Bionano Access® Software User Guide

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Revision History

Revision	Notes

N	Update for Bionano Access 1.7
	• Removed references to Iris line of products
	• Updated various legacy terms and associated procedures to conform with Saphyr terms
	• Updated Jobs Menu
	• Included EnFocus Fragile X Analysis Options to menu
	• Added NxClinical Bundle Download feature
	• Added Chips Metrics Dashboard feature
	• Added Perform Duo and Trio Analysis execution steps
	• Enhanced explanation of the following features
	  ○ Circos Plot visualization
	  ○ Enhanced Whole Genome visualization
	  ○ Curated Variant List
	• Added Visualization Reporting functionality
	• Updated View option settings
	• Updated Settings section to reflect Access 1.7 updates

Introduction

The Bionano Access® Software enables users to view Saphyr® run results in real time and perform a variety of bioinformatics analyses.

Saphyr users can perform bioinformatics operations including, but not limited to, the following:

Operation	Description
***In silico* digestion of data sequences**	Perform *in silico* digestion by transforming a FASTA file into a Bionano CMAP file format. Calculates the estimated label density of FASTA file.
Merge molecule data sets	Merge two or more molecule jobs into a single molecule job.
Filter molecule jobs	Filter molecules jobs by length and total amount with random selection or enrich for longest molecules.
Generate *De Novo* assemblies	Assemble single molecules into consensus maps for structural variant (SV) detection and hybrid scaffold applications.
Perform rare variant analysis	Detect structural variants at low allelic fraction.
Generate EnFocus™ FSHD analysis	Analyze regions relevant to facioscapulohumeral muscular dystrophy (FSHD).
Generate EnFocus™ Fragile X syndrome analysis	Analyze regions relevant to Fragile X syndrome
Align maps	Compare two different maps or molecules to maps by aligning them to each other.
Build hybrid scaffolds	Merge Bionano maps with sequence assemblies to produce contiguous hybrid scaffolds that represent the chromosome structure.
Annotate structural variants	Provide variant annotation for downstream filtering and prioritization of SVs based on Bionano internal human database or custom control database. Perform dual or trio comparisons.
Convert SMAP to VCF file	Convert insertion, deletion, duplication, inversion, and translocation breakpoint calls in an SMAP file to dbVar-compliant VCF v4.2 format.

Saphyr users can additionally create experiments to run on their Saphyr Instrument and monitor chip runs in real time.

Compatibility

Bionano web Access 1.7 is compatible with Google Chrome web browser. Other web browsers are not validated and using web browser other than Google Chrome may cause Bionano Access malfunction.

Bionano Access Terms

Cluster — Compute servers are required to perform bioinformatic analysis operations. Multiple compute servers can be organized into a cluster to work as unit and complete large amounts of work efficiently. Bionano sells Saphyr and Bionano Compute systems that can be combined into a cluster scaled to meet the customers' computing requirements. Customers can also utilize Bionano Compute On Demand, a cloud-based computing platform, which is optimized for Bionano pipelines to perform bioinformatics analyses. The bioinformatics pipelines may also work on custom clusters; however, custom configurations may be needed.

Jobs (Also known as "Objects" in 1.6.1 or earlier version) — Whenever Bionano Access performs a job, it may generate a variety of output data depending on the operation, such as molecule data, map alignments, assemblies, hybrid scaffold results, variant annotations, and others. Each of these distinctive sets of output files is a job (a set of output files). The types of jobs include, but are not limited to:

- Alignment
- (Annotated) *De Novo* Assembly
- (Annotated) Rare Variant Analysis
- EnFocus™ FSHD Analysis
- EnFocus™ Fragile X Syndrome Analysis
- Variant Annotation Results
- Hybrid Scaffold
- Consensus CMAP files
- Molecule BNX files
- FASTA file
- BED file

Please go to "Bioinformatics Analysis" section for more details.

Prep — The information of the sample, reference, and enzyme in one flow cell and related to experiment setup. This is also known as a Molecule Set.

Cohort — The number of subgroups that each scan is divided into for real-time analysis. One scan contains multiple cohorts, and we generate run metrics for each cohort.

Consensus CMAP — The Bionano Genomics CMAP file is a raw data file which provides location information for label sites within a genome map or an *In Silico* digestion of a reference or sequence data. The CMAP is a tab-delimited text based file. Please go to *P/N 30039, CMAP File Format Specification Sheet* for more details.

BNX — The Bionano Genomics BNX file is a raw data view of molecule and label information and quality scores per channel identified during a run. BNX v1.3 supports one or two label channels (colors). Please go *to P/N 30038, BNX File Format Specification Sheet* for more details.

Login to Bionano Access

User Roles

There are four distinct user roles in Bionano Access. Users with administrator privileges can assign roles to other users.

Roles	Description
Administrator	The Administrator can set the security standards, assign user roles, manage user accounts and the access to projects, manage references and projects, delete projects permanently, or restore deleted projects.
Project Lead	The Project Lead can give users access to view projects, create**,** manage projects, and manage references. All project leads can view and edit all projects in the Projects list.
User	The User can view the projects that they have access to and edit experiments.
Read Only	The Read Only user can view the projects that they have access to.

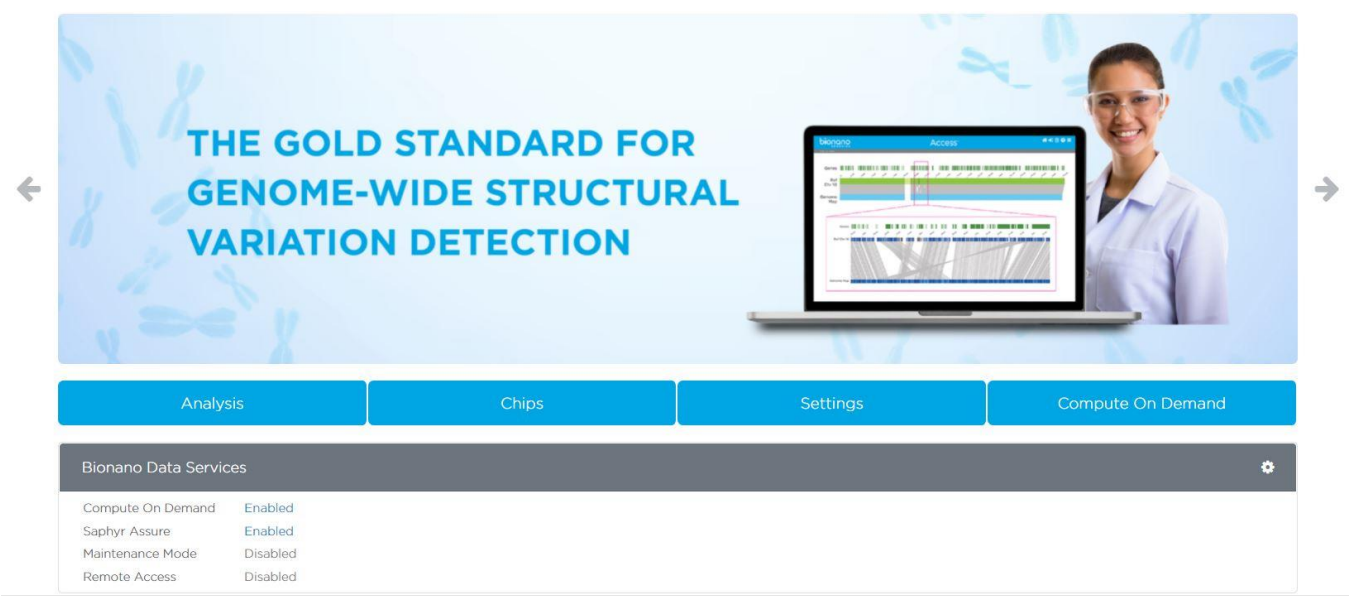
Login

After installation of the Bionano Access software on the server, a URL is needed for login. Standalone user can use *localhost:3005* to open web Bionano Access. To log in, you must first have a user account. Users with the Administrator role can create new accounts. If you do not have an account, please contact your administrator to create a new account. If you forgot your password, click Forgot Password, and you will receive an email to reset your password.

1. Navigate to the Bionano Access web page.
2. Enter **User Name** and **Password**.
3. Click **Login**.

Bionano Access Modules

The Bionano Access *Home* page lists the following modules:



Note: Depending on the types of User Roles (see below) and the types of Access install instances (see details in 30170 Bionano Access Installation Guide), not all the modules are available on the *Home* page.

Module	Description	Availability	Note
Analysis	This module lets users create, view, edit, and manage projects. Additionally, users can import and export data.	All user roles.	Name changed from "**Projects**" to "**Analysis**" in Access 1.7
Chips	This module lets users set up and manage Saphyr experiments, view templates, and track chip run progress.	Only for systems integrated with a Saphyr Instrument.	Name changed from "**Experiments**" to "**Chip**" in Access 1.7
***In Silico* Digestion**	This module lets users perform *in silico* digestion, update its settings, and view results.	All user roles.	Moved to **Settings** in Access1.7
Settings	This module lets users create and manage user accounts, add references, configure software, upload BED files and Control Databases, and enable maintenance mode, enable Compute On Demand and test the connection.	Only available to "Administrator" and "Project Lead" user roles.	
Compute On Demand	This module lets users redeem and transfer tokens, check balance and transaction of tokens.	Only available for systems enabled with Compute On Demand.	

Help

To get a better understanding of Bionano Access, users can click the **Help** icon.

The “Help” page has the following information:

- Software version information of Bionano Access and Solve.
- Compute On Demand version information of Bionano Tools and Solve (if enabled).
- Tutorial Videos.
- Security patch information.
- Support contact information.

User Profile

Users can change the email address or password, enable, or disable the email notifications after the first-time logging in.

In Bionano Access, click the **User Profile** icon.

1. At the **User Name** field, user name displays.
2. At the **Full Name** field, full name displays.
3. At the **User Role** field, user role displays.
4. At the **Email Address** field, type the email address.

   By default, the **Send email notifications** check box is selected. You can uncheck this box to disable email notifications.
5. At the **Current Password** field, type the current password.
6. At the **New Password** field, type the new password.
7. At the **Confirm Password** field, type the new password again.
8. At the **Expiration date** field, the expiration date of the password is displayed.

Message Center

In Bionano Access, click the **Message Center** icon to read and delete individual messages. Users can also mark all messages as read and delete all read messages.

When there are new messages about job status in Access ready to be read, a red circle with a number will show

at the message center icon . By default, the system will attempt to notify users via email and the message center. Users can disable email notifications on the user profile page.

Alerts

Bionano Access sends alerts to notify system administrators of errors or system conditions that require attention. This is the foundation of proactive system diagnostics where the system can monitor itself and alert those responsible when action is needed. This feature works similarly to the messaging and message center. Messages are notifications sent to specific users regarding job status and other system events. Alerts are broadcast to all users with the 'Administrator' role. The Alerts icon will appear in the header of Bionano Access next to the existing Message Center Icon. The system will also post alerts sent by the instrument that pertain to a chip run on the dashboard.

Menu

User can click the **Menu** to access to different Bionano Access modules, include **Home**, **Analysis**, **Chips**, **Settings, Compute On Demand**, **Profile**, **Help** and **Logout** directly.

User can use **Job ID Search** to access to specific job directly. System will go to the project and highlight the specific job.

Analysis

In the Analysis module, users can create new projects, manage projects, edit, import, and download jobs. Users can also filter the projects by project name and export all the jobs in the project by clicking the select-all option. Additionally, users can perform bioinformatics analysis such as alignment, molecules merge, hybrid scaffold, and other commands for individual jobs in the project. Jobs can be shared across all projects.

Create Project

Users in the Project Lead or the Administrator role can create projects.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Click **Create Project**.

   The *Project* dialog box appears.

3. At the **Project Name** field, type the name of the project.

4. [Optional] At the **Key** field, type a key (e.g. a project code) to associate with the project.

5. [Optional] At the **Description** field, type a brief description.

6. Click **Submit**.

   The project appears in the Projects list.

Sample Search

Users can find which projects contain certain samples.

1. From the Bionano Access main menu, select **Analysis.**

   The *Projects* window appears.

2. At the **Sample Name** field, type the name of the sample.

   *The search for sample name is case sensitive.

3. Click **Search**.

   The project list will show the projects that contain the sample.

Export Project

Users can export a project containing multiple jobs, such as molecules, assembly, SV merge and variant annotation pipeline.

Only users in the Project Lead or the Administrator role can export projects.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Click the **Export** icon of the project.

   The *Export Data* window appears.

3. At the **Export File Name** field, type in the name.

4. [Optional] At the **Description** field, type the description.

5. Select the jobs (i.e. assembly, molecules, map, SV merge and variant annotation pipeline).

6. Click **Submit**.

7. The exported file (**.bng**) will be automatically saved to the “export” folder which is in the “webServerShare” directory, as configured in the Access configuration file. For more details, please go to *P/N 30170 Bionano Access Installation Guide*.

Import Project

Users can import an exported project containing multiple jobs, such as molecules, assembly, and variant annotation pipeline.

Only in the Project Lead or the Administrator role can import project.

1. Copy the exported file (**.bng**) to the “import” folder, which is in the “webServerShare” directory, as configured in the Access configuration file. For more details, please go to *P/N 30170 Bionano Access Installation Guide*.

2. Go to Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

3. Click the **Import** icon of the project.

   The *Import Data* window appears.

4. At the **Import File Name** field, select the one you would like to import.

5. Click **Submit**.

Delete Project

Users in the Project Lead or the Administrator role can delete projects. Deleting a project is a two-step process. As the first step, a project lead can delete the project from the project list. However, only the administrator can delete the project permanently from Bionano Access, as the second step. For more details, see the *Deleted Projects* section.

1. From the Bionano Access main menu, select **Analysis**.

The *Projects* window appears.

2. Click the **Delete** icon of the project to delete.

   The *Confirm* dialog box appears.

3. Click **Yes**.

   The project is deleted from the list.

There are several rules that would prevent a user from deleting a project.

1. If the project contains experiments, you cannot delete it because experiments cannot be deleted.
2. If the samples and jobs in the project are not removed, you cannot delete the project.

Thus, before deleting the project as a whole, make sure this project does not contain experiments and the samples and jobs are already deleted. Please see *Samples* section for more details.

Edit Project

User in the Project Lead or the Administrator role can edit project information.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select one project from the project list.
3. Click **Edit**.

   The *Project* dialog box appears.

4. At the **Project Name** field, type the name of the project.
5. [Optional] At the **Key** field, type a key (e.g. a project code) to associate with the project.
6. [Optional] At the **Description** field, type a brief description.
7. Click **Submit**.

Give Users Access to View Projects

Users in the Project Lead or the Administrator role can give User and Read-only role to access and view projects.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Click the **Access** icon of the project.

   The *Project Access Control* screen appears.

3. In the *All Users* pane, select the users to give access, and then click **Grant Access**. Users in the Project

Lead or the Administrator role are not listed in the pane.

The users appear in the *Users with Access* pane.

Deleted Projects

Users must have administrator privileges to perform this task. Users can restore a project that was deleted by a project lead.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Click the **Deleted Projects** icon at the top right-corner of the screen.

   The *Deleted Projects* screen appears.

3. At the project to restore, click the **Restore** icon.

   The software restores the project and adds it back to the project list.

4. At the project to permanently delete, click the **Delete** icon.

   The project would be permanently deleted.

Jobs

When users select a project, the *Project Browser* page opens. The page contains menu options, the list of job(s), the selected-job details, and the options and operations for the selected job.

Menu

Menu	Description
Import	To import output files from Saphyr clusters or Bionano files from another system.
Remove	To remove a job from the project.
Copy	To copy data from the job and paste it in another project.
Edit	To edit job name, sample, reference, tags, and description.
Jobs	To view a list of jobs and their state (Active, Working, Complete, Cancelled, Failed) for a project.
Samples	To view a list of samples associated with the jobs in this project.
Reset	To remove all filters currently applied in the job list.

Jobs List

All the jobs that are listed belong to the selected project.

Info	Description
Sample	The sample name.
Type	The job type (e.g. *De Novo* assembly, molecules, map, scaffold, FASTA file).
Name	The user-defined name for the job.
Curation	The curation status for the job
Tag	The Tag feature allows users to create and edit keywords that are tagged to this job. Users can filter jobs with the same keywords to easily manage jobs in a project. 1. To create or edit tags for the job, select the job, and then click **Edit**. 2. At the Tags field, type the keyword, and then press Tab. Repeat to add more keywords. Click the delete “x” icon to delete the keyword.
Created	The date when the job was created.
	Click this icon to sort and filter the information in the columns.

Sample Details

Info	Description
Name	The sample name that is assigned to the job
Sample ID	System generated global unique identifier
Created	Date job was created

Job Details

Info	Description
Name	The job name should be unique in a project. The same job can have different names in different projects.
Reference	[Optional] The reference map.
Description	[Optional] The description of the job.
Created	The date the job was created.
Operation	The type of job performed.
Status	The current status of the job.
User	The user who created the job.
Job ID	The identifier of the job. Icon of Job Error Reporting shows there is returned error message.
Command	Detailed information about the job and the Bionano Tools command issued to compute servers.

Job Options

The *Options* pane contains a list of options that can be visualized or downloaded with the selected job. The options are dependent on the type and current status of the job. The links in the *Options* pane may be disabled and greyed out if a previous operation is still in progress.

Alignment Options

- Download Alignment Job
- View Maps Alignment
- View Molecules Alignment

(Annotated) *De Novo* Assembly Options

- *De Novo* Assembly Informatics Report
- Molecules to Maps
- Maps to Reference with SV
- Download *De Novo* Assembly
- Download NxClinical™ Bundle
- Download VCF file

(Annotated) Rare Variant Analysis Options

- Rare Variant Analysis Informatics Report
- Maps to Reference with SV
- Download Rare Variant Analysis
- Download NxClinical™ Bundle
- Download VCF file

Variant Annotation Pipeline Options

- View variant annotation results
- Download SV Annotation file
- Download Variant Annotation Analysis

EnFocus™ FSHD Analysis Options

- View EnFocus™ FSHD Analysis
- View EnFocus™ FSHD Informatics Report
- Maps to Reference with SV
- Go to Molecules Job
- Download EnFocus™ FSHD File
- Download EnFocus™ FSHD JSON File
- Download VCF file

EnFocus™ Fragile X Analysis Options

- View EnFocus™ Fragile X Analysis
- View EnFocus™ Fragile X Informatics Report

- Maps to Reference with SV
- Go to Molecules Job
- Download EnFocus™ Fragile X File
- Download EnFocus™ Fragile X JSON File
- Download VCF file

Bed Option

- Download BED file

FASTA Option

- Download FASTA file

Map Options

- Download Map

Molecules Options

- Download Molecules File
- Show Molecule Quality Report (MQR)

Scaffold Options

- Hybrid Scaffold Report
- Maps to Next Generation Sequencing (NGS) with Conflicts
- Maps and Next Generation Sequencing (NGS) to Hybrid Scaffold
- Download Hybrid Scaffold
- Export NCBI Package

Job Operations

The *Operations* pane contains a list of operations that can be performed with the selected job. The *Compute On Demand* pane also contains the same list of operations that can be performed using tokens. The operations are dependent on the type and current status of the job. The links in the *Operations* pane may be disabled and greyed out if a previous operation is still in progress.

(Annotated) *De Novo* Assembly Operations

- Generate Hybrid Scaffold
- Generate 2-Enzyme Hybrid Scaffold
- Generate Variant Annotation – Single
- Generate Dual Analysis
- Generate Trio Analysis
- Align Maps

(Annotated) Rare Variant Analysis Operations

- Generate Variant Annotation – Single
- Generate Dual Analysis
- Generate Trio Analysis

EnFocus™ FSHD Analysis Operations

- Generate EnFocus™ FSHD Analysis Report

EnFocus™ Fragile X Analysis Operations

- Generate EnFocus™ Fragile X Analysis Report

Map Operations

- Generate Hybrid Scaffold
- Generate 2-Enzyme Hybrid Scaffold
- Align Maps

Molecules Operations

- Filter Molecule Job
- Merge Molecule Jobs
- Align Maps
- Generate Molecule Quality Report (MQR)
- Generate *De Novo* Assembly
- Generate Rare Variant Analysis
- Generate EnFocus™ FSHD Analysis

- Generate EnFocus™ Fragile X Analysis

Import Job

Users can import data from the following:

- Data output files that are generated from the Saphyr cluster, which are based on user-defined commands.
- Data from another system that is in the acceptable Bionano file format

Import Molecules

Users must have Bionano molecules data in ***.bnx** or ***.bnx.gz** file to import to Bionano Access.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select **Molecules**.
5. At the **Molecules Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file or **New** to add a new Sample. Once click **New**, the *Sample* dialog box appear.
   a) At the **Sample Name** field**,** type a name for this sample.
   b) [Optional] At the **Sample Type** field, type a type of the sample.
   c) [Optional] At the **Indication filed**, type a brief indication.
   d) [Optional] At the **Description** field, type a brief description.
   e) [Optional] At the **Comment** field, type a brief comment.
7. [Optional] At the **Reference for Channel 1** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Molecules File** field, and click **Browse** to choose the molecules file (.bnx or .bnx.gz).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data is imported.

Import Consensus Map

Users must have consensus map data in ***.cmap** file to import to Bionano Access.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.
4. At the **Job Type** field, select **Consensus Map.**
5. At the **Consensus Map Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Consensus Map File** field, click **Browse** to choose the cmap file (.cmap).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data is imported.

Import an Alignment Job

Users must have all three of these files to import an alignment to Bionano Access: **.xmap, _r.cmap, and _q.cmap**

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, **Alignment** is auto selected.
5. At the **Alignment Name** field, type the name of the alignment.
6. At the **Sample** field, select the sample from the drop-down list.
7. [Optional] At the **Reference** field, select a reference from the drop-down list.
8. [Optional] At the **Tags** field, type the keywords to associate with the alignment job.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Alignment Type** field, choose one of the following options for Bionano to display the files in:
    - **Anchor to Genome Maps**— Display a consensus map as contigs. Select this option if the alignment is between two consensus maps.
    - **Anchor to Molecules**—Display a consensus map as molecules. Select this option if the alignment is between molecule file to a reference or assembly consensus map.
11. At the **Reference Map** field, click **Browse** to choose the reference cmap file (_r.cmap).
12. At the **Alignment Map** field, click **Browse** to choose the alignment xmap file (.xmap).
13. At the **Query Map** field, click **Browse** to choose the query cmap file (_q.cmap)
14. Click **Import**.
15. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

16. The **Upload Completed** shows up when the upload is finished.

17. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data is imported.

Import FASTA

Users must have fasta data in ***.fasta**, ***.fa** or *.**fna** file to import to Bionano Access.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select **FASTA**.
5. At the **FASTA Name** field**,** type a name for this job.
6. [Optional] At the **Tags** field, type the keywords to associate with the file.
7. [Optional] At the **Description** field, type a brief description.
8. At the **FASTA** field, click **Browse** to choose the fasta file (.fasta, .fa or .fna).
9. Click **Import**.
10. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

11. The **Upload Completed** shows up when the upload is finished.
12. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import Scaffold

Users must have the hybrid scaffold data in a ***.zip** file to import to Bionano Access.

Important: Hybrid Scaffold result generated by Bionano Solve through the command line MUST have output directory set in ***/output**.

1. From the Bionano Access main menu, select **Analysis**.

The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.
4. At the **Job Type** field, select **Scaffold**.
5. At the **Hybrid Scaffold Name** field, type a name for the scaffold.
6. At the **Sample** field, select the sample from the drop-down list.
7. [Optional] At the **Tags** field, type the keywords to associate with the alignment job.
8. [Optional] At the **Description** field, type a brief description.
9. At the **Hybrid Scaffold File** field, click **Browse** to choose the scaffold file (.zip).
10. Click **Import**.
11. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

12. The **Upload Completed** shows up when the upload is finished.
13. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import *De Novo* Assembly

Users must have *De Novo* Assembly or Annotated *De Novo* Assembly data generate by Bionano Solve in ***.gz** or ***.zip** file to import to Bionano Access.

Important: *De Novo* Assembly result generated by Bionano Solve through the command line MUST have output directory set in ***/output**.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select ***De Novo* Assembly**.
5. At the **Assembly Name** field, type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **De Novo assembly File** field, click **Browse** to choose the assembly file (.zip).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import BED

Users must have BED data in ***.bed** file to import to Bionano Access.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.
4. At the **Job Type** field, select **BED**.
5. At the **Name** field, type a name for this job.
6. [Optional] At the **Tags** field, type the keywords to associate with the file.
7. [Optional] At the **Description** field, type a brief description.
8. At the **BED File** field, click **Browse** to choose the bed file (.bed).

9. Click **Import**.
10. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

11. The **Upload Completed** shows up when the upload is finished.
12. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import Cytoband

Users must have cytoband data in ***.bed** file to import to Bionano Access.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.
4. At the **Job Type** field, select **Cytoband**.
5. At the **Cytoband Name** field, type a name for this job.
6. [Optional] At the **Tags** field, type the keywords to associate with the file.
7. [Optional] At the **Description** field, type a brief description.
8. At the **Cytoband File** field, click **Browse** to choose the cytoband file (.bed).
9. Click **Import**.
10. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

11. The **Upload Completed** shows up when the upload is finished.
12. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import Rare Variant Analysis

Users must have rare variant analysis or annotated rare variant analysis data generate by Bionano Solve in ***.zip** file to import to Bionano Access.

Important: Rare Variant Analysis result generated by Bionano Solve through command line Must with output directory set in ***/output**.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select **Rare Variant Analysis**.
5. At the **Rare Variant Analysis Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Rare Variant Analysis File** field, click **Browse** to choose the analysis file (.zip).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import Variant Annotation

Users must have variant annotation analysis data generate by Bionano Solve in ***.zip** file to import to Bionano Access.

Important: Variant Annotation Pipeline Analysis result generated by Bionano Solve through the command line MUST have output directory set in ***/output.**

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select **Variant Annotation**.
5. At the **VAP Analysis Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Variant Annotation Pipeline** field, click **Browse** to choose the VAP analysis file (.zip).
11. Click **Import**.
12. The **File Upload Status** shows up.

    The progress bar indicates the status of the data uploading to the server.

    Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import FSHD Analysis

Users must have FSHD analysis data generated by Bionano Solve in ***.zip** file to import to Bionano Access.

Important: FSHD Analysis result generated by Bionano Solve through the command line MUST have output directory set in ***/output**.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

   The *Import* dialog box appears.

4. At the **Job Type** field, select **FSHD Analysis**.
5. At the **FSHD Analysis Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **FSHD Analysis File** field, click **Browse** to choose the FSHD analysis result file (.zip).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Import Fragile X Analysis

Users must have Fragile X analysis data generated by Bionano Solve in ***.zip** file to import to Bionano Access.

Important: Fragile X Analysis result generated by Bionano Solve through the command line MUST have output directory set in ***/output**.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Import**.

The *Import* dialog box appears.

4. At the **Job Type** field, select **Fragile X Analysis**.
5. At the **Fragile X Analysis Name** field**,** type a name for this job.
6. At the **Sample** field, click the drop-down list to select the sample associated with file.
7. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
8. [Optional] At the **Tags** field, type the keywords to associate with the file.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Fragile X Analysis File** field, click **Browse** to choose the Fragile X analysis result file (.zip).
11. Click **Import**.
12. The **File Upload Status** shows up.

The progress bar indicates the status of the data uploading to the server.

Important: Do not close the page until the upload is finished. If users close the page while the data is uploading, the uploading process may be interrupted.

13. The **Upload Completed** shows up when the upload is finished.
14. Click **Ok**.

The screen switches back to the *Project Browser* page. Bionano Access sends an email notification when the data are imported.

Download NxClinical™ Bundle

Use can download a NxClinical™ Bundle file for *De Novo* Assembly and Rare Variant Analysis that can be imported into NxClinical™.

1. From the Bionano Access main menu, select **Analysis.**

   The *Projects* window appears

2. Select the project to view from the list.
3. In the Jobs list**,** select the *De Novo* Assembly or Rare Variant Analysis job.
4. In the *Operations* pane, click **Download NxClinical™ Bundle**.

   A *.zip file will be downloaded. User can import the *.zip file to NxClinical platform.

Remove Job

Users can remove jobs from a project. Removing an job from a project does not affect that same data that are

shared/copied with other projects. To remove an job that exists in several projects, users would need to remove it from each project.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the job to remove.
4. Click **Remove**.

   The *Remove Job from Project* dialog box appears.

5. Click **Yes**.
6. When jobs are deleted in the Project Browser they go into the trash. To permanently remove an job or restore a previously deleted job in a project, select the project and click on Deleted Jobs icon at the top-right corner of Access webpage to manage deleted jobs. Administrators can permanently delete jobs in the trash. There is a button to allow Administrators to delete all the jobs in the trash, so the jobs do not have to be deleted one at a time.

Copy Job

Users can copy jobs from one project and add it to another project.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select an job (i.e. assembly, molecules, map) to share.
4. Click **Copy**.

   The *Copy Job to Project* dialog box appears.

5. At the **Select the target project** field, click the drop-down list to another project.
6. Click **Submit**.

   Users can view the data from the project that was selected to share.

Edit Job

Users can edit job's name, sample reference, tags and description information.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. Click **Edit**.

   The *Edit Job* dialog box appears.
4. At the **Name** field, click the drop-down list to select the sample associated with file.
5. [Optional] At the **Reference** field, click the drop-down list to select the reference that was used.
6. [Optional] At the **Tags** field, type the keywords to associate with the file.
7. [Optional] At the **Description** field, type a brief description.
8. Click **Submit**.

Jobs

Users can view all recently (default: last 30 days) submitted jobs' status, cancel running jobs, sort and filter the columns of job listing.

Info	Description
Project	The project name of the job.
Job ID	The unique job number auto generated by Bionano Access server when a job is created.
Operation	The operation of the job, such as Alignment, Import Molecule, DLE-1 ***De Novo*** Assembly, Variant annotation.
Name	The created job name.
User	The user name who submitted the job.
Created	The date when the job was created/submitted.
State	The current state of the job. - **Active: the job is running.** - **Working**: copying input files; **job has not** started - **Complete: job completed successfully** - **Cancelled:** job cancelled - **Failed**: job failed
Status	The current status of the job. System reads **status.xml file** in the compute server for each job.
Server	**The IP address** or hostname **of your assembly server.**
Actions	To view job details or cancel the job. **Bionano Access automatically cancels jobs that are inactive for more than 10 days. Jobs that are inactive for longer than 10 days are assumed to have been interrupted or orphaned.**

Samples

Menu

Menu	Description
Add	To add a new sample.
Remove	To remove a sample. Note: to remove a sample, one has to remove all the associated jobs.
Copy	To copy sample from the job and paste it in another project.
Edit	To edit sample name, description and comment.

Users can view all samples' information, sort and filter the columns of sample list.

Info	Description
Sample	The sample name that is assigned to jobs.
ID	System generated global unique identifier
Description	The description of the sample.
Type	The type of the sample was created.
Indication	The indication of the sample was created
Comments	The comment of the sample was created.
Created	The date the sample was created.

Reset

After applying filters to the job list, users can clean all the filters applied.

Deleted Job

Users must have administrator privileges to perform this task.

1. From the Bionano Access main menu, select **Projects**.

   The *Projects* window appears.

2. Select the project that contains the job to delete.

3. Click the **Deleted Jobs**  icon at the top right-corner of the screen.

   The *Deleted Jobs screen* appears.

4. Click **Delete all jobs** if it is desired to delete all the jobs permanently in all projects.

5. Select the job from the list to restore, click the **Restore** icon.

The software restores the project and adds it back to the job list.

6. Select the job from the list, and then click the **Delete** icon.

   The software permanently deletes the job from the project. If the job is shared with other projects, the job still exists in those projects.

To permanently delete the same job that is in other projects, repeat steps 1 through 4 for the other projects.

Download Job

1. From the Bionano Access main menu, select **Projects**.

   The *Projects* window appears.

2. Select the project to view from the list.

3. In the Jobs list, select the job to download.

4. In the *Options* pane, click the download [job type] option.

   The software downloads the data onto the workstation running the browser.

Chips

In the Chips module, users can add new projects, manage projects, track run performance, and view real-time metrics. Additionally, users can view and edit templates. In Access 1.7, each chip can now be associated to more than one project. Before inserting the Saphyr Chip into the instrument, create a project or projects to associate with the chip. The run results are posted for the project or projects that are associated with the chip.

A project created on Bionano Access is in *pending* status until it is associated with a Saphyr chip. Users can modify or delete pending projects. Once a project is associated with a scan on the Saphyr, users cannot modify or delete the project.

Add Chip

Only users in the Administrator or Project Lead role can create chip run.

1. From the Bionano Access main menu, select **Chips**.

   The *Chips* list appears. Here is a description of Chip Runs list.

Info	Description
Start	Time that the chip run was started.
Finish	Time that the chip run was completed.
Name	The chip name.
Instrument	The name of the Saphyr instrument.
Chip Run	The chip run ID generated by the system.
Serial Number	The serial number of the chip.
Operator	The user name who set up the chip run.
Action	To view dashboard.

Click Expand Icon to get more information about each experiment as below:

Info	Description
Location	The location of Flow Cell on each chip. FC – 1, FC – 2 or FC – 3.
Project	The name of the project.
Sample	The sample origin. Human samples or others.
Label	The label color. Green 01 or Red 01.
Enzyme	The enzyme name.

Reference	The reference name.
Auto Assemble	False or true.
Auto Rare Variant Analysis	False or true.
Auto FSHD	False or true.
Auto Fragile X	False or true.
Primary Label	Only applicable for dual label samples.

2. Select **Add Chip**.

   A dialog box appears.

3. At the **Chip Name** field, type the name of the chip that is associated with the experiment.
4. At the **Chip Part Number** field, select the correct Chip Part Number.
5. Click **Next.**
6. At the **Workflow** field, click the drop-down list to select “Single Sample”, “Sample Multiplex” or “Dual Labeled Sample”.

   Please see section “Red Labeled Experiment” below for details to setup “Sample Multiplex” and “Dual Labeled Sample” experiments. The instruction below describes setup for Single Sample.

7. At the **Throughput Target (Gbp)** field, type the target throughput. This field only applies to Saphyr instruments running ICS 4.8 or greater. For more detail, please refer to *P/N 30173 Data Collection Guidelines*.
8. At the **Molecule Job Name** field, type the name of molecule job. You can also leave it blank for default naming.
9. At the **Project** field, click the drop-down list to select a project or click **New** to a create a new project**.**
   - When creating a new project, type a name under **Project Name**. **Key** and **Description** are optional. Click **Submit**
10. At the **Sample** field, click the drop-down list to select a sample or click **New** to create a new sample.
    - When creating a new sample, type the **Sample Name**. **Sample Type**, **Indication**, **Description**, and **Comment** are optional. Click **Submit**
11. At the **Label** filed, click the drop-down list to select “Green 01” (green labeled) or “Red 01” (red labeled) for Single Sample.
12. At the **Recognition Enzyme** field, click the drop-down list to select an enzyme or click **New** to add a new enzyme.
13. At the **Reference** field, click the drop-down list to select a genome reference for the experiment.

- If hg19 (DLE1, BSSSI or BSPQI), or hg38 (BSSSI or BSPQI) is chosen as reference, **Auto *De Novo* Assembly** and **Auto Rare Variant Analysis** options will be available. Check the box to enable automatic pipeline submission.
- If hg38 (DLE1) is chosen as reference, **Auto *De Novo* Assembly**, **Auto Rare Variant Analysis**, **Auto EnFocus FSHD** and **Auto EnFocus Fragile X** options will be available. Check the boxes to enable automatic pipeline submission.

14. [Optional] At the **Isolation Kit Lot Number** field, type the lot number of the isolation kit used.
15. [Optional] At the **Labeling Kit Lot Number** field, type the lot number of the labeling kit used.
16. Click **Add to Flowcell 1**.

    To better distinguish between dual labeled samples and multiplexed flow cells, Prep Type is added to each flow cell listing in the experiment design module. To edit the information for Flowcell 1, click **Remove** under Flowcell 1 in the *Chip* pane, and then make changes.
17. Repeat steps 6 through step 15 for Flowcell 2 and 3, if applicable.
18. Click **Add to Flowcell 2 (or 3)**.

    To edit information for either Flowcell 2 or 3, click **Remove** under the corresponding Flowcell in the *Chip* pane, and then make changes.
19. Click **Save Chip**.

Create an Experiment Template

Users can create an experiment template if the same enzyme and reference are used every time. Only users in the Administrator or Project Lead role can create experiment templates.

1. From the Bionano Access main menu, select **Chips**.

   The *Chips* list appears.
2. Select **Templates**.

   By default, there are 3 templates for FSHD Analysis using Chip Part Number 20319, 20366 and 20367.
3. Repeat steps 2-17 in the **Add a Chip** section.
4. [Optional] Click **Save as Template.**

   A window for Experiment Template appears.
5. At the **Template Name** field, type in the name of template.
6. At the **Label for sample slot** field, type in the name of slot for different samples.

   The experiment template will be saved, and user can select it.

7. Click **Ok**.

Monitor Run Progress

After the instrument scans the chip for 15 to 20 minutes and raw images are being detected, users can monitor the progress of the run and view real-time metrics from the Bionano Access web site.

1. From the Bionano Access main menu, click **Chips**.
2. The latest chip run appears at the top of the list. Here is a description of Chip Runs list.

Info	Description
Start	The date and time when the chip run was started.
Finish	The date and time when the chip run was finished.
Name	The chip name
Instrument	The instrument name.
Chip Run	The chip run ID generated by the system.
Serial Number	The serial number of the chip.
Operator	The user name who set up the chip run.
Action	To view dashboard.

3. [Optional] Click the Filter icon to filter data in the columns.
4. Select the experiment or chip run to monitor, and then click **View Dashboard**. For more detail information, please refer to *P/N 30304 Bionano Access: Dashboard Guidelines.*

   The *Dashboard* screen appears showing:

   - Run information
   - Analysis graphs
   - Run metrics table

Run Information

Info	Description
Chip	The Saphyr Chip bar code.
Run ID	The system-generated run identifier.
Experiment	The experiment name.
Instrument	The instrument serial number.

Min Length	The minimum length of molecules that are used for analysis. The setting for this parameter is system generated.
Min Labels	The minimum labels per molecule that are used for analysis. The setting for this parameter is system generated.
Start Time	The run start time.
End Time	The run end time.

Analysis Graphs

Map	Description
DNA per Scan (Gbp)	This graph shows the amount of DNA per flowcell that is detected per scan.
Map Rate (%)	This graph shows the percentage of molecules that map to the reference genome. The map rate cannot be calculated unless there is a minimum number (1000) of molecules and labels. If the minimum threshold is not acquired, the map rate is defaulted to zero. If no reference genome is provided, the map rate is defaulted to zero.

Run Metrics Table

Metric	Description
Plot	The color used in the plot and check box for each flow cell.
Flowcell	The scanned flowcell.
Prep	The names of the sample, channel, enzyme, type, and reference
Avg N50 (>=150kbp) (Mbp)	The molecule length N50 for all molecules that are ≥ 150 kbp in length.
Avg N50 (>=20kbp) (Mbp)	The molecule length N50 for all molecules that are ≥ 20 kbp in length. Molecules that are less than 20 kbp are considered noise by the image detection algorithm.
Avg Label Density (per 100 kbp)	The number of labels that are detected by the image detection algorithm per 100 kbp of DNA length for molecules ≥ 150 kbp.
Avg Map Rate	The percentage of molecules that map to the reference for molecules ≥ 150 kbp. If no reference genome is provided, the metric is blank.
Estimated Effective Coverage	The coverage number is calculated as follows: Average Map Rate * Total DNA / length of the reference For human structural variation detection, we recommend at least 80X effective coverage.
Avg PLV	Average Positive Label Variance: Percentage of molecule labels absent in reference labels.

Avg NLV	Average Negative Label Variance: Percentage of reference labels absent in molecule labels.
DNA Collected (Gbp)	The total amount of DNA that is detected per flowcell during the run.
Scan Count	The scans “Read” number reflects the number of scans submitted by Saphyr Control Software to Bionano Access. The Scans “Mapped” number reflects the number of scans that have had metrics data generated.
Cumulative DNA (Gbp)	**Collected:** The total amount of DNA that is detected in this flowcell across all runs of this chip. **Target:** Target throughput of this flowcell as set in the “Experiment.” **Percent:** Percent of target throughput collected.

*Please refer to Bionano Access Dashboard Guidelines 30304 for more details.

Chip Metrics Dashboard

User can see chip metrics for last **30**, **60** or **90** days. It contains chip runs information include **Total DNA (>=150kbp)**, **N50 (>=150kbp)**, **Average label density (>=150kbp)**, **Map rate (%)**, **DNA per scan (Gbp)** and **Longest molecule (kbp)**. Each data point represents one flow cell run in a chip.

1. From the Bionano Access main menu, select **Chips**.

   The *Chips* list appears.

2. Select **Metrics**.

- User can click on data points to open corresponding dashboard.

Bioinformatics Analysis

Analysis Types

Users can perform the following analyses in Bionano Access:

Analysis	Description
Molecules Merge	Merge two or more molecules jobs into a single molecules job.
Filter molecule jobs	Filter molecules jobs by length and total DNA.
***De novo* Assembly**	Assemble single molecules into consensus maps for SV detection and hybrid scaffold applications.
Rare variant analysis	Identify rare variants at low frequencies.
EnFocus™ FSHD analysis	Analyze FSHD relevant regions.
1-Enzyme Hybrid Scaffold	Use a set of Bionano maps and a sequence assembly to build hybrid scaffolds.
2-Enzyme Hybrid Scaffold	Use two sets of Bionano maps and a sequence assembly to build two-enzyme hybrid scaffolds
Alignments	Compare two different maps or molecules to maps by aligning them to each other.
Variant Annotation Pipeline	Annotate SV calls for applications such as identification of rare and potential ***De Novo*** SVs for trio (mother, father, and proband) or for cancer research.
Convert SMAP to VCF file	Convert insertion, deletion, duplication, and inversion and translocation breakpoint calls in an SMAP file to dbVar-compliant VCF v4.2 format.
EnFocus™ Fragile X analysis	Analyze Fragile X relevant regions

Filter Molecule Jobs

User can filter BNX files based on molecules length and total DNA. The Bionano Genomics BNX file is a raw data view of molecule and label information and quality scores per channel identified during a run or runs if data from multiple runs are merged. BNX v1.3 and above supports one or two label channels (colors). If a user has a chip run that generated more data than recommended for the application, it is possible to down sample the data before running a *De Novo* assembly. Users can also filter the data on label count or length. On filtering a BNX file, a new BNX file is generated so that the raw molecules file is always saved as a copy.

To filter a molecule job:

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select a Molecules job to filter.
4. In the *Operations* pane, select **Filter Molecule Job**.

   The BNX Filter screen appears.

5. At the **Filtered Molecule Name** field, type the new name for the molecules job.
6. At the **Channel Information** field, Channel ID, the enzyme motif, name, and reference name are displayed.
7. At the **Reference** field, an automatically selected reference is shown up.
8. [Optional] At the **Tags** field, type the keywords to associate with the assembly.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Filter Settings** field, the following options are available for user to select.

    - [Optional] At the **Channel to Keep** field, select one of 'Keep Only Channel 1', 'Keep Only Channel 2', or 'Keep Both Channels' (**Only available on dual-label molecule job**).
    - [Optional] Select the check box of **Label Count** to type value of **Minimum** label to keep.

      By default, Bionano Access automatically uses 10 as Minimum.

    - [Optional] Select the check box of **Length (kbp)** to type values of **Minimum** and **Maximum**.

      By default, Bionano Access automatically uses 100 kbp as Minimum and 5000 kbp as Maximum.

    - [Optional] Select the check box of **Total DNA (Gbp)** and select **Random Seed** to randomly selected the molecules or select **Keep Longest** to keep the longest molecules.

      If a dataset is larger than necessary for a desired application, we recommend to down sample it in Bionano Access. If you want to down sample the volume of DNA, you can type the desired volume here.

      This operation offers base filtering options which will be expanded in the future. Molecules are sampled based on a pseudorandom process, which requires a random seed. A random integer is expected. If the same seed is used, the same molecules would be output.

      By default, Bionano Access automatically uses 300 Gbp as Total DNA and 17 as Random Seed.

11. Click **Submit**.

Users will receive an email when the filtering is complete.

Merge Molecule Jobs

Users may want to combine data from multiple runs to create a super set of their best data or sometimes it may take more than one run to acquire the amount of coverage required to perform an assembly. In such cases, users can merge these molecules file generated from different flow cells for assembly.

The program will automatically select Molecule jobs with the same sample name and recognition enzymes for users to merge. The user can still choose other molecule jobs that do not have the same sample name, but they must have the same recognition enzymes.

- **Merge molecule jobs that have the best quality data.**
- **Use BNX files that are with the same sample and reference.**
- **Do not merge BNX files that have different levels of quality data.**
- **Do not merge BNX files from 1st generation Saphyr System and 2nd generation. Please contact Bionano Genomics Technical Support for assistance if needed.**
- **Do not merge BNX files generated from Bionano Access v1.2 and greater with those generated from Bionano Access v1.0 or v1.1. Please contact Bionano Genomics Technical Support for assistance.**
- **Dual labeled BNX files can only be merged with dual labeled BNX files with matching recognition enzymes.**

Best Practices:

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. In the Jobs list, select a Molecules job to merge.
4. In the *Operations* pane, select **Merge Molecule Jobs**.

   A dialog box appears.
5. At the **Merge Molecule Name** field, type the new name for the molecules job.
6. At the **Sample** field, select the sample from the drop-down list.
7. [Optional] At the **Reference** field, select the genome reference from the drop-down list.
8. [Optional] At the **Tags** field, type the keywords to associate with the assembly.

9. [Optional] At the **Description** field, type a brief description.
10. Select the check boxes of the molecule jobs to merge with the molecules job that was selected in step 3 from the list of molecules jobs. the description of the information is shown in the list below:

Info	Description
Name	The molecules job name
Sample	The sample name.
Reference	The reference.
Map Rate (%)	The percentage of molecules that map to the reference for molecules ≥ 150 kbp. If no reference genome is provided, the metric is blank.
N Mol	The total number of molecules
Coverage	The coverage number is calculated as follows: Total DNA Throughput / length of the reference
PLV %	Average Positive Label Variance: Percentage of molecule labels absent in reference labels.
NLV %	Average Negative Label Variance: Percentage of reference labels absent in molecule labels.
SR	The quadratic term in sizing error relative to reference.
SF	The minimum expected sizing error relative to reference.

By default, Bionano Access automatically selects the molecule jobs in the project that are a match with the molecule job selected to merge with. For example, it selects the molecule jobs with the same reference and sample information. There is also a “select-all” option.

11. Click **Submit**.

Users will receive an email when the merge is complete.

Align Maps

Users can use map or assembly jobs to generate alignment data. If a user has two independent sets of maps, say each from a different nicking enzyme, it is possible to align the maps to each other in order to understand the similarities or differences between the two maps.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the map or assembly to use as a reference.

4. In the *Operations* pane, select **Align Maps**.

   The *Alignment* screen appears.

5. At the **Alignment Name** field, type the name of the alignment.
6. [Optional] At the **Tags** field, type the keywords to associate with the alignment job.
7. [Optional] At the **Description** field, type a brief description.
8. At the **Alignment p-value** field, type the p-value for the alignment. You can also check the boxes of *Output Best Alignment* and *Swap Anchor - Query*.
9. At the **Sample** field, the name of the sample is displayed.
10. At the **Source Anchor Map** field, the name of the selected consensus map or *De Novo* assembly results is displayed.
11. At **Target Query Map**, select the map, assembly, or reference to generate an alignment.
12. Click **Submit**.

    Users will receive an email when the alignment is complete.

Users can also use molecules jobs to generate alignment result.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the molecules job to use for alignment.
4. In the *Operations* pane, select **Align Maps**.

   The *BNX Alignment* screen appears.

5. At the **Alignment Name** field, type the name of the alignment.
6. [Optional] At the **Tags** field, type the keywords to associate with the alignment job.
7. [Optional] At the **Description** field, type a brief description.
8. At the **Sample** field, displays the name of the sample.
9. At the **Source Molecules** field, displays the name of the selected molecules job.
10. At **Target Map** field, select the map, assembly, or reference to generate an alignment.
11. At **Configuration** field, select or edit the configuration file.

    - Select “Saphyr data” if the data is collected from Saphyr instrument.

- Select “Irys data” if the data is collected from Irys instrument.

12. Click **Submit**.

    Users will receive an email when the alignment is complete.

Generate Molecule Quality Report

Users can generate molecule quality report with molecules data. Bionano Access will automatically generate a molecule quality report after importing molecule files. Please refer to *P/N 30223 Saphyr Molecule Quality Report Guidelines* for detail MQR description.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the molecules job to generate molecule quality report.
4. In the *Operations* pane, select **Generate Molecule Quality Report (MQR)**.

   The *Generate Molecule Quality Report* screen appears

5. At the **Name** field, displays the name of the molecules job.
6. At the **Sample** field, displays the name of the sample.
7. [Optional] At the **Reference** field, displays the reference of the selected molecules job.
8. At **Enzyme of primary molecule channel** field, select the enzyme.
9. Click **Submit**

   Users will receive an email when the molecule quality report is complete.

Generate *De Novo* Assembly

De Novo assembly algorithm is built on the overlap-layout-consensus (OLC) strategy with a maximum likelihood model for scoring alignments. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.

Users can generate *De Novo* assembly with molecules data.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the molecules job to perform *De Novo* assembly.

4. In the *Operations* pane, select **Generate *De Novo* Assembly**.
5. At the **Primary Channel to Assemble** field, select the channel to use for assembly. (**Only available on dual-label molecule job**).
6. At the **Assembly Name** field, type the name of the assembly.
7. At the **Estimated Genome Size (Gbp)** field, type the estimated genome size.
8. [Optional] At the **Tags** field, type the keywords to associate with the assembly.
9. [Optional] At the **Description** field, type a brief description.
10. The **Selected BNX** field shows the name of selected BNX job.
11. The **Reference** field shows the name of selected reference
12. The **Organism** field shows the name of selected organism (Human or non-human).
13. The following options in the **Advanced Assembly Options** are **optional** to change the default setting.
    a. The **Expected CN Baseline File** is used as the basis for calling genomic regions with abnormal CN states. It is recommended to select "Default Expected CN Baseline" for human samples. Users can upload and select their own file for human or non-human samples. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.
    b. The **Control CNV File** is used to reduce variation unrelated to true CNV events in raw coverage data. It is recommended to select "Default CNV control database" for human samples. Users can also upload and select their own file for human or non-human samples. Please refer *to P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.
    c. The **CNV Mask Bed File** filters CNVs from regions of the genome that are included in the mask. It is recommended to select the corresponding CNV mask bed file for human samples. Users can also upload and select their own CNV mask bed files for their human or non-human samples. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.
    d. The **SV Mask Bed File** filters SVs from regions of the genome that are included in the mask. It is recommended to select the corresponding SV mask bed file for human samples. Users can also upload and select their own mask bed files for their human or non-human samples. Refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.
    e. At the **Use Custom Config** field,
       - **if user select "No**", you can use the dialog to automatically determine the correct configuration to use.
         1) At the **Add Pre-Assembly** field, select "yes" to add this step to *De Novo* assembly process to help with samples that have no reference or if the reference is poor. This option will be turned on automatically if the sample has no reference. If you have a reference, you will have the

option to enable pre-assembly.

2) At the **Assembly Type** field, select one from “haplotype”, “non-haplotype with extend and split”, “non-haplotype without extend and split”. Haplotype is recommended for human samples and non-haplotype without extend and split for non-human samples.

3) At the **Cut CMPR (Complex Multi-Path Regions)** field, select “yes” or “no”. Recommend this to be set to ‘Yes’ for most applications of human samples. Refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.

- **If user select “yes”,** then the list of configuration files will be displayed. You can select the configuration file from the default list or select a customized configuration.

1) At the **Add Pre-Assembly** field, select “yes” to add this step to *De Novo* assembly process to help with samples that have no reference or if the reference is poor. This option will be turned on automatically if the sample has no reference. If you have a reference, you will have the option to enable pre-assembly.

2) To customize a configuration, click the **Edit** icon in one of the default configurations. The configuration dialog box appears.

3) Define the settings for *De Novo* assembly, and then click **Save As**.

4) Type the name of the configuration, and then click **OK**.

The customized configuration appears in the list of configurations.

f. At the **VCF Experiment Id** field, type the Id number for the VCF result.

g. Select **Disable VCF Breakpoint Uncertainty** check box will remove uncertain breakpoint in VCF result.

14. The following default setting for annotation pipeline in the **Advanced Variant Annotation Pipeline Settings** are optional to change when **Run Variant Annotation with control SV database** is enabled.

a. Select the SV control database for the annotation pipeline. User can create their own control database. For more detail information, please refer to ***P/N*** *30190 Bionano Solve Theory of Operation: Variant Annotation Pipeline.*

b. At the **Known genes** field, select a bed file from the drop-down list.

c. Use the default values or enter the values to use for the parameter fields of variant annotation.

15. Click **Next**.

16. User can run the *De Novo* Assembly with **Original BNX**, or **Recommended**, which is down sampled to 80X effective coverage.

Depending on the volume of data and coverage, the *De Novo* assembly run time may vary. Refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details. If Variant

Annotation is selected, only one annotated *De Novo* assembly job will be generated.

17. Click **Submit.**

    Users will receive an email when the assembly is complete.

Generate Rare Variant Analysis

The Rare Variant Analysis (RVA) is designed specifically to identify variants at low variant allele frequencies in heterogeneous samples such as cancers or samples with allele mosaicism. For more information on how the algorithm runs, please refer to the *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling*. Users can generate rare variant analysis with molecules data.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select a molecule job to perform rare variant analysis.
4. In the *Operations* pane, select **Generate Rare Variant Analysis**.
5. At the **Rare Variant Analysis Name** field, type the name of the analysis.
6. [Optional] At the **Tags** field, type the keywords to associate with the analysis.
7. [Optional] At the **Description** field, type a brief description.
8. The **Selected BNX** field shows the name of selected BNX job.
9. The **Reference** field shows the name of selected reference.
10. The following options in the **Advanced Rare Variant Analysis Options** are optional to change the default setting.
    a. At the **Expected CN Baseline File** field, select “Default Expected CN Baseline” or customer’s own baseline file from the drop-down list.
    b. At the **Control CNV File** field, select “Default CNV control database” for human sample, “mm10 control CNV” for mouse sample, or customer’s own CNV control database from the drop-down list.
    c. At the **CNV Mask Bed File** field, select a bed file from the drop-down list.
    d. At the **SV Mask Bed File** field, select a bed file from the drop-down list.
    e. At the **Configuration** field, select the Arguments
11. The following default setting for annotation pipeline in the **Advanced Variant Annotation Pipeline Settings** are optional to change when **Run Variant Annotation with control SV database** is enabled.
    a. Select the SV control database for the annotation pipeline. User can create their own control

database. For more detail information, please refer to *P/N 30190 Bionano Solve Theory of Operation: Variant Annotation Pipeline*

b. At the **Known genes** field, select a bed file from the drop-down list.

c. Use the default values or enter the values to use for the parameter fields of variant annotation.

12. Click **Submit**.

    Users will receive an email when the assembly is complete.

Generate EnFocus™ FSHD Analysis

EnFocus™ FSHD Analysis targets the regions of the genome related to FSHD. Please refer to *P/N 30321 Bionano Solve Theory of Operation: Bionano EnFocus™ FSHD Analysis* for more details. The EnFocus™ FSHD Analysis pipeline cannot run from merged BNX, and only can be run on BNX files that come from one flowcell or one chip run. It is only applicable for human samples using hg38 as the reference.

1. From the Bionano Access main menu, select Analysis.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select a molecule job to generate FSHD analysis.
4. At the field of **FSHD Analysis Background Information**, you can check more information about this analysis.
5. At the **FSHD Job Name** field, type the name of the analysis.
6. [Optional] At the **Tags** field, type the keywords to associate with the analysis.
7. [Optional] At the **Description** field, type a brief description.
8. The **Sample Name** field shows the name of selected sample.
9. The **Selected BNX** field shows the name of selected BNX job.
10. The **Reference** field shows the name of selected reference.
11. The **Show Additional information** field is enabled by default.
12. Click **Submit**.

    Users will receive an email when the assembly is complete.

Generate EnFocus™ Fragile X Analysis

EnFocus™ Fragile X Analysis targets the region of the genome related to Fragile X. Please refer to *P/N 30457 Bionano Solve Theory of Operation: Bionano EnFocus Fragile-X Analysis* for more details. The EnFocus™ Fragile X Analysis pipeline cannot run from merged BNX, and only can be run on BNX files that come from one flowcell or

one chip run. It is only applicable for human samples using hg38 as the reference.

1. From the Bionano Access main menu, select Analysis.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select a molecules job to generate Fragile X analysis.
4. At the field of **Fragile X Analysis Background Information**, you can check more information about this analysis.
5. At the **Fragile X Job Name** field, type the name of the analysis.
6. [Optional] At the **Tags** field, type the keywords to associate with the analysis.
7. [Optional] At the **Description** field, type a brief description.
8. The **Sample Name** field shows the name of selected sample.
9. The **Selected BNX** field shows the name of selected BNX job.
10. The **Reference** field shows the name of selected reference.
11. Click **Submit**.

    Users will receive an email when the assembly is complete.

Generate Hybrid Scaffold

Users can merge Bionano map or assembly jobs with sequence assemblies to produce long hybrid scaffolds that represent the chromosome structure for analysis. We recommend that users do not use haplotype assemblies to generate a hybrid scaffold.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.

2. Select the project to view from the list.
3. In the Jobs list, select the map or assembly to use as a reference.
4. In the *Operations* pane, select **Generate Hybrid Scaffold**.

   The *Scaffold* screen appears.

5. At the **Scaffold Name** field, type a name for the scaffold.
6. [Optional] At the **Tags** field, type the keywords to associate with the hybrid scaffold job.
7. [Optional] At the **Description** field, type a brief description.
8. At the **Selected Map** field, by default the software shows the map to use for scaffolding.

9. At the **Enzyme Selection** field, select the enzyme from the drop-down list.
10. At the **FASTA** field, select the file from the drop-down list.
11. At the **Conflict Resolution** field, for best practices, select **Resolve Conflicts** for both Bionano Assembly and Sequence Assembly. For more information, see more *in P/N 30073 Bionano Solve Theory of Operation: Hybrid Scaffold*.
12. At the **Trim Overlapping Sequence Contigs** field, turn it on or off. For more information, see more in *P/N 30073 Bionano Solve Theory of Operation: Hybrid Scaffold*.
13. At the **Configuration** field, select the configuration file from the default list or select a customized configuration.
    a. To customize a configuration, click the **Edit** icon in one of the default configurations. The configuration dialog box appears.
    b. Define the settings for hybrid scaffold, and then click **Save As**.
    c. Type the name of the configuration, and then click **OK**.

    The customized configuration appears in the list of configurations.
14. Click **Submit**.

    Users will receive an email when the hybrid scaffold is complete.

Generate 2-Enzyme Hybrid Scaffold

Users can also generate a hybrid scaffold using two enzymes. If DLE-1 is one of the enzymes, the DLE-1 assembly or CMAP must be selected first.

1. From the Bionano Access main menu, select **Analysis**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. In the Jobs list, select the map or assembly to use as a reference.
4. In the *Operations* pane, select **Generate 2-Enzyme Hybrid Scaffold**.

   The *2 Enzyme Scaffold* screen appears.
5. At the **Scaffold Name** field, type a name for the scaffold.
6. [Optional] At the **Tags** field, type the keywords to associate with the hybrid scaffold job.
7. [Optional] At the **Description** field, type a brief description.
8. At the **First Map** and **Enzyme** field, by default the software shows the map to use for scaffolding.

9. At the **Second Map** field, select the second map to use for scaffolding. The **Enzyme** field will automatically show up after that.
10. At the **Fasta** field, select the file from the drop-down list.
11. At the **Trim Overlapping Sequence Contigs** field, turn it on or off. For more information, see more in *P/N 30073 Bionano Solve Theory of Operation: Hybrid Scaffold*.
12. At the **Configuration** field, select the configuration file from the default list or select a customized configuration.
    a) To customize a configuration, click the **Edit** icon in one of the default configurations. The configuration dialog box appears.
    b) Define the settings for hybrid scaffold, and then click **Save As**.
    c) Type the name of the configuration, and then click **OK**.

    The customized configuration appears in the list of configurations.
13. Click **Submit**.

    Users will receive an email when the two-enzyme hybrid scaffold is complete.

Perform Variant Annotation - Single

The purpose of the variant annotation pipeline (VAP) is to enable users to determine if a Bionano structural variant (SV) call is relevant to certain physical or disease traits in humans. It can help to identify if a variant is overlaps with annotated genes or is a potential false positive call.

1. From the Bionano Access main menu, select **Projects**.

   The *Projects* window appears.
2. Select the project to view from the list.
3. In the Jobs list, select the map or assembly to use as a reference.
4. In the *Operations* pane, select **Generate Variant Annotation - Single**.
5. At the **Name** field, type the name for the variant annotation pipeline.
6. [Optional] At the **Tags** field, type the keywords to associate with the job.
7. [Optional] At the **Description** field, type a brief description.
8. At the **Control SV database** field, select the control SV database from the drop-down list.

   The control SV database is used to estimate the percentage of similar SVs found in Bionano control samples. It is recommended to select the corresponding control SV database file for human or mouse samples. Users can also upload and select their own control SV bed files for their human or non-human

samples. User can also run variant annotation pipeline with no control SV database file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

9. At the **Known genes** field, select a bed file from the drop-down list.

   The gene annotation bed file is used to annotate SVs. It is recommended to select the corresponding gene bed file for human or mouse samples. Users can upload and select their own file for their human or non-human samples. Users can also run variant annotation pipeline with no gene bed file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

10. The following default settings for annotation pipeline in the **Advanced Variant Annotation Pipeline Settings** are optional to change. Use the default values or enter the values to use for the following parameter fields.

    For more details, see the *P/N 30190 Bionano Solve Theory of Operations: Variant Annotation Pipeline* for guidance on setting these parameters.

11. Click **Submit**. The spinning arrows indicates that the pipeline is uploading data to the server.

12. When the spinning arrows disappear, click **Close**.

    Bionano Access sends an email to notify the user when the variant annotation pipeline is complete.

Perform Dual Analysis

The purpose of the dual variant annotation pipeline is to identify variant differences in two samples, such as somatic in tumor-normal pair studies.

1. From the Bionano Access main menu, select **Projects**.

   The *Projects* window appears.

2. Select the project to view from the list.

3. In the Jobs list, select the map or assembly to use as a reference.

4. In the *Operations* pane, select **Generate Dual Analysis**.

5. At the **Name** field, type the name for the variant annotation pipeline.

6. [Optional] At the **Tags** field, type the keywords to associate with the job.

7. [Optional] At the **Description** field, type a brief description.

8. At the **Control assembly** field, select the control sample assembled result from the drop-down list.

9. At the **Control SV database** field, select the control SV database from the drop-down list.

   The control SV database is used to estimate the percentage of similar SVs found in Bionano control samples. It is recommended to select the corresponding control SV database file for human or mouse samples. Users can also upload and select their own control SV bed files for their human or non-human

samples. User can also run variant annotation pipeline with no control SV database file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

10. At the **Known genes** field, select a bed file from the drop-down list.

    The gene annotation bed file is used to annotate SVs. It is recommended to select the corresponding gene bed file for human or mouse samples. Users can upload and select their own file for their human or non-human samples. Users can also run variant annotation pipeline with no gene bed file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

11. The following default setting for annotation pipeline in the **Advanced Variant Annotation Pipeline Settings** are optional to change. Use the default values or enter the values to use for the following parameter fields.

    For more details, see the *P/N 30190 Bionano Solve Theory of Operations: Variant Annotation Pipeline* for guidance on setting these parameters.

12. Click **Submit**. The spinning arrows indicates that the pipeline is uploading data to the server.

13. When the spinning arrows disappear, click **Close**.

    Bionano Access sends an email to notify the user when the variant annotation pipeline is complete.

Perform Trio Analysis

This analysis let users identify rare and potential *De Novo* SVs for trio Variant Analysis Pipeline (mother, father, and proband).

1. From the Bionano Access main menu, select **Projects**.

   The *Projects* window appears.

2. Select the project to view from the list.

3. In the Jobs list, select the map or assembly to use as a reference.

4. In the *Operations* pane, select **Generate Trio Analysis**.

5. At the **Name** field, type the name for the variant annotation pipeline.

6. [Optional] At the **Tags** field, type the keywords to associate with the job.

7. [Optional] At the **Description** field, type a brief description.

8. At the **Father *De Novo* assembly** field, select the assembly from the drop-down list.

9. At the **Mother *De Novo* assembly** field, select the assembly from the drop-down list.

10. At the **Control SV database** field, select the control SV database from the drop-down list.

    The control SV database is used to estimate the percentage of similar SVs found in Bionano control

samples. It is recommended to select the corresponding control SV database file for human or mouse samples. Users can also upload and select their own control SV bed files for their human or non-human samples. User can also run variant annotation pipeline with no control SV database file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

11. At the **Known genes** field, select a bed file from the drop-down list.

    The gene annotation bed file is used to annotate SVs. It is recommended to select the corresponding gene bed file for human or mouse samples. Users can upload and select their own file for their human or non-human samples. Users can also run variant annotation pipeline with no gene bed file selected. Refer to *P/N 30110 Bionano Solve Theory of Operations: Structural Variant Calling* for more details.

12. The following default setting for annotation pipeline in the Advanced Variant Annotation Pipeline Settings are optional to change. Use the default values or enter the values to use for the following parameter fields.

    For more details, see the *P/N 30190 Bionano Solve Theory of Operations: Variant Annotation Pipeline* for guidance on setting these parameters.

13. Click **Submit**. The spinning arrows indicate that the pipeline is uploading data to the server.

14. When the spinning arrows disappear, click **Close**.

    Bionano Access sends an email to notify the user when the variant annotation pipeline is complete.

Visualization Features

Navigate to the Viewer

Users can navigate to the *Viewer* screen from the project page.

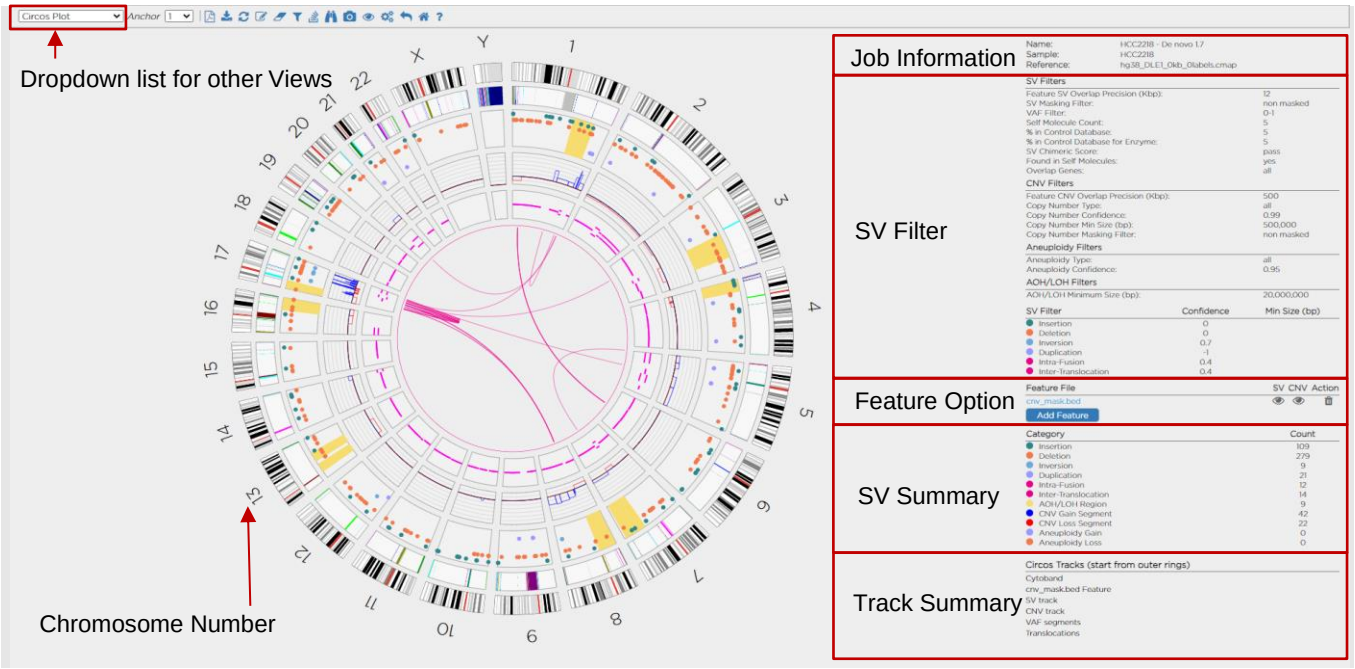
1. From the Bionano Access main menu, select **Analysis**. The *Projects* window appears.
2. Select the project to view from the list.
3. In the Jobs list, select an assembly, alignment, rare variant analysis, variant annotation pipeline, or scaffold job. Users can view these jobs on the *Viewer* screen.
4. In the *Options* pane, depending on the job selected, these are possible options to get to *Viewer* screen:
    - Molecules to Maps
    - Maps to Reference with SV
    - View variant annotation results
    - View EnFocus™ FSHD Analysis
    - View EnFocus™ Fragile X Analysis
    - Maps to NGS with Conflicts
    - Maps to NGS with Hybrid Scaffold

Circos Plot visualization

The Circos Plot is an interactive visualization tool to facilitate the identification and analysis of similarities and structural variants in genomics studies. The Circos plot is displayed by default for human assemblies, rare variant analysis, and variant annotations.

Note: For more mouse and keypad short cuts to maneuver the Circos plot, click on the ? icon which is the last icon on the top panel. For more detail, please follow Key and Mouse shortcuts section.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. The **Circos plot** is the default viewer for human sample. User can select **Circos Plot** from the drop-down list at upper-left corner of the “Viewer Screen” from the viewer. Here is an example of human variant annotation pipeline visualization page:



Circos Plot visualization example.

- The Circos Plot is displayed as the circular plot on the left, with a detail information of the job, SV, CNV and an aneuploidy calls' Filter Criteria, SV Filter Confidence scores, Add Feature, and color legend on the right.
- To zoom on the Circos Plot, rotate your mouse-wheel forward (scroll it down). The user can also click and drag the plot around using the mouse. In the Circos Plot each chromosome is positioned end-to-end-in a large circle. Each track represents a different type of annotation or result.
- User can change **SV filter** setting to filter the SV calling. For more detail, please follow the SV filter section.
- User can add current displayed SV calling to Curated List. For more detail, please follow Adding SV to Curated Variant List/SV report for Circos Plot.
- The outermost numerical track corresponds to the chromosome number, with cytoband information shown in the black-and-white banding pattern. New in Bionano Access 1.7, Chromosomes 1 through 22 (the autosomes) are designated by their chromosome number. The sex chromosomes are designated by X or Y for human sample.
- Relevant genome annotations are used for either data filtering or visualization, it can be included in the Circos Plot as a separate track. Each feature in the file is a separate vertical line. Users can zoom in and hover over the lines to get more information about the feature.
- The next track includes the detected SVs as represented by color-coded dots. A user can hover over the dot to view info about a given SV call. AOH/LOH regions also show in this track indicated with a yellow-colored block.

- The next track shows copy number calls. For the copy number track, the baseline state of autosomes in a diploid genome is a copy number of 2 for human sample. Three color coded lines are used to visualize the copy number calls. Black is the baseline, blue is CNV gain, and red is CNV loss. If a region shows a copy number gain, the line will move outward from 2 (colored blue), reflecting an increase in the local copy number state. If the region has a loss, it will move inward (colored red).
- The next track shows **Variant Allele Fraction (VAF)** segments. The segmentation smooth line helps distinguish changes in the VAF pattern across the genome. By default, the VAF smooth lines will only display in *De Novo* assembly result. For RVA results, the VAF track is not displayed by default. User can change this setting in View Options for Circos Plot.
- Click on any SV dots, CNV, AOH/LOH region or cytoband in the Circus Plot will navigate to **Genome Browser Viewer**. Click on VAF smooth line to navigate to **Whole Genome Viewer.**
- Shift + Left Click of the **Feature** in the feature track to annotate the corresponding name. Clear feature name(s) by clicking **Clear Feature Annotations**  icon.

Note: Please refer to the SV Workflow video on *https://bionanogenomics.com/support-page/bionano-access/* to see how the following features can be used in visualizing, filtering for and reporting SVs of interest.

Add Feature

1. Click the **Add Features** icon,

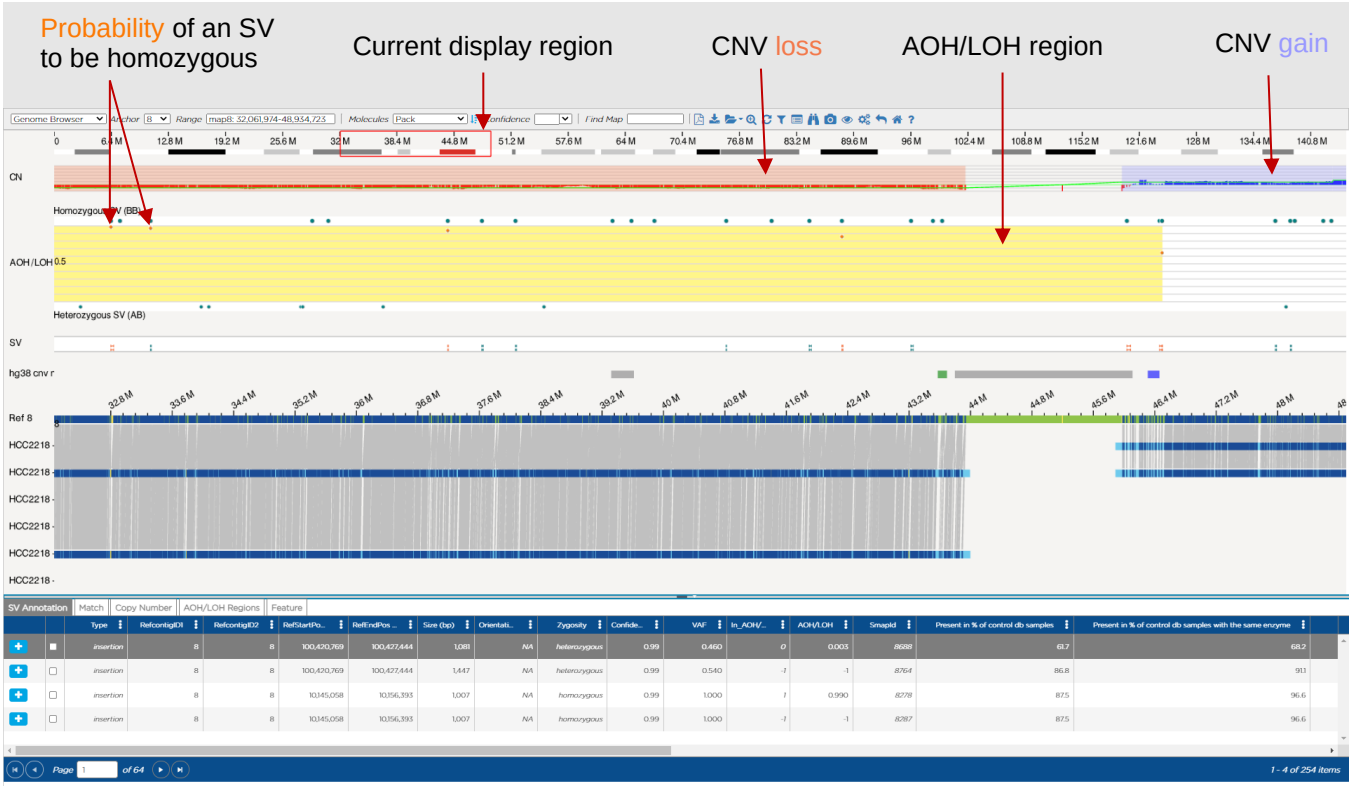
   Feature Filter Settings dialog box appears.

2. At the **Feature Selection** field, click the drop-down list and select one bed file.
   - To add new feature file to Access, please follow the **System Features** setting.
3. At the **Structural Variant Overlap Precision (Kbp)** field, type the value. **12** is the system default.
4. At the **Structural Variant Filter Setting** field, select one from **Show all structural variants**, **Show structural variants that overlap BED Regions** and **Hide structural variants that overlap BED Regions**. **Show all structural variant** is the system default.
5. At the **CNV Segment Overlap Precision (Kbp)** field, type the value. **500** is the system default.
6. At the **CNV Segment Filter Setting** field, select one from **Show all CNV segments**, **Show CNV segments that overlap BED Regions** and **Hide CNV segments that overlap BED Regions**. **Show all CNV segments** is the system default.
7. Click **OK**.
8. The **Feature** track will be added into Circos Plot.

Genome Browser visualization

The genome browser view is an interactive visualization tool for analyzing variants on a chromosome. The central viewing region includes tracks that display information for a given chromosome.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Genome Browser** from the drop-down list.
3. Genome Browser viewer shows up.



**Genome Browser visualization example.*

- At the **Anchor** field, change the view of other contigs and chromosomes in the same sample.
- At the **Range** field, user can set the range of the coordinates on the same chromosome.
- At the **Molecules** field, user can sort the molecules in the order of pack, start, end, confidence, length, label density**.**
- At the **Confidence** field, user can view molecules in the genome maps depending on their levels of confidence. Users can also enter a specific value manually.
- At the **Find Map** field, user can highlight the genome map by typing Genome Map ID here.
- The **Cytoband** information is shown in the black-and-white banding pattern with centromere in red. The displayed range is marked in red box.

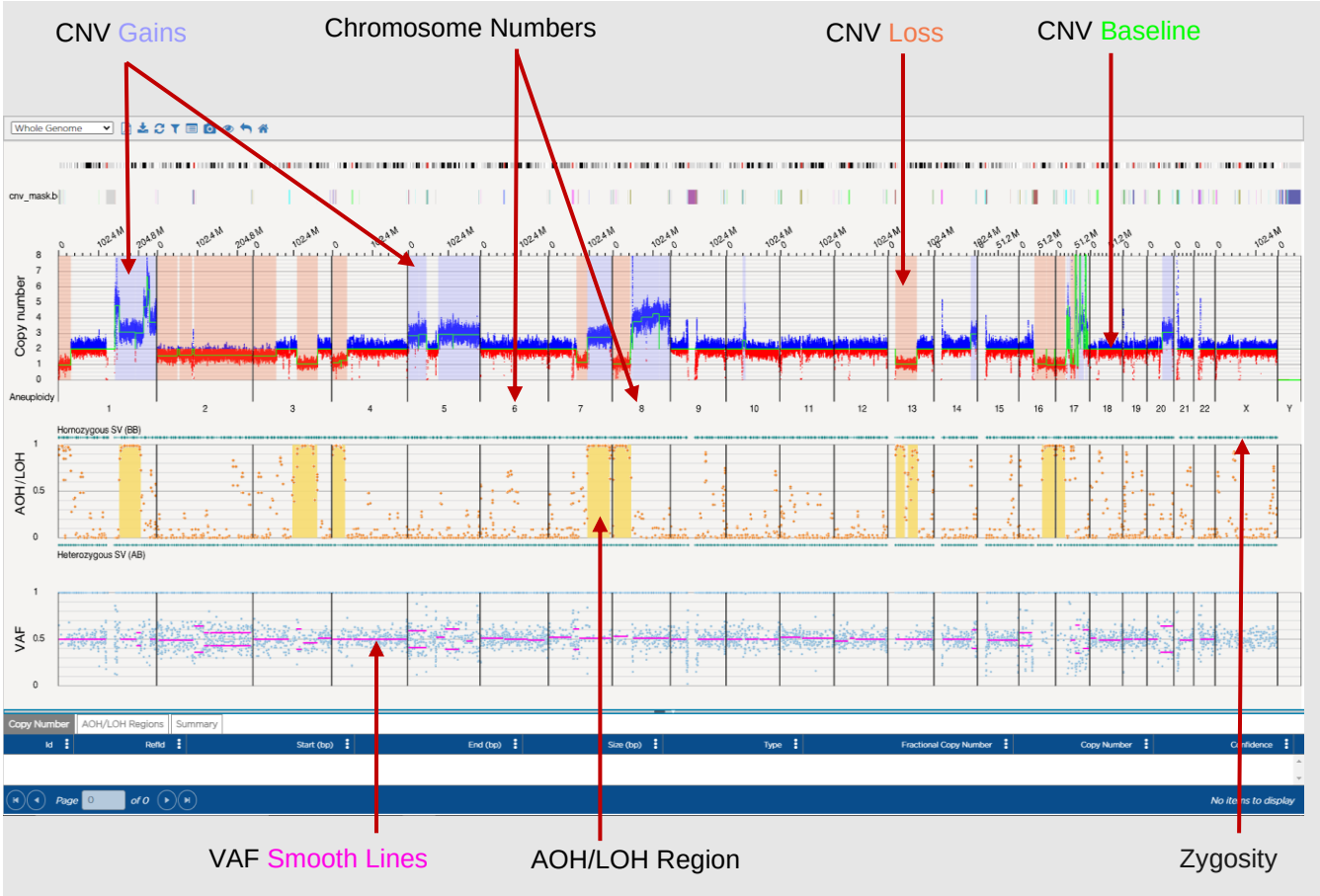
- By default, **CN (Copy Number)** track is shown as a smooth light blue line with highlighted regions for deletions and duplications. After zooming in, it shows the individual copy number values for each label with high copy number in blue and low copy number is red by default.
- **SV** track marks the location of each SV.
- The **AOH/LOH** track shows AOH/LOH calls, and marks as yellow region. User can show all AOH/LOH probability data points in yellow dot (disabled by default). To enable this option, please follow View Options for Genome Browser.
- **Ref** track displays the reference information of the selected chromosome or contig. By default, it is shown as green map with dark blue labels.
- **Map** track displays the genome maps that are aligned to the selected chromosome or contig. By default, it is shown as blue background with aligned labels in dark blue and unaligned labels in yellow.
- User can change **SV filter** setting to filter the SV calling. For more detail, please follow the SV filter section.
- User can add current displayed SV calling to Curated List. For more detail, please follow Adding SV to Curated Variant List/SV report for Genome Browser.
- If match groups or maps are hidden, click the **Show All**  icon to view them again.
- Right click **Ref** or **Map** track to select the following options:

Option	Description
Align Maps to Current Label (Ref track only)	**Adjust** all **map**s position **by** align all aligned labels to the selected **label** in reference.
Hide	Hide the selected reference or genome map.
Hide the Others	Hide the other reference or genome map(s).
Collapse	Collapse multiple Map tracks into one.
Hide (Show) Ruler	Hide or Show ruler of the reference or genome map.
Hide (Show) Matchgroups	Hide or Show matchgroups of the reference or genome map.
Show Feature (Ref tack only)	Select the feature file.
Remove All Features	Remove all added features.
Invert	Invert the orientation of the selected reference or genome map.

Whole Genome visualization

The whole genome view in Bionano Access helps visualize genomic locations across all chromosomes with **Copy number**, **AOH/LOH** and **Variant Allele Fraction (VAF)** in three separate plots. AOH/LOH is plotted only in *the De Novo* analysis workflow. For each graph, Bionano displays the data identifier, one or more attributes, and the data.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Whole Genome** from the drop-down list.
3. Whole Genome view shows up.



**Whole Genome visualization example.*

- The **Copy Number** plot represents the CNV profiles across the chromosomes. The Y axis shows copy number for each of the chromosomes plotted on the X axis. Molecules showing regions with increased copy number from the calculated baseline (green) are shown in blue and regions with decreased copy number are shown in red.
- The **AOH/LOH** graph represents regions of AOH/LOH is calculated for structural variants identified in the *De Novo* workflow. The X axis represents chromosomes, and the Y axis represents the range of zygosity for these genomic locations. The teal dots represent zygosity of the variant. Users can also plot the probability of the SV to be present in the LOH/AOH region is (orange dot). The regions of the genome that

have a consistently high AOH/LOH calls will be indicated with a yellow-colored block. AOH/LOH graph is only available on *De Novo* Assembly result.

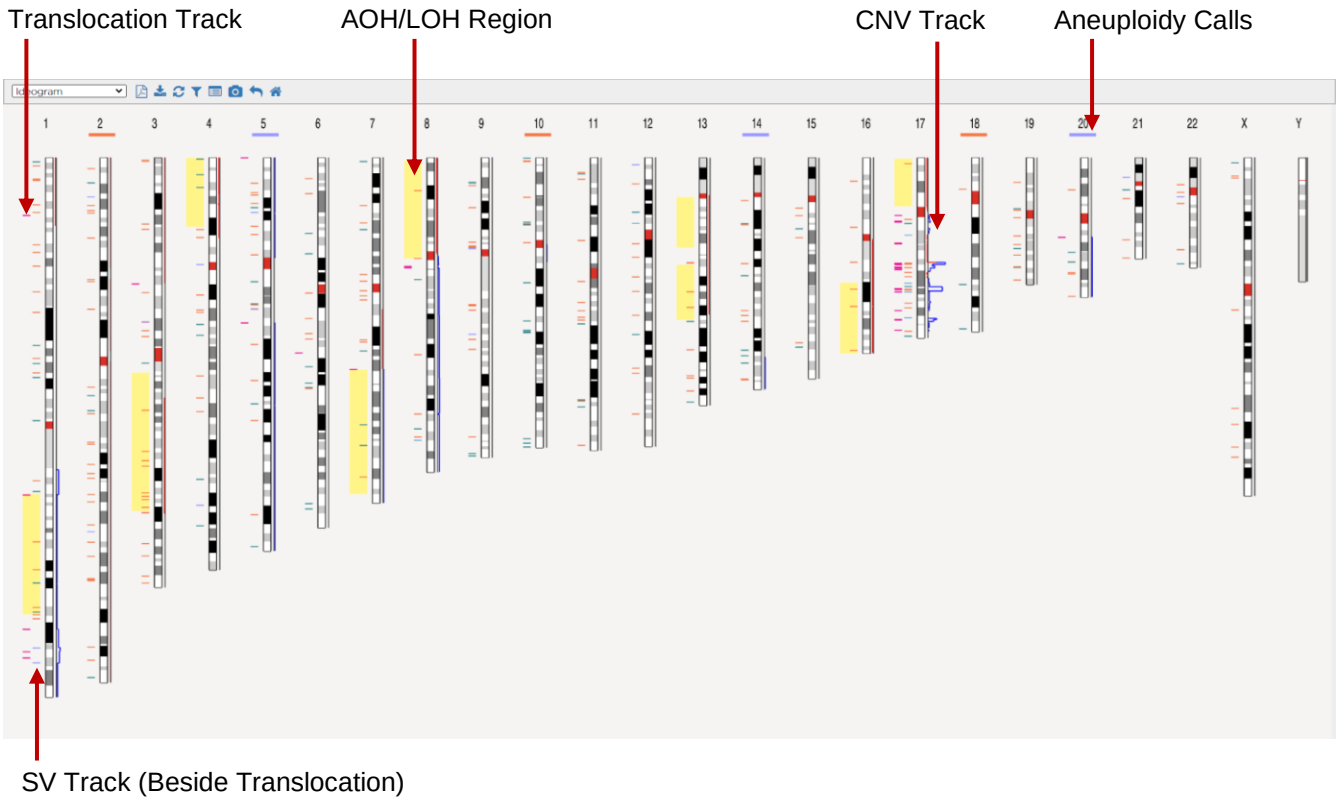
- The **Variant Allele Fraction (VAF)** plot represents the fraction of copies that are of a particular allele in a defined population. The VAF track provides a visual illustration of this difference with a scatter plot of allele frequencies for a given variant. The Y axis represents the range of the allelic frequency across the different chromosomes plotted on the X axis. The mean of the frequency rests around .5 for diploid genome and is color-coded magenta. If the data show fluctuations in the ploidy, the mean value changes and is visualized by more than one magenta lines across the region of the genome that shows polyploidy. Chromosomes or regions of the genome that Variant Allele frequency calculated for all SVs detected in both Rare Variant Analysis and *De Novo* Analysis Pipelines. Users can choose to filter variants based in their allelic frequency.

Ideogram visualization

Ideograms provide a schematic representation of chromosomes. They are used to show the relative size of the chromosomes and their characteristic banding patterns. Bionano Access 1.7 now have an interactive viewer to show the location of the structural variants, copy number variants and loss of heterozygosity on human chromosomes. SVs, CNVs, AOH/LOH detected from either different resources that can be chosen to display on chromosomes. All chromosomes and cytobands can be viewed via Ideogram.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Ideogram** from the drop-down list.

3. Ideogram view shows up.



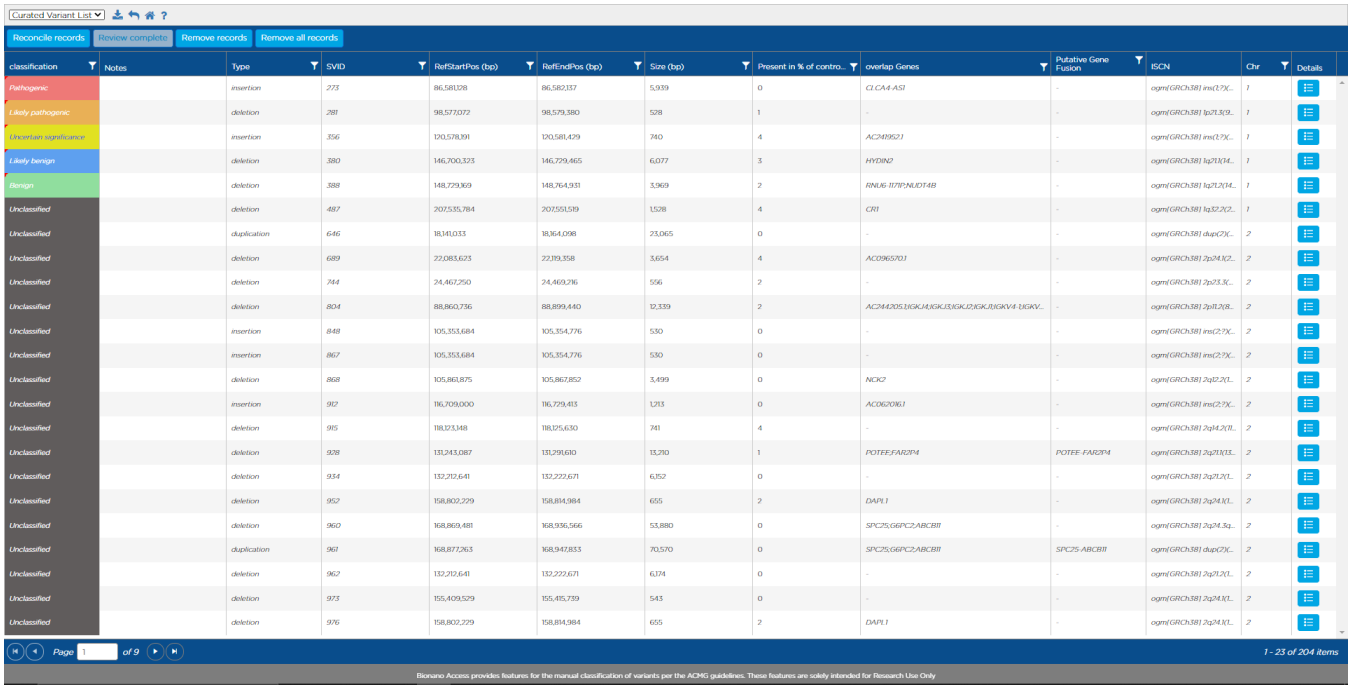
** Ideogram visualization example.*

- The Cytoband information is shown in the black-and-white banding pattern with centromere in red.
- 2 SV tracks are on the left and CNV track is on the right of each chromosome.
- Aneuploidy information is also marked on the top.
- The yellow blocks in the SV tracks represent the AOH/LOH regions.

Curated Variant List

The curated variant list view contains the SV calls that the user added to the curated list. It contains the SV details and classification made in the variant classifier view. Administrators and project leaders can generate an SV report with the classification information after the review is completed. Bionano Access provides features for the manual classification of variants per the ACMG guidelines. These features are solely intended for Research Use Only.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Curated Variant List** from the drop-down list.
3. Curated Variant List view shows up.



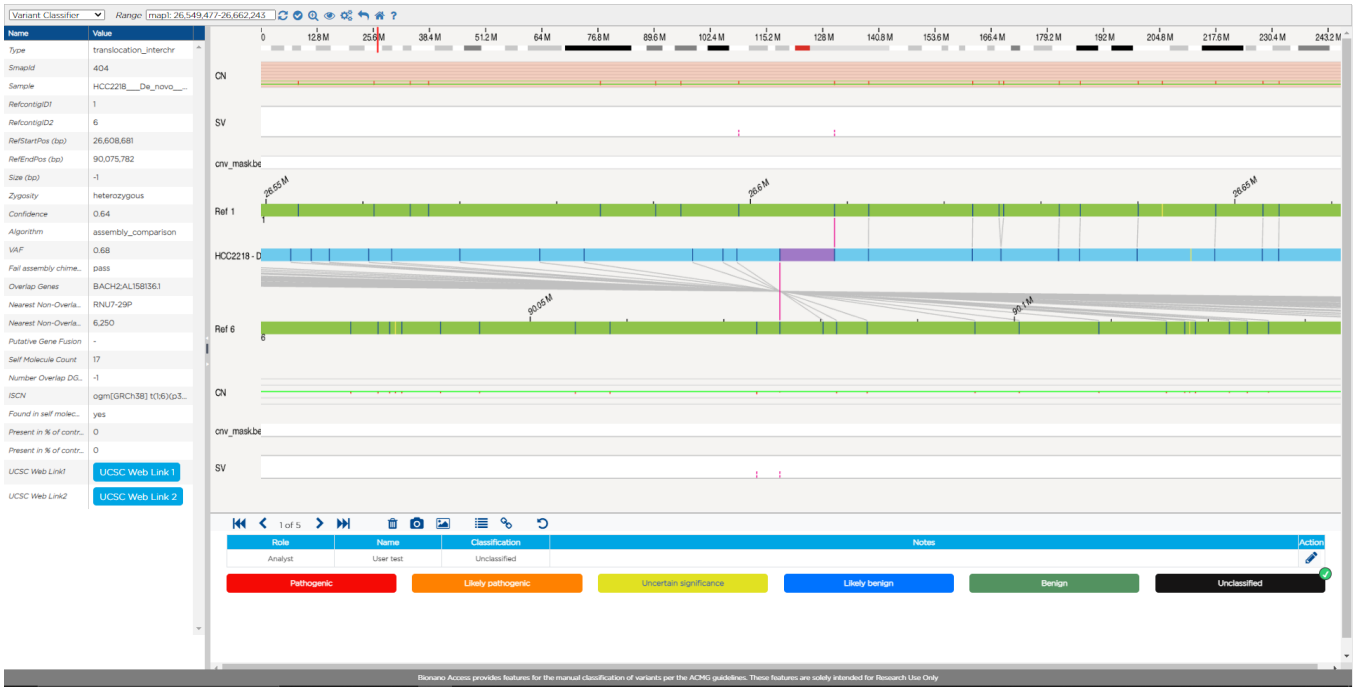
**Curated Variant List visualization example.*

- Users can remove variants from the curation list by selecting the row or rows to remove and then clicking the Remove Records button.
- Users can clear the entire curation list by clicking the Remove all records button.
- User with access to the project will be assigned as Analyst and submit the classification result to the supervisor for review by clicking  icon.
- Administrators and project leaders will be assigned as supervisor. Supervisor account can reconcile the records and change the job curation status to 'In Review' after clicking  icon.
- After the supervisor makes final classifications, clicking  icon will allow for SV report with classification information to be generated. The job curation status will change to "Review Complete".
- After the review is complete, supervisor can download the SV report with classification information by clicking  icon. In the **Summary** field, type the summary for the SV report. The job curation status will change to "Published".

Variant Classifier

The variant classifier view is an interactive view that allows users to curate and visualize one variant in the curated variant list at a time. Bionano Access provides features for the manual classification of variants per the ACMG guidelines. These features are solely intended for Research Use Only.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Variant Classifier** from the drop-down list.
3. Variant Classifier view shows up.



Variant Classifier visualization example.

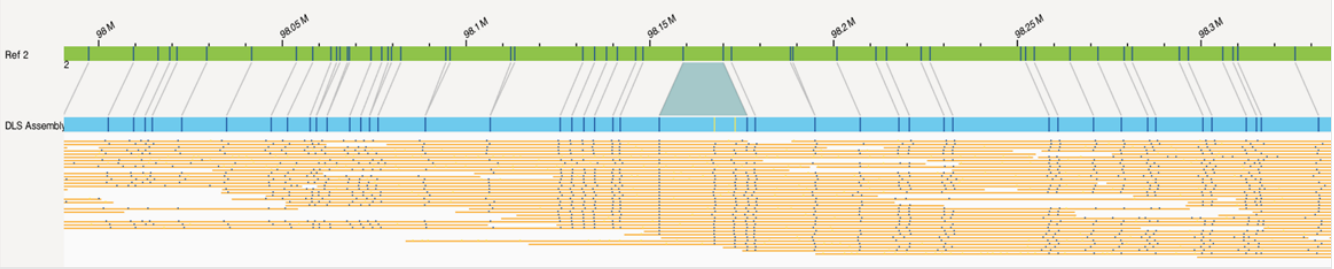
- Table on the left includes information about a single variant. The rows have information from the Variant calling algorithm that is also found in the Genome browser view and SMAP file.
- Genome map in the middle includes the cytoband, SV track, feature track, reference map and the sample specific assembled maps in the viewing region information.
- The lower panel includes navigation tools that allow a user to view a different variant, remove a variant, create images, or add to an existing list.
- User can re-capture the SV image for the SV report by clicking icon. Click icon to show captured image.
- User can remove the SV from the curated variant list by clicking icon.
- User can go back to Curated Variant List view by clicking icon or go back to Genome Browser view by clicking icon.
- User can add note to the SV by clicking icon.
- In this view, the user can review the ACMG categories assigned to a variant during curation and change them if necessary. The options are **Pathogenic**, **Likely pathogenic**, **Uncertain significance**, **Likely**

benign, **Benign** and **Unclassified**. The Variant classifier view is specifically designed for variant curation.

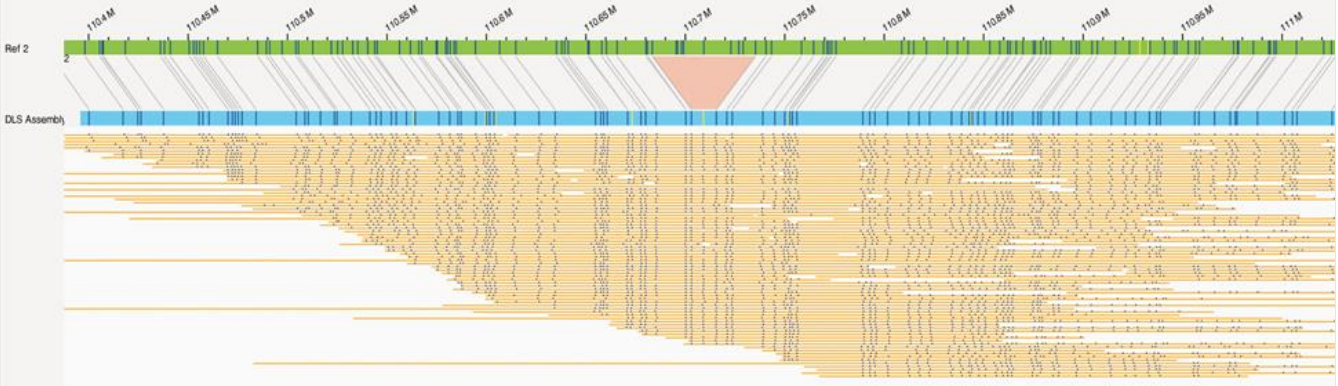
- Administrators and Project leaders (Supervisor role) can see the classification made by Analyst (user role) after they click classification complete and make a final classification.

Structural Variant example

Insertion

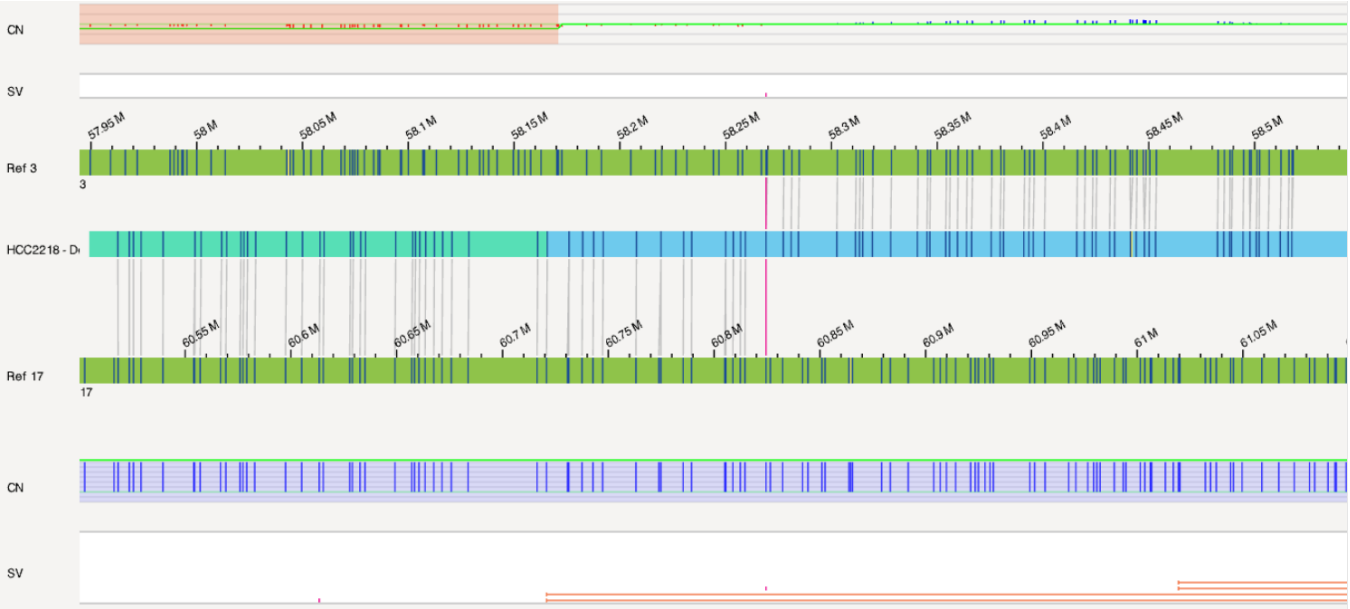


Deletion

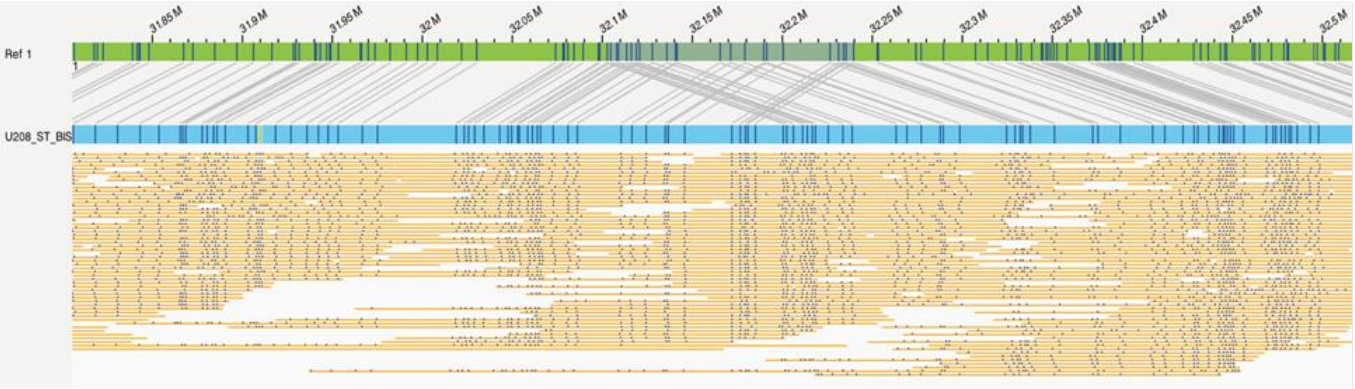


Inversion

Translocation

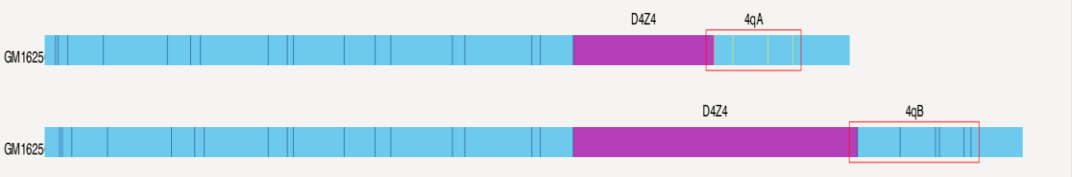


Duplication



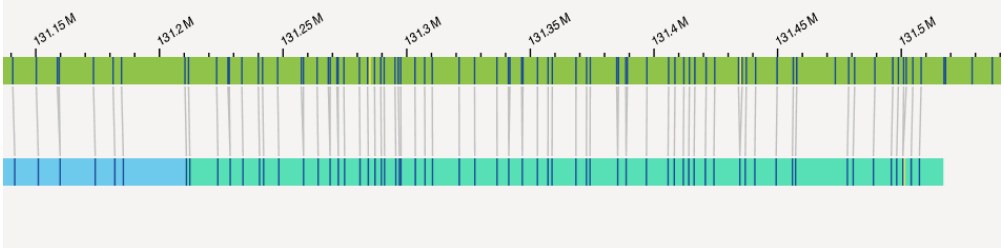
FSHD Visualization

The genome browser has been customized for FSHD results. It will automatically focus on the areas of interest on either chromosome 4 or 10. The consensus maps are shown in blue. The repeat region of interest (labeled as D4Z4) and the haplotype-specific region (labeled as either 4qA or 4qB) are highlighted. Below is an example:



Complex Multi-Path Region Visualization

Bionano Access highlights Complex Multi-Path Regions (CMPR). For CMPR detail information, please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details. By default, it is shown in mint green on the assembled map, as below:



Generate SV Report

Reporting features have been added to the visualization page to allow users to share their findings with others d.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Circos Plot, Genome Browser, Whole Genome, Ideogram** from the drop-down list.
3. Click the **Generate SV Report** icon.
4. At the **Generate PDF report** field, select one from **Filtered Variants** and **Curation Variant List**. **Filtered Variants** are structural variants based on filter criteria. **Curation Variant List** are the SVs were added to the Curated Variant List.

The PDF generated will include a screen shot of the Circos plot complete with the legend, a screen shot of each structural variant, details on each structural variant, and general information about the dataset.

Download Files

User can download filtered SV files

1. At the **Download Files** field, select one from **Filtered Variants** and **Curation Variant List**. **Filtered Variants** are structural variants based on filter criteria. **Curation Variant List** are the SVs were added to the Curated Variant List.
2. Check one or more boxes of **Copy Number Variants (CSV Format)**, **Structural Variants (SMAP Format)**, **Annotated Structural Variants (SMAP Format)**, **Aneuploidy File (.txt)**, **Informatics Report** and **Structural Variants (VCF Format)**.
3. Click **OK** to generate and download reports.

   The files will be generated and downloaded to your system.

Refresh

Users can refresh the current view.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Click the **Refresh** icon.
3. Refresh the current view.

Annotate Genomic Features

User can annotate specific gene or cytoband on the Circos Plot.

1. In Bionano Access, navigate to the Circos Plot screen viewer of the job to analyze.
2. Click the **Annotate Genomic Features** icon.
3. At the **Feature Name** field, type the gene name (i.e. FMR1) on the feature user added, or the chromosome region (i.e. 4q11) for the cytoband.
4. Click **Annotate**. And the feature will be annotated on the Circos Plot.

Clear Annotations

User can clear all annotated features on the Circos Plot.

1. In Bionano Access, navigate to the Circos Plot screen viewer of the job to analyze.
2. Click the **Clear Annotations** icon.
3. All annotated features will be removed.

SV Filter

SV Filter icon is used to set up your own customized filter criteria. After setting, the applied filter criteria is displayed on the top right corner of Circos plot view. Filter settings will be saved on user's profile, and each individual user can save different SV filter settings.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Click the **SV Filter** icon on the Circos Plot view, Genome Browser view or Whole Genome CNV view.

   The *Filter Settings* Window appears.

Filter by SV Type

User can filter SVs by SV Type, confidence score and minimum size. For more details, please refer to Theory of Operation SV calling.

- At the **Insertion** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended for 1.6 (in Access 1.7)** or your customized filter from the drop-down list and type SV minimum size (bp).
- At the **Deletion** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended for 1.6 (in Access 1.7)** or your customized filter from the drop-down list and type SV minimum size (bp).
- At the **Inversion** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended for 1.6 (in Access 1.7)** or your customized filter from the drop-down list.
- At the **Duplication** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended for 1.6 (in Access 1.7)** or your customized filter from the drop-down list and type SV minimum size (bp).
- At the **Intra-Translocation** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended Prior for 1.6 (in Access 1.7)** or your customized filter from the drop-down list.
- At the **Inter-Translocation** field, check the box to display, select one from **All**, **Recommended**, **Recommended Prior to 1.6, Recommended Prior for 1.6 (in Access 1.7)** or your customized filter from the drop-down list.

General SV Filters

User can filter SVs by chromosome and SV Masking.

- At the **Chromosomes to Display on Circos Plot** field, select one from **All chromosomes**, **Only chromosomes that have structural variants**, and **Only chromosomes from this range**. **All chromosomes** is the system default.
- At the **SV Masking Filter** field, select one from **All Structural Variants**, **Masked Structural Variant Only** and **Non-Masked Structural Variant Only**. **Non-Masked Structural Variant Only** is the recommended for human analysis if mask bed file is selected during *De Novo* assembly and rare variant analysis.
- At the **VAF filter min** and **VAF filter max** field, type the minimum and maximum value (between 0 to 1) for the variant allele frequency to filter SVs by their variant allele frequency.

Variant Annotation Filters

User can filter SVs by percentage in Bionano control samples, chimeric score filter, self/control/parent molecule/assembly check, overlapping genes and self molecule count. It is only available for variant annotation pipeline. For more details, please refer to Theory of Operation Variant Annotation Pipeline 30190.

- At the **SV in less than this % of the Bionano control samples** field, type the value. **100** is the system default.

- At the **SV in less than this % of the Bionano control samples with the same enzyme** field, type the value. **100** is the system default.
- At the **SV chimeric score filter** field, select one from **All SVs**, **Show Failed Chimeric Score**, and **Show Not Failing Chimeric Score**. It is only available for variant annotation of *De Novo* assembly.
- At the **SV control assembly check** field, select one from **All SVs**, **SV found in control assembly**, and **SV not found in control assembly**. It is only available for dual variant annotation pipeline.
- At the **SV control molecule check** field, select one from **All SVs**, **SV found in control molecules**, and **SV not found in control molecules**. It is only available for dual variant annotation pipeline.
- At the **SV parent assembly check** field, select one from **All SVs**, **SV found in both parent assemblies**, **SV found in father assembly**, **SV found in mother assembly** and **SV not found in parent assemblies**. It is only available for trio variant annotation pipeline.
- At the **SV parent molecule check** field, select one from **All SVs**, **SV found in both parent molecules**, **SV found in father molecules**, **SV found in mother molecules** and **SV not found in parent molecules**. It is only available for trio variant annotation pipeline.
- At the **SV self molecule check** field, select one from **All SVs**, **SV found in self molecules**, and **SV not found in self molecules**.
- At the **SV overlapping genes filters** field, select one from **All SVs**, **SV with overlapping genes**, and **SV with no overlapping genes**.
- At the **Self Molecule Count** field, type the value. **5** is the system default.

Copy Number Variant Filters

User can filter copy number calls by type, confidence score and minimum size. For more details, please refer to Introduction to Copy Number Variation.

- At the **Copy Number Variant Type** field, select one from **All**, **Deletion**, and **Duplication**.
- At the **Copy Number Variant Confidence** field, select one from **All**, **Recommended**, **Recommended Prior to 1.6,** or **Recommended for 1.**6 **(in Access 1.7)** from the drop-down list.
- At the **Copy Number Variant Minimum Size (bp)** field, type the value. **500000** is the system default.
- At the **Copy Number Variant masking filter** field, select **All Copy number variants, Masked Copy Number Variants Only,** or **Non-Masked Copy Number Variants Only** to filter CNV.

Aneuploidy Filters

- At the **Aneuploidy Type** field, select **All**, **Gain** or **Loss** to show the aneuploidy calls.
- At the **Aneuploidy Confidence** field, select one from **All**, **Recommended**, **Recommended Prior to 1.6,**

or **Recommended for 1.6 (in Access 1.7)** from the drop-down list.

AOH/LOH Filters

- At the **AOH/LOH Minimum Size (bp)** field, type the minimum size in bp to filter AOH/LOH calling region. 25,000,000 is the system default.

3. Click **Apply** to apply the filter criteria.
4. Click **Reset Filter** to reset to the default settings.
5. Click **Show All SVs** to show all the SVs.

SV Summary

User can check SV summary on Genome Browser viewer, Whole Genome viewer and Ideogram Viewer by click the SV summary  icon. In Genome Browser viewer, the SV summary will display the SV summary for the current chromosome. In Whole Genome viewer and Ideogram Viewer, the SV summary will display the full SV summary.

Search Genomic Features

User can search different features on the Genome Browser viewer.

1. In Bionano Access, navigate to the Genome Browser screen viewer of the job to analyze.
2. Click the **Search Genomic Feature**  Icon.
3. Use one of the following options to search features.
    - [Optional] At the **Feature Name** field, type the feature name in the corresponded feature in the drop-down list.
    - [Optional] At the **Smap Id** field, type the smap Id.
    - [Optional] At the **CNV Id** field, type the CNV Id.
4. Click **Search**. Access will direct to the corresponded feature.

Export to JPEG

Users can download the current view in JPEG file format.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Click the **Export to JPEG**  icon.

    The dialog box appears.

3. At the **Filename** field, type the name. By default, it is AccessViewer.
4. Click **OK**.

View Options

The View Options contain different setting on Circos Plot, Genome Browser and Whole Genome visualization

Circos Plot

- Click the **View Options** icon.

  The dialog box appears.
- At the **Show Curation Variant List** option, switch on or off to display SV in curated variant list only.
- At the **Show AOH/LOH regions** option, switch on or off to display AOH/LOH calling regions.
- At the **Show Copy Numbers Masked BED** option, switch on or off to display CNV mask track.
- At the **Show SV Masked BED** option, switch on or off to display SV mask track.
- At the **Show CytoBand on Circos Chromosome** option, swtich on or off to display cytoband detail on the cytoband track.
- At the **Show Alternated Transcripts** option, switch on or off to display alternated transcript.
- At the **Show VAF Smooth Lines** option, select Hidden, *De Novo* Assembly Only, Rare Variant Assembly Only or Both to show VAF smooth lines on the corresponded analysis results. This setting effect on all analysis result for the that user account. Default is on *De Novo* Assembly Only.
- Click **Close** to apply the setting.

Genome Browser

- Click the **View Options** icon.

  The dialog box appears.
- At the **Show Curation Variant List** option, switch on or off to display SV in curated variant list only.
- At the **Show Unmatched Labels** option, switch on or off to show unmatched labels.
- At the **Show Matched Labels** option, switch on or off to show matched labels.
- At the **Grouping Highlighted Molecules** option, switch on or off to enable or disable.
- At the **Auto Align Highlighted Molecules** option, switch on or off to enable or disable.
- At the **Show Molecule Matchlines** option, switch on or off to enable or disable .
- At the **Show Molecule Coverage** option, switch on or off to enable or disable.

- At the **Show Feature Track In Overlapping Mode** option, switch on or off to enable or disable.
- At the **Show Feature Name In Non-Overlapping Mode** option, swtich on or off to enable or disable.
- At the **Show AOH/LOH Track** option, swtich on or off to show the track.
- At the **Show AOH/LOH Data Point** option, swtich on or off to show the AOH/LOH probability data points.
- At the **Show SV Track** option, switch on or off to show the track.
- At the **Show SV Query Individually** option, switch on or off to enable or disable.
- At the **Show Copy Numbers** option, switch on or off to enable or disable.
- At the **Show Copy Numbers Masked BED** option, switch on or off to display CNV mask track.
- At the **Show SV Masked BED** option, switch on or off to display SV mask track.
- At the **Show CytoBand** option, switch on or off to show the cytoband.
- At the **Show Tooltips** option, switch on or off to enable or disable.
- At the **Show Alternated Transcripts** option, switch on or off to display alternated transcript.
- At the **Show Molecule Labels** option, click the drop-down list, and select one from **UnStretch**, **Stretch**, **and Stretch Matchgroup**. **UnStretch** is the system default.
- At the **Show Match Lines in** option, click the drop-down list, and select **Enzyme Color**.
- Click **Close** to apply the setting.

Whole Genome

- Click the **View Options** icon.

  The dialog box appears.
- At the **Show Curation Variant List** option, switch on or off to display curated variant list only.
- At the **Show AOH/LOH Data Point** option, switch on or off to show the AOH/LOH probability data points.
- At the **Show Copy Numbers Masked BED** option, switch on or off to display CNV mask track.
- At the **Show SV Masked BED** option, switch on or off to display SV mask track.
- At the **Show Alternated Transcripts** option, switch on or off to display alternated transcript.
- At the **Hide VAF Outliers** option, switch on or off to hide the VAF outliers data point.
- At the **Show VAF Smooth Lines** option, select Hidden, *De Novo* Assembly Only, Rare Variant Assembly Only or Both to show VAF smooth lines on the corresponded analysis results. This setting effect on all analysis result for the that user account. Default is on *De Novo* Assembly Only.
- Click **Close** to apply the setting.

View Settings

Users can customize the options settings, such as setting enzyme colors, SV colors, map (reference, genome or NGS map) colors, minimum and maximum map height value, molecule and copy number height value, and others. In Bionano Access, navigate to the Viewer screen of the project to analyze.

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Click the **View Settings** icon.

   The dialog box appears.
3. At the **Minimum map height (6-20 px)** field, type the value. By default, the value is 10.
4. At the **Maximum map height (20-100 px)** field, type the value. By default, the value is 40.
5. At the **Molecule height (1-40 px)** field, type the value. By default, the value is 2.
6. At the **Copy number height (30-150 px)** field, type the value. By default, the value is 40.
7. At the **Non-overlapping BED row height (30-400px)** field, type the value. By default, the value is 150.
8. At the **Copy number zoom threshold (30-400px)** field, type the value. By default, the value is 5.
9. At the **Max copy number to display (0 - 100)** field, type the value. By default, the value is 8.
10. At the **Track name width (80 px)** field, type the value. By default, the value is 80.
11. At the **Gap between molecules (0-40 px)** field, type the value. By default, the value is 2.
12. At the **SV row height (2-10 px)** field, type the value. By default, the value is 2.
13. At the **Minimum gap between rows (10-40 px)** field, type the value. By default, the value is 10.
14. At the **Maximum gap between rows (40-200 px)** field, type the value. By default, the value is 70.
15. At the **Font size (%)** field, type the value. By default, the value is 100.
16. At the **BED color opacity (%)** field, type the value. By default, the value is 60.
17. At the **SV color opacity (%)** field, type the value. By default, the value is 40.
18. At the **Raw copy number opacity (%)** field, type the value. By default, the value is 100.
19. At the **Ruler text rotation (degree)** field, type the value. By default, the value is 30.
20. At the **Hybrid cut flashing duration (msec)** field, type the value. By default, the value is 5000.
21. At the **Circos plot SV size** field, type the value. By default, the value is 0.6.
22. At the **Circos plot translocation width** field, type the value. By default, the value is 0.5.
23. At the **Hybrid cut color** field, select the color from the palette.

24. At the **Background color** field, select the color from the palette.
25. At the **Highlight row color** field, select the color from the palette.
26. At the **Selected map border color** field, select the color from the palette.
27. At the **Selected label color** field, select the color from the palette.
28. At the **Molecule panel color** field, select the color from the palette.
29. At the **Molecule color** field, select the color from the palette.
30. At the **Reference map color** field, select the color from the palette.
31. At the **Genome map color** field, select the color from the palette.
32. At the **NGS map color** field, select the color from the palette.
33. At the **Matchline color** field, select the color from the palette.
34. At the **Label coverage color** field, select the color from the palette.
35. At the **Lasso color** field, select the color from the palette.
36. At the **Highlight molecule color** field, select the color from the palette.
37. At the **Highlight label/matchline color** field, select the color from the palette.
38. At the **Highlight matchgroup color** field, select the color from the palette.
39. At the **Highlight molecule matchline color** field, select the color from the palette.
40. At the **SV track color** field, select the color from the palette.
41. At the **SV insertion color** field, select the color from the palette.
42. At the **SV deletion/CNV loss color** field, select the color from the palette.
43. At the **SV inversion color** field, select the color from the palette.
44. At the **SV translocation color** field, select the color from the palette.
45. At the **SV duplication/CNV gain color** field, select the color from the palette.
46. At the **SV end color** field, select the color from the palette.
47. At the **AOH/LOH color** field, select the color from the palette.
48. At the **AOH/LOH region color** field, select the color from the palette.
49. At the **VAF color** field, select the color from the palette.
50. At the **VAF Segment color** field, select the color from the palette
51. At the **CMPR color** field, select the color from the palette.

52. At the **Copy number color** field, select the color from the palette.
53. At the **High copy number color** field, select the color from the palette.
54. At the **Low copy number color** field, select the color from the palette.
55. At the **Nt.BspQI enzyme** field, select the color of matched label from the first palette, and unmatched from the second one.
56. At the **Nb.BbvCI enzyme** field, select the color of matched label from the first palette, and unmatched from the second one.
57. At the **Nb.BsrDI enzyme** field, select the color of matched label from the first palette, and unmatched from the second one.
58. At the **Nb.BssSI enzyme** field, select the color of matched label from the first palette, and unmatched from the second one.
59. At the **DLE-1 enzyme** field, select the color of matched label from the first palette, and unmatched from the second one.

    You may see more enzymes if other enzymes are added into your system.
60. Click **Save**.

    The new settings are saved.
61. [Optional] To revert back to the original settings, click **Reset Options**.

Return to Project Browser

Users can return to Project Browser.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Click the **Return to Project Browser** icon.
3. Return to Project Browser.

Home Page

Users can return to Home Page.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Click the **Home Page** icon.
3. Return to Project Browser.

Key and mouse shortcuts

Users can use key and mouse shortcuts when viewing results.

1. In Bionano Access, navigate to the *Viewer* screen of the project to analyze.
2. Click the **Key & Mouse Shortcuts** ? icon.

   The dialog box appears.
3. Here is the list of highlighted features and descriptions. You can find more information in Access.

Feature	Description
Customize the view of the samples	In the first ruler, press down on the mouse, and then drag left or right to highlight the samples to view. A red box indicates the selected samples that are displayed on the viewer.
Move maps horizontally	Left click on mouse; move left or right.
Move maps vertically	Press Shift; move up or down.
Map options	Right-click on map. • Hide—Hide the selected map from the viewer. • Hide the others—Hide the other genome maps from the viewer except for the selected map and reference map. • Collapse—Collapse the genome maps to a single row next to the reference map. • Show Ruler—Attach the ruler to the selected genome map. • Show Molecule—Show all the aligned molecules that are related to the selected genome map. If there are no molecule alignments associated with a map in the viewer the 'Show Molecules' option in the right click menu will no longer be available. • Show Molecule for—Show all the aligned molecules that are related to Proband, Mother and Father for variant annotation results. • Hide Matchgroups—Remove the gray line connecting to the selected genome map from the reference map. • Invert—Invert the orientation of the selected map.
Zoom in or out	Use the mouse to scroll zoom in or zoom out.

SV Tab

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Genome Browser** from the drop-down list at upper left corner of the Viewer.
3. Click the **SV** Tab. This tab is only available in *De Novo* assembly and rare variant analysis. This table includes Sample, Global_ID, Smap_ID, Type, Zygosity, Confidence, Ref1_ID, Ref2_ID, Ref_Start, Ref_End, Size, Qry_ID, Qry_Start,and Qry_End. The columns can be sorted. Please refer to 30041 SMAP File Format Specification Sheet for more details.

SV Annotation Tab

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Genome Browser** from the drop-down list at upper left corner of the Viewer.
3. Click the **SV Annotation** Tab. This tab is only available in annotated *De Novo* assembly and rare variant analysis or variant annotation pipeline. This table includes Sample, Type, Algorithm, RefcontigID1, RefcontigID2, RefStartPos, RefEndPos, Size, Zygosity, Score, SmapId, link SmapId, Present in % of BNG control samples, Present in % of BNG control samples with the same enzyme, Fail assembly chimeric score, Overlap Genes, Nearest Non-overlap Gene, Nearest Non-overlap Gene Distance, Putative Gene Fusion, Self Molecule Count, Found in self molecules. The columns can be sorted. Please refer to SV Annotation Pipeline File Format Specification Sheet for more details.

Adding SV to Curated Variant List/SV report

Circos Plot

User can add current remain SVs to the Curated Variant List to do variant classifier.

1. In Bionano Access, navigate to the Circos Plot screen viewer of the job to analyze.
2. Click the **Add to Curated List**  icon.
3. All current remain SVs will be added to the Curated Variant List.

Genome Browser

User can select SV of interested to the Curated Variant List for Variant Classifier, Generate SV report, or Download Files.

1. In Bionano Access, navigate to the Viewer screen of the job to analyze.
2. Select **Genome Browser** from the drop-down list at the upper left corner of the Viewer screen.
3. To add an SV, click **Add to Report**  icon of an SV in the SV or SV Annotation tab.
4. Select **Curated Variant List** or **Variant Classifier** from the drop-down list at the upper left corner of the Viewer screen. All added SV will be showing there.

Match Tab

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Genome Browser** from the drop-down list of **Circos Plot**.
3. Click the **Match** Tab. This table includes XmapId, RefId, QryId, Ref_Start (Kbp), Ref_End (Kbp), Qry_Start (Kbp), Qry_End (Kbp), Orientation, Confidence. The columns can be sorted and filtered. Please refer to XMAP File Format Specification Sheet for more details.

Copy Number Tab

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Genome Browser** or **Whole Genome CNV** from the drop-down list.
3. Click the **Copy Number** Tab. It contains columns of Id, Start (Kbp), End (Kbp), Size, Type, Fractional Copy Number, Copy Number and Confidence. The columns can be sorted. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.

Aneuploid Tab (For Whole Genome View)

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Genome Browser** or **Whole Genome CNV** from the drop-down list of **Circos Plot**.
3. Click the **Aneuploid** Tab. It contains per-chromosome aneuploidy call and includes columns of Type, Length, Score and FractCN. The columns can be sorted. It is only available for Access 1.4 or higher. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.

Summary Tab (For Whole Genome View)

1. In Bionano Access, navigate to the Viewer screen of the project to analyze.
2. Select **Whole Genome CNV** from the drop-down list.
3. Click the **Copy Number** Tab. It contains columns of RefId, % Deletion and % Duplication. The columns can be sorted. Please refer to *P/N 30110 Bionano Solve Theory of Operation: Structural Variant Calling* for more details.

Repeat Tab (For EnFocus™ FSHD analysis and EnFocus™ Fragile X analysis)

1. In Bionano Access, navigate to View EnFocus™ FSHD Analysis of the project to analyze.
2. Click the **Repeat** Tab. It is only generated for human FSHD samples and includes columns of Qry Id, Repeat Count (units), Haplotype, Repeat spanning coverage (X). The columns can be sorted and filtered. Please refer to Theory of Operation EnFocus FSHD Analysis for more details.

Conflict Resolutions Tab (For Hybrid Scaffold analysis)

1. In Bionano Access, navigate to Maps to NGS with Conflicts of the hybrid scaffold job in any project to analyze.
2. Click the **Conflict Resolutions** Tab. It is generated from hybrid scaffold jobs and includes columns of RefId, Enzyme, RefLeftBkpt, RefRightBkpt, RefLeftBkptToCut, RefRightBkptToCut, RefToDiscard, QryId, QryLeftBkpt, QryRightBkpt, QryLeftBkptToCut, QryRightBkptToCut, QryToDiscard. The columns can be

sorted. Please refer to Hybrid Scaffold Conflict Cut Status File Format for more details.

Settings

User Accounts | Banners | User Account Settings
References | Control Databases | System Status
System Features | Configurations | System Warning
System Services Settings | Queue Status | Named Filters
In Silico Digestion

User Accounts

Users must have administrator privileges to see this setting.

All user accounts are listed in the User Accounts screen, and the user list can be exported by click **Export to Excel**.

User can **Edit**, **Delete** or **Lock/Unlock** account in the list.

New User

All users should have their own user account with a valid email address. Bionano Access notifies users via email when their job is complete. Do not use shared accounts.

1. From the Bionano Access main menu, select **Settings**.
2. Select **User Accounts**.
3. Click **New User**.

   Create User Window appears.
4. At the **User Name** field, type the user name.

5. At the **Full Name** field, type the full name.
6. At the **Email Address** field, type the email address.
7. At the **Password** field, type the password.
8. At the **Confirm Password** field, type the password again to confirm.
9. At the **Role** field, choose one of the following:
    - User
    - Project Lead
    - Administrator
    - Read Only
10. At the **User Status** field, chose one of the following:
    - Active: The user account is active; the user can log on to Bionano Access.
    - Disable: The user account is disabled; the user cannot log on to Bionano Access.
11. Click **Submit**.

    The new user account appears on the *User Accounts* screen.

Banner

User can add login banner to the login page of Bionano web Access or add download message that users must acknowledge before files can be downloaded. Users must have administrator privileges to perform this task.

1. From the Bionano Access main menu, select **Settings**.
2. Select **Banner**.
3. At the **Login Banner** **or** **Download Banner** field, type and edit the information.
4. Click **Submit**.

User Account Settings

At the **User Account Settings** field, user can edit the following options:

- **Idle User Session Expiration [minutes]** – Bionano Access will automatic logout when no action was performed in a period time. Default is120 minutes.
- **Maximum User Concurrent Sessions** – The maximum number of session user can open. Default is 2.
- **Maximum Login Attempts** – The Maximum login attempts user can enter before Access lock the account. Default is 3 times.

At the **Password Retention** field, user can edit the following options:

- **Password Retention Checks –** Enable/Disable password retention check. Default is on.
- **Previous Password Retention [month]** – How long does Access save old password to prevent the password can be re-used. Default is 3 months.
- **Password Expiration [days]** -- Bionano Access will ask user to change password after setting time. Default is 90 days.

At the **Password Settings** field, user can edit the following options:

- **Minimum Characters** – minimum characters are required in password. Default is 1.
- **Minimum Numerals** – minimum number are required in password. Default is 0.
- **Minimum Special Characters** – minimum special characters are required in password. Default is 0.
- **Minimum Case Changes** – minimum Case changes are required in password. Default is 0.

References

Users must have administrator or project lead privileges to perform this task.

Users can add references (CMAP files) to use for map alignments.

1. From the Bionano Access main menu, select **Settings**.
2. Select **References**.

   The *Reference List* page appears.
3. Click **Add Reference**.

   A dialog box appears.
4. At the **Reference Name** field, type the name for the reference.
5. At the **Enzyme** field, select the enzyme from a list of enzymes already known in the system. You can also click the **Add Enzyme** button to add a new enzyme if needed. You can select more than one enzyme in the list if needed.
6. At the **Genome Build** field, type the name of the genome.
7. If the reference is human, select the **This is a human reference** check box.
8. At the **Reference File (.cmap)** field, click **Choose File**, and then browse to select the CMAP file to use.
9. Click **Open**.
10. Click **OK**.
11. Select one reference to **Download File**, **Delete** or **Edit**.

Control Database

Users must have administrator or project lead privileges to perform this task. For Control Database format, please go to *P/N 30190 Bionano Solve Theory of operation: Variant Annotation Pipeline* for more detail.

1. From the Bionano Access main menu, select **Settings**.
2. Select **Control Database**.
3. Click **Add Control Database**.

   Add Control Database window appears.
4. At the **Control Database Name** field, type the name.
5. At the **Genome Build** field, select the genome build from the list.
6. At the **Control Type** field, select one from **SV**, **CNV** or **ECNB**.

   SV Control Database is used for SV calling during *De Novo* assembly or Rare Variant Analysis job.

   CNV Control Database is used for CNV calling during *De Novo* assembly or Rare Variant Analysis job.

   ECNB (Expected Copy Number Baseline) is used for CNV calling to set the baseline of copy number during *De Novo* assembly or rare variant analysis job.
7. At the **Operation Type** field, select one from **Assembly** or **RareVariant**. It is only applicable if **SV** is selected for **Control Type**.
8. At the **This is a human control database** field, check the box if it is a human control database.
9. [Optional] At the **Description** field, type a brief description.
10. At the **Control Database File (*.txt)** field, click **Choose File**, and then browse to select the file (*.bed) to use.
11. Click **Open**.
12. Click **OK**.

System Status

Administrator can check Bionano Access Server (BAS) or customer's own system status by viewing the System Status. It includes Uptime, system version, Disk Usage, Memory statistics, SSH status, I/O Utilization, CPU information and Processes Running.

System Features

Administrator, project lead or user privileges to perform this task. Users can add or select features to automatically display on jobs if the Genome Build matches the sample being viewed. Access can accept BED and GTF files.

For BED file format, please go to *P/N Bionano 30164 BED File Format Specification Sheet* for more details.

Add new features

1. From the Bionano Access main menu, select **Settings**.
2. Select **System Features**.
3. Click **Add**.

   A dialog box appears.
4. At the **Name** field, type the name for the bed.
5. [Optional] At the **Description** field, type a brief description.
6. At the **Feature Type** field, select one from Generic, Mask, CNVMask or Genes.

   Generic bed files are used for SV and CNV Filter in *De Novo* assembly, Rare Variant Analysis and Variant Annotation Pipeline job.

   Mask bed files are used for masking putative false positive translocation breakpoint calls, reference nbase gaps (for insertions and deletions) or complicated regions, such as segmental duplications during *De Novo* assembly or Rare Variant Analysis.

   CNVMask bed files are only used for masking coverage variable regions during copy number analysis.

   Genes bed files are used to annotate SVs during Variant Annotation Pipeline job.
7. At the **Genome Build** field, select the genome build from the list.
8. At the **File (*.bed, *.gtf)** field, click **Choose File**, and then browse to select the file to use.
9. Click **Open**.
10. Click **OK**.

Auto Display

1. From the Bionano Access main menu, select **Settings**.
2. Select **System Features**.
3. Check the **Auto Display** option, system will Auto Display the feature file if the Genome Build matches the sample being viewed.

   Locks are user specific not system wide.

Configurations

Users must have administrator or project lead privileges to perform this task.

When generating a *De Novo* assembly or hybrid scaffold, users are required to select a configuration that contains a set of pre-defined parameters. Users can select a configuration from:

- A list of default configurations in Bionano Access
- A list of customized configurations based on the existing configurations in Bionano Access
- Configuration files (***.xml**) that users upload to Bionano Access

Bionano Access does not validate the formatting or parameter settings of the upload XML file. If the uploaded file does not contain proper parameters or formatting, the file may cause the system to crash. Users can also download the configuration files in the system.

For details on customizing configurations based on existing and validated configurations in Bionano Access, see the following tasks:

- *Generate De Novo Assembly*
- *Generate rare variant analysis*
- *Generate Hybrid Scaffold*

1. From the Bionano Access main menu, select **Settings**.
2. Select **Configurations**.
3. Click **Add Configuration.**

   A dialog box appears.
4. At the **Configuration Name** field, type the name for the configuration.
5. At the **Configuration Type** field, select the operation type to use this configuration file from the drop-down list.
6. [Optional] At the **Description** field, type a brief description.
7. At the **Configuration File** field, click **Choose File**, and then browse to the configuration file (*.xml) to use.
8. Click **Open**.
9. Click **OK**.
10. Select one configuration file to **Download File**, **Delete** or **Edit**.

System Warning

Users must have administrator privileges to perform this task.

When administrator want to post a warning message to all user, system warning can show the message to all users.

1. From the Bionano Access main menu, select **Settings**.

2. Select **System Warning**.
3. At the **System Warning** field, type the information.
4. Click **Submit**.

System Services Settings

Users must have administrator privileges to perform this task.

1. From the Bionano Access main menu, select **Settings**.
2. Select **System Services Settings**.
3. At the **System Level Services** field, please find the following options:
4. At the **Compute On Demand & Saphyr Assure** field, opt in or out Compute On Demand and Saphyr Assure.

   The Compute On Demand service provides pay per use elastic computing resources to supplement and accelerate your analysis needs. Together with the Saphyr Assure service you can monitor the health of your entire workflow. This is the recommended option. Internet access is required to connect to the Bionano Compute on Demand solution. You can test the connection by clicking the **Test Connection** button after enabling the service.
5. At the **Saphyr Assure Only** field, opt in or out Saphyr Assure. Use this option if you want to ensure optimal performance of your instrument but are not interested in the Compute On Demand service.
6. At the **Maintenance Mode** field, toggle maintenance mode.

   Toggle maintenance mode to prevent non-administrator level users from accessing the system during maintenance windows. In Maintenance Mode, only users with the administrator role can log into the system. This allows administrators to check system operations after upgrades before opening the system to general use.
7. At the **Remote Access** field, opt in or out Remote Access.

   This option is only available in Bionano Access software, which is installed in a Bionano Access Server with HTTPS (Hypertext Transfer Protocol Secure, the encrypted version of HTTP) enabled. *P/N 30377 How to enable HTTPS in Bionano Access* for more details.

   By default, this option is off (opt-out). Once opt-in, it allows Bionano support personnel to directly access Bionano Access Server for remote support and troubleshooting.

Queue Status

Users must have administrator privileges to perform this task.

The queue status page will list all jobs currently being managed by SGE. If you have more than one SGE cluster

in your Access configuration, Queue Status page will list the status of each SGE cluster. This feature offers a quick way to better understand the compute load on your systems.

Named Filters

Users must have administrator or project lead privileges to perform this task.

1. From the Bionano Access main menu, select **Settings**.
2. Select **Named Filters**.
3. Select **Add Filter**.

   The *Create Filter* window appears.
4. At the **Filter Name** field, type the name.
5. At the **Insertion Confidence**, type the confidence score cutoff for insertion calls.
6. At the **Deletion Confidence**, type the confidence score cutoff for deletion calls.
7. At the **Inversion Confidence**, type the confidence score cutoff for inversion breakpoint calls.
8. At the **Duplication Confidence**, type the confidence score cutoff for duplication calls.
9. At the **Intra-Translocation Value**, type the confidence score cutoff for intra-translocation breakpoint calls.
10. At the **Inter-Translocation Value**, type the confidence score cutoff for inter-translocation breakpoint calls.
11. At the **Copy Number Value**, type the confidence score cutoff for copy number calls.

In Silico Digestion

In Silico digestion is used to create a genome reference consensus map for any sequence file. This tool uses a FASTA file and the recognition sequence of a labeling enzyme to create a CMAP file that can be used as a reference. The *In Silico* digestion results are accessible by all users. Bionano Access saves the FASTA files that are uploaded; users can reuse the files to generate different CMAP files with different enzymes or enzyme-to-channel combinations.

The *In Silico* Digestion page contains an accordion menu that contains three panes: Create New Run, Runs in Progress, Completed Runs. Click the arrow on the pane to expand or collapse the view.

Configuration Settings

Before performing *in silico* digestion, define the following settings:

1. From the Bionano Access main menu, select **Settings**.
2. Select ***In Silico* Digestion**.
3. Click the **Configuration Settings** icon in the top right corner.

The *Configuration* dialog box appears.

4. At the **Minimum Labels** field, set a value between 0–20 (default as 0).
5. At the **Minimum Length** field, set a value between 0–250 kbp (default as 0).
6. At the **Number of Channels** field, set the number of channels to digest. The channel refers to the laser color used.
7. At the **Number of Enzymes per Channel** field, set the number of enzymes per channel to digest.
8. Click **Save**.

   The software displays the settings in the *Create New Run* pane.

Enzyme Management

Users can add and edit labeling enzymes and recognition sequences; however, the default enzymes listed in Bionano Access are not editable.

Users must have administrator privileges to perform this task.

1. From the Bionano Access main menu, select **Settings**.
2. Select ***In Silico* Digestion.**
3. Click the **Enzyme Management** icon in the top right corner.

   The *Manage Enzymes* dialog box appears. Here is a description of Enzyme list.

Info	Description
Name	The enzyme name.
Recognition Sequence	The recognition sequence.
Type	Bionano or user identified.
Matched Label Color	The color.
Unmatched Label Color	The color.
Experiment count	The count of experiments using this enzyme.

4. Click **Add**.

   The *Add New Enzyme* dialog box appears

5. At the **Enzyme Name** field, type the enzyme name.
6. At the **Enzyme Sequence** field, type the recognition sequence of the labeling enzyme (ACGT). The maximum length for the enzyme sequence is 20 nucleotides.
7. At the **Matched Label Color** field, select the color from the palette.

8. At the **Unmatched Label Color** field, select the color from the palette.
9. Click **Save**.

   The software adds the enzyme to the list.
10. Select one enzyme with the Type as User from the list. Then click **Edit**.

    The *Edit Existing Enzyme* dialog box appears.
11. At the **Enzyme Name** field, type the enzyme name.
12. At the **Enzyme Sequence** field, type the recognition sequence of the labeling enzyme (ACGT). The maximum length for the enzyme sequence is 20 nucleotides.
13. At the **Matched Label Color** field, select the color from the palette.
14. At the **Unmatched Label Color** field, select the color from the palette.
15. Click **Save Changes**.
16. Select one enzyme with the Type as User from the list. Then click **Delete**.

    The *Delete Existing Enzyme* dialog box appears.
17. Click **Yes** to delete this enzyme.

FASTA Management

Users can use *in silico* Fasta Files Management to manage FASTA files that have been uploaded into the *In Silico* digestion tool.

Users must have administrator privileges to perform this task.

1. From Bionano Access main menu, select **Settings**.
2. Select ***In Silico* Digestion.**
3. Click the **Fasta Management** icon in the top right corner.

   The *Fasta Files* list and *In Silico Digestion Runs* for every fasta file appears. Here is a description of Run list for every fasta file.

Info	Description
Run Details	System-generated number for the run.
Date	The date the file is uploaded.
Minimum Labels	The user-defined setting for the minimum number of labels per map.
Minimum Length	The user-defined setting for the minimum map length.
Enzyme(s) Channel 1	The enzyme(s) that are used in Channel 1.

Enzyme(s) Channel 2	The enzyme(s) that are used in Channel 2.
Enzyme(s) Channel 3	The enzyme(s) that are used in Channel 3.
Action	To Delete the run.

4. Click **Delete Fasta** to delete the selected Fasta.

   The *Confirm* dialog box appears.

   Click **Yes** or **Cancel**.

5. Click **Delete All Runs** to delete all runs of the selected Fasta.

   The *Confirm* dialog box appears.

   Click **Yes** or **Cancel**.

Create New Run

Depending on the digestion tool settings, users may have one or more label channels parameters to select.

Bionano Access saves the FASTA files that have been digested. Bionano Access only checks if there are duplicate file names. If a user uploads a FASTA file that Bionano Access already has saved, the software will point to the existing file. For best practices, give FASTA files unique names to easily track them.

The following task is an example of an *In Silico* digestion for one label channel.

1. From the Bionano Access main menu, select **Settings**.
2. Select ***In Silico*** **Digestion.**
3. In the *Create New Run* pane, at the **Select file** field, browse to the FASTA file to digest, and then click **Open**.
4. At the **Label Channel 1**, select the enzyme from the drop-down list.
5. [Optional] At the **Label Channel 2**, select the enzyme from the drop-down list.
6. Click **Launch**.

   A message appears **to indicate** that the file is uploading if it is new, and then the software initializes the run. The digestion progress appears in the *Runs in Progress* pane. Once the run is complete, the digested data appears in the *Completed Runs* pane.

   If this FASTA file was previously uploaded to the server, this message appears: "*This FASTA has been uploaded to the server. To use it, click OK. To use a new version of this FASTA, click Cancel, rename your FASTA, upload, and try again.*"

Runs in Progress

Expand the *Runs in Progress* pane to view the following statistics:

Statistic	Description
Run	System-generated number for the run.
Date	The date the file is uploaded.
FASTA	The FASTA file name.
Min Labels	The user-defined setting for the minimum number of labels per map.
Min Size	The user-defined setting for the minimum map length.
Enzyme(s)	The enzyme(s) that are used.
Progress	The progress of the run.
Cancel	Click x to cancel the file upload.

Completed Runs

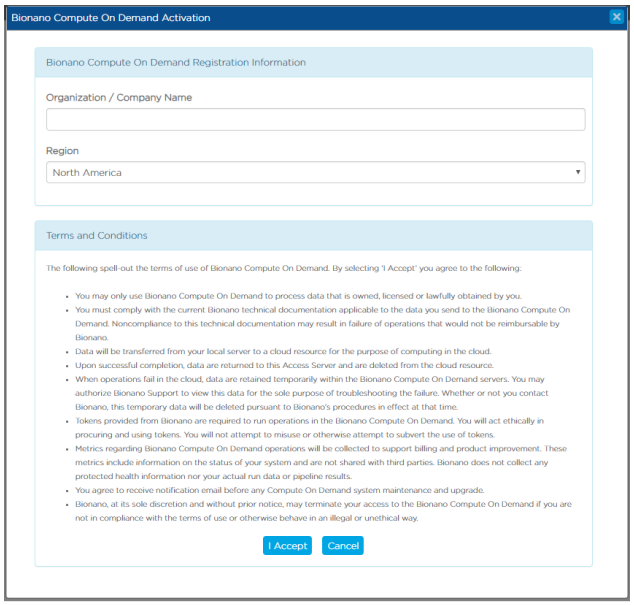
Expand the *Completed Runs* pane to view the following statistics:

Statistic	Description
Run ID	System-generated number for the run.
Date	The date the CMAP is generated.
Files FASTA	The FASTA file name.
Files Cmap	The CMAP file that can be downloaded.
Files Key	A file to track CMAP IDs that are associated with the FASTA sequence map names.
Files Gap	The GAP file can be downloaded. The GAP file track N-base gaps in sequence.
Files Summary	A complete summary of the results that can be downloaded.
Enzyme(s)	Enzyme(s) that are used to create the CMAP.
#Maps	Number of maps in the digested sample.
N %	Percent of genome that has N bases.
Sites / 100 kbp Ch1 Sites	The number of labels per 100 kbp in Channel 1.
Action **"Add to References"**	Clicking on this will automatically copy the newly digested CMAP file to the reference pool. Users do not need to manually download the cmap file and import to Reference pool.

Compute On Demand

How to enable Compute On Demand

To enable the Bionano Compute On Demand within Bionano Access, you can opt in Compute On Demand in System settings (see above for more details). After that, it will pop up Bionano Compute On Demand Activation window:



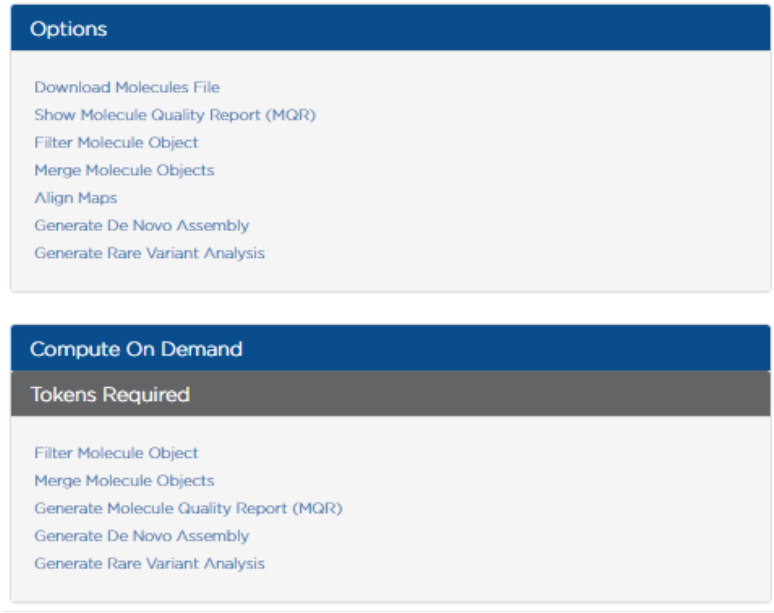
If you are not ready to complete the opt-in dialog, you can click the 'Cancel' button, but Administrators will continue to be prompted each time they login until they complete the opt-in dialog or Compute On Demand is disabled. Once the opt-in has been completed, the dialog will no longer appear. Please carefully follow the steps below to fill the 2 fields in the Compute On Demand setting page. Please note that once the information of these fields are submitted by clicking on "I Accept" button, it is not possible to change them easily.

The first prompt is for Organization / Company Name. We recommend setting this value to the domain in your email. For example, if your email was jsmith@bionanogenomics.com, we would recommend setting your organization to 'bionanogenomics'.

The second prompt is for the computing region. We currently offer 4 computing end points: North America, Canada, Europe, and Germany. Germany server is intended only for customers who are in Germany and Europe server is for customers in Europe but not in Germany. Additional regions will be added in the future based on demand. The region is where the servers will reside that will execute your compute job. Users are not restricted to selected region but selecting a region closest where your system resides is best for security and performance reasons. Tokens must be purchased with the same server as registration.

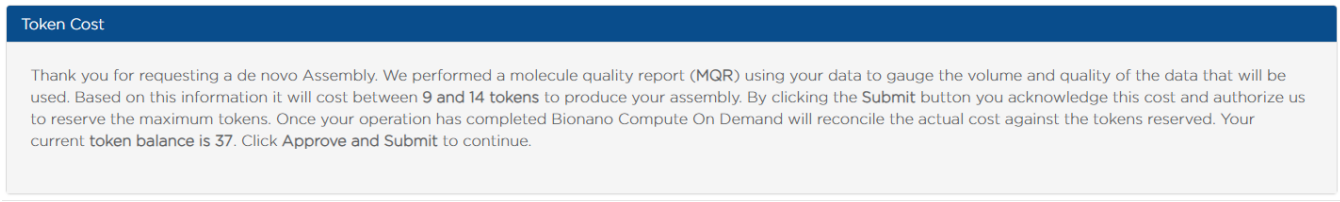
Compute On Demand Operations

Once the opt-in process described above has been completed, Bionano Compute On Demand operations will appear in the Project Browser. In the Project Browser the operations panel will display different operations users can perform based on the job type selected. When Bionano Compute On Demand is enabled, there will be an additional panel visible below the Options panel (image below). The Bionano Compute On Demand options are listed separately since they require tokens. Like the existing Operations panel the hyperlinks on the Compute On Demand panel will change depending on the type of job selected. If a local Saphyr Compute is not configured, then the Compute On Demand operations will be the only ones displayed. Users can check the status of Compute On Demand by clicking “Compute On Demand Test” in “Settings” to see whether the service is online or not.



Tokens

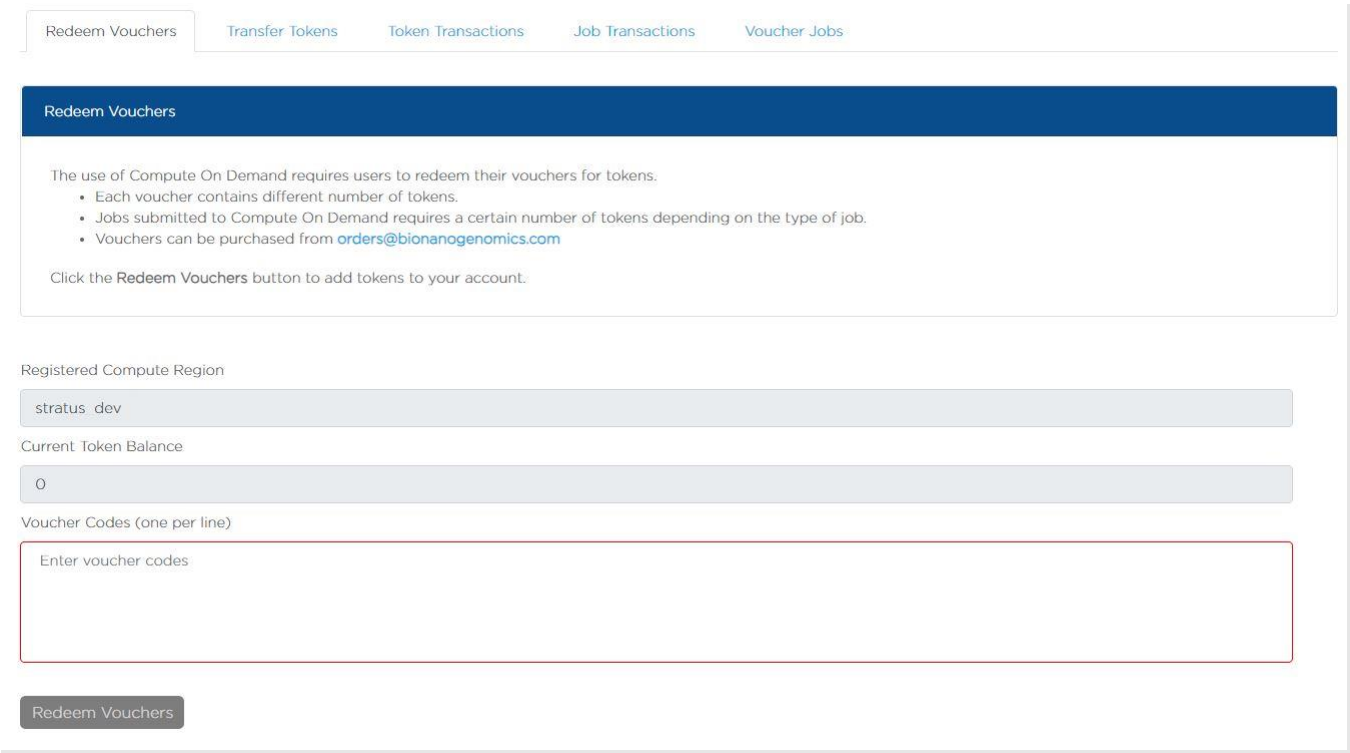
When performing a Bionano Solve operation locally, users first will select the required inputs. When performing a Bionano Compute On Demand operation, the inputs are the same as the inputs with one addition, the token cost (image below). To proceed users must have enough tokens and must approve the token cost estimates. The token cost estimates will be a range. In the example below, the cost estimate is between 9 and 14 tokens. When users approve the operation the maximum token cost (14 in this example) will be deducted from your token balance. When the job completes, if the cost was less than the maximum token estimate, the token difference will be refunded. For example, if the job below were to cost 12 tokens upon completion, users would receive a message informing those 2 tokens have been returned to token balance. Users are guaranteed that the cost will not exceed the maximum token cost quoted.



Tokens to execute jobs on our Bionano Compute On Demand service can be purchased through your designated sales person or by contacting orders@bionanogenomics.com.

Compute On Demand Options

From the Bionano Access main menu, select **Compute On Demand**.



Redeem Vouchers

1. **Redeem Vouchers** is selected by default.
2. At the **Registered Compute Region** field, display the region.
3. At the **Current Token Balance** field, display the current token balance.
4. At the **Voucher Codes (one per line)** field, input one voucher coder per line.

   When users purchase tokens, users will receive one or more voucher codes. When a voucher is redeemed, all the tokens for that voucher are granted to the account for the user who is logged in.
5. Click **Redeem Vouchers** to redeem.

Transfer Tokens

1. At the **Your Current Token Balance** field, display your current token balance.
2. At the **Organization Token Balance** field, display all the users and their token balance, email address.
3. At the **Select Token Recipient** field, select the user you want to transfer the token to.

   Any user who owns tokens can transfer them to other user accounts (except those with Read-Only access) on the same server. Read-Only accounts cannot have tokens because they cannot perform operations. Tokens currently cannot be transferred between Bionano Access servers.
4. At the **Token to Transfer** field, type the token amount that you want transfer.
5. Click **Transfer** or **Reset**.

Token Transactions

User can view the transferred tokens from each user to a recipient.

Job Transactions

User can view the reserved and actual cost of the tokens for each job.

Voucher Jobs

User can expand the voucher row to see the corresponding jobs for every voucher. Use this view to understand how tokens for a given voucher were spent

Token Use Recommendation

- Only use as much amount of data as needed for the application. Please refer to 30173 Data Collection Guidelines for more details
- Use the data down sampling tools available within Bionano Access to help achieve a targeted coverage
- Provide good quality references; this reduces costs by eliminating the need for the pipeline to first generate a rough assembly

Appendix

Red Labeled Sample Experiment

Bionano Access supports experiments that use the red laser (if applicable) in the Saphyr instrument. These workflows are disabled by default. To enable this operation, users need to contact Bionano Genomics Technical Support (support@bionanogenomics.com).

Red Only Workflow

If the capability of running red labeled sample has been enabled, when users open the Single Sample workflow, they will have the choice of selecting green or red. If red labeled sample setup has not been enabled, the system will only generate experiments using green laser.

Dual Labeled Workflow

If red is enabled, the Dual Labeled workflow in the experiment design module will be available. The Dual Labeled workflow is designed to process a single sample with motifs in the DNA labeled using either green or red colors. The system will generate label-related metrics on the dashboard for each color. Users can provide separate references for each color, but only one reference will be associated with the final merged BNX file. The user is prompted to select which label (the primary) would retain its reference. The Dual Labeled workflow will generate a single BNX file that contains both green and red labels information.

When users import a dual-labeled BNX file, the reference will automatically be associated with channel 1, as specified in the header or the BNX file. Users can select the Edit option to designate either channel for the reference. When users run an assembly using a dual labeled BNX file, they will be prompted to select labels from which channel they want to use for assembly. After the assembly is done, when users choose to show molecules in the viewer, the viewer system will show labels for both labeled motifs.

Multiplex Workflow

If red is enabled, the Sample Multiplex workflow will be enabled in the Experiment Design module. This workflow is designed to process one sample using green and a second sample using red in the same flowcell. This workflow will generate separate dashboard metrics for each color. This workflow will also generate a separate BNX file for each color. It is possible to use the same sample for both red and green, but the molecules loaded should be labeled with only red or green, but not both.

ISCN Symbols and Abbreviated Terms

Symbols and abbreviated terms used in the description of chromosomes and chromosomal abnormalities are listed below. For a detailed discussion of ISCN notation produced by the Solve pipeline, see Bionano Solve Theory of Operation Variant Annotation Pipeline (PN 30190).

Consider the following when searching for chromosomal abnormalities:

Symbol/abbreviation	Description
amp	amplication of interval where copy number is greater than 4
approximate sign (~)	denotes intervals and boundaries of a chromosome segment or number of chromosomes, fragments, or markers; denotes a range of number of copies of a chromosomal region when the exact number cannot be determined
brackets, square ([ ])	surround number of cells or genome build
dup	duplication
fus	fusion between regions of the same chromosome
ins	insertion
inv	inversion
ogm	optical genome mapping data
p	short arm of chromosome
parentheses ( )	surround structurally altered chromosomes and breakpoints; surround chromosome numbers, X, and Y in normal and abnormal results; surround coordinates (or nucleotide positions) in abnormal result
q	long arm of chromosome
question mark (?)	insertion of unknown sequence
t	translocation
x	copy number

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

Type	Contact
Email	**support@bionanogenomics.com**
Phone	**Hours of Operation:** **Monday through Friday, 9:00 a.m. to 5:00 p.m., PST** **US: +1 (858) 888-7663**
Website	**www.bionanogenomics.com/support**

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