



SMRT Link v5.0 Update

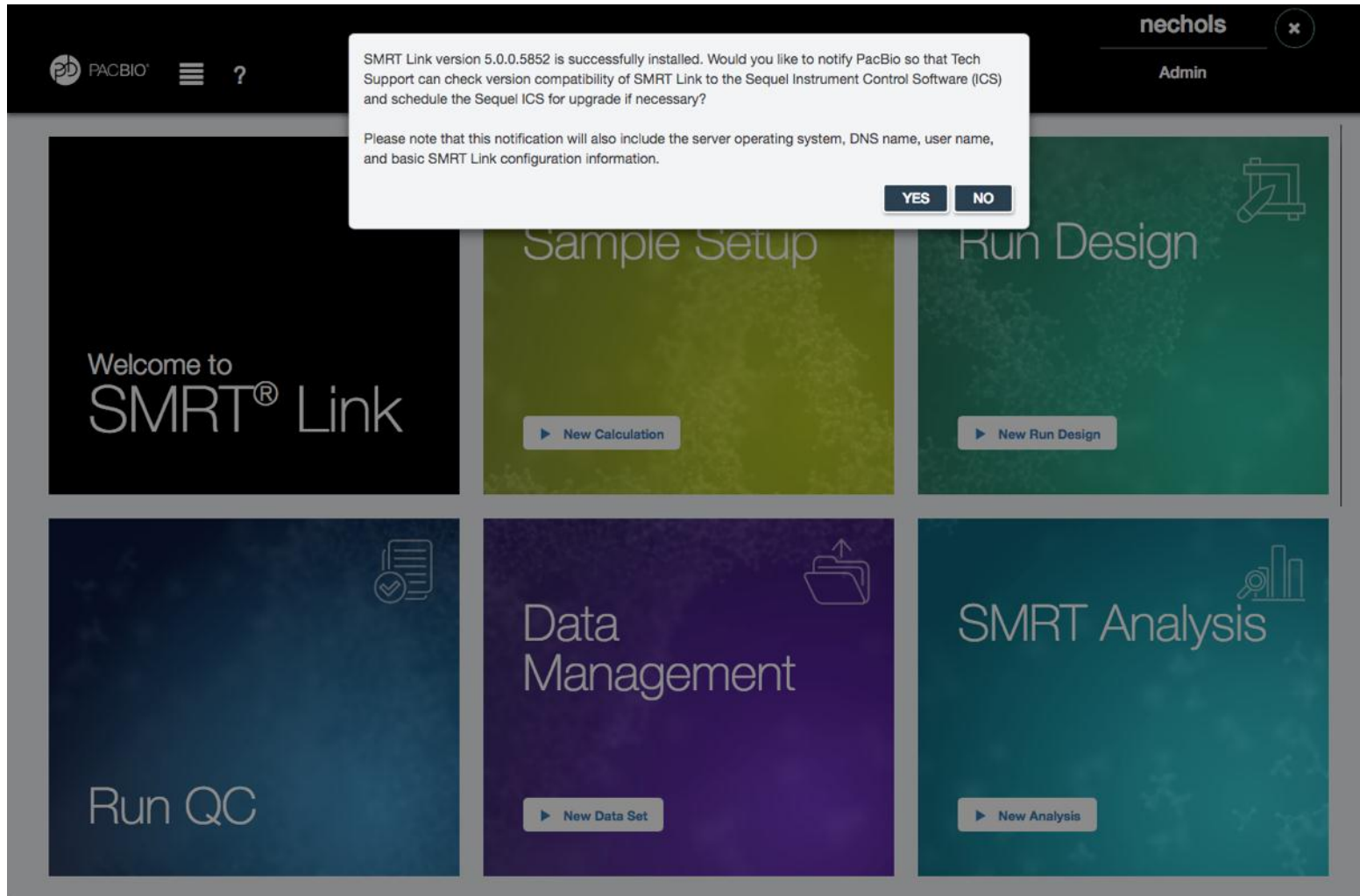
AGENDA

- SMRT Link platform new features
- SMRT Link module-based new features
 - Data Management
 - SMRT Analysis
- Known issues
- Fixed issues

SMRT LINK V5.0 PLATFORM NEW FEATURES

- Notify Tech Support about readiness for ICS upgrade
- Easy update of SMRT Link compatibility with new chemistries which does not require reinstalling SMRT Link
- Persists configuration of LDAP and SSL Certificate if you have them integrated with SMRT Link v4.0
- Ability to open multiple different browser tabs with SMRT Link, share URLs, and use the Back browser button
- Support for the new 384 barcodes set for Sequel and RS II

NOTIFY TECH SUPPORT OF READINESS FOR ICS UPGRADE



The screenshot shows the SMRT Link software interface. At the top left is the PACBIO logo and a menu icon. At the top right, the user name "nechols" and the role "Admin" are displayed. A central notification dialog box is open, containing the following text:

SMRT Link version 5.0.0.5852 is successfully installed. Would you like to notify PacBio so that Tech Support can check version compatibility of SMRT Link to the Sequel Instrument Control Software (ICS) and schedule the Sequel ICS for upgrade if necessary?

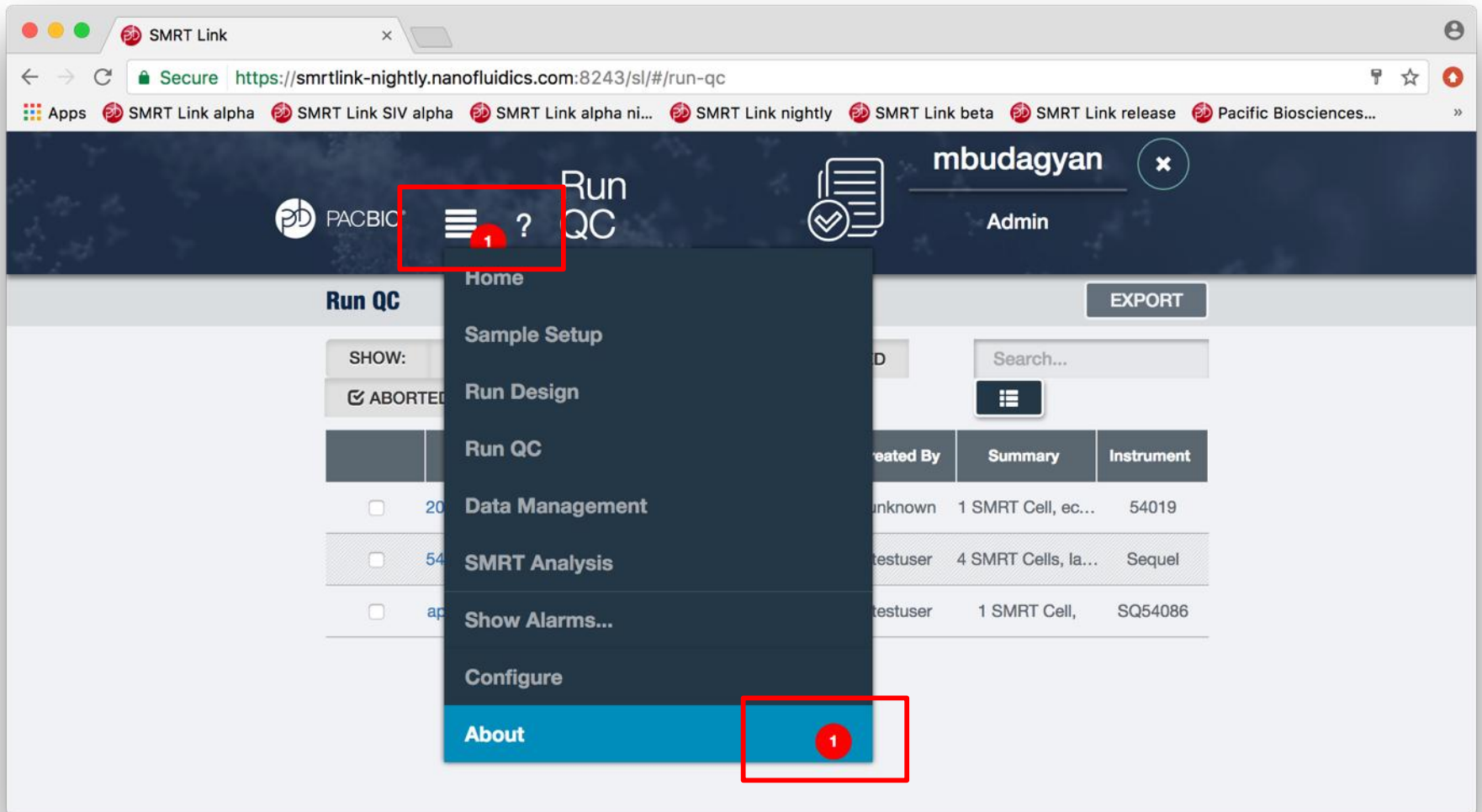
Please note that this notification will also include the server operating system, DNS name, user name, and basic SMRT Link configuration information.

At the bottom of the dialog box are two buttons: "YES" and "NO".

The background interface is divided into six main sections:

- Welcome to SMRT® Link**: A large black panel with white text.
- Sample Setup**: A green panel with a "New Calculation" button.
- Run Design**: A teal panel with a "New Run Design" button.
- Run QC**: A dark blue panel with a checkmark icon.
- Data Management**: A purple panel with a folder icon and a "New Data Set" button.
- SMRT Analysis**: A teal panel with a bar chart icon and a "New Analysis" button.

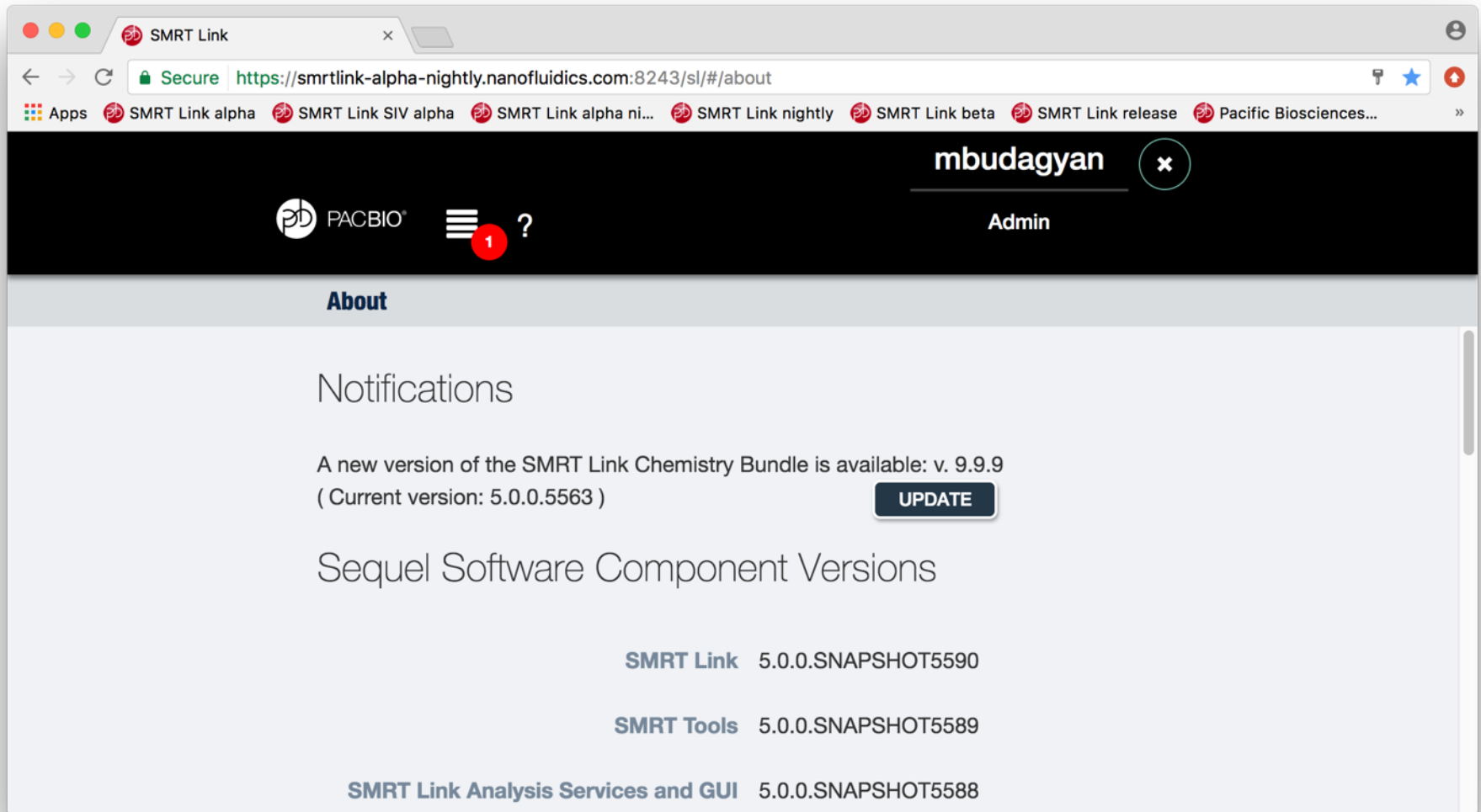
UPDATE OF CHEMISTRY WITHOUT SMRT LINK UPGRADE



The screenshot shows the SMRT Link web interface. The browser address bar displays the URL: <https://smrtlink-nightly.nanofluidics.com:8243/sl/#/run-qc>. The user is logged in as 'mbudagyan' with 'Admin' privileges. A navigation menu is open, listing the following options: Home, Sample Setup, Run Design, Run QC, Data Management, SMRT Analysis, Show Alarms..., Configure, and About. Two red boxes with the number '1' highlight the hamburger menu icon in the top left and the 'About' option at the bottom of the menu.

Created By	Summary	Instrument
unknown	1 SMRT Cell, ec...	54019
testuser	4 SMRT Cells, la...	Sequel
testuser	1 SMRT Cell,	SQ54086

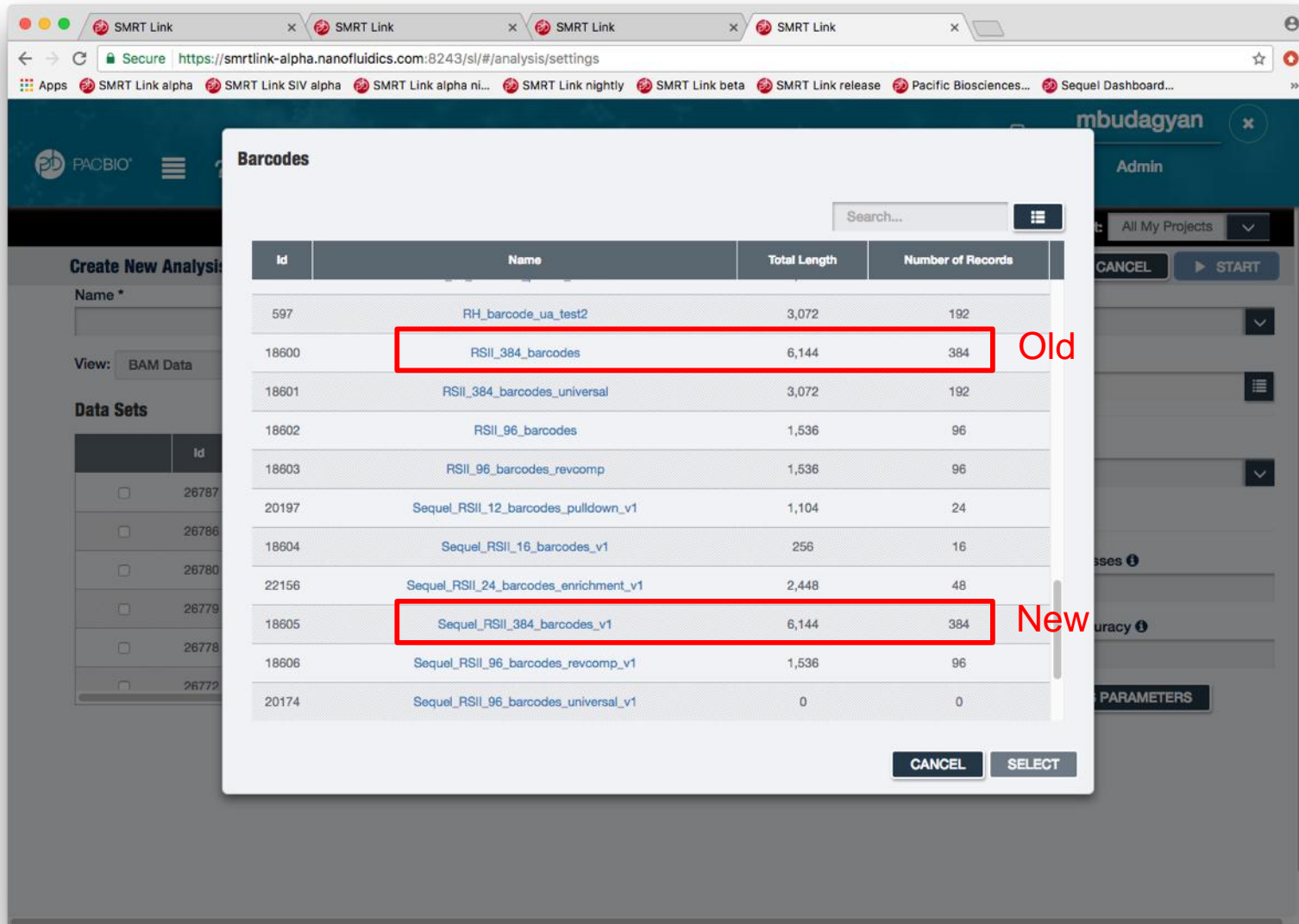
UPDATE OF CHEMISTRY WITHOUT SMRT LINK UPGRADE



The screenshot shows a web browser window with the URL `https://smrtlink-alpha-nightly.nanofluidics.com:8243/sl/#/about`. The user is logged in as `mbudagyan` with an `Admin` role. The page title is `About`. A notification banner states: "A new version of the SMRT Link Chemistry Bundle is available: v. 9.9.9 (Current version: 5.0.0.5563)" with an `UPDATE` button. Below the notification, the "Sequel Software Component Versions" are listed:

Component	Version
SMRT Link	5.0.0.SNAPSHOT5590
SMRT Tools	5.0.0.SNAPSHOT5589
SMRT Link Analysis Services and GUI	5.0.0.SNAPSHOT5588

SUPPORT FOR THE NEW 384 BARCODES SETS



The screenshot shows the SMRT Link interface with a 'Barcodes' modal window. The modal contains a table with the following data:

Id	Name	Total Length	Number of Records
597	RH_barcode_ua_test2	3,072	192
18600	RSII_384_barcode	6,144	384
18601	RSII_384_barcode_universal	3,072	192
18602	RSII_96_barcode	1,536	96
18603	RSII_96_barcode_revcomp	1,536	96
20197	Sequel_RSII_12_barcode_pull_down_v1	1,104	24
18604	Sequel_RSII_16_barcode_v1	256	16
22156	Sequel_RSII_24_barcode_enrichment_v1	2,448	48
18605	Sequel_RSII_384_barcode_v1	6,144	384
18606	Sequel_RSII_96_barcode_revcomp_v1	1,536	96
20174	Sequel_RSII_96_barcode_universal_v1	0	0

The row with ID 18600 (RSII_384_barcode) is highlighted with a red box and labeled 'Old'. The row with ID 18605 (Sequel_RSII_384_barcode_v1) is highlighted with a red box and labeled 'New'. The modal also includes a search bar, a 'CANCEL' button, and a 'SELECT' button.



PACBIO®

Data Management

NEW FEATURES IN DATA MANAGEMENT

- Projects – View and One Click access
- Project Ownership and Management
- Share Projects with SMRT Link Users
- Data Sets:
 - Delete
 - Copy
 - Export

VIEW AND ACCESS PROJECTS

Two ways to access Projects – through “View” and One Click access

The interface is titled "Data Management" and is accessed by user "tkerelska". It features several action buttons: "+ CREATE DATA SET", "+ CREATE PROJECT", "VIEW OR IMPORT SEQUENCE DATA", and "EXPORT DATA SETS".

View: BAM Data

Id	Name	Run Name	Cell Index	Total Length of Subreads (bp)	Date Created
25111	RP21D24_11k-060117	RP21D24_11k-060117	4	85,831,090	6/2/2017, 8:38:21 AM
25110	RP21D24_11k-060117	RP21D24_11k-060117	3	77,941,539	6/2/2017, 8:37:35 AM
25109	RP21D24_11k-060117	RP21D24_11k-060117	2	99,507,912	6/2/2017, 8:37:16 AM
25107	RP21D24_11k-060117	RP21D24_11k-060117	0	154,889,915	6/2/2017, 8:36:31 AM
25106	RP21D24_11k-060117	RP21D24_11k-060117	4	85,831,090	6/2/2017, 7:33:03 AM
25105	06-01-2017_EL_RC_48kB_LambdaBel... 06-01-2017_EL_RC_48kB_LambdaBel...	06-01-2017_EL_RC_48kB_LambdaBel...	1	56,599,312	6/2/2017, 5:28:43 AM
25104	RP21D24_11k-060117	RP21D24_11k-060117	3	77,941,539	6/2/2017, 4:17:06 AM

VIEW PROJECTS

Once a project is selected, its contents can be edited from users with proper access permissions

The screenshot displays the PACBIO Data Management interface. The top navigation bar includes the PACBIO logo, a menu icon, a user profile for 'tkerelska' with 'Admin' permissions, and a 'Project:' dropdown menu currently set to 'All My Projects'. Below the navigation bar, there are four main action buttons: '+ CREATE DATA SET', '+ CREATE PROJECT', 'VIEW OR IMPORT SEQUENCE DATA', and 'EXPORT DATA SETS'. A 'View: Projects' dropdown is highlighted with a red box. Below this is a table of projects:

Id	Name
15	Project X
14	test 2
13	test
1	General Project
3	Test

An 'Edit Project' modal window is overlaid on the bottom right, also highlighted with a red box. It contains the following sections:

- Project Name:** Test Project Share
- Description:** Demo sharing projects
- Associated Data Sets:** A search button 'SELECT DATASETS' and two entries: 'AppsLab_SC_54026_T052517' and 'AppsLab_SC_54086_T052517', each with a close icon.
- Members:**
 - Access for All SMRT Link Users:** None
 - Access for Individual SMRT Link Users:**

Tzvetana Kerelska (tkerelska@pacificbiosciences.com)	Owner	✕
Mary Budagyan (mbudagyan@pacificbiosciences.com)	Owner	✕
Marty Badgett (mbadgett@pacificbiosciences.com)	View	✕
Brittany Gigliotti (bgigliotti@pacificbiosciences.com)	View	✕

At the bottom of the modal, there is a search input field 'Enter partial user name here' and a 'SEARCH BY NAME' button.

SHARE PROJECTS WITH SMRT LINK USERS

Share Projects with All SMRT Link Users – None, View or View/Edit

The screenshot shows the 'Data Management' interface in the PACBIO system. The user 'tkerelska' is logged in as an Admin. The current project is 'All My Projects'. The 'Create Project' section is active, with 'Test Project Share' as the project name and 'Demo sharing projects' as the description. Two associated data sets are listed: 'AppsLab_SC_54026_T052517' and 'AppsLab_SC_54086_T052517'. The 'Members' section is highlighted with a red box, showing the 'Access for All SMRT Link Users' dropdown menu open. The menu options are 'None', 'View', and 'View/Edit'. The 'View/Edit' option is selected. Below the menu, a table lists individual SMRT Link Users with their roles and access levels. A search bar is present to find users by name.

Members

Access for All SMRT Link Users

- None
- View
- View/Edit

Individual SMRT Link Users	Role	Access
tkerelska (tkerelska@pacificbiosciences.com)	Owner	View/Edit
mbudagyan (mbudagyan@pacificbiosciences.com)	View/Edit	View/Edit
mbadgett (mbadgett@pacificbiosciences.com)	View	View/Edit

SEARCH BY NAME

User Name: Marty Badgett
Email: mbadgett@pacificbiosciences.com

ADD SELECTED USER

SHARE PROJECTS WITH SMRT LINK USERS

Share Projects with specific SMRT Link Users – Owner, View, View/Edit

The screenshot shows the 'Data Management' interface for user 'tkerelska'. The 'Create Project' form is active, with 'Test Project Share' as the project name and 'Demo sharing projects' as the description. The 'Associated Data Sets' section shows two datasets: 'AppsLab_SC_54026_T052517' and 'AppsLab_SC_54086_T052517'. The 'Members' section is highlighted in red and contains the following information:

- Members**
- Access for All SMRT Link Users: None
- Access for Individual SMRT Link Users:

User Name	Role	Action
Tzvetana Kerelska (tkerelska@pacificbiosciences.com)	Owner	[Dropdown] [X]
Mary Budagyan (mbudagyan@pacificbiosciences.com)	Owner	[Dropdown] [X]
Marty Badgett (mbadgett@pacificbiosciences.com)	View	[Dropdown] [X]
Brittany Gigliotti (bgigliotti@pacificbiosciences.com)	View	[Dropdown] [X]

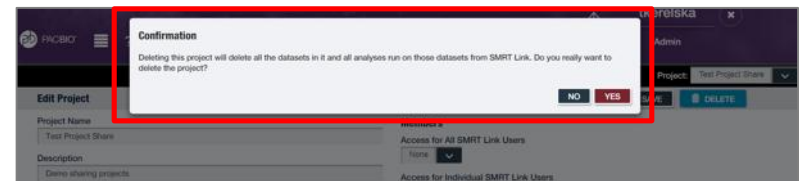
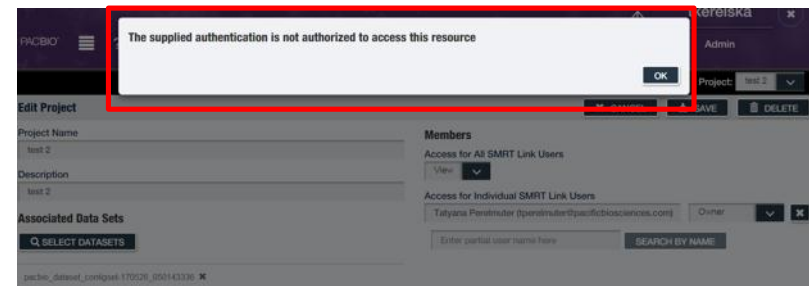
A search dropdown menu is open, showing 'SEARCH BY' with options: Owner, View, and View/Edit. The 'View/Edit' option is highlighted in blue. Below the table is an 'ADD SELECTED USER' button.

Note: SMRT Link will seemingly let user delete the only project owner, but when project is saved, the owner is not deleted.

PROJECT OWNERSHIP AND MANAGEMENT

Permissions and available actions

- User creating the project is project owner
- Unless shared with other SMRT Link users, project is only accessible to the user that creates it
- Project must have one or multiple owners
- Only owner/s can delete a project
- Deleting a project deletes all data sets and analyses in it

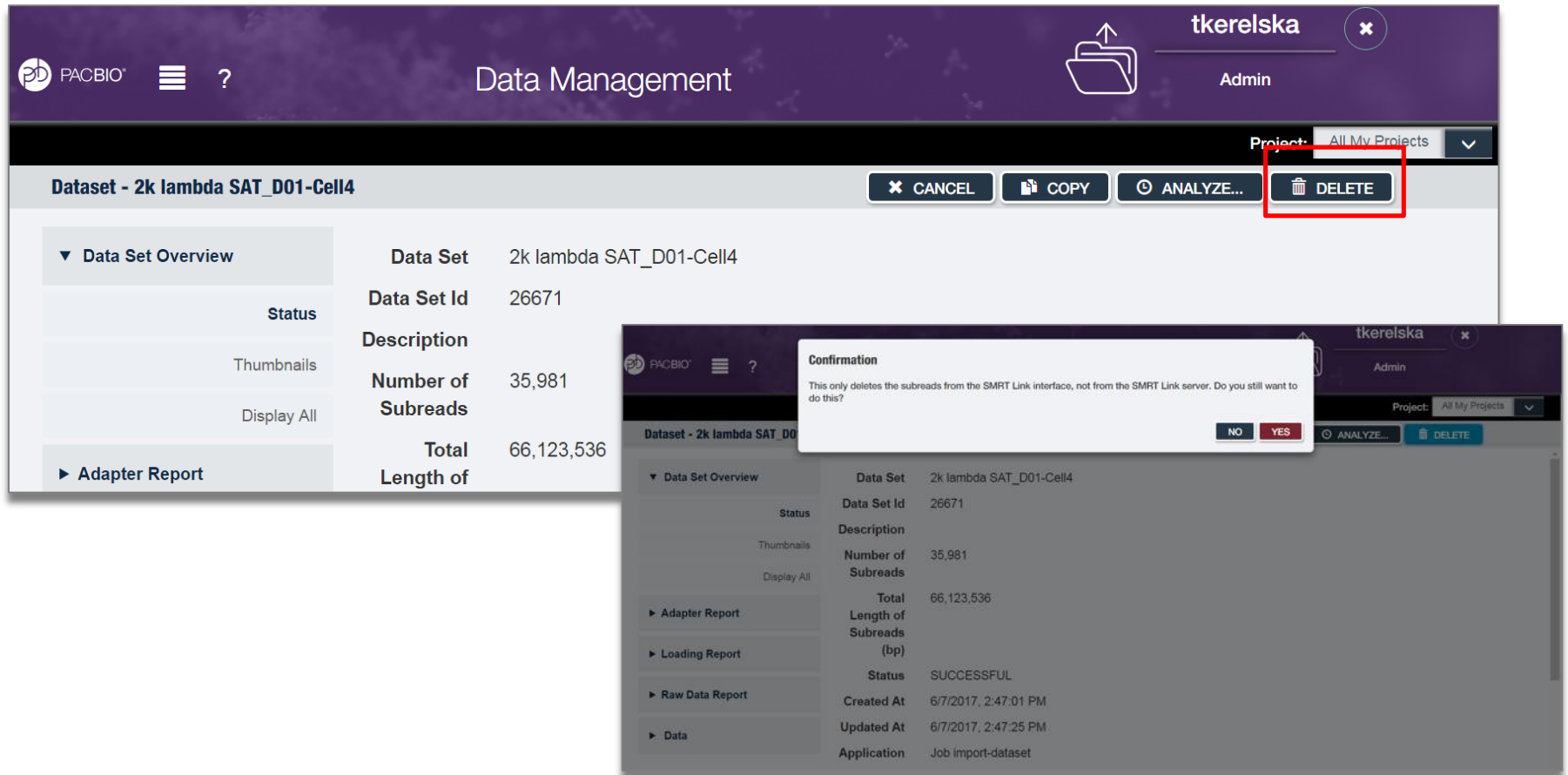


Note: If the Project Owner has set access permissions for all SMRT Link users through the “Access for All SMRT Link users” option, s/he can increase user's access level by adding him/her to the individual member list through “Access for Individual SMRT Link Users”. However, user access can not be decreased through described mechanism.

Example: If "Access for All" is "View" and Owner individually gives user A "View/Edit", then user A will be able to edit. But if "Access for All" is "View/Edit" and owner gives user A "View", user A will still be able to edit the Project.

DELETE A DATASET

Dataset is only deleted from SMRT Link, not from the network server



The screenshot shows the PacBio Data Management interface. The user is logged in as 'tkerelska' (Admin). The current project is 'All My Projects'. The dataset being managed is 'Dataset - 2k lambda SAT_D01-Cell4'. The 'DELETE' button is highlighted with a red box. A confirmation dialog is open, asking for confirmation to delete the dataset.

Dataset - 2k lambda SAT_D01-Cell4

Data Set Overview	Data Set	2k lambda SAT_D01-Cell4
Status	Data Set Id	26671
Thumbnails	Description	
Display All	Number of Subreads	35,981
Adapter Report	Total Length of Subreads (bp)	66,123,536
	Status	SUCCESSFUL
	Created At	6/7/2017, 2:47:01 PM
	Updated At	6/7/2017, 2:47:25 PM
	Application	Job import-dataset

Confirmation
This only deletes the subreads from the SMRT Link interface, not from the SMRT Link server. Do you still want to do this?

Buttons: NO, YES

EXPORT DATASETS

Datasets are compressed and exported in selected folder.
 Optionally datasets can be deleted from SMRT Link at the time of export

The screenshots illustrate the export process in the PACBIO Data Management interface:

- First Screenshot:** The 'Data Management' dashboard with the 'EXPORT DATA SETS' button highlighted in red.
- Second Screenshot:** The 'Data Export' dialog box with the 'EXPORT SELECTED DATA' button highlighted in red. Below the button is a table of data sets:

Id	Name	Run Name
<input checked="" type="checkbox"/>	2k lambda SAT_C01-Cell3 (filtered)	SAT_A12_DarkCal_06062017
<input checked="" type="checkbox"/>	2k lambda SAT_C01-Cell3	SAT_A12_DarkCal_06062017
<input type="checkbox"/>	2k lambda SAT_B01-Cell2	SAT_A12_DarkCal_06062017
<input type="checkbox"/>	2k lambda SAT_A01-Cell1	SAT_A12_DarkCal_06062017

- Third Screenshot:** The 'Export Destination' dialog box showing a file tree with folders like 'SRV', 'sys', 'tmp', 'usr', 'var', and files like 'initrd.img', 'vmlinuz', and 'vmlinuz.old'. The 'Selected File' field shows '/home' and 'Export-subreads-06_07_2017_22_49_54.zip'. The 'Delete data set files after export' checkbox is highlighted in red, and the 'EXPORT' button is also highlighted in red.

COPY DATASET

Useful when a user needs to make changes to a dataset without impacting other users working with same dataset

Dataset - DatasetCopyTest

CANCEL COPY ANALYZE... DELETE

Created At 6/9/2017, 3:54:23 PM
Updated At 6/9/2017, 3:54:30 PM
Application Job merge-datasets
Inputs BAM Data
 BAM Data
 BAM Data
Dataset Import Path /pbi/dept/secondary/siv/smrlink/smrlink-alpha/smrtsuite_170220/userdata/jobs_root/015/015131

Data Management

+ CREATE DATA SET + CREATE PROJECT VIEW OR IMPORT SEQUENCE DATA EXPORT DATA SETS

View: BAM Data

Search...

Id	Name	Run Name	Cell Index	Total Length of Subreads (bp)	Date Created
27077	DatasetCopyTest (copy)	SAT_A12_DarkCal_06062017	2	6,530,082,690	6/9/2017, 3:54:56 PM
27076	DatasetCopyTest	SAT_A12_DarkCal_06062017	2	6,530,082,690	6/9/2017, 3:54:30 PM



SMRT Analysis

SMRT ANALYSIS: NEW FEATURES

- Minor Variant Analysis Application (Beta) (also with Barcoding)
 - Identifies and phases minor single nucleotide variants in complex populations
 - Generic Minor Variant application for any target organism or gene
 - Includes example configuration for the HIV *pol* coding region
- Structural Variant Analysis Application (Beta)
 - Identifies large insertions and deletions (default ≥ 50 bp) in a sample relative to a reference from whole genome sequence data
- In case of failed analysis, send log files to PacBio Tech Support
 - Send analysis log files to PacBio tech support in case of failed analysis
 - Send system status files to PacBio tech support in case of system error

NEW APPLICATIONS IN SMRT LINK V5.0

Create New Analysis - Settings

Name *

View: BAM Data

Data Sets

	Id	Run Name	Weight
<input type="checkbox"/>	142	20170123_SMS_SB_A6_3150601_HIV_MinorVariants	SMS_SB_A6_3150601_
<input type="checkbox"/>	136	20170403_NGAT_SB_A6_3250031_Minor_Variant_phi29_Asymm_Mixes	NGAT_SB_A6_32500
<input type="checkbox"/>	134	unknown	
<input type="checkbox"/>	133	unknown	
<input type="checkbox"/>	132	unknown	
<input type="checkbox"/>	122	2015-07-31_42161_1362_NGAT_modA10_OFF_Lexogae	E0 no

Analysis Application *

- Iso-Seq Classify Only
- Iso-Seq with Mapping
- LAA with Barcoding
- Long Amplicon Analysis (LAA 2)
- Minor Variants Analysis [Beta]**
- Minor Variants Analysis with Barcoding [Beta]**
- Resequencing
- Site Acceptance Test (SAT)
- Structural Variant Calling [Beta]**

MINOR VARIANT ANALYSIS

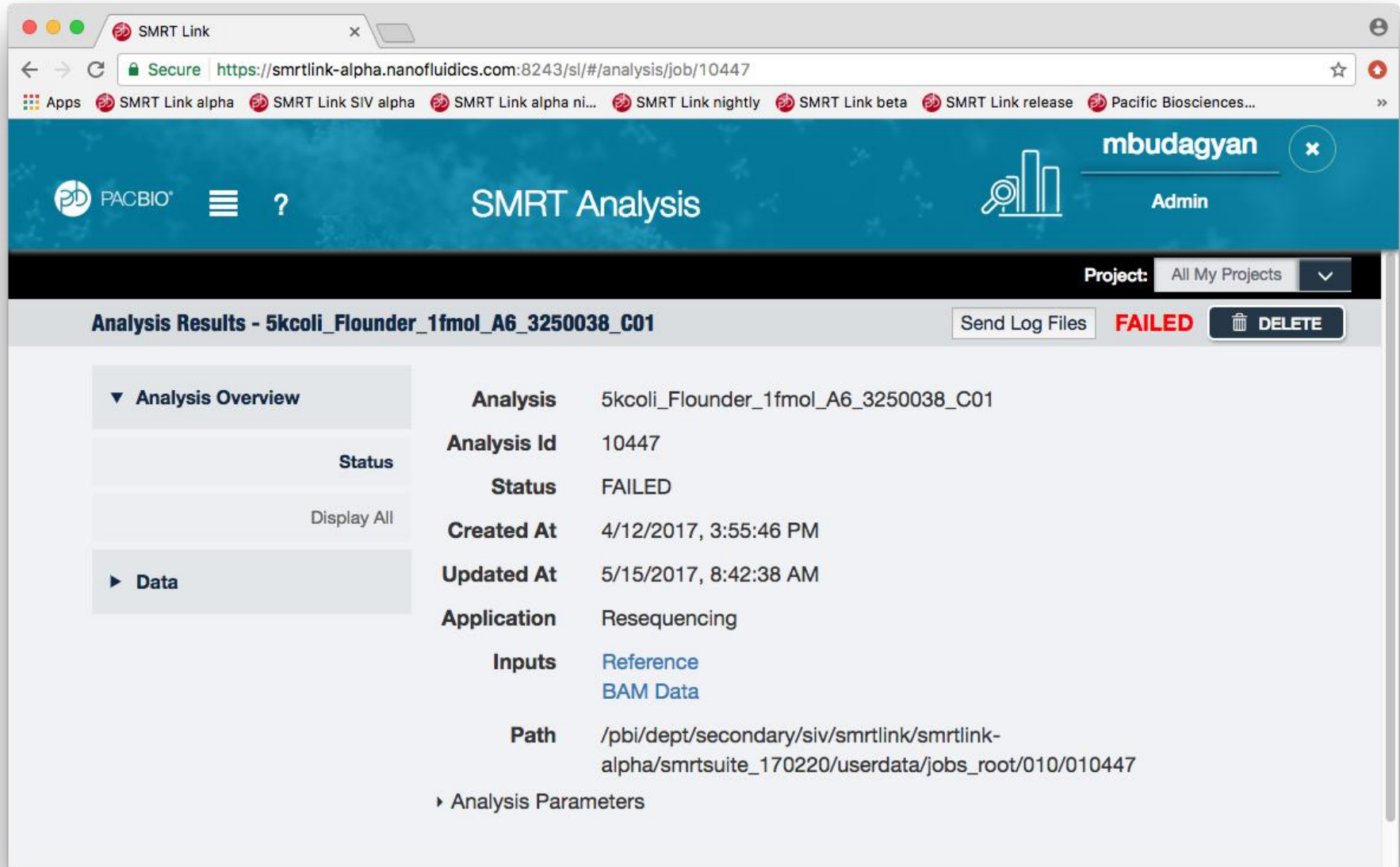


- **Minor Variant Analysis in SMRT Link (powered by Juliet)**
 - Reference-based codon amino acid calling (Indel variants disallowed)
 - $\leq 4\text{kb}$ amplicon (fully spanned by long reads)
 - Supported application: HIV *pol* coding region
 - Great results on viral HIV, oncology BCR-ABL and multigene cancer panel, synthetic biology Phi29 Enzymes
- **Reliable 1% minor variant detection**
 - With 6000 high-quality CCS reads per sample:
 - False negatives below detection (order 1/10,000)
 - $\leq 1\%$ false-positive rate (PCR heteroduplexes)
- **Two analysis applications provided with and without barcodes**
- **Drug resistance annotation for clinicians**
 - Pulled from publicly available databases
- **One-click application**

STRUCTURAL VARIANT ANALYSIS

- Structural Variant Caller in SMRT Link (powered by psv)
 - Reference-based structural variant calling (insertions and deletions ≥ 50 bp)
 - High sensitivity (88%) and low false discovery rate (5%) even at modest sequencing coverage (10-fold)
 - Assign genotypes and annotate variants for repeat content
 - Designed for human genome
- Outputs standard file formats
 - BAM for mapped reads
 - VCF and BED for variant calls
- Provides summary reports
 - Histogram of variant lengths
 - Table of counts by variant types and annotation
- One-click application

SEND FAILED ANALYSIS FILES TO TECH SUPPORT

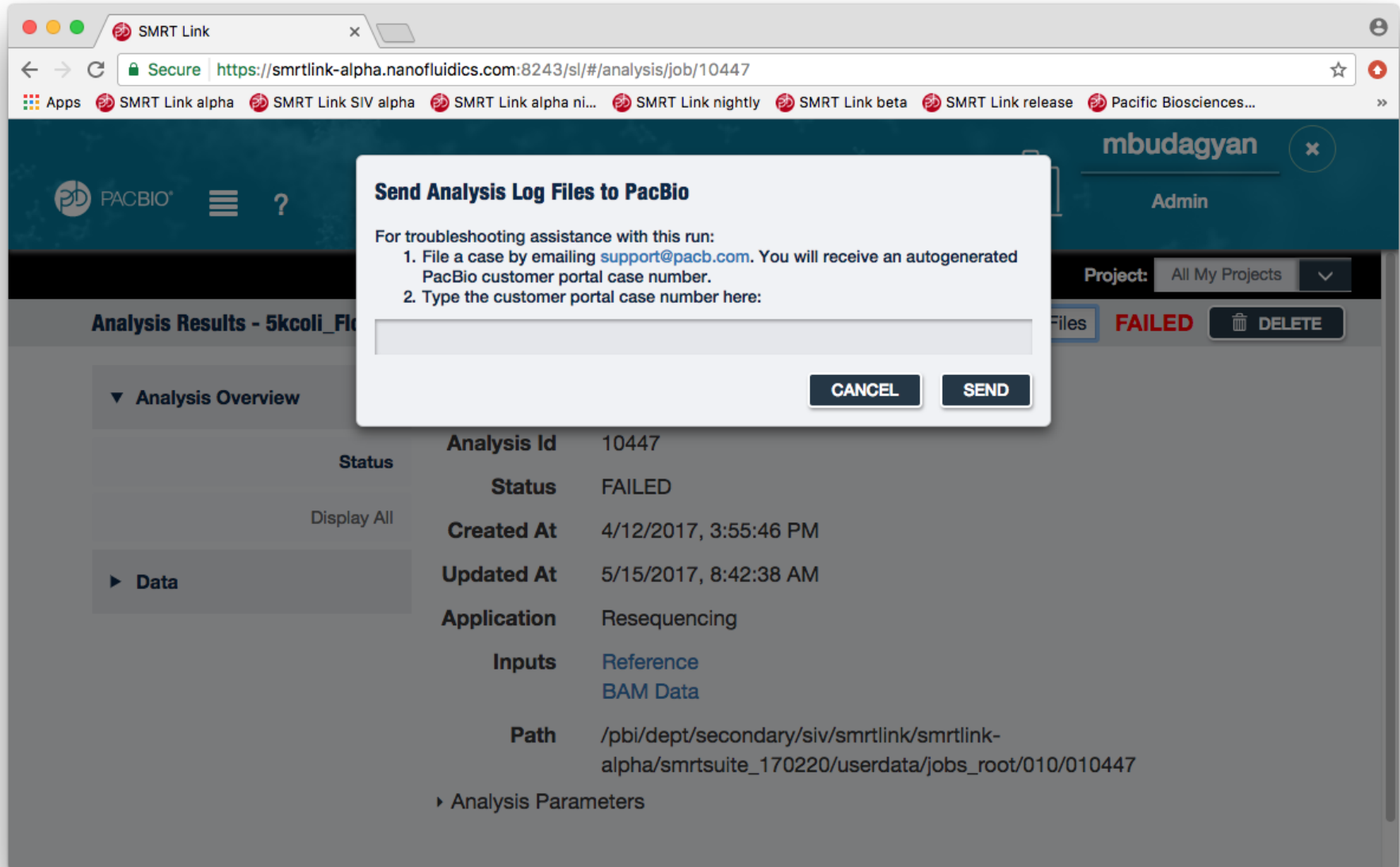


The screenshot shows a web browser window with the URL `https://smrtlink-alpha.nanofluidics.com:8243/sl/#analysis/job/10447`. The page title is "SMRT Analysis" and the user is logged in as "mbudagyan Admin". The main content area displays "Analysis Results - 5kcoli_Flounder_1fmol_A6_3250038_C01" with a "Send Log Files" button, a red "FAILED" status indicator, and a "DELETE" button. A sidebar on the left contains "Analysis Overview", "Status", and "Data" sections. The main content area lists the following details:

Analysis	5kcoli_Flounder_1fmol_A6_3250038_C01
Analysis Id	10447
Status	FAILED
Created At	4/12/2017, 3:55:46 PM
Updated At	5/15/2017, 8:42:38 AM
Application	Resequencing
Inputs	Reference BAM Data
Path	/pbi/dept/secondary/siv/smrtlink/smrtlink-alpha/smrtsuite_170220/userdata/jobs_root/010/010447

Below the details, there is a link for "Analysis Parameters".

SEND FAILED ANALYSIS FILES TO TECH SUPPORT



Send Analysis Log Files to PacBio

For troubleshooting assistance with this run:

1. File a case by emailing support@pacb.com. You will receive an autogenerated PacBio customer portal case number.
2. Type the customer portal case number here:

CANCEL **SEND**

Analysis Results - 5kcoli_Fl

▼ Analysis Overview

Status

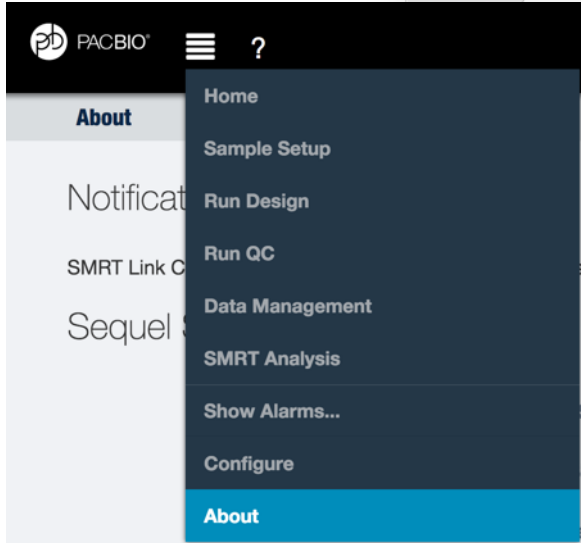
Display All

► Data

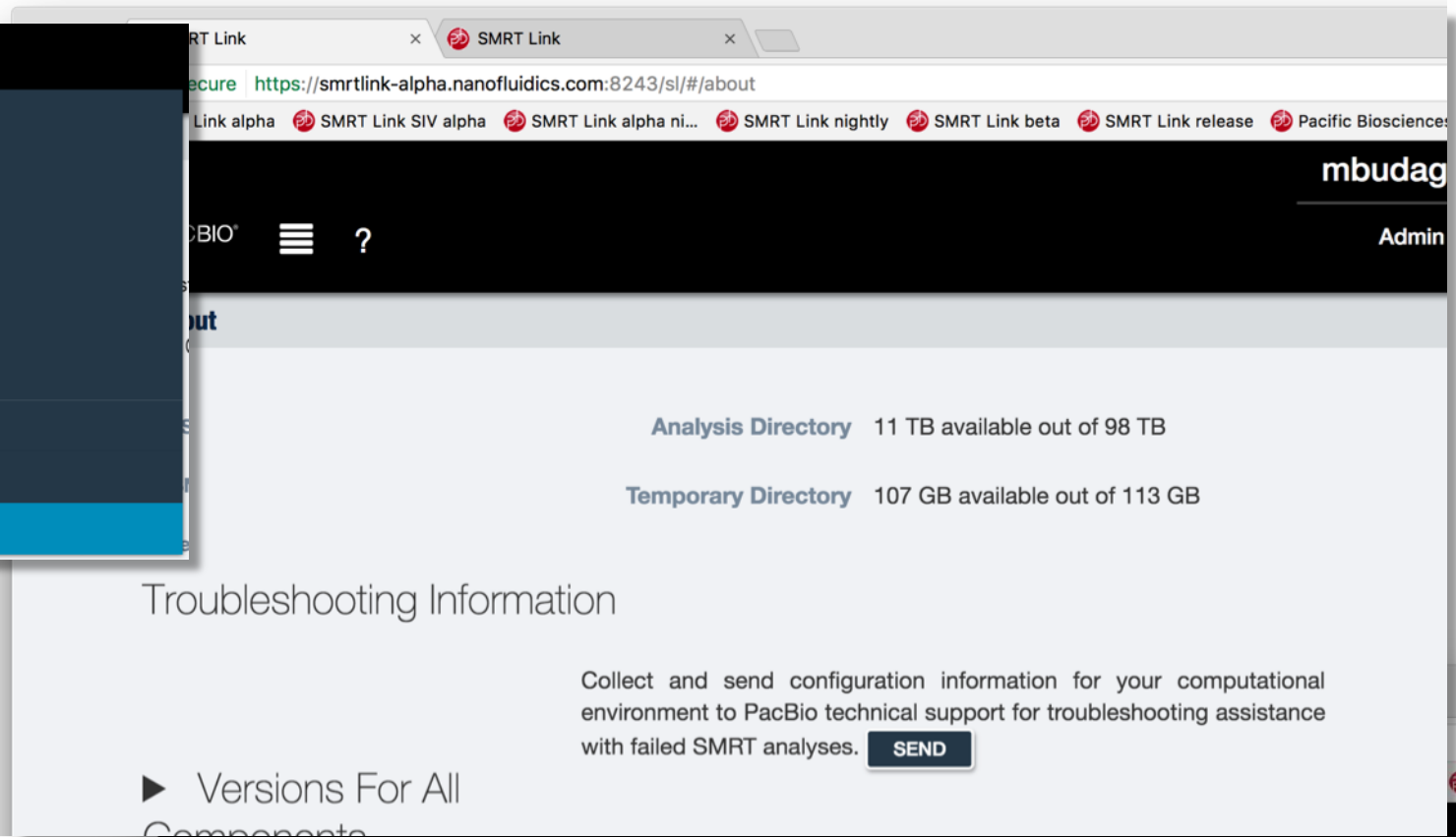
Analysis Id	10447
Status	FAILED
Created At	4/12/2017, 3:55:46 PM
Updated At	5/15/2017, 8:42:38 AM
Application	Resequencing
Inputs	Reference BAM Data
Path	/pbi/dept/secondary/siv/smrtlink/smrtlink-alpha/smrtsuite_170220/userdata/jobs_root/010/010447

► Analysis Parameters

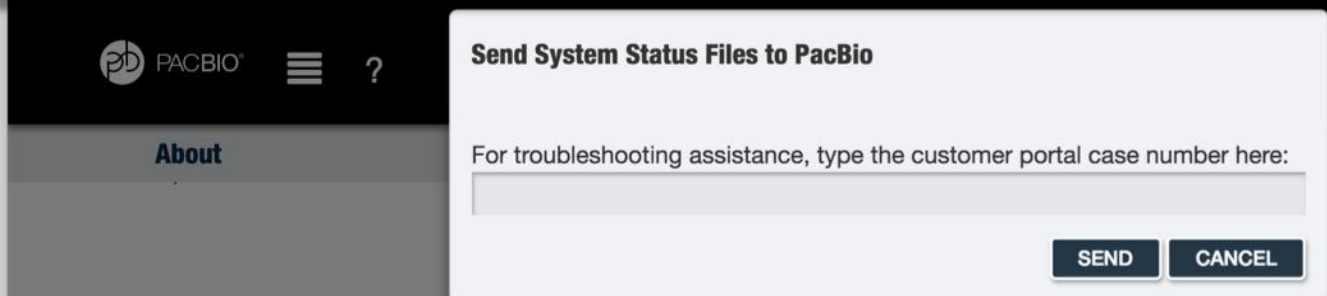
SEND SYSTEM LOG FILES TO TECH SUPPORT



A navigation menu for the PACBIO system. The menu is dark blue with white text. The 'About' option is highlighted in a lighter blue. The menu items are: Home, Sample Setup, Run Design, Run QC, Data Management, SMRT Analysis, Show Alarms..., Configure, and About.



A screenshot of the SMRT Link dashboard. The browser address bar shows the URL: <https://smrtlink-alpha.nanofluidics.com:8243/sl/#/about>. The dashboard displays storage information: **Analysis Directory** 11 TB available out of 98 TB and **Temporary Directory** 107 GB available out of 113 GB. Below this, there is a section titled 'Troubleshooting Information' with the text: 'Collect and send configuration information for your computational environment to PacBio technical support for troubleshooting assistance with failed SMRT analyses.' and a **SEND** button.



A dialog box titled 'Send System Status Files to PacBio'. It contains the text: 'For troubleshooting assistance, type the customer portal case number here:' followed by a text input field. At the bottom right, there are two buttons: **SEND** and **CANCEL**.

SEND LOG FILES TO TECH SUPPORT

- Files sent:
 - Failed analysis:
 - Any file within a job directory that matches these extensions: "sh", "stderr", "stdout", "log", "json", "html", "css", "png", "dot")
 - System status bundle:
 - All dirs under userdata "config", "log", "generated", "user_jmsenv"
 - Any log file only will take the last 2000 lines to keep the size of the total TGZ bundle small
- Destination of files: Internal event server
- Review process: Tech Support reviews emails posted to server

Note: ONLY emails with Salesforce Case Number get reviewed

KNOWN ISSUES

- Cannot delete references and barcodes through the GUI; they can be deleted only through the command line.
- In a SMRT Analysis results page, Data - File Downloads section, the Analysis Log file is correctly populated and downloadable, but file size is always shown as 0 bytes.

Analysis Results - rid1803_basemods_test

	File	Size	Type
▶ Analysis Overview			
▶ Modified Base Motifs	Analysis Log	0 bytes	log
	Motifs and Modifications	725,531 bytes	gff
▶ Base Modifications	Motifs Summary	169 bytes	csv

- At browser window sizes smaller than 1600x900, certain features may be inaccessible.
 - Also when run name is very long

FIXED ISSUES

- See Release Notes for complete list
- SL-527 Barcode 'names' in barcode report do not match CCS report names
- SL-617 Barcoded HGAP4 application - filtered subreadset is used for assembly, but total dataset is used for polishing.
- SL-1169 SMRT Link administrator cannot change password in WSO2. Fixed by providing a command-line tool to enable changing the password at post-install configuration.
- SL-1287 Several analysis applications used to output filtered data sets named identically to the input data sets. Now those output datasets are annotated with a "(filtered)" suffix.
- SL-1155 SFDC #116205 CCS2 with barcoding – min barcode score cannot be set
- SL-1156 When demultiplexing barcodes by running “dataset split –barcodes” at the command line, the output dataset chunks were formerly named according to descending order of read abundance. The behavior was fixed to name the chunks according to barcode index. This issue did not affect barcode analyses in the UI.
- SL-723 – In certain configurations of the compute and storage environment, the available server space displayed on the About page is incorrect.
- SL-944 SForce #57039 SMRT Link v4.0 change request: Remove nanofludics users from SMRT Link installation
- SL-1091 SMRT Analysis - Data Set creation too slow
- SL-1157 downloadable CCS2 with barcoding files need to be double-unzipped
- SL-1164 All Iso-Seq applications: Double negative in settings- “Ignore Poly A”
- SL-1182 Ability to delete stuck analysis jobs through the command line
- SL-41 Reduced frequency of analyses getting stuck in CREATED state
- Run Design and Run QC: By default, entries are not sorted by date. To sort entries, click the column header.
- Pbsmrtpipe does not handle properly string parameters that default to None.
- BLASR produces inconsistent gap placement in homopolymer stretches.
- For completed CCS mapping analyses, Mapping Report>CCS Mapping Statistics Summary incorrectly swaps the following values: Number of CCS Bases (mapped) and CCS Read Length N50 (mapped). Note: Mapping Report > Summary Metrics displays the same values correctly.



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