IrysView® v2.5.1
Software Training Guide

Document Number: 30035

Document Revision: G

Table of Contents

1 About this Manual

This manual describes how to install and use the genomics analysis software, IrysView®, to analyze and visualize data generated by the Irys® instrument. Review the release notes to best determine what features have been added or modified since our last release.

2 Installation and Release Notes

Please use the installation guide for installation:

http://www.bnxinstall.com/training/docs/IrysView_Software_Installation_Guide_251.pdf

Click the following link to view the new features and changes for IrysView 2.5.1:

http://www.bnxinstall.com/training/docs/IrysView_ReleaseNotes_251.pdf

3 Demo

3.1 Public Datasets

Beyond the training datasets noted in the following sections there is also a collection of public data available on the Bionano Genomics corporate website in the Science section. We provide these datasets to enable the community to better understand and interact with NGM data.

3.2 Demo Data

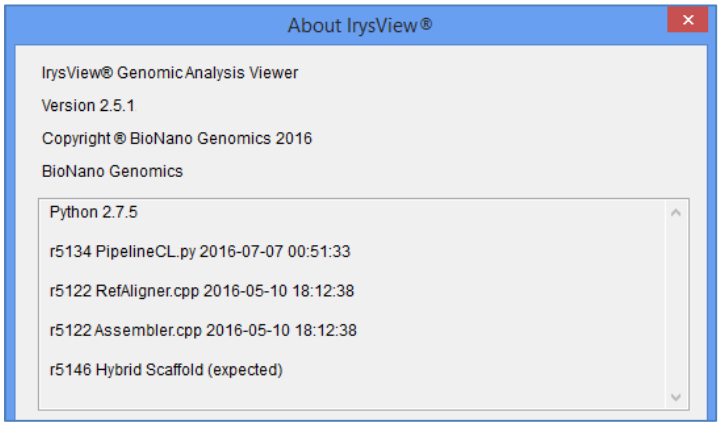
- Download from: http://www.bnxinstall.com/training/DataSet/2012-08.zip
- Extract the 2012-08 folder to *C:\Users\username\Documents*.

3.3 *E coli MG1655* References

- FASTA File: http://www.bnxinstall.com/training/DataSet/MG1655.zip
- FASTA File (double): http://www.bnxinstall.com/training/DataSet/Ecoli_MG1655_double.zip
- CMAP File (edited to introduce artificial SVs): http://www.bnxinstall.com/training/DataSet/MG1655_normal_bspqI_SVs.zip

4 Version Verification

- Open the IrysView Application. *Select Help | About IrysView* from the menu bar. Windows (not remote Linux) versions should be as listed below:
    - IrysView: 2.5.1 or higher
    - PipelineCL.py: 5134 or higher
    - RefAligner: r5122 or higher
    - Assembler: r5122 or higher
    - Hybrid Scaffold: r5146 or higher
- The following commands in their respective installation paths will give you the current version installed:
    - *~/tools → ./RefAligner –version*
    - *~/tools → ./Assembler –version*
    - *python -V*
    - *~/scripts → python pipelineCL.py –v 2*
    - *~/scripts/HybridScaffold → perl hybridScaffold.pl –v*
- If the correct IrysView version is not displayed, close the IrysView application. Uninstall IrysView through *Control Panel → Program and Features* and then re-install from: http://www.bnxinstall.com/IrysView/IrysView.htm



5 IrysView GUI

5.1 Interface Layout

The main pane of IrysView is divided into a right and left section. The left section contains the *Runs*, *Maps* and *Comparisons* tabs for importing datasets, toggling selected runs, and launching analysis jobs. The right section contains the *Reports*, *Data* and *Analysis* tabs for visualizing the corresponding analysis results.

This matrix describes the three main steps of the workflow along the first blue column. The green columns denote the types of analysis output.

Higher Level Analysis

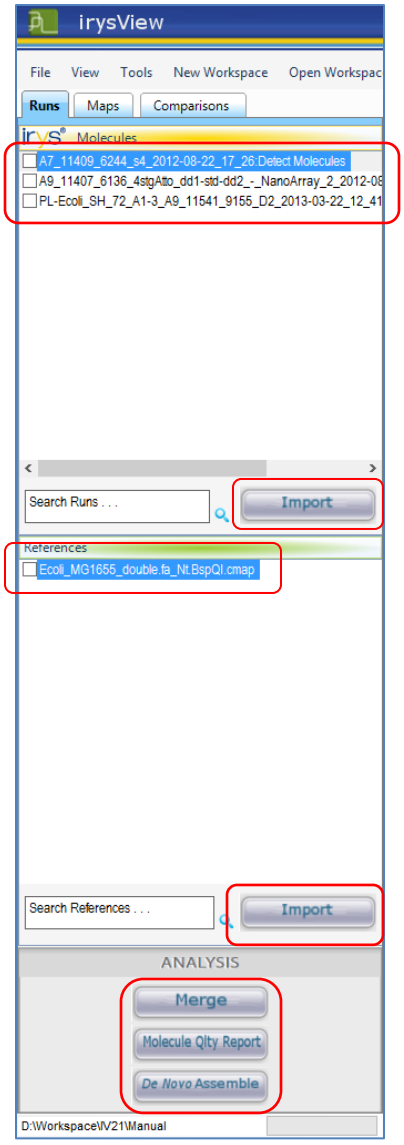
Higher Processed Data

Inputs \ Outputs	Report	Data	Analysis
Runs	• Run Report • Molecule Quality Report	• Molecule images • Statistics / Graphs	Reference Mapping Browser ○ Molecule-to-Ref
Maps	• Assembly Report • Map Quality Report	Assembly Browser ○ Consensus Maps and corresponding molecules	Reference Mapping Browser ○ Maps-to-Ref
Comparisons	• Comparison Report*	Comparison Browser ○ Query Map relative to Anchor Map	3 Level View* ○ Reference – Maps - Molecules

5.1.1 Input Tabs

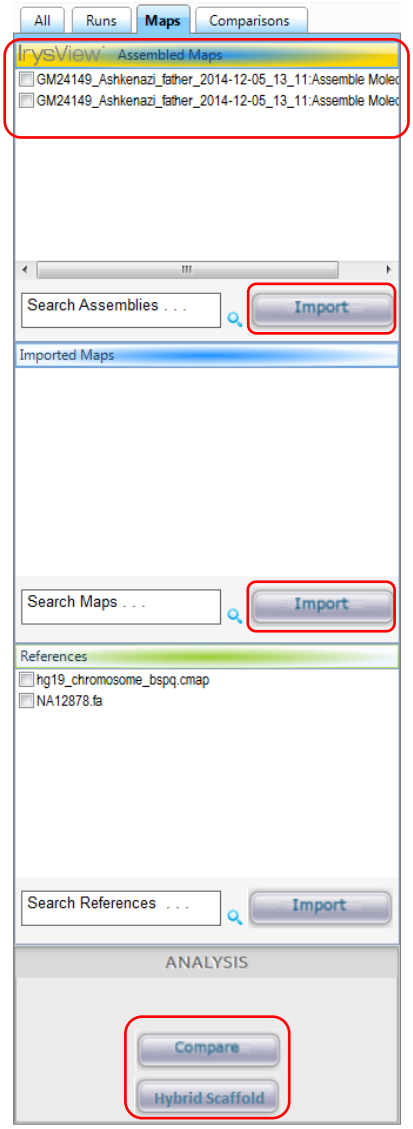
Runs

- *Molecules* pane: Maintains a list of all the imported datasets and BNX files.
- *References* pane: Allows import of externally generated CMAPs, or raw FASTA file. The latter will be digested by a user provided enzyme into CMAP.
- *Analysis* pane: Buttons for *Merge*, *Molecule Quality Report* and *De Novo* Assembly.



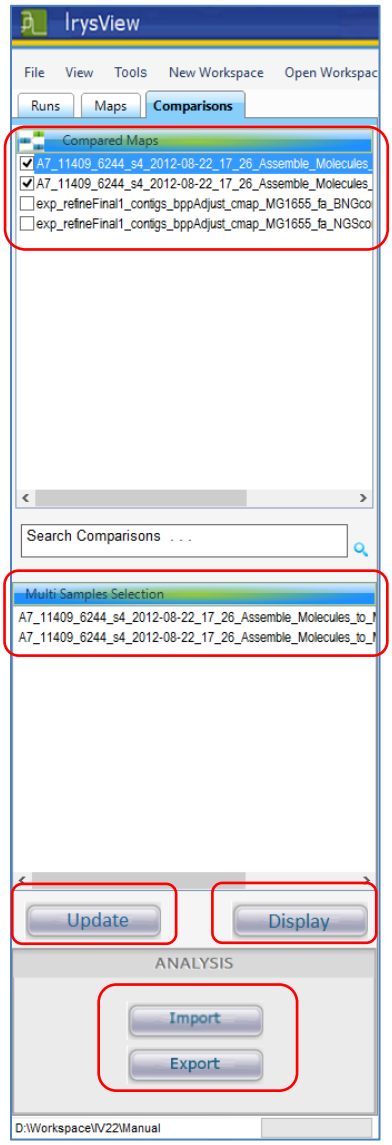
Maps

- *Assembled Maps* pane: Lists the assembled genome maps from *de novo* assembly. The *Import* button allows the import of externally generated De Novo runs.
- *Imported Maps* pane: Allows import of externally generated CMAPs, or FASTA file. The latter will be digested by a user provided enzyme into CMAP.
- *References* pane: Allows import of externally generated CMAPs, or raw FASTA file. The latter can be optionally digested by a user provided enzyme into CMAP.
- *Analysis* pane: Buttons for generating a comparing CMAPs and performing hybrid scaffolding.



Comparisons

- *Compared Maps* pane: Lists the pair of compared maps (*.xmap) generated from *Compare* buttons in the previous *Maps* tab.
- *Multi Sample Selection* pane: list of selected samples to be displayed in the Analysis tab.
- *Analysis* pane: Allows Import of XMAP files and Export of *FASTA, BED, and AGP* files.


IrysView
File
View
Tools
New Workspace
Open Workspac
Runs
Maps
Comparisons
Compared Maps
A7_11409_6244_s4_2012-08-22_17_26_Assemble_Molecules_
A7_11409_6244_s4_2012-08-22_17_26_Assemble_Molecules_
exp_refineFinal1_contigs_bppAdjust_cmap_MG1655_fa_BNGco
exp_refineFinal1_contigs_bppAdjust_cmap_MG1655_fa_NGSco
Search Comparisons . . .
Multi Samples Selection
A7_11409_6244_s4_2012-08-22_17_26_Assemble_Molecules_to_
A7_11409_6244_s4_2012-08-22_17_26_Assemble_Molecules_to_
Update
Display
ANALYSIS
Import
Export
D:\Workspace\IV22\Manual

5.1.2 Output Tabs

Report

- Accessed from *Runs* tab: Loads *Run* and *Molecule Quality Reports.*
- Accessed from *Maps* tab: Loads the *Assembly* and *Map Quality Report*s.
- Accessed from *Comparisons* tab: Loads the *Comparison Report*.

Data

- Accessed from *Runs* tab: Loads molecules, labels, images and repeats data.
- Accessed from *Maps* tab: Loads Molecules to assembled Genome Maps alignments.
- Accessed from *Comparisons* tab: Loads assembled Genome Maps to Anchor alignments.

Analysis

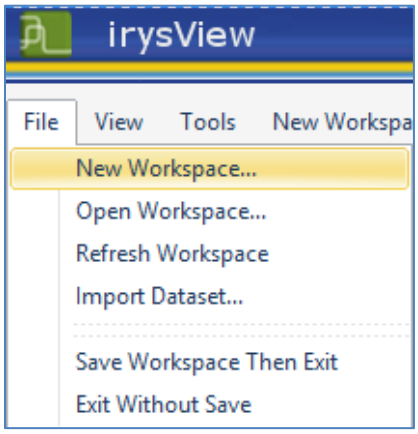
- Accessed from *Runs* tab: Loads Molecules to Anchor alignments from Molecule Quality Report.
- Accessed from *Maps* tab: Loads assembled Genome Maps vs Anchor alignments.
- Accessed from *Comparisons* tab: Loads 3-level Molecules to assembled Genome Maps to Anchor alignments (if a reference is included during *De Novo* assembly)

All

- Shows a tree view of the entire workspace.
- Accessed from *View* → *Workspace* pull-down menu.

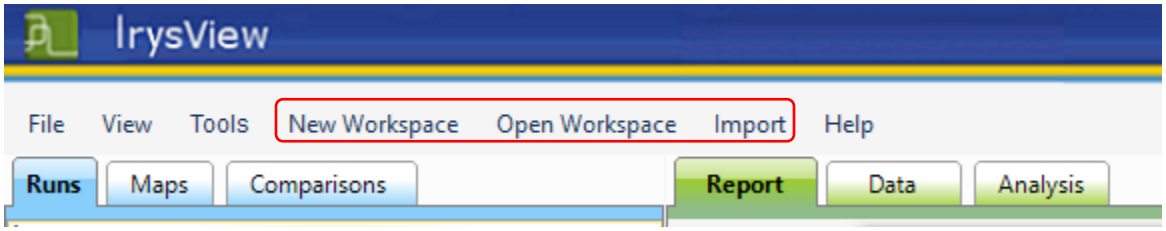
5.2 Menu Items

File



- *New Workspace* – Create a new workspace.
- *Open Workspace* – Open an existing workspace.
- *Refresh Workspace* – Manually update the display of workspace items.
- *Import Dataset*- Import pre-detected dataset.

Note: These menu items can also be accessed directly from the toolbar.

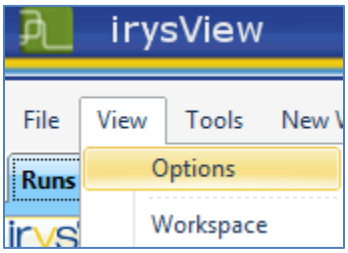


- *Save workspace Then Exit* – Save all changes to the workspace before exit. This may take some time for a large workspace.

Warning: Keep workspaces clean, free of unused datasets, ambiguous naming or multiple versions of similar analyses. Large and overly complicated workspaces are susceptible to data corruption. Please see Workspace Section 6 for further details.

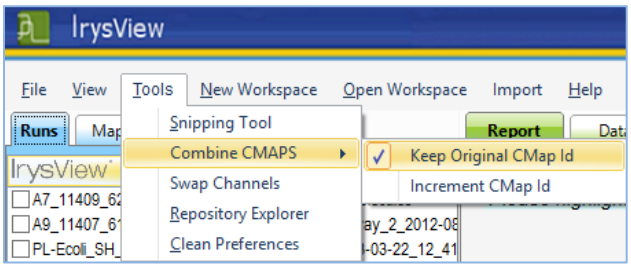
- *Exit without Save* – Exit IrysView immediately.

View



- *Options* - popup window of configuration and analyses settings.
- *Workspace* – full directory structure and files of a workspace.

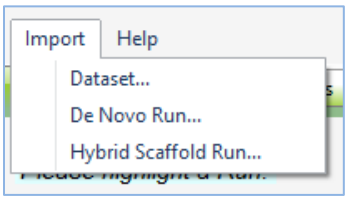
Tools



- *Snipping Tool* – Screen capture Cut & Paste tool (Windows 7 and later).
- *Combine CMAPS* – Concatenate multiple CMAP files into one CMAP. The first option "*Keep Original CMap Id*" will concatenate the 2 CMAPs into one without changing the CMap Id. The "*Increment CMap Id*" option will increment the CMap Id so that they are unique throughout the file.
- *Swap Channels* – Swap the color channels of a BNX file.
- *Repository Explorer* – Table of importable datasets in the parent directory of datasets. This same table also appears when importing datasets.
- *Clean Preferences* – Clean all configuration files (Clean Tool).

Import

A dropdown menu that allows a user to import existing data into IrysView:



- Dataset… - Import pre-detected dataset (same as Import Dataset… from the File menu).
- De Novo Run… - Import an already finished De Novo run. This option allows the user to import a completed De Novo run into IrysView. A dialog is displayed which allows the user to select the location of the De Novo run either on the local computer or on the remote server:

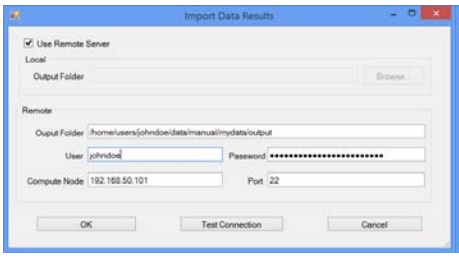


Fig. 1

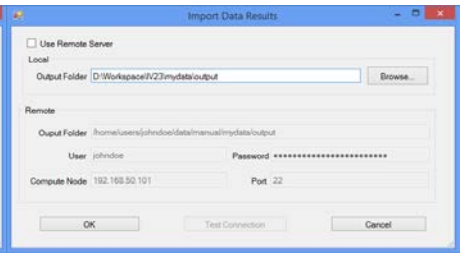
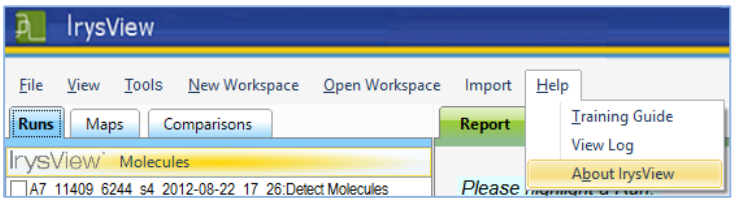


Fig. 2

  - If the data is on a remote server then “check” the Use Remote Server box (as shown in Fig. 1). This will enable the remote section of the dialog where the user shall enter the location of the data (this should include the “output” folder). Also, all the necessary information for connecting to the server should be filled in. A test button can be used to test the connection to the server. Click OK to begin the import of the De Novo run.
  - The user can also import the results from the local computer and in this case the user should “uncheck” the Use Remote Server box (as shown in Fig. 2). This will enable the Local section of the dialog. The user can use the Browse… button to locate the De Novo data (this should include the “output” folder). Click OK to begin the import of the De Novo run.
  - If the “all.bnx” file exists, IrysView will create a new dataset in the Runs tab and then import the data into this run.
  - If the “all.bnx” file is missing, then a dialog shall be displayed to get the name of the folder to be used for the data import.

- Hybrid Scaffold Run… - Import an existing Hybrid Scaffold run into IrysView. The same dialog box for the import of a De Novo run is displayed. The user shall enter the location of the Hybrid Scaffold data including the “output” folder. The user has the option to select a local or remote import.

Help

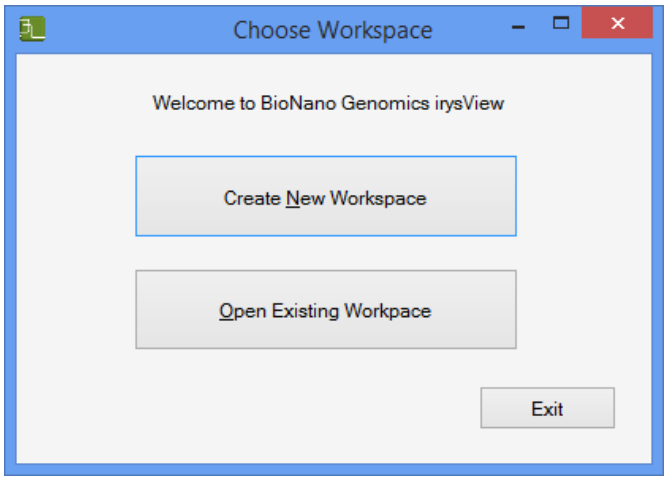


- *Training Guide* – Hyperlinks to the current training guide.
- *About IrysView* – Shows all supporting software versions and the workspace directory.
- *View Log* – Allows the user to Copy to Clipboard or Export to a file the contents of the IrysView log files.

6 IrysView Workflow

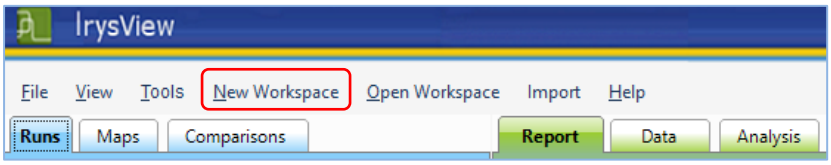
6.1 Choose Workspace

- When opening IrysView, choose between *Open Existing Workspace* and *Create New Workspace*. Note that a workspace must be created to view maps.

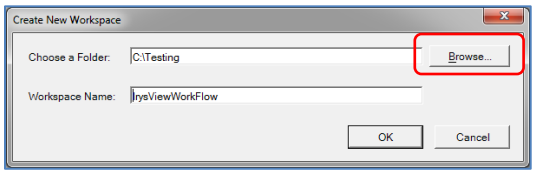


6.2 Create a New Workspace

- Click *New Workspace* from menu.

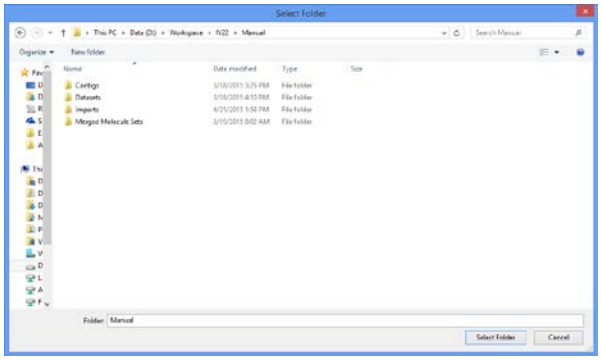


- Click *Browse* to choose a destination folder to save the workspace.
- Type in a name for workspace and click **OK.**



6.3 Open Existing Workspace

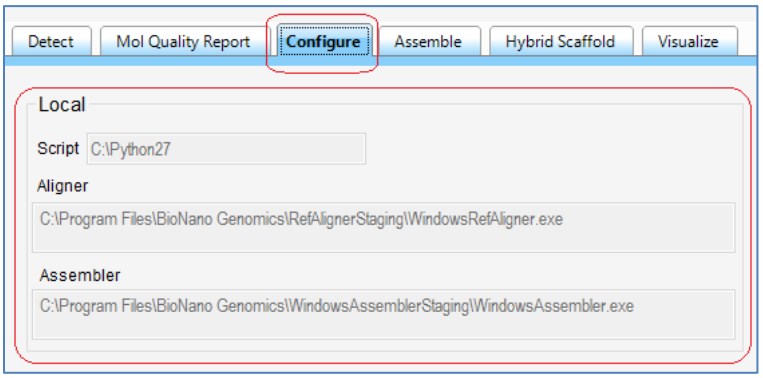
- Navigate to the workspace folder. This is the *parent* directory of four auto-generated folders: *Contigs, Datasets, Imports and Merged Molecule Sets*.



6.4 Options

- From the *View* menu, click *Options*.
- Choose *Configure* tab.

- For assembly on the local workstation, the paths of *Python* scripts, *RefAligner*, and *Assembler* executables are shown. These are default installation Windows paths during installation and are not editable.



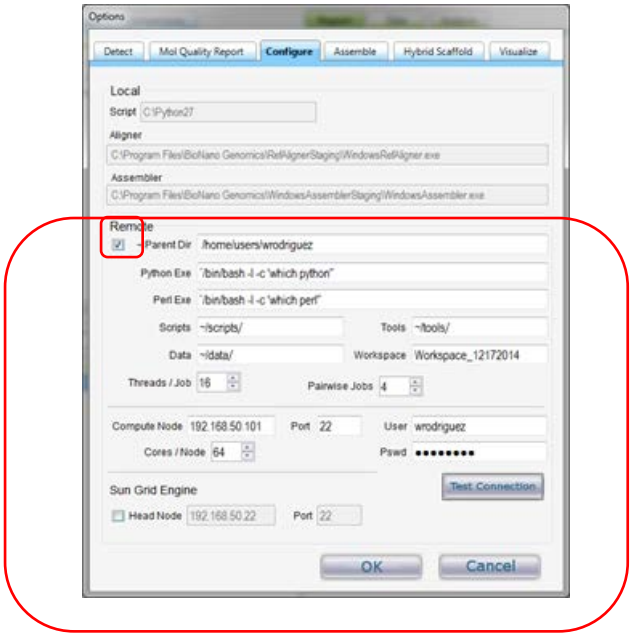
- For assembly on a remote Linux server.
  - Check the *Remote* box.
  - Verify the Python executable for your Python 2.7 installation, e.g. *python* or *python27.* By default, the Python version specified by the user's bash shell will be used.
  - Verify the Perl executable for your Perl installation, e.g. *perl* or *perl516.* By default, the Perl version specified by the user's bash shell will be used.
  - Verify the parent '~' directory, e.g. */home or home/users/username*.
    Note: Unlike earlier versions of IrysView, this parent directory can be entirely *independent* from the current login user. It simply needs full read/write/execute access.
  - Verify the *scripts* and *tools* paths, where '~' can denote the parent directory. It is recommended to use the full UNC-style path to each folder. Paths to symlinks should end with */,* e.g. *~/tools/* where *tools* is a symlink to */mnt/scratch/tools*
  - Symbolic links will require a backlash at the end of the path.
  - Verify the *data* path, e.g. *~/data/ or /mnt/scratch/data.*
  - Verify the *Workspace* name. All data from this workspace will be stored under this folder name under the *data* folder, e.g. *~/data/ecoli_demo_workspace/*
  - Set hardware-specific *Cores per Node*
  - Choose optimal *Threads Per Job* and *Pairwise Jobs* values.

- Set *Threads per Job* to 16 for large genomes (>1 Gbp) and 8 for mid-size genomes (<1Gbp), assuming a minimum RAM size of 2 Gbp per core. The optimal settings are determined by your server/cluster configuration. Please contact support@bionanogenomics.com for further guidance.
- Set *Pairwise Jobs* to be at least *Cores Per Node* divided by *Threads Per Job.*

o Enter IP, Port, Username and Password for a worker node.

o (Optional) Check and set Head Node IP/Port for Sun Grid Engine. Use of SGE requires configuration of the *scripts/clusterArguments.xml* file. For more information please contact support@bionanogenomics.com.

o Click *Test Connection* button to verify communication and installed files on the server.



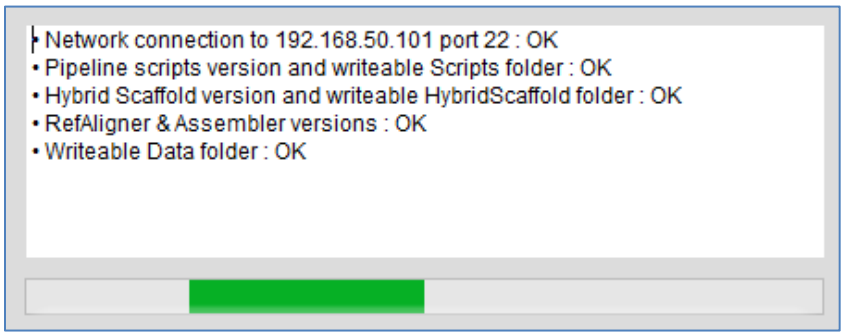
o The Test Connection button will test for the following:

1. Check that a connection to the compute (and head node) server can be made with the user provided credentials.
2. Check that the *scripts* folder exists and has full read/write/execute permissions
3. Check that the pipeline version is at least the minimum version listed in *About IrysView* screen.

4. Check that the *tools* folder exists and has full read/write/execute permissions
5. Check that the *RefAligner* executable exists and has at least the minimum version.
6. Check that the *Assembler* executable exists and has at least the minimum version.
7. Check that the *data* directory exists and has full read/write/execute permissions
8. Check that the *scripts/HybridScaffold* folder exists and has full read/write/execute permissions
9. Check that the *Hybrid Scaffold* version has at least the minimum version.

   Note: *Hybrid Scaffold* errors are non-fatal and do not prevent assembly from launching

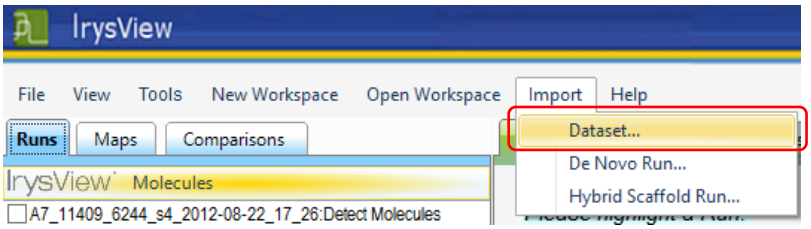
- An example of a successful test connection (without “Head node” selection) is shown below:



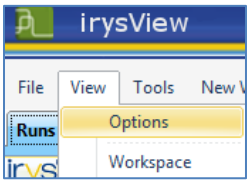
6.5 Import Data

There are two ways to import data into the *Runs* Tab: *Import->Dataset…* from Import Menu Option and Drag 'n Drop Datasets.

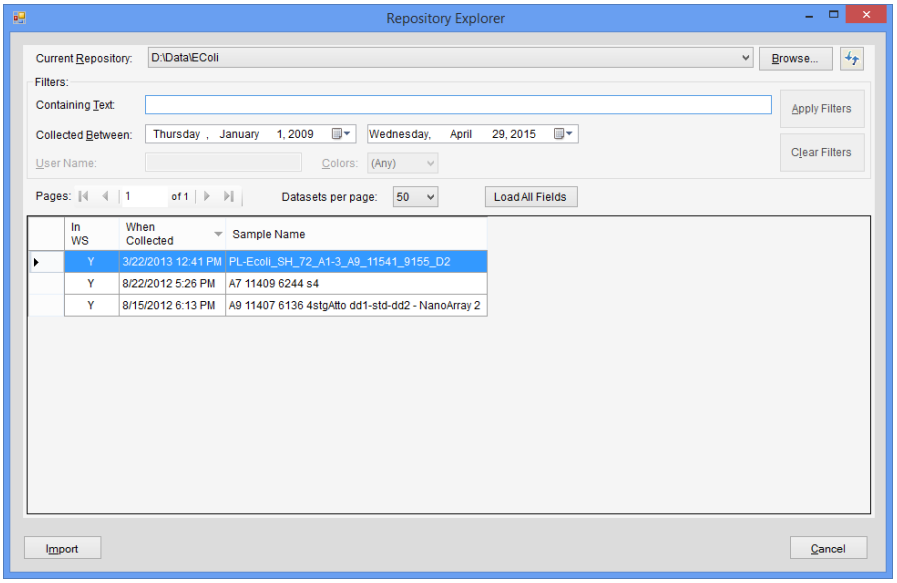
6.6 Import dataset from menu



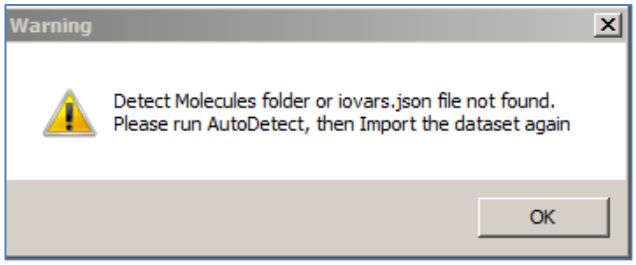
Note: Global filtering by molecule size and label SNR is automatically applied to all imported datasets. The filter type and values can be changed in *View/Options menu.*



- Choose the parent directory of the date folders (e.g. 2012-08) from the top drop down list or from *Browse*.
- Sort by *When Collected* or *Sample Name,* and Filter by using *Containing Text* to find your data.



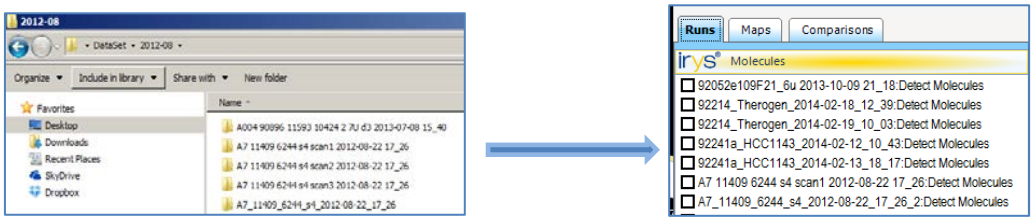
- Choose one or more datasets (holding down Shift or Control key) and press *Import* on the bottom left.
- If a dataset is not ready for import, run AutoDetect first and then try again.



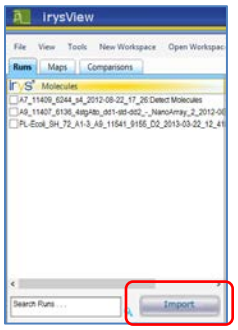
- Once data has been imported a *Y* will appear under the *In WS* column.
- Click *Cancel* once the data has been imported.

6.7 Drag n' Drop Dataset

- Datasets can be directly imported by dragging its run folder into the *Runs* tab.



- Run data without *.tiff* images can also be imported. This is particularly useful for sharing run data whose image files are too large for transfer. Click the *Import* button and then navigate to a *RawMolecules.bnx* file and click *Open.*



6.8 De Novo Run…

- De Novo Run… - Import an already finished De Novo run. This options allows the user to import a completed De Novo run into IrysView. A dialog is displayed which allows the user to select the location of the De Novo run either on the local computer or on the remote server:

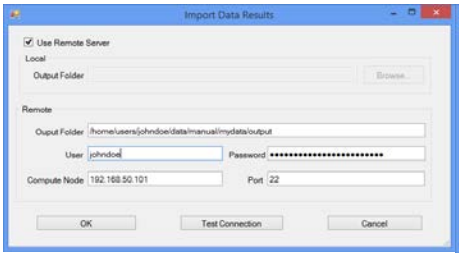


Fig. 1

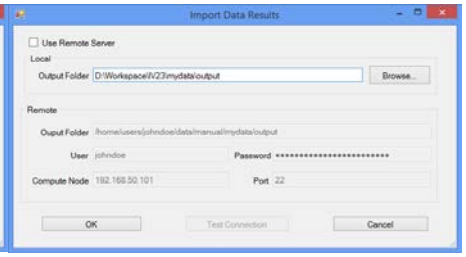


Fig. 2

  - If the data is on a remote server then “check” the Use Remote Server box (as shown in Fig. 1). This will enable the remote section of the dialog where the user shall enter the location of the data (this should include the “output” folder). Also, all the necessary information for connecting to the server should be filled in. A test button can be used to test the connection to the server. Click OK to begin the import of the De Novo run.

- The user can also copy the results to the local computer and in this case the user should “uncheck” the Use Remote Server box (as shown in Fig. 2). This will enable the Local section of the dialog. The user can use the Browse… button to locate the De Novo data (this should include the “output” folder). Click OK to begin the import of the De Novo run.
- If the “all.bnx” file exists, IrysView will create a new dataset in the Runs tab and then import the data into this run.
- If the “all.bnx” file is missing, then a dialog shall be displayed to get the name of the folder to be used for the data import.

6.9 Hybrid Scaffold Run…

- Hybrid Scaffold Run… - Import an existing Hybrid Scaffold run into IrysView. The same dialog box for the import of a De Novo run is displayed. The user shall enter the location of the Hybrid Scaffold data including the “output” folder. The user has the option to select a local or remote import.

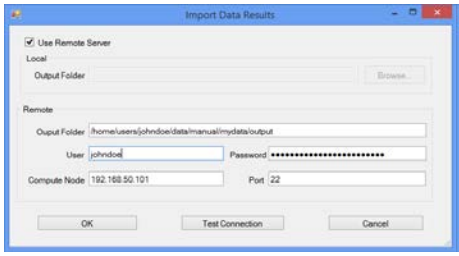


Fig. 1

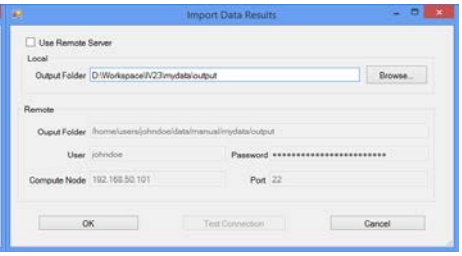
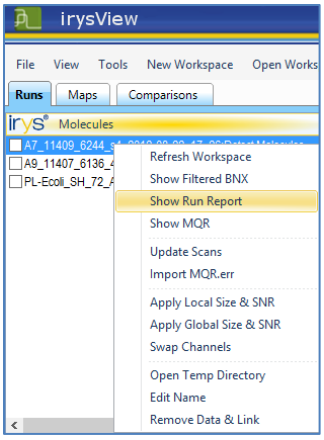
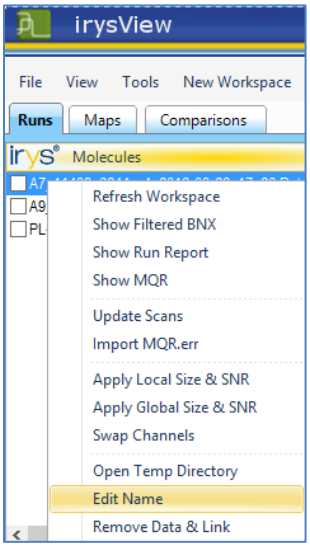


Fig. 2

- *Show Run Report* – Use this menu to display an already generated Run Report.



- *Edit Name* - Rename a run.

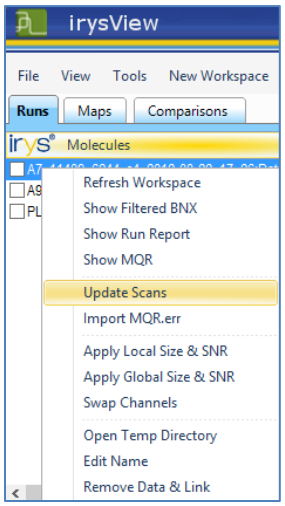


- *Open Temp Directory* - Opens the local temporary folder of *De Novo* assembly outputs.

6.10 Update Scans

As soon as scans become available from the *Irys* instrument after being processed by *AutoDetect*, the user can pull them into IrysView.

- Click *Runs* tab. Right click on a Run and choose *Update Scans.*

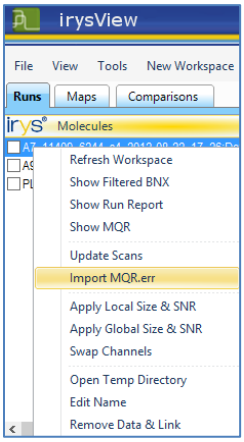


6.11 Show MQR

Force the display of *Molecule Quality Report* after it was computed.

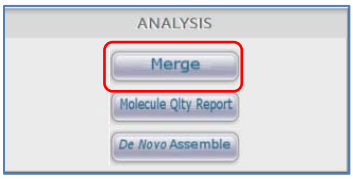
6.12 Import MQR.err

An externally generated *Molecule Quality Report* by command line *RefAligner* can be imported and shown in *IrysView* in conjunction with *Run Report*.

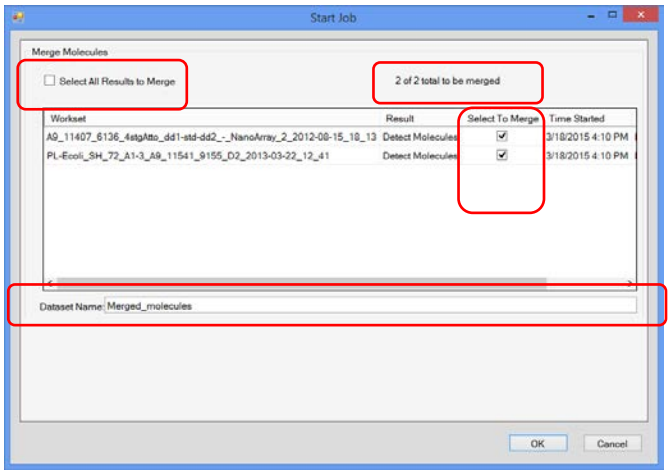


6.13 Merge Runs

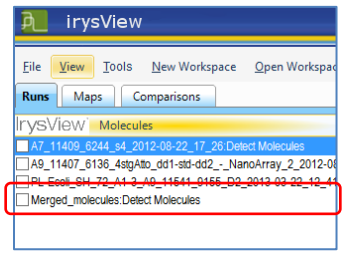
1. Click *Runs* tab.
2. Click (*not* checkmark) *one* of the runs then press *Merge* button on the *Analysis* pane.



- From the *Merge Molecules* dialog, *Select All Results to Merge* or checkbox *Select To Merge* individual runs. The number of runs selected is displayed in the top right corner of the dialog. The user can set the name of the merged dataset in the Dataset Name field just below the list of runs.

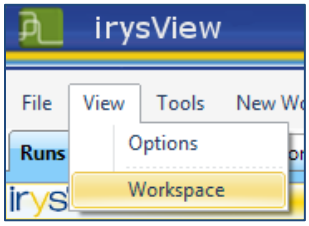


3. Once merging is completed a *Merged Molecule Set* will be created in the *Runs* tab.



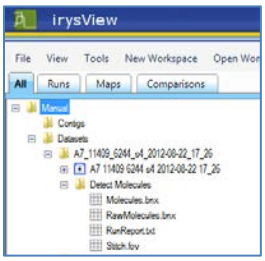
6.14 Workspace

1. To view the workspace directory, choose *Workspace* from the *View* pull down menu.

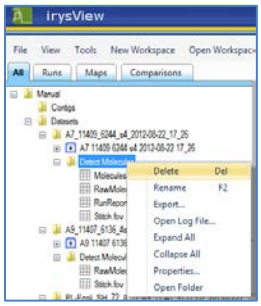


Warning: A workspace is *not* a regular file system. It is a custom database. All interactions with the workspace *must* be initiated within *IrysView* to maintain integrity. A non-expert user should not directly manipulate this folder or any of its content. Should corruption occur, running the *CleanIrysViewConfigs* tool outside of IrysView and creating a fresh workspace should fix the problem.

2. The *All* tab on the left shows the workspace file content.

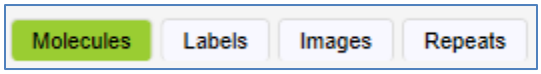


3. Workspace content can be renamed or deleted by IrysView. Outdated or duplicated analysis folders should be regularly deleted as a recommended practice to maintain performance and data integrity.

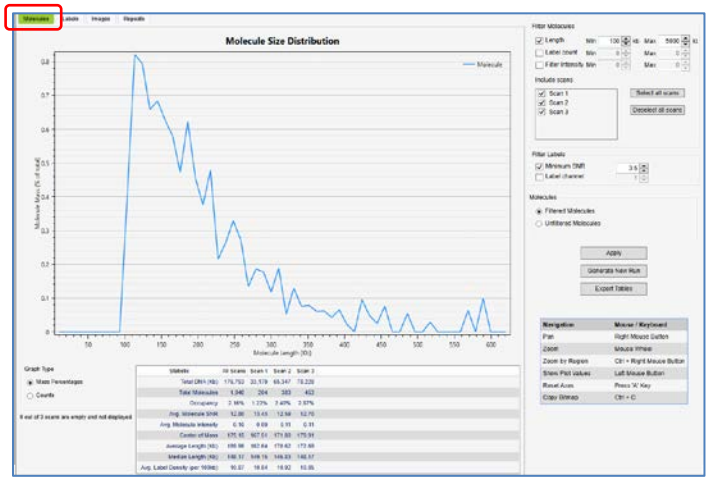


6.15 Explore Detection Results

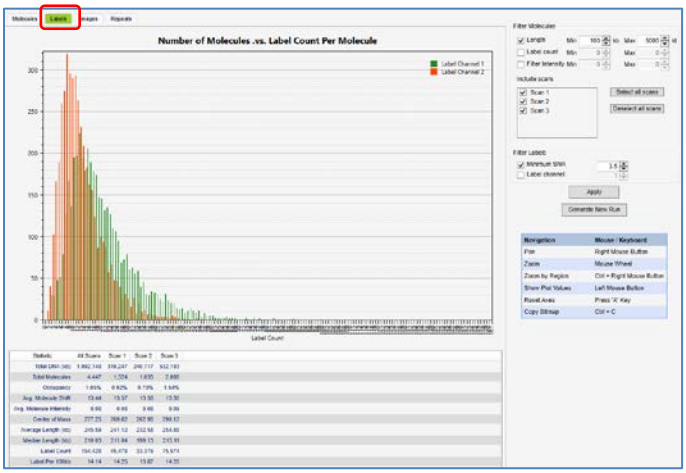
1. Select a Run from the *Runs* tab.
2. Click on the result *Data* tab to see the detection results.
3. Molecule, Label statistics, Images and Repeats can be selected from the top toolbar.



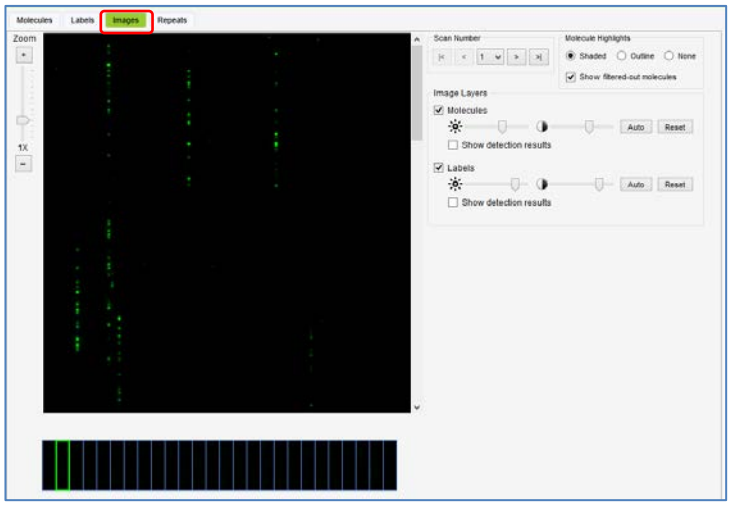
4. Select *Molecules* for the overall molecule distribution and statistics.



5. Select *Labels* for the overall label distribution and statistics. The labels channels are hardcoded to show Green (channel 1) and Red (channel 2).

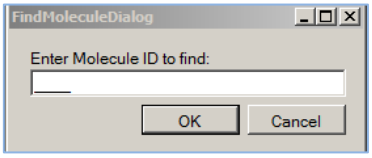


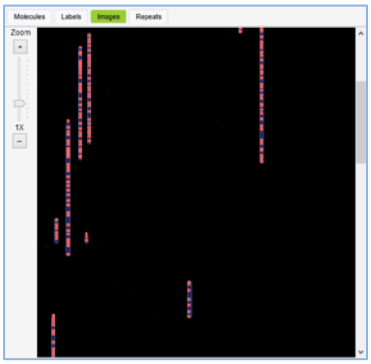
6. Select *Images* for the image of each scan.



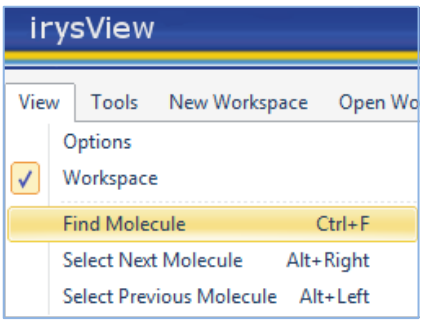
(Note: This feature is intended for use with bnx files that contain a single run.)

a. Find any molecule by clicking *Ctrl+F*, or *View menu + Find Molecule,* and enter the molecule *ID* and click *OK.* The image will center to that molecule.





b. Find the Next or Previous Molecule, by *Alt+Right Arrow*, or *Alt+Left Arrow.*



7. Filter

   a. Molecules and labels can be filtered by Length, Intensity, SNR and Channel.

      Warning: This filtered data will *not* be used with any other analyses, such as *Molecule Quality Report* or *De Novo* Assembly. It is for data exploration *display* purpose only.

   b. Filtered and Unfiltered molecule data can also be displayed by selecting the appropriate selection in the Molecules group.

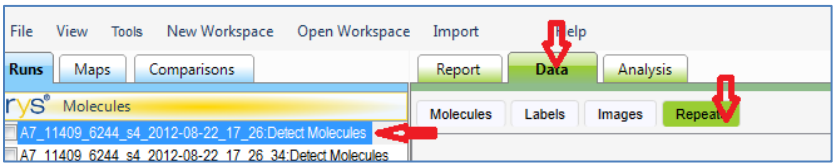
   c. The user can now save the tabular data into a tab delimited text file.

8. Repeats

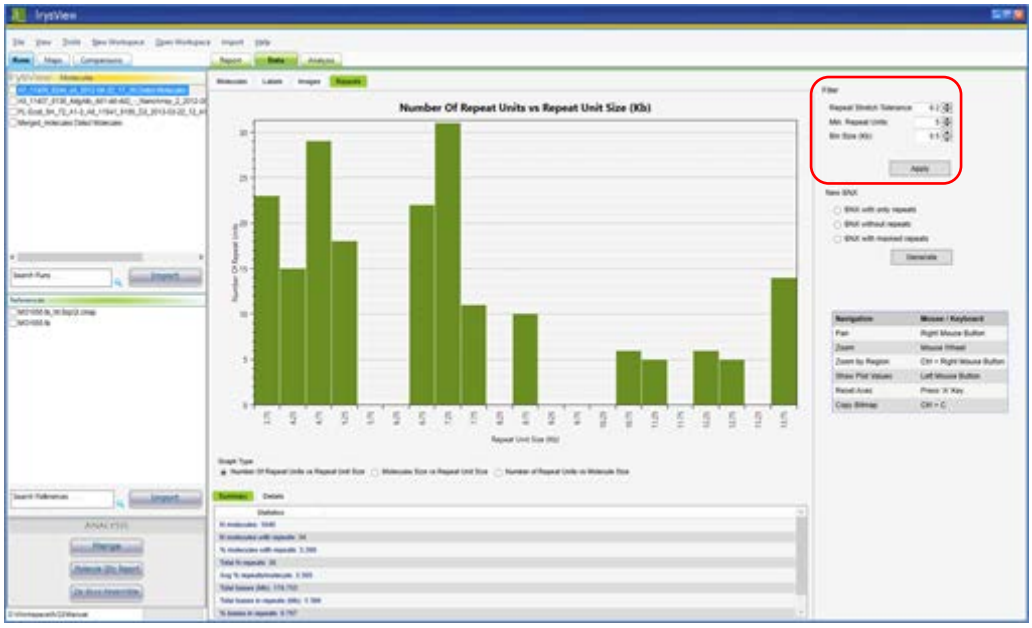
   Detected molecules can have a repeating series of equally spaced labels. Repeating labels fall into two categories: single-label and compound multi-label repeats. A single label repeat is a set of consecutive label sites along the molecule that are equally spaced, for example 2 kbp or 5 kbp. This version of IrysView computes single-label repeats with more complex repeat detection anticipated in future versions.

   a. Select a Run.

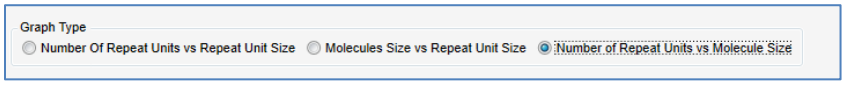
b. Navigate to the *Data* tab, then the *Repeats* tab.



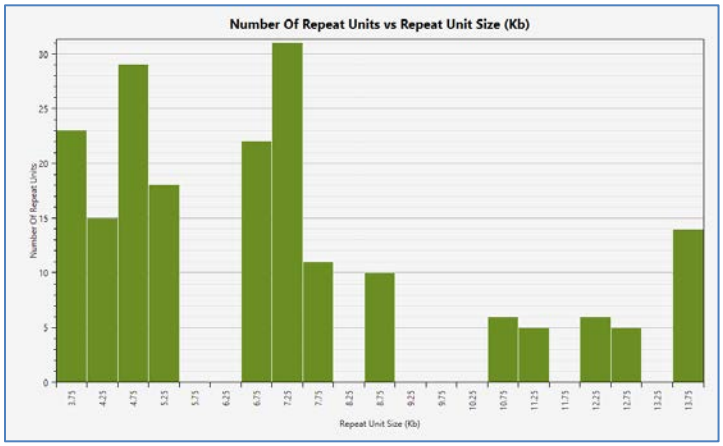
c. Select the appropriate filter *Repeat Stretch Tolerance* value. Click *Apply*.



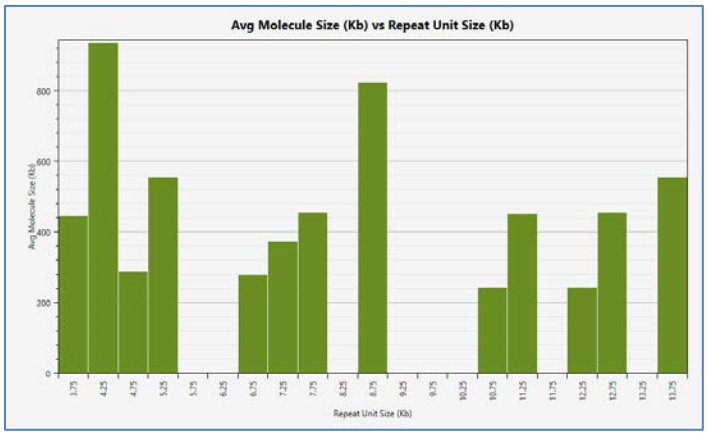
d. Select among three types of *Repeats* statistics radio buttons.



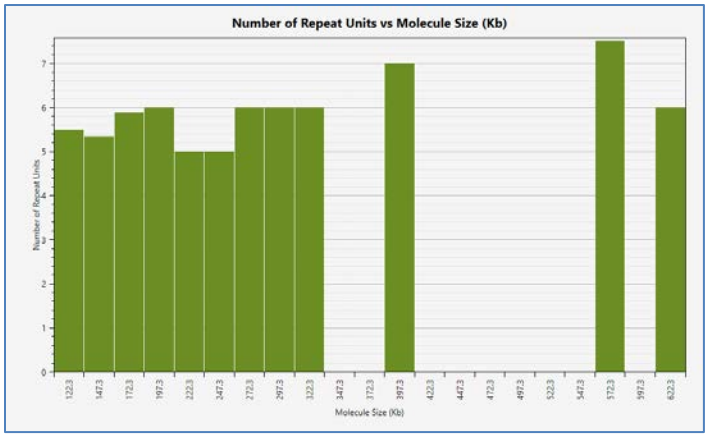
- *Number of Repeat Units* vs *Repeat Unit Size (kbp)*



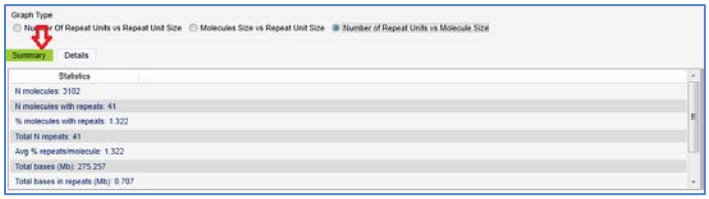
- *Molecules Size vs Repeat Unit Size (kbp)*

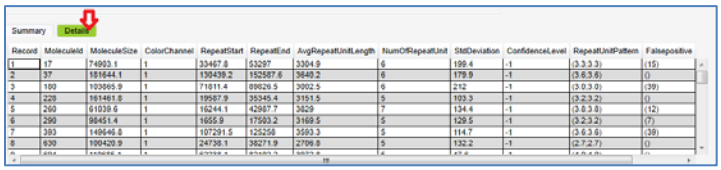


- *Number of Repeat Units vs Molecules Size (kbp)*

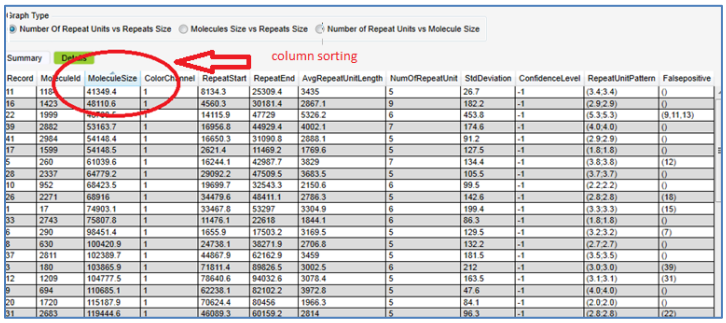


- *Summary* and *Details* tabs provide additional molecule level details.





Record	MoleculeId	MoleculeSize	ColorChannel	RepeatStart	RepeatEnd	AvgRepeatUnitLength	NumOfRepeatUnit	StdDeviation	ConfidenceLevel	RepeatUnitPattern	Falsepositive
1	17	74993.1	1	33467.8	53297	3304.9	6	199.4	-1	(3:3:3:3)	(15)
2	37	161644.1	1	130439.2	152587.6	3640.2	6	179.9	-1	(3:6:3:6)	()
3	180	193065.9	1	71811.4	89626.5	3002.5	6	212	-1	(3:0:3:0)	(39)
4	228	161461.8	1	19587.9	35345.4	3151.5	5	103.3	-1	(3:2:3:2)	()
5	260	61039.6	1	16244.1	42987.7	3829	7	134.4	-1	(3:6:3:6)	(12)
6	290	98451.4	1	1655.9	17503.2	3169.5	5	129.5	-1	(3:2:3:2)	(7)
7	393	149946.8	1	107291.5	125256	3593.3	5	114.7	-1	(3:6:3:6)	(39)
8	630	100420.9	1	24738.1	36271.9	2706.8	5	132.2	-1	(2:7:2:7)	()



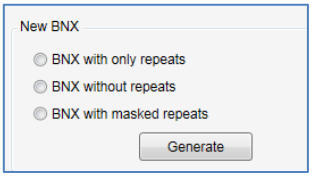
Record	MoleculeId	MoleculeSize	ColorChannel	RepeatStart	RepeatEnd	AvgRepeatUnitLength	NumOfRepeatUnit	StdDeviation	ConfidenceLevel	RepeatUnitPattern	Falsepositive
11	115	41349.4	1	8134.3	25309.4	3435	5	26.7	-1	(3.4:3.4)	()
16	1423	48110.6	1	4560.3	30181.4	2867.1	9	182.2	-1	(2.9:2.9)	()
22	1999	[illegible]	[illegible]	14115.9	47729	5326.2	6	453.8	-1	(5.3:5.3)	(9,11,13)
39	2882	53163.7	1	16956.8	44929.4	4002.1	7	174.6	-1	(4.0:4.0)	()
41	2984	54148.4	1	16650.3	31090.8	2888.1	5	91.2	-1	(2.9:2.9)	()
17	1599	54148.5	1	2821.4	11469.2	1769.6	5	127.5	-1	(1.8:1.8)	()
5	260	61039.6	1	16244.1	42987.7	3829	7	134.4	-1	(3.8:3.8)	(12)
28	2337	64779.2	1	29092.2	47509.5	3683.5	5	105.5	-1	(3.7:3.7)	()
10	952	68423.5	1	19699.7	32543.3	2150.6	6	99.5	-1	(2.2:2.2)	()
26	2271	68916	1	34479.6	48411.1	2786.3	5	142.6	-1	(2.8:2.8)	(18)
1	17	74903.1	1	33467.8	53297	3304.9	6	199.4	-1	(3.3:3.3)	(15)
33	2743	75807.8	1	11476.1	22618	1844.1	6	86.3	-1	(1.8:1.8)	()
6	290	98451.4	1	1655.9	17503.2	3169.5	5	129.5	-1	(3.2:3.2)	(7)
8	630	100420.9	1	24738.1	38271.9	2706.8	5	132.2	-1	(2.7:2.7)	()
37	2811	102389.7	1	44867.9	62162.9	3459	5	181.5	-1	(3.5:3.5)	()
3	180	103865.9	1	71811.4	89826.5	3002.5	6	212	-1	(3.0:3.0)	(39)
12	1209	104777.5	1	78640.6	94032.6	3078.4	5	163.5	-1	(3.1:3.1)	(31)
9	694	110685.1	1	62238.1	82102.2	3972.8	5	47.6	-1	(4.0:4.0)	()
20	1720	115187.9	1	70624.4	80456	1966.3	5	84.1	-1	(2.0:2.0)	()
31	2683	119444.6	1	46089.3	60159.2	2814	5	96.3	-1	(2.8:2.8)	(22)

e. Filtering of Repeats

- Filter by *Repeats Stretch Tolerance*, *Min. Repeat Units*, *Bin Size* to be displayed on the graph and *Color Channel.*

f. New BNX

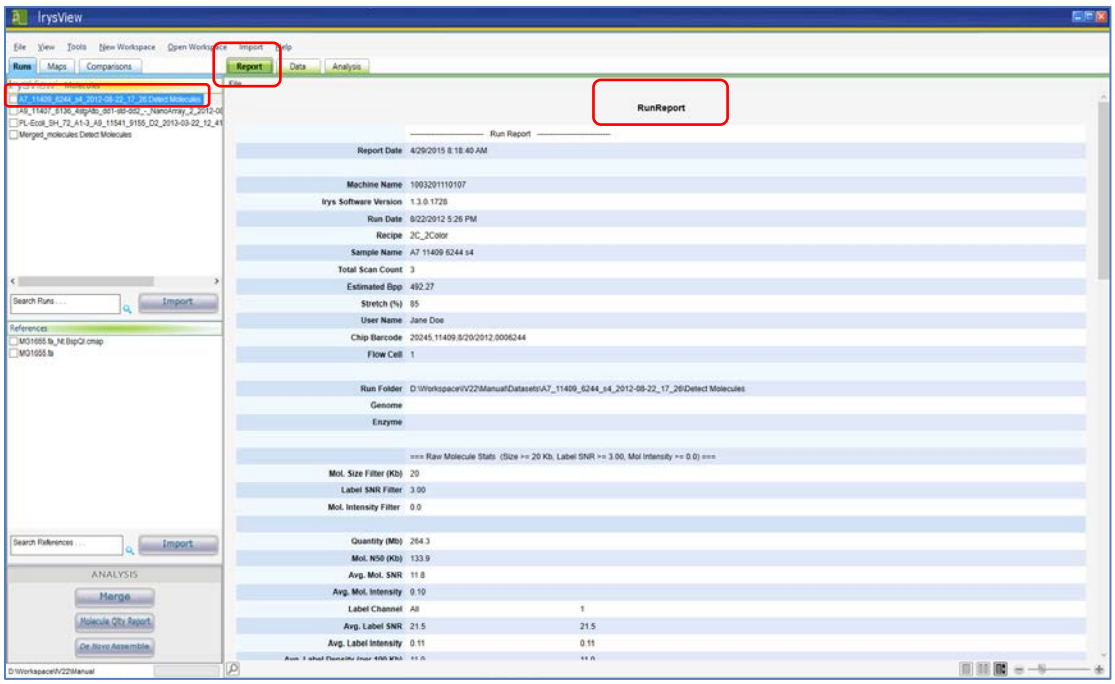
- A new *Run* can be created using either the default or custom filter values.
- There are three types of runs: *BNX with only repeats*, *BNX without repeats* and *BNX with masked repeats*.
- Click *Generate* to apply the filter. The new run is automatically imported into the *Runs* tab.



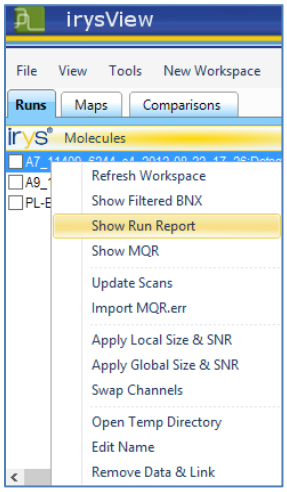
6.16 Run Report

1. Select the *Runs* tab.

   Select a Run from the *Molecules* pane. A *Run Report* will load in the *Report* tab.



2. To refresh the report view, Right-click on the run and select the Show Run Report option:

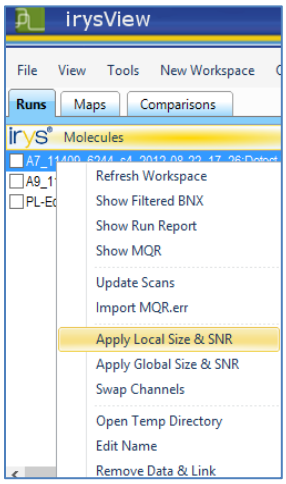


6.17 Data Filter

Molecules and Labels can be filtered by Size and SNR before *De Novo* assembly. There are 2 filtering options: *Local* filter sets the parameters for one selected dataset, and *Global* filter applies to more than one datasets. When set from the *View-Options* menu item, the filter values will globally affect all subsequently imported datasets.

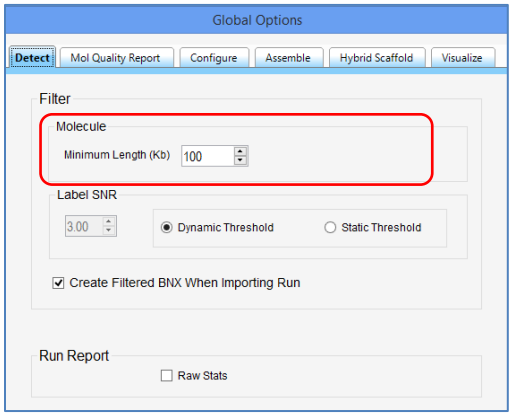
To filter the runs already imported into the workspace, follow the steps below.

1. For local filter, right click on a Run and choose *Apply Local Size & SNR*.



a. Molecule_Length

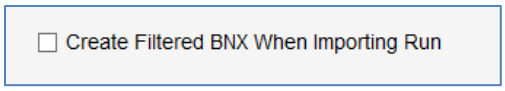
From the pop up *Options* window and *Detect* tab, select a minimum cut-off molecule length in kbp. By default, 100 kbp is used as the minimum length cut-off. For larger, complex genomes, 150 kbp cutoff is recommended.



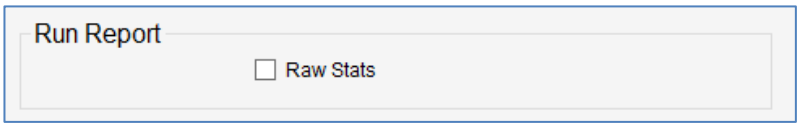
b. Label SNR

- *Dynamic Threshold*. An optimal Label SNR is calculated by IrysView. The original data remains unchanged in a *RawMolecules.bnx* file. *Dynamic Threshold* is the default.
- *Fixed Threshold*. The user can enter any fixed value between 2.0 and 8.0. For most instruments, the recommended value is 2.75.

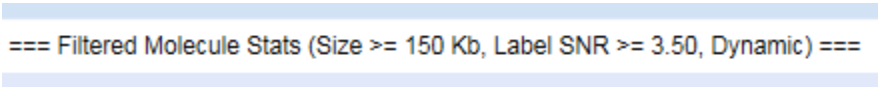
c. *Filter BNX on import*. Check the box to automatically filter the BNX file when importing a Run.



2. Include unfiltered raw molecule statistics in the Run Report.

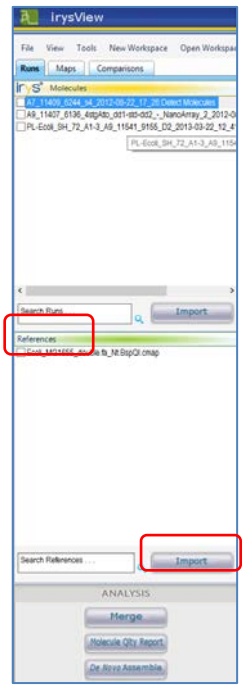


3. By default a *Run Report* uses the global filter threshold:

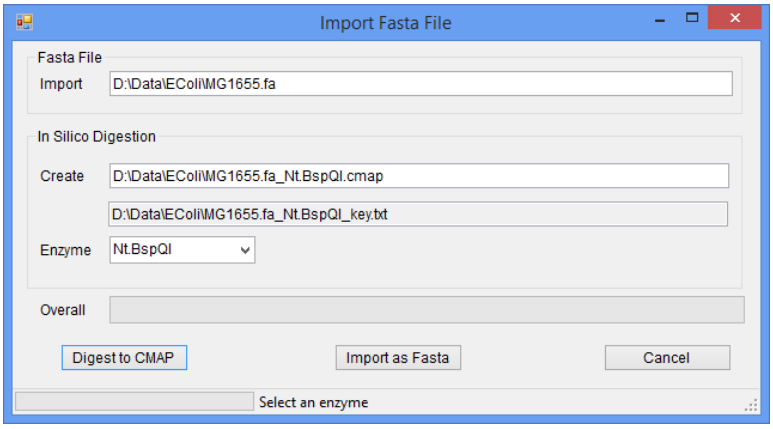


6.18 Molecule Quality Report

1. Import a Reference
   a. Under the *References* pane press *Import*.



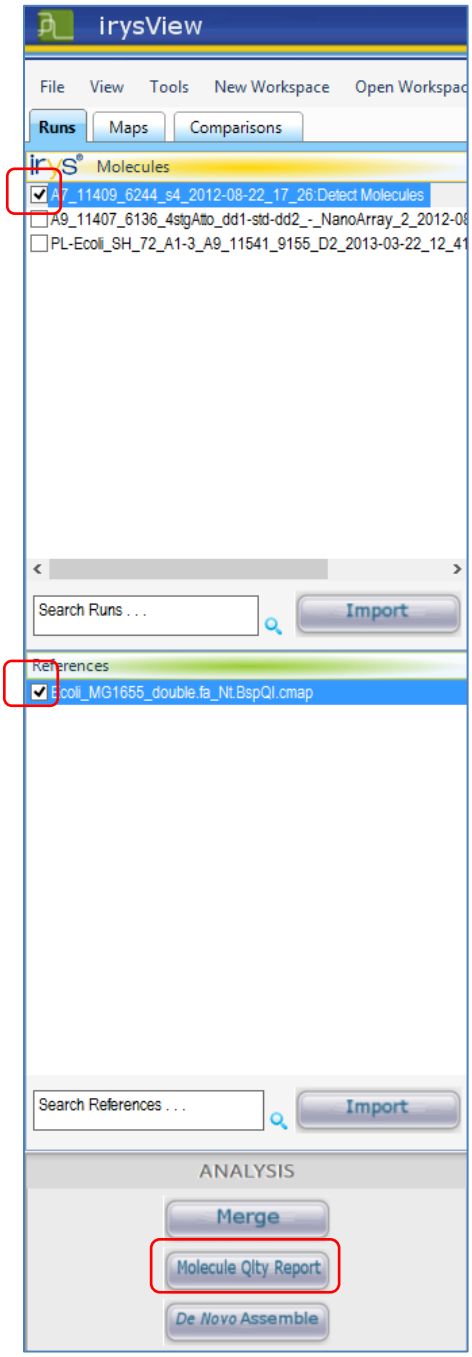
   b. Search for file types *CMAP, FASTA*, or *FA*. For example, *MG1655.fa.*
   c. In the *Import Fasta File* window, verify the files.



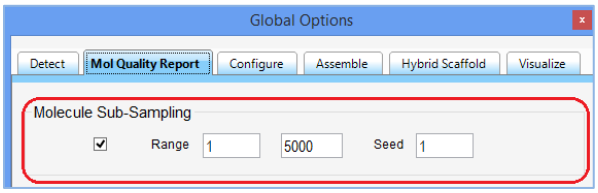
d. Use the drop down list to choose the appropriate nickase *Enzyme* (ex. *Nt.BspQI*). To generate a dual nick cmap (with both *NtBspQI* and *NbBbvCI*), please see the IrysView installation guide for the link to download the *Knickers* tool. Knickers should be used for Genomes that are greater than 100 Mbp.

e. Press *Digest to CMAP* to create the CMAP file with in-silico digestion of FASTA to CMAP using the selected enzyme or *Import as Fasta* to import raw FASTA without in-silico digestion. The *Digest to CMAP* file will generate a key file that is required for exporting the results of hybrid scaffold xmap in the *Comparisons* tab as FASTA.

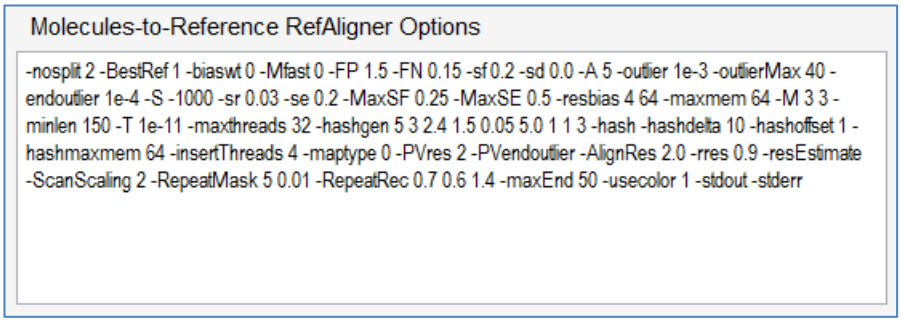
2. Checkmark a *Run* and the imported *Reference Map*, then press *Molecule Quality Report.* A pop up Options Dialog is displayed.



3. Sub-Sampling range
   a. For large datasets, sub-sampling can speed up generation of *Mol Quality Report.*
   b. From *Options* menu and *Mol Quality Report* tab, set sub-sampling values. Each randomized sub population can be reproducibly sampled by keeping the non-zero *Seed* integer constant.



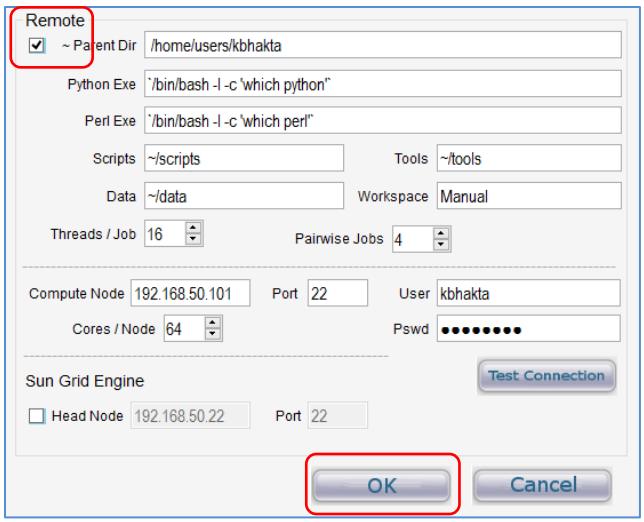
4. Alignment Options
   a. These command line argument values affect important metrics such as Mapping Rate (%), FP and FN. The recommended default values have been provided. For more information on these parameters use the '-help' argument on the command line.



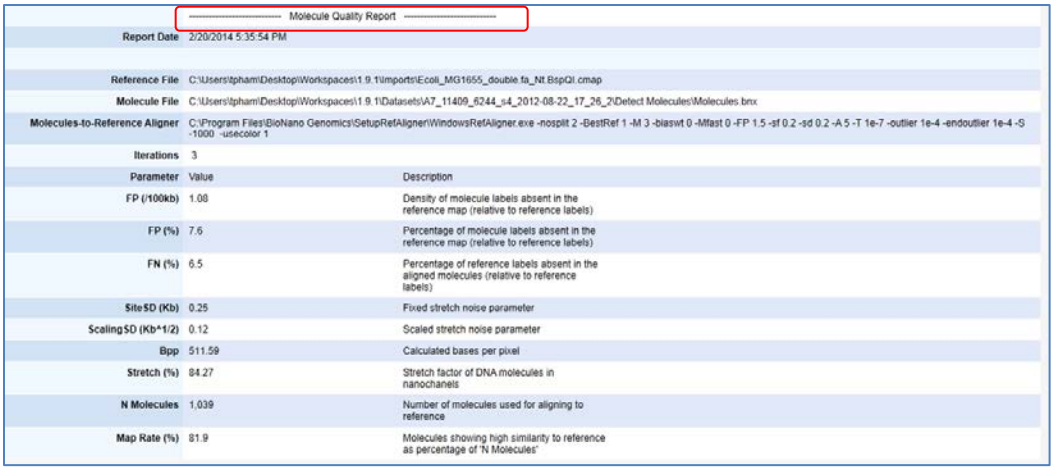
5. *Local* or *Remote*

   Execution time can be reduced with a fast multicore remote system, if available.

   a. From *Options* menu and *Configure* tab, check the *Remote* checkbox and click OK.



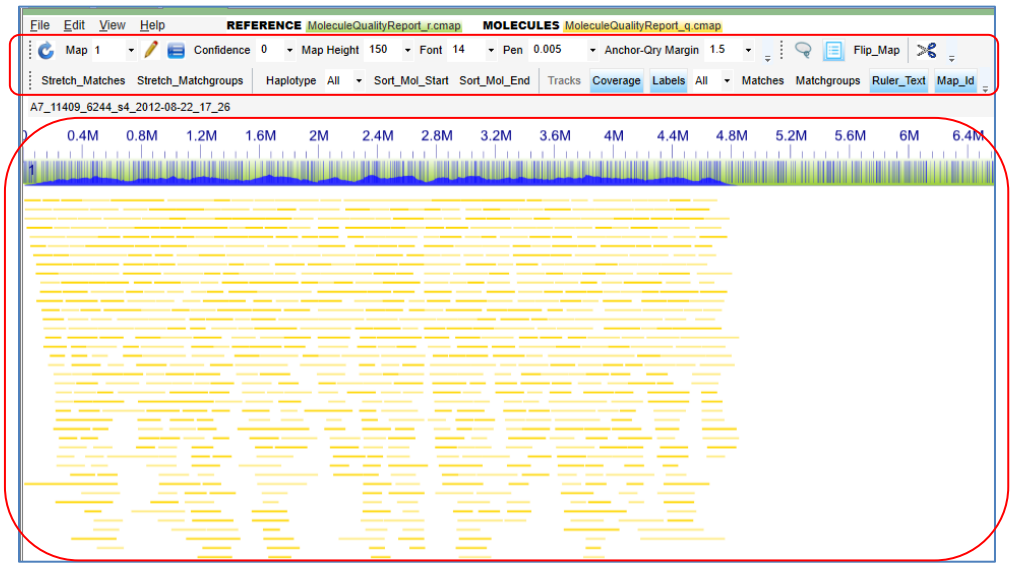
6. The *Molecule Quality Report* will be appended to the end of the *Run Report*.



Molecule Quality Report

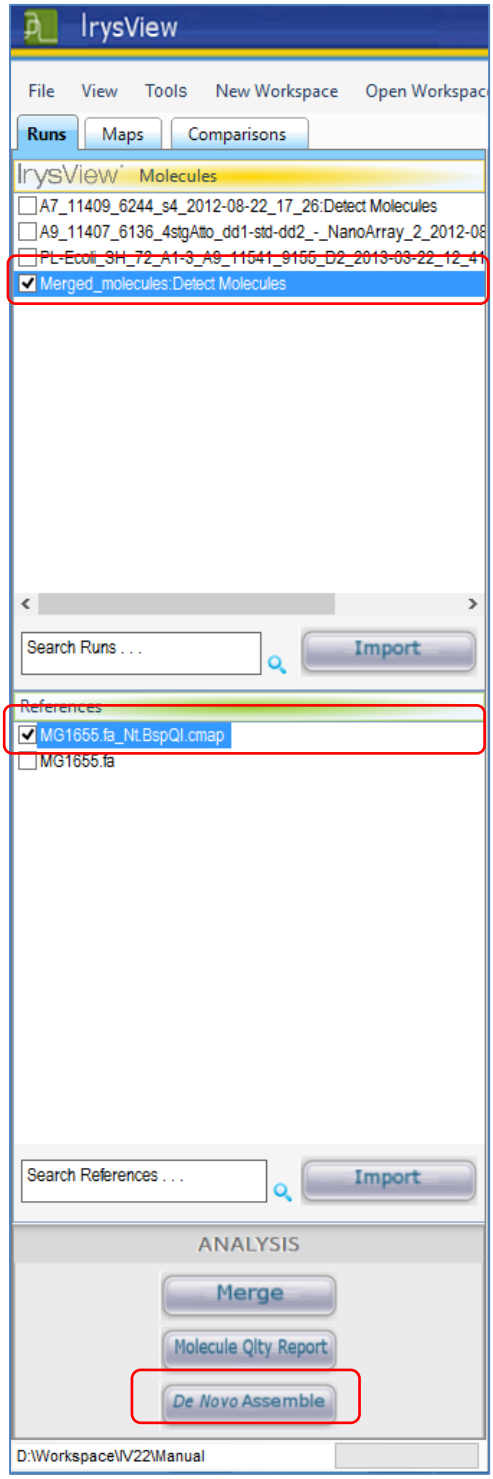
Report Date	2/20/2014 5:35:54 PM	
Reference File	C:\Users\tpham\Desktop\Workspaces\1.9.1\Imports\Ecoli_MG1655_double.fa_Nt.BspQI.cmap	
Molecule File	C:\Users\tpham\Desktop\Workspaces\1.9.1\Datasets\A7_11409_6244_s4_2012-08-22_17_26_2\Detect Molecules\Molecules.bnx	
Molecules-to-Reference Aligner	C:\Program Files\BioNano Genomics\SetupRefAligner\WindowsRefAligner.exe -nosplit 2 -BestRef 1 -M 3 -biaswt 0 -Mfast 0 -FP 1.5 -sf 0.2 -sd 0.2 -A 5 -T 1e-7 -outlier 1e-4 -endoutlier 1e-4 -S -1000 -usecolor 1	
Iterations	3	
Parameter	Value	Description
FP (/100kb)	1.08	Density of molecule labels absent in the reference map (relative to reference labels)
FP (%)	7.6	Percentage of molecule labels absent in the reference map (relative to reference labels)
FN (%)	6.5	Percentage of reference labels absent in the aligned molecules (relative to reference labels)
SiteSD (Kb)	0.25	Fixed stretch noise parameter
ScalingSD (Kb^1/2)	0.12	Scaled stretch noise parameter
Bpp	511.59	Calculated bases per pixel
Stretch (%)	84.27	Stretch factor of DNA molecules in nanochanels
N Molecules	1,039	Number of molecules used for aligning to reference
Map Rate (%)	81.9	Molecules showing high similarity to reference as percentage of 'N Molecules'

7. Molecules aligned against the *in silico* digested Reference will appear under the *Analysis* tab. Please refer to the *View Comparison* section below for details on the toolbar display options.

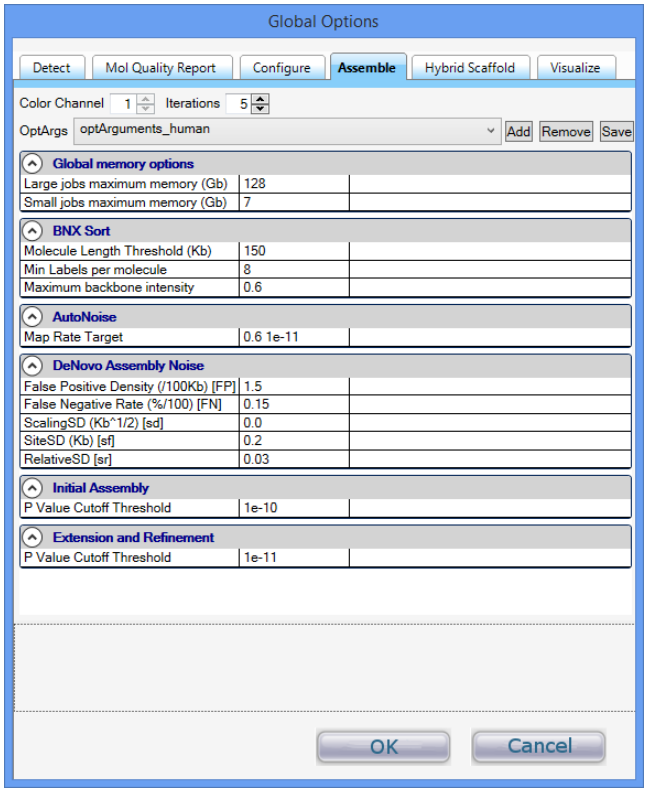


6.19 *De Novo* Assembly

1. Checkmark (not highlight) a Run from the *Runs* tab. Optionally, checkmark a Reference from the *References* tab. Then press *De Novo Assemble.*



2. From the *Options* pop-up window and *Assemble* tab, configure the arguments to be used during *De Novo Assembly*.



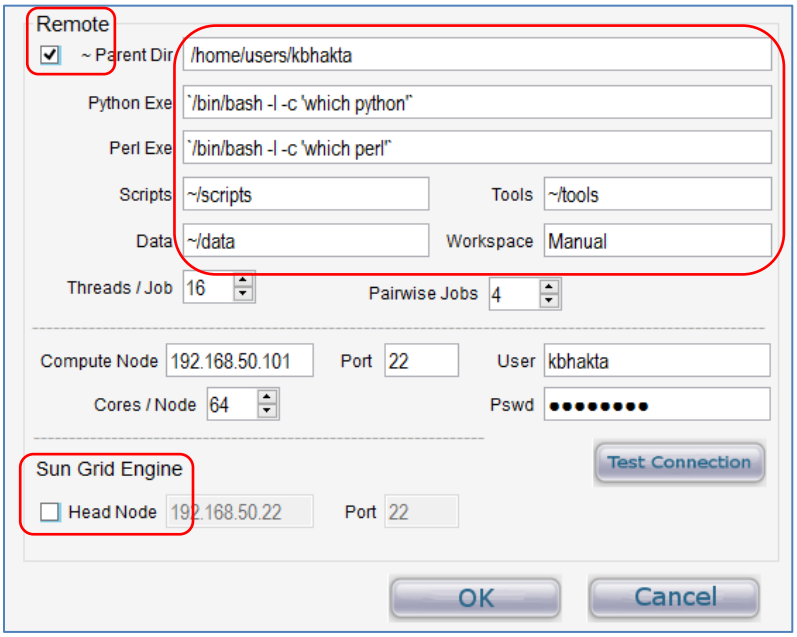
- *Iterations* -The number of assembler iterations greater than or equal to 1. Recommended value is 5.
- *AutoNoise* – If a reference is selected and available.
- *OptArgs* – Three different optArguments files containing generic alignment and assembly parameters are provided by default:
    - optArguments_small.xml contains parameters generally suggested as a starting point for small genomes, <10 Mbp in size such as *E. coli.*
    - optArguments_medium.xml contains parameters generally suggested as a starting point used for medium genomes, 10-1000 Mbp in size such as *Drosophila.*
    - optArguments_human.xml contains parameters generally recommended for large genomes, >1Gbp in size such as human.

Note: The parameters provided in the default optArguments files are suggested starting points for each genome size. The optimal values for each parameter vary based on organism and application.

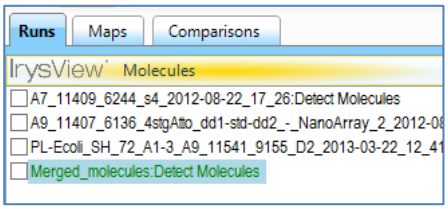
- Edit the specific values in the table. Click on each parameter to see more explanation at the bottom.

- *Save* - Overwrite the selected optArguments file, or save to a new file.
- *Add* - Import any arguments.xml file to include in the dropdown.
- *Remove* - Delete the currently selected arguments.xml from the dropdown list.

3. From the *Configure* tab, checkbox *Remote* to execute remotely or leave unchecked to execute locally.
   - Verify the paths of the *Parent*, *Scripts*, *Tools* and *Data* directories.
   - *Workspace* – default current workspace or custom Data subdirectory name
   - Check *SGE* if headnode Sun Grid Engine has been configured.
4. Press OK to start *De Novo* Assembly.

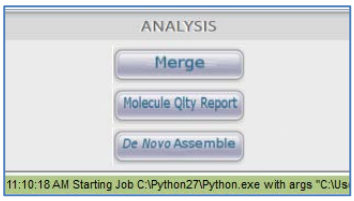


5. Status
   - The highlighted Run will turn green during assembly.

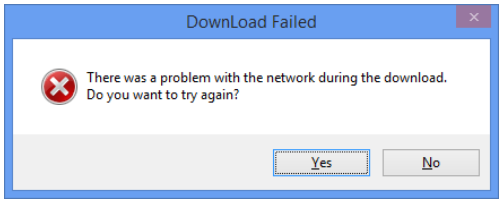


- An olive progress bar on the bottom will update with progressing details.

  Note: De Novo assembly takes some time. Overnight assembly is recommended.

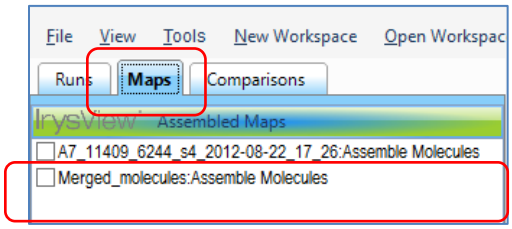


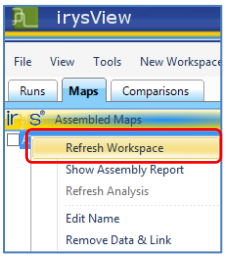
- During the download of the results from the server, IrysView now does an MD5 check of the files. IrysView will retry a maximum of 10 times if the MD5 check fails for the file. When the maximum number is reached a dialog box is displayed as shown below:



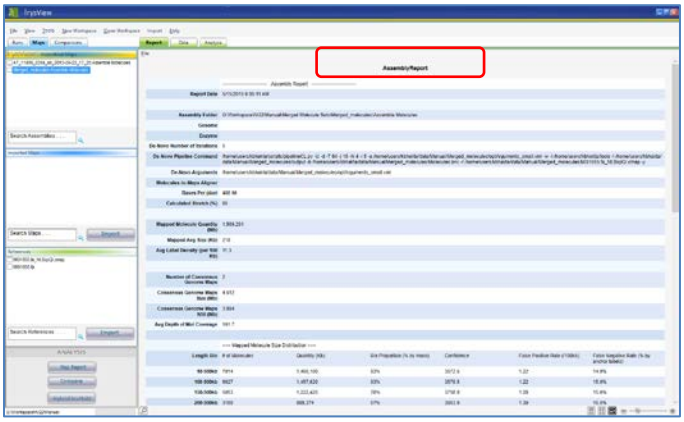
- You can contact your network administrator to check your network and then click "*Yes*" to continue, or you can click "*No*" and the download will fail resulting in a failed run.

6. Once assembly has completed, the highlighted Run background turns white, and an *Assembled Map* appears in the *Maps* tab. If the assembly does not appear, right click on the panel to *Refresh Workspace.*

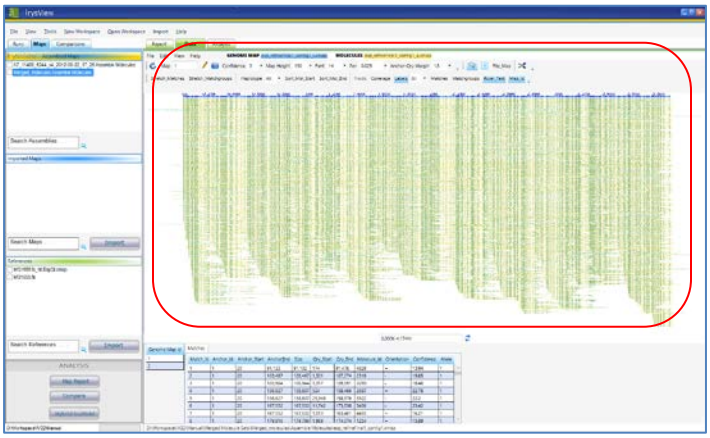




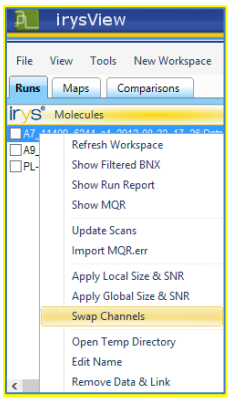
a. Click to highlight (not checkmark) to select the assembly under the *Assembled Maps* pane.
b. An *Assembly Report* will load in the *Report* tab.



7. Molecule “pile ups” aligning to the *De novo* assembly genome maps will load in the *Data* tab. Please refer to the *View Comparison* section below for details on the toolbar display options.

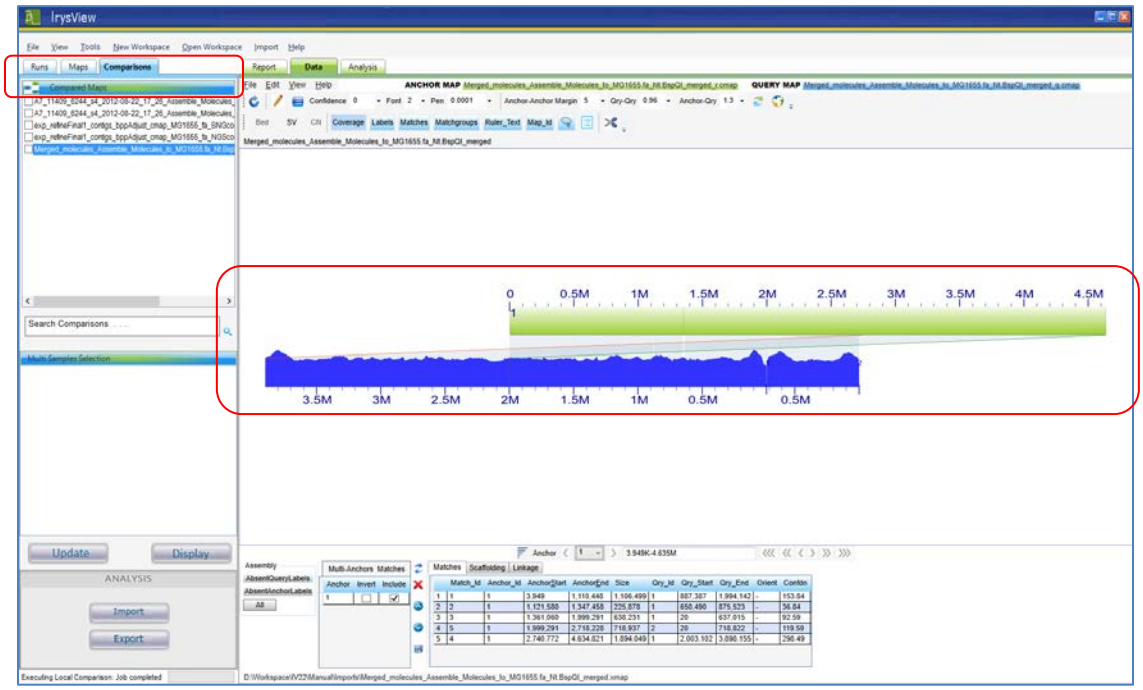


8. Swap color channel.
    - Right-click *Swap Channels* on a Run in the Runs tab to swap the colors in the bnx file and automatically imports the swapped data back into IrysView.



6.20 View Comparison

1. Select the *Maps* tab and choose two maps to compare.
2. Checkmark an Assembled Map or an imported map as well as a reference to launch the alignment:
   a. *Assemblies Maps*: e.g. *Merged_molecules:Assemble Molecules*
   b. *References Map*: e.g. *MG1655.fasta _Nt.BspQI.cmap*
3. Click *Compare* button.
4. From the pop-up *Select Anchor and Query* window, verify:
   a. Anchor: MG1655.fasta _Nt.BspQI.cmap
   b. Query: Merged_molecules:Assemble Molecules
5. Select the *Comparisons/Data* result tab.
6. Click on the item *Compared Maps: Merged_molecules_Assemble_Molecules_to_MG1655.fasta _Nt.BspQI* to view the comparison. The Query map appears in Blue while the Anchor is in Green.



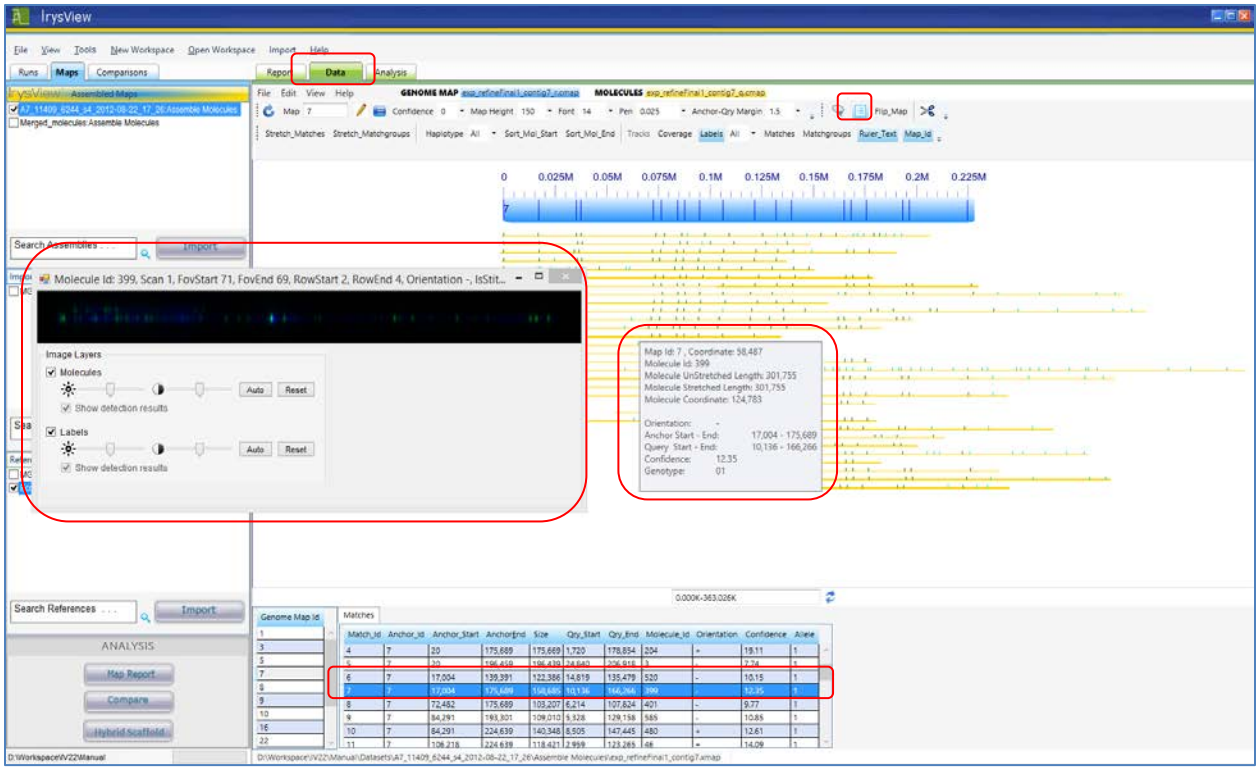
6.21 Molecules-to-Reference

1. From the *Runs* tab, checkmark a *Molecules* Run and a *References* map then press *Molecule Quality Report*.
2. Click the *Analysis* tab to see the Reference vs. Molecules visualization.

3. In general, Reference-to-Molecule XMAPs can also be loaded directly from the *File* / *Open* menu of the viewer in the *Analysis* tab of *Runs*.

6.22 Molecules-To-Maps

1. On the *Maps* tab, click on an Assembled map.
2. Click the *Data* tab to see the Genome Map vs. Molecule visualization.

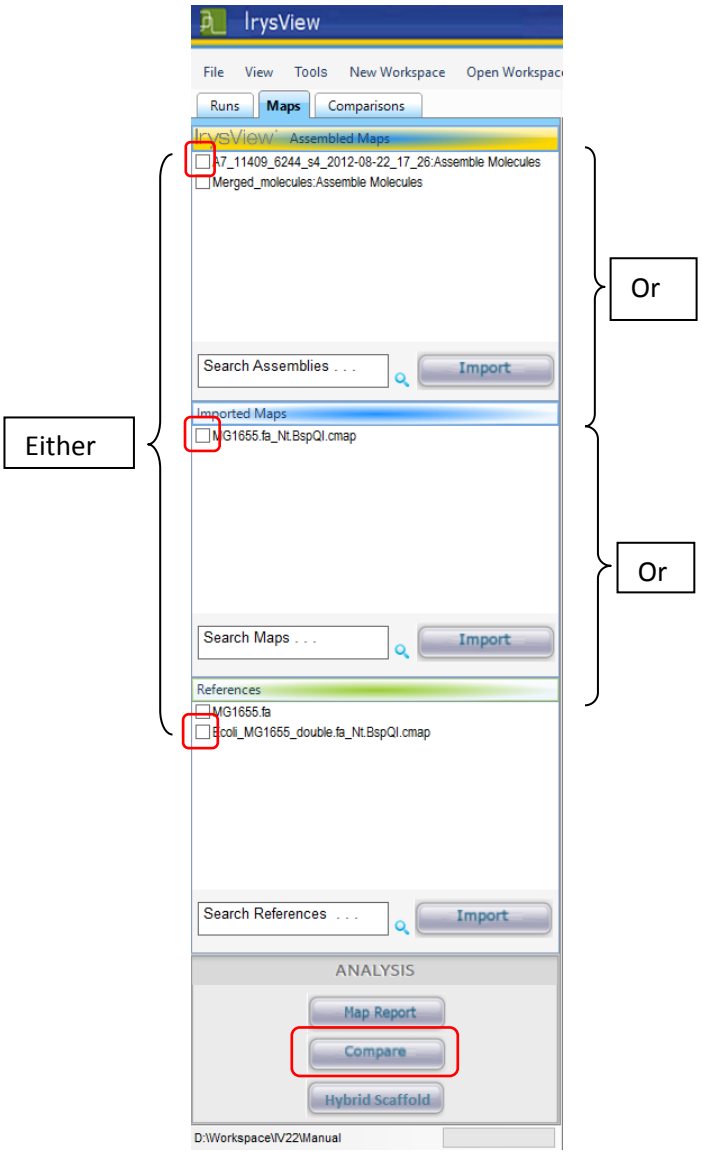


a. Toggling *Molecule Details* button  and hovering over each molecule show additional details (e.g. ID, orientation).

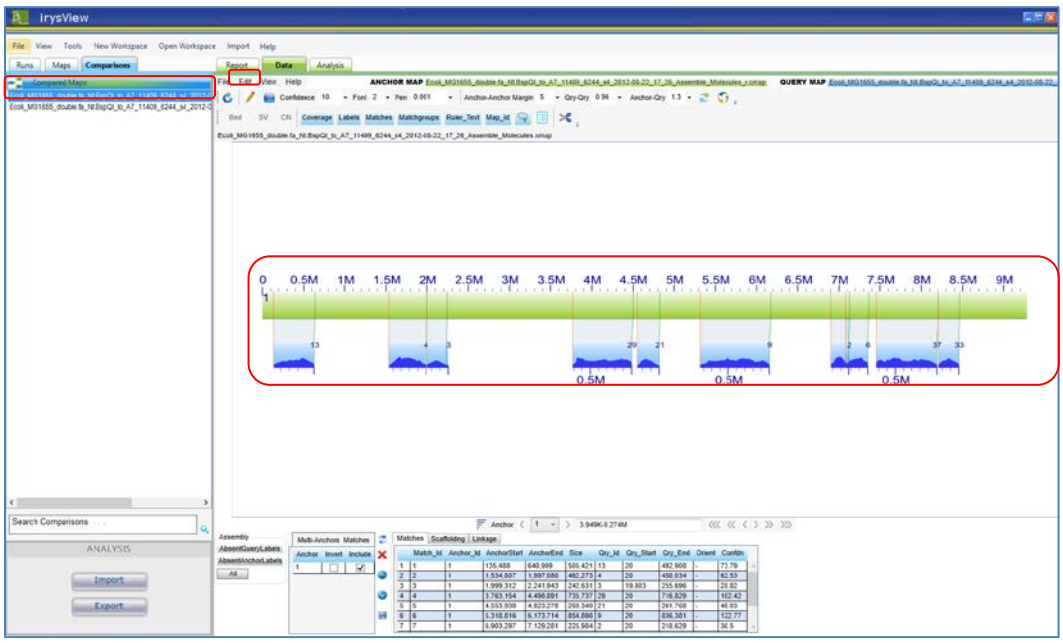
b. To view the raw image, click on a molecule, or on any row in the table of matches.

6.23 Query-to-Anchor

1. On the *Maps* tab, checkmark any pair of maps, such as *Assembled Map* and *Reference,* or *Imported Map* and *Reference,* or *Assembled Map* and *Imported Map*, then press *Compare*.

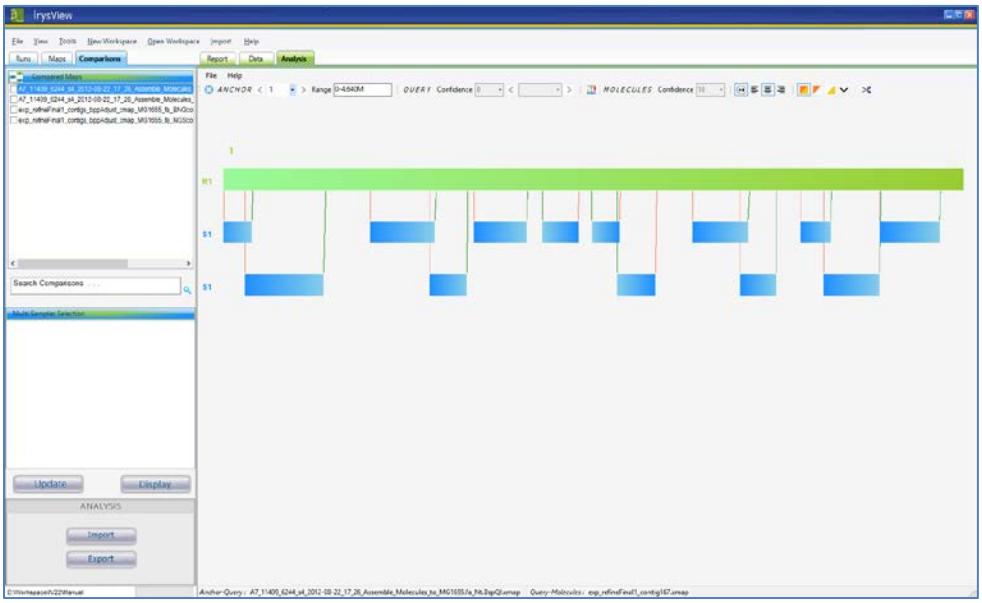


2. Click the *Comparisons* / *Data* tab to see the Anchor vs. Query visualization.
3. In general, Anchor-to-Query XMAPs can also be loaded from the *File* / *Open* menu of the viewer in the *Data* tab of *Comparisons.*

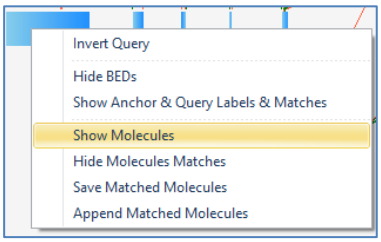


6.24 Molecules-to-Maps-to-Reference

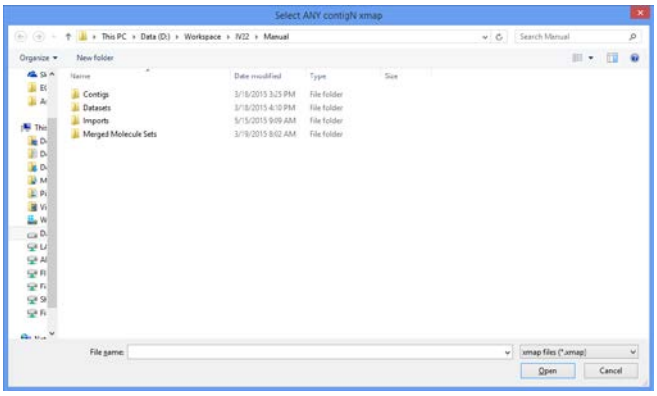
1. Under the *Comparisons* tab, click any comparison node.
2. Click the *Analysis* tab to see the Reference vs. Maps visualization.



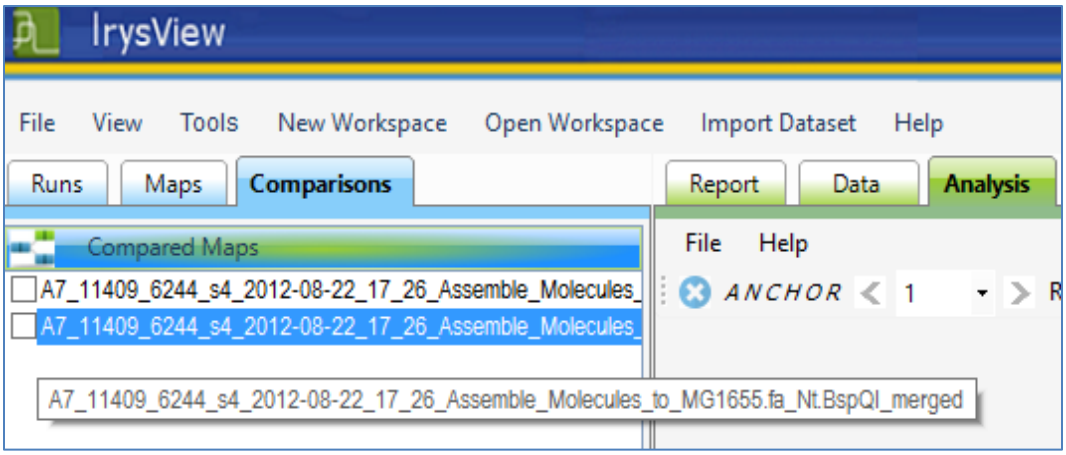
3. Select the anchor from the dropdown list to step through the anchors.
4. To view molecules in the Comparisons->Analysis tab, IrysView needs to know where the molecules data resides:
   i. Right click on the query whose molecules are to be viewed to display a popup menu and select Show Molecules.



   ii. A file browser is displayed for you to navigate to the folder with the molecules to maps alignments:

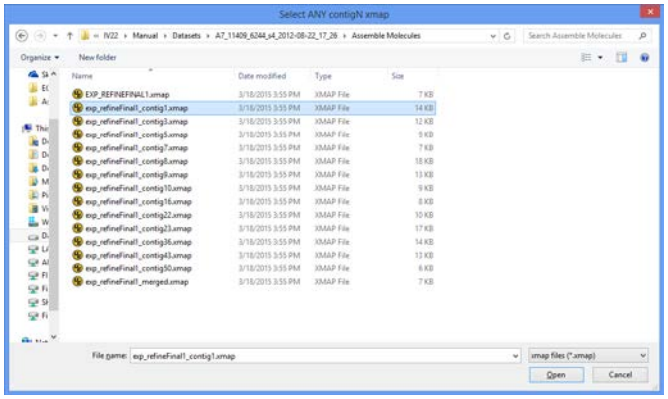


   iii. The location of the molecules can be found by hovering over the selected dataset For example the selected dataset is *A7_11409_6244_s4_2012-08-22_17_26_Assemble_Molecules_to_MG1655.fa_Nt.BspQI_merged.*



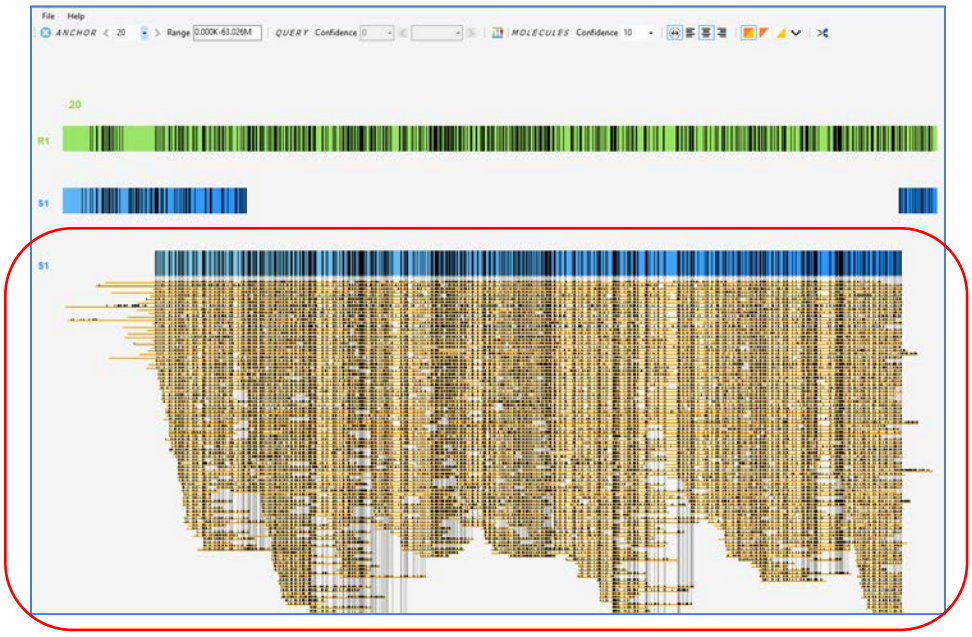
iv. Select the corresponding dataset folder (eg: *A7_11409_6244_s4_2012-08-22_17_26*).

v. Within this dataset folder, select the corresponding Assemble Molecules folder. In this example it is *Assemble_Molecules*.



vi. Select any .xmap file in the folder. IrysView will automatically select the appropriate file for the selected query. Click the *Open* button to display the molecules.

vii. Note: The folder location for the molecules only needs to be loaded once per viewing session per dataset. Subsequent session viewing of molecules queries will only require selecting the *Show Molecules* menu option in (i).

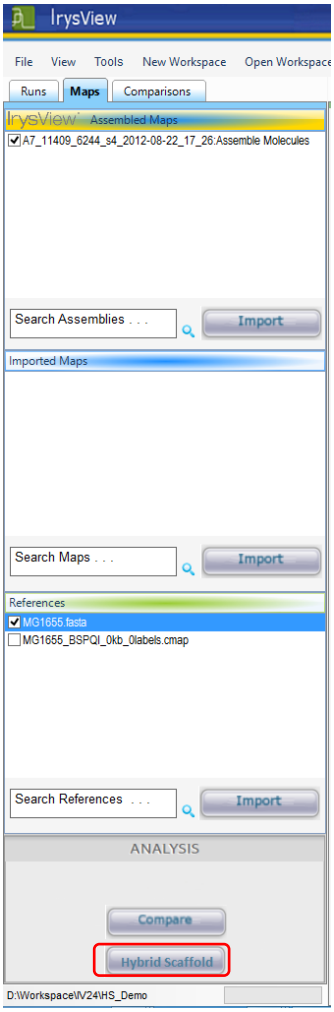


5. Please refer to section 6.26 for additional display and navigation options.

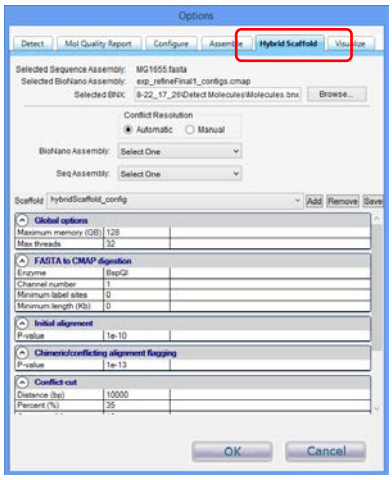
6.25 Hybrid Scaffold

Hybrid scaffold is a process in which one scaffolds sequence contigs/scaffolds and BioNano genome maps to generate a more contiguous assembly. Adjacently aligned sequence genome maps are anchored by a genome map, while adjacently aligned genome map are anchored by a sequence genome map. These alignments can be visualized and can be exported in AGP or FASTA format if desired.

1. From the the *Maps* tab, checkmark an assembly (either Assembled Map or Imported Map) and a reference (fasta only). The latter must be selected from the *References* pane of the *Maps* tab.

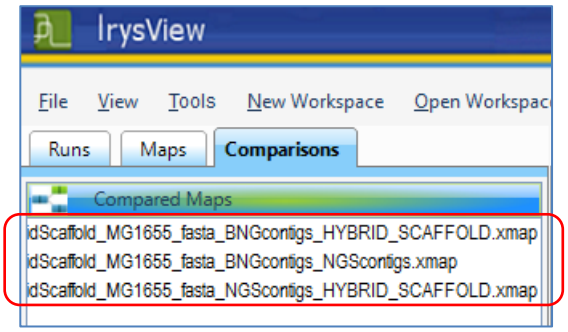


2. Click the “Hybrid Scaffold” button to display the input Hybrid Scaffold dialog.
3. Refer to the *Hybrid Scaffolding Theory of Operations* for the parameter settings.

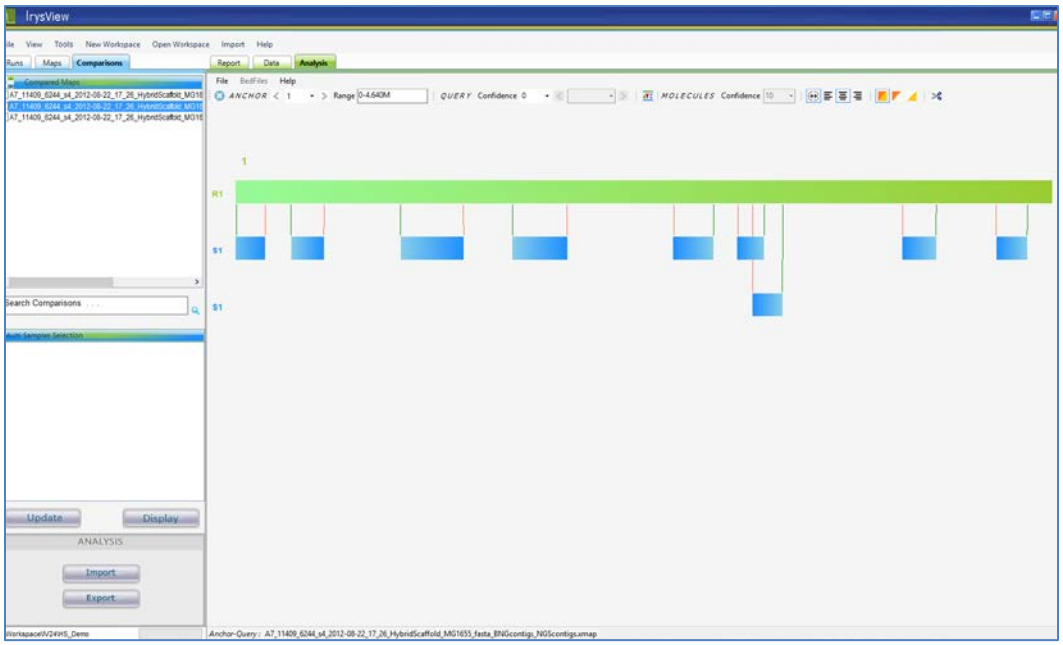


- You must perform an *Automatic* conflict resolution before attempting manual.
- Select an option from the *BioNano Assembly.*
- Select an option from the Seq Assembly.
- Hybrid Scaffold can now take a bnx file as an input.

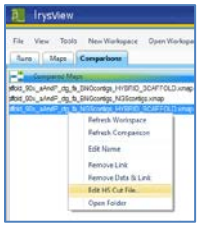
4. After an automatic run has been done, the user can edit the cuts made and rerun Hybrid Scaffold by selecting the *Manual* option.
5. Click an alignment of either the BNG to NGS, BNG to Hybrid Scaffold, or NGS to the Hybrid Scaffold in the *Comparisons* tab to display the result in the *Data* tab.



6. The hybrid scaffold(s) appear as anchors, while either the NGS sequence or BioNano assembled genome maps appear as query maps. Click  to render anchors in Blue.



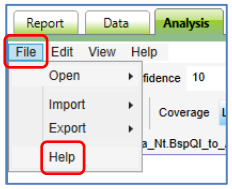
7. Select *Edit HS Cut File…* option from the menu by right clicking on the dataset.



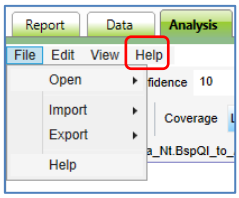
8. The cuts made by Hybrid Scaffold show up as a bed feature when the BNG_NGS dataset is selected.

6.26 Viewer Navigation

1. Click *Help* from any of the viewers.



or



2. Zooming and Panning can be done with keyboard and mouse, or by keyboard alone.

HOW TO NAVIGATE

Navigation	Using Mouse	Using Keyboard
Centering	Mouse Double Click	
Lasso ON	Left Mouse Click + Drag	
Lasso OFF	Left + Right Mouse Clicks	
ZOOM - in place	Point with mouse + Wheel	Point with mouse + Key I / K
PAN - Horizontal	(Lasso OFF) Left Mouse Click + Drag , or Control + Wheel	Key J / L
PAN - Vertical		Key U / N
Home	Right Mouse Click	Key Comma
STRETCH - Horizontal	Alt + Wheel	Key S / F

3. Three-Level viewer navigation.

HOW TO NAVIGATE

Navigation	Using Mouse	Using Keyboard
Lasso	Ctrl + Click + Drag	
Move Track (Vertical)	Shift + Drag track + Drop	
Pan Queries (Vertical)	Mouse wheel (inside Qry region)	
Pan (Horizontal)	Click + Drag (outside Mols region)	J or L key
Zoom (Horizontal)	Ctrl + mouse wheel (outside Mols region)	
- - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Show Molecules	Right click query + 'Show Molecules'	
Pan Molecules (Vertical)	Mouse wheel (inside Mols region)	V or B key
- - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Highlight Matches	Mouse over map label	
Highlight Molecules	Click map label	
Clear highlighted labels		X key
Go Home		Space or Escape key
- - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Options Menu	Right mouse click	

6.27 Molecules-To-Reference View

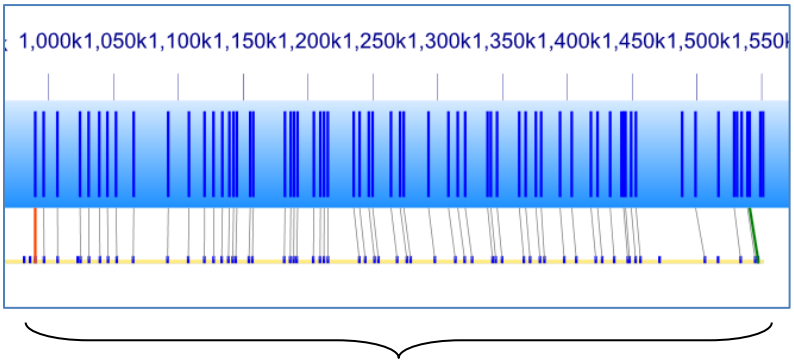
Common tab Controls

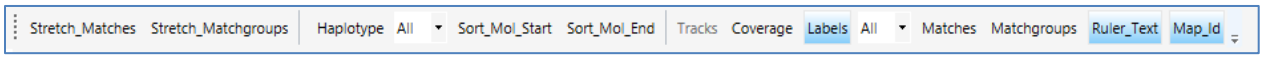
- Refresh the display.
- Redraw immediately after changing either font, pen or margin values (default).
- Redraw as a batch after changing one or more display options.
- Toggle Select Zoom
- Toggle Molecule Details
- Snip tool
- Swap Anchor/Query
- Switch Colors
- Text Font size for Labels
- Pen size

Runs – Analysis tabs

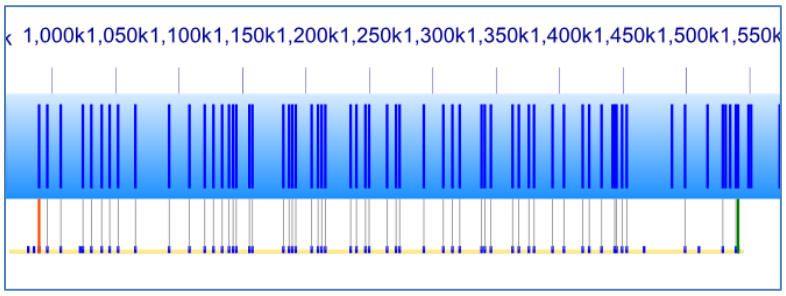
- *Map* - Choose a Reference map based on its ID.
- *Confidence* - Choice of confidence threshold to filter out low confidence alignment.
- *Map Height* – Height of Anchor map.
- *Anchor-Qry Margin* – Vertical spacing between Anchor and Query maps.
- Toggle *Lasso* selection. When *Off*, clicking/dragging the left mouse button will horizontally pan the view.
- Toggle between *Single Molecule Image* (if available) and double-click centering.
- *Snipping* tool to select and cut any visual for export. Available on Windows 7.
- *Flip_Map* - Reverse the head and tail orientation of the maps.

Definition - A Matchgroup is a group of contiguous matching labels between an anchor and a query map according to the alignment algorithm. There can be discordant labels flanked by matching labels within this group.

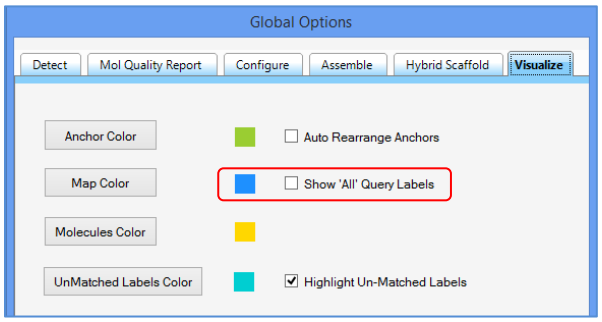




- *Stretch Matches* - Stretch the positions of labels *inside* (Dark blue) a Matchgroup to vertically line up with the matching anchor labels.

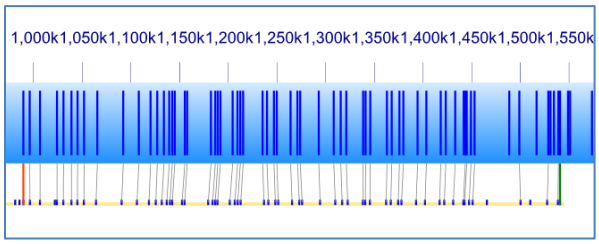


  - *Unmatched* labels *inside* a Matchgroup are colored Turquoise if *Show All Query Labels* is checked.



  - *Unmatched* labels *outside* a Matchgroup are Dark Blue.

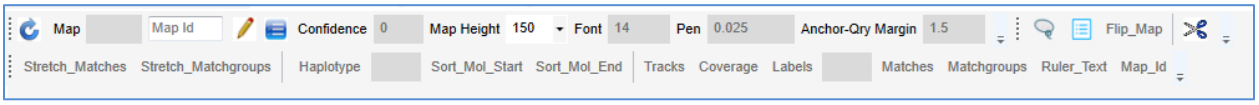
- *Stretch Matchgroups* - Stretch *only* the start (green) and end (red) query label position of a Matchgroup to vertically line up with the matching anchor labels, and not the matches inside the Matchgroup.



- *Haplotype* – currently not supported.
- *Sort_Mol_Start* - Left-justified layout of query map starts.
- *Sort_Mol_End* - Right-justified layout of query map ends.
- *Tracks* - Show Track features from imported .Bed files.
- *Coverage* - Depth of Coverage.
- *Labels* - Show the Labels.
- *Matches* - Show the Match lines between anchor and query map labels.
- *Matchgroups* - Show groups of matches.
- *Ruler Text* - Show Ruler text.
- *Map_Id* - Show Map Id.
- *Flip Map* - Reverse the head and tail orientation of the maps.

6.28 Molecules-To-Maps View

Maps - Data tabs



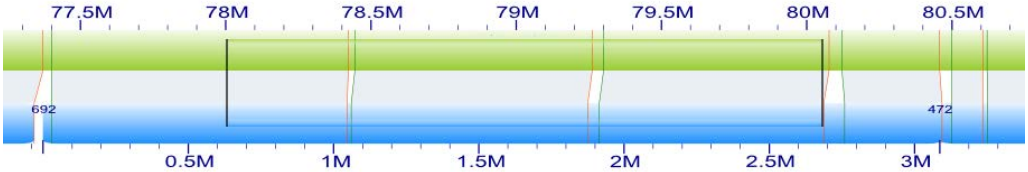
- Identical to the Reference-to-Molecules View above.

6.29 Maps-To-Anchors View

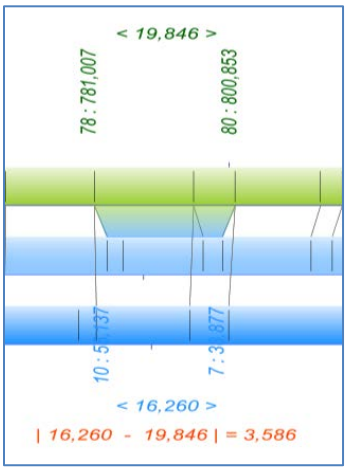
Comparisons - Data tab

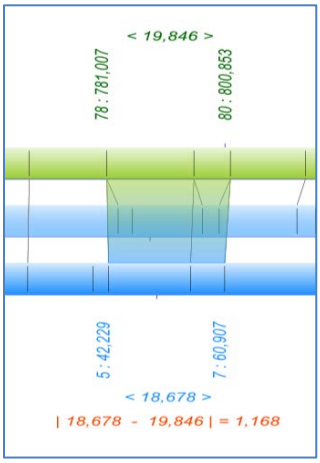
- The whole genome view of query maps as ordered by their anchor id. Left mouse click to select any one anchor map.

- Holding and dragging the left mouse button displays the distance in bases. The lasso can be turned off using the toggle button , to enable drag/drop horizontal panning of the view.

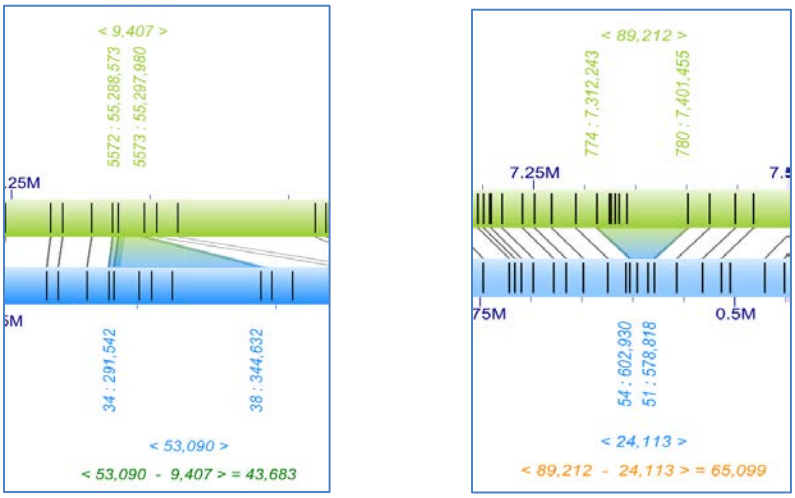


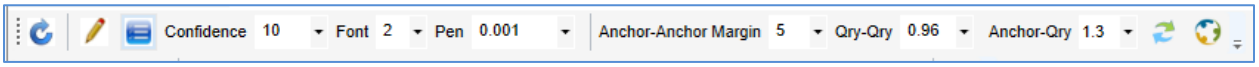
- Lasso selection contains multiple information.
    - Releasing the left mouse button *on a particular query map* displays the enclosed anchor and query matches for that query map.



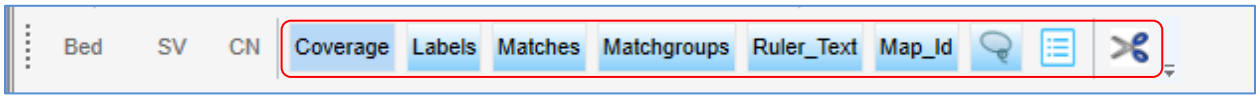


- The top green numbers correspond to the left and right *anchor* label *id:position* in bases, and their separating distance.
- The bottom blue numbers correspond to the left and right *matching query* label *id:position* in bases, and their separating distance.
- The lowest number corresponds to the difference between the anchor separation and the query separation. This typically corresponds to the size of a structural variation. It is colored green for ‘insertion’ and orange for ‘deletion’.

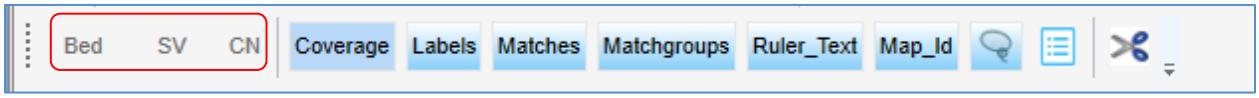




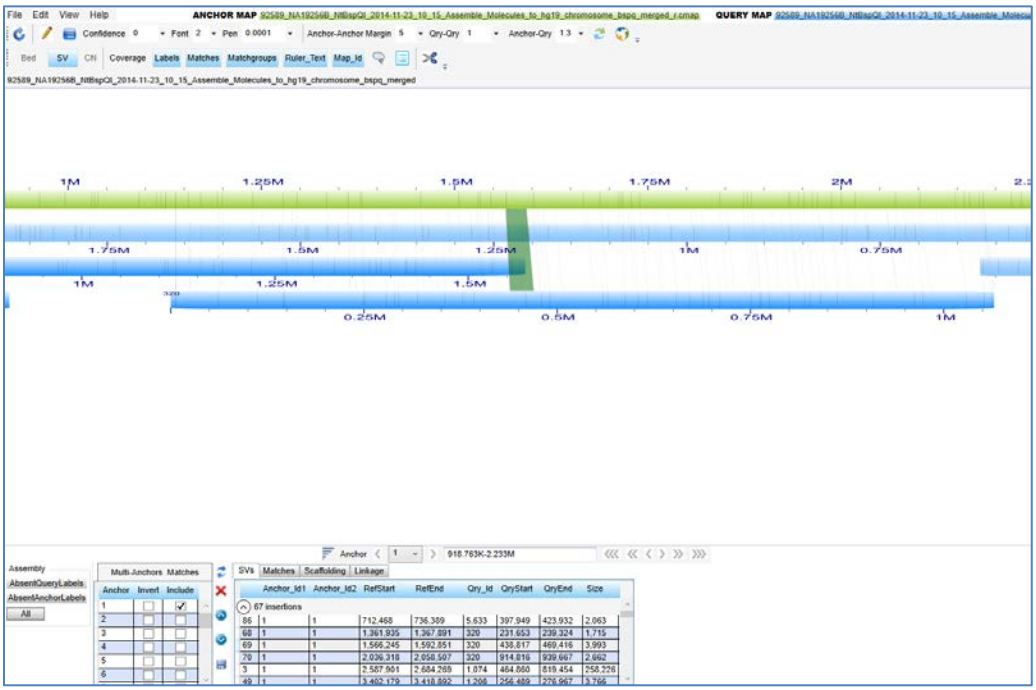
- Refresh the display.
- Redraw immediately after changing either font, pen or margin values (Default).
- Redraw as a batch after changing one or more display options.
- Choice of confidence threshold to filter out low confidence alignment.
- Text Font size
- Pen size
- Horizontal spacing between anchor maps.
- Horizontal spacing between query maps. This is particularly useful to vertically separate out the overlapping query maps by increasing this parameter.
- Vertical spacing between anchor and query maps. Swap the colors of anchor and query maps.

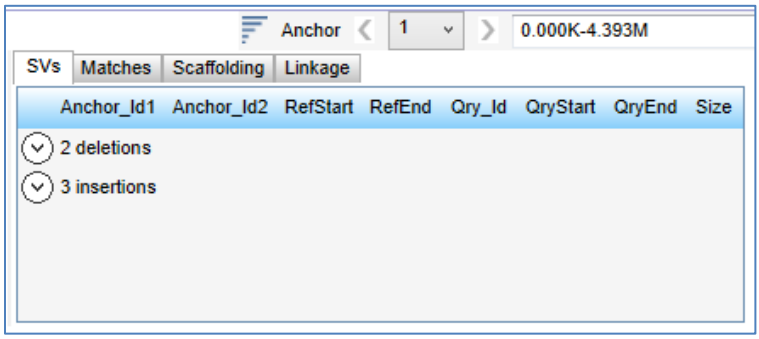


- Show Coverage
- Show Labels
- Show Match lines between anchor and query map labels.
- Show groups of matches
- Show Ruler text
- Show Map Id
- Toggle Lasso selection. When *Off*, clicking/dragging the left mouse button will horizontally pan the view.
- Snipping tool to select and cut any visual for export. Available with Windows 7.

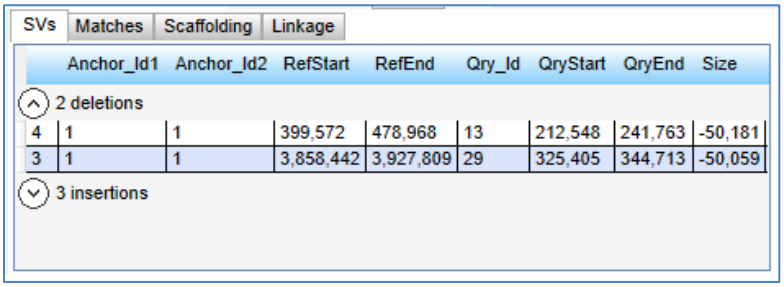


- Show imported BED, SV and CN features. These overlays will only appear in single selected anchor, not in the whole genome, view.





Structural Variation (SV) Table



Structural Variation (SV) ExpandedTable showing deletions

Anchor_Id1 - Reference map ID (XmapID1).

Anchor_Id2 - Reference map ID (XmapID2 -- these two are always the same for insertions, deletions, and inversions).

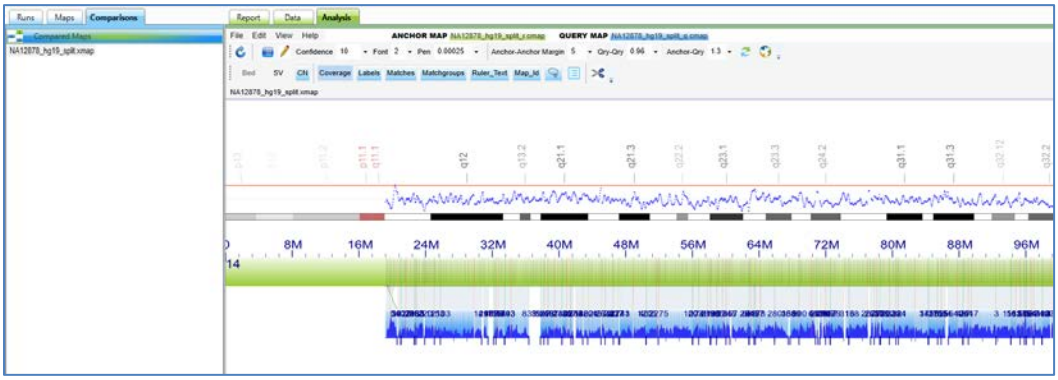
RefStart - Reference start of SV (the end of the first alignment on the query for indels).

RefEnd - Reference end of SV (start of the second alignment on the query for indels).

Qry_Id - Query map ID (same id as in the xmap file).

QryStart - Query start of SV (the end of the first alignment on the query for indels).

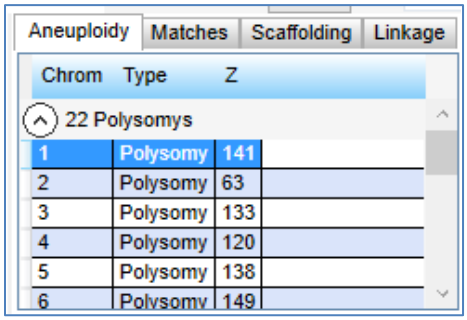
QryEnd - Query end of SV (start of the second alignment on the query for indels).



Copy Number (CN)

X axis - PhysicalPosition

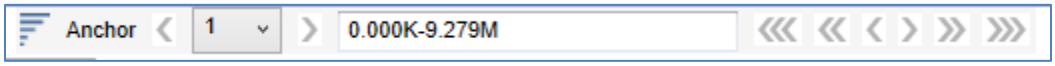
Y axis - CopyNumber. The solid red line represents a baseline copy number of 2.



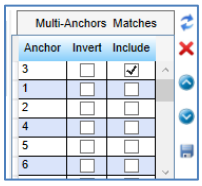
Aneuploidy table for Copy Number (CN)

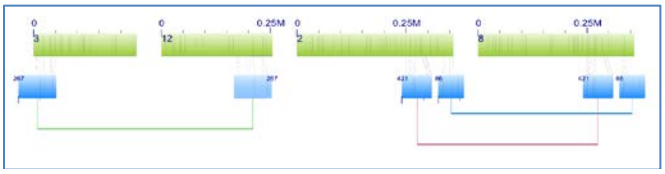
Type - Polysomy (if ZValue > 10) or Monosomy (if ZValue < -10)

Z - Mean Absolute Deviation (MAD) of the affected chromosome's representation from the median value observed in euploids, divided by the MAD of euploid chromosomal representations.

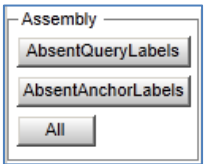


- Display the whole genome view of all anchors.
- Backward and forward selection of anchor map, or choose any Anchor by Id.
- Jump directly to any specified Coordinates Range for the selected Anchor.
- Backward and forward panning by 100%, 50% or 10% of the current coordinate range.
- Zoom factors per mouse wheel click.
- The *Multi-Anchors Matches* toggle button layouts anchor maps horizontally to clearly show multiple queries matching one anchor map.

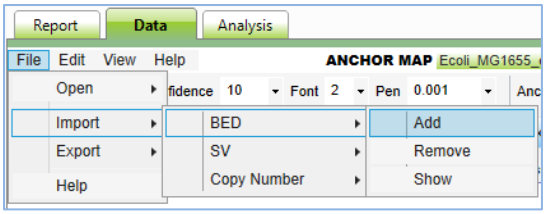




- For further customized layout of anchors, the user can choose and reorder any subset of anchors.
  - The refresh button re-layouts the multi-anchor queries view.
  - The button selects or deselects all anchors in the list.
  - The buttons move a highlighted anchor up and down the list.
  - The button saves the current layout in a new xmap.



- Toggle labels present in Anchor but absent in Query maps.
- Toggle labels present in Query but absent in Anchor maps.
- Toggle all visual overlays, including discordance and tracks.



- Import external BED files that represent overlay visuals on *Anchor* maps. To improve rendering time, include '# Min Size: *Tab N*' in the .bed file header.
- Remove imported BED files from the reference .cmap file, and the current .xmap directory. The source .bed file will not be deleted if it is from a different directory.
- Show the imported text-based BED files.
- Similarly, for SV and Copy Number files.

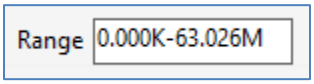
6.30 Molecules-To-Maps-To-Anchors View

Comparisons - Analysis tab

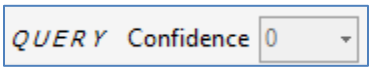
The 3-level view displays any one or more samples to a common anchor aligned against their query maps with their molecules pile-up in one integrated view.

- BED structures can also be imported from *File* → *Import* → *BED* and are overlaid over each anchor (Note: to view molecules please refer to section 6.24.4 item 4).

- Display options

-  Reset the display to its default values.
- 

Filter by a smaller range of anchor coordinates, e.g. *110M-120M.*

- 

Query Confidence.
- 
Click to display the strutural variation data into a popup window
- 
Query selection (enabled when viewing Putative-Translocations).
- 

Molecules confidence.
- 
Layout option as left justified, centered, or right justified.
- 
Stretch Matches
- 
Filter the Molecules
    - 
Show All Molecules
    - 
Show Matched Molecules
    - 
Show UnMatched Molecules

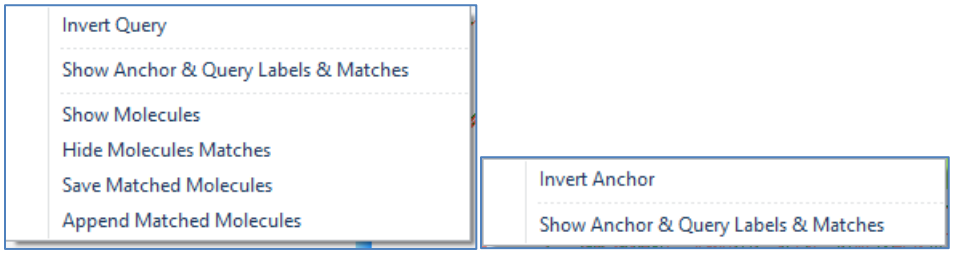
o Trace a match from anchor to query to molecules by hovering over any anchor or map label (Note: to view molecules please refer to section 6.18.4 item 4).

o Highlight only matched molecules for selected labels by successively clicking multiple map labels. Only those molecules sharing *all* of the selected labels are highlighted in red (Note: to view molecules please refer to section 6.18.4 item 4).

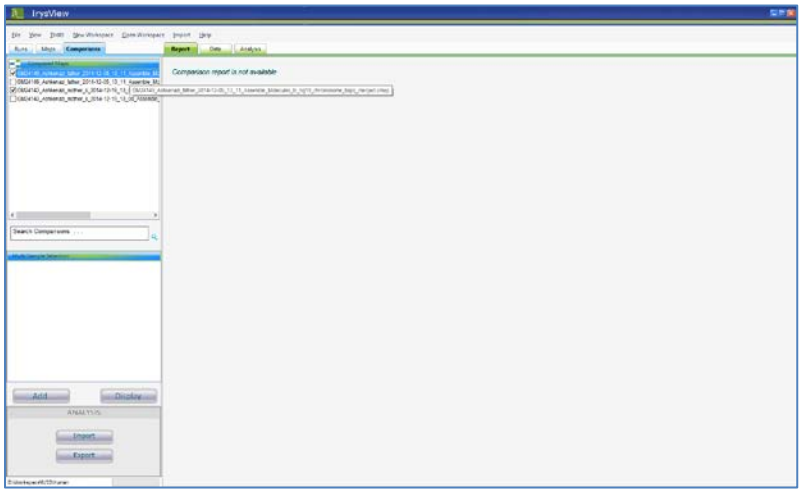
o Show only matched molecules for selected labels by successively clicking multiple map labels. Only those molecules sharing *all* of the selected labels are displayed (Note: to view molecules please refer to section 6.18.4 item 4).

o Show all matched molecules excluding those molecules for selected labels by successively clicking multiple map labels. All molecules except those sharing *all* of the selected labels are displayed (Note: to view molecules please refer to section 6.24.4 item 4).

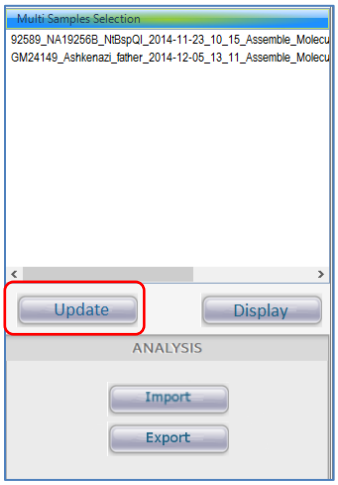
o Show and Hide other overlays by right click on a map or reference to display a menu (Note: to view molecules please refer to section 6.24.4 item 4).



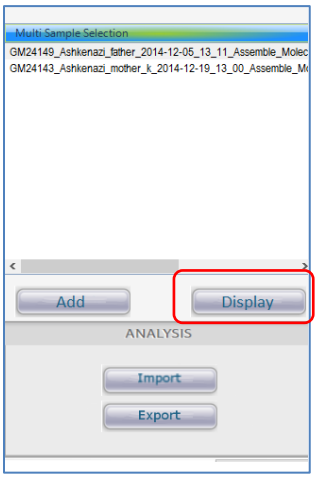
o Select (check) multiple samples to be displayed.



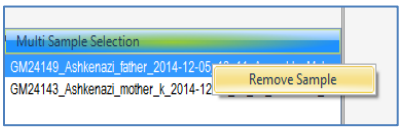
o Click the “Update” Button to populate the selected samples from the Compared Maps pane into the Multi Sample Selection pane. This is the collection of samples that are to be displayed in the 3 Level view.

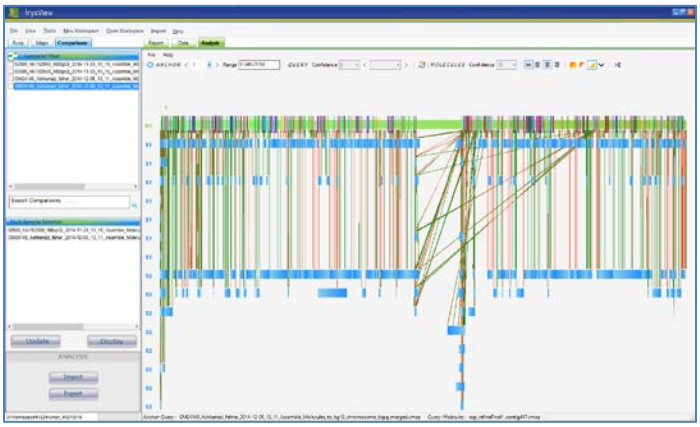


o Click the “Display” button to load the selected samples into the 3 level view.

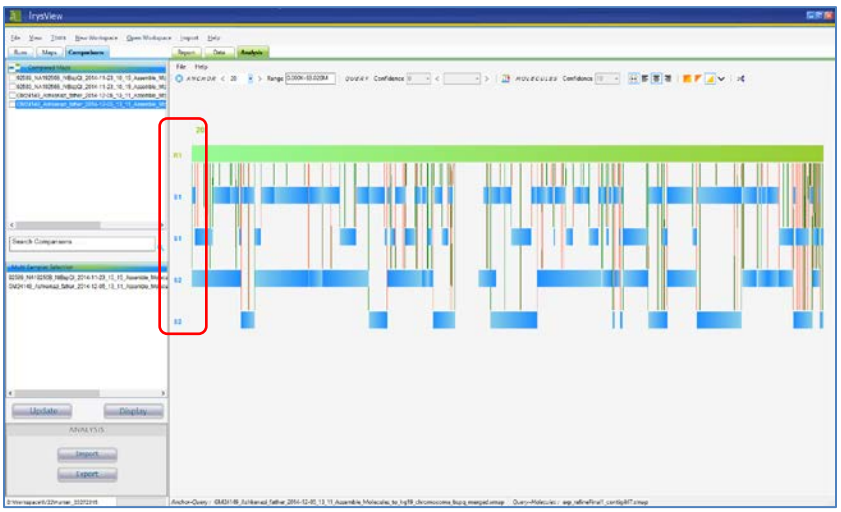


o To remove the dataset from the Multi Sample Pane, right click and select the “Remove Sample” and then click the “Display” button to redraw the updated list of samples.



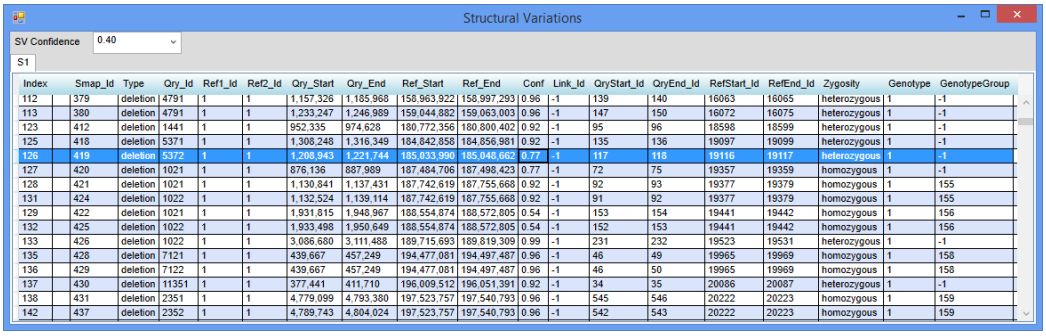


- Select the appropriate anchor. In this case we have chosen anchor 20.



- Hover over the label marked S1 etc. on the left to display a tool tip for the name of the sample.

- Click  icon to display the table of structural variations. Structural Variations are only displayed for single datasets.



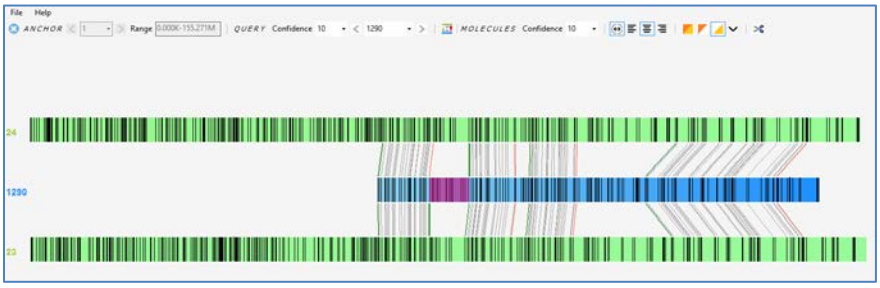
Structural Variations

SV Confidence 0.40

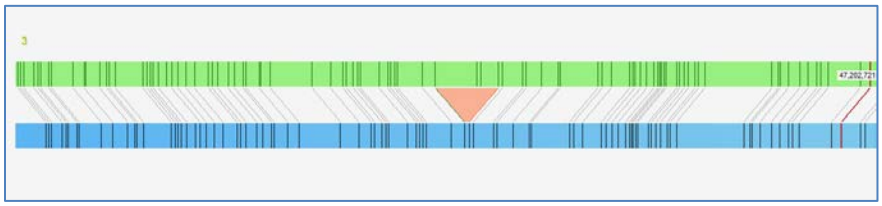
S1

Index	Smap_Id	Type	Qry_Id	Ref1_Id	Ref2_Id	Qry_Start	Qry_End	Ref_Start	Ref_End	Conf	Link_Id	QryStart_Id	QryEnd_Id	RefStart_Id	RefEnd_Id	Zygosity	Genotype	GenotypeGroup
112	379	deletion	4791	1	1	1,157,326	1,185,968	158,963,922	158,997,293	0.96	-1	139	140	16063	16065	heterozygous	1	-1
113	380	deletion	4791	1	1	1,233,247	1,246,989	159,044,882	159,063,003	0.96	-1	147	150	16072	16075	heterozygous	1	-1
123	412	deletion	1441	1	1	952,335	974,628	180,772,356	180,800,402	0.92	-1	95	96	18598	18599	heterozygous	1	-1
125	418	deletion	5371	1	1	1,308,248	1,316,349	184,842,858	184,856,981	0.92	-1	135	136	19097	19099	heterozygous	1	-1
126	419	deletion	5372	1	1	1,208,943	1,221,744	185,033,990	185,048,662	0.77	-1	117	118	19116	19117	heterozygous	1	-1
127	420	deletion	1021	1	1	876,136	887,989	187,484,706	187,498,423	0.77	-1	72	75	19357	19359	homozygous	1	-1
128	421	deletion	1021	1	1	1,130,841	1,137,431	187,742,619	187,755,668	0.92	-1	92	93	19377	19379	homozygous	1	155
131	424	deletion	1022	1	1	1,132,524	1,139,114	187,742,619	187,755,668	0.92	-1	91	92	19377	19379	homozygous	1	155
129	422	deletion	1021	1	1	1,931,815	1,948,967	188,554,874	188,572,805	0.54	-1	153	154	19441	19442	homozygous	1	156
132	425	deletion	1022	1	1	1,933,498	1,950,649	188,554,874	188,572,805	0.54	-1	152	153	19441	19442	homozygous	1	156
133	426	deletion	1022	1	1	3,086,680	3,111,488	189,715,693	189,819,309	0.99	-1	231	232	19523	19531	heterozygous	1	-1
135	428	deletion	7121	1	1	439,667	457,249	194,477,081	194,497,487	0.96	-1	46	49	19965	19969	homozygous	1	158
136	429	deletion	7122	1	1	439,667	457,249	194,477,081	194,497,487	0.96	-1	46	50	19965	19969	homozygous	1	158
137	430	deletion	11351	1	1	377,441	411,710	196,009,512	196,051,391	0.92	-1	34	35	20086	20087	heterozygous	1	-1
138	431	deletion	2351	1	1	4,779,099	4,793,380	197,523,757	197,540,793	0.96	-1	545	546	20222	20223	homozygous	1	159
142	437	deletion	2352	1	1	4,789,743	4,804,024	197,523,757	197,540,793	0.96	-1	542	543	20222	20223	homozygous	1	159

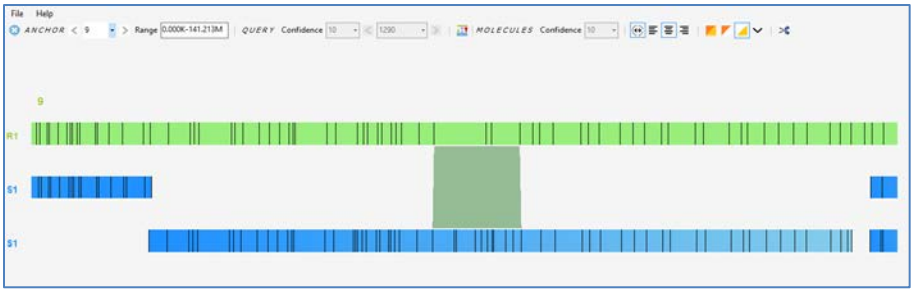
- The SV Confidence dropdown list filters the table by the “Conf” column. The range for the confidence is between 0 and 1.
- Open the appropriate structural variation category and click a row of the data to visualize.
- Example of Putative_Translocation



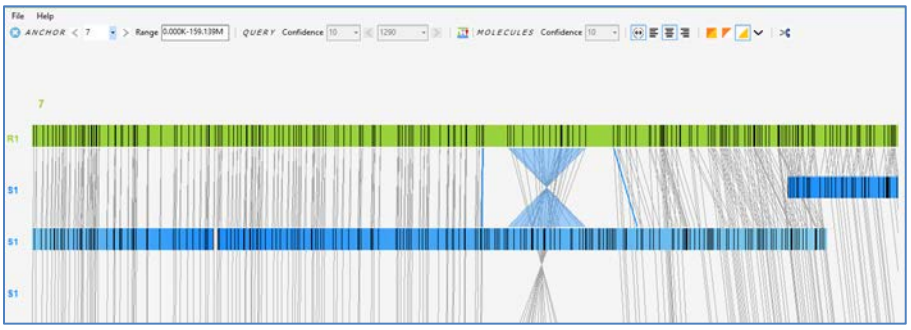
- Example of Deletion



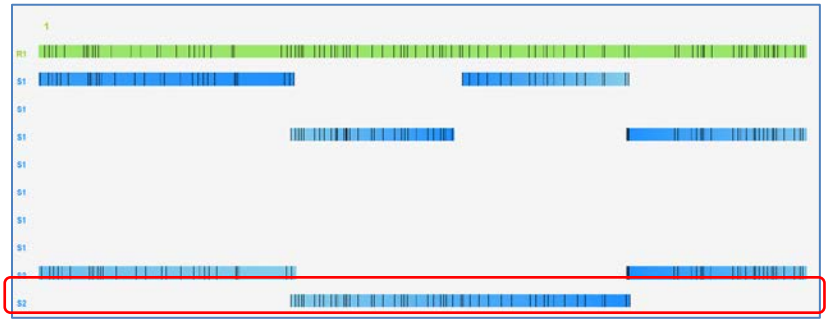
- Example of Insertion



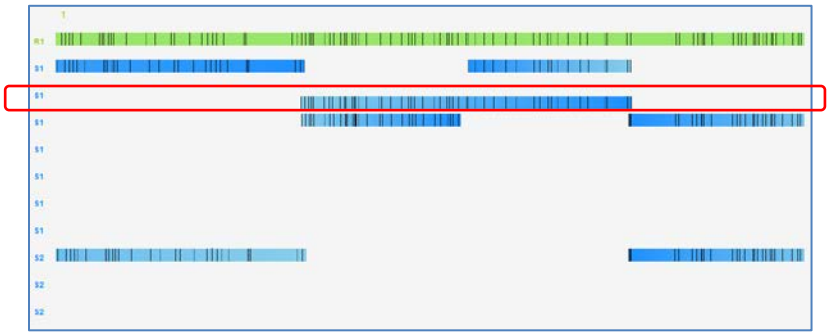
- Example of Inversion



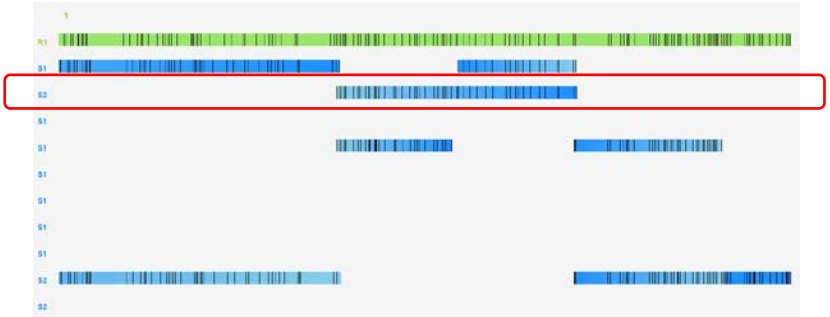
- Individual tracks can be moved by holding down the shift key and dragging the track to its new location.



Before Move of track S2

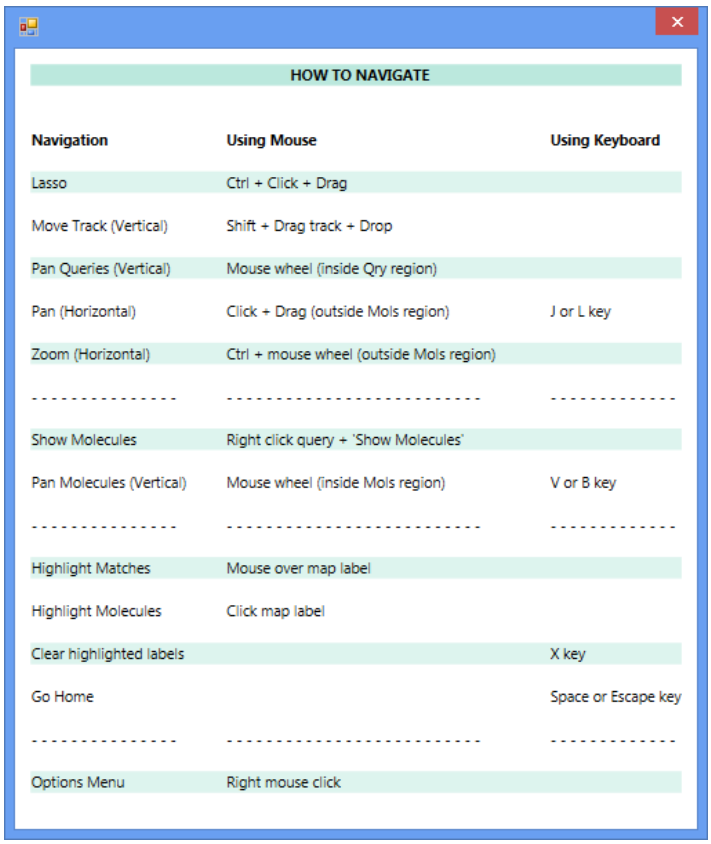


During move of track S2



After Move of track S2

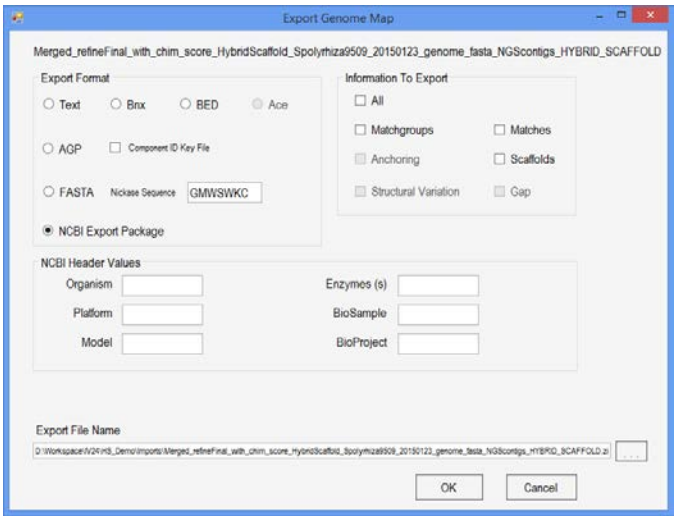
- Click *Help* for a navigation guide using mouse and keyboard.



HOW TO NAVIGATE

Navigation	Using Mouse	Using Keyboard
Lasso	Ctrl + Click + Drag	
Move Track (Vertical)	Shift + Drag track + Drop	
Pan Queries (Vertical)	Mouse wheel (inside Qry region)	
Pan (Horizontal)	Click + Drag (outside Mols region)	J or L key
Zoom (Horizontal)	Ctrl + mouse wheel (outside Mols region)	
- - - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Show Molecules	Right click query + 'Show Molecules'	
Pan Molecules (Vertical)	Mouse wheel (inside Mols region)	V or B key
- - - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Highlight Matches	Mouse over map label	
Highlight Molecules	Click map label	
Clear highlighted labels		X key
Go Home		Space or Escape key
- - - - - - - - - - - - - - -	- -	- - - - - - - - - - - - -
Options Menu	Right mouse click	

6.31 Export Options



6.31.1 Text

Use this option to export the data as a simple text file. One can export *Matchgroups* and/or *Matches* information. The *All* checkbox selects both options.

6.31.2 BNX

Use this option to export the BNX data file. No other option is available.

6.31.3 BED

Use this option to export the BED formatted file. One can export the *Anchoring* information into the BED file.

6.31.4 Export AGP

Use this option to export an AGP format file. One can export the *Scaffolds* information into the AGP file. The AGP export takes an optional *Component ID* key file as input. The key file maps FASTA ID and cmap ID.

6.31.5 Export FASTA

Use this option to export FASTA format file. The export requires a backing fasta file and a *Component ID* key file. The key file is either created by *Knickers* or by *IrysView* if a reference FASTA file was imported and converted to a CMAP.
The nickase enzyme sequence will appear in the exported FASTA at each cmap label position.

6.31.6 Export NCBI

An NCBI Export package option has been added to the export dialog for comparison maps. The zip file will contain the coordinate map file, AGP file, FASTA files, and Hybrid Scaffold CMAP files. The NCBI Header Values provided will be written into the appropriate file headers.

7 Frequently Asked Questions (FAQ)

Can I close IrysView when a local job is running?

IrysView cannot be closed while a local job is running. If IrysView is closed while running a local job, the running process will be terminated.

Can I run more than one instance of IrysView?

You can run multiple instances of IrysView ONLY FOR VIEWING OF DATA.

Use **ONLY ONE** instance of IrysView if you are **PERFORMING ANY DATA ANALYSIS** such as MQR, De Novo Assemble, etc.

What happens to an already running remote job when I close IrysView?

The remote job will continue to run. IrysView remembers that a remote job was running. The following will happen when IrysView starts up:

1. IrysView will copy over the files from the remote server if the remote job completed.
2. IrysView will monitor the progress of the job and download the files from the remote server.

Why is my .xmap dataset not visible in the Comparison tab?

You need to first select the dataset in the *Maps* tab in order for the .xmap to appear in the *Comparison* tab. This selection enables *IrysView* to drill-down from the selected Query (from the Comparison tab) to the related Molecules (in the Maps tab).

Can I run Hybrid Scaffold on a local machine?

No. Hybrid Scaffold was validated to run on a remote server with perl 5.10.X, 5.14.X, or 5.16.X installed.

Why can I not see my Structural Variations from my previous runs when I load .xmap in the Comparison tab?

IrysView 2.3 and later requires that the SMAP entry be in the XMAP file. Runs generated with previous versions of RefAligner placed the SMAP into the *r_cmap file. Use the File->Import->SMAP from within the Comparisons->Analysis to import the SMAP. This will automatically add an entry into the XMAP file for you so the next time you open the same XMAP file the SMAP will be loaded.

Can I run Copy Number Profiles on a local machine?

No. Copy Number Profiles was validated to run on a remote server with R 2.14.1 or greater installed.

What is the recommended system that I should run “Human” on?

It is recommended that “Human” be run on a remote cluster running Sun Grid Engine. Please see the Irys Site Preparation guide for recommend cluster configurations.

Can I import Structural Variation file into an imported .xmap file?

You can import an SV file into IrysView using the File->Import->SV->Add in the Comparisons->Data tab. This will place an entry into the associated *_r.cmap file of the loaded .xmap file. In the Comparisons->Analysis tab use the File->Import->Smap option to import the SV into the loaded .xmap file. Note that in the Comparisons->Data tab the updated file is the associated *_r.cmap whilst the Comparisons->Analysis tab the updated file is the *.xmap file.

Will applying Unfiltered Molecules disable the Length, Label count and Filter Intensity filters?

No. Unfiltered Molecules will display the data from the RawMolecules.bnx file instead of Molecules.bnx.

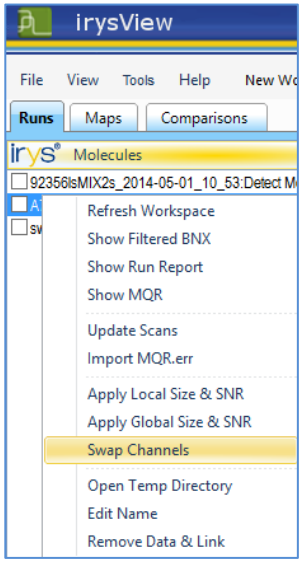
Can more than one person import the same data at the same time from the server?

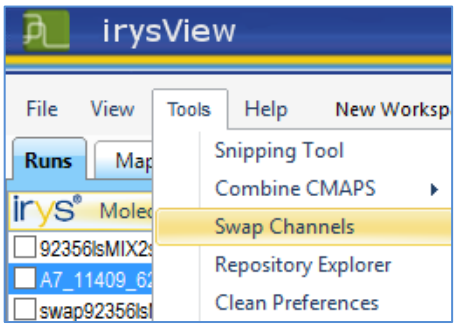
No. Please set the permissions to allow other users to create files in the folder and subfolders of the directory that contains the data. To speed up the transfer time and the number of files being transferred, IrysView compresses the files on the server in the folder(s) where the data resides; therefore it is recommended that not more than one person transfer the data at any given time.

How do I use dual label color BNX

Using dual label color BNX data requires the user to swap the color channels as IrysView currently displays alignments using channel 1 only.

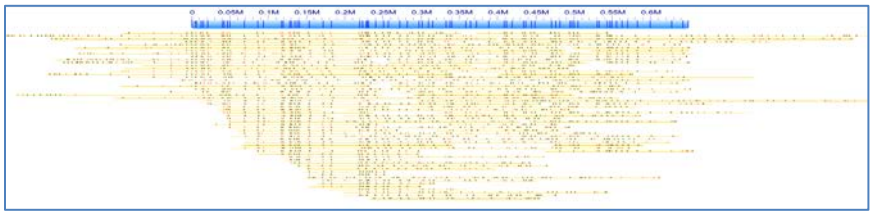
You can use the right-click menu option on the data set in the *Runs* tab to swap the color channels and automatically load the resulting BNX file back into *IrysView*. Optionally you can use the option under the *Tools* menu to swap the color channels and then you can manually load the resulting BNX file back into IrysView.

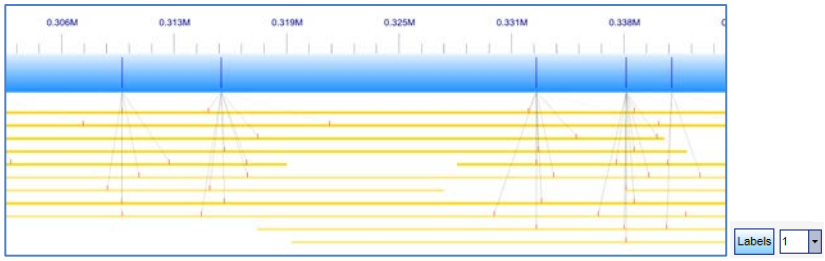


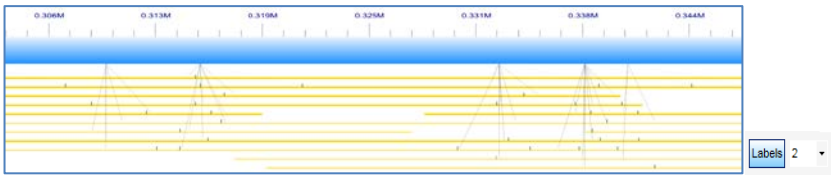


The following images show the results before and after swapping the channel colors:

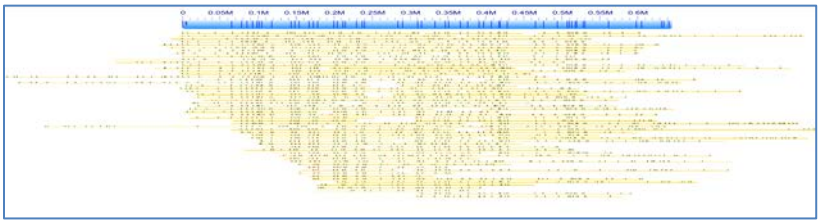
Before swap (use color 1)

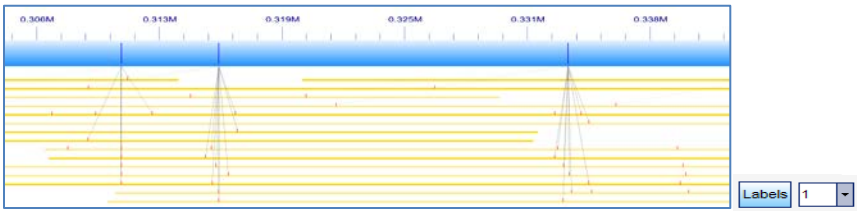






After Swap (use color 1):





8 Known Issues

Running a job on the server from with IrysView and then changing workspace can cause data to be downloaded into the newly opened workspace

Starting a job with IrysView and then changing workspace can cause the download of the finished job into the workspace that is currently open in IrysView. To prevent this from happening, it is recommended that you close IrysView and then reopen IrysView and then navigate to the workspace. The closing of IrysView closes all threads associated with IrysView.

Aneuploidy table is empty when I selected to view Copy Number (CN) from the menu.

Do the following if the Aneuploidy grid does not populate in the Comparisons->Data tab after selecting the CN button:

1. Select any single Chromosome.
2. Toggle the CN button off. This should populate the Aneuploidy table with data.
3. Toggle the CN button again to display the CN track.

Exporting

A comparison (.xmap) must be loaded into the Comparisons-Data tab before exporting can proceed. Note that the data displayed in the Data tab is what will be exported regardless of the current comparison map highlighted.

Zoom in 3 Level View

In the 3 Level View, you zoom into a region by holding down the control-key and holding down the left mouse button and then drag the mouse to select the zoom region. You then should release the mouse button and then the Control-Key to zoom. If you release the Control-Key before releasing the mouse button keeps the zoom region in the view.

Hovering on labels in Anchor does not highlight molecules in Multi Sample Level View

In Multi Sample Level View, if you have molecules displayed, and you hover the mouse over the labels in the Reference track, the molecules do not highlight. The workaround is to hover the mouse on the labels of the query.

Multi Sample Level View with multiple references displayed does not scroll appropriately

Currently scrolling queries in Multi Sample Level View works correctly when there is a single common reference. Scrolling of queries does not function correctly when you have more than one reference:

Move the bottom reference to the top.

Scrolling of queries leaves ghost lines for the samples associated with the reference that has been moved.