PG-Seq™

Software

TECHNICAL DATA SHEET

Version 1.0

40-46 West Thebarton Road
Thebarton, South Australia, 5031

PG-Seq™ Software User Guide Version 1.0 April 2018

PG-Seq™ is a Research Use Only product and is not to be used for diagnostic procedures

PG-Seq™ is for Research Use Only and should not be used in diagnostic procedures. You are responsible for ensuring that you accurately follow the protocols provided in this Technical Data Sheet (TDS) and analysing and interpreting the results you obtain. PerkinElmer Health Sciences (Australia) Pty Ltd does not guarantee any results obtained.

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1. PG-Seq™ Software: General Overview and PerkinElmer Health Sciences (Australia) parameters

1.1 Download and install PG-Seq™ Software

PerkinElmer Health Sciences (Australia) Pty Ltd includes access to the PG-Seq™ Software as part of the PG-Seq™ kit.

The software provided is installed onto a single stand-alone computer that has the following hardware requirements:

- Operating system: 64-bit platform (Windows/OSX/Linux)
- Memory: Minimum 4GB (recommended 8GB)
- Hard-drive: Minimum 500GB

Users of PG-Seq™ are provided with a unique software licence activation code, a weblink to install and activate the software, and detailed instructions. Follow the detailed instructions to install and activate the software.

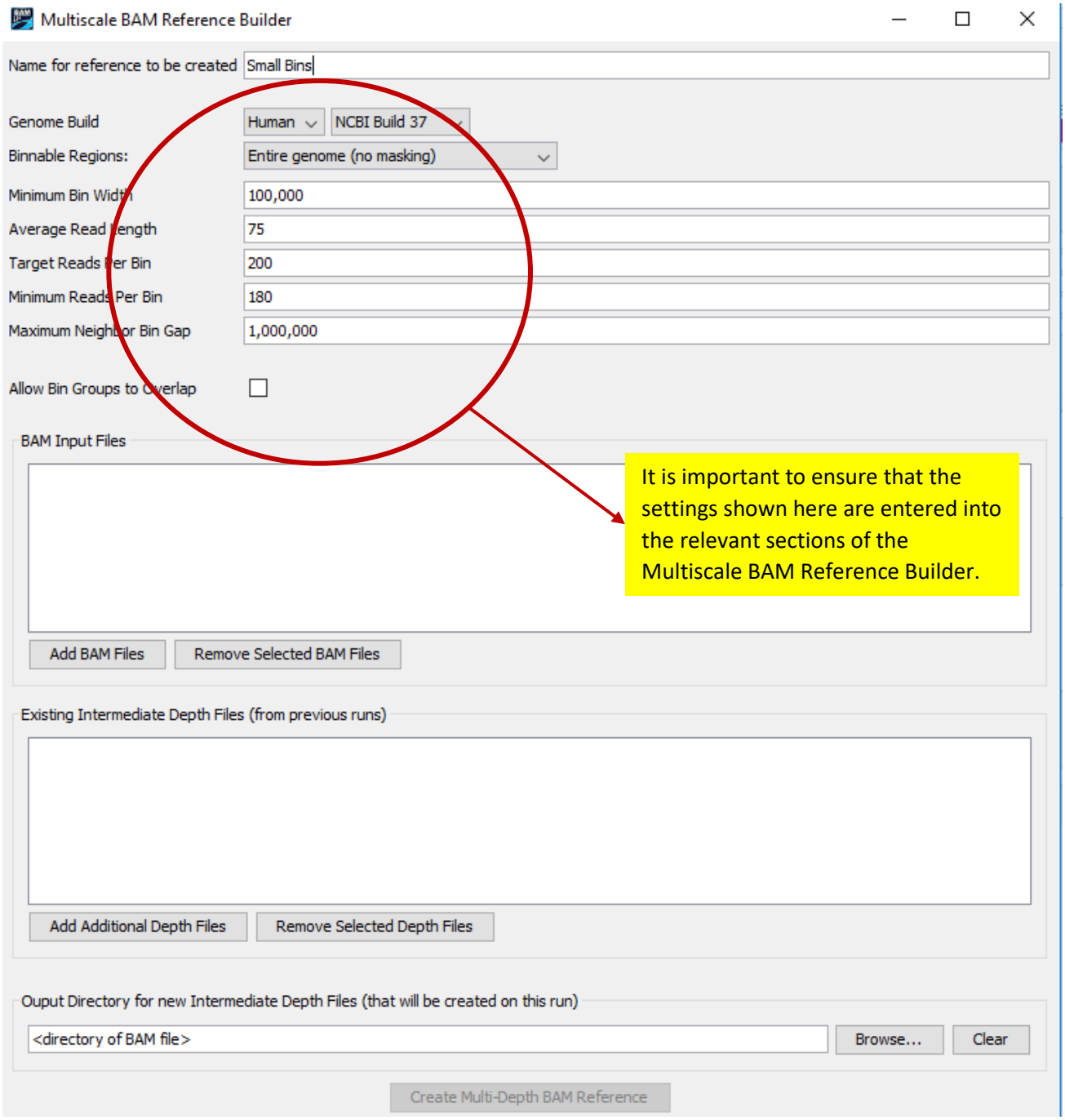
Please contact PerkinElmer Health Sciences (Australia) Pty Ltd or support@rhsc.com.au if experiencing issues with installation or activation of the PG-Seq™ Software.

2. Create a Reference (optional)

It is recommended that a reference is made from minimum 10 euploid male BAM files which have been processed with the same conditions as your sample files, e.g. same WGA, library preparation and sequencing parameters. Some abnormal sample BAM files can be included in this reference as long as no samples have the same abnormality.

2.1 Open Multiscale BAM Reference Builder.

2.2 Name the reference file then enter the settings according to the below figure.



2.3 Use the "Add Files" button under the BAM Input Files section to select the BAM files you wish to include in your reference pool.

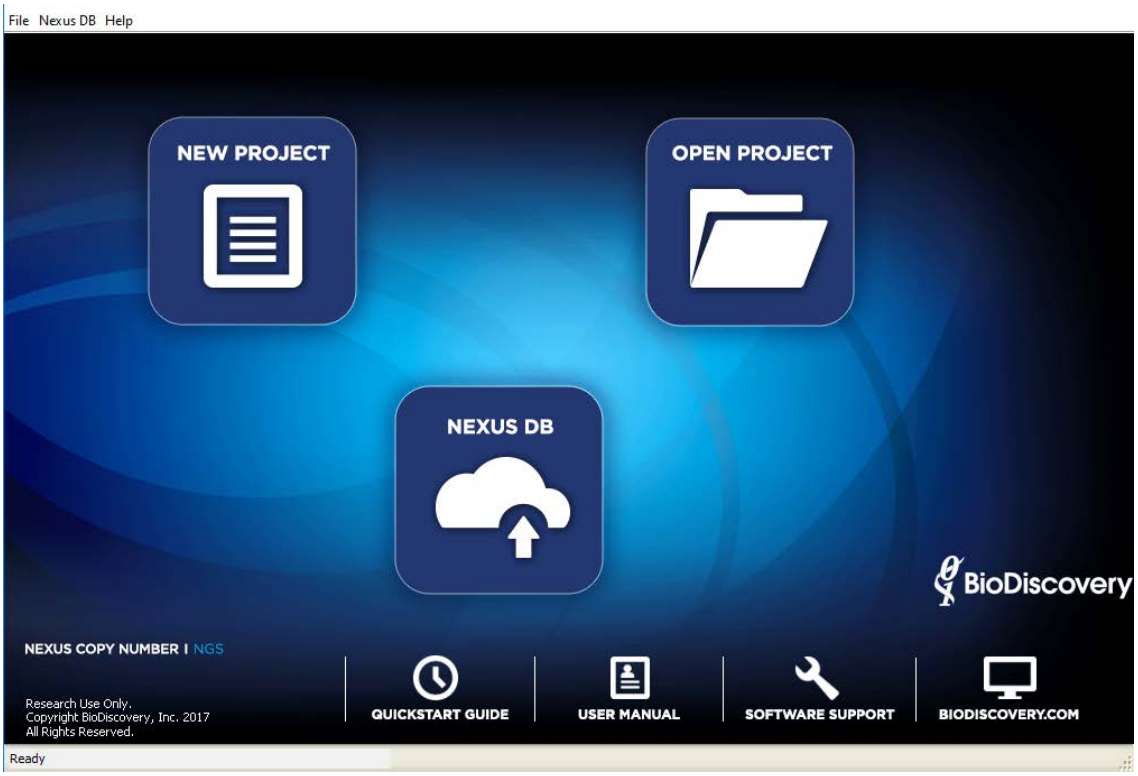
2.4 Press "Create Multi-Depth BAM Reference".

The parameter “Target Reads Per Bin” can be adjusted to create higher or lower resolution results. The parameter “Minimum Reads Per Bin” should be set at 90% of the “Target Reads Per Bin” value.

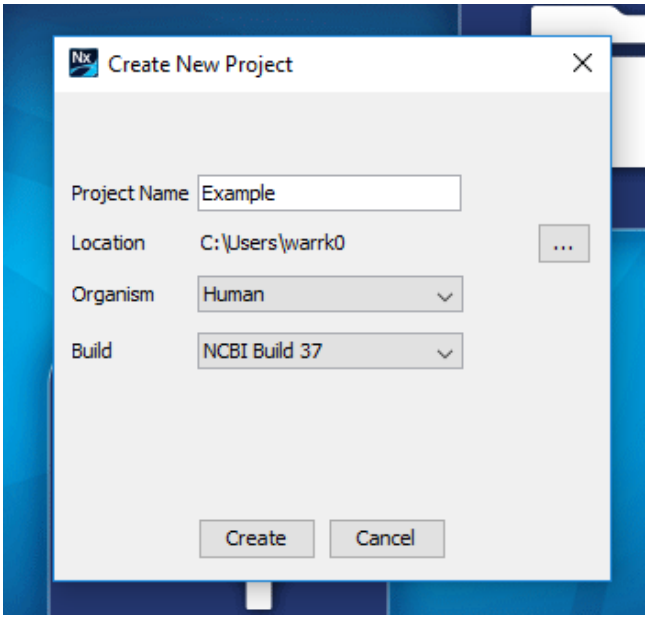
3. Apply the PerkinElmer Health Sciences (Australia) recommended settings

3.1 Open PG-Seq™ software.

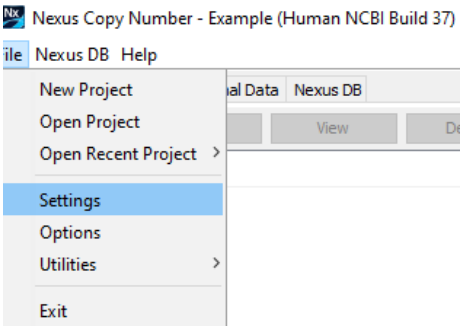
3.2 Select New Project.



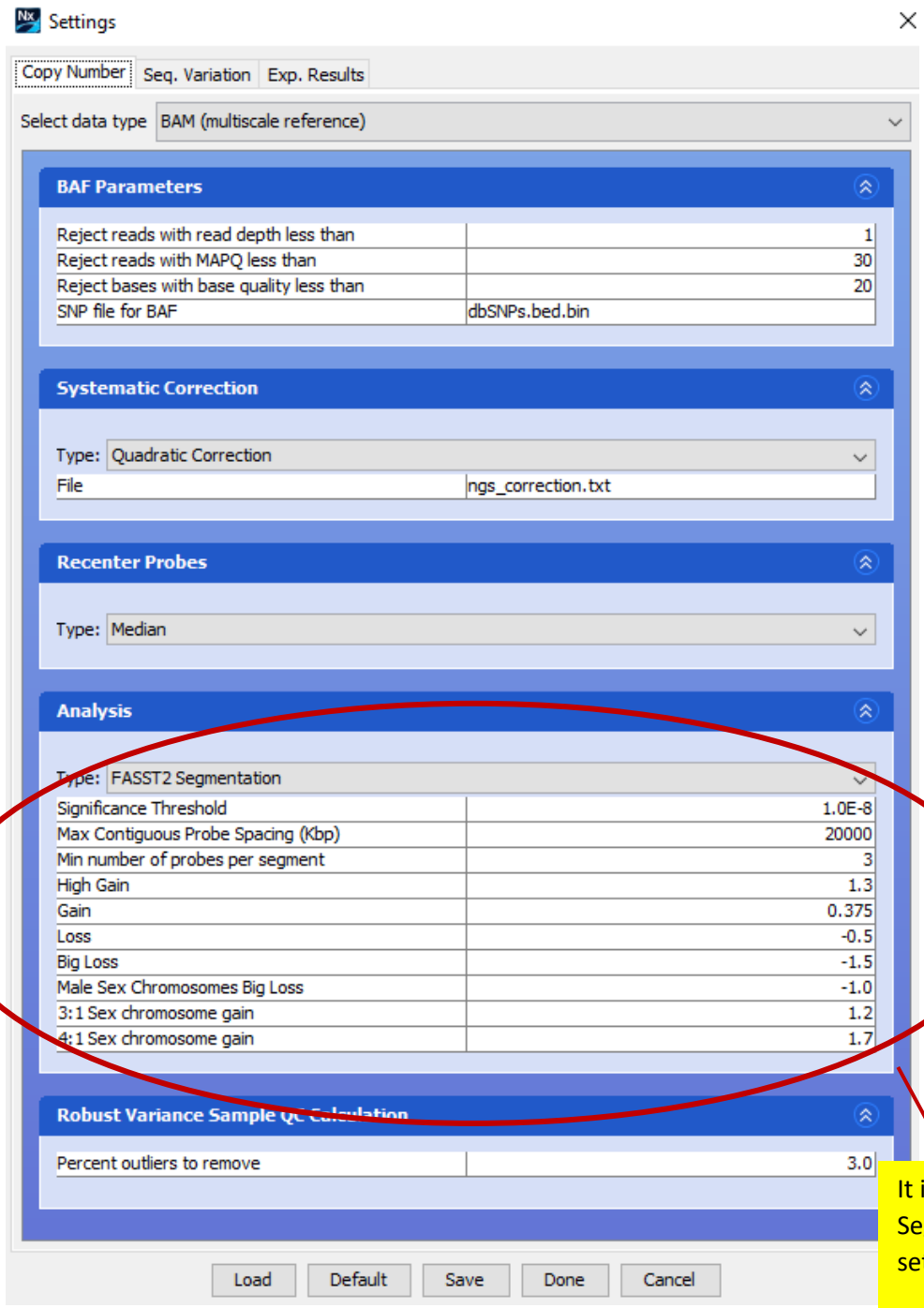
3.3 Enter a project name and enter the following settings:



3.4 Navigate to the settings menu by selecting “File – Settings”.



3.5 Under the tab “Copy Number”, enter the following settings:

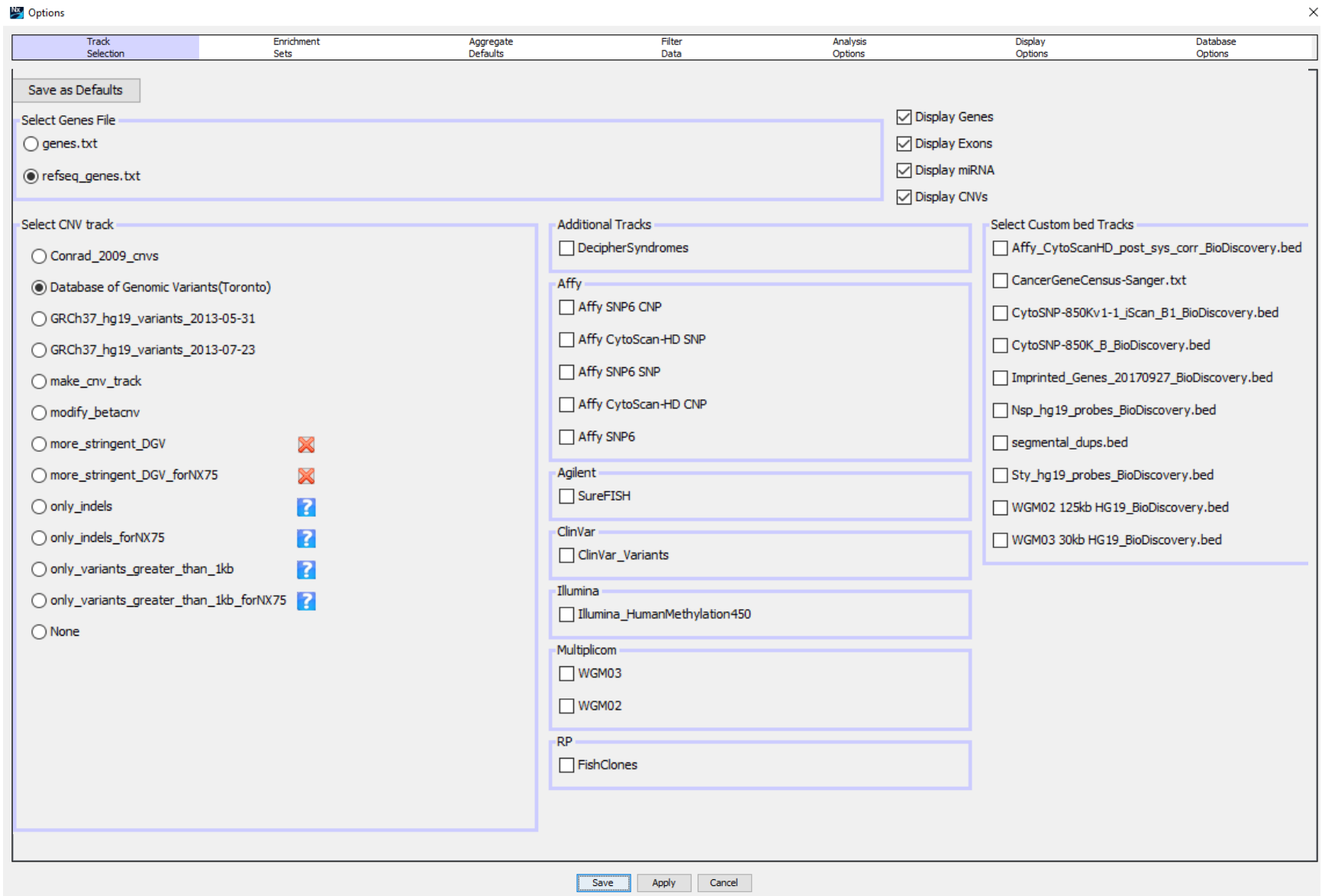


It is important to ensure FASST2 Segmentation is selected and the settings are entered as shown here.

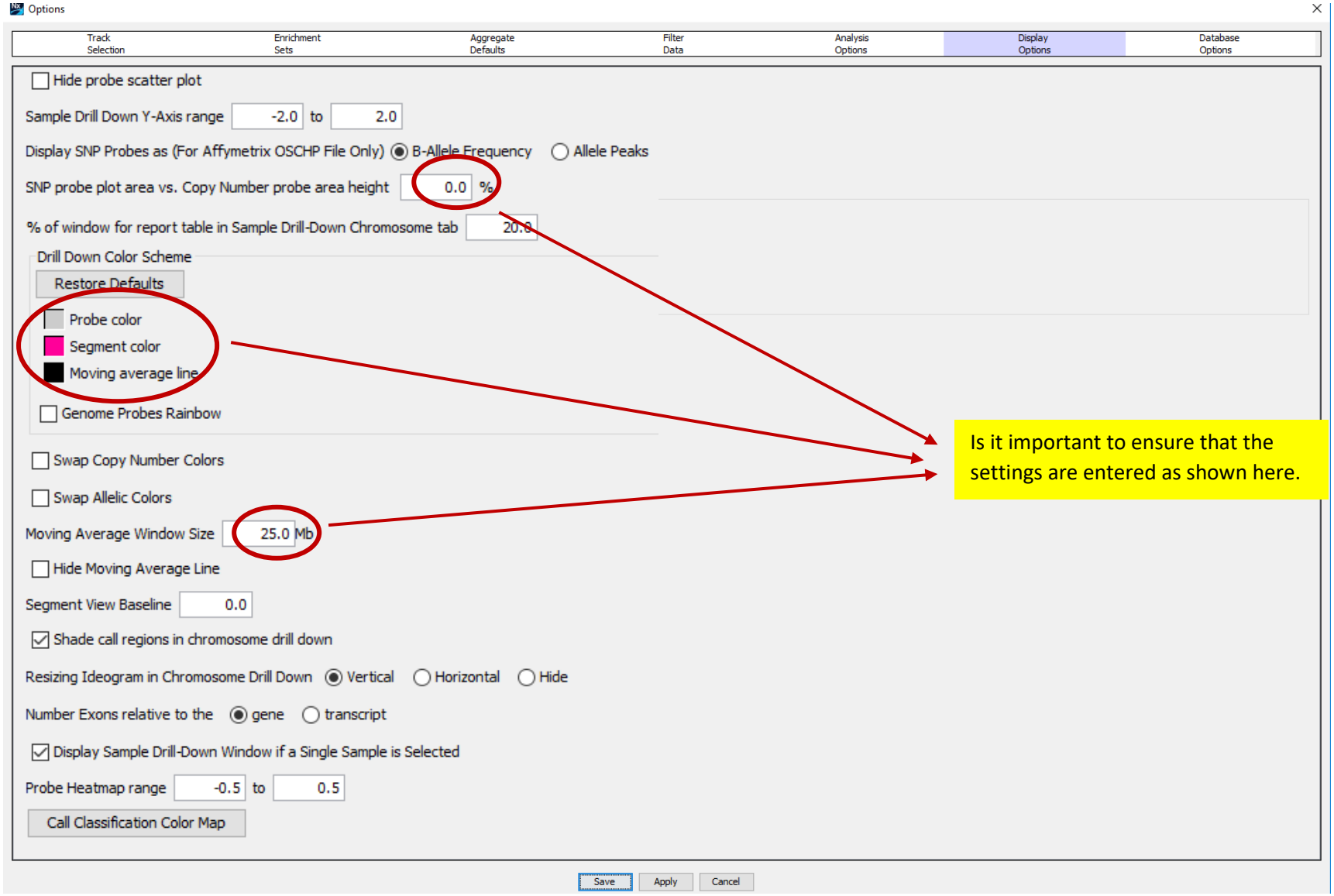
3.6 Press “Done”.

3.7 Navigate to the Options menu by selecting “File – Options”.

3.8 Under the tab “Track Selection” use settings according to the following figure



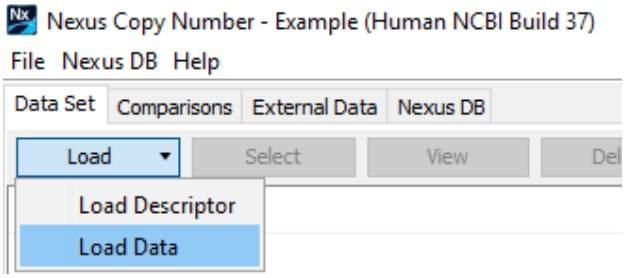
3.9 Under the tab “Display Options” use settings according to the below figure



3.10 Press “Apply”

4. Load and analyse samples

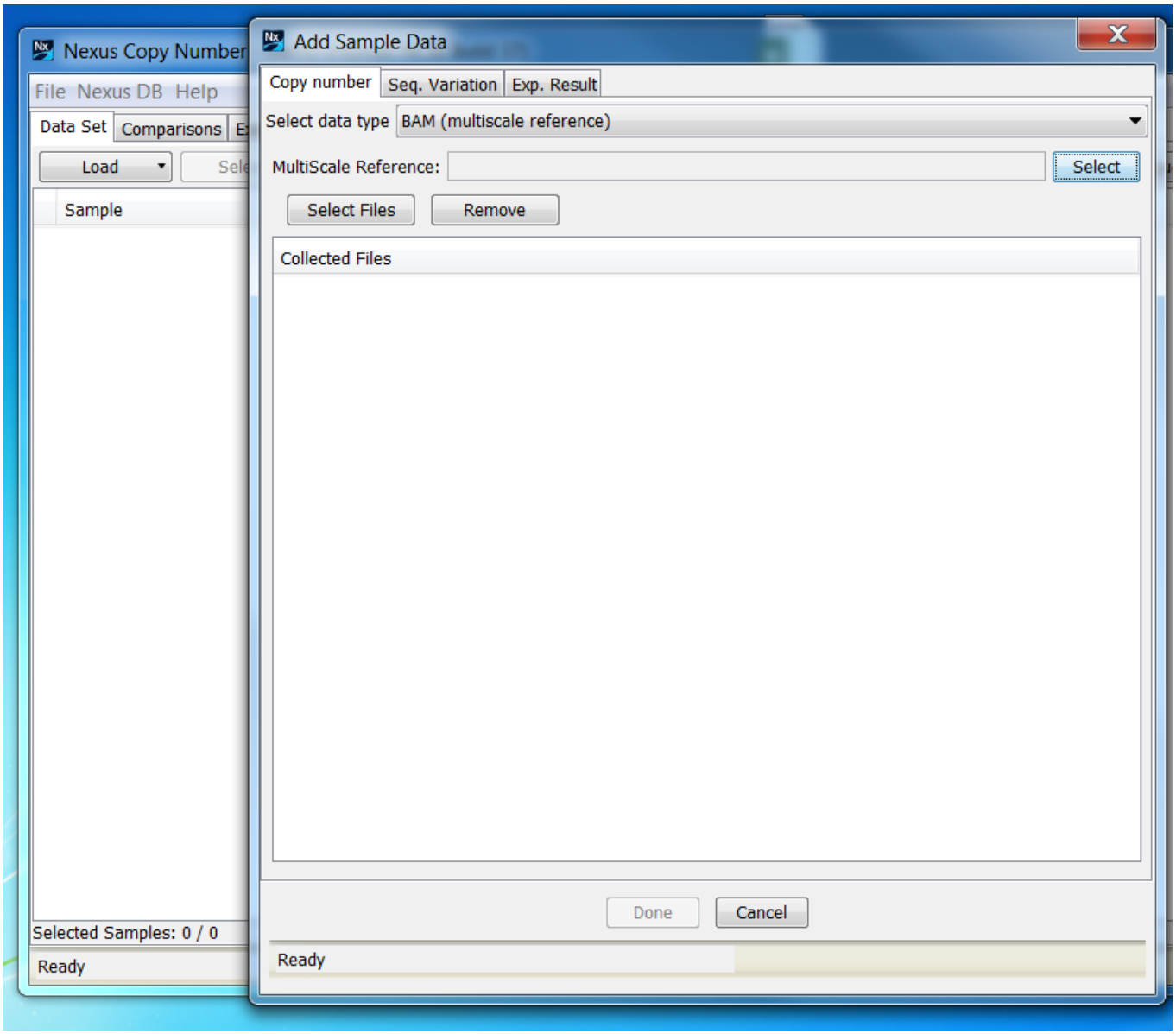
4.1 On the main screen, select "Load – Load Data".



4.2 For the option Data Type select: BAM (multiscale reference).

4.3 For the option Multiscale Reference select the reference you made previously.

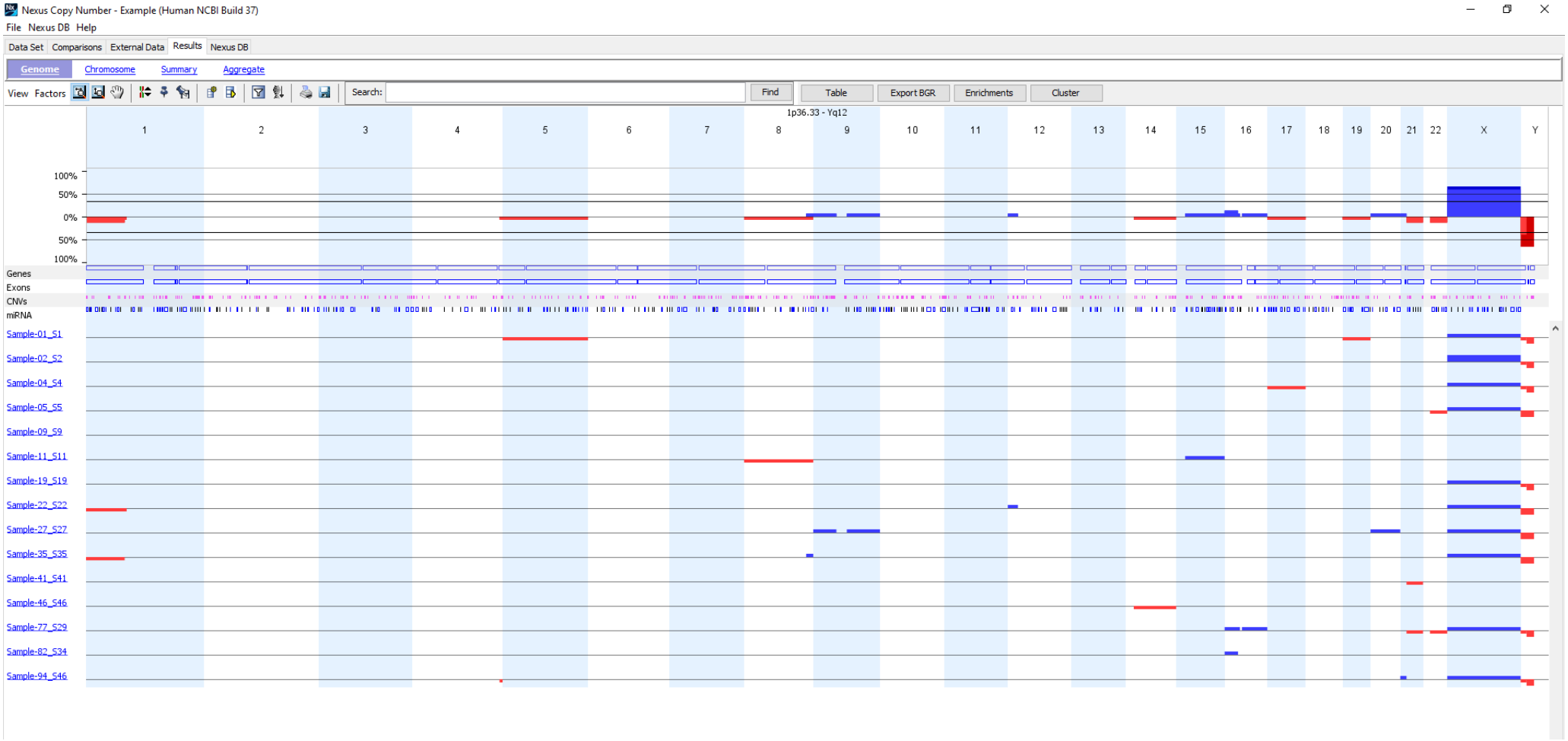
4.4 Press "Select Files" to choose the samples you wish to analyse.



4.5 Select "Open" and then "Done".

5. Results analysis and Interpretation

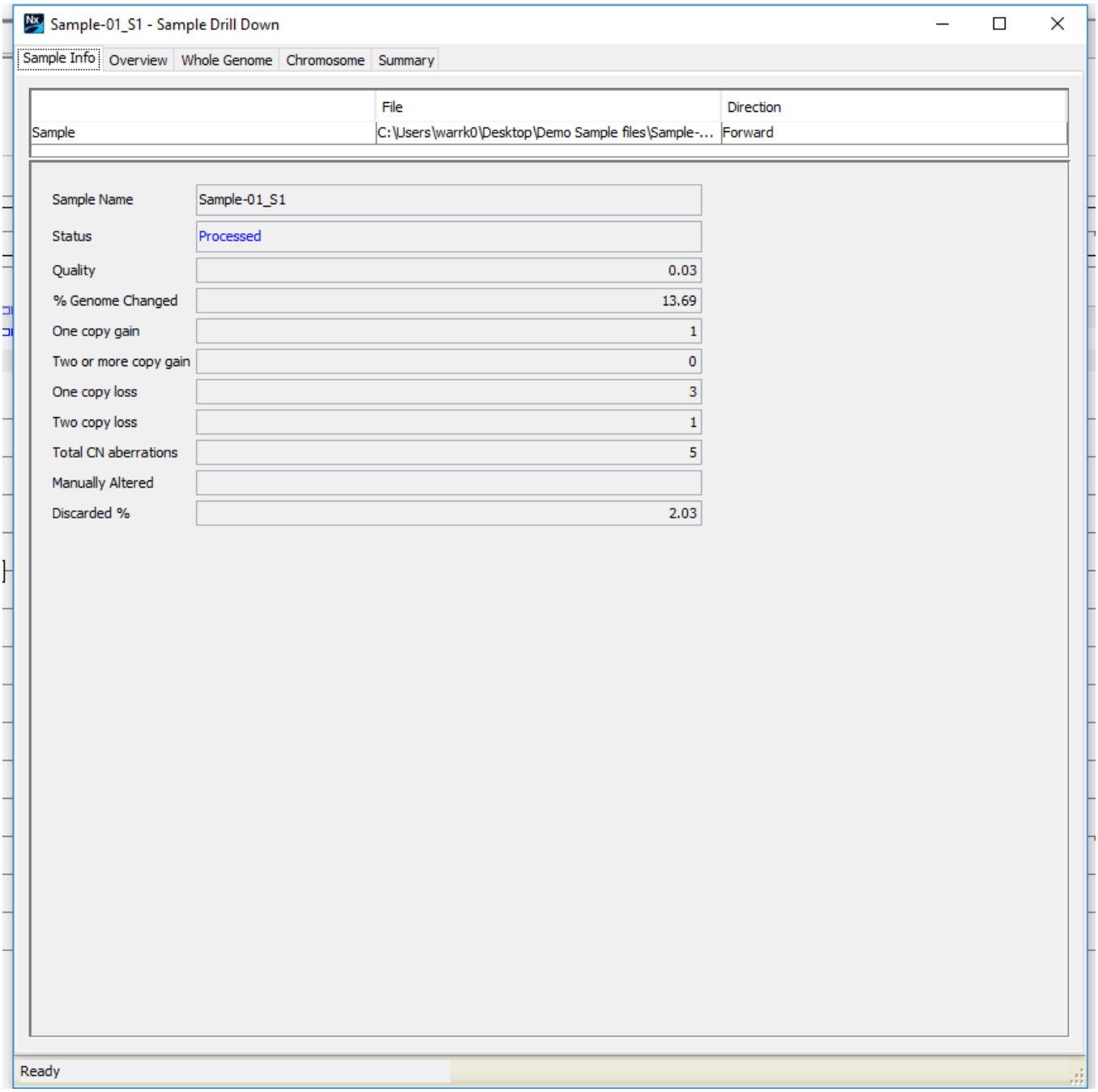
Once the analysis is completed you will see an overview screen displaying the samples along the y-axis and chromosome number on the x-axis. Copy number gains are highlighted blue and losses in red.



You can select an individual sample to view it in more detail by clicking on the sample name hyperlink in the first column on the y-axis to the left of the screen.

This will take you to the individual sample drill down menu where you have the following tabs:

a) Sample Info tab:

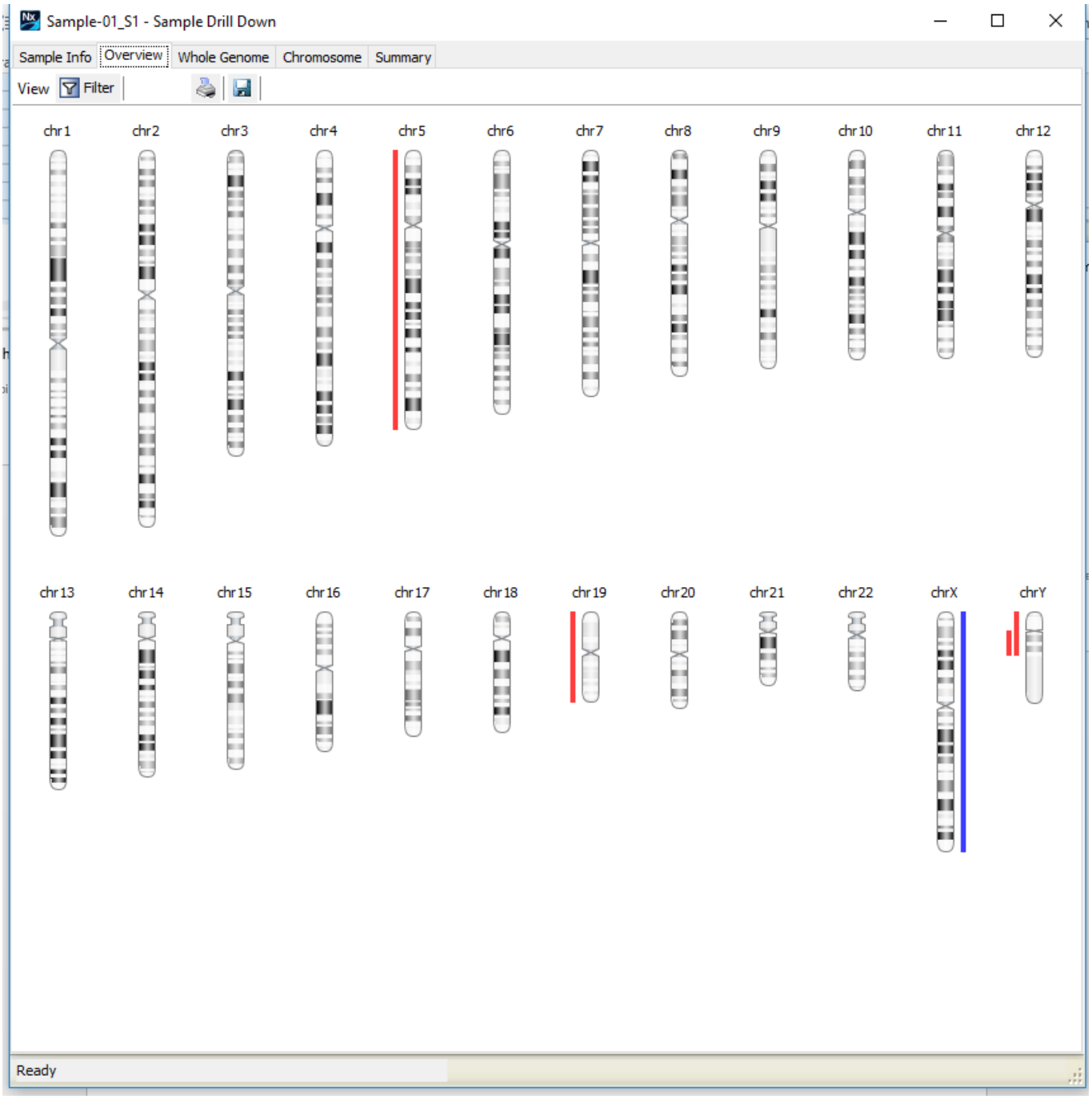


This tab displays information such as the sample file location, sample file name, the quality score and the number of copy gains or losses.

Saving Images

All images in the sample drill down can be saved by selecting the save button. These images can then be inserted into reports.

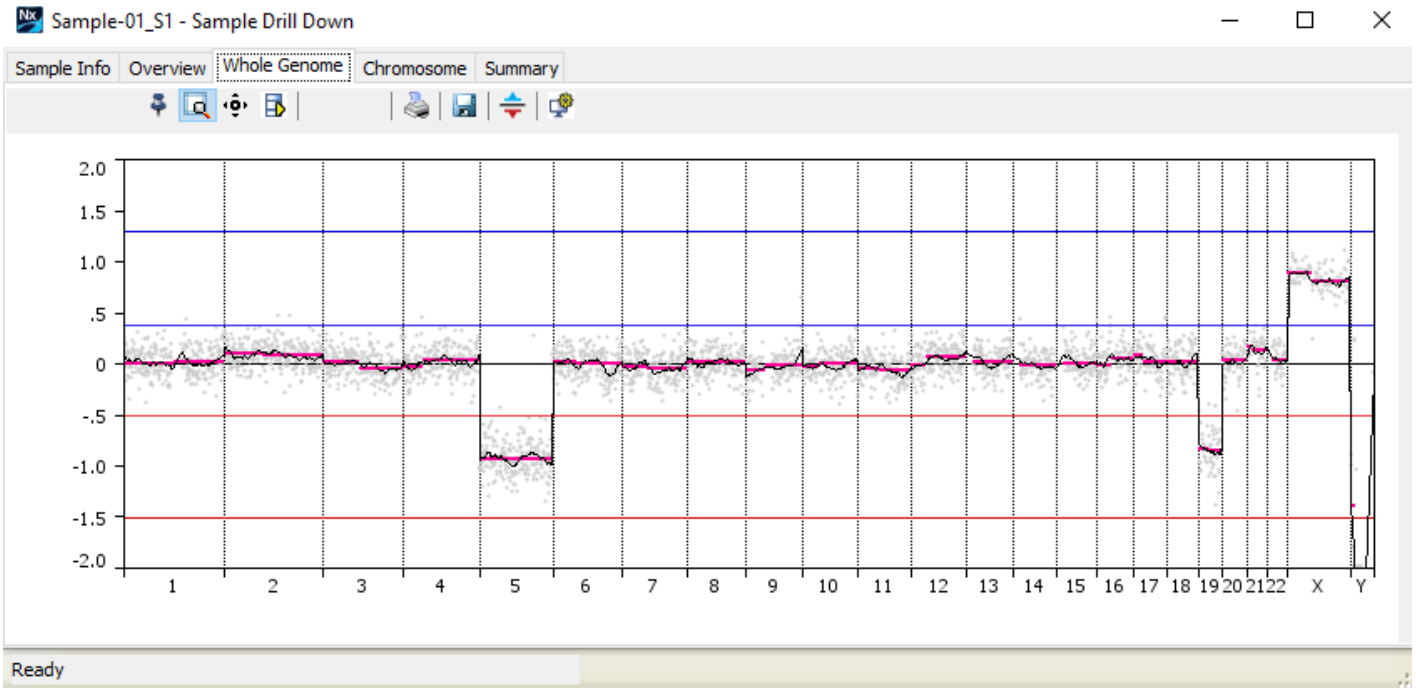
b) Overview tab:



The overview tab displays an ideogram with chromosome gains highlighted in blue and chromosome losses highlighted in red. A single red or blue line indicates one copy gain or loss whereas two lines indicates two copy gains or losses.

1. In this example there are two blue lines next to chromosome X, indicating two additional copies of Chromosome X.

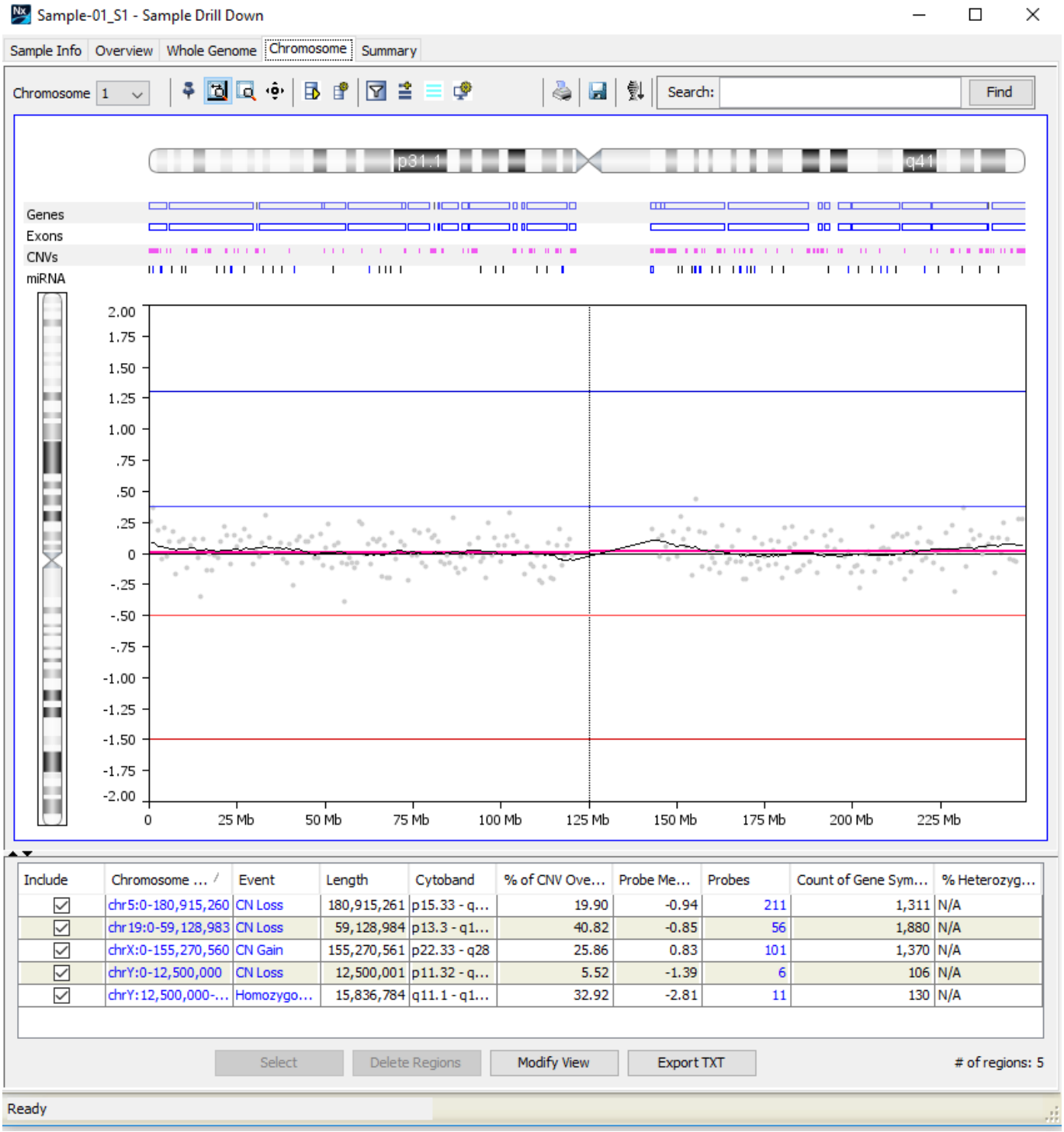
c) Whole Genome tab:



The whole genome tab is a graphical display of the chromosome profile. Chromosome numbers are listed on the x-axis and a log2 ratio of each normalised bin on the y-axis. Each grey dot or bin represents a position on the genome and the value of each bin is determined by calculating the number of reads in that location in comparison to the reference samples.

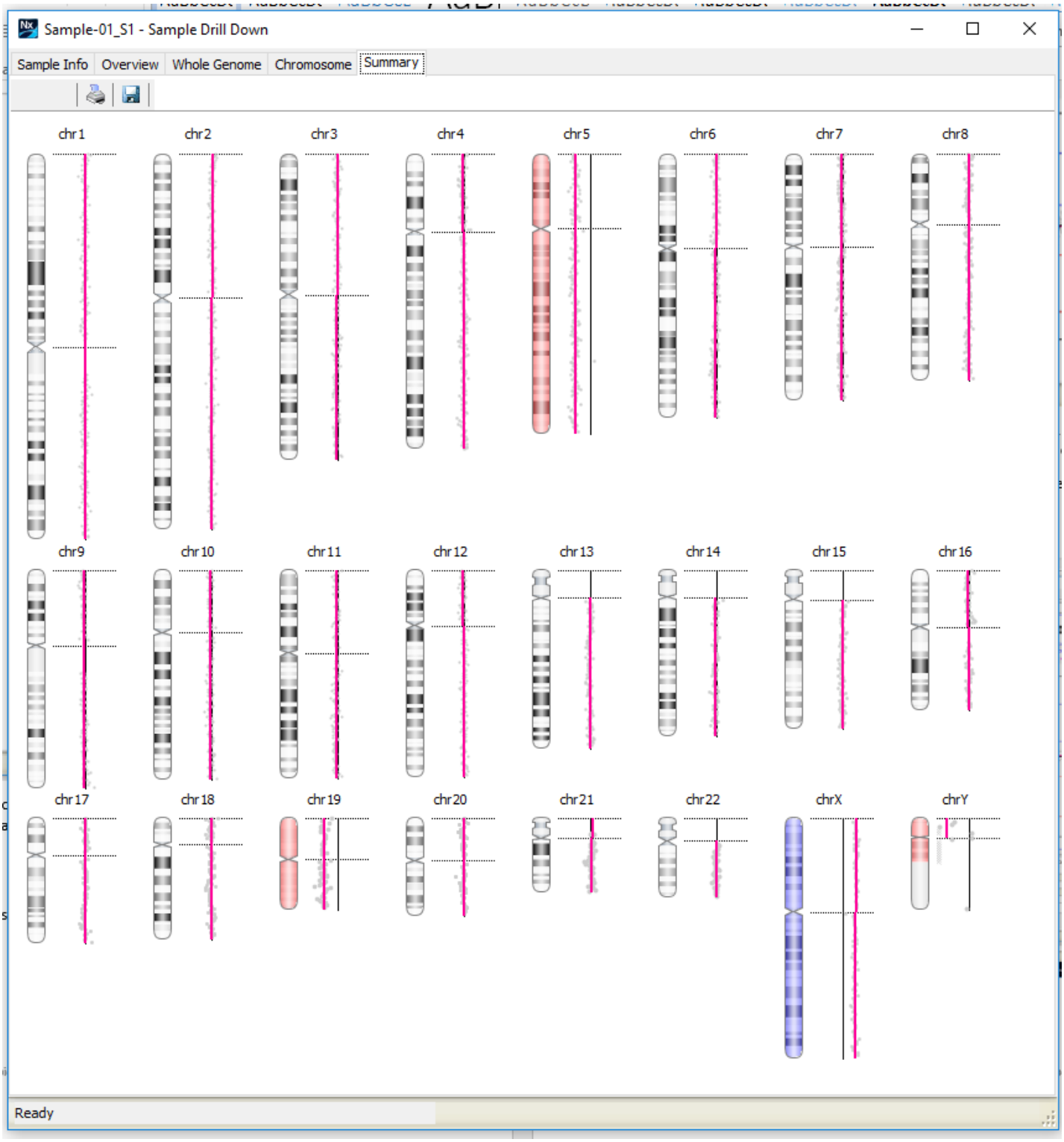
The pink segment line represents the call for each chromosome or part of chromosome and the black line is the moving average. When the segment line is above the first blue threshold line it is called as an additional copy (in comparison to a 46,XY reference), when it is above the second higher blue line it is called as two additional copies. Similarly when the segment is below the first red line it represents one copy loss, whereas when it is below the second red line it is two copy losses.

d) Chromosome tab:



The chromosome tab allows you to view each chromosome in more detail. Different chromosomes can be selected by using the drop down menu in the top left of the screen. As well as providing a zoomed image of each chromosome, all copy number events across all chromosomes for the sample are listed in the table at the bottom. Each event has additional details such as the length and cytoband position.

e) Summary tab:



The summary tab provides an ideogram similar to the overview tab which displays gains in blue and losses in red. This tab also includes a small graphic of the bins in relation to the Euploid threshold line.

6. Software Demo

For a software demonstration please contact PerkinElmer Health Sciences (Australia) Pty Ltd.

7. Ordering Information

Please contact your local distributor or PerkinElmer Health Sciences (Australia) Pty Ltd for ordering information. For more information on the products use, limitations, and licenses: www.perkinelmer.com

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PerkinElmer Health Sciences (Australia) Pty Ltd (ABN 84 010 126 708)
40-46 West Thebarton Road THEBARTON SA AUSTRALIA 5031
E: info@rhsc.com.au Ph: +61 88152 9383