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Welcome	Welcome to Genome Studio: Performing CNV Analysis using cnvPartition. This short module reviews the process of utilizing Illumina's cnvPartition software plug in order to perform copy number variation, or CNV, analysis.
Navigation	Take a moment to familiarize yourself with the navigation for this course. You can access these instructions at any time by clicking Help in the top right corner of the page.
Course Objectives	By the end of this course, you will be able to: • Understand the basis for cnvPartition's calculations of copy number • Install the Illumina cnvPartition plug-in software • Carry out a CNV analysis on a GenomeStudio genotyping project using cnvPartition • Visualize and report the results of the CNV analysis using Illumina GenomeViewer
What is cnvPartition?	cnvPartition is a plug-in software program provided by Illumina that works from within the GenomeStudio Genotyping module to identify regions of copy number variation in a genotyping project's sample, based on the intensity and allele frequency data contained in the project. It also works with GenomeStudio's GenomeViewer to visualize copy number regions graphically and present copy number analysis results in table form.
How does cnvPartition calculate copy number?	Regions of copy number variation exhibit particular characteristics with regards to the allele frequencies and assay intensities contained in them. In these graphs, the B-allele frequency and the normalized intensity as represented by the Log R ratio are displayed in the context of copy number, denoted by "CN".
	The cnvPartition algorithm estimates copy number by comparing the observed log R ratio (LRR) and B allele frequency (BAF) for each locus to predict LRRs and BAFs of different copy number scenarios. The algorithm also takes into account some options set by the user when initializing cnvPartition, which will be reviewed

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	later in this training module For a detailed review of how copy number is calculated consult the Technical Notes “DNA Copy Number Analysis Algorithms” and “Interpreting Infinium Assay Data for Whole-Genome Structural Variation”. These calculations are also reviewed in the CNV Analysis Basics online course.
Requirements for cnvPartition?	In order to carry out CNV analysis using cnvPartition, you need: A current version of GenomeStudio; The GenomeStudio cnvPartition plug-in software; and an Infinium genotyping project. Note that CNV analysis with cnvPartition is intended for Infinium genotyping datasets generated from high-density chips, such as the Omni family of microarrays. Low density arrays, arrays targeted at rare variants such as the Human Exome BeadChip, and GoldenGate projects are not recommended for CNV analysis. Low density chips do not provide sufficient resolution to accurately detect CNVs. The chips that target only rare variants do not display sufficient heterozygotes’ genotypes in the B allele frequency graphs to detect copy number.
Demo 1: Downloading the cnvPartition plug-in through MyIllumina	This demo shows how to download and install the cnvPartition plug-in. Click play to begin. You can obtain the most up-to-date version of GenomeStudio cnvPartition plug-in software from the Illumina Website. After logging in to MyIllumina, hover your mouse over the Services button. Click IlluminaConnect under the heading Other Services. Scroll down the page to the most current version of cnvPartition. Click the associated link to download the zipped file containing the software. After unzipping the downloaded file, you will see the user guide and the installer file. Double-click the installer file to install the software. We recommend that you install the files in the default directories suggested by the installer.
Demo 2: Starting and Running cnvPartition	This demo shows how to use the cnvPartition plug in. Click play to begin. To open the data set in GenomeStudio, select File, followed by Open Project. Navigate to an existing GenomeStudio workspace

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and select **Open**.

In the samples table, select the samples that you wish cnvPartition to analyze. Click the **Analysis** menu, and select **CNV analysis**.

The CNV analysis window will open. In the drop-down option box, under the heading **Create New CNV Analysis**, click the arrow on the right side. If cnvPartition or any other CNV analysis plug-ins have been installed, they will appear in the drop-down menu. Select **cnvPartition**.

After you have selected an analysis method, a default analysis name will appear. You can modify the name if you wish.

Check the box **Calculate Only Selected Samples** if you wish to perform CNV analysis only on samples previously selected and highlighted in blue in the samples table. If you do not check this box, cnvPartition will analyze all samples in the project.

Several options will appear below the analysis name. These settings allow the user to adjust the search parameters that cnvPartition uses when detecting CNV regions. If you click an option, a brief description will appear in the smaller pane immediately below.

For a complete description of these settings, please consult the cnvPartition user guide that was included in the software download package from the IlluminaConnect webpage. Each option can be edited by clicking the column to the right and either overtyping or selecting a different option from the drop down menu that appears.

You can alter the color coding used to represent the various copy number states in the CNV region display by using the drop-down menus on the left side of the window.

When the parameters have been set to your satisfaction, click **Calculate New CNV Analysis** at the top right of the window.

A pop-up window will appear, indicating the progress of the analysis. This will take up to a few minutes per sample.

When the cnvPartition plug-in has completed its analysis, the **CNV Region Display** window appears. This is a graphic display of the detected CNV regions across all samples selected for the analysis. The regions are color coded by copy number as determined during the options selection. Hover the mouse cursor over any colored bar to see the sample name, chromosome, coordinate, and copy number information for the CNV across the bottom.

Save the GenomeStudio project to ensure that the CNV analysis is

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	saved.
Demo 3: Viewing cnvPartition Results	In addition to viewing the results of a cnvPartition analysis in the CNV Region Display, you can view results: In the full data table of the GenomeStudio Genotyping project; As a graphic representation in GenomeViewer; Or as a list of CNVs in the Bookmark Analysis table. Click play to watch any of the demos below.
Viewing cnvPartition results in the full data table	To view your CNV results in the full data table of the GenomeStudio, click the Column Chooser button. The Column Chooser pop-up window will appear. In the lower right pane, under Hidden Sub columns, select CNV Value and CNV Confidence, and click the Show button. The items will transfer from the Hidden Sub columns pane to the Displayed Sub columns pane. Click OK. Two new sub columns will appear for each sample in the full data table – CNV Value and CNV Confidence. These columns remain blank and will not be populated unless cnvPartition has been run. Once run, normal regions will have a CNV value of 2 and a blank CNV confidence. Markers associated with detected CNV will be denoted with values in the CNV confidence column and varying numbers in the CNV Value column. Copy-neutral CNV are denoted by CNV values of 2 accompanied by values in the CNV confidence column.
Viewing cnvPartition results in GenomeViewer	To access GenomeViewer, go to the Tools menu in the main GenomeViewer screen and in the drop-down menu, select Show Genome Viewer. The Add Favorite Data Plots window will appear. From the Sample list, click the checkboxes for all the samples for which you wish to view plots. In the Sub Columns list, select the plots that you wish to be generated for each sample. In general, the most useful plots for CNV analysis are the B allele Frequency and Log R ratio plots. Next, click Add to favorite. Your selections will appear on the right, under the list Favorite Data Plots. Ensure that the plots you have selected appear in the Favorites Data Plots window before

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continuing.

When you have added all the plots that you wish to access in GenomeViewer, click **OK**.

The views you selected will now be listed in the **Data Plots** tab. The plots to be displayed will have checkmarks by them. However, none of these will be displayed in GenomeViewer until you click the **Update** button above the **Data Plots** tab. You can change which of the available plots are displayed by clicking or unclicking the checkboxes. By default, any subsequent changes you make in the selected data plots will not be displayed in the viewer until you click the update button. Click the **Immediate Mode** button if you wish changes in the selected data plots to be automatically updated in the display.

GenomeViewer defaults to the GenomeViewer view, in either: the Whole Genome View mode, which simultaneously displays all chromosomes, or the chromosome slide show mode, which displays plots for a number of chromosomes as designated by the user. However, these views only display graphs for a single sample at any given time, regardless of how many sample views are checked under the data plots tab. In order to view plots for multiple samples, switch to the Chromosome Browser view. Click the **View** menu, and in the drop-down, select **Chromosome Browser**.

In the graphics pane, a **Chromosome Browser** tab appears. The view is divided into three segments. The upper segment displays all plots selected in the data plots tab. The middle segment contains graphical representations of the chromosome including cytoband segments, along with features, such as genes, contained in the area of the chromosomes being viewed. The red box on the chromosome drawing highlights the region of the chromosome currently being viewed in both the graphic pane and the data plots. The viewing range can be scaled and moved by resizing and dragging the red box to a different location on the chromosome.

The third segment displays detailed information regarding any of the genes selected by the user in the middle segment. Click any of the genes to obtain specific information for that gene.

For further information about the Genome Viewer and Chromosome Browser, consult the GenomeStudio Framework User Guide.

The CNV regions detected by cnvPartition can be graphically displayed as shaded regions in the chromosome plots. To do this, click the **View** menu and select **CNV Analysis as Bookmarks**.

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	The Display CNV Analysis window will appear which will give you options such as color, fill style, and opacity, regarding how Genome Viewer displays these regions. Click OK after editing these options. Detected CNV regions now appear as shaded areas in either the GenomeViewer or Chromosome Browser. In this example a small region of copy neutral loss of heterozygosity was detected in the Q arm, as shown by the small green shaded region. A large portion of the Q arm is duplicated, as shown by the blue shaded area. In addition, if you hover your mouse cursor over any shaded region, a pop-up pane appears with information about the detected CNV region being pointed to.
Reporting CNV regions in table format	Detected CNVs can be displayed in table form. In the View menu, select Bookmark Viewer. The Bookmark Analysis window appears. If there have been multiple CNV analyses run on the data in the project, they will be listed in the top pane. You can select which analysis you wish to have displayed by clicking on it. The pane below lists all the CNVs detected by cnvPartition, listing sample IDs, bookmark types (i.e. copy number), location in the genome, and size of the variation. The confidence score appears in the comment field. Above the table, appear many buttons corresponding to the standard buttons available in the main GenomeStudio window. The data in the table can be sorted, filtered, and manipulated in much the same manner as data in any GenomeStudio table. The bookmarks are hot linked to the GenomeViewer. If you right-click any CNV on the list, and select Go to Bookmark, the graphic display in GenomeViewer will jump to that CNV. The data displayed in the Bookmark Analyses window can be exported. To export the data table, click the Export displayed data to a file button. The table data will be exported into a tab-delimited text file, which can be viewed in Microsoft Excel.
Summary: Topics Covered	In this training module you learned: • How to obtain a copy of and install the GenomeStudio cnvPartition plug-in software • How to start a cnvPartition analysis, adjust the analysis parameters, and complete the analysis • How to visualize the results of the analysis in the full data

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	table of a GenomeStudio project; in the GenomeViewer; and as a bookmark analysis table. Finally, you learned how to export the data from the Bookmark Analysis table to a *.csv file, viewable by Microsoft Excel
Course Completion	Congratulations! You have completed this course.