listed in the tables).

ge	Sheared and Concentrated DNA Amount	Ligation	DNA Damage Repair
	5 µg	Blunt	Required

to perform titrations to ensure optimal loading.

A

ear your DNA sample using the following conditions. Note that , approximately 20% to 50% sample loss is to be expected as a result . Therefore, be sure to have sufficient amounts of starting DNA in concentrated DNA (140 ng/µL) for the Damage Repair reaction.

ance can vary with each shearing assembly, we recommend optimiz- assembly is used.

	Speed Code	Cycles	Shearing Volume
	SC8 - SC10	20	200 µL

PACIFIC BIOSCIENCES®

Procedure & Checklist - Low-Input 10 kb Library Preparation and Sequencing (MagBead Station)

Before You Begin

To perform this procedure, you must have the PacBio®:

- DNA Template Prep Kit (verify you have the correct kit for your insert size)
- DNA/Polymerase Binding Kit
- MagBead Kit
- DNA Sequencing Kit
- DNA Control Complex
- SMRT® Cells for standard sequencing.

This procedure can be used to prepare 10 kb libraries from 1µg up to 5 µg of sheared and concentrated DNA. If preparing libraries with DNA input amounts great than 5 µg, scale all the reaction volumes proportionally (e.g., if the input amount of DNA is 10 µg, which is double the maximum input amount set forth in this procedure, then double all the reaction volumes listed in the tables).

Insert Size Target	Insert Size Range	Sheared and Concentrated DNA Amount	Ligation	DNA Damage Repair
10 kb	8 kb to 12 kb	1 to 5 µg	Blunt	Required

For DNA sequencing, it is recommended to perform titrations to ensure optimal loading.

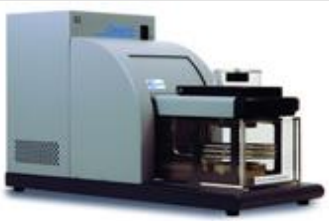
Fragment and Concentrate DNA

Use a Covaris® g-TUBE™ to shear your DNA sample. The most up-to-date guidance on how to use the g-TUBE, along with recommended centrifuges and centrifugation speeds, can be found in the g-TUBE user manual available for download from the Covaris website. Depending upon the quality of your sample, approximately 20% to 50% sample loss is to be expected as a result of the shearing and concentration process. Therefore, be sure to have sufficient amounts of starting DNA in order to have at least 1 µg of sheared and concentrated DNA (30 ng/µL) for the Damage Repair reaction.

Note that a Hydroshear® Shearing Device can also be used to shear your DNA sample. However, because Hydrodynamic shearing performance can vary with each shearing assembly, we recommend optimizing the shearing whenever a new shearing assembly is used.

Lab equipment and supplies

- **Covaris**
  or Hydroshear

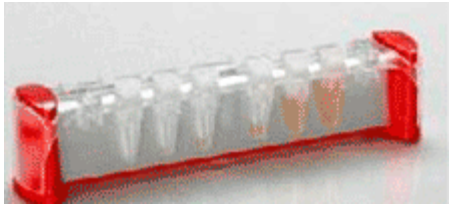


or

- **Agilent 2100 Bioanalyzer**

- **Qubit™ Quantitation Platform**
- **Fluorometer**

- **Agencourt ® AMPureXP® Beads**



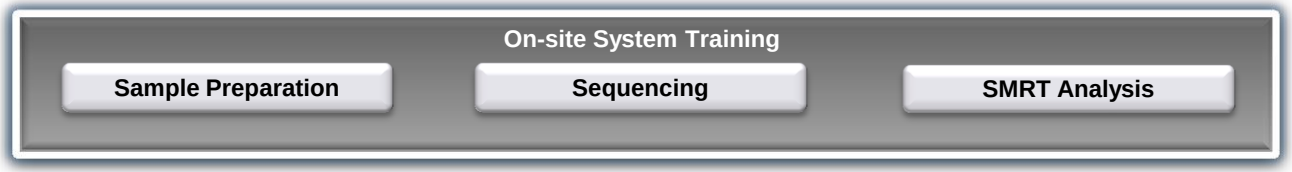
- **Low bind tubes**
- **PCR System**
- **Centrifuge (Plate)**
- **Vortex Genie**

P6/C4 reagents&Consumables

厂家	类型	货号	描述
PCB	耗材	000-448-888	Sample Plate (50 pack)
		000-882-901	Sample Plate Septa (50 pack)
		000-947-365	Mixing Plate (160 pack)
		100-243-500	Mixing Plate (40 pack)
		100-237-900	Sequencing plate septa
		001-292-541	Tube septa
		100-154-200	Pipet Tips (10 racks)
		001-252-068	Pipet Tips (50 racks)
	试剂	100-171-800	SMRT Cell 8Pac V3 (8 Cells)
		100-209-300	SMRT Cell Oil
		100-133-600	MagBead Kit
		100-265-900	AMPure PB (5ml)
		100-259-100	SMRTbell Template Prep Kit 1.0
		100-286-300	SMRTbell HT Template Prep Kit 1.0
		100-356-200	DNA Sequencing Reagent 4.0
		100-356-400	DNA Sequencing Reagent 4.0 (10 package)
		100-372-700	DNA/Polymerase Binding Kit 6.0 V2
		100-356-500	DNA Internal Control Complex (P6)
	其他	100-514-900	SMRTbell™ Barcoded Adapter Complete Prep Kit - 96
		100-465-900	SMRTbell™ Damage Repair Kit
		100-466-100	Barcoded Universal F/R Primers Plate - 96

On-site System Training

- Duration: 5 days
- Covers the end-to-end workflow:



- Primary Users
  - Scientists
  - Research Associates
  - Bioinformaticians

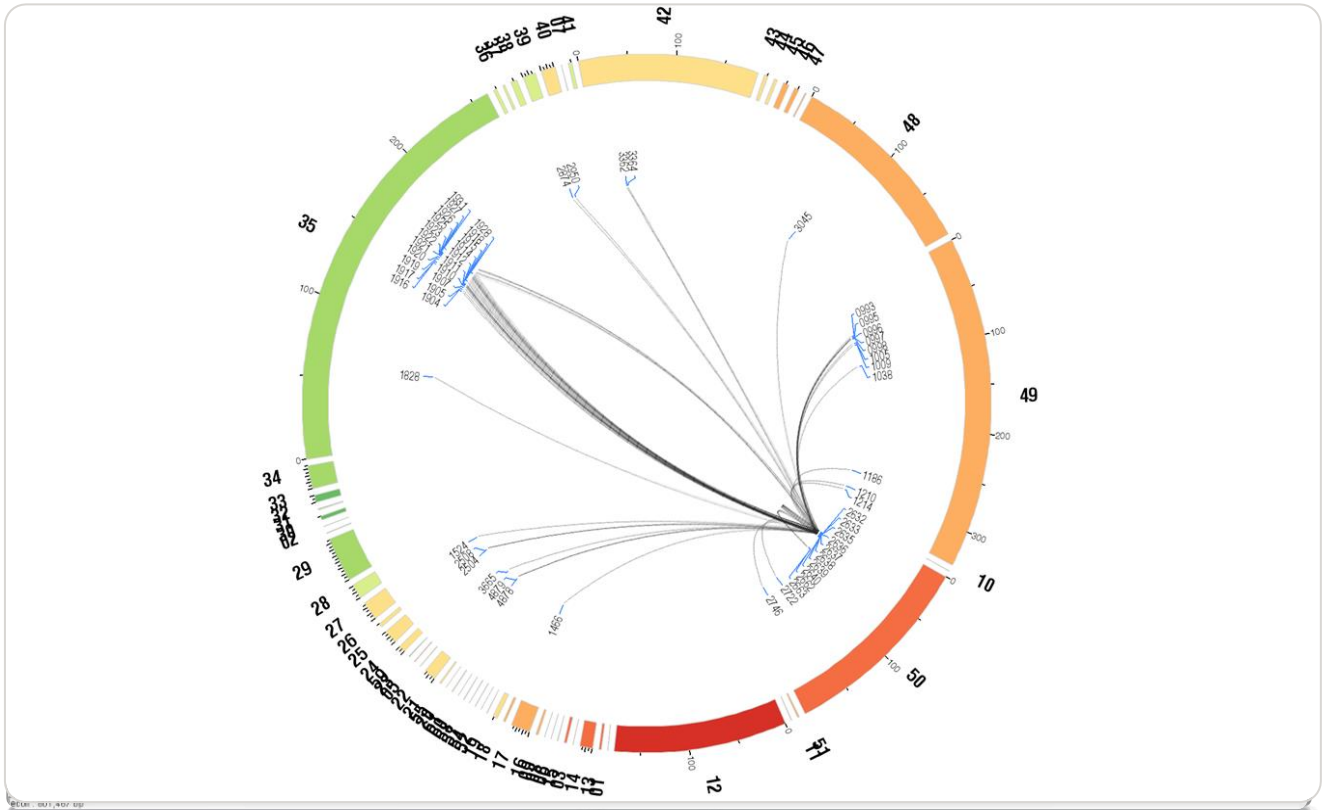
- Standard Training
  - Lambda only
  - 10 kb
  - 5 days total
  - Standard Training Kit
  - Help customers with their samples once training is complete

Training Agenda:

Time	Day 1 (Monday)	Day 2 (Tuesday)	Day 3 (Wednesday)	Day 4 (Thursday)
	Training Overview	10 kb Library Construction (Contd.)	Annealing/Bindinfg	Data Analysis
9:00 AM	Technology Overview	Heat Kill Ligase	Data Analysis Overview	•SMRT Portal •SMRT View
9:30 AM	Workflow Overview	Exo Treatment		
10:00 AM		Double Ampure Purification	Calculator Overview	
10:30 AM	10 kb Lambda Library Shearing	Quantitation, Bioanalyzer, Break		
11:00 AM		Quantitation, Bioanalyzer, Break	Hands On Pactice Calculator	Metrics Review
11:30 AM	Purification of sheared DNA	MBS Binding		
12:00 PM	Lunch Break		Lunch Break	Lunch Break
1:00 PM	Quantitation (Qubit, nanodrop). BioAnalyzer	Training Run RS Remote Set Up	Troubleshooting Overview	Instrument RS Overview
1:30 PM				
2:00 PM	10Kb Library prep		RS remote Overview	Laboratory Setup overview
2:30 PM	PreCR	Instrument Run		
3:00 PM	End Repair		Hands On Pactice RS remote	
3:30 PM	Purify	Additional Training		
4:00 PM	Quantitation/Quality			
4:30 PM	Blunt Ligation (safe stopping point), Store at 4 C overnight			
5:00 PM	Review: Day 1	Review: Day 2	Review: Day 3	Review: Day 4

Sequencing Workflow

Template Preparation | Run Design | Polymerase Binding | Instrument Run | Primary Analysis | Secondary Analysis | Tertiary Analysis



SMRT Portal Circular View

After Training

- Sign-off training completion form
- Complete the quiz
- Follow up on bioinformatics by PacBio Tech Support
  - Technical bioinformatics issues
  - Q&A
- Contact PacBio Tech support
  - Toll Free Telephone +1 (877) 920-7222
  - Email techsupport@pacificbiosciences.com
  - Logging a case on PacBio Portal

File Edit View Favorites Tools Help

DISTRIBUTOR PORTAL

Case Edit

Search

Search All

search

Advanced Search...

Create New...

Recent Items

- 2011-05-23 Office Hour - Low Yield Troubleshooting, Error Messages, PM
- 2011-05-02 Office Hour - Web Based Calculator
- 2011-04-18 Office Hour - Release v1.2.1 Field Webinar
- 2011-03-28 Office Hour - SMRT Cell Loading
- Repair stage pi...
- 2011-03-16 Office Hour - Annealing and Binding Calculator
- Cholera Webinar by Eric Schadt
- Recorded Webinar - Release v1.2 End-User Training

https://na5.salesforce.com/500/e

Case Edit | Submit | Save & Close | Submit & Add Attachment | Save & New | Cancel

Submit & Add Attachment

= Required Information

Case Information

Case Owner: Yuanjie Zhou

Account Name: Click lookup icon...

Contact Name:

Parent Case:

Case Manager: --None--

Visible in Customer Portal:

Status: Submitted

Solution Release: --None--

Description Information

Type: Complaint

Case Reason: --None--

Case Reason Detail: --None--

Subject:

Description:

Internal Comments:

Other Information

PACIFIC BIOSCIENCES

SMRT® Technology

SMRT® Cell

Thank you for attention