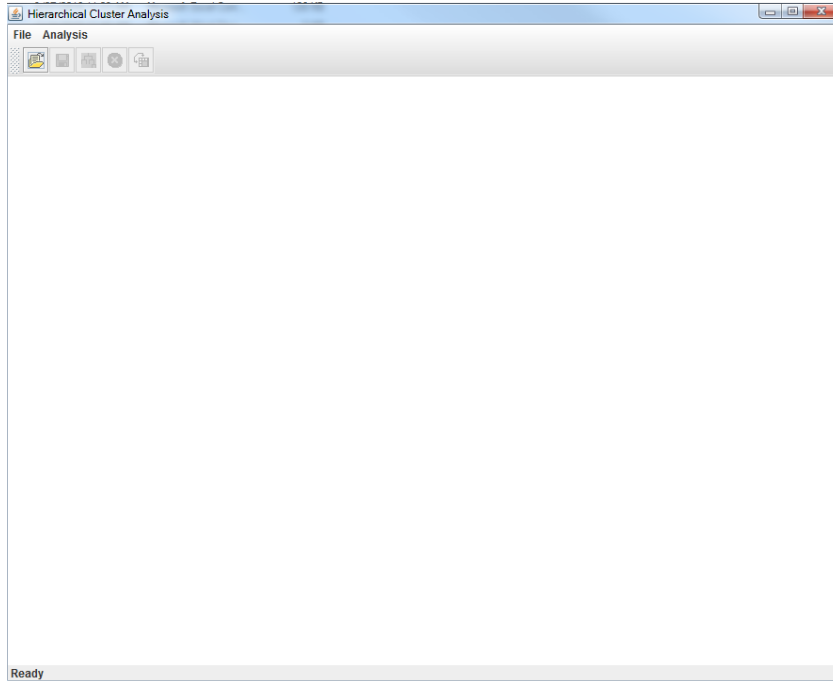


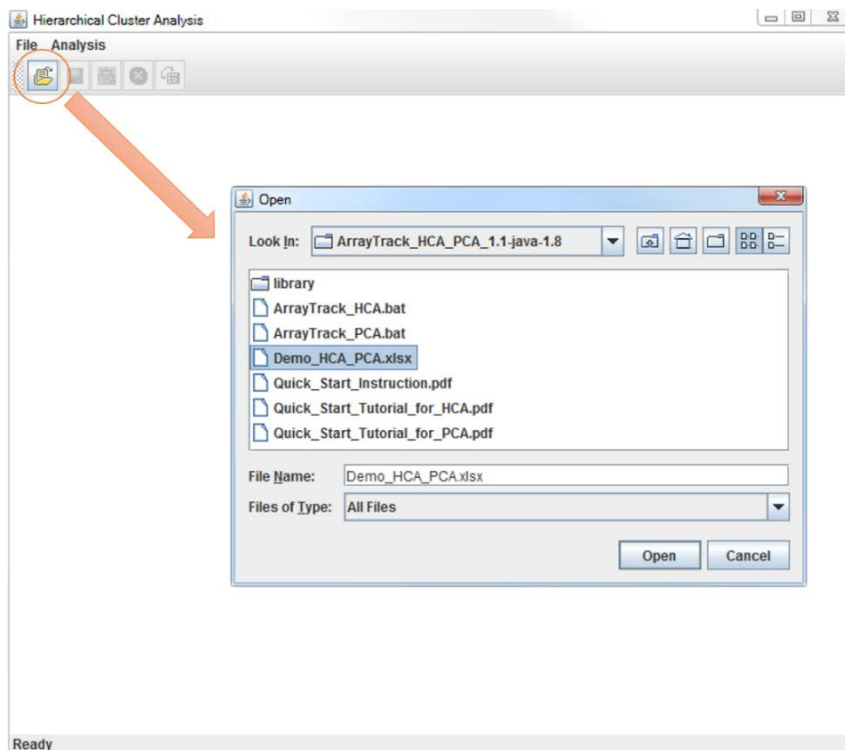
# For Windows OS

## Part 1. Load Data

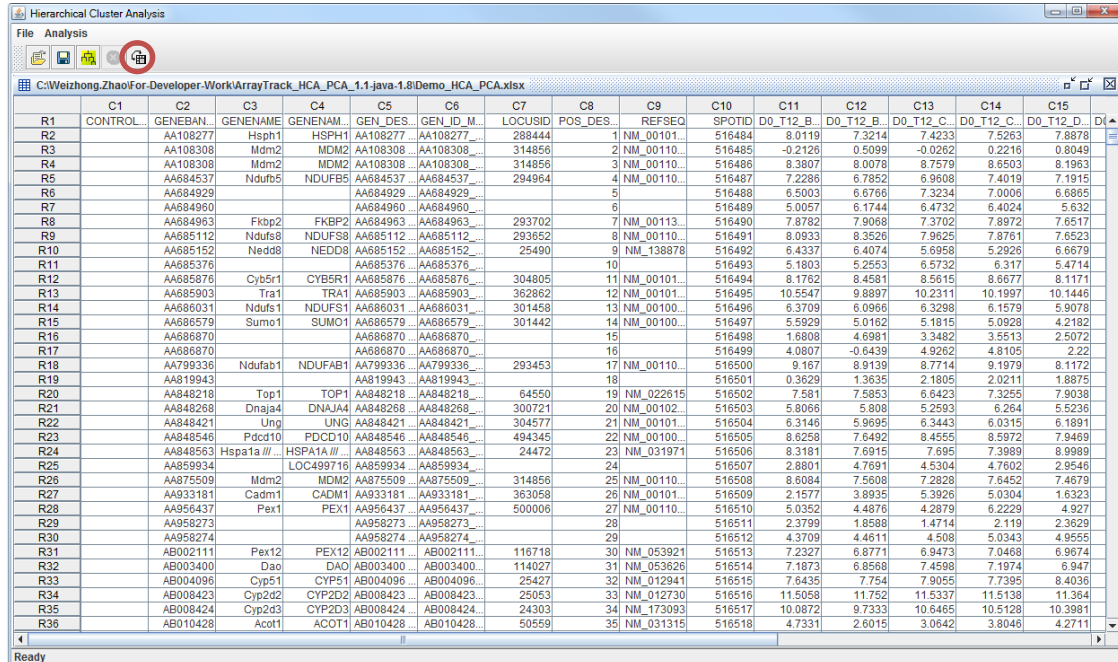
Step1. Double click the batch script file “ArrayTrack\_HCA.bat” to open HCA tool.



Step2. Click the “Open File” button (marked with a red circle) to open a file browser window.



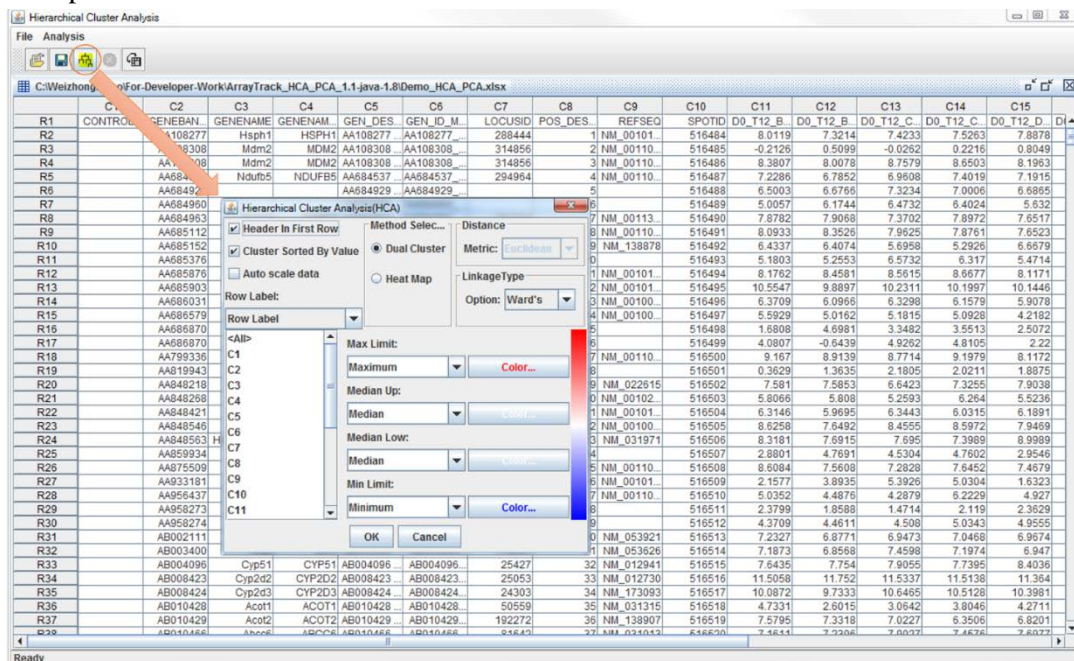
Step3. Find the sample data set (“Demo\_HCA\_PCA.xlsx” in this tutorial) by the file browser window, load the data by selecting the “Open” button, and get the window as shown. In default, each row represents a sample.



	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
	CONTROL	GENEBAN	GENENAME	GENENAM	GEN_DES	GEN_ID_M	LOCUSID	POS_DES	REFSEQ	SPOTID	D0_T12_B	D0_T12_D	D0_T12_C	D0_T12_C	D0_T12_D
R1		AA108277	Hsp1	HSPH1	AA108277		288444	1	NM_00101...	516484	8.0119	7.3214	7.4233	7.5263	7.8878
R2		AA108308	Mdm2	MDM2	AA108308		314856	2	NM_00110...	516485	-0.2126	0.5099	-0.0262	0.2216	0.8049
R3		AA108308	Mdm2	MDM2	AA108308		314856	3	NM_00110...	516486	8.3807	8.0078	8.7579	8.8503	8.1963
R4		AA684537	Nduf5	NDUF5	AA684537		294964	4	NM_00110...	516487	7.2286	6.7852	6.9608	7.4019	7.1915
R5		AA684929			AA684929			5		516488	6.5003	6.6766	7.3234	7.0006	6.6865
R6		AA684960			AA684960			6		516489	5.0057	6.1744	6.4732	6.4024	5.632
R7		AA684963	Fkbp2	FKBP2	AA684963		293702	7	NM_00113...	516490	7.8782	7.9068	7.3702	7.8972	7.6517
R8		AA685112	Ndufs8	NDUFS8	AA685112		293652	8	NM_00110...	516491	8.0933	8.3526	7.9625	7.8761	7.6523
R9		AA685152	Nedd8	NEDD8	AA685152		25490	9	NM_138878	516492	6.4337	6.4074	5.6958	5.2926	6.6679
R10		AA685376			AA685376			10		516493	5.1803	5.2553	6.5732	6.317	5.4714
R11		AA685876	Cyb5r1	CYB5R1	AA685876		304805	11	NM_00101...	516494	8.1762	8.4581	8.5615	8.6677	8.1171
R12		AA685903	Trt1	TRT1	AA685903		362862	12	NM_00101...	516495	10.5547	9.8897	10.2311	10.1997	10.1446
R13		AA686031	Ndufs1	NDUF51	AA686031		301458	13	NM_00100...	516496	6.3709	6.0966	6.3298	6.1579	5.9078
R14		AA686579	Sumo1	SUMO1	AA686579		301442	14	NM_00100...	516497	5.5929	5.0162	5.1815	5.0928	4.2182
R15		AA686870			AA686870			15		516498	1.6808	4.6981	3.3482	3.5513	2.5072
R16		AA686870			AA686870			16		516499	4.0807	-0.6439	4.9262	4.8105	2.22
R17		AA799336	Ndufab1	NDUFAB1	AA799336		293453	17	NM_00110...	516500	9.167	8.9139	8.7714	9.1979	8.1172
R18		AA819943			AA819943			18		516501	0.3629	1.3635	2.1805	2.0211	1.8875
R19		AA848218	Top1	TOP1	AA848218		64550	19	NM_022615	516502	7.581	7.5853	6.6423	7.3255	7.9038
R20		AA848268	Dnaj4	DNAJ4	AA848268		300721	20	NM_00102...	516503	5.8066	5.808	5.2593	6.264	5.5236
R21		AA848421	Ung	UNG	AA848421		304577	21	NM_00101...	516504	6.3146	5.9695	6.3443	6.0315	6.1891
R22		AA848546	Pdcd10	PDCD10	AA848546		494345	22	NM_00100...	516505	8.6258	7.6492	8.4555	8.5972	7.9469
R23		AA848553	Hspa1a	HSPA1A	AA848553		24472	23	NM_031971	516506	6.3181	7.6915	7.695	7.3989	8.9989
R24		AA859934			AA859934			24		516507	2.8801	4.7691	4.5304	4.7602	2.9546
R25		AA875509	Mdm2	MDM2	AA875509		314856	25	NM_00110...	516508	8.6084	7.5608	7.2828	7.6452	7.4679
R26		AA933181	Cadm1	CADM1	AA933181		363058	26	NM_00101...	516509	2.1577	3.8935	5.3926	5.0304	1.6323
R27		AA956437	Pex1	PEX1	AA956437		500006	27	NM_00110...	516510	5.0352	4.4876	4.2879	6.2229	4.927
R28		AA958273			AA958273			28		516511	2.3799	1.8588	1.4714	2.119	2.3629
R29		AA958274			AA958274			29		516512	4.3709	4.4611	4.508	5.0343	4.9555
R30		AB002111	Pex12	PEX12	AB002111		116718	30	NM_053921	516513	7.2327	6.8771	6.9473	7.0468	6.9674
R31		AB003400	Dao	DAO	AB003400		114027	31	NM_053626	516514	7.1873	6.8568	7.4598	7.1974	6.947
R32		AB004096	Cyp51	CYP51	AB004096		25427	32	NM_012941	516515	7.6435	7.754	7.9055	7.7395	8.4036
R33		AB008423	Cyp2d2	CYP2D2	AB008423		25053	33	NM_012730	516516	11.5058	11.752	11.5337	11.5138	11.364
R34		AB008424	Cyp2d3	CYP2D3	AB008424		24303	34	NM_173093	516517	10.0872	9.7333	10.6465	10.5128	10.3981
R35		AB010428	Acot1	ACOT1	AB010428		50559	35	NM_031315	516518	4.7331	2.6015	3.0642	3.8046	4.2711

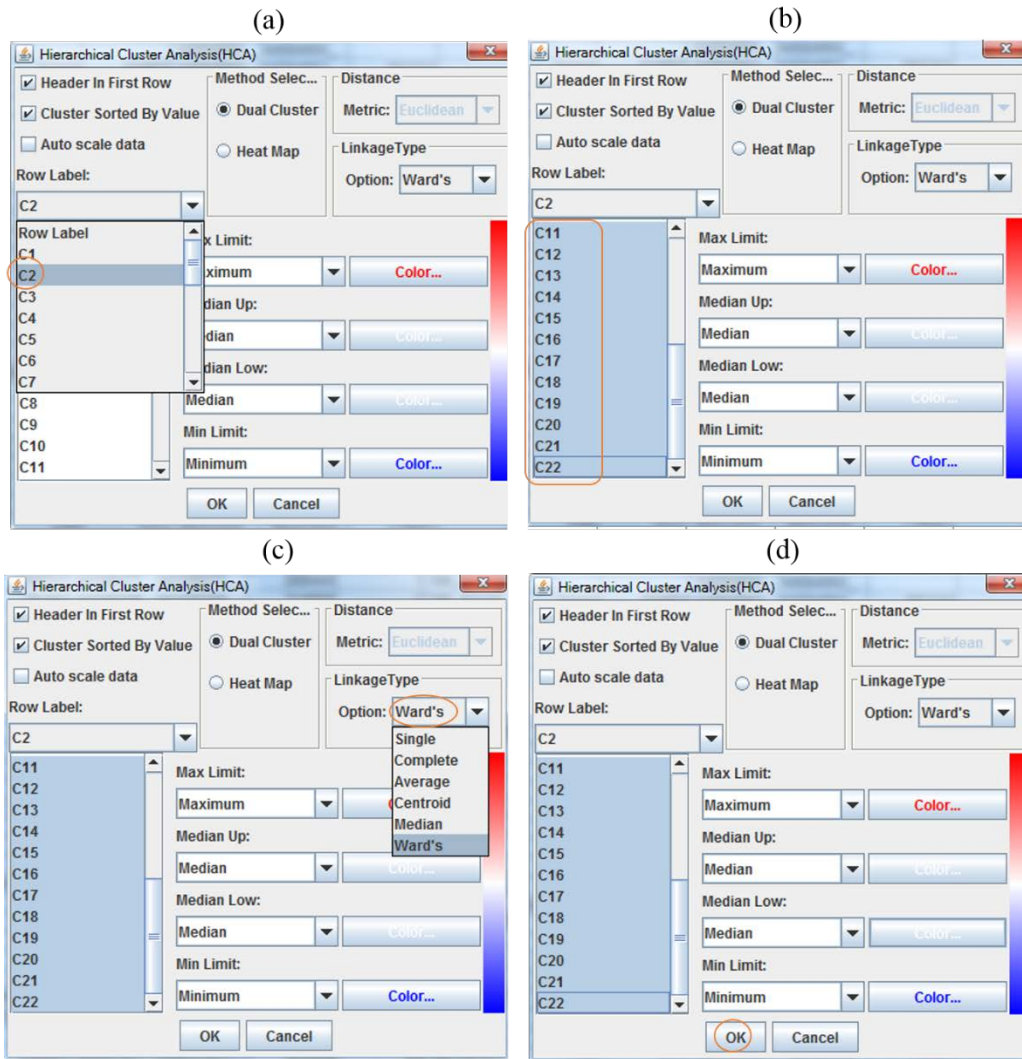
## Part 2. HCA Analysis Parameter Setting

Step1. Click the “Hierarchical Cluster Analysis” button (marked with a red circle) to open the HCA setup window.

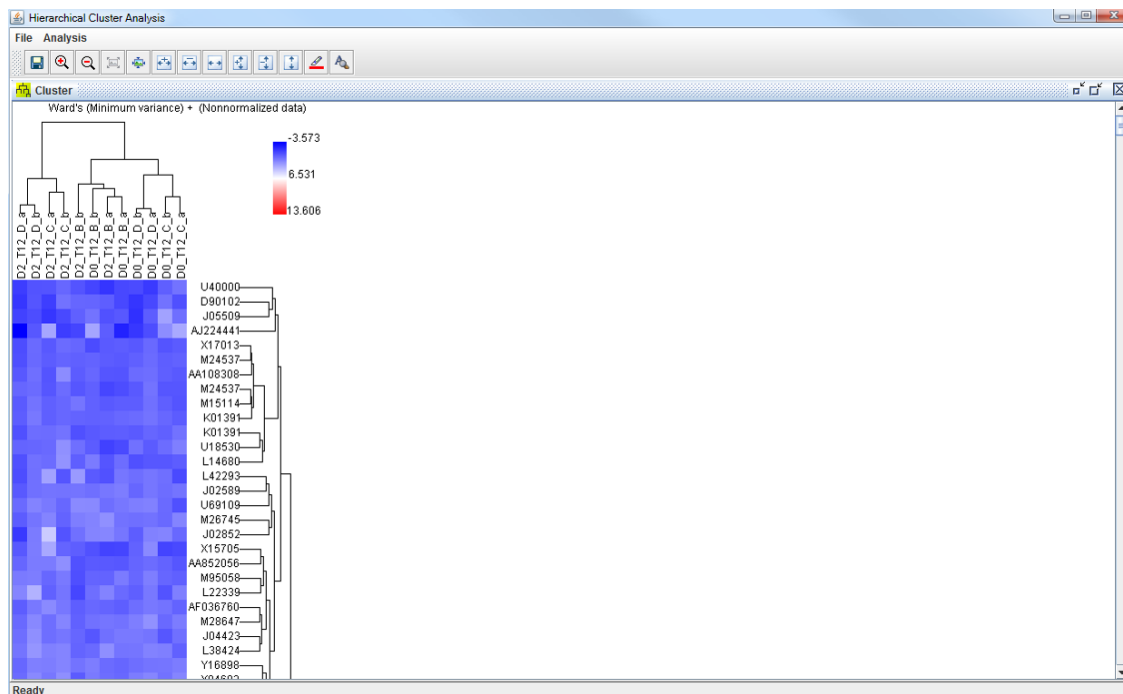


The screenshot shows the 'Hierarchical Cluster Analysis (HCA)' dialog box. The 'Header In First Row' checkbox is checked. The 'Cluster Sorted By Value' checkbox is checked. The 'Auto scale data' checkbox is checked. The 'Method Selection' section has 'Dual Cluster' selected. The 'Linkage Type' is set to 'Ward's'. The 'Metric' is set to 'Euclidean'. The 'Option' is set to 'Ward's'. The 'Max Limit' is set to 'Maximum'. The 'Median Up' checkbox is checked. The 'Median Low' checkbox is checked. The 'Min Limit' is set to 'Minimum'. The 'Color' button is highlighted.

Step2. To setup the parameters: (a) Pick the Row Label (column C2 in the demo data) which will not be used for HCA analysis; (b) Choose the expression values (columns C11-C22 in the demo data) to do HCA; (c) Choose the “Linkage Type” (“Ward’s” as default; more details of Linkage Type can be found in Wikipedia [https://en.wikipedia.org/wiki/Hierarchical\\_clustering](https://en.wikipedia.org/wiki/Hierarchical_clustering)) in HCA analysis; (d) Click “OK” button to start the HCA analysis.

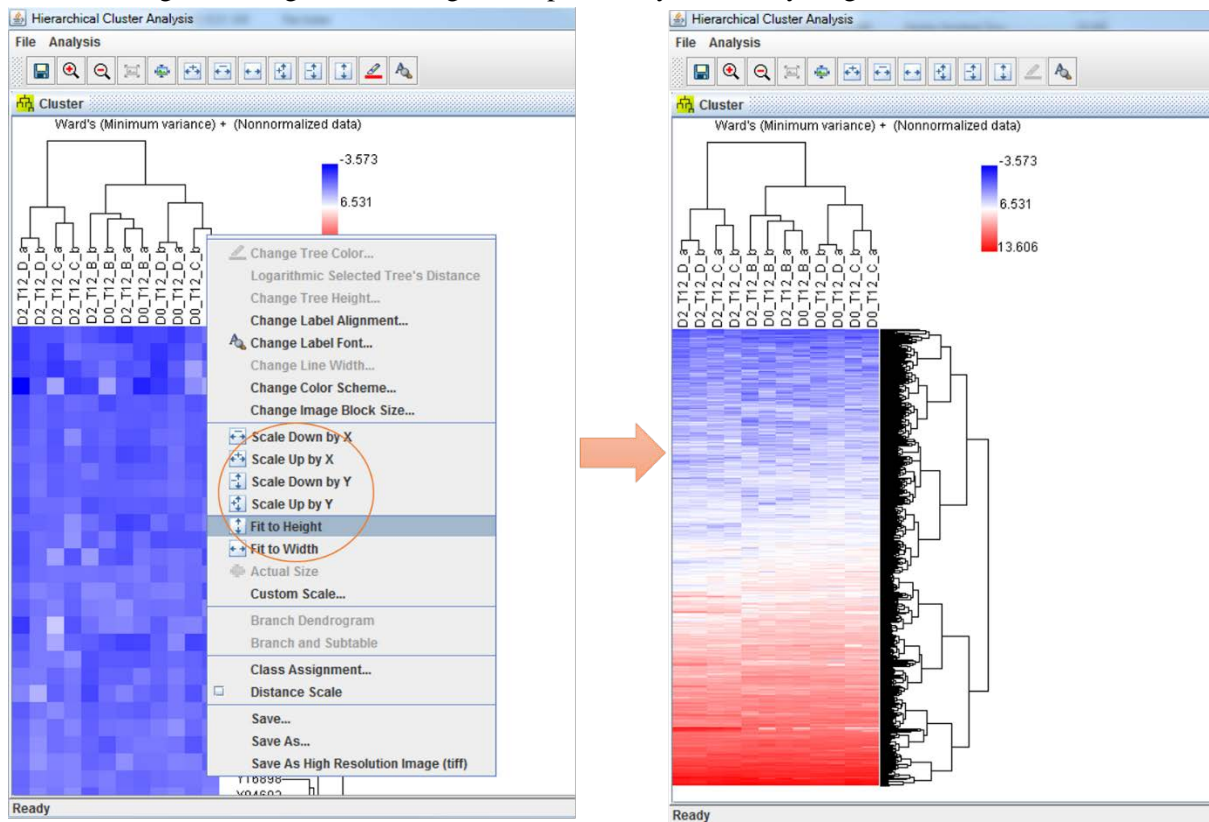


Step3. After HCA analysis has finished, results will be shown (in default setting) as follows.

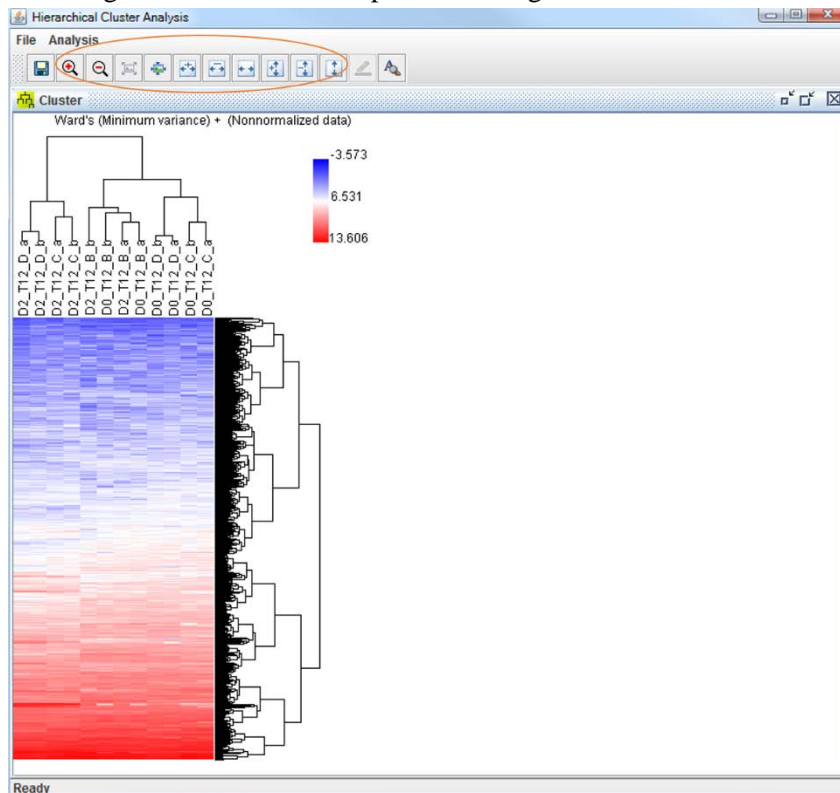


### Part 3. Result Presentation

Step1. Right click the result figure to access the context menu. Use the context menu to change the default settings of the figure, including scale up/down by axis, fit by height/width, custom scale, etc.

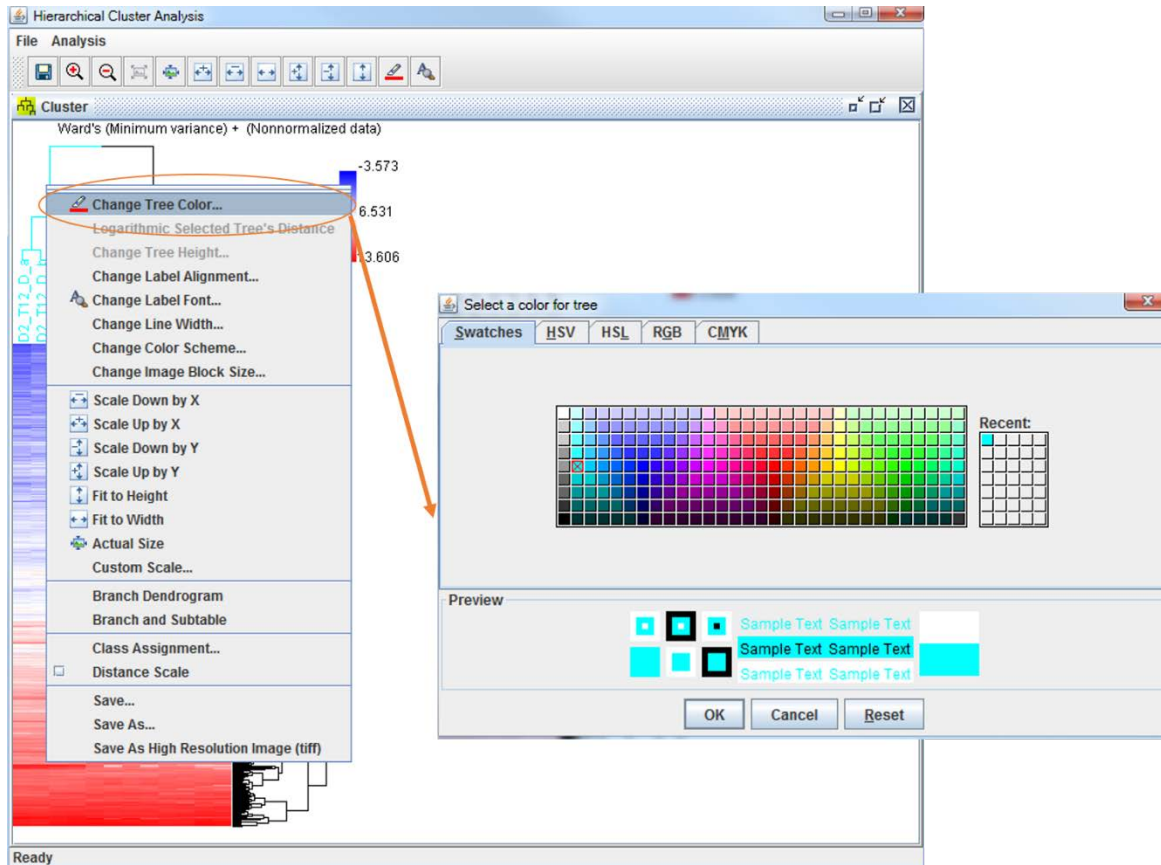


Step2. The default setting of the visualization can be changed by using buttons on the tool bar, including zoom in/out, scale up/down, change label font, etc.

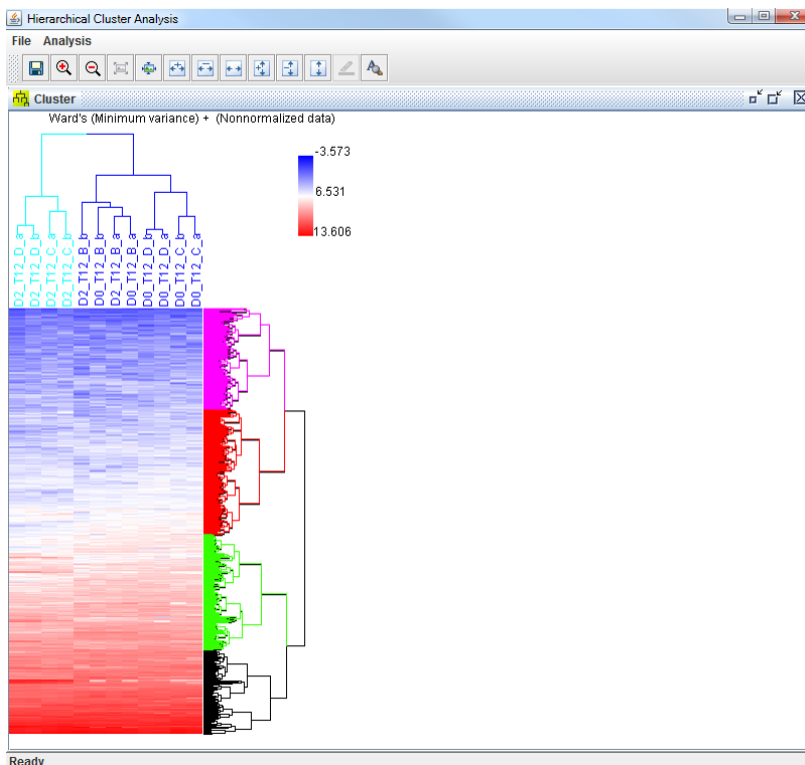




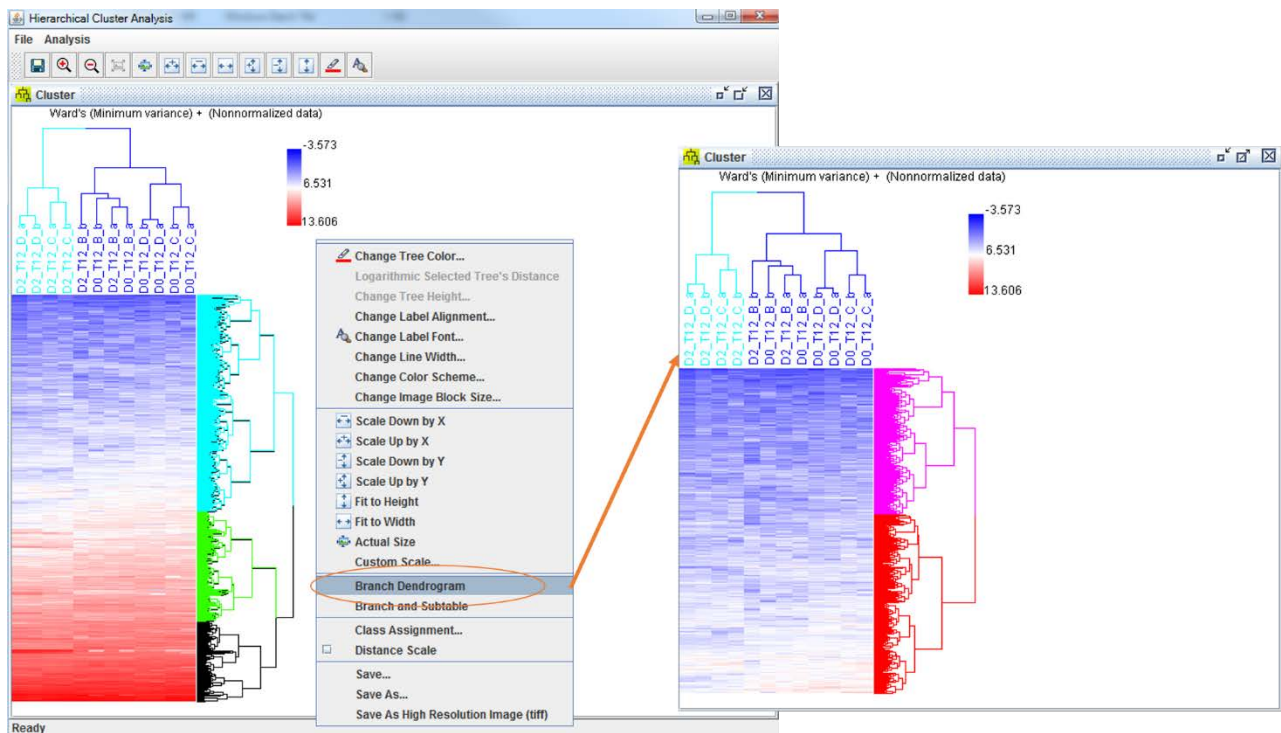
Step3. To change the default color (black) for each sample/feature group: (a) Click the branch of the group to change in the dendrogram tree, the color will highlight blue when it is selected; (b) Right click the selected branch and use the “Change Tree Color” in the popup menu to set the specific color the selected group.



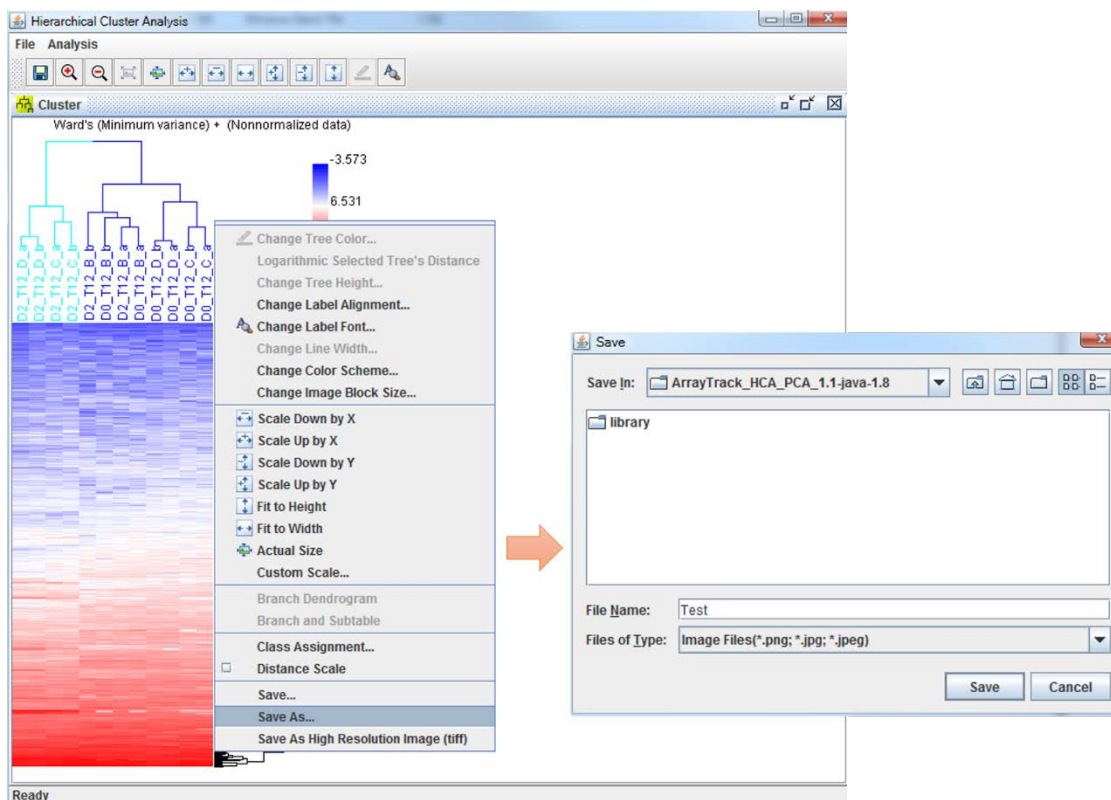
Step4. After changing the colors for the sample/feature groups, the figure will appear as follows.



Step5. To present a specific sample/feature group as a single dendrogram: (a) Click the branch of the group in dendrogram tree to select the group; (b) Right click the selected branch and select “Branch Dendrogram” in the context menu to display the specific group in a new figure.



Step6. Right click the result figure and click the “Save As” menu to save the result as a “.png / .jpg / .jpeg” file. Right click the result figure and click the “Save As High Resolution Image (tiff)” menu to save the result as a “.tiff” file.



#### **Part 4. Exit**

Step1. Click the “Close” button to close the HCA window to exit the tool.