TO PROCESS AGILENT (CGH+SNP ARRAY) DATA GENERATED WITH FEATURE EXTRACTION:

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, a place to save it on your computer, a genome and then the appropriate genome build based on the mapping information for your probes.

NK (Create New Project	×
Destanting	- [1
Project Nam	e	
Location	C:\	
Organism	~ ~]
Build	v]
	Create Cancel	

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

>>	Nexu	is Copy Number - My project (Human NCBI Build 37) 🗧	. 🗆	×
File Nex	us DB Help			
Data Set	Comparisons E	xternal Data Nexus DB		
Loa	d 🔹 Selec	t View Delete Reset Duplicate Factors	•	Modify Vie
Lo	ad Descriptor	Sample		t ₽
Lo	oad Data			^
	13			
Ready				

4. In the Add Sample Data window, select Agilent SNP FE from the "Select data type" drop down in the "Copy

Number" tab. A number of options will be displayed. Clicking on the icon will provide more details on the parameters.

Market Add Sample Data	×
Copy number Seq. Variation Exp. Result	
Select data type Agilent SNP FE	v
Reference European Male (NA12891_v1).txt	*
Set processing settings based on the following and process	
 For mosaic samples such as cancer (possible increased false-positive rate) For non-mosaic samples 	
Stringency: Lenient Average Stringent	
Perform Systematic Correction	
Array Types:	
Select Files Remove	
Collected Files	
Done Cancel Ready	

a. Select the reference file you wish to use. If the reference file you require is not listed, contact BioDiscovery Support (support@biodiscovery.com) to obtain the necessary files.



b. The option **Set processing settings based on the following and process** uses default parameters for the type of data you are loading and immediately begins processing after data loading is complete.

For the **Agilent SNP FE** data type, **you must uncheck the box** as you need to add a factor to the samples after data loading and before processing can commence.



c. Click Select Files to select your Agilent Feature Extraction files to load into Nexus Copy Number. You can select any number of files but they must all be Agilent Feature Extraction files from Agilent CGH+SNP arrays. 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:

Click **Done** after you have selected all your files.

5. Samples will be loaded but not processed and will appear in the Data Set tab with Status as "Unprocessed":

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File Nexus DB	Help								
Data Set Compa	arisons External Data Nexus DB								
Load 🔹	Select View	Delete	Reset Dup	olicate	Factors 🔹	Modify View Que	ery	Tools	•
	Sample	Status	Data Type	Quality	Discarded %	Agilent SNP Reference			₽
✓	1_CytoCGH_0209_4x	Unprocessed	Agilent SNP FE		0.0	AgilentSNPReferenceFile	s\European M	lale (NA12	~
✓	2_CytoCGH_0209_4x	Unprocessed	Agilent SNP FE		0.0	AgilentSNPReferenceFile	s\European M	lale (NA12	
					×				~
<								>	
Selected Samples:	2/2								
Ready									

6. You must add a factor called **Control Gender** and specify the control gender for your samples. Without this value, samples will not be processed. From the Data Set tab, click on the **Factors** button, select **Add Factor** and an Input Dialog will open asking you to give the factor a name. Enter **Control Gender** and click **OK**.

	Factors Modify View		
	Add Fact A	Input	×
Т	Load Factors		
	Make Factor	Factor Name	
	Make Trio factors	Control Gender	
	Delete Factors	OK Cancel	
	Delete Empty Factors		

The new column shows up at the end of the table. You can either enter values manually by clicking on each cell and typing or you can copy and paste a column from a spreadsheet. See the User Manual for additional details on adding factors.

Nx		Nexu	is Copy Nun	nber - My	y Project (Hu	man NCBI Bi	uild 37)	-	•	×
File	Nexus DB	Help								
Data	Set Comp	arisons E	kternal Data N	exus DB						
	Load	Select	tView	Delete	Reset	Duplicate	Factors	s 🔻	Modify	y Vie
	Sample	Status	Data Type	Quality	Discarded %	Agilent SNP Ref	ference	Contro	l Gen	₽₽
✓	1_CytoC	Unproce	Agilent SNP FE		0.0	AgilentSNPRefer	enceFil	Male		^
v	2_CytoC	Unproce	Agilent SNP FE		0.0	AgilentSNPRefe	renceFil	Male		
										\mathbf{v}
Selec	ted Samples	:2/2								
Read	dy									:

- 7. It is recommended to apply Systematic Correction (GC wave correction). **Systematic Correction** corrects the wavy pattern often present in the probe distribution. Since the correction is based on probes used, the specific array is needed.
 - a. Open the Settings window via the File menu.



b. Scroll down to the Systematic Correction section. Choose the **Type** of correction (linear, quadratic, etc.) from the dropdown.

Systematic Correction	۲
Type: Quadratic Correction	~
File not selected	

- c. Select the correction file to use by clicking on the field displaying "not selected". A file chooser window will pop up. Navigate to the Systematic Correction folder (based on the organism and build for your project) in the Nexus Copy Number installation directory.
 - E.g. ...\Nexus 8.0\Organisms\Human\NCBI Build 37\SystematicCorrection\Agilent SNP

	Open					
Look in:	🌗 Agilent SNP 🗸 🦻 📴 🖬 🗸					
Recent Items	Catalog_Agilent_Cancer_CGH+SNP_180K_030587_20120515 Catlg_Agilent_CGH+SNP_400K_028081_20120726					
Desktop	File name: not selected Q Files of type: All Files Ca)pen ancel				

Select the appropriate file and click **Open**. If a correction file for your array type is not here, please contact BioDiscovery Support (<u>support@biodiscovery.com</u>) to obtain the necessary files. You can continue to process without performing the correction; just uncheck the box. You can reprocess the samples later with systematic correction applied.

d. To adjust other settings from the default parameters, see the section **Settings** in the **User Manual** for more details. Once all settings are adjusted, click **Done** to apply those settings. Then click on **View** in the Data Set tab to process and see the results.