applied biosystems
by Thermo Fisher Scientific

CytoScan XON data analysis in Chromosome Analysis Suite (ChAS) software

A quick reference guide for CytoScan XON data analysis using ChAS software

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Software version

For analysis of Applied Biosystems™ CytoScan™ XON microarray files, you must install:

- Applied Biosystems™ ChAS software v3.3 or higher
  - **Link to download ChAS v3.3 or higher**
- CytoScan XON library files
- Browser/NetAffx™ Genomic Annotations for **hg19** and/or **hg38** (you can only use one at a time)

Supported sample types

Default QC metrics are for postnatal blood samples and could vary for prenatal and cancer specimens.

Suggested files to use

CytoScan XON Suite (.xnchp) sample file(s): Open
(ensure that NetAffx Genomic Annotations version is correct)

XON Region Levels: On or **Off** per user preference (can use **Filters** to select gene levels 1–4)

Default Histogram/XON DGV Histogram: On (CytoScan XON_aDGV is derived from 1,855 peripheral blood samples from phenotypically normal individuals)

Genes or OMIM® Genes: On

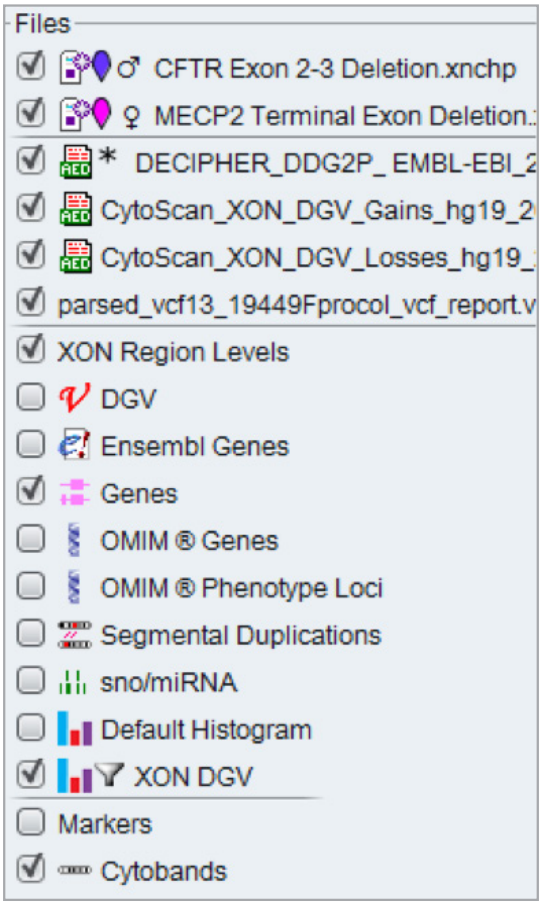
AED/BED files: On if relevant to phenotype or NGS results. In addition to disease-specific AED/BED files, the following Database of Genomic Variants AED files may be useful:

- CytoScan_XON_DGV_Gains_hg19_20180521.aed
  CytoScan_XON_DGV_Losses_hg19_20180521.aed

  Or

  CytoScan_XON_DGV_Gains_hg38_20180521.aed
  CytoScan_XON_DGV_Losses_hg38_20180521.aed
- If desired, you can load one or more additional AED/BED files. Right-click on each file individually and select "**Include in CytoRegions**". Multiple AED/BED files will be treated as one large CytoRegion, with vertical gray bars running through the data, indicating clinically significant gene regions.
- To perform truly targeted analysis, you can restrict the analysis to only those CytoRegions, and all other regions will be hidden from the view:
  - **View** menu > **Restrict to CytoRegions** (**or**  **button**)

VCF files: On if NGS findings are relevant. (VCF files must contain only genotype or indel data.)



Database: These aDGV XON files can be useful for comparison to segment calls in your samples:

- ChASDB_XON_aDGV_20180626.backup
  ChASDB_XON_aDGV_20180628_hg38.backup

  Or

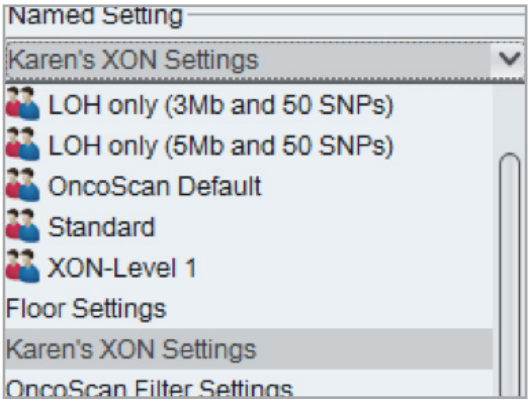
  You may prefer to use your own database of abnormal segments in conjunction with the XON_DGV_Gains.aed and XON_DGV_Losses.aed files mentioned above.

- If you use the XON_aDGV as the database, you can hover your mouse over the histogram (**Default Histogram**), and it will show the number and percentage of samples in the database with that same segment.

- If you use the XON_DGV_Gains/Losses.aed files, the frequency of positive samples will appear above the segment in the AED file, but only if the frequency is greater than 1%. Thus, if you use both the database and the AED files, there may be a discrepancy in frequency.

Named Setting section

Select **XON-Level 1** or user-defined **Named Setting** as shown here.



Data Types section

Gain (XON Region) and **Loss (XON Region): On**

Log2 Ratio: On

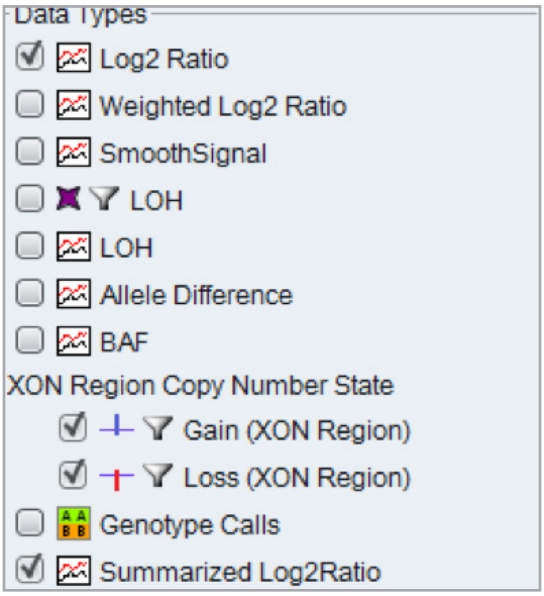
Summarized Log2Ratio: On

- If you have **XON Merging** turned **On** in the **User Configuration**, you won't see the **Summarized Log2Ratio** values for merged segments, because **Summarized Log2Ratio** is specific to each exon region.

LOH: Off, unless looking for large regions (>5 Mb)

SmoothSignal: Off, unless looking for larger regions, because there's no distinct difference for exon-level changes. If you choose to have **SmoothSignal On**, adjust the graph display to **Bar** (rather than **Line**) for easier visualization and interpretation.

Allele Difference and **BAF** (B Allele Frequency)**: Off**, unless looking for large regions

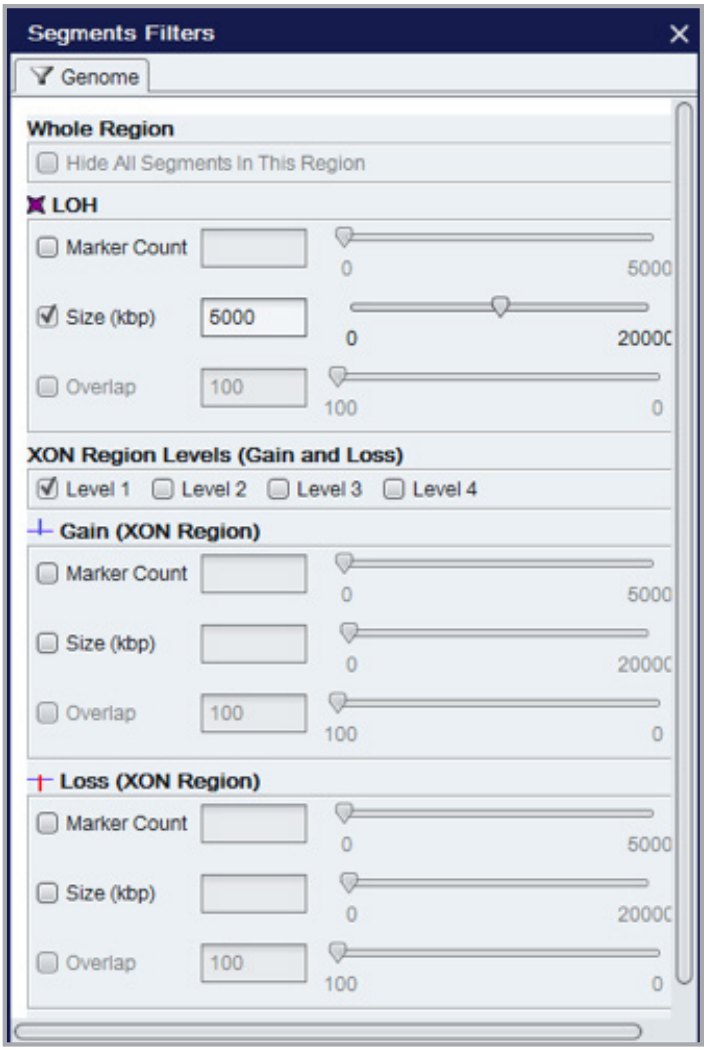


Segments Filters

Gene levels:

- Level 1: Genes of high clinical relevance
- Level 2: ClinVar genes not in Level 1
- Level 3: Other OMIM genes
- Level 4: Other regions from RefSeq, UCSC, Ensembl, and/or Leiden Open Variation Database (LOVD)

- **Start with Level 1 genes to minimize number of calls for analysis**
  - In ChAS, **Level 1** genes are displayed in **yellow blocks**
  - In ChAS, **Levels 2–4** are displayed in **brown blocks**
- **Gain:**
  - Marker Count: **Off**
  - Size (kbp): **Off**
- **Loss:**
  - Marker Count: **Off**
  - Size (kbp): **Off**
- **LOH:**
  - Size (kbp): **On**, minimum of 5 Mb



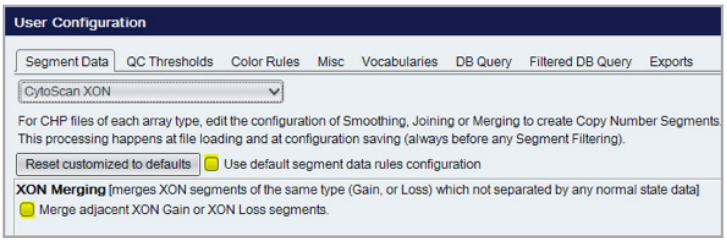
User Configuration

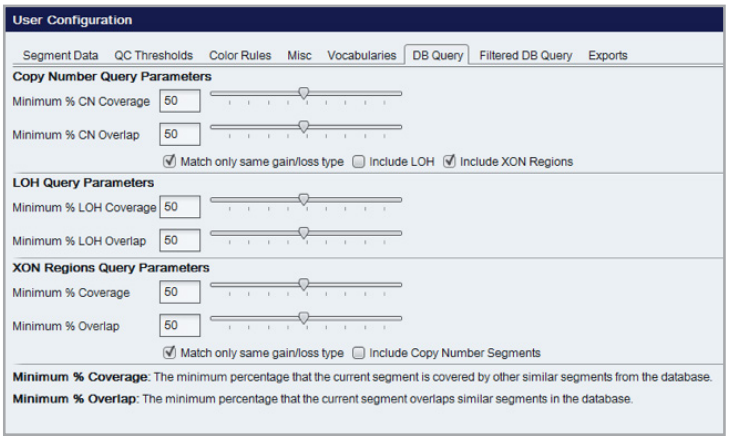
Segment Data tab:

- **Use default segment data rules configuration: Off**
- **XON Merging: Off**

DB Query tab:

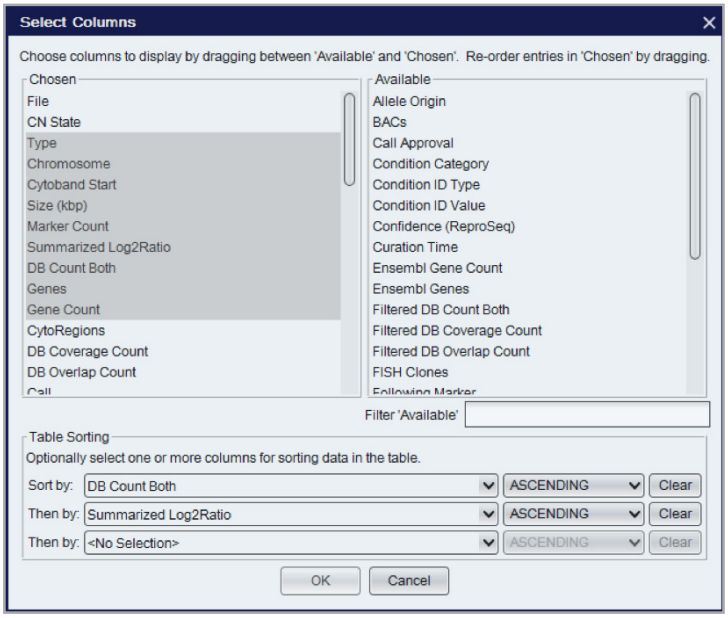
Additional information on coverage and overlap functions can be found in the ChAS User Guide.





Segments Table column selection

- Ensure that **Summarized Log2Ratio** and **DB Count Both** are in the **Chosen** section (left in the screenshot at right) for visualization in the table view.
- Set up **Table Sorting** as shown at right (or in a user-defined way).



CytoScan XON sample analysis

Open a CytoScan XON sample file:

1. Look at the **QC Metrics**; thresholds are shown below.
   - MAPD ≤0.20
   - SNPQC ≥10
   - Waviness SD ≤0.08
2. Go to **Filter Settings** and turn **On** **"Level 1 Genes"** only (check box).
3. Go to **Whole Genome View** to see if there's anything obvious in the tracks (large **Loss/Gain**). 
4. Go to **Segments Table**.
   - There are two new columns in this table:
     - **Summarized Log2Ratio**
     - **XON Region Level**
   - Note that the software doesn't give **Copy Number State**. It only calls **Gain** or **Loss**.
   - Note the number of calls in the upper right corner of the **Segments Table**: 

 is shown as an example
   - **Sort on DB Count Both**: Sort on "0"s first (large arrow up); focus on losses/gains that are <10% of what's in the DB. In the aDGV database of 1,855 phenotypically normal samples, that would be ~18.
   - **Sort on Summarized Log2Ratio**: Sort on most negative values (small arrow up) for **Loss**; values below 0.5 might be more "real". Sort on most positive values (further down the list) for **Gain**. The larger the number, the more likely it is to be a true call.
5. Apply **Gene Lists/AED/BED** files (DECIPHER, disease-specific files, etc.), especially when there's a distinct phenotype. Right-click on the name of the file and select **Set a CytoRegion for Targeted XON analysis**.
6. If you've set a CytoRegion for targeted analysis (step 5), you can also restrict the view to these areas using the **Restricted Mode** button in the toolbar, or go to the **View** menu > **Restrict to CytoRegion**. This will hide all data with the exception of what's in the targeted region file.

7. Using **Edit** mode  , you can manually edit the calls to merge exon segments into one larger call, or adjust the breakpoints. You can assign a copy number if you feel confident about it, but it's not necessary. As mentioned previously, merging segments will eliminate the **Summarized Log2Ratio** for an exon call.

8. Some probes (SNPs) are used for **Allele Difference** or **B Allele Frequency**, but not for XON calls. These probes are shown as gray dots in the tracks, not as colored dots.

9. **Smoothing** and **Joining** are optional and should be turned **Off** in **User Configuration**.

General comments

- For a **targeted approach**, as an orthogonal method to confirm NGS or data from other microarrays:
  - Search for a gene or region, and/or load targeted **AED/BED** file(s), and right-click on file name to create a CytoRegion for targeted XON analysis.
  - Restrict the region of analysis as described above, if desired.
  - Scan through the **Loss** and **Gain** segments in the vertical gray bars, examining the size, number of markers, number of genes, gene significance, etc., and confirm whether the calls are significant.
  - Examine any large (≥5 Mb) regions of LOH to determine relevance.
- For a **nontargeted approach**, for samples where you may be blinded to other findings, or there's no accompanying case history or phenotype:
- Ultimately, you should sort data based on **DB Count Both (ascending)** and then on **Summarized Log2Ratio (ascending)**. You can do this in two different ways:
  - Click on the **Table** icon at the top right corner of the **Segments Table** tab:  The **Select Columns** table appears, in which you can drag attributes from the right (**Available**) column to the left (**Chosen**) column. You can then drag the attributes up or down to place them in the desired order in the **Segments Table**.
  - Alternatively, you can click on the column header and double-sort by dragging the columns to the desired location.
  - Ultimately, you should focus on those segments that have a small **DB Count Both**, but a large **Summarized Log2Ratio** (most positive and most negative numbers) to help gauge whether it's likely to be a true call vs. a false-positive call, or a normal copy number variant. Opening other samples (.xnchp files) at the same time can provide additional insight as to veracity.
  - **Hint:** For exploratory studies, use an arbitrary cutoff value of 15–18 counts for **DB Count Both** when using the aDGV as the database. (This would represent less than 1% of the samples in the database.)

Notes

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