

CytoScan[®] Cytogenetics Suite

1. What is CytoScan Cytogenetics Suite?
 - CytoScan Cytogenetics Suite consists of CytoScan[®] arrays and CytoScan[®] Reagent Kit, GeneChip[®] Command Console[®] Software (AGCC), and Chromosome Analysis Suite (ChAS). It enables the performance of high-resolution, genome-wide DNA copy number analysis and also provides genotyping information for the detection of copy neutral loss/absence of heterozygosity (LOH/AOH), which can be used to detect uniparental isodisomy (UPD). The combination of high-resolution DNA copy number data and the ability to detect gains, losses, and UPDs on a single array makes CytoScan Cytogenetics Suite ideal for cytogenetics studies.

Equipment requirements

2. What Affymetrix equipment is required for the CytoScan[®] assay?
 - The CytoScan assay requires either GeneChip[®] Scanner 3000 7G System with Workstation and AutoLoader or GeneChip[®] System 3000Dx v.2 and a GeneChip[®] Hybridization Oven 645.
3. What thermal cycler can be used in for the CytoScan assay?
 - Applied Biosystems[™] GeneAmp[™] PCR System 9700 (silver or gold-plated silver block only; do not use an aluminum block)
 - Applied Biosystems[™] Veriti[™] 96-Well Thermal Cycler
 - Bio-Rad[®] DNA Engine[®] PTC-200 Thermal Cycler
 - Eppendorf[®] Mastercycler[®] Pro S Thermal Cycler
 - Applied Biosystems[™] 2720 (pre-PCR room only)
4. How can one tell the difference between a silver or aluminum thermal cycler block?
 - Look at the serial number on the block. An "A" in the serial number indicates the block is aluminum; an "S" indicates it is silver. In addition, an aluminum block has a honeycomb appearance between the wells, whereas a silver block is smooth.
5. How should the thermal cycler settings be programmed?

Setting	Applied Biosystems Veriti 96-Well	Bio-Rad DNA Engine PTC-200	Eppendorf Mastercycler Pro S
Lid temperature	103°C	103°C	103°C
Temperature mode	N/A	Calculated	Safe
TSP heated lid	N/A	N/A	Yes
Switch off lid at a low block temperature	N/A	N/A	No

6. What is required for processing and viewing CytoScan array CEL files?
 - CytoScan array CEL files are processed and analyzed in ChAS. This is a free download from the Affymetrix web site. For processing the CEL files, a 64-bit computer with a minimum of 8 GB of RAM, is required. For viewing the resulting CYCHP files, either a 32-bit or 64-bit computer is required. For 32-bit computers, it is important to note that the minimum amount of RAM needed is 3 GB. The recommended system specifications are included in the *Chromosome Analysis Suite User Manual* (Part number 702943).
7. Is it required to have all equipment current on their calibration and maintenance status?
 - For successful execution of the CytoScan assay, it is important that equipment be properly maintained and calibrated per the manufacturers' specifications. Please be sure that the centrifuges, pipettes, thermal cyclers, and Affymetrix equipment, including the 645 hybridization oven, fluidics station(s), and scanner, have had their recommended maintenance prior to running the assay. For lab equipment, it is typically a yearly calibration. For Affymetrix equipment, there is typically a yearly preventative maintenance.

8. Can electronic pipettes be used in the CytoScan assay instead of multichannel manual pipettes?
 - Pipetting viscous solutions using electronic pipettes may be very challenging and result in ineffective pipetting that could contribute to overall poor QC values. Therefore, the use of electronic pipettes is not recommended.
9. Which magnetic racks are supported for the CytoScan assay?
 - The use of the following magnetic racks is supported:
 - DynaMag™-2 Magnet (Thermo Fisher Scientific; Catalog number 123-21D)
 - MagnaRack™ (Thermo Fisher Scientific; Catalog number CS15000)
 - PureProteome™ Magnetic Stand (Millipore™; Catalog number LSKMAGS08)

General CytoScan® assay

10. What sample types are validated on the CytoScan assay?
 - The CytoScan assay has been validated with blood and cell line samples.
11. What samples are in the Reference Model file?
 - The Reference Model file in CytoScan Cytogenetics Suite includes 380 samples, which were run as part of a larger set of microarrays by nine operators. These operators processed ~48 unique samples in two rounds each, with random placement of sample DNAs across the PCR plates and with random use of instruments and reagents. The source DNA includes the following samples:
 - 284 HapMap samples including at least one replicate of each of 270 HapMap samples: 90 from each of the Yoruban, Asian, and Caucasian ethnic groups, from cell line-derived DNAs from the Coriell Institute of Medical Research
 - 96 DNA samples from blood of phenotypically healthy male and female individuals obtained from BioServe Biotechnologies
12. What are the general genomic DNA requirements for the CytoScan assay?
 - DNA must be double-stranded genomic DNA.
 - DNA must be free of PCR inhibitors.
 - DNA must not be contaminated with other human genomic DNA sources or with genomic DNA from other organisms.
 - DNA must not be degraded.
 - DNA should have an A260/A280 between 1.7–2.1 (numeric rounding allowed).
13. What is the recommended genomic DNA extraction methods used for the CytoScan assay?
 - Both blood and cell line sample sources have been tested by Affymetrix with the CytoScan assay.
 - Methods that include boiling or strong denaturants are not acceptable because the DNA would be rendered single-stranded. Genomic DNA extracted using the following methods have been tested at Affymetrix:
 - QIAGEN – Genra Puregene® Kit
 - 5 PRIME – PerfectPure™ DNA Blood Kit
 - The CytoScan assay requires genomic DNA concentration ≥ 50 ng/ μ L. Therefore, the elution volumes for each of the kits will need to be adjusted accordingly to achieve the desired concentration.
14. How many freeze/thaw cycles are validated for CytoScan Reagent Kit (24 samples)?
 - CytoScan Reagent Kit (Part number 901808) is validated for five freeze/thaw cycles.
15. How many samples should be processed at a time using the CytoScan assay?
 - It is recommended to run no more than 24 samples at a time. The assay is a four-day protocol; however, the technician can use an optional three-day protocol after becoming comfortable with the assay.
16. What other reagents are required for the CytoScan assay?
 - The Clontech Titanium® Taq PCR Kit (300 reactions, Part number 639240 **or** 400 reactions, Part number 639243) and absolute ethanol (Sigma; Part number 459844) are also required. All other reagents are included in CytoScan Reagent Kit.
17. What are the fill volumes of the reagents in CytoScan Reagent Kit?
 - Only the volume that is indicated on the label can be guaranteed. Since Affymetrix's assurance guarantee is only provided for the label volume, it is advised not to plan experiments or standard operating procedures based on potential overage volumes.

18. Can different sub kits from different lots of the modules from the CytoScan assay kit be used for a particular experiment?
- It is highly recommended not to mix different sub kit lots from different modules because functional testing is done based on sub kits from the same lot.
19. Can reagents from Affymetrix® SNP 6 Core Reagent Kit be used in the CytoScan assay?
- No. The CytoScan assay has been optimized for performance with only CytoScan Reagent Kit.
20. Does the expiration date apply to the date of opening or date of manufacturing?
- The expiration date applies to the date of manufacturing. For CytoScan Reagent Kit, the reagents can be used for up to one year from the date of manufacturing.
21. What are the stopping points in the assay?
- After any stage in the assay (including fragmentation), samples can be stored at -20°C if one is not proceeding directly to the next step. However, once a stage has been initiated, it must be completed before storage of the samples.
22. How is the CytoScan assay different from the assay used with Affymetrix® Genome-Wide Human SNP Array 6.0?
- The CytoScan assay has significantly fewer pipetting steps and requires less hands-on time than the Genome-Wide Human SNP 6.0 assay. The CytoScan assay uses only the Nsp I restriction enzyme and has been optimized for cytogenetics applications. The CytoScan assay is not intended for genome-wide association studies.
23. Can someone buy just the reagents or arrays alone?
- No, the reagents and arrays are sold as bundles for 24 reactions (Part number 901835).
24. What is the purpose of including a positive and negative genomic DNA control in each assay, and what would be the passing and failing results for each control type?
- **Positive control:** This sample has prior proven performance and represents a pristine sample, meeting all genomic DNA input requirements. The performance of this sample helps to assess the quality of the assay steps and final product. In troubleshooting cases, performance of this control sample versus that of non-control samples can help to parse out possible assay failure culprits.
 - **Negative control:** For this assay, the negative control is simply reduced EDTA TE Buffer and acts as a control for the process up to the PCR quantitation and PCR gel. If no smear is seen on the PCR gel, it is considered passing, indicating that there is no DNA contamination in any of the reagents that could lead to amplification of other targets. A failing result will have a smear in the PCR gel. Note that the presence of PCR primer dimers is expected.
25. Why do the pre- and post-PCR areas need to be separated?
- The post-PCR lab contains amplicons, whereas the pre-PCR area should not. These amplicons can be inadvertently transferred back to the pre-PCR area, thus contaminating the lab and posing a contamination threat to all reactions set up in the pre-PCR lab. Because all samples are amplified using the same primers and adaptors, contaminating DNA can also be amplified.
26. What are the 10 assay processing steps listed in order?
- The 10 assay processing steps, as listed in the *CytoScan Assay User Manual* (Part number 703038) are the following:
 1. Genomic DNA Preparation
 2. NSP1 Restriction Enzyme Digestion
 3. Ligation
 4. PCR
 5. PCR Product Purification
 6. Quantitation
 7. Fragmentation
 8. Labeling
 9. Hybridization
 10. Wash, Stain, Scanning

Digestion and ligation

27. What happens during the digestion and ligation steps?
- The DNA is digested with Nsp I restriction endonuclease and ligated to adaptors that recognize the cohesive four-base pair (bp) overhangs. All fragments resulting from restriction enzyme digestion, regardless of size, are substrates for adaptor ligation.
28. How long can the ligated products be stored on the thermal cycler?
- A plate can be held in thermal cycler at 4°C for up to 60 hours.
29. How long can the ligated products be stored at -20°C?
- A plate can be frozen at -15 to -25°C for up to one week.

PCR

30. What can cause faint or absent bands on the PCR gel?
- Several things can cause this problem. To troubleshoot this problem, first determine if the positive control worked properly. Common reasons for this failure include incomplete digestion of genomic DNA or inefficient ligation of adaptors, ligation samples that are not properly diluted or mixed, and degraded DNA (if only the positive control worked). See the troubleshooting section of the *CytoScan Assay User Manual* (Part number 703038) for more information.
31. In a hypothetical assay failure in which PCR fragment size distribution observed on the PCR gel is significantly greater than 2,000 bp DNA smear, what processes in the assay could play a role in the failure?
- The digestion step could be root cause. If the reaction did not go to completion, there may be, on average, longer fragments at the end of the reaction. These longer fragments then pass on to ligation and PCR reactions.
 - Insufficient amounts of PCR primer were added to facilitate suppression PCR. The primer concentration shifts the PCR reaction equilibrium toward larger fragment distributions, in this case, by increasingly unfolding (linearizing) the stem-loop structure at increasing primer concentration when the same adaptor ends hybridize.
32. Does the PCR have to be set up in a separate pre-PCR clean room?
- Using two separate rooms greatly reduces the risk of sample contamination by previously amplified PCR products. If only one room is available, designate one area of the room as the pre-PCR clean area and a separate area as the post-PCR clean area. If a one-room configuration is being used, it is highly recommended to use a laminar flow cabinet for the pre-PCR clean area. See the *CytoScan Assay User Manual* (Part number 703038) for more details about the recommended laboratory setup.
33. What should someone do if they see a smear in their negative control on their PCR gel?
- A smear in the negative control indicates there has been a contamination. If the negative control band is a light smear, it could be a low-level environmental bacterial contaminant introduced through plastic surfaces. This has shown not to have a material impact on the CytoScan HD data. If a bright band or a smaller smear is seen, then that could be a real cross-contaminant from a sample, and the samples would need to be checked for contamination. If a smear is seen in the negative control well, the negative control should be re-run to check if it was not a result of sample bleed over from gel loading.

PCR product purification

34. Is there a plate purification option for the PCR product purification step?
- No, only the single-tube purification process is validated with the assay.
35. Can a different magnetic rack be used instead of the recommended MagnaRack™ magnetic stand?
- Yes, the assay is currently validated for the following magnetic racks:
 - DynaMag™-2 Magnet (Thermo Fisher Scientific; Catalog number 123-21D)
 - MagnaRack™ (Thermo Fisher Scientific; Catalog number CS15000)
 - PureProteome™ Magnetic Stand (Millipore™; Catalog number LSKMAGS08)

36. What is the typical yield range from PCR product purification?
- The minimum recommended yield is 2.5 µg/µL for a sample. Yields can range from 3.0–4.5 µg/µL, and the average yield for seven or more samples processed in a run (not including the negative control) should be greater than 3.0 µg/µL. If the average yield is below this, consult the troubleshooting section of the *CytoScan Assay User Manual* (Part number 703038).
37. What is the role of purified PCR product?
- The purification process is required to remove all the non-amplified DNA after the PCR process.
38. Can someone stop after the PCR process and continue the next day with the purification step?
- Yes, the purified PCR products can be stored at –15 to –25°C, and one can continue with the purification step later.
39. How long can the purified PCR products be stored at –20°C?
- The purified PCR products can be stored at –15 to –25°C for 10 days.

Fragmentation

40. What is the purpose of the fragmentation step?
- Probes on the array are 25-mers. The fragmentation step reduces the purified PCR product size (150–2,000 bp) to an even smaller size range (25–125 bp), making them more optimal for hybridization to the array probes. Ideally, 25 bp-sized PCR products hybridize to 25-mer probes on the array; however, optimal hybridization can be achieved with 25 to 125 bp-sized PCR products.
41. What should be done if a refrigerated centrifuge is not available for the fragmentation step?
- A refrigerated centrifuge is highly recommended. If one is not available, a plastic plate rack that has been stored at –20°C may be used. It is recommended to keep the samples chilled and work quickly prior to initiating the incubation at 37°C. See the *CytoScan Assay and Data Analysis Training Video* for more details about required equipment and consumables.
42. Do the samples need to be run on a gel and the Bioanalyzer during fragmentation QC?
- No, the routine QC includes running samples only on a gel. Some samples will be saved for additional analysis on the Bioanalyzer, should that become necessary.
43. What can cause over-fragmentation?
- Common causes of over-fragmentation include excess enzyme due to pipetting errors or an incorrect volume of enzyme used based on the unit activity. In addition, warming up of the assembled reaction prior to initiating the 37°C incubation can lead to over-fragmentation. See the troubleshooting section of the *CytoScan Assay User Manual* (Part number 703038) for more information about over-fragmentation.
44. What can cause under-fragmentation?
- Common causes of under-fragmentation include improper storage or handling of the enzyme, an incorrect volume of enzyme used based on the unit activity, and improper mixing of fragmentation master mix. See the troubleshooting section of the *CytoScan[®] Assay User Manual* (Part number 703038) for more information about under-fragmentation.

Hybridization and washing/staining

45. How long can the labeled products be stored at –20°C?
- The labeled products can be stored at –20°C for no more than 10 days.
46. What is the hybridization time for CytoScan arrays?
- The hybridization time is 16–18 hours. If there are a limited number of fluidics stations, the hybridization process can be staggered by two hours. For example, if there is only one fluidics station and eight arrays, the hybridization for four arrays can be started at one time, and then the hybridization for the other four arrays started two hours later. After 16 hours, the first four arrays can be placed on the fluidics station, and then the next four arrays placed on the fluidics station two hours later. Thus, all eight samples will have hybridized for 16 hours. Another option is to wash the first set of samples at 16 hours and the second set at 18 hours.
 - It is not recommended to stagger more than three wash/stain cycles in an eight-hour workday. Please consult the local Field Application Specialist for more details on the hybridization process.

47. What is the hybridization temperature and rotation speed for CytoScan arrays?
- The hybridization temperature is 50°C. The rotation speed is 60 rpm.
48. Can samples be re-hybridized?
- No, this has not been validated at this time.
49. After hybridization, how long can arrays be stored before washing and staining?
- Arrays must be put onto the fluidics station immediately after removal from the hybridization oven. Do not remove arrays from the oven you are ready to wash them.
50. How long can arrays filled with holding buffer be stored before scanning?
- After the washing and staining step, arrays can be stored for up to 24 hours at 4°C before scanning.
51. What type of water should be used on the fluidics stations?
- Deionized (DI) water should always be used on the fluidics stations for all protocols, including the shutdown and bleach protocols.
 -
52. Are any shutdowns run after bleaching the fluidics station?
- Yes, it is recommended to run two water shutdown protocols after bleaching the fluidics station.

CytoScan HD Array

53. What is the array format?
- CytoScan HD Array uses a 49 format and a 5-µm feature size.
54. What is the array content?
- CytoScan HD Array covers both constitutional and cancer genes with
 - Overall intragenic coverage at 1 marker/880 bases
 - ClinGen (previously CCG and ISCA) constitutional coverage at 1 marker/384 bases
 - Complete cancer gene coverage at 1 marker/553 bases
 - 14,000 OMIM genes at 1 marker/723 bases
 - >36,000 RefSeq genes at 1 marker/880 bases
 - Backbone (non-gene) coverage at 1 marker/1,737 bases across genome for breakpoints
 - Overall (gene and non-gene backbone) coverage at 1 marker/1,148 bases
55. How many single-nucleotide polymorphism (SNP) and copy number (CN) probes are included on the array?
- CytoScan HD Array includes 750,000 genotype-able SNPs and 1.9 million non-polymorphic probes.
56. How long does CytoScan HD Array take to scan?
- Each array takes ~32 minutes to scan.
57. What is the correct fluidics script for CytoScan HD Array?
- The correct fluidics script is CytoScanHD_Array_450.
58. What file sizes are associated with CytoScan HD Array?
- The CEL file is ~66 MB, and the CYCHP file is ~119 MB.

CytoScan® 750K Array

59. What is the array format?
- CytoScan 750K Array uses a 64 format and a 5-µm feature size.
60. What is the array content?
- CytoScan 750K Array covers both constitutional and cancer genes with
 - Overall intragenic coverage at 1 marker/1,737 bases
 - ClinGen (previously CCG and ISCA) constitutional coverage at 1 marker/1,292 bases
 - Complete cancer gene coverage at 1 marker/1,269 bases
 - 14,000 OMIM genes at 1 marker/1,445 bases
 - >36,000 RefSeq genes at 1 marker/2,525 bases
 - Backbone (non-gene) coverage at 1 marker/6,145 bases across genome for breakpoints
 - Overall (gene and non-gene backbone) coverage at 1 marker/4,127 bases

61. How many SNP and CN probes are included on the array?
 - CytoScan 750K Array includes 200,000 genotype-able SNPs and 550,000 non-polymorphic probes.
62. How long does CytoScan 750K Array take to scan?
 - Each array takes ~7 minutes to scan.
63. What file sizes are associated with CytoScan 750K Array?
 - The CEL file is ~46 MB, and the CYCHP file is ~33 MB.
64. What software is available for analysis?
 - For CytoScan 750K Array, all genotyping and copy number analysis is done with ChAS 1.2 or higher. For ChAS versions prior to 2.0, there is an update installer required to install the 750K-specific files. The installer is available for download from the Affymetrix website; the file name is "CytoScan750K_ChASSupportUpdate.exe". ChAS 2.0 or higher does not require this update. This product is not intended for genome-wide association studies.

Data analysis and management

65. What software is available for analysis?
 - All genotyping and copy number analysis is done with ChAS. CytoScan Cytogenetics Suite is not intended for genome-wide association studies.
66. What are the definitions of the array QC metrics terms "MAPD" and "SNPQC"; what does each metric measure; and how are they used in assessing array performance?
 - **MAPD:** Median of the Absolute values of all Pairwise Differences is a per-microarray estimate of variability, like standard deviation (SD) or interquartile range (IQR). It measures the variability in log₂ ratios by looking at the pair difference of all probes and taking a median value. The effect of an occasional big difference in log₂ ratios between probes is removed by taking a median value and not a mean. This variability can come from different sources:
 - Intrinsic variability in the starting material, hybridization cocktail preparation, microarray, or scanner.
 - Apparent variability induced by the fact that the reference may have systematic differences from the sample on this microarray.

Regardless of the source of variability, increased variability decreases the quality of the CN calls. A high MAPD can be attributed to any of the above factors and indicates that CN calls may be inaccurate, leading to a higher false positive/negative rate.
 - **SNPQC:** This is a measure of how well genotype alleles are resolved in the microarray data. In other words, it estimates the distributions of homozygous AA, heterozygous AB, and homozygous BB alleles and calculates the distance between them. The better the separation of these distributions, the better the ability to identify a genotype based on its cluster position. The larger the difference between the peaks and the troughs, the better the resolution of homozygotes and heterozygotes and the higher the SNPQC metric is. If the three peaks are not well resolved, the difference between peaks and troughs will be low, resulting in a lower SNPQC value. A low SNPQC value indicates that quality of the SNP allele data is compromised, due to higher noise within the array, which compromises the overall quality and clarity of results.
67. What is Waviness-SD?
 - Waviness-SD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. Based on an empirical testing dataset, array data with Waviness-SD >0.12 has either sample or processing batch effects that will reduce the quality of the copy number calls. Elevated Waviness-SD is not always an indication of too much noise. Elevated Waviness with good MAPD and SNPQC metrics can occur in samples with many copy number changes or very large regions of change. It is therefore advised to check the data when observing elevated Waviness with good MAPD and SNPQC. The Waviness-SD metric is applicable to blood and cell line data. The Waviness-SD metric is not intended for alternative sample types such as solid tumor or FFPE samples in which the results may vary as a result of the biological complexity. For these sample types, it is recommended to use the ndwavinessSD.

68. What are the array QC metrics for the CytoScan arrays?
- The CytoScan arrays use the following QC metrics:
 - SNPQC ≥ 15
 - MAPD ≤ 0.25
 - Waviness-SD ≤ 0.12
 - These QC metrics have been fine-tuned for blood-derived constitutional samples. The SNPQC and Waviness-SD metrics are based on an assumption of a relatively normal diploid genome for which the majority of the genome is not mosaic. For hematological malignancy samples, the baseline assumption pertaining to constitutional samples does not meet the normal aberration frequency pattern and high levels of mosaicism, which will likely trigger the SNPQC and Waviness-SD metrics to fail. Only the MAPD metric should be considered for non-constitutional samples. A failure of any one of these metrics for constitutional samples is a failure for that array result. There is no direct correlation between the absolute passing numeric value for any one of the metrics and the quality of a sample. For example, a sample that has an SNPQC=30 is not necessarily a better quality sample than one that has an SNPQC=20.
69. What can cause SNPQC failures?
- SNPQC failures can be caused by contamination of samples, equipment, or reagents. Other possible causes include over- or under-fragmentation of samples or a hybridization oven that is out of calibration. See the troubleshooting section of the *CytoScan[®] Assay User Manual* (Part number 703038) for more information.
70. What can cause MAPD failures?
- MAPD failures can be caused by assay drift due to variation in assay execution or over-fragmentation. See the troubleshooting section of the *CytoScan Assay User Manual* (Part number 703038) for more information.
71. What can cause Waviness-SD failures?
- Waviness-SD failures can be caused by degraded DNA, an incompatible sample type, or a sample-specific effect. See the *CytoScan Assay User Manual* (Part number 703038) and the *Chromosome Analysis Suite User Manual* (Part number 702943) for more information.
72. Are individual genotypes generated from the CytoScan arrays?
- Yes, genotypes can be generated in ChAS. The software uses BRLMM for genotyping, which uses a Bayesian model based on prior clusters. This product is not intended for genome-wide association studies.
73. What are the recommended graph settings for data interpretation in ChAS?
- It is recommended to change the weighted log₂ ratio graph from the default to a minimum at -1.5 to a maximum at 1.5, using data type as points.
 - It is recommended to change the allele peaks graph from the default to a minimum at -2 to a maximum at 2, using data type as points.
 - The other graphs can stay with the default values.
74. Which genotype is presented in each allele difference track?
- AA markers would be distributed about +1.
 - AB markers would be distributed about 0.
 - BB markers would be distributed about -1.
75. What samples are in the Reference Model file?
- The Reference Model file in the CytoScan HD Array and CytoScan 750K Array set of NA32 Analysis includes 380 microarrays, which were run as part of a larger set of microarrays by nine operators. These operators processed ~48 unique samples in two rounds each, with random placement of sample DNAs across the PCR plates and with random use of reagents and instruments. The source DNA includes the following:
 - 284 HapMap samples including at least one replicate of each of 270 HapMap samples: 90 from each of the Yoruban, Asian, and Caucasian ethnic groups, from cell-line derived DNAs from the Coriell Institute of Medical Research
 - 96 DNA samples from blood of phenotypically healthy male and female individuals obtained from BioServe Biotechnologies

The CytoScan HD Array samples that were used to create the Reference Model file were chosen to be run by different operators and with different kits and reagents, while still covering all the HapMap cell line ethnic groups, plus the normal bloods of both sexes.

76. Are there additional databases to refer to that will help in data analysis?
- Yes, refer to the CAGdb database, ICCG, and DECIPHER for additional public databases that may assist in data analysis, and for which we do not have a direct link within ChAS.
77. How does someone export LogRatios for the whole genome?
- They can export the Whole Genome View as a PNG file. To do this, click on **File** → **Export window PNG**
78. Where are the library files located on the website?
- They are located on the ChAS web page and also on the array product pages under the technical documentation tab.
79. How does someone unzip the library file if they have to install it manually?
- Right click the zip folder and click extract all. They can also use an already installed software that unzips files (e.g., Winzip, 7-zip, or any other equivalent software). Browse to the location to save the folder and click Extract. Go to the folder to find the files.
80. What is the library file location for ChAS?
- The library file location for ChAS 2.1 and above is C:\Affymetrix\ChAS\Library.
81. What is the mosaic call determination in ChAS?
- The algorithm is designed to detect only mosaicism between ~30–70% for CNs between 1 and 3 for regions on the order of 5,000 markers in size or larger.
 - The endpoint location of mosaic segments is less precise than the CN segmentation, with endpoint variation within 500 markers being typical for segments of 5,000 markers or larger.
 - Some regions of full integer CN 1 or 3 below 5,000 markers in length may be incorrectly called as mosaic segments.
 - Some regions of CN below 1 or above 3, mosaic or otherwise, less than 5,000 markers in length may be incorrectly called as mosaic segments.
82. What data files are produced during assay and analysis process?
- A total of six files are produced that are key to the process:
 - ARR file: this file includes sample information.
 - AUDIT file: this file is a log of the sample history.
 - DAT file: this file is the raw data from the scanner.
 - CEL file: this file is the gridded and processed data.
 - CYCHP file: this file is the output of ChAS and contains all of the analysis data.
 - CHPCAR file: this file stores user-annotated calls, interpretations, and modifications made to CHP file segment data (ChAS 2.0 and higher).
83. Is it important for someone to back up their data?
- Out of the six data files mentioned in the preceding question, it is important to backup and archive the ARR, CEL, CYCHP, and CHPCAR files at a minimum. This will allow someone to maintain the ability to either reanalyze from the CEL file or re-visualize the results using the CYCHP/CHPCAR files.

Affymetrix, Inc: (US) +1-888-362-2447, +1-408-731-5000 ▪ (EU) +44-(0)1628-552550 ▪ (JP) +81-(0)3-6430-4020 ▪ (CN) +86-21-63915511
eBioscience Products: (US) +1-888-999-1371, +1-858-642-2058 ▪ (EU) +43 1 796 40 40 305 ▪ (JP) +81-(0)3-6430-4020
USB Products: (US) +1-800-321-9322, +1-216-765-5000 ▪ (EU) +44-(0)1628-552600

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