

HOW TO LOAD (UNMATCHED) NGS DATA USING BAM MSR (MULTISCALE REFERENCE) DATA TYPE:

Nexus Copy Number can load and process data from WES, WGS, or targeted panels. It works well on normal coverage data as well as low pass sequencing. The software loads BAM files and can also compute BAF from these files using the BAM MSR (multiscale reference) data type. It requires use of a reference file that needs to be built before samples can be processed. For matched tumor-normal pairs, please see the guide **How to Load (matched) NGS Data**.

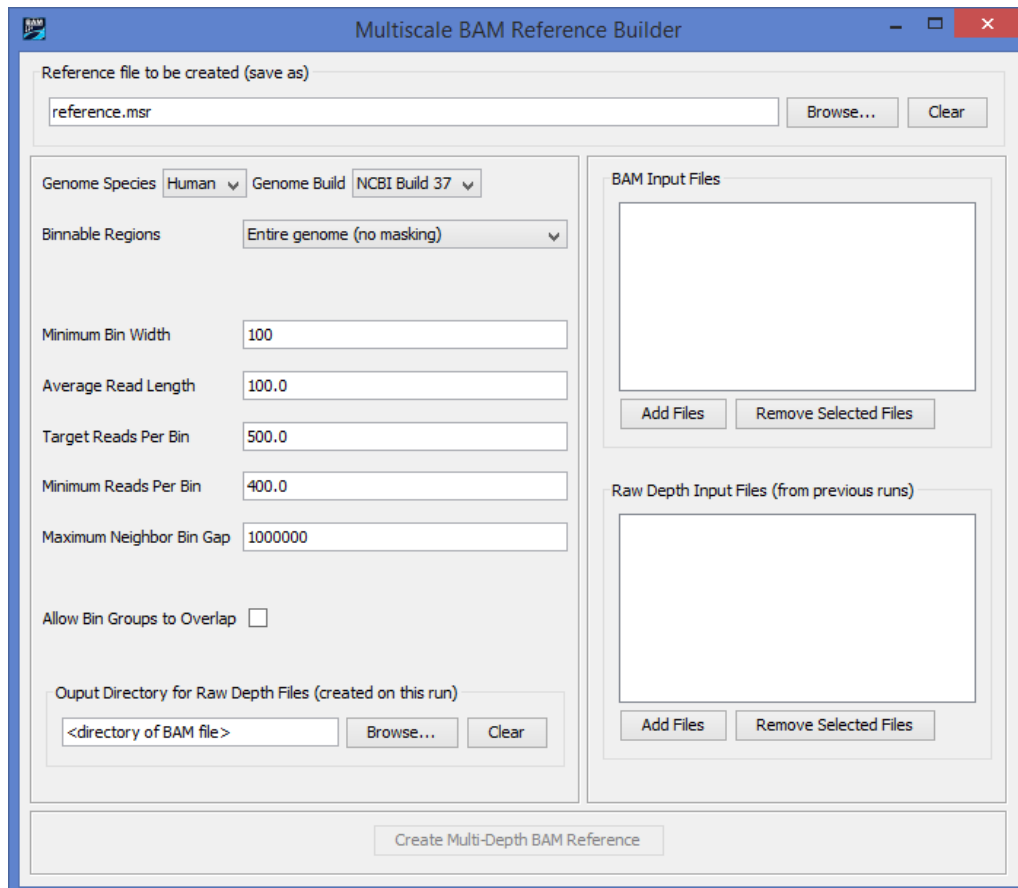
Note: *It is recommended to increase the memory allocated to the Multiscale BAM Reference Builder to 50% of total memory available; at minimum, the reference builder requires 4GB of memory. You need to edit the “MultiScale BAM Reference Builder.lax” file in the installation directory. E.g. for win64 machines, to increase the memory to 8GB, edit the line to*

`lax.nl.java.option.additional=-Xms500m -Xmx8000m`

If you need assistance with this, please contact [BioDiscovery Support](#).

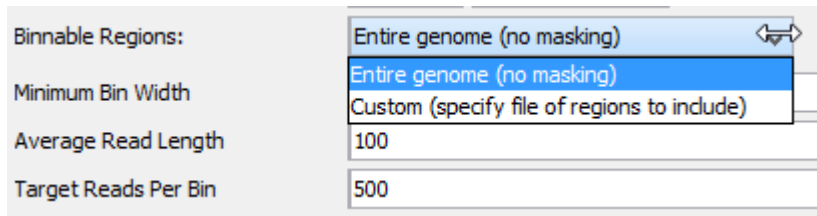
BUILDING THE REFERENCE FILE

Double click on the Multiscale BAM Reference Builder icon to open the window below.



Hovering over the field labels will provide more details on the parameters.

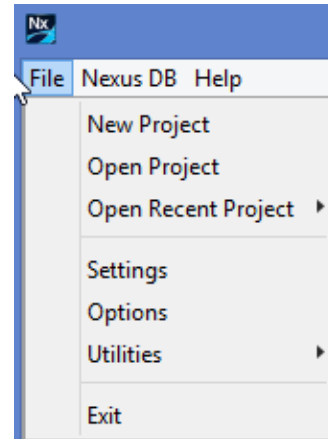
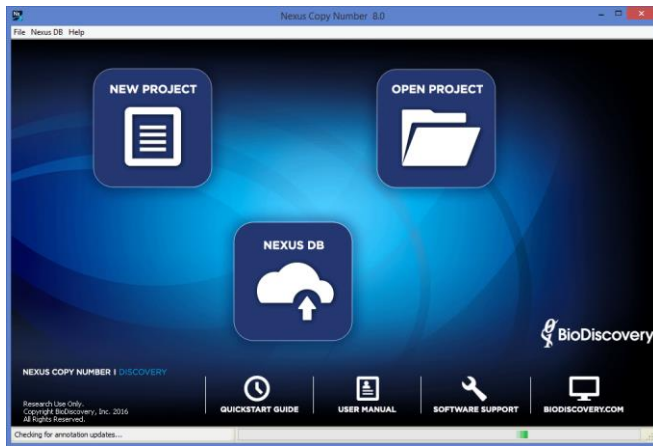
1. Give a name to your reference file in the **Name for reference to be Created** field. You will later select this file from a dropdown when loading your BAM files. Select the folder where you want to save this file. This is the name that will appear in the dropdown to select the reference file when loading data of the type BAM (multiscale reference) in Nexus Copy Number.
2. Select the appropriate **Genome Build**.
3. Select **Entire genome (no masking)** to get copy number estimation over the entire genome. If you wish to limit results to only specific regions of the genome, select “Custom (specify file of regions to include)” to specify a BED file to use to create a meaningful reference file. The file must list genomic regions over which copy number variation should be estimated. The first three columns should specify chromosome, start, and stop positions, respectively.



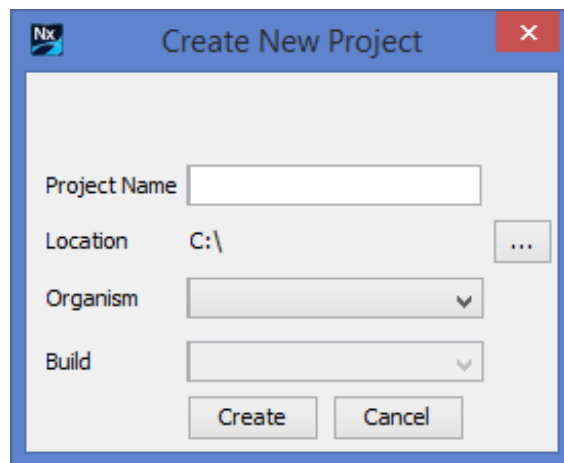
4. Leave the default values in the **Minimum Bin Width**, **Average Read Length**, **Target Reads Per Bin**, and **Maximum Neighbor Bin Gap** fields. Leave the **Allow Bin Gaps to Overlap** box unchecked.
5. Select an Output Directory for storing the Raw Depth Files which are intermediary files
6. Click on **Add Files** in the **BAM Input Files** field and select your BAM files via the File Chooser. A minimum of 10 normal samples are recommended to create the reference file. Highlight a file and click “Remove Selected Files” if you want to remove a file from the list.
7. For the first time creating a reference file, leave the **Raw Depth Input Files** field empty; these intermediary files are typically not needed. For more information on depth files, please review the User Manual.
8. Click **Create Multi Depth BAM Reference**. The reference file will be automatically saved to the selected folder.

LOADING AND PROCESSING BAM FILES

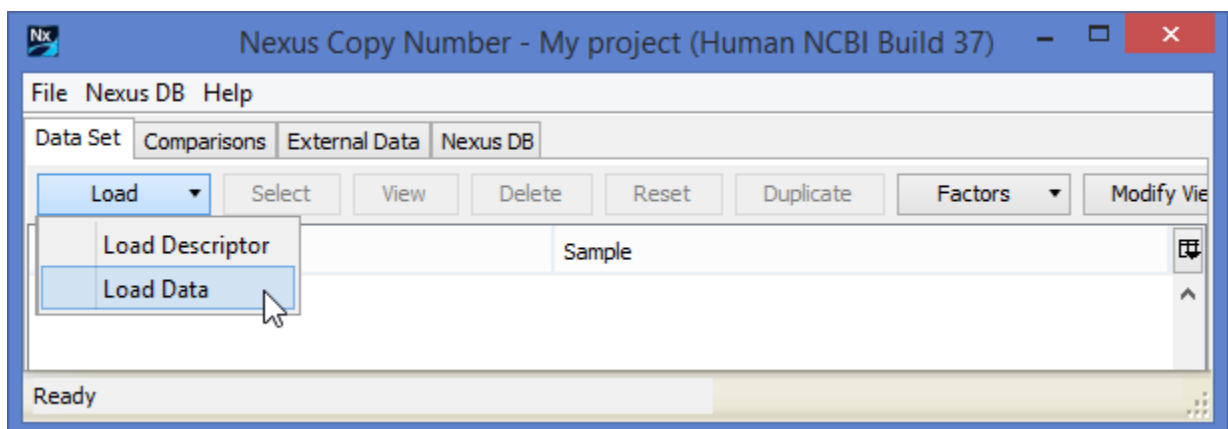
1. Launch Nexus Copy Number. Create a new project or open an existing project from the start page. You can also open and create projects from the **File** menu.



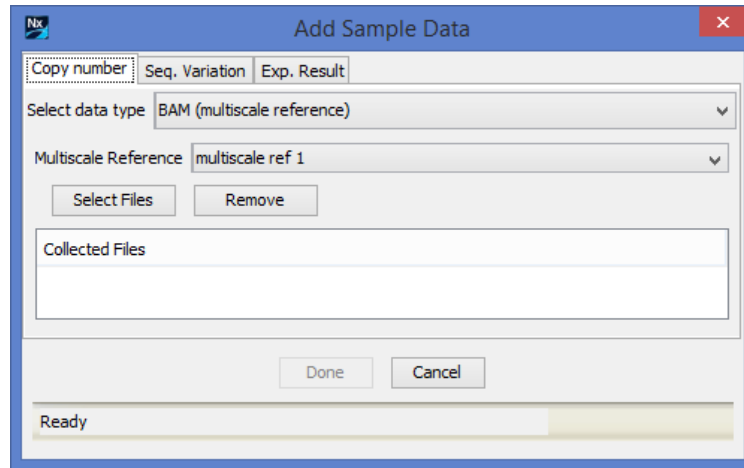
- 2. Specify a name for your project and a place to save it on your computer. Select the appropriate genome and build, based on the mapping information for your probes.



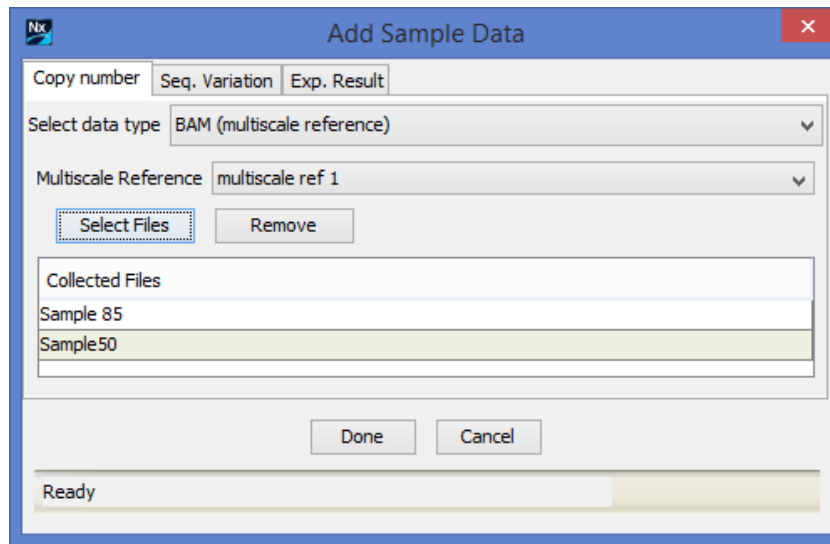
- 3. Select **Load** -> **Load Data** from the Data Set tab.



- 4. In the **Add Sample Data** window, select **BAM (multiscale reference)** from the **Select data type** drop down in the **Copy Number** tab.



- a. From the **BAM (multiscale reference)** drop down, select the reference file you just created.
- b. Click **Select Files** to select your **BAM** files to load into Nexus Copy Number. You can select any number of files but they must all be BAM files. Once you have finished selecting files via the File Chooser, click **Open** and the sample file names will be listed in the **Collected Files** section.



- c. Click **Done** after you have selected all your files.
5. Samples will now be loaded and processed. The Data Set tab will be displayed with a progress bar at the bottom showing the loading/processing status of the samples. The **Status** column of the table will also change from **Unprocessed** to **Processed** upon sample processing completion. Once processing is complete, results will be displayed in the window.