Agilent Technologies

Release Note

Agilent Genomic Workbench 7.0

Associated Products and Part Number

G3794AA – G3799AA - DNA Analytics Software Modules

New for the Agilent Genomic Workbench 7.0

- Support for CGH+SNP analysis of mosaic and genetically complex samples such as hematological cancers.
- Support for CGH+SNP analysis of complete/constitutional triploid or tetraploid samples
- Reporting of clonal fraction of the largest aberrant clone for mosaic samples with more than one clone.
- New Reference Correct QC metric to inform user that the correct reference sample is assigned in the software.
- New cnLOH fraction metric to inform user the fraction of genome having copy neutral LOH region once LOH analysis performed.
- Improved access to CGH and SNP QC metrics and their thresholds to more easily understand the quality of the sample analysis.
- Access to distribution plot (Normalized log ratio distribution, CGH and SNP fit distribution) of the data.
- Additional to centralization, diploid peak centralization provided to set diploid state as the new point zero.
- Ability to generate only LOH report apart from aberration and LOH report when LOH analysis is performed.
- Ability to import/export, generate and use of probe ID list in the analysis so as to exclude or include specified probes from analysis.
- Improved access to aberration filter conditions to allow more specific way for filtering amplification and deletion calls.
- Inclusion of nesting filter as a part of aberration filter.
- Access to LOH filter to include significant LOH regions only.
- Improved access to peak reassignment so that adding, deleting or modifying default peaks will re-trigger the analysis as per the peak changes by user.

Preloaded Data in Agilent Genomic Workbench 7.0

Default designs and samples:

Type	AMADID	Genome Build	# Probe (Size)	FE Files
CGH	14698	Hg19	99931	None
CGH	14693	Hg20	238143	None
CGH	14950	Hg21	43118	None
CGH	18897	Hg22	254881	None
CGH	18898	Hg23	258476	None
CGH	28081	Hg24	294974	US23502418_252808110002_S01_CGH_109_Feb10_1_1 US23502418_252808110002_S01_CGH_109_Feb10_1_2 US23502418_252808110004_S01_CGH_109_Feb10_1_1 US23502418_252808110006_S01_CGH_109_Feb10_1_1
CGH	29830	Hg25	115234	None
ChIP	14706	Hg26	237279	None
ChIP	14707	Hg27	240982	None
CH3	14791	Hg28	201731	None
Expression	14850	Hg29	42898	None
Expression	14868	Hg30	42983	None

Default Tracks available

Following Tracks are provided upon installation

Hs_hg17_CNV_20100203
Hs_hg17_CpGIsland_20100203
Hs_hg17_PAR_20100203
Hs_hg18_CNV_20120403
Hs_hg18_CpGIsland_20120403
Hs_hg18_miRNA_20120403
Hs_hg18_PAR_20120403
Hs_hg19_CNV_20120403
Hs_hg19_CpGIsland_20120403
Hs_hg19_miRNA_20120403
Hs_hg19_PAR_20120403
Mm_mm7_CpGIsland_20090908
Mm_mm8_CpGIsland_20090908
Mm_mm8_miRNA_20090908
Mm_mm9_CpGIsland_20090908
Mm_mm9_miRNA_20090908
Rn_rn3_CpGIsland_20080510
Rn_rn4_CpGIsland_20091012
Rn_rn4_miRNA_20091012
Agilent_022837_Regions
Allchr_hg18
No_XY_hg18

Reference Genotypes

YORUBA MALE (NA18507_V1)
EUROPEAN MALE (NA12891_V1
YORUBA FEMALE (NA18517_V1
CHINESE FEMALE (NA18579_V1)
EUROPEAN FEMALE (NA12878_V1)
AGILENT EURO FEMALE
AGILENT EURO MALE

System Requirements for Agilent Genomic Workbench 7.0 Software

Windows and Linux System Requirements:

Operating System:	Windows XP, Vista Windows 7 or later; Red Hat Linux 5
Processor:	2GHz CPU or greater, Intel Core 2 Duo or better
Available memory (RAM):	64-bit: 4GB RAM minimum (need more when working with 244K or higher microarrays) 32- bit: 2GB RAM
Hard disk space:	40 GB (For analysis of large datasets, more space is required)
Display resolution:	1280x768 Display minimum
Internet connectivity:	T1 or T3 connection (1.5 Requirements: Mps) for use of eArray

Mac System Requirements:

Operating System:	Mac OS X v10.6.x (Snow Leopard or later)
Processor:	3 GHz Intel Core 2 Duo CPU or better
Available memory (RAM):	4 GB
Hard disk space:	40 GB (For analysis of large datasets, more space is required)
Display resolution:	1280 x768 or higher
Internet connectivity:	1 Mbps or faster

Tips on using Agilent Genomic Workbench 7.0 Software

Win 7/Vista: need to change install directory

Symptom: The software will not install correctly on Win7/Vista machines without the correct install directory. **Workaround**: User needs to make an install directory, with full permissions.

1. Win 7/Vista: user.home property not set correctly

Symptom: Sometimes, the user home directory value is wrongly set on Win7/Vista. Due to this, many processes like workflow will not function as they require user settings file which is located at user home directory. http://bugs.sun.com/bugdatabase/view_bug.do?bug_id=6519127.

Workaround: User needs to get the user home directory corrected by authorized system administrator.

2. Change memory settings if necessary

Symptom: Sometimes, the user can change the memory settings to speed up the processing.

Workaround: If you want to maximize the speed of processing, you can change the memory setting for the "heap size" of several processes. The heap size is controlled by two flags, –Xms<size> and –Xmx<size>. JVM starts with -Xms amount of memory and can grow to a maximum of -Xmx amount of memory. The 32-bit machine JVM does not support over 1400MB.

To change memory settings for running DNA Analytics 7.0

1. In Notepad, open the file ".../Program Files\Agilent\Agilent Genomic Workbench 7.0.1.0/Agilent Genomic Workbench 7.0.1.0.lax".
2. Find the line "lax.nl.java.option.additional=-Xms1024m -Xmx1400m -Dsun.java2d.noddraw=true".
3. Change the flags to your preferable memory setting. For example, if you have 2 GB RAM, change the line to read "lax.nl.java.option.additional=-Xms1400m -Xmx1400m -Dsun.java2d.noddraw=true".

Note: *Make sure the letter m is present at end of size and there is no space between the number and m.*

To change memory settings for Workflow mode

1. In Notepad, open the file ".../Program Files\Agilent\Agilent Genomic Workbench 7.0.1.0/config/config_workflow.properties".
2. Find the property, HEAP_SIZE=-Xmx1200m.
3. Change "1200" to your preferable memory setting. For example, if you have 2 GB RAM, change the property to HEAP_SIZE=-Xmx1400m.

To change memory settings for the background process for importing FE data files

1. In Notepad, open the file ".../Program Files\Agilent\Agilent Genomic Workbench 7.0.1.0/config/config_FEImport.properties".
2. Find the property, HEAP_SIZE=-Xmx512m.
3. Change "512" to your preferable memory setting as you did for Workflow mode.

3. User may encounter an error message "Could not create Java Virtual Machine Error"

Symptom: There is a known incompatibility between Agilent Genomic Workbench (AGW) versions 6.0 and later and Microsoft's Application Compatibility Toolkit (ACT) Version 5.5 (MS ACT). Installing MS

ACT will result in an error, "Could not create the Java virtual machine", which is displayed when attempting to launch AGW.

Workaround: Uninstalling MS ACT 5.5 will return functionality to AGW.
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Bug fixes in Agilent Genomic Workbench 7.0

- When probes are filtered, the signal intensity should also be grayed along with logratio in table.(TT_054849)
- When we restore experiment generated by workflow, by default one sample should be selected. (TT_054893)
- During installation, "Create icons for all user" option should be selected by default.(TT_131267)
- 'Show intensity bar chart' feature in gene view is not working.(TT_154977)
- SAF import feature overrides the extraction status attribute of samples generated from extraction workflow. (TT_155827)
- If workflow having design file path provided is exported, design path is not saved. (TT_158821)
- Critical error while generating genotype reference in one corner case. (TT_158825)
- Creating CNVR results in a new result node do not save already calculated SNP results. (TT_158827)
- Size of interval in CNVR report is 1 probe less than actual size. (TT_158829)
- If Image workflow is exported, input type is not saved in xml file.(TT_158830)
- Changing threshold for ASCN or LOH algorithm does not re-trigger analysis. (TT_158849)
- Generating Cyto report on selected experiment mode, make SNP results invalid for selected experiment in workspace.(TT_158851)
- "Computing SNP CN" message is displayed on the progress bar CH3 application (TT_158905)
- SNP QC metrics from CGH application are displayed in other application mode like CH3, ChIP. (TT_158906)
- Displaying annotations in the table view, removes LOH visualization in table view (TT_159713)
- Genotype report link is not created for multiple sample in workflow (TT_160442)
- In plot distribution , Label displayed for normalized plot is confusing.(TT_160550)
- In the home tab, gene view cannot be seen when the views are re-sized and return to home tab after switching the tab. (TT_160989)
- In a export dialog, previously exported path is showed while exporting tracks. (TT_160996)
- SNP analysis fails when multiple samples are used and combine inter array selected. (TT_161046)
- Manually reassign peaks ON/OFF notification should be displayed in the task bar. (TT_161379)
- Go to gene/genomic location dialogue accepts null value. (TT_161380)
- When SNP analysis failed for one of the sample, genotype reports are not generated for any array in the experiment.(TT_162422)
- Only Tracks of same genome build which is set in workspace should be allowed to select. (TT_126627)
- Combined replicate probe shows NaN value if one of the replicate probes is filtered or has NaN value. (TT_165114)
- Extraction Status in SAF rows should be ignored when SAF file is imported in the application.(TT_165792)
- Analysis goes in infinite loop with centralization threshold changed.(TT_161399)
- When SNP genotype report per chromosome generated, column headers are missing in the report.(TT_167025)
- In the open application page, 'Help' button is not working. (TT_156887)
- When saved results are restored, results are not same always.(TT_165112)

- Microarray id being shown instead of name while adding same array in experiment. (TT_165113)
- Experiment from 5.0 version are not imported in 6.5. (TT_165116)
- In a workflow console, hyperlink for Probe penetrance is not generated.(TT_165117)
- On the highest zoom level gene view displays size in 'pt' and not ‘bp’. ((TT_165118)
- SNP analysis fails if design contains SNP probes on some chromosomes only. (TT_165120)
- Normalization plot is not same if already analyzed sample is reimported and analyzed together in same experiment.(TT_165122)
- LOH call is not same in one corner case. (TT_165123)
- Corrected CGH line fit plot that did not show line passing through all points. (TT_171036)
- Sample with diploid autosome and triploid in Chr X shows homozygous calls in Chr X.
- Male samples display LOH region for entire ChrX.(TT_184148)

Known Issues in Agilent Genomic Workbench 7.0

1. Combine Track logic

Symptom	In this release operations for combining tracks work by assuming the track to be sets of intervals but do not perform any operations on intervals themselves. For example if a track consists of intervals e1, e2 and another of interval e2 , e3 then the logic of intersection would result in a track with just e2 . However if a track has interval e1 and another track has interval e2 where e2 is contained in e1 then the intersection would not give common part e2 but rather result would be empty and a message no annotations found would be shown
Fix/Workaround	None
Notes	None (TT_075144)

2. Cyto Report keeps running for Use Workspace Settings on HMM

Symptom	Steps: 1. Create an Experiment and apply Aberration with HMM Algorithm. 2. Create a Cyto Template with option "Use Workspace Settings" selected in Analysis Settings tab. 3, Run the same cyto report. The report keeps running
Fix/Workaround	None
Notes	None (TT_075661)

3. If any gene list is applied, Aberration in all the views gets updated except in the table

Symptom	Apply gene list functionality is not working on the data table. The gene list only gets applied on the aberrations in the views.
Fix/Workaround	None
Notes	Apply gene list only influences the display and not the actual aberration result. (TT_076300)

4. Application becomes very slow after you apply lowess OR variance stabilization with intra array.

Symptom	Chip Context > Application becomes very slow after you apply lowess OR variance stabilization with the intra-array combine algorithm. If you turn off the intra-array algorithm, the application again works properly. Another observation: In case all the normalizations are applied in one step, then it takes a very long time. Instead if you apply all the normalizations one by one, it takes much less time.
Fix/Workaround	None
Notes	None (TT_115165)

5. Issues with high density ChIP designs:

Symptom	If a million features ChIP-on-chip design has most of the probes on a few chromosomes only, then analysis may have certain issues. 1. With those designs ChIP workflow also may fail. The probe report may not be generated and application may yield “Out of Memory” error. 2. Fusing two or more such designs will cause analysis to fail.
Fix/Workaround	None
Notes	None (TT_118009)

6. Colored filled circle looks like a square.

Symptom	Colored filled circle symbols in gene view looks like squares in the scatter plot views
Fix/Workaround	None
Notes	None (TT_117963)

7. OutOfMemoryError while restoring auto created experiment from workflow with one hundred 1-million feature arrays

Symptom	OutOfMemoryError while restoring auto created experiment from workflow with 100 million feature data
Fix/Workaround	None
Notes	None (TT_118045)

8. Aberrations which are not common are also seen in the Text Summary of Common Aberration and also in UI.

Symptom	Aberrations which are not common are also seen in the Text Summary of Common Aberration and also in UI.
Fix/Workaround	None
Notes	None (TT_076245)

9. Workflow with one hundred 1-Million feature arrays with Z-score and penetrance reports fails.

Symptom	Workflow with 100 Million feature data with Z-score and penetrance reports fails.
Fix/Workaround	None
Notes	None (TT_118044)

10. Cyto report header has incorrect text.

Symptom	Cyto report header has incorrect text if some special characters are present in

	the report header section in the cyto report template.
Fix/Workaround	None
Notes	None (TT_133074)

11. ManualQCFlag status not reflected in Sample Manager immediately.

Symptom	If ManualQCFlag is set for any array from QC Metric table, it is not updated in the Sample Manager in same session
Fix/Workaround	Quit application and restart it.
Notes	None (TT_133163)

12. If “Overwrite if file exists” option is not checked in the workflow for reports, workflow will fail if we run more than one workflow continuously.

Symptom	If “Overwrite if file exists**”** option is not checked in the workflow for reports, workflow will fail if we run more than one workflow continuously. Cause: when 1st workflow is running, the 2nd workflow is in waiting state. When 1st workflow completes and 2nd one starts, the report file is already present.
Fix/Workaround	None
Notes	None (TT_132376)

13. Workflow Console screen blank at Completion.

Symptom	Sometimes at the conclusion of a workflow run, the console log screen is blank.
Fix/Workaround	Switching to Summary Console, then back to the workflow console will show the log correctly.
Notes	None (TT_130489)

14. When doing an extraction in the workflow mode, you may get a security alert concerning Java binary files.

Symptom	When doing an extraction in the workflow mode, you may get a security alert concerning Java binary files.
Fix/Workaround	When prompted, choose "Unblock" to allow the workflow to continue. You may resolve this by changing your firewall security options
Notes	None (TT_115733)

15. When multiple arrays analyzed interactively, application closes sometimes.

Symptom	When multiple samples (>30-35) are analyzed interactively with SNP analysis settings, application closes on its own.
Fix/Workaround	Run analysis on 64 bit machine if available.
Notes	None (TT_166981)

16. Hyperlinks generated for reports in Reports manager show incomplete path.

Symptom	Hyperlinks generated for different reports like LOH, Aberration And LOH or Genotype reports in Reports manager show incomplete path.
Fix/Workaround	None
Notes	None (TT_179825)

17. Navigator pane get reset to default settings.

Symptom	Navigator pane in the application gets reset to default settings when user switches to workflow/sample manager tab and again comes to home tab.
Fix/Workaround	None
Notes	None (TT_182611)

18. Connection to URL of Locuslink is not working

Symptom	When tried to connect LocusLink through right click in table view, it does not open the LocusLink page.
Fix/Workaround	None
Notes	None (TT_182923)

19. Imported aberration filter from earlier version cannot be updated in application.

Symptom	When exported aberration filters from earlier versions of Agilent Genomic Workbench are imported in 7.0 application, updating of the settings is ineffective.
Fix/Workaround	Try to create new filter providing conditions.
Notes	None (TT_183508)

20. Sometimes when already present samples are tried for import, the color highlight still present even after renaming samples.

Symptom	When multiple samples which are already present in application tried to import, the color highlight for duplicate global display name is not getting removed even after renaming.
Fix/Workaround	User can still import the renamed samples in the application.
Notes	None (TT_183539)

21. Sometimes connection to UCSC from application does not work for proxy based internet connection.

Symptom	Connecting to UCSC from application doesn`t work for CGH, CH3 ChIP or TE application.
Fix/Workaround	Try to set proxy host and port settings in ‘UCSCConfig.properties’ file located in ‘config’ folder of the installation directory.
Notes	None (TT_184284)

Important Notes: AGW 7.0

1. CGH aberration results will be different between AGW7.0 and AGW6.5 or earlier version as following changes included in AGW 7.0.

    a) Order of normalization : In previous versions of AGW GC correction was applied before centralization but in 7.0 normalization order is changed as centralization > GC correction > Diploid centralization

    b) Order of filters and normalization: In previous versions of AGW filters are applied after normalization but in 7.0 filters are applied before normalization algorithm.

    c) Splitting of P Q arm: AGW 7.0 includes fixes for calculating length of p and q arm.

    d) Sorting of replicate probes: In AGW 7.0 replicate probes are sorted according to their feature number. The sorting of probes was not there in earlier version so intervals which are having replicate probes at the edge of it might have difference.

    e) Bug fixes in GC correction: AGW 7.0 includes fixes in the calculation of co-efficient for GC correction algorithm.

2. cnLOH fraction metric is available only if LOH analysis is performed. Sex chromosomes are excluded from the calculation of the metric value.

3. Nesting level of child intervals is shifted one level up if parent of that child is filtered and if nesting in legacy mode is OFF.

4. Aberration filter with HMM and Z score : The aberration intervals reported after aberration filter applied, may include intervals which seems not satisfying aberration filter probe condition but still present in the report. This is because in case of these algorithms filtering works on probe level and not intervals as such.

5. Split parent in aberration filter: This option is set FALSE in the software by default. This split happens when absolute height of child is less than absolute height of parent interval and child interval is getting filtered by any condition of aberration filter. In this case, the parent interval is split into two separate intervals and these splits are reported as separate intervals. In such cases the results of aberration filter would be different from previous version of the software.

6. Suboptimal analysis of complete triploid/tetraploid sample: When diploid centralization and SNP CN is OFF, algorithm may not give the optimal results for these samples.

7. Probe id list versions: In case existing probe id list is updated, no intermediate versions maintained but final updated list. However, when peak reassignment performed after updating list, original version of list is used for re-analysis.