Analysis Quick Start: Annotated Rare Variant Calling

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bionano

Table of Contents

Revision History

REVISION	NOTES
A	Initial release.
B	Update screenshots and candidate gene analysis description.

Rare Variation Detection - Introduction

Bionano Access supports different workflows for the detection of structural variants (SV) in the human genome (**Figure 1**). As per Bionano technology, a rare variant is defined as one that is present in low abundance in the sample and is not present in the reference molecule. To identify such variants effectively, a dedicated bioinformatics pipeline is used that locally aligns molecules to a reference, assembles them with putative differences into consensus maps, and identifies the structural variations with less computational burden. For more information on each of these workflows, please see *Bionano Solve Theory of Operation: Structural Variant Calling* (CG-30110), *Bionano Solve Theory of Operation: Variant Annotation Pipeline* (CG-30190), and *Bionano Solve Theory of Operation: EnFocus FSHD Analysis* (CG-30321). To obtain information on data coverage targets, which can vary depending on the analysis, see Data Collection Guidelines (CG-30173). To obtain information on how Bionano determines quality control of raw data, please refer to *Bionano Access Dashboard and Chip Metrics Guidelines* (CG-30304) and *Bionano Access Molecule Quality Report Guidelines* (CG-30223).

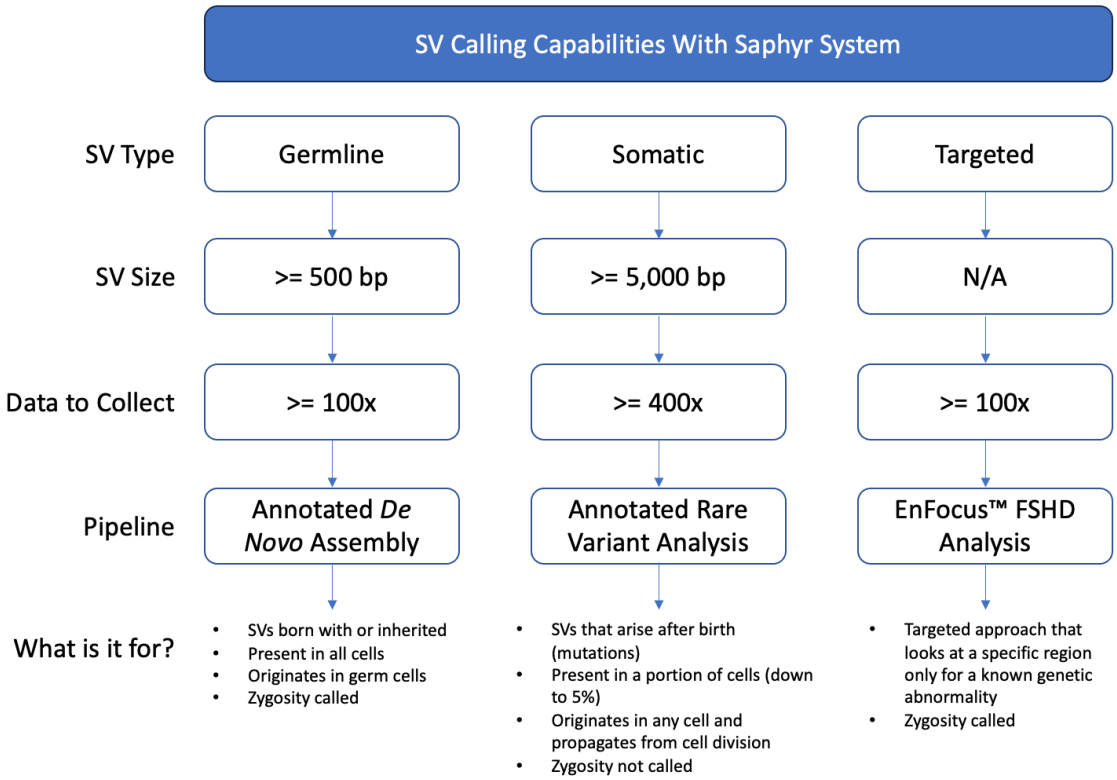


Figure 1. Schematic representation of the Bionano structural variant detection workflows. Variant Analysis Pipeline - includes first constructing a *de novo* map and then comparing the resulting map with a reference which is highly sensitive in detecting SVs.

Workflow Overview

This workflow, shown in **Figure 2**, discusses how to filter and narrow a search for high confidence SVs that were identified using the Rare Variant Analysis pipeline (RVA). Once DNA is isolated and labeled, Bionano chips are loaded and run on the Saphyr instrument. Molecules and labels imaged on the Saphyr instrument are digitized and saved as in a BNX format (raw data). This serves as input for downstream analyses. RVA detects SVs by the "split-read" analysis, where initial molecule alignment and molecule extension refinement allow identification and detection of somatic variants and identifies variants that are 5,000 bp or longer in size. For more information on the Variant Annotation Pipeline and relevant file formats, please refer to *Bionano Solve Theory of Operation: Variant Annotation Pipeline* (CG-30190) and *OGM File Format Specification Sheet* (CG-00045)..

To access the results from an RVA analysis, the user begins by logging into the web server location which contains user data. Once logged in, the user navigates to the project which contains the data for the RVA experiment of interest. RVA can be used for a single sample, duo (matched tumor-normal, for example) or trio (parents-child, for example) analysis. For any individual experiment, clicking on the sample data will take the user to a Circos plot which has a global overview of the mapped genome.

The user may then perform filtering of the rare variants and identify genes of interest that might have been affected by a rare variant event. Additional filtering and masking of low confidence calls may be performed using a BED file. Once the dataset has been filtered appropriately the user can visually explore the structural variants, create a report, and then export the data for further bioinformatic analysis. For more details on thresholds and experimental designs refer to *Bionano Solve Theory of Operations: Variant Annotation Pipeline* (CG-30190).

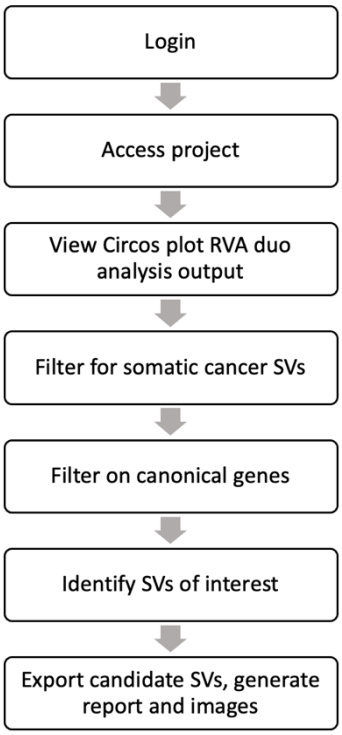


Figure 2. Bioinformatic workflow for post RVA data analysis in Bionano Access

Accessing Bionano Data and Filtering with Recommended Settings

Goal of the Tutorial

This quick start guide directs users on how to view annotated rare variants, which are detected from Bionano's genome mapping data. It can be used to evaluate a user's own data or to familiarize users with the software using a pre-loaded demo dataset. The demo dataset is provided by Bionano to help users get familiarized with the Bionano Access software interface that enables analysis, visualization, and data export. To walk through the analysis, follow the numerical prompts in this guide. Text outside of the numerical steps is provided to facilitate learning. This guide assumes all necessary analyses pipelines have been run on user samples, and so this tutorial will guide users through selecting the proper data, and performing filtering based on current Bionano guidance before exporting the data for presentations or further analysis outside of Bionano Access. The filter sets used here represent current recommended settings but may be adjusted to fit the needs of a project. In the hands-on section of the tutorial, users will be walked through the analysis of tumor (HCC2281) and non-tumor (HCC2281BL) cell lines established from the same patient with breast cancer. The goal is to identify rare somatic variants in the HCC2281 breast carcinoma cell line when compared to its matched normal cell lines.

To obtain information on data coverage targets, which can vary depending on the analysis, see *Data Collection Guidelines* (CG-30173). To obtain information on how Bionano determines quality control of raw data, please refer to *Bionano Access Dashboard and Chip Metrics Guidelines* (CG-30304) and *Bionano Access Molecule Quality Report Guidelines* (CG-30223). To obtain information on ways by which bioinformatic analysis of annotated structural variants are recommended, see *Bionano Solve Theory of Operation: Variant Annotation Pipeline* (CG-30190).

Entering Bionano Access

1. Using a web browser (Google Chrome is recommended), navigate to the web address of the user's Bionano Access data (e.g., https://us.bionanoaccess.com). Users with an installed Saphyr™ System will utilize an Access Installation hosted on their local Bionano Access Server. Enter the appropriate **User Name** and **Password**, then press **Login** (**Figure 3**). To access the demo dataset or user's sample data generated by the Bionano Commercial Services Lab, a user will need login credentials as provided by a Bionano representative.

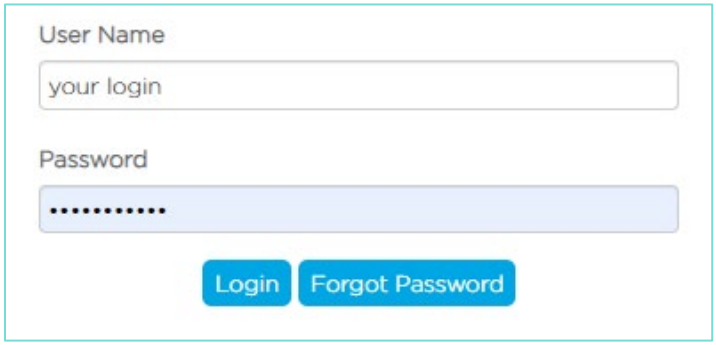


Figure 3. Login to Bionano Access

2. To open user sample data or demo project data, click on **Projects** on the Home page (**Figure 4**). A Project is the central repository for all objects such as experiments and any bioinformatic analysis that is performed.

Figure 4. Bionano Access login screen.

3. In the **Projects** view, click on the hyperlinked project name corresponding to the submitted project, or **BionanoAccess_somatic** to follow along with this demo dataset (**Figure 5**). This will open the list of data types (Objects) within that **Project** (**Figure 6**).

Figure 5. Bionano Access Home page - contains data repository in **Projects**.

The **Project Data** page (**Figure 6**) will list the various data types for a given sample or analysis. For rare variants with the variant annotation pipeline, a project may contain some or all the following file **Types**.

a) **Molecules:** the raw, unassembled, molecules data (also known as a .bnx filetype). A Molecules file is generated by the image analysis software. It contains single-molecule optical mapping data generated on a Bionano Saphyr instrument and is the first data type produced. These may or may not be present and are not essential for visualization.

b) ***De novo* Assembly:** the assembled consensus genome maps (also known as a .cmap filetype). The *de novo* Assembly file contains the assembled consensus genome maps which are then compared against a reference assembly (e.g., hg19 or hg38) to produce un-annotated SV calls. These may or may not be present.

c) **Rare Variant Analysis:** The results from the rare variant annotation pipeline are output to a text file with/without annotation for each SV detected for each sample.

d) **Annotated Rare Variant Analysis:** This file is unique to a duo analysis. The important thing about this filetype is that it is 1) annotated 2) compared to the internal control database. For duo analysis, the calls are then compared to each other and contain the rare variants identified in the cancer sample compared to its matched control samples.

On highlighting each file type, different operations become visible under the **Options** pane on the right.

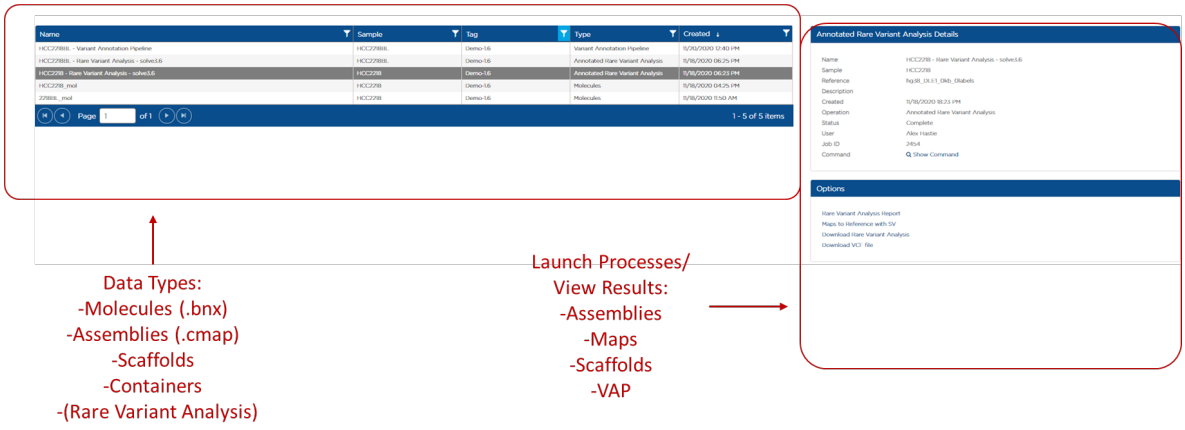


Figure 6. **Project Data** page. This contains a list of objects and a list of operations (options) that can be performed with the selected object.

Variant Annotation Pipeline

In this tutorial, users will find the Project contains at least three different files, as seen in **Figure 7**:

1. HCC2218 - Rare Variant Analysis (Solve3.6).
2. HCC2218BL - Rare Variant Analysis (Solve3.6).
3. HCC2218BL - Variant Annotation Pipeline

The rare variant calls from each sample (HCC2218 and HCC2218BL) are saved as separate files. The third file includes the dual annotation file which includes all rare variants identified in the samples compared to an internal database of more than 200 normal, apparently healthy individuals. In this tutorial, the dual analysis file is used to identify rare somatic variants.

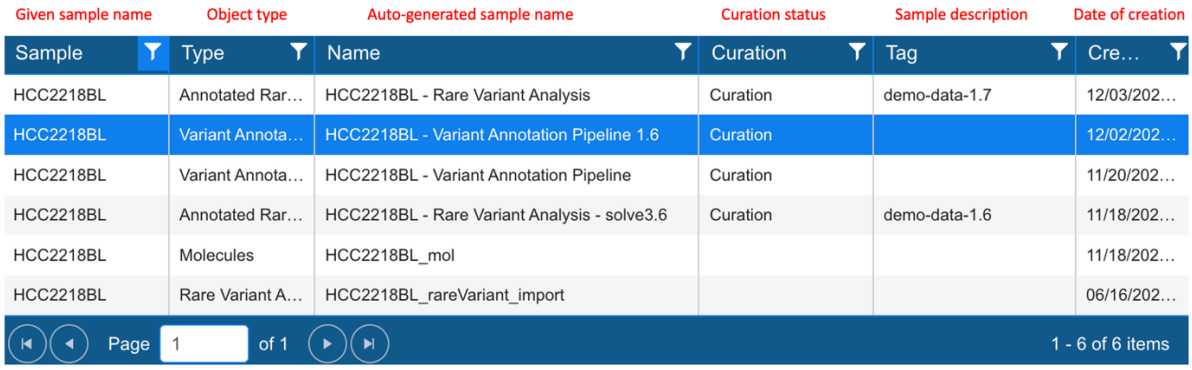


Figure 7. Selecting the Variant Annotation Pipeline data type (file type). Each column lists the auto-generated sample name for a given sample

To view a sample, click on the sample name. Depending upon the experimental design, the content may be different. To identify the files used to generate the dual analysis, **click,** and highlight **HCC2218BL-Variant Annotation Pipeline** as shown in **Figure 8**. Check the sample name under "Content." For more information see *Bionano Solve Theory of Operation: Variant Annotation Pipeline* (CG-30190).

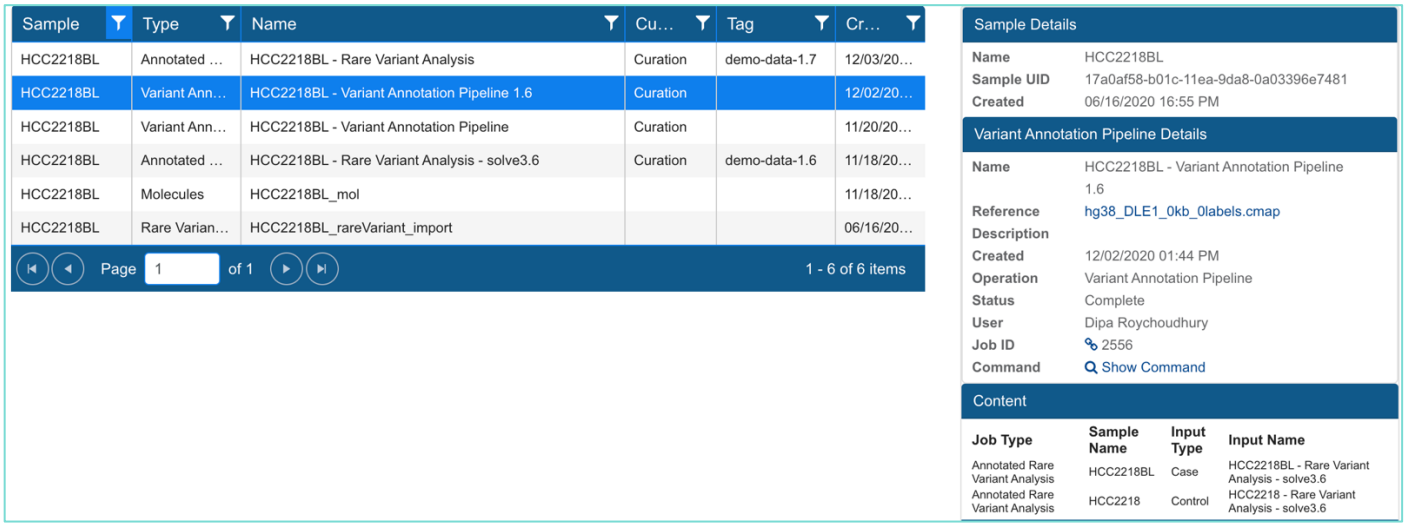


Figure 8. Dual Analysis File-The content lists the samples compared in this analysis. The first operation under **Options** is **View variant annotation results**. This launches the Circos plot.

4. Click on **View Results** to launch the Circos plot.

Circos Plot View

Users can view one sample or file type at a time in the Circos plot view. In a duo analysis, all SVs detected in normal versus tumor samples that is compared against the control database can be viewed on a Circos plot. Using the filer settings as discussed below, users can display SVs unique to tumor or normal.

In Bionano Access, the Circos plot (**Figure 9**) is displayed by default for human assemblies and variant annotations. The Circos plot is displayed as the circular plot on the left, with **Filter Criteria**, **SV Filter Confidence** scores, **BED Files**, and **Samples** on the right. To zoom on the Circos plot, rotate the mouse-wheel forward (scroll down). The user can also click and drag the plot around using the mouse. **NOTE**: For more mouse and keypad short cuts used to maneuver the Circos plot, click on the **?** icon, which is the last icon on the top panel.

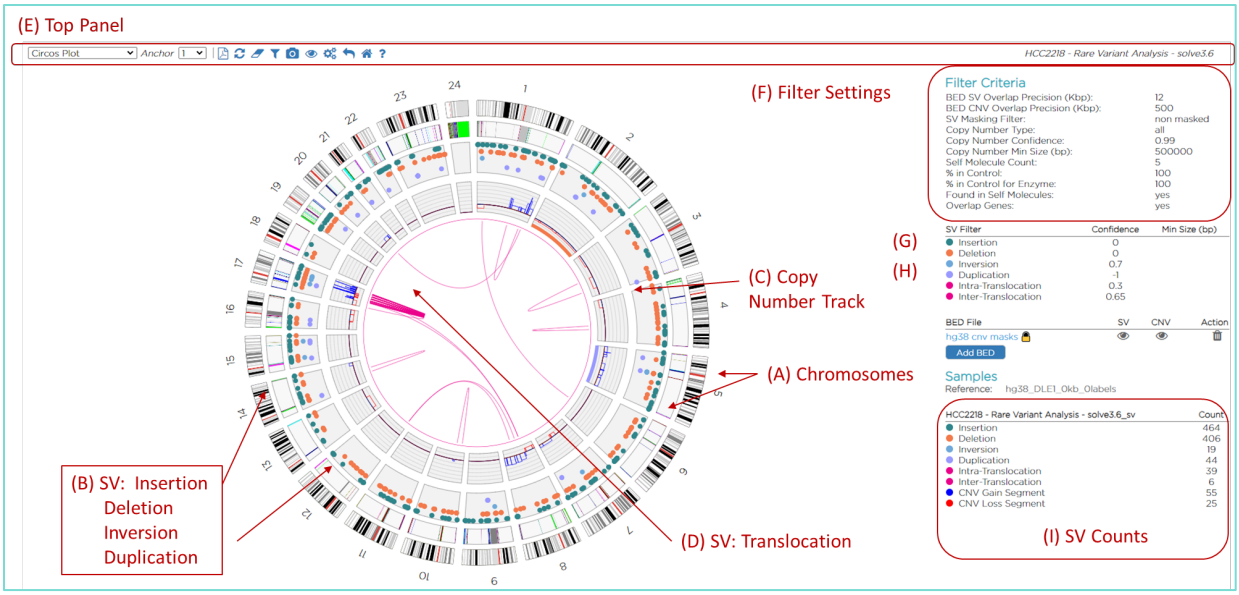


Figure 9. Circos plot view includes a top panel with filtering options (E), options to add BED files (G), Sample Name (H), summary SV counts (I), and the central Circos plot with the different tracks (A-D).

In a Circos plot, each chromosome is positioned end-to-end in a large circle. Each track represents a different type of SV or annotation result. Clicking on the cytoband, SV, or copy number track will open the genome browser, and show the user a detailed view of a Bionano consensus genome map for that locus. The user can navigate back to this Circos plot from other views by clicking on the circle icon on the Top Panel (E) toolbar. The rings of the Circos plot are described from the outermost track to the inner most track.

- The outermost numerical track (A) corresponds to the chromosome number, with cytoband information shown in the black-and-white banding pattern. Currently, chromosome 23 corresponds to X and chromosome 24 to Y.
- The next track (B) includes the detected SVs as represented by color-coded dots. A user can hover over the dot to view information about a given SV call.
- The next track (C) shows copy number calls. For the copy number track, the baseline state of autosomes in a diploid genome is a copy number of 2. If a region shows a copy number gain, the line will move outward from 2 (colored blue), reflecting an increase in the local copy number state. If the region has a loss, it will move inward (colored red).

- The innermost track (D) contains information about translocations, both inter-chromosomal and intra-chromosomal (intra-chromosomal translocations are defined by an abnormal fusion within one chromosome with breakpoints at least 5 Mbps apart). The lines (colored magenta) going from one chromosome to another are drawn to show chromosomal rearrangements.
- The top panel (E) of **Figure 9** includes **Anchor** which corresponds to the chromosome number.

NOTE: Range (which corresponds to coordinate ranges on the genome) allows the user to view SVs of interest by specifying genomic coordinates.
By default, when using Access for the first time, all SVs detected can be visualized in the Circos plot and the **Filter Criteria** (F) lists the default settings used to detect rare variants in the analysis pipeline. Once changed, they will remain at that setting going forward until they are changed again or reset to default.

NOTE: In Bionano Access, “masking” refers to a genomic sequence that has been scanned for either N-base gaps, sub-centromeric and sub-telomeric regions that are prone to generating putative false positive translocation breakpoint calls. Apart from these, regions with putative SVs such as translocation breakpoints can be masked during the alignment step in Solve. Access allows users to view these annotations and regions during the SV filtering steps.

In this tutorial, the default settings including the **SV Masking** option were used.

Visual Comparison of Circos Plot (optional)

5. To compare the SVs detected in each sample, highlight the cancer sample HCC2218 (**Figure 10**).

6. Under the **Options** section on the right, click on the second option **View Results**. This will launch the Circos plot.

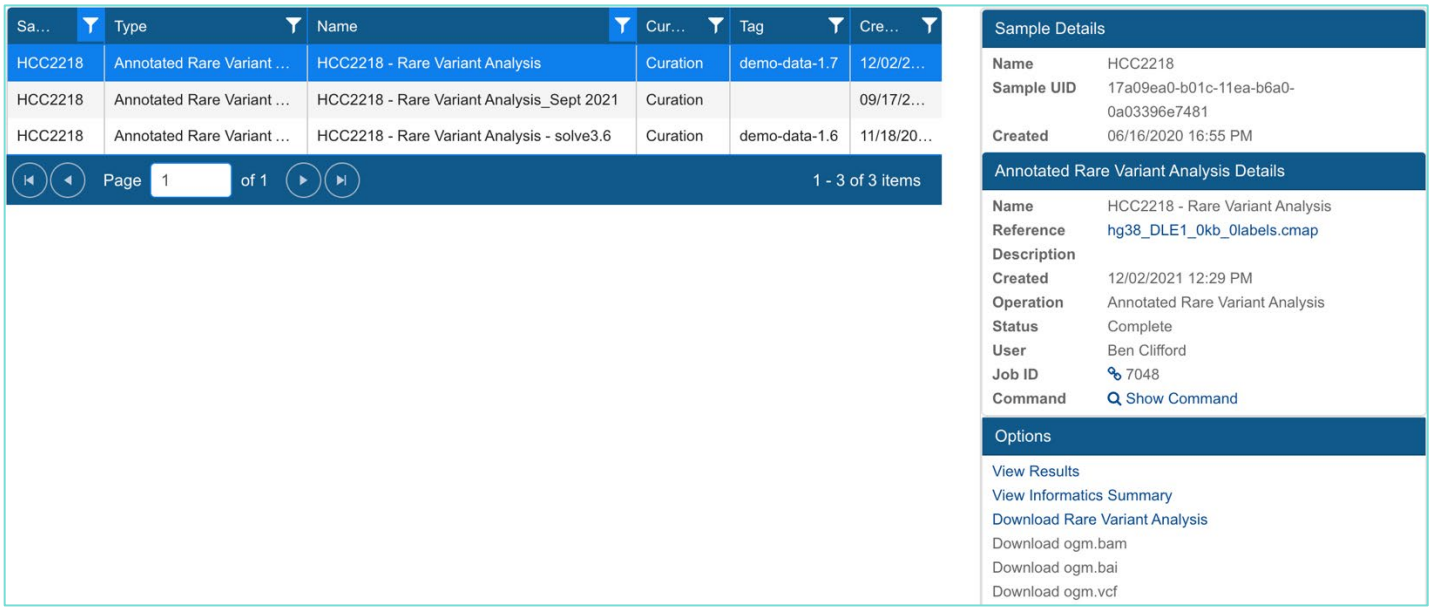


Figure 10. Launching the Circos plot for HCC2218.

7. Similarly, to view the Circos plot for the normal sample (HCC2218BL), first click on the back arrow icon on the top panel. This will navigate the user back to the **Project** page.

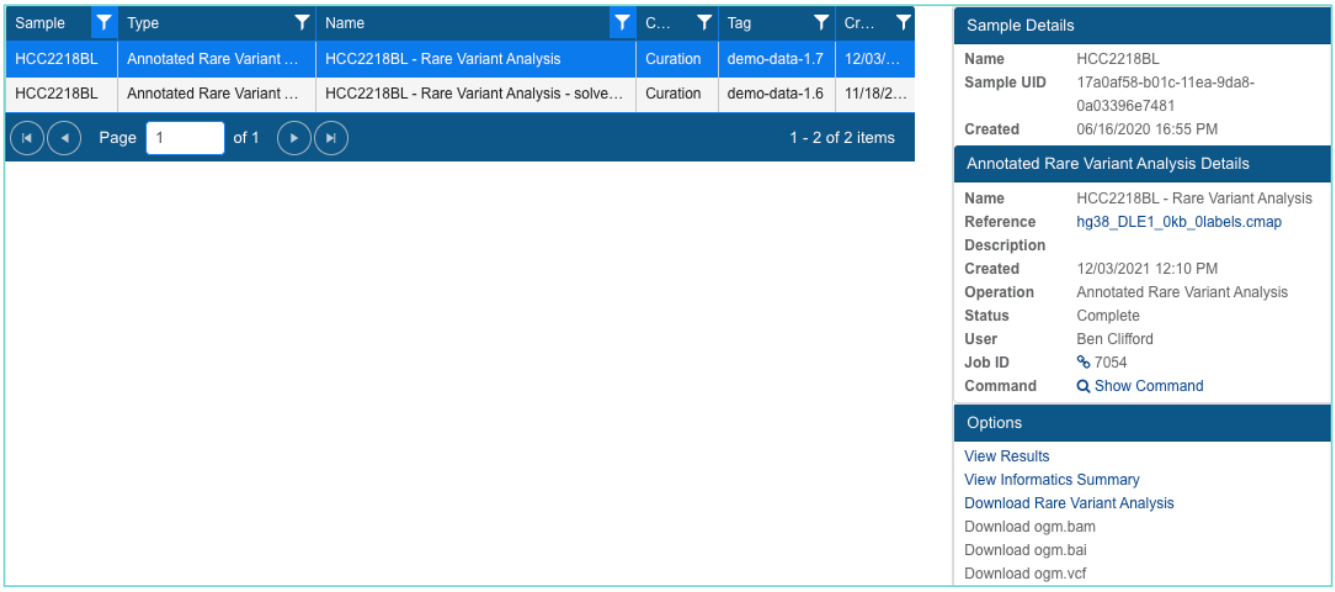


Figure 11. Launching the Circos plot for HCC2218BL

8. Click on the sample HCC2218BL to highlight it. From the **Options** section, click on **View Results** to launch the Circos plot as seen in **Figure 11**.

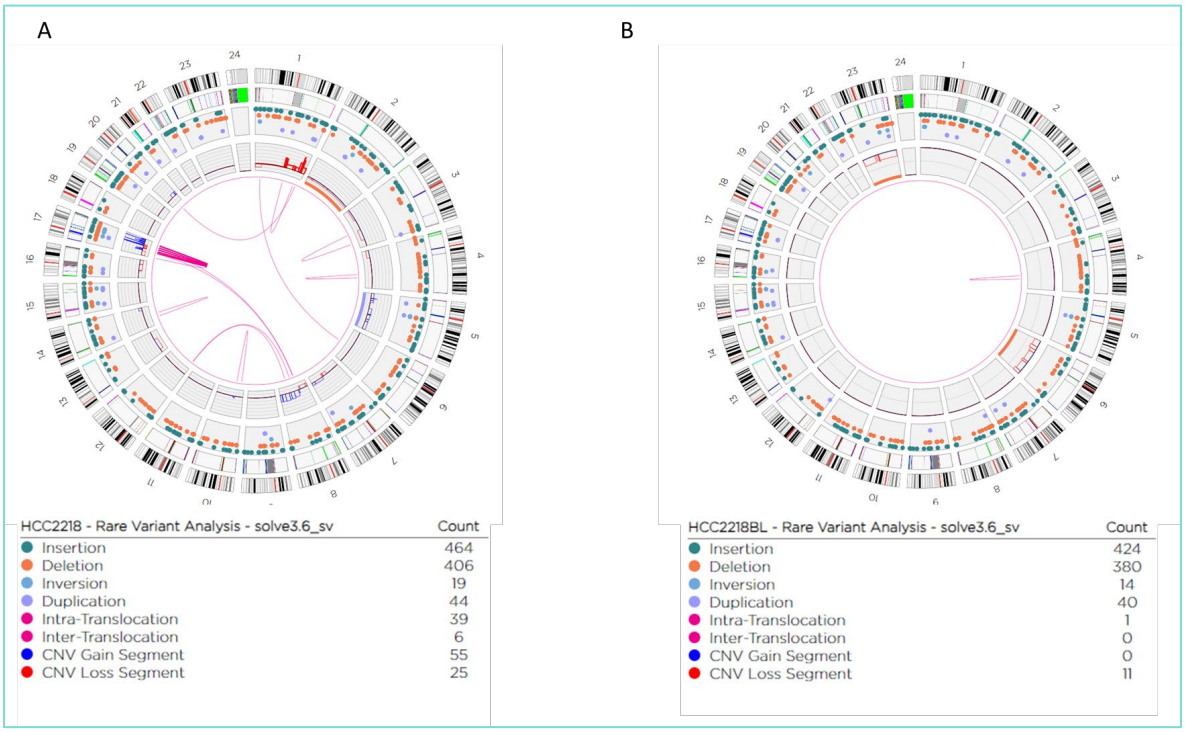


Figure 12. Comparison of the Circos plots from two samples with Tumor cell line (A) and Normal cell line (B)

For ease of comparison, two Circos plots are shown side by side in **Figure 12**. Note the difference in the nature of the distribution of rearrangements between the two cell lines depicted. The whole genome profile on the left (A) has 147 translocation rearrangements that are heavily clustered on Chromosome 17. In contrast, no such rearrangements were found in the normal (B) cell line present in the profile on the right-hand side.

NOTE: Users can customize the enzyme colors, SV colors, map (reference, genome) colors, minimum and maximum map height value, molecule and copy number height value, and others.

9. To change the size of the SVs detected in the Circos plot, click on the **Settings** icon in the top panel as shown in **Figure 13**. Scroll down to find **Circos plot SV size** and change **Circos plot translocation width** size to 1 (**Figure 14**). This increases the size of variants in the SV track and the translocations in the innermost track. Click **Save**.

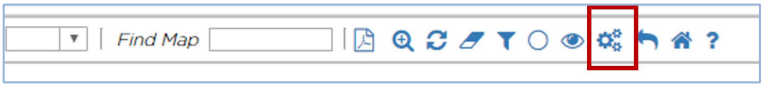


Figure 13. The **Settings** icon in the red box customization of the Circos plot includes **Anchor** and **Range** to navigate across the chromosomes and **Molecules**, **Confidence** and **Find Map** to customize the visualization area. The **Settings** icon in the red box customization of the Circos plot.

NOTE: Changed settings parameters are saved under the username and across datasets, then retained when Bionano Access is opened later. Select **Reset Options** to return to original default filters.

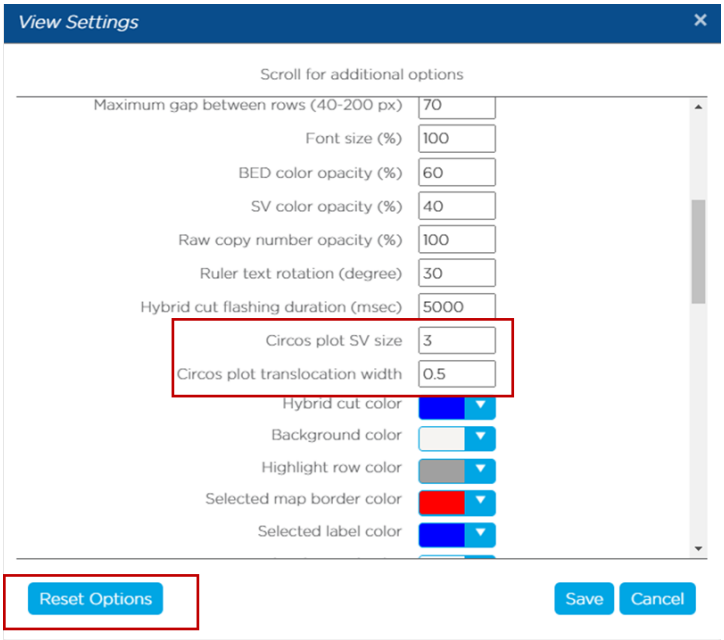


Figure 14. **Viewing SV events**. Customizable options that allow better visualization of the tracks are listed. Select **Reset Options** to return to default settings.

Identify Somatic Variations in a Tumor - Normal Comparison

In general, SV filters can be customized to identify structural variants that are *de novo* or proband-specific in family studies or somatic SVs in cancer datasets. They can also be used to identify rare variants found infrequently in the control database within single individuals, or variants that overlap with annotated genes. In this

tutorial, cancer-specific somatic variants are filtered for. The dual analysis for RVA filters only those cancer sample specific variations. To remove germline calls, Access filters can be adjusted to identify tumor-specific somatic variations from a dual annotation file called “HCC2218_rareVariant_import_annotationDual file”.

1. Click on either the back arrow or the home page to navigate back to the **Project Browser** (**Figure 15**).

Figure 15. Navigate back to the **Project Data** page to find the container file "HCC2218_rareVariant_import_annotationDual file”.

2. Follow instructions as described earlier to launch the Circos plot page for “HCC2218BL-Variant Annotation Pipeline.”

TIP: To ensure none of the additional filtering options are active, check the filter criteria listed in **Figure 16** and click on **Reset Filters** before the recommended filters are chosen.

3. To choose filtering options, click the SV **Filter** funnel icon, shown in **Figure 17**.

4. The filter dialog window opens showing the **Filter by SV Type** options (**Figure 18**).

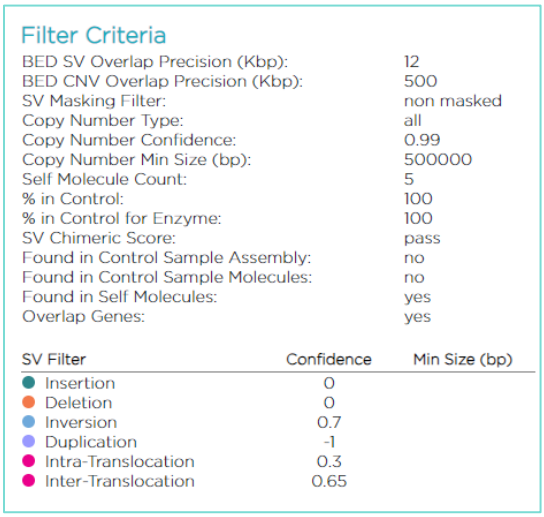


Figure 16. Filter Criteria for SVs are filtered on user defined criteria. By default, stringent confidence score settings are used to detect high confidence SVs.

Figure 17. **Filter** icon for SVs

In general, this wizard allows the user to set rules for identifying variants relevant to their data based on confidence of a call and the length of the detected SV. The confidence score of a detected SV is defined as the probability of the SV being a true positive call.

The filter dialog pop-up window contains four filter tabs:

- The **Filter by SV Type** tab allows basic filtering of type of SV using size cutoff and “recommended” or “all” confidence cutoffs. “Recommended” confidence cutoffs balance false positives and false negatives when calling SVs.
- The **General SV Filters** utilize the masking BED file, allowing users to select which chromosomes to display, the precision of the BED file overlap with called SVs and how the desired masking should occur (either by showing the areas that are masked or unmasked).
- The third tab is for controlling the Variant Annotation filtering and allows users to search for SVs present in a given percentage of the samples in the control database as well as determining whether SVs that are present in the control sample should be shown.
- The fourth tab is for filtering copy number variants.

5. For this demo dataset, choose the default or **Recommended** setting for SV Confidence and leave blank for SV Minimum Size (bp). In short, make no changes for the **Filter by SV Type** tab. Then click on **General SV Filters**.

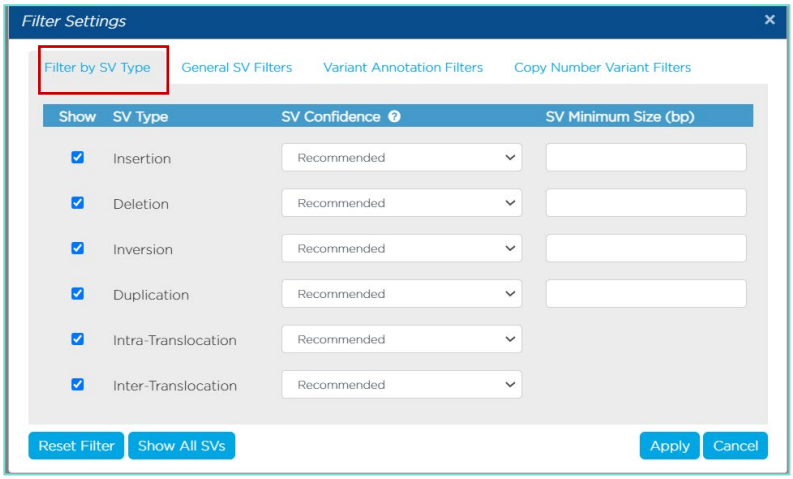


Figure 18. Filter Settings include four different tabs.

6. In the **General SV Filters** tab (**Figure 19**), select the **All chromosomes** radio button. Leave **SV BED Overlap Precision** to 12 kbp to identify SVs overlapping a region of interest when using a BED annotation file to filter data. Select **Non-Masked Structural Variants Only** from the dropdown box under the **SV Masking Filter**.

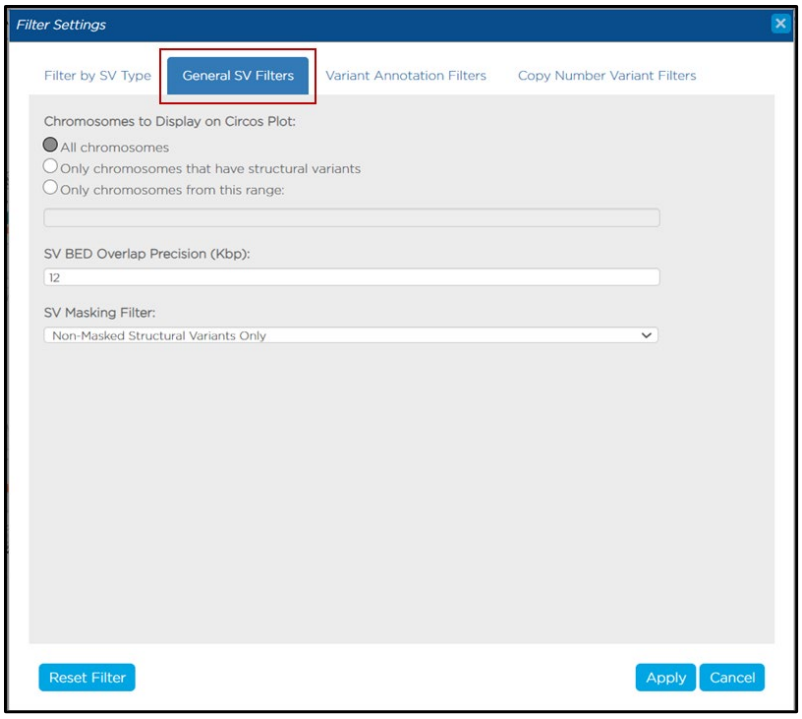


Figure 19. General SV Filters.

7. To filter for somatic variations unique to the cancer sample make the following changes in the **Variant Annotation Filters** tab, seen in **Figure 20**:

- SV in less than this percentage of the Bionano control samples: 100
- SV in less than this percentage of the Bionano control samples with the same enzyme: 100
- SV chimeric score filter: Show Not Failing Chimeric Score
- SV control sample assembly check: SV not found in control assemblies.
- SV control sample molecule check: SV not found in control molecules.
- SV self molecule check: SV found in self molecules.
- SV overlapping genes filter: SV with overlapping genes.
- Self Molecule Count: **5**

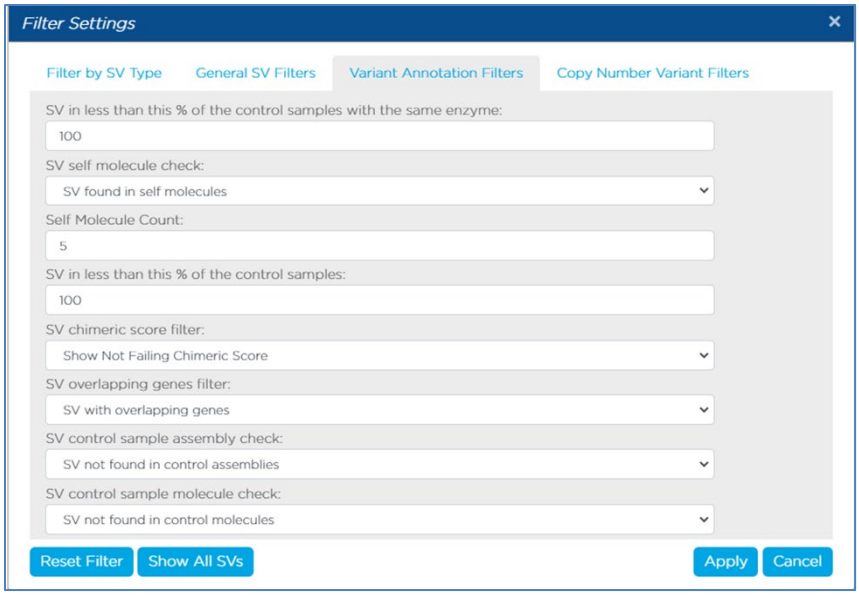


Figure 20. RVA Variant annotation filtering options. The filters in the variant annotation tab have eight different options that can be grouped in six different categories. (A) Check against somatic variants detected in control database of healthy individuals, (B) Check for Spurious alignment, (C) Check for against one control sample (for duo analysis) or two parental control samples (for trio analysis. Not shown), (D) Check for sample-specific assembly or molecule, (E) Check for overlapping genes.

The first option filters for somatic variants detected in a control database of healthy individuals. By setting these values to “100”, the software will display structural variants which are present in any of the individuals in the control database. The control database is not exhaustive and does not include all variants. But the control database may help to identify pathogenic SVs that could be present in a control sample, those of which may not be “healthy” at the time of sample collection. The SV chimeric score filter ensures that regions on the Bionano maps from which SVs are detected have sufficient molecule support to create a confident consensus genome map. The next set of options shows filters to remove any variants detected in the matched normal sample. This is only applicable for a paired sample or a trio analysis. The SV **self molecule check** and **Self Molecule Count** options show filters to include either molecules that align only to the cancer sample (HCC2218) or limit the number of supporting molecules, respectively. The higher number of molecules supporting a call, the greater the confidence of that SV call. The option **SV overlapping genes** filter helps identify the biological importance of the calls by identifying genic information. For more details on any of the settings, please see *Bionano Solve Theory of Operation: Structural Variant Calling* (CG-30110). For more details, see the *Bionano Solve Theory of Operation: Variant Annotation Pipeline* (CG-30190) for guidance on setting these parameters.

8. After clicking **Apply** the user will be returned to the Circos Plot. Confirm that the SV counts lists three insertions, 36 deletions, 9 inversions, 9 duplications, 64 Intra-Translocation, 17 Inter-Translocation, 55 copy number gains and 25 copy number losses (**Figure 21**). These are represented by different colors on the SV track of the Circos plot. **NOTE**: the SVs detected after the filter is applied is lower than the SVs initially detected in sample HCC2218 (**Figure 12A**).

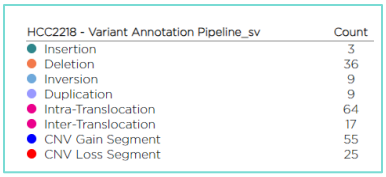


Figure 21. Structural Variants. Color coded by the different types.

There are many ways to identify a set of rare variants that might be of interest to a user. Detailed study is therefore needed to identify rare variants that are biologically important. One way to make sense of the data is to identify relevant, publicly available information for the region, type of SV or gene of interest. The next section describes how to apply a BED file to identify conserved genic regions in the overlapping genomic coordinates.

Practice Session – Identifying Potentially Pathogenic SVs, VAP Filter

This section can be used to facilitate learning about how to find a specific SV of interest. Basic filtering to identify potential somatic variants that are rare among phenotypically “normal” individuals can be applied. Then a masking BED file to identify SVs specific to a gene of interest can be used. ATCC describes HCC2218 and HCC2218BL cell lines as patient derived lymphoblastoid cell lines that are highly positive for expression of Her2/neu and p53. HCC2218BL is an EBV transformed cell line from the same patient. The Dependency Map (DepMap) portal lists large numbers of mutations and gene fusion events for HCC2218 and also reported around 500 SNPs and 36 inter and intra-chromosomal translocation events. Of all the genes known to have a translocation, INTS2 genes was reported to have six different intra-chromosomal rearrangements. The cancer cell line HCC2218 can be checked to identify INTS2 CNV gain as reported by DepMap**.**

As a proof of principle, simple filtering criteria can be applied to identify the ZNF652-INTS2 translocation in the HCC2218 cancer sample. To identify the genomic coordinates of the SVs impacting them, annotations to the SV calls should be added by providing overlapping gene information. The same filtering options can be applied to identify other variants rare among phenotypically “normal” individuals that may overlap with annotated genes or are potential false positive calls.

Filter Based on Gene Annotations

To visualize genic regions on a chromosome, users can import annotations from public resources such as the UCSC Browser or use a pre-loaded BED file available in the software. A BED file (.bed) is a tab-delimited text file that defines a feature track. Canonical, or known genes, datasets are constructed by identifying a single transcript or isoform for a gene that has many reported isoforms. For ease of analysis, only the longest isoform is chosen for a gene and its chromosomal coordinates are included in the BED file. Because the chromosomal coordinates are reported in a BED file, genes can be visualized as tracks in a Circos plot or genome map. Click here to read on how canonical genes are identified. In this tutorial, known genes corresponding to human build hg38 (“hg38 known canonical.BED”) is used for analysis since raw data was aligned using the hg38 genome. This BED can be easily customized and has been manually curated to include recent genes.

Adding BED Files for Filtering

1. To add a BED file, click the Add BED button on the Circos plot display page as shown in **Figure 22**; this will prompt the user to select from the numerous reference genome-specific BED files that can be easily added to any analysis. “hg38 known Canonical” will also be used to filter structural variants to just those that overlap known genes and view them in the Circos plot or the Genome Browser.

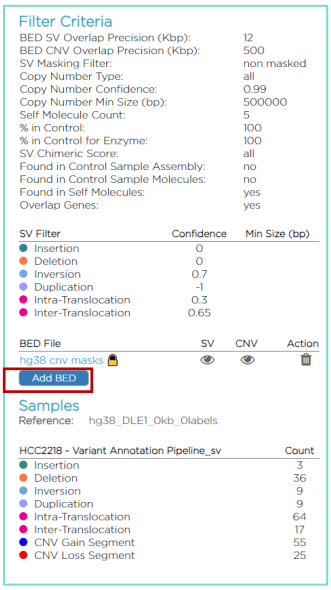


Figure 22. Filtering settings and BED (masking) file.

2. In the BED Configuration window, select the **hg38 Known Canonical** file from the dropdown as shown in **Figure 23**.

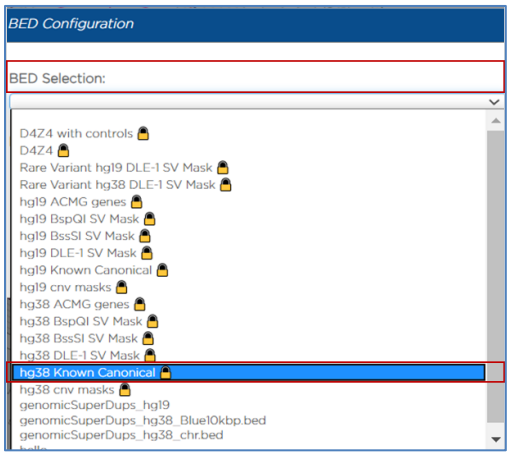


Figure 23. Selecting hg38 Known Canonical

3. Choose the button for **Show structural variants that overlap BED Regions** as highlighted (**Figure 24**) and then press **Ok**.

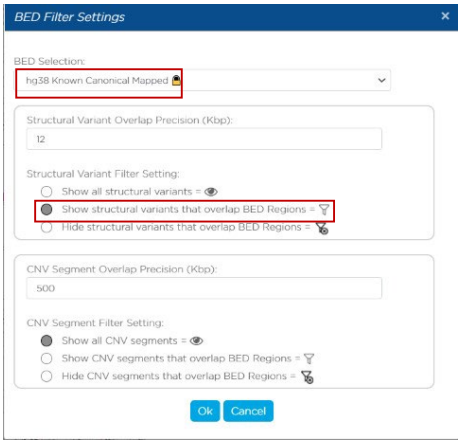


Figure 24. Selecting a BED file. Preloaded bed files can be used filter out structural variation in non-specific regions.

Since the visualization is interactive, the Circos plot will now show an extra annotation track. **Figure 25** now shows the known canonical genes on the second track right next to the cytobands. In this track, each vertical line represents a gene.

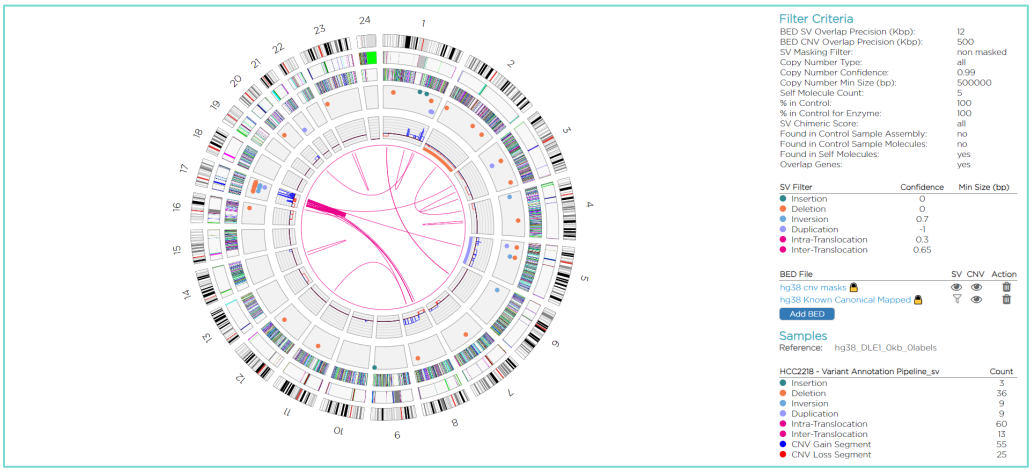


Figure 25. Rare variants overlapping known genes - Circos plot with BED track.

The dataset has now been filtered according to Bionano's recommended filter settings and a BED masking file has been applied to the dataset so that only SVs that overlap with known genes will be visible. Confirm the SV counts as shown in **Figure 25**. These are represented by different colors on the SV track of the Circos plot. **NOTE** Upon implementing the filters, the lowest number of rare variants is identified.

Now the user can confirm whether the SVs can be found on chromosomes 17 in the regions specified by the DepMap database.

4. Zoom in on the Circos plot region for chromosome 17 using the mouse wheel or track pad (**Figure 26**).

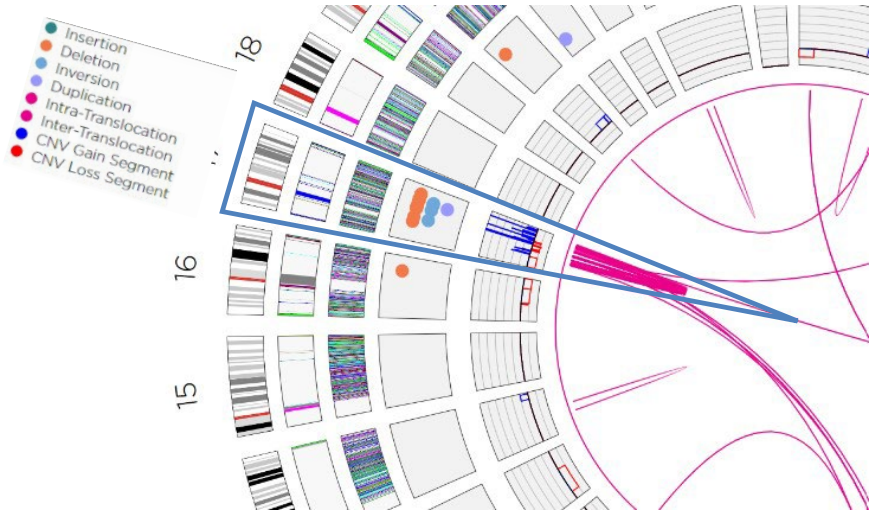


Figure 26. Circos plot with Variant Annotation filters applied. Zoom in and mouse over SVs on chromosome 17.

5. Hover the mouse cursor over the SV track for chromosome 17, which also has a large copy number change associated with that region.

6. Click over the deletion on chr 17. Confirm that the position is roughly 41,109,560-67,483,840 according to the Bionano data.

7. Click on the intrachromosomal fusion to open the **Genome Map** view, seen in **Figure 28**. Alternatively, choose **Genome Browser** and Chr 17 for **Anchor** from the dropdown as shown in **Figure 27**. This will launch the Genome browser view. This genome map view shows an intrachromosomal fusion event on chromosome 17.

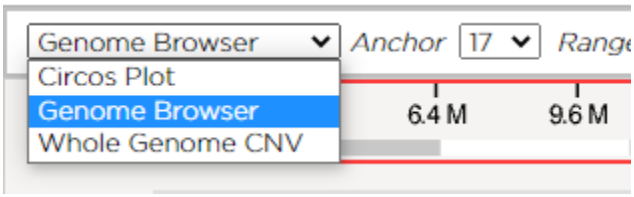


Figure 27. Dropdown from the top panel allows navigation to **Genome Browser** view, **Anchor** represents chromosome number.

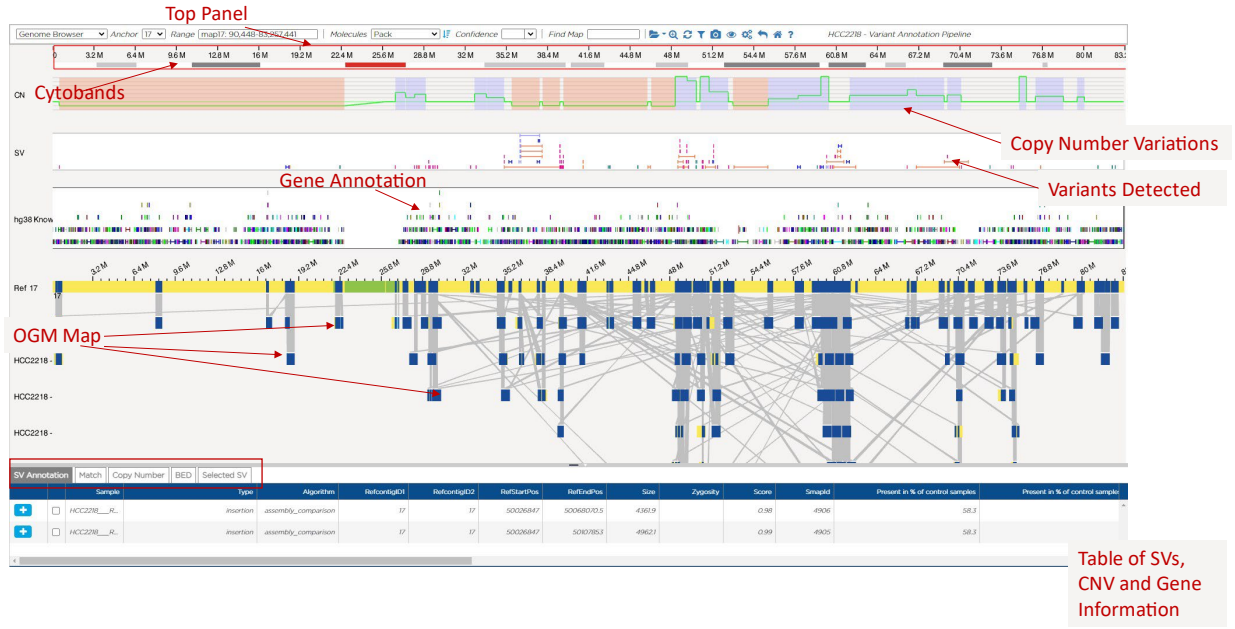


Figure 28. **Genome Map View** visualization

This zoomed out genome map view shows chromosome 17. The top panel of the browser is consistent with the Circos plot view. The cytoband is the top track. The **Anchor** and **Range** show the precise coordinates on top reference that is being viewed. The SV can be viewed in more detail in the central region of the map.

In **Figure 28**, the small red box in this track indicates the location of the intrachromosomal fusion. The second track includes the SVs detected (red box) which in this example is an intrachromosomal fusion. Known genes from hg38 are added in the view as a reference. The vertical lines on the reference map and assembled genome map are the recognition sequence of the labeling enzyme and the connections between maps (grey lines) are alignments. If the labels on the assembled genome maps match with the reference map, then no structural variations are reported in a region of interest. In this example, by comparing the enzyme marks on the Bionano map (blue) and reference genome locations (green), one can see there are alignment marks from the assembled genome that matches marks of chromosome 17. The abnormal alignment marks (pink lines) show the region of the map that skips contiguous alignment to the reference map suggesting a translocation event. Using the mouse, draw a box on the cytoband track around 41 Mb to 44 M region to zoom in. In the tabular section of the view, click on the header of each column to sort it. Use the mouse to scroll zoom in or zoom out. Please refer to *OGM File Format Specification Sheet* (CG-00045). for more details about each column.

8. Use the scroll bar at the base of the table (**Figure 29**) to navigate to the **Overlap Genes** column. This lists the overlapping genes and nearest non-overlap genes. The first few rows in the table lists the SVs detected in this region.

9. Click on the header column to sort the overlapping gene names. This will list all the SV events for a fused gene called ZNF652 - INTS2.

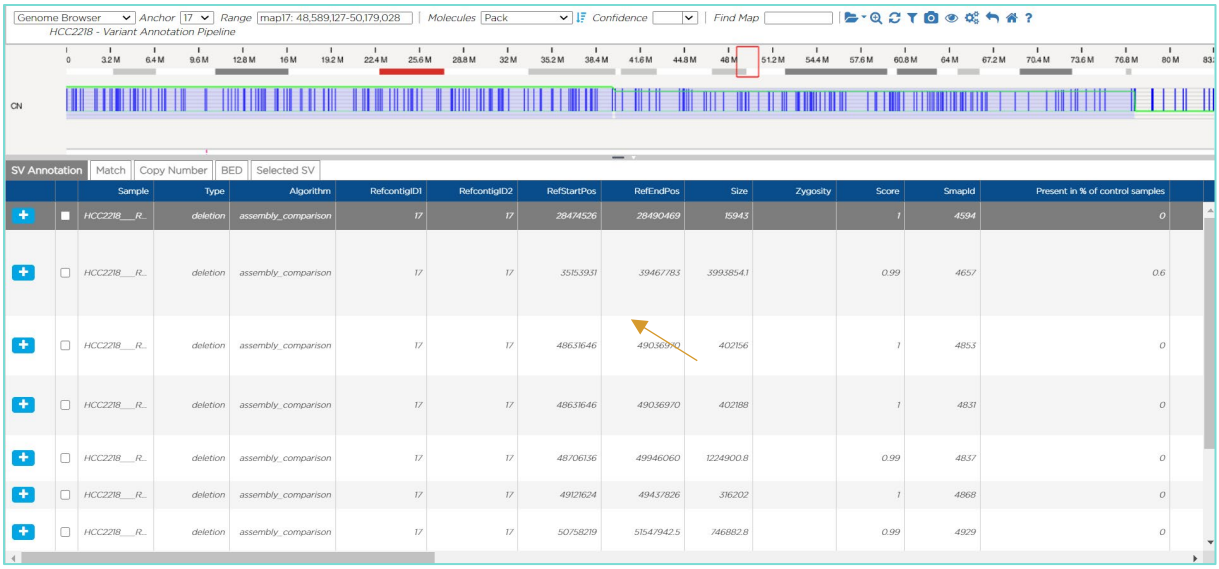


Figure 29. Table at the bottom of the Genome Map lists the SVs for genes in the region.

10. Click on the first row to automatically navigate into this region with the SV overlapping the gene ZNF652 or INTS2. The table at the lower half lists all the SVs, CNVs and their associated annotations. The highlighted row lists “translocation_intrachromosomal” under the **Type** column. The next few columns provide information about the algorithm used to call the SV, contig information and breakpoint coordinate information for the reference map, among others.

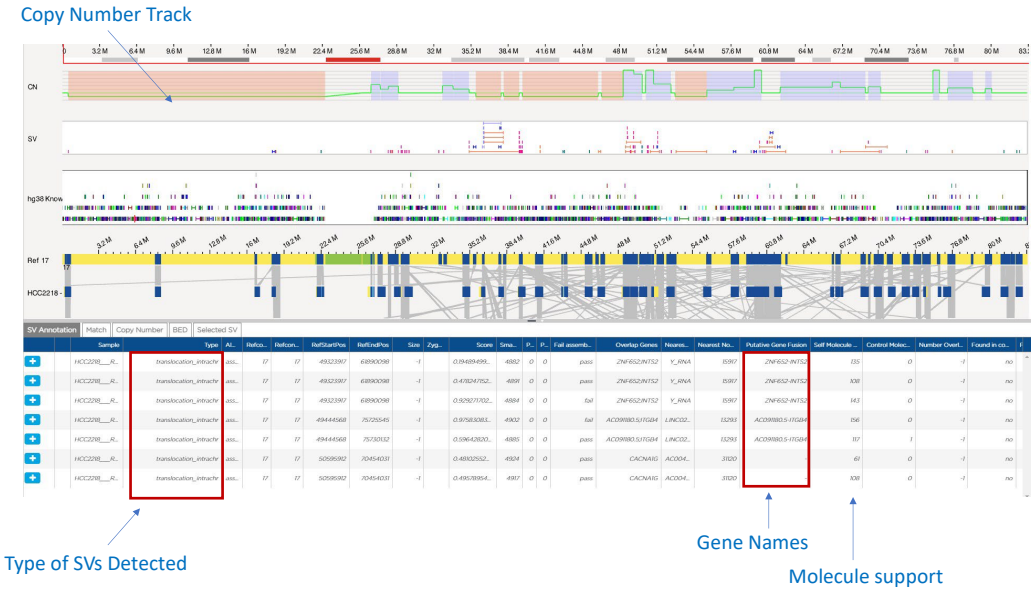


Figure 30. ZNF652 gene showing several structural variations in this region.

11. Zoom in to the region map17: 49,100,615-49,570,725 to view the ZNF652 gene. This region shows several structural variants including translocations events in the region as well as copy number change. When regions located on the same chromosome fuse, they are called an intrachromosomal

rearrangement. These intrachromosomal rearrangements are well known characteristic of malignancies. For more details on translocation calls detected using Bionano optical mapping techniques, refer to *Bionano Solve Theory of Operations: Variant Annotation Pipeline* (CG-30190). Additionally, SV track and the table on the lower half also suggest two large deletion events spanning the region.

12. Scroll to the right to find the column **Self Molecule Count**. For the list of translocation events the self-molecule count is listed in the range of 135 to 143, suggesting that the breakpoints for this translocation event was supported by molecules that line up to the breakpoint coordinate. While hovering over the table, users can press the left and right arrow keys to navigate to the additional cells or use the trackpad to drag the table left or right (**Figure 31**).

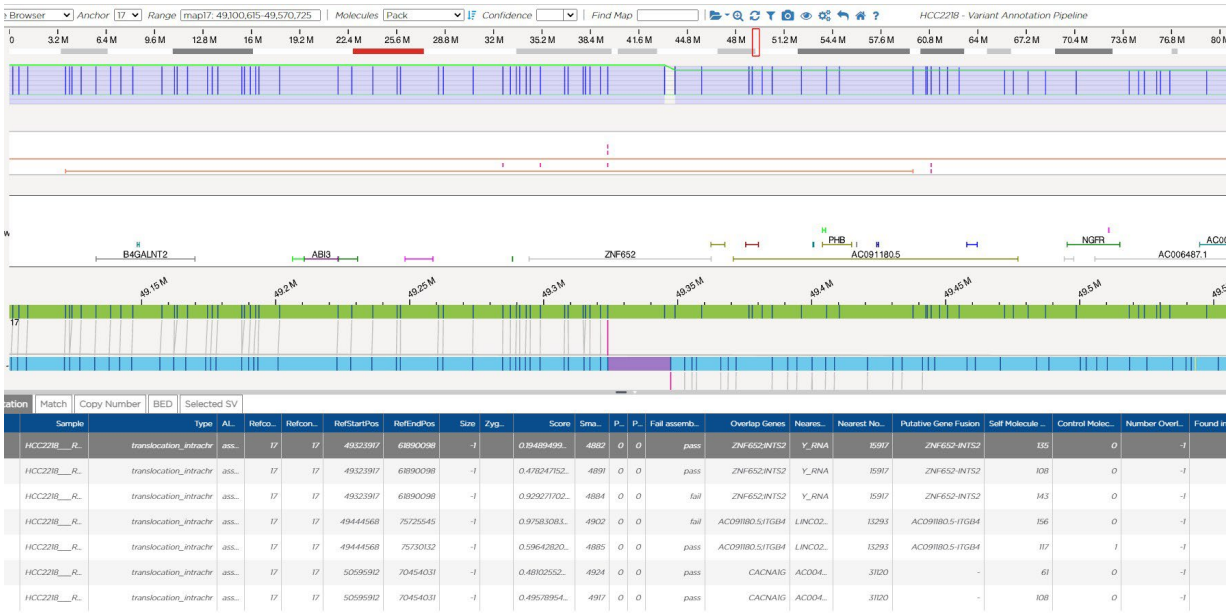


Figure 31. **Genome** view with the copy number track at the top.

13. Click on the “+” sign to the left of the row that has the intrachromosomal translocation SV of interest. This will add it to the “Selected SV’s list, and the SV report to be printed out later.

14. Right-click on the assembled genome map (light blue) to list options to show the molecule pileup (**Show Molecules**) used to find the variants (**Figure 32**).

Figure 32. Right click options to add molecules or ruler to the genome map view

Thus, optical mapping confirms the translocation call. In addition, optical mapping also reports a large deletion (color coded orange) and copy number increase (in the copy number track) as well as other translocations in this region. To view the any other SV call, click on the variant and it will launch the optical map supporting the call and the molecules supporting. Similar filtering criteria can also be applied to a user's own dataset. The Circos plot and genome map navigation will be applicable to all datasets analyzed in Access. In this figure, the CNV track is shown with the SV track, gene information track and the assembled Bionano map with the reference map. To find more information about copy number calls, click on the **Copy Number** tab. For more information on how CNVs are calculated see *Bionano Solve Theory of Operations: Structural Variant Calling* (CG-30110).

15. To view the whole genome copy number view, use the dropdown on the top panel to navigate to the WGCNV view as shown in the **Figure 33**. Optical mapping also shows aneuploidy and dramatic copy number changes in the genome.

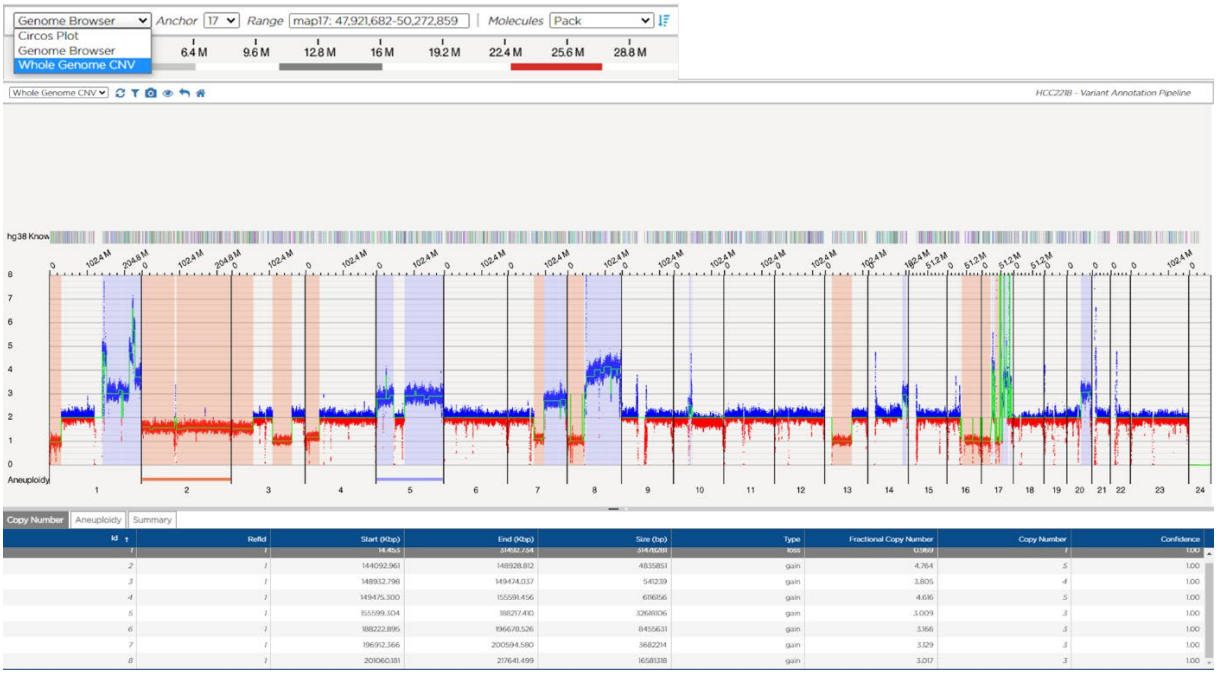


Figure 33. Whole Genome Copy Number View. The individual label copy number data points are plotted in blue or red. The segmented copy number line is green, and the copy number variant segments identified are shaded according to the variant type. Aneuploidy information is also marked on the bottom. Zoom in/out to see the scale of chromosome.

Report Generation, Exporting Data, and Creating Images

Report Generation and Data Export

Data export can be useful for record keeping or for tertiary analysis. To learn more about the different data files, read the *OGM File Format Specification Sheet* (CG-00045). Results from the rare variant annotation pipeline are output to a text file with annotation for each SV detected for each sample. Exporting data from Access allows the user to share their findings with others or for record keeping. Access users can create a pdf report with analysis details, as well as scalable vector graphics (SVG) or screen captures for the genome map views or Circos plots. These figures are also a part of the pdf generated from Access. The image in the report will reflect what is projected on the screen when the SV is selected in the data grid by default.

1. To generate a PDF SV annotation report containing the SVs that have been added, make sure the Circos plot view is selected and then select the **Generate Report** icon (pdf icon and the pdf dialog box will pop up (**Figure 34**).

Figure 34. Generating a pdf report. The icon in the red box launches the PDF generating dialog box.

2. To download all the filtered variants, check the box according to the file format desired (or check all of them), ensure **Filtered Variants** is selected in the dropdown box and then click **Ok** (**Figure 35**). This command will save a zipped file in the default folder (downloads or desktop).

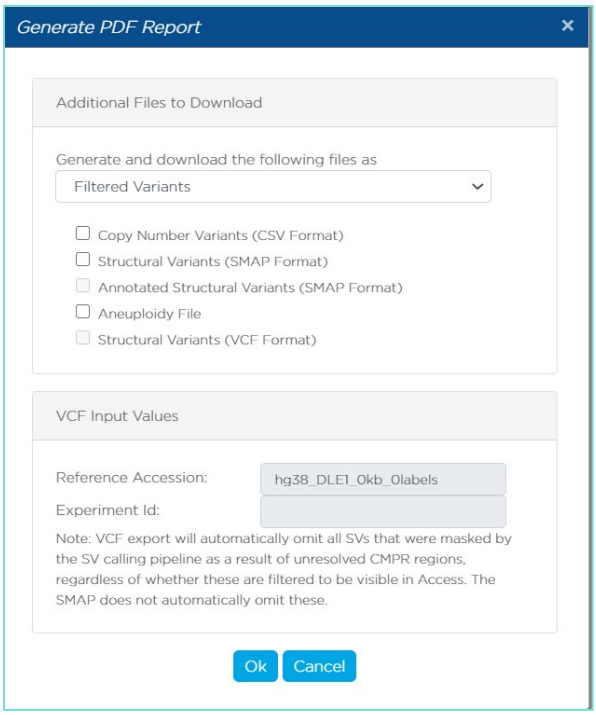


Figure 35. Generating a pdf report.

This file is called “HCC2218_rareVariant_import_annotationDual_879_6_30_2020_2_33_18.zip.” This folder will contain a PDF report, a VCF file and two CSV files of the selected variant (**Figure 36**).

TIP: To download the variants detected in each sample, choose either “HCC2218” or “HCC2218BL,” listed as **Rare Variant Analysis** under the column **Type**. For each of these files, click on **Download Rare Variant Analysis**.

NOTE: To generate a close-up of the Circos plot, zoom in to the desired location before selecting **Generate Report**.

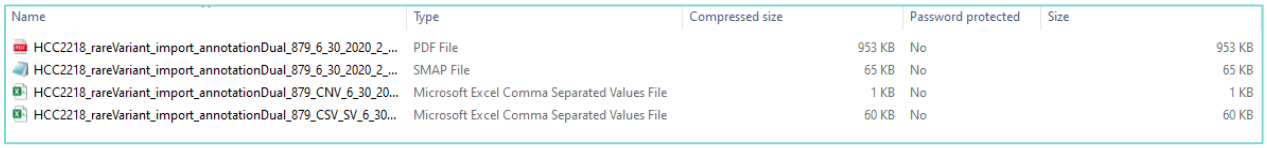


Figure 36. Rare variant downloaded folder showing the different files downloaded as a part of the report

3. To view the files, Unzip the folder. Double-click on PDF to read the file.

   This report includes a screen shot of the Circos plot complete with the legend, a screen shot of each structural variant, details on each structural variant, and general information about the dataset and any comments that were included during the analysis. To obtain more information about the SMAP file, refer to *OGM File Format Specification Sheet* (CG-00045). **NOTE:** Another option to export files or the whole project for the sample analyzed is to download the SV annotation files. To do this, first click on **Home** or the **Back** icon to go back to the project data page (**Figure 37**).

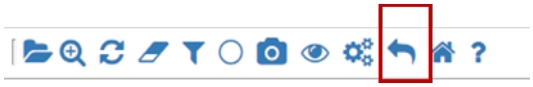


Figure 37. Relaunching the project. The back icon in the red box relaunched the project browser

Creating Images

Bionano Access is equipped with a tool to generate publication quality images in Jpeg file format in Access version 1.6. Preserving graphical quality is essential in publishing a reported finding.

4. To capture an image of a structural variant, navigate to the SV of interest.

5. Click on the camera icon displayed in the genome browser (**Figure 38**). A preview icon is also available to allow the user to view the image that was captured.

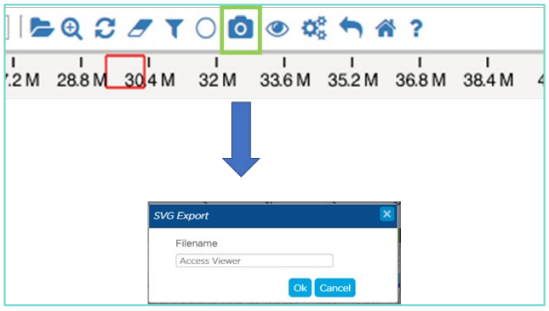
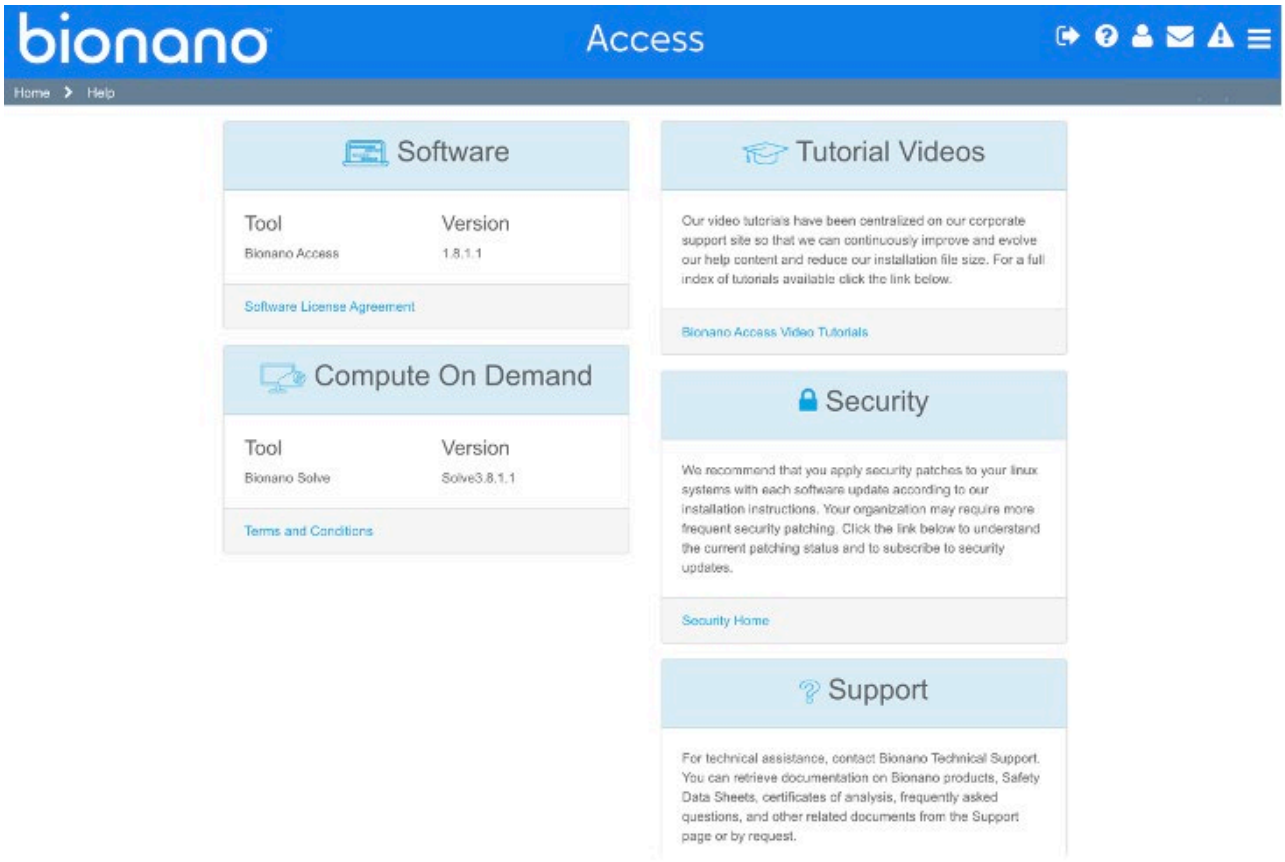


Figure 38. Generating images of regions of interest. Inset shows camera icon that launches the SVG export wizard.

6. This launches an interactive "AccessViewer.svg" file from the local download. This image can be further edited where it can scale to any dimension without losing quality.

Resources and Information

For more information about software and data analysis, click on the **Help** button (question mark icon) on the **Home** page. This opens the **Support** page.



Click on the video tutorials for short videos to learn about specific analysis steps:
http://www.bnxinstall.com/Videos/TutorialIndex.html

- For Structural Variants, Copy Number Variations and Rare Variant Analysis, refer to:

Bionano Solve Theory of Operation: Structural Variant Calling

- For Variant Annotation Pipeline, refer to:

Bionano Solve Theory of Operation: Variant Annotation Pipeline

- For hybrid scaffolding pipeline details, refer to:

Bionano Solve Theory of Operation: Hybrid scaffold

- When using command line, refer to:

Guidelines for Running Bionano Solve Pipeline on the Command Line

You can also find the latest versions of these documents on Bionano's Data Analysis Documentation page (https://bionano.com/software-and-data-analysis-support-materials/).

Technical Assistance

For technical assistance, contact Bionano Technical Support.

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

TYPE	CONTACT
Email	support@bionano.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663 Monday through Friday, 9:00 a.m. to 5:00 p.m., CET UK: +44 115 654 8660 France: +33 5 37 10 00 77 Belgium: +32 10 39 71 00
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