

BlueFuse Multi v4.5

Software Guide



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Revision History

Document	Date	Description of Change
Document # 15053620 v04	December 2018	Made Duo/Trio Analysis content more robust. Removed references to VeriSeq PGS 2.0. Removed references to obsolesced CytoChip products. Updated document for BlueFuse Multi v4.5.
Document # 15053620 v03	January 2017	Reworked Importing Using a Batch File section into two sections, Import 24sure and BeadArray Experiments and Importing VeriSeq PGS Experiments. Added Genotype Analysis (Duo/Trio Analysis) section to chapter 2 CytoChip Module. Removed references to obsolesced CytoChip products (CytoChip, CytoChip Focus Constitutional, CytoChip Oligo). Removed references to Bluefish tracks. Updated document for BlueFuse Multi v4.4.
Document # 15053620 v02	January 2016	Replaced 24sure Single Channel content. Removed "K" barcode ends in Image File Barcode section.
Document # 15053620 v01	November 2015	Reorganized document into 3 chapters—Getting Started, CytoChip Module, and Karyomapping Module. Updated document for BlueFuse Multi v4.3 and BlueFuse Server v1.7.
Document # 15053620 Rev. C	January 2015	Removed the Karyomapping Genome Build section from the User Interface chapter. Additional minor updates for BlueFuse Multi v4.2 and BlueFuse Server v1.6.
Document # 15053620 Rev. B	December 2014	Updated document for BlueFuse Multi v4.2 and BlueFuse Server v1.6.
Document # 15053620 Rev. A	July 2014	Initial release.

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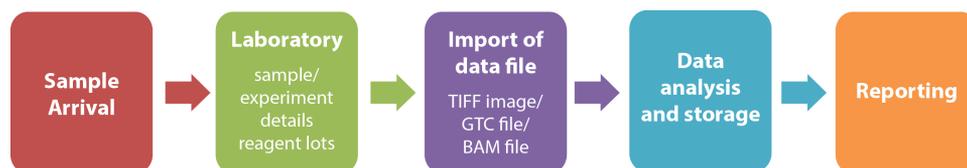
Chapter 1 Getting Started

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Overview

BlueFuse® Multi analyzes microarray and sequencing data from supported Illumina® platforms, utilizes quality control measures, and stores experiment information in one easy-to-use software package.

Figure 1 BlueFuse Multi Workflow



This guide describes the functions, algorithms, and advanced features of the software, with separate sections for the CytoChip™ and karyomapping modules. This guide refers to the latest release of the software, BlueFuse Multi v4.5 and BlueFuse Server v1.7.

Additional Resources

The [BlueFuse Multi support pages](#) on the Illumina website provide additional resources. These resources include software, training, compatible products, and the following documentation. Always check the support pages for the latest versions.

Resource	Description
BlueFuse Multi v4.5 Installation Guide (part # 15053614)	Provides installation instructions.
BlueFuse Workflow Manager Quick Ref Guide (part # 15056206)	Information on creating sample sheets.
Walkthrough Video Guides	BeadChip Array, 24sure, 24sure+, VeriSeq PGS, Karyomapping, and BlueFuse Server Installation walkthrough video guides.

Modes of Deployment

BlueFuse Multi v3.0, or later, can be deployed in the following modes:

- ▶ **Classic Mode**—Multiple installations of BlueFuse Multi can access a single database, but are limited to read-only activity.
- ▶ **Server Mode**—Multiple users have read-write access to a database. If another user has an experiment open, users are notified. The status bar shows the number of users viewing an experiment at any time. Experiment reports can be viewed in a web browser. If many users require read-write access, use BlueFuse Multi in server mode.

Changing Read-Write Access in Classic Mode

- 1 To change read-write access, select **Users | Set Read-Only** or **Users | Set Read-Write**.

Activating Web Access through Server Mode

If you are on the same network as your server, you can access experiment reports using a web browser. You do not need BlueFuse Multi installed on your computer to view web reports.

The web reporting functionality is disabled by default. Enable web reporting using the server configuration file. Before enabling web reporting, consider data privacy and security issues with your IT department. If web reporting is enabled, valid login credentials are required to access the reports.

- 1 On the server computer, open the configuration file using a text editor.
- 2 In the [WebReports] section of the configuration file, change the Enabled setting to 1.
- 3 In the same section, add a database path and alias entry in the format `Database = [UNC database path]|[database alias]`. The path is a full network UNC path to the database. The alias is shown as the database name on the web reporting login page. You can add multiple database entries.
- 4 Save the changes and then close the file.
- 5 In the **Control Panel**, select **Admin Tools** and then **Services** to restart BlueFuse Multi or reboot the server computer.

To view the web reports, open your web browser to the URL *yourserver:<port_number>/reports*. For example, if your BlueFuse Server computer name is *mainserver* and you are using the default port, then the URL is *http://mainserver:12345/reports*.

The web reports are static. To update the web report with changes from the database, refresh the page.

Create a BlueFuse Multi Database

A database stores the information required to run the software and saves the data generated from BlueFuse Multi analysis.

Create the database on the local computer drive or on a networked drive. Make sure that there is enough free disk space in the chosen database location to contain all the image and result files for your experiment.

The top-level folder of the BlueFuse Multi database contains the following hidden folder hierarchy.

- 📁 [Top-Level Database Folder]
 - 📁 Attached Documents
 - 📁 BeadArray™ Files
 - 📁 Database Files
 - 📁 GAL and Clone Files
 - 📁 Image Files
 - 📁 Report Files
 - 📁 Sequencing Files
 - 📄 MOVING FOLDERS,FILES README.txt

When an experiment is created, files are automatically copied to the relevant folder and are stored in the database. Sequencing files are not copied by default. To retain sequencing files, see [Retain BAM Files in the Sequencing Folder on page 5](#). Saved queries, imported tracks, comments, and customized settings are also stored in the database.

The top-level folder containing the folder hierarchy can be copied, backed up, and moved to a different machine. All data files within the folder hierarchy are accessed using relative paths, not absolute paths. Do not delete or rename any content within these folders.

The most recently used list of database locations are saved for easy access.

Create a Database in Classic Mode

- 1 Select **Tools | Change Database Path**.
- 2 Browse to the directory for the new database. Use the full UNC path that is used for locating databases to improve speeds across the network.
- 3 Select **Make New Folder**, and then name the new database.
- 4 Fill in the administrator details.
- 5 Select **OK**.
- 6 Import an annotation database. For more information, see *Import a New Annotation Database on page 6*.

Create a Database in Server Mode

- 1 Select **Tools | Change Database Path**.
- 2 Select **Connect to Database Server**.
- 3 Enter the Host directory path for the server.
- 4 Enter the Port directory. The default is 12345.
- 5 Browse to the directory for the new database.
- 6 Select **Make New Folder**, and then name the new database.
- 7 Fill in the administrator details.
- 8 Select **OK**.
- 9 Import an annotation database. For more information, see *Import a New Annotation Database on page 6*.

Create and Manage Users

All users require a valid login to use BlueFuse Multi and access databases. The administrator can create, delete, or change user details. The administrator can also promote users to administrator or supervisor.

If multiple computers have BlueFuse Multi installed, you can use this user name and password to access the database.

- 1 When logged in to a database as the Administrator, select **Users | Configure Users**.
- 2 The following procedures can be performed:
 - ▶ Create a user—Select **Add User**, and then enter the user details.
 - ▶ Edit an existing user—Select the user name, select **Edit User**, and then make the necessary changes.
 - ▶ Delete a user—Select the user name, and then select **Delete User**.
- 3 Select **Close**.

Database Permissions

Refer to the following table for database access permissions.

Level	Description	Example
User	<ul style="list-style-type: none"> • Can import and process samples and experiments • Can sign off cases to level 1 • Cannot delete content from the database or configure any settings 	Laboratory Technician
Supervisor	<ul style="list-style-type: none"> • Multiple supervisors allowed • Can perform all functions of a user, in addition to the following: <ul style="list-style-type: none"> • Configure algorithm settings for reporting and pick lists • Delete experiments • Sign off cases to level 2 • Create and manage genome masks • Cannot configure users, custom views and shortcuts, or custom reports 	Laboratory Supervisor
Administrator	<ul style="list-style-type: none"> • Multiple administrators allowed • Creator of the database • Can perform all functions of a supervisor, in addition to the following: <ul style="list-style-type: none"> • Create, delete, or change user details. Can promote and downgrade users to administrator or supervisor. • Algorithm settings for reporting • Custom views and shortcuts • Custom reports 	Laboratory Head

Switch Users

- 1 Log in to BlueFuse Multi using your user name and password.
- 2 To switch between different users of a database, select **Login** or select **Tools | User Login**.
- 3 Select your user name from the drop-down menu.
- 4 Enter your password, and then select **OK**.
Until BlueFuse Multi is closed or another user logs in, you remain logged in as that user. The application title bar shows the user name of the logged in user.

Change Passwords

- 1 When logged in to a database, select **Users | Change Password**.
- 2 Confirm your existing password, then enter and confirm a new password.
If a user password is forgotten, the Administrator can reset all passwords.
- 3 Select **OK**.

Reset the Administrator Password

- 1 Select **Users | Reset Admin Password**.
- 2 A new, random password is generated. Select **Copy to Clipboard**, and email the password to [Illumina Technical Support](#).
- 3 Illumina support returns an email with a text string. Enter the text string in the verification box.
- 4 Select **OK**.

Changing to a Different Database

To view a particular database, open the module that was used to create that database. If you switch from one module to another, you are prompted to switch to the database that is relevant to that module.

- 1 Select **Tools | Change Database Path**.

- 2 Browse to the directory, and then select the database.
- 3 Select your user name and enter your password.
- 4 Select **OK**.

Back Up a Database

If other users are using the database, do not copy or move it. Large databases can take a long time to copy. Perform the process overnight.

For VeriSeq PGS, store the original sequencing run folder to have a record of all raw data. If necessary, you can use this data to reimport data into BlueFuse Multi.

- 1 Select the top-level database folder.
- 2 Right-click, and then select **Copy**.
- 3 Paste the folder in the destination location.

Retain BAM Files in the Sequencing Folder

By default, the Retain Sequencing Files option is not enabled. Before selecting this option, make sure that there is sufficient disk space. Consult your IT partners to prevent unauthorized access to the BAM file folder.

BlueFuse Multi does not retain all raw data, such as sample sheets and summary files.

- 1 Select **Tools | Sequencing Configuration | File Retention Configuration**.
- 2 Select the **Retain Sequencing Files** checkbox.
- 3 Select **OK**.

Annotation Database

BlueFuse Multi uses an annotation database to store the most recent information about the human genome. This information includes gene content, implication in disease, common genetic variants, and the genomic locations of Illumina microarrays and the bins for sequencing assays. The annotation database provides the information that appears in the Decision Tracks pane of the software, along with history tracks.

The information provided the annotation databases is taken from the major public databases, including:

- ▶ Ensembl and UCSC for gene annotation
- ▶ OMIM for inherited diseases
- ▶ DGV and Decipher for genetic healthy or disease variants

Import an annotation database into each BlueFuse Multi database. To download annotation databases, go to accounts.illumina.com and select **BlueGnome** from the destination drop-down.

Available Annotation Databases

A GRCh37 annotation database is available for all supported experiment types. A GRCh38 annotation is available for use only with Illumina BeadChip experiments in the CytoChip module.

Annotation databases are updated inline with the work of the international scientific communities, for example, to include updates to the genome build. Annotation databases can be imported into existing BlueFuse Multi databases if they pertain to the same version of the human genome as previously imported annotations. This process lets existing and new data get processed and viewed with the most current annotation information.

The side pane of the software shows the current annotation database used in BlueFuse Multi. It is important to have the latest version of BlueFuse Multi software to use the latest annotation database. The current annotation databases are available for download on our website.

Import a New Annotation Database

- 1 Select **Help | About** to check the version of your software. Make sure that the downloaded annotation database is compatible with the software version.
- 2 Select **Tools | Import New Annotation**.
- 3 Browse to the downloaded, extracted annotation database file, and then select **Open**.

Reanalyze with a More Recent Annotation Database



NOTE

This can only be done with an annotation database of the same build.

- 1 Create a copy of the reported cases processed with the original annotation database.
- 2 Make sure that you have the latest version of the software and import the most recent annotation database.
- 3 Select an experiment from the Selection group in the side pane.
- 4 Select **File | Edit**, or select **Edit**.
- 5 On the screen where the annotation database is specified, select the latest annotation database file.
- 6 Click through the wizard.
When complete, the experiment icon reverts to orange.
- 7 To reprocess the experiment, select **Run Batch Processing** or select the BlueFuse Multi algorithm button.

Using or Migrating to a GRCh38 Annotation

The GRCh38 annotation can be used with CytoChip module databases that exclusively contain experiments using the latest versions of Illumina BeadChip arrays.



NOTE

A GRCh38 annotation cannot be imported into a database into which a GRCh37 annotation has previously been imported, and vice versa.

A GRCh38 annotation can be imported into a newly created database. For more information, see [Import a New Annotation Database on page 6](#). Future updates to the GRCh38 annotation are importable into this database.

Use the following procedure to migrate from GRCh37 to GRCh38 while maintaining access to historical data.

- 1 Export tracks as BED files from the existing database. For more information, see [Export Tracks on page 24](#).
- 2 To map the regions to the GRCh38 assembly, use a tool like UCSC liftOver (genome.ucsc.edu/cgi-bin/hgLiftOver)
 - ▶ Be sure to specify the minimum ratio of bases that must remap (0.5 recommended).
- 3 Check the UCSC liftOver error log for regions that did not successfully remap.
- 4 Create a database and import the GRCh38 annotation.

5 Import at least one experiment.



NOTE

This import must be done to be able to view the track imported in step 6.

6 Import the BED files containing the output from step 2 that are then displayed as tracks in the DecisionTrack view. For more information, see *Importing BED files on page 24*.

7 Use this database to import and process future experiments as described in *Experiments and Samples on page 31*. For more information on Duo Analysis, see the *Migration of Tracks from a BlueFuse Multi GRCh37 database to a GRCh38 database* bulletin on the [BlueFuse Multi Software support page](#).

Module Selector

Use the following table to determine the module to use when switching from the standard CytoChip module to the Karyomapping module.

Module	Description
CytoChip Module	Used to process the following array types: <ul style="list-style-type: none"> • CytoSNP-850K • CytoSNP-12 • 24sure • 24sure+ The CytoChip module is also used to process VeriSeq PGS data.
Karyomapping Module	Used to analyze karyomapping cases using the HumanKaryomap-12 BeadChip.

CytoChip Module

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Supported Experiment Types

When you add an experiment to a BlueFuse Multi database, select an experiment type based on the microarray design and laboratory protocol. The experiment type determines the algorithm. For more information, see *Algorithm Steps for Each Experiment Type on page 8*.

The following experiment types are supported in the CytoChip module. This table does not include all supported array types. For a full list, contact Illumina Technical Support.

Experiment Type	Description	Illumina model numbers
24sure Single Channel	24sure arrays run with the single channel (v3) format	No longer available for sale.
24sure	24sure and 24sure+ arrays run with the standard dual channel format	No longer available for sale.
BeadArray	<ul style="list-style-type: none"> • Infinium CytoSNP-850K v1.1 BeadChip Kit (8 samples) • Infinium CytoSNP-850K v1.1 BeadChip Kit (16 samples) • Infinium CytoSNP-850K v1.1 BeadChip Kit (48 samples) • Infinium CytoSNP-850K v1.1 BeadChip Kit (96 samples) • HumanCytoSNP-12 v2.1 BeadChip Kit (12 samples) • HumanCytoSNP-12 v2.1 BeadChip Kit (24 samples) • HumanCytoSNP-12 v2.1 BeadChip Kit (48 samples) 	<ul style="list-style-type: none"> • WG-322-1101 • WG-322-1102 • WG-322-1103 • WG-322-1104 • WG-320-2101 • WG-320-2102 • WG-320-2103
VeriSeq PGS	NGS solution for PGS on MiSeq®	• RH-101-1001

BlueFuse Multi Algorithms

Algorithm Steps for Each Experiment Type

BlueFuse Multi performs end-to-end analysis of data from supported Illumina platforms. Each analysis consists of several steps, depending on the experiment type that is selected. For information about the algorithms used for VeriSeq PGS experiments, see *VeriSeq PGS Algorithms on page 12*.

Algorithm Step	24sure Single Channel	24sure Dual Channel	BeadArray
Grid Alignment	√	√	
Quantification	√	√	
Single Channel Processing	√		
Normalization	√	√	
Exclusion	√	√	
Fusion	√	√	
Smoothing	√	√	√
CGH Region Calling			√
SNP Processing			√
LOH Calling			√
Region Assessment			√
Aneuploidy Calling	√	√	
Reporting	√	√	√

Algorithm Descriptions

Algorithm	Description
Grid Alignment	<p>This algorithm is for a fully automated and robust grid alignment, which has been tested on tens of thousands of arrays.</p> <p>The algorithm allows for some rotation of the image due to small imperfections in positioning of the slide on the scanner bed.</p> <p>The GAL file is used to construct an initial grid configuration. A template-matching procedure compares the configuration to the image and produces a score for that configuration. The configuration is then adjusted in various ways and rescored. The configuration which yields the highest score is selected as the final configuration. Adjustments to the grid configuration can involve shifting or stretching of the whole grid. On printed arrays, individual blocks can also shift independently.</p> <p>The score calculated for each grid configuration is insensitive to various kinds of image artifacts (eg, uneven backgrounds, poor signal to background ratios, dye blobs). Otherwise, these image artifacts can cause grid alignment to fail.</p> <p>In routine practice, grid alignment only fails if the microarray image is cropped incorrectly and there is insufficient margin around all edges of the printed area of the microarray.</p>
Quantification (BAC array)	<p>This algorithm is used for image quantification of printed microarrays and uses a Bayesian model to represent statistical variability in the spot formation and noise processes.</p> <p>Each spot is isolated in a small square tile from the image. Within each tile, the statistical model determines whether each pixel belongs to the spot or to the background around the spot.</p> <p>The signal for a spot is calculated by combining together the intensities of the spot pixels. Small numbers of relatively bright or dark pixels do not bias the result. These signal values are calculated separately for the Cy3 and Cy5 channel data. Similar summary values are calculated for the background pixels in each channel and are subtracted from the signal values.</p>
Normalization	<p>Hybridized against itself on a microarray, some spread in the log₂ ratios of the probes centered on the 0 line is expected. The spread is due to irreducible noise and several factors that systematically bias the log₂ ratio data. The systematic biases can be modeled and their effects can be subtracted, causing a reduction in the spread of log₂ ratio data and making profiles appear less noisy.</p>

Algorithm	Description
Spatial Bias Correction	Probes are randomly allocated to addresses on the array. In most experiments, most probes are expected to have a log ₂ ratio close to 0. Large areas where all the adjacent spots have ratios above or below 0 are not expected. However, in practice, these regions can be observed for various reasons. Pin bias in printed arrays and uneven hybridization can lead to areas of the array where the spots have higher or lower log ₂ ratio values than expected. The spatial bias correction algorithm models the log ₂ ratio data from an array as a smooth nonlinear function of spot position. This estimate is subtracted from the observed log ₂ ratio of each spot to remove bias.
Intensity Bias Correction	Due to variability in scanner laser powers, labeling, and dye efficiencies, the log ₂ ratios measured can be biased away from 0. Furthermore, the degree of bias can vary with the overall spot intensity. This bias can be visualized by looking at a plot of log ₂ ratio against log ₂ product. Ideally this plot shows no trends. In practice, this plot often has a slope or even a curve. Intensity bias correction models the trend between log ₂ ratio and log ₂ product and then subtracts this trend from the data.
GC Base Content Bias Correction	GC-rich and GC-poor sequences have different hybridization characteristics. In array CGH data, a characteristic waviness can sometimes be seen in the profile of a sample. This bias can be visualized by looking at a plot of log ₂ ratio against GC content. Ideally this plot shows no trends. In practice, this plot often has a slope or even a curve. GC bias correction models the trend between log ₂ ratio and GC content and then subtracts this trend from the data.
[Optional] Chromosome Recentering/ Renormalization	Renormalization is helpful when a profile has several different copy number levels across multiple chromosomes. If karyotype data are available, 1 or more normal chromosomes can be identified and entered into the software. BlueFuse Multi shifts the log ratios for all chromosomes so that the normal chromosomes have the expected median log ₂ ratio of 0. The log ₂ ratios in the profile can then be interpreted directly as gains and losses.
Exclusion	Exclusion only applies to BAC array experiments. Clone inclusion rates and data quality can be improved with a set of exclusion rules, which are developed with the knowledge that arrays are manufactured to a consistent standard. These rules can be summarized as follows: <ul style="list-style-type: none"> • Detection and exclusion of outlier replicates for each clone. • Removal of low intensity spots. • Exclusion of replicates in an individual subarray, which are inconsistent according to standard deviation. • Exclusion of clones with insufficient replicates. • Removal of control spots.
Fusion	Probe replication can reduce the noise in an array CGH profile. When more than 1 replicate result remains for a probe after exclusion, the data from the replicates are fused to produce a single final result for that probe. A robust average is taken over the replicates.
Smoothing	The log ₂ ratio measurements made by individual probes can be noisy, but neighboring probes are expected to produce similar values. Smoothing reduces the noise in an array CGH profile by pooling information from neighboring probes. Smoothing is controlled by picking a window size <i>k</i> . To calculate the smooth log ₂ ratio for a probe, BlueFuse Multi calculates the median log ₂ ratio of the probe and the <i>k</i> neighbors on either side. As the window size <i>k</i> is increased, the profile becomes less noisy. However, there is a corresponding decrease in resolution. Generally, after smoothing with a window size <i>k</i> , only regions with <i>k</i> +1 or more probes are visible and any smaller regions are suppressed.
CGH Region Calling	Region calling algorithms are optimized in terms of the array technology and the application. The main difference is between the aneuploidy algorithm used with the 24sure arrays, and the algorithm developed for detecting imbalances in BeadChip arrays.
Aneuploidy (24sure+, 24sure)	The 24sure arrays are optimized for the detection of whole chromosome aneuploidy. The region calling algorithm works by determining the majority behavior of each chromosome. If most of the probes in a chromosome have a log ₂ ratio consistent with typical log ₂ ratios expected for gains or losses, the chromosome is called as a gain or loss. A confidence metric is also calculated for each call. Confidence values range from 0.5 to 1. A value of 0.5 indicates that the chromosome is on the boundary between no change and either a gain or loss. Values close to 1 indicate that the affected chromosome has shifted away from the expected ratio.

Algorithm	Description
CGH and LOH Region Calling (BeadChip Arrays)	<p>This algorithm simultaneously detects regions of copy number imbalance and regions of copy number neutral LOH. Each copy number, LOH, and mosaic state is associated with a specific pattern in both the logR ratio chart and the B allele frequency chart. This algorithm simultaneously uses the direction and magnitude of the logR ratio intensity signal, along with the distribution of B allele frequency signal. Therefore, the algorithm can segment the genome into regions where the joint logR ratio and B allele frequency patterns differ.</p> <p>To identify regions of copy number imbalance on the sex chromosomes, the result of the algorithm is compared to the expected result for the gender of the sample. The final outcome can depend on whether the sample gender has been set by the user. If the gender is not provided, then BlueFuse Multi attempts to estimate the sample gender based on the presence or absence of a Y chromosome. If a Y chromosome is detected, the predicted gender is male. Otherwise, the predicted gender is female.</p> <p>Using both the logR ratio and B allele signals together results in more sensitivity to detect smaller regions of imbalance and increases the accuracy of the region boundaries.</p> <p>The algorithm can take a long time to call BeadChip array experiments with noisy data and can produce large amounts of false positive calls. In this instance, BlueFuse Multi automatically switches to simple calling (whole chromosome calling). BlueFuse Multi uses simple calling for any samples with a normalized LogR Dev of greater than 0.3. BlueFuse Multi uses the normalized LogR Dev to trigger the simple calling, but the value reported in the Median Log R Deviation report is based on raw data. This is why the value reported can be above 0.3, but simple calling has not been triggered because the normalized value is below the threshold. Both normalized and raw values are detailed in the logs during processing. For more information on overriding this switch to simple calling, see Array Algorithm Settings on page 13.</p>
LOH Calling (Bead)	<p>LOH regions identified on Illumina BeadChips are called automatically. The algorithm simultaneously detects regions of copy number imbalance and regions of copy number neutral LOH. For more information, see CGH and LOH Region Calling (BeadChip Arrays)</p>
Region Assessment (postnatal CGH Regions only)	<p>Fully assessing the clinical significance of a copy number imbalance requires a high level of expertise and full information of the clinical context. BlueFuse Multi provides summaries of data relevant to the decision. BlueFuse Multi also applies simple rules to that data to suggest a region assessment in certain cases where the evidence appears to be clear.</p> <p>To make an automated region assessment, the software first looks for clear evidence that the region is benign. A benign region has the following characteristics:</p> <ul style="list-style-type: none"> • Fully covered by significant CNVs from the DGV database <i>or</i> fully covered by benign CNVs from the ISCA/dbVar database of the same type <i>and</i> not overlapping a known disease region <i>and</i> not overlapping 80% or more of any ISCA/dbVar pathogenic region of the same type. • Does not overlap a known disease region. • Does not overlap more than 80% of an ISCA (GRCh37) <i>or</i> dbVar (GRCh38) pathogenic CNV. <p>If the region cannot be classified as benign, it is assessed for evidence that it is pathogenic. A pathogenic region has the following characteristics:</p> <ul style="list-style-type: none"> • Less than 80% covered by DGV regions of the same type <i>and</i> is at least 1Mb in size <i>or</i> overlaps a known disease reason, <i>or</i> an ISCA pathogenic region of the same type is >80% overlapped by the region. • Is at least 1 Mb in size, overlaps a known disease region, <i>or</i> overlaps 80% or more of any ISCA (GRCh37) <i>or</i> dbVar (GRCh38) pathogenic region. <p>If there is no evidence that the region is pathogenic, it is classified as unknown.</p> <p>Software assessments are always marked with a “?” symbol to indicate that these assessments are suggestions, not definitive results. Do not rely on these results directly. Always investigate and confirm the results using full clinical information regarding the case. After a region has been investigated and confirmed, modify the assessment status using the Change Assessment context menu option.</p> <p>GRCh38 databases do not include the Disease track and the region assessment logic simply excludes disease regions in its calculation.</p>

VeriSeq PGS Algorithms

Algorithm	Description
Filtering	Filtering algorithms only use reads that align uniquely to the genome, are not duplicates of other reads, and reach a sufficient base quality.
Binning	Based on its alignment position, every read is assigned to a bin across the genome. Bins are approximately 1 MB in size for VeriSeq PGS and are comparable to the genomic positions of clones or probes tiling the genome on microarrays. The number of reads assigned to each bin is the raw data measured for VeriSeq PGS.
Amplification Protocol Bias Correction	Certain genomic regions are overrepresented or underrepresented based on the whole genome amplification protocol. This bias is corrected automatically for each bin. Deviations from the recommended protocol make this correction less effective and introduce noise.
GC Base Content Bias Correction	Similar to hybridization on microarrays, genomic regions with low or high GC base-composition have different characteristics. In sequencing-based experiments, they are overrepresented or underrepresented after the whole genome amplification protocol. This bias is corrected automatically.
[Optional] Chromosome Recentering/ Renormalization	A profile can have several different copy number levels across multiple chromosomes. Similar to array experiments, renormalization can be applied by selecting euploid chromosomes manually.
Smoothing	Smoothing allows you to reduce the noise in a VeriSeq PGS profile by pooling information from neighboring bins. Similar to array experiments, smoothing is controlled by selecting a window size k . As the window size k is increased, the profile becomes less noisy. However, there is a corresponding decrease in resolution.
Rescaling	VeriSeq PGS data can be shown directly on a copy-number scale. Euploid autosomes and X chromosomes in female samples are expected to have a copy number of 2. Male samples are expected to have a copy number of 1 for X and Y chromosomes.
CNV Region Assessment and Aneuploidy Calling	Similar to 24sure arrays, VeriSeq PGS is optimized for the detection of whole chromosome aneuploidy. The region calling algorithm works by determining the majority behavior of each chromosome, based on the counts per bins of a chromosome. A confidence metric is calculated for each call as before. Confidence values range from 0.5–1. A value of 0.5 indicates that the chromosome is on the boundary between no change and either a gain or loss. Values close to 1 indicate that the affected chromosome is shifted away from the expected ratio. Subchromosomal CNVs or mosaic chromosomes have lower confidence scores.

Change Algorithm Settings for Microarray Experiments

Changes made using these procedures affect algorithm settings for all microarray experiments. For more information on general parameters and settings, see [Array Algorithm Settings on page 13](#).

- 1 Select **Tools | Array Configuration | Array Algorithm Settings**.
- 2 Select general parameters.
- 3 Select the microarray type.
- 4 Edit the settings.
- 5 Select **Save Current** or **Save All**.

Change Algorithm Settings for VeriSeq PGS

Changes made using these procedures affect algorithm settings for all VeriSeq PGS experiments.

- 1 Select **Tools | Sequencing Configuration | Sequencing Algorithm Settings**.
- 2 Modify the backbone smoothing parameter. The default value is 3 bins.

- 3 Select **Save Current** or **Save All**.

Changing Algorithm Settings for a Selected Experiment

Changes made using these procedures affect algorithm settings for only the selected experiment.

- 1 Select an experiment from the selection group in the side pane.
- 2 Select **File | Edit** or **Edit Sample/Experiment/Query**.
- 3 Click through the wizard.
- 4 Deselect **Use Default Algorithm Settings**.
- 5 Edit the settings. For more information on general parameters and settings, see *Array Algorithm Settings on page 13*.
- 6 Chromosomes for Recentering—Select chromosomes with normal copy numbers to recenter the data, and then select the + button. This option is beneficial for complex copy number profiles.
- 7 Select **Next**, and then **Finish**.
- 8 Select **OK** to apply the changes.
- 9 Reprocess the experiment using the BlueFuse Multi algorithm button in the side pane.

Array Algorithm Settings

You can modify the following algorithm settings for microarray experiments.

General Parameters	Description
Run Sex-Mismatched Calling	Select this parameter when a sex-mismatched hybridization is performed (male vs female). This setting is not relevant for 24sure v3, BeadChip arrays, or sequencing applications.
Disable Simple Calling	This setting is for BeadChip arrays only. Simple calling is automatically used with noisy data. Select this setting to prevent simple calling and to use normal BeadArray calling.
Region Size Settings	Description
Minimum Del Size—Exclusively Backbone (Kb)	Modify the minimum region size parameters for deletions, duplications, or LOH regions to include or exclude regions from the report. These settings do not affect the calling regions (ie determining whether they are a gain or loss). These settings also do not prevent regions from appearing in the history track.
Minimum Del Size—Including Disease (Kb)	
Minimum Dup Size—Exclusively Backbone (Kb)	
Minimum Dup Size—Including Disease (Kb)	
Minimum LOH Region Size (Mb)	
Significant Clones Settings	Description
Minimum Number of Clones—CGH Region	Modify the minimum number of significant clones parameters for CGH and LOH regions to affect the sizes of regions that BlueFuse Multi calls.
Minimum Number of Clones—LOH Region	
Smoothing Parameter—Backbone	Smoothed regions can be set as the number of clones included or region size in bps. Values ≤ 20 are considered clones and values > 20 are considered base pairs. Calling is performed on the smoothed data. Therefore, modifications to the smoothing parameters can affect the regions that are called.
Smoothing Parameter—Disease	

File Types

BlueFuse Multi uses different file types during installation, experiment import, and data processing. Data are generated from the NextSeq 550 system, MiSeq system, iScan®, and other non-Illumina scanners.

The annotation database file is required for the analysis of all array and sequencing experiment types. For more information, see *Annotation Database on page 5*.

GAL File

GAL (*.gal) files are issued for every array design, except for BeadArray. A GAL file contains information about the DNA and their locations on the microarray slide. BlueFuse Multi uses this information to extract the information from the scanned microarray image file. BlueFuse Multi then plots the position of the DNA probe on the genome against the fluorescent ratio value of the hybridized labeled DNA. After the GAL files are used in BlueFuse Multi, they are stored in the database.

To download GAL files, go to accounts.illumina.com and select **BlueGnome** from the destination drop-down. The download center contains GAL files for each lot of printed BAC arrays (24sure and 24sure+).

BAC array GAL files are specific to each lot of slides because the arrays of spots are assessed for quality after the arrays are printed. The GAL files accommodate any spots that failed to print.

Image File

Scanned microarray images are saved in a TIFF (*.tif) file. Store all scans—up to 2 prescans (Cy3 and Cy5) and 2 high-resolution scans—in a single, multiframe TIFF file.

When you name the image file, include the barcode in the first or second string of the file name. The barcodes in the following examples are bolded.

- ▶ Correct examples:
 - ▶ **252458510481**_S01_D.tif, US23502418_**252458510481**_S01_D.tif
 - ▶ **064068B**_BOTTOM.tif
- ▶ Incorrect example: chaslab_2010-07-16_12h38m_**252458510481**_CY3-34627_CY5-34759_D.tif

From the barcode, BlueFuse Multi can automatically identify the type of array and assign the correct settings and files.

Image File Barcode

Refer to the following table for more information about the image file barcode. The delimiter used in the file name can either be an underscore (_), dash (-), or space (.). If the image file name is not extracted correctly, make sure that the correct experiment type is selected.

Array Type	Example File Name	File Name Section	Description
24sure	064068T_BOTTOM.tif	064	Lot number—Relates to the printed lot. Use this lot number with the array type letter to select the GAL file, ie 24sure_Lot064T.gal.
		068	Position during the print run
		T	Array type <ul style="list-style-type: none"> • K/L/M—24sure • T/U—24sure+
		BOTTOM	Position on the array (TOP or BOTTOM)
BeadArray	9324519002_R08C01.gtc	9324519002	Barcode—The number of the array. Barcodes can be 10, 11, or 12 digits long.
		R08C01	Position on the array <ul style="list-style-type: none"> • R—Row • C—Column

Configure BlueFuse Multi to Recognize the Image File Name

By default, BlueFuse Multi expects the barcode to be in the first string of the image file name. You can alter the default settings so that the software expects the barcode in the second string.

- 1 Select **Tools | Array Configuration | Experiment Configuration**.
- 2 Select **Ignore character before first delimiter**.

Image Files in 24sure Experiments

Multiframe TIFF files usually have two or four frames. The following frames are used by default.

- ▶ Two-frame files—The first frame is used as the Cy5 image and the second frame is used as the Cy3 image.
- ▶ Four-frame files—The fourth frame is used as the Cy3 image and the third image is used as the Cy5 image.

BlueFuse Multi automatically assess the experiment content of the database according to the Cy3 or Cy5 dye. If a sample is loaded into the software that appears to contradict the rest of the database, the software issues a warning.

BeadArray File Types

The following file types are used to process and analyze BeadArray experiments.

File	Description
GTC File	Incorporates the fluorescent image file information. The iScan software can be configured to create the GTC file automatically as part of scanning. Alternatively, you can use the Illumina Beeline software to convert IDAT files to GTC files after scanning.
SNP Manifest File	Contains the SNP probe information and is specific for each BeadArray design. The file extension is *.bpm. Download from the Illumina support site.
SNP Cluster File	Contains the reference information used for analyzing BeadArray. The file extension is *.egt. Download from the Illumina support site.
Annotation Database	The annotation database, BG_Annotation_Ens70_20130904.db or above, is required to process BeadArray experiments.

BlueFuse Multi can automatically extract information from the GTC file name to identify the type of array, the barcode and specific subarray location on the slide, and assign the correct settings. For more information, see *Image File Barcode on page 14*.

After the SNP manifest and SNP cluster files are used in BlueFuse Multi, they are stored in the database.

NGS/ VeriSeq PGS File Types

The following file types are used to process and analyze VeriSeq PGS data.

- ▶ **BAM File**—Contains the aligned sequencing data for a sample.
- ▶ **Sample Sheet**—Created using the BlueFuse Workflow Manager and contains run and sample information. For more information on creating sample sheets, see the *BlueFuse Workflow Manager Quick Ref Guide (part # 15056206)*.
- ▶ **Summary.xml and RunParameters.xml Files**—Contain sequencing run and QC data. The sequencer produces these files, which BlueFuse Multi can also read.
- ▶ **Annotation Database**—The annotation database BG_Annotation_Ens74_20160609.db or above is required to process VeriSeq PGS data in BlueFuse Multi. VeriSeq PGS only supports GRCh37 annotation databases.

User Interface

BlueFuse Multi uses a standard Windows user interface. The interface comprises of six main components:

- ▶ **Menus**—Provides access to all functions in BlueFuse Multi.
- ▶ **Toolbar**—Contains icons for quick access to commonly used functions.
- ▶ **Side pane**—Contains options to select samples, experiments, queries, views, and experiment action buttons.
- ▶ **Genome build**—A dropdown to view experimental data against multiple builds of the human genome.
- ▶ **Views**—The different ways of viewing the experiment data.
- ▶ **Module Selector**—A dropdown to select CytoChip or karyomapping module.

Icons

Refer to the following table for descriptions of the icons in the CytoChip module. To change the size of the toolbar icons, click **View | Large Toolbar Icons**.

Toolbar Icons	Toolbar Icon Description	Side Pane Icons	Side Pane Icon Description
	Add an Experiment (Express)		Sample
	Add a Sample		Experiment—Unprocessed
	Add an Experiment		Experiment—Processed
	Add a Query		Experiment—Manually Modified but Not Signed Off

Toolbar Icons	Toolbar Icon Description	Side Pane Icons	Side Pane Icon Description
	Edit the Sample/Experiment/Query		Experiment—Signed Off to level 1
	Delete the Sample/Experiment/Query		Experiment—Signed Off to level 2
	Run Batch Processing		BlueFuse algorithm has been run or the experiment has been signed off
	Export Results to File		BlueFuse algorithm has not been run or the experiment has not been signed off
	Show/Hide the Side Pane		Query—Temporary
	Apply Filter to Selection		Query—Saved to Database
	Log in to a Database		
	History Icons—Forwards, Backwards		

View Recent History

To navigate through your viewing history of samples and experiments, select the forward and backward history icons on the toolbar. Alternatively, select the dropdowns on the history icons to view the five experiments or samples in the viewing history. The current view is bolded. Select an experiment or sample from the list to view.

The view history is stored for each user of each database. The view history is saved after logging out of the database.

Side Pane

Group	Description
Selection	<p>Opens the list of all samples and experiments or a list of all queries that have been created, in the order of most recently modified.</p> <p>This list can be filtered so that items that meet specified criteria are displayed. For more information, see <i>Filtering Experiments and Samples on page 38</i>.</p> <p>The Sample ID is the top level of organization for an experiment or set of experiments. In a PGS (24sure/VeriSeq) setting, the Cycle ID is equivalent to the Sample ID and the Embryo ID is equivalent to the Experiment description.</p>
Algorithm	<p>Includes algorithm options and is visible when an experiment is selected. The following algorithms are available:</p> <ul style="list-style-type: none"> • BlueFuse Multi—Use to process and analyze all experiments. • Sign Off—Use to record manual changes and sign off experiments. For more information, see <i>Filtering Experiments and Samples on page 38</i>.

Group	Description
Shortcuts	Lists the common configurations of views. Each option opens 1 or more views from the View group box, except for the Close All option, which closes all views. An administrator can customize shortcuts and save the shortcuts within the database. For more information, see Set a Custom Shortcut View on page 30 . The current shortcut is maintained when you change between the selected sample, experiment, or query in the Selection group box.
View	Lists all possible views that can be opened within the application for the experiment. The current view, including chart zoom levels, is maintained when you change between the selected sample, experiment, or query in the Selection group box.
Details	Shows basic information about the selected item

Views

This section describes the options for viewing your experiment. The software views are also described in the Quickstart and Walkthrough video guides found in the Help menu.

Different configurations of views are stored as shortcuts such as Genome View and Chromosome View. Customized shortcut views can be saved to the database. For more information, see [Set a Custom Shortcut View on page 30](#).

All views are fully linked. If an imbalance is selected in a table, the same region is displayed in any open chart views. Views are linked when zoomed.

Chart Views

You can view experiment results in various chart views. The following table includes descriptions about the main chart views.

Chart	Description
Raw Chart	Shows fluorescent data from the microarray with no postprocessing.
Fused Chart	Shows the postprocessed results that are normalized to correct for spatial, intensity, and dye-related effects. For BAC microarrays, results are also normalized to correct for combined replicates.
CGH Chart	Opens a vertical, fused chart alongside the chromosome ideogram. A green or red bar is displayed to indicate regions of gain or loss, respectively.
Karyotype Chart	Offers an overview of each experiment and opens all chromosome ideograms in the standard configuration. A green or red bar is displayed to indicate regions of gain or loss, respectively. No individual data points are shown.

The data displayed in the fused, CGH, CNV, and karyotype charts are segmented and called. The calling step takes the fused results and splits them into regions of imbalance classified as gains, losses, or no change. For VeriSeq PGS, the calling step takes the binned sequencing data.

From any chart view, you can select a region to search the current database for other experiments that have an imbalance (gain/loss/no change) in the same region. Use the Create Region Query context menu option. If a region or spot is selected, it is displayed in yellow and yellow shading extends into the DecisionTrack™ pane.

BAC microarray (24sure) chart spots are colored using confidence flags. The confidence estimate flag is based on the microarray spot quality in both fluorescent channels, the signal intensity, and the consistency between the channels.

Spot Color	Description
Green	A—High Confidence
Light Blue	B
Dark Blue	C
Orange	D
Red	E—Low Confidence

The colors for BeadArray chart spots are colored according to genotype. Probes that can be called are cyan and probes that cannot be called are orange.

Chart View Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Change Type	Allows you to change the type of an incorrectly called existing region. This change is recorded in the manual change column.
Chart Properties	Allows you to modify the following: <ul style="list-style-type: none"> • Minimum and maximum values of the axis of the currently displayed chart. • Toggle between autoscale and nonautoscale mode. If autoscale is selected, the minimum and maximum values in the chart are used to determine the axis values. • Select or deselect the following options: View Copy Lines, View Guidelines, View Additional Stem/Line Series (24sure Single Channel Protocol or dye swaps only), View Lines Connecting Probes, Small Spot Size, View Region Shading, BAF Dup. Guide Lines, and BAF Del. Guide Lines.
Copy Clone IDs	Copies the clone IDs for all currently selected spots as a comma-separated list to the clipboard.
Copy/Print/Save	Allows you to copy the chart to the clipboard, print, or save to a file in *.jpeg or *.bmp formats.
Create Region	Allows the manual creation of a region of imbalance in the CGH/CNV and Fused charts and to create a region of LOH in the Fused SNP Copy Number chart for SNP experiments. You can select and manually create a region from a series of clones that have not been automatically called. Specify whether the region is a gain, loss, or no change (LOH, heterozygous, hemizygous, or nullizygous for SNP experiments). If the selected clones are already included in a region, the options for the region type are grayed out. Multiple existing regions can be merged into a single region. This context menu option is essential for 24sure+ users.
Create Region Query	Allows you to search the current database for other experiments that contain the same imbalance. For more information, see Queries on page 40 .
CytoChip Login	Opens a browser window to the account login page.
Links to web resources	Provides links to relevant web resources. When a region or clone is selected (displayed in yellow) in a Fused Chart, use the web link to search for that clone or region in an internet browser window. If no region or clone is selected, the web links are inaccessible. Make sure that the genome build, region extents, and the gene or disease symbol match the expected information.
Save All	Saves *.jpeg files of all 24 chromosomes to a specified location. Applicable to the CGH Chart view.
Save Genome	Saves 3 separate *.jpeg files, each with 8 chromosomes, to a specified location. Applicable to the CGH Chart view.
Zoom Options	Different ways of navigating the experiment results. For more information, see Chart View Zoom Options on page 20 .

Change Chart Axes

In addition to the options in Chart Properties, you can select a type of chart axes using the dropdowns in the chart toolbar. Axes that cannot be changed are grayed out. For the certain types of data, you can select the following options.

Data type	Options
24sure	Amplitude Channel 1, Amplitude Channel 2, Ratio, Log ₂ Ratio Ch1/Ch2, Log ₂ Product, Confidence, Unsmoothed L2R, Chromosomal Position
BeadArray	Y-axis—LogR Ratio or Unsmoothed LogR Ratio
VeriSeq PGS	Y-axis—Copy Number or Raw Counts

Chart View Zoom Options

Use following options provide to view the experiment results.

- ▶ Right-click and drag to zoom into a region in either the x direction only, y direction only, or both directions.
- ▶ Context menu zoom options include:
 - ▶ **Zoom Out**—Doubles the zoom width/height or both around the center of the zoomed x/y axis.
 - ▶ **Zoom Reset**—Zooms out to the whole genome.
 - ▶ **Zoom to Chromosome**—Zooms to the whole of the selected chromosome.
 - ▶ **Advanced Zoom**—Zoom to a specified region. Specify a region by genomic position, chromosome band, or HGNC gene name.
- ▶ Double-click any chromosome in the Karyotype Chart to zoom to that chromosome in all displayed views.

Table Views

You can view the experiment data in various table views. The following table includes details about the main table views.

Table	Description
Raw Table	Opens the raw data with clones listed by genomic location. For BAC arrays, if clones are replicated, the table includes data for multiples of the same clone. The disease clones are colored green and the remaining clones are colored black.
Fused Table	Shows results that are normalized to correct for spatial, intensity, and dye-related effects. For BAC microarrays, results are also normalized to correct for combined replicates. The disease clones are colored green and the remaining clones are colored black.
CGH Table/ CNV Table	Contains identified regions of copy number change. This CGH calling step takes the fused results and splits them into regions or imbalance classified as gains, losses, or no change. By default, gains are listed first, followed by regions or loss, and then regions with no change. The CNV table is used for VeriSeq PGS applications and is equivalent to the CGH Table.

The following table contains descriptions of the CGH table and CNV table columns.

Column	Description
Number	Number of the region, in sequential order along the genome.
Start Cyto	Cytogenetic band at the start of the imbalance.
End Cyto	Cytogenetic band at the end of the imbalance.
Type	Type of imbalance—Gain, Loss, or No Change.
Region Confidence	A confidence measure indicating the quality of the call.
Assessment	Calling of the imbalance—Benign, Pathogenic, or Unknown. The automated assessments are assigned through the application of certain rules and comparison to DGV, ISCA/dbVar, and Illumina CNV databases). The automatically assigned assessments do not represent a diagnosis. Manually confirm all automated assessments or modify the automated assessments using the Change Assessment context menu option.
Report	Whether the imbalance is included in the report.
Size	The size of the imbalance in base pairs.
Copy #	Copy number—A log ₂ ratio value.
Chromosome	Number of the chromosome, X=23, Y=24
Start	Base pair location of the start of the imbalance.
End	Base pair location of the end of the imbalance.
Man Change	Whether the imbalance has had a manual change.
Stddev	The standard deviation of all the clones included in the region.
Included	The number of clones included in the region.
Excluded	The number of clones excluded from the region.
% Included	The % of included clones.

Table View Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Change Assessment	Available for the CGH, CNV, and SNP LOH table. Allows you to validate the automated assessments and change the assessment to remove the "?" symbol. This change is recorded in the manual change column.
Change Type	Available for the CGH or CNV table. Allows you to change the type of an incorrectly called existing region. This change is recorded in the manual change column.
Create Region Query	Allows you to search the current database for other experiments that contain the same imbalance. For more information, see Queries on page 40 .
Column Configuration	Allows users you customize the display of the columns in a table. Columns can be removed, replaced, and changed in order. To change column sizes, hover over the column header separators. When the cursor changes to a resizing cursor, click and drag to the desired size.
Copy	Copies the selected text to the clipboard. To select multiple rows, press Shift for a continuous selection, or press Ctrl to select any row. The maximum number of rows that can be copied is 200, except for the Query Results, Overlapping Regions, and Reported Genes tables.
Mark Reported/Not Reported	Available for the CGH table. Allows you to select a region to include or remove from the report. If a region is marked as "not reported," the imbalance is removed from the report and no longer displayed in the Karyotype Chart (Reported). This change is recorded in the manual change column.
Links to web resources	Provides links to relevant web resources. Make sure that the genome build, region extents, and the gene or disease symbol matches the expected information.
Sort	Opens a window that allows you to define up to 2 sorting options. If you set both columns to <None>, the columns revert to the default sorting.
Table Configuration	Opens a window that allows you to select the region characteristics to display in the table.

Table View Zoom Options

Double-click any imbalance in table views to zoom to that region of imbalance in all displayed views.

DecisionTrack View

DecisionTrack view incorporates information from different sources into a single pane. For a full explanation and color coding of each track, see the BlueFuse Multi Color Key PDF. To view the file, select **Help | Colour Keys**. You can also hover over a track to view information about the track.

DecisionTrack Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Change Assessment	Available when a region is selected in the current track. Allows you to validate the automated assessments and change the assessment to remove the "?" symbol. This change is recorded in the manual change column.
Change Type	Allows you to change the type of an incorrectly called existing region. This change is recorded in the manual change column.

Option	Description
Configure Tracks	Allows you to configure track settings in the following ways: <ul style="list-style-type: none"> • Select the Visible checkboxes to configure the tracks that are visible. • Select a track and click the up and down arrows to modify the order of the tracks. • If applicable, select the Expanded checkboxes.
Copy/Print/Save	Allows you to copy the DecisionTrack to the clipboard, print, or save to a file in *.jpeg or *.bmp formats.
Create Region Query	Allows you to search the current database for other experiments that contain the same imbalance. For more information, see Queries on page 40 .
Email Region	Available when a region is selected. Incorporates experiment and region information into an email.
Links to web resources	Provides links to relevant web resources for the selected track region. Make sure that the genome build, region extents, and the gene or disease symbol matches the expected information.
Mark Reported/Not Reported	Available when a region is selected. Allows you to select a region to include or remove from the report. If a region is marked as "not reported," the imbalance is removed from the report and no longer displayed in the Karyotype Chart (Reported). This change is recorded in the manual change column.
Zoom Options	Different ways of navigating the experiment results. For more information, see DecisionTrack View Zoom Options on page 23 .

DecisionTrack View Zoom Options

Use the following options provided to view the experiment results.

- ▶ Right-click and drag to zoom into a region in the x direction only.
- ▶ Context menu zoom options include:
 - ▶ **Zoom Out**—Doubles the zoom area.
 - ▶ **Zoom Reset**—Zooms out to the whole genome.
 - ▶ **Zoom to Chromosome**—Zooms to the whole of the selected chromosome.
 - ▶ **Advanced Zoom**—Zoom to a specified region. Specify a region by genomic position, chromosome band, or HGNC gene name.

Track Sharing

Track sharing facilitates sharing of data between BlueFuse Multi databases, or between laboratories and interested groups. A whole database or a filtered subset of experiments can be exported and stored as a single file that other users can email and import. Alternatively, you can use this feature to subset your own data according to certain criteria, eg disorder type.

The import and export of .db and .bed file formats are supported.

Import Tracks

Before importing tracks, make sure that the contributing data are derived from the same genome build as the annotation database in the BlueFuse Multi database.

- 1 Select **Tools | Import/Manage Tracks**.
- 2 Select **Import**, and then navigate to the file.
- 3 Add a display name.
- 4 **[Optional]** Add a short description of the track.

- 5 Select **OK**.
- 6 The track appears in the Import/Manage Tracks window. Select **Close** to load the track into the DecisionTrack pane.

Hover the mouse point over the imported track to reveal summary information about the data. To configure the track view, right-click the DecisionTrack pane to view the context menu, and then select **Configure Tracks**.

Export Tracks

Create a filtered list to export or use an unfiltered list. For more information, see *Filtering Experiments and Samples* on page 38.



NOTE

The .bed export functionality is recommended for use when inputting into genome migration tools such as UCSC liftOver. The .bed files exported from BlueFuse Multi do not contain the standard .bed header. Use the .db export option for the general transfer of tracks between databases.



NOTE

When BeadArrays are exported, two files are created. One that contains CNVs (<filename>_CNV.db or <filename>_CNV.bed) and one that contains LOH regions (<filename>_LOH.db or <filename>_LOH.bed).

- 1 Select **Tools | Export Tracks**.
- 2 **[Optional]** Select the fields to export—**Assessment, Sex, and Assay Type**.
- 3 Select whether to export the whole database or a filtered subset.
- 4 Select the data type—**Oligo, BAC, Bead, or Sequencing**.
- 5 Add a brief description of the data.
- 6 Save the file to a chosen location, and then select **OK**.

Importing BED files

A BED file (.bed) is a tab-delimited text file that defines a feature track. The BED file provides a flexible way to import data tracks into BlueFuse Multi, that are shown in the DecisionTrack pane. This method allows external genomic data to be shown alongside the cases in the current BlueFuse Multidatabase. For more information on the BED file format and creating a BED file, see *BED File Format* on page 24.

For more annotation files in BED format, go to accounts.illumina.com and select **BlueGnome** from the destination drop-down. URLs can be incorporated into BED files and websites launched from the DecisionTrack context menu.

BED files are imported in the same way as tracks as detailed in *Track Sharing* on page 23.



NOTE

The masking feature uses a mask BED file. For more information, see *Genome Masking* on page 49.

BED File Format

BED (.bed) files can be created in any text editor and consist of a track line and an array of data that display in the DecisionTrack pane.

The track line is the first line in the file and defines how to display the data in the BED file. The track line must begin with the word *track* and use the following parameters:

- ▶ name – Unique name to identify the track. Only the first six or seven characters can be fully displayed next to the track.
- ▶ description – Long description of the content.
- ▶ db – Defines the genome assembly. Either GRCh37 (hg19) or GRCh38 (hg38).

For example

```
track name="Exons" description="Ensembl exons" db="GRCh37"
```



NOTE

If there are spaces in the parameter definitions, the text must be contained in quotation marks.

Following the track line is the array of data that must be presented in columns and rows. Each row represents a region or feature.

The first three columns are required and are defined as follows:

- ▶ chrom – Name of the chromosome (eg chr3, chrY).
- ▶ chromStart – Starting position of the feature in the chromosome. The first base in a chromosome is numbered 0.
- ▶ chromEnd – Ending position of the feature in the chromosome. The chromEnd base is not included in the display of the feature. For example, the first 100 bases of a chromosome are defined as chromStart=0, chromEnd=100, and span the bases numbered 0–99.

The chromStart and chromEnd positions must be valid in the assembly (db) specified in the track line.

Two additional optional columns can be included as needed:

- ▶ name – Name of the feature. A link to a website can be included using the following format:

```
<name for tooltip>|<url>
```

where <url> is the full web address

For example:

```
BMP8A: Atrial Natriuretic Factor|http://www.ncbi.nlm.nih.gov/pubmed/17903293
```

- ▶ color – Number that represents the desired display color:
 - ▶ 1 = green
 - ▶ 2 = light blue
 - ▶ 3 = dark blue
 - ▶ 4 = orange
 - ▶ 5 = red

The following is an excerpt from a BED file with the three required columns and the optional fourth column for the feature name.

```
track name="Treat-ID" description="ID gene defects that are treatable, from http://www.treatable-id.org" db="GRCh37"
```

```
chr1 11845780 11866977 I.o. MTHFR Deficiency (MTHFR)|http://www.treatable-id.org/page81/MTHFR%20Deficiency.html
chr1 24128375 24165110 HMG-CoA Lyase Deficiency (HMGCL)|http://www.treatable-id.org/page71/HMGCoA%20Lyase%20Deficiency.html
chr2 73114489 73119287 R deficiency (Biotpterin deficiency) (SPR)|http://www.treatable-id.org/page46/SPR%20deficiency.html
chr3 49454211 49460186 I.o. Non-Ketotic Hyperglycinemia (AMT)|http://www.treatable-id.org/page35/NonKetotic%20Hyperglycinemia.html
chrX 38211798 38280703 I.o. OTC Deficiency (OTC)|http://www.treatable-id.org/page33/OTC%20Deficiency.html
```

Report Views

The following table includes descriptions about the main report types.

Report	Description
Experiment Report	Available when an experiment is selected. Contains all experiment information, including details about the platform, files used in the analysis, the genome build, consumable information, batch numbers, and labeling QC data.
Sample Report	Available when a sample name is selected. Lists only subject and sample information. Manually enter the fields.
Report	Available when an experiment is selected. A full report available for each experiment. Includes the results and all the information from the sample and experiment reports. If enabled, the report can be viewed in a web browser. For more information, see Activating Web Access through Server Mode on page 2 .

Reports View Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Increase Font/Decrease Font	Allows you to change the font size.
Select All/Copy	Click Select All and then Copy to copy the whole view to the clipboard. Alternatively, select specific regions, and then click Copy .
Print	Allows you to print the report.

Image View

The composite images created from the combination of the cy3 and cy5 channel TIFF images can be displayed in Composite Image, displayed as part of the Subarray QC shortcut. The composite image is useful to assess the quality of the array. For example, the user can see any major artifacts and wash problems, and can also determine whether BlueFuse Multi has successfully fitted the grids during processing.

Image View is linked to other views. For example, a clone selected in a fused table highlights the clones on the image.

Image view is not relevant for BeadArray or VeriSeq PGS.

Image View Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Copy/Print/Save	Lets you copy the image to the clipboard, print, or save to a file in *.jpeg or *.bmp formats. The whole array image is saved and is not zoomed or gridded.
Settings	Lets you change the image contrast. Click Reset to return the contrast the default setting.
View Grids	After the images are processed, you can toggle the visibility of the grids and select spots. BAC arrays—Grids are colored according to the confidence flag of each spot. For more information, see Chart Views on page 18 .
Zoom In Zoom Out	Increase or decrease the zoom level. When zoomed in, use the scroll bars to move around the image.

Region View

The Region View is part of the Genome View shortcut for cytogenetic experiment types. This view populates only when an imbalance is selected. The following tabs are available.

Tab	Description
Detailed Region Summary	Opens the information for the imbalance, including the Type, Assessment, Reported status, Position, Band, Size, and Manual Change. Comments are recorded and saved to the database and included in the report with the user name and the time and date of the comment.
Overlapping Regions	Lists any other samples in the current database that share the current selected imbalance. Overlapping regions include imbalances within the selected region and the imbalances that overlap the region. This function provides similar information to a region query.
Overlapping LOH regions	Lists any other samples in the current database that share the region of LOH with the currently selected region. Overlapping LOH regions include LOH regions within the selected region and the LOH regions that overlap the region.
Reported Genes	Lists any genes or portions of genes that are included in the selected imbalance. Any OMIM genes are shown in green.

Region View Context Menu Options

Right-click to open the context menu and access additional functions.

Option	Description
Increase Font/Decrease Font	Allows you to change the font size.
Mark Reported/Not Reported	Allows you to set genes as reported or not reported.
Select All/Copy	Click Select All and then Copy to copy the whole view to the clipboard. Alternatively, select specific regions and then click Copy .
Print	Allows you to print the report.

BeadChip Array Views

The following charts and tables are specific to BeadChips.

View	Description
Charts	<ul style="list-style-type: none"> • LogR Chart—Similar to the Fused Chart and shows the equivalent copy number imbalance data. Called imbalances are identified and shown in the CGH table. For more information on the LogR chart calculations, see the following definitions table. • B-Allele Chart—Equivalent to the Fused SNP Copy Number Chart and shows the SNP calls for the experiment. B-allele frequency duplication and deletion guidelines can be enabled for this chart through Chart Properties. After selecting a region, you can create a region using the context menu. For more information on the B-allele frequency, see the following definitions table. • Karyotype Chart—Shows regions of LOH/UPD as light blue blocks.
Tables	<p>The SNP LOH Table contains the type of identified regions, which are automatically called as nullizygous, LOH, heterozygous, or hemizygous. You can change the region type using the context menu.</p>
DecisionTrack	<p>The following additional SNP tracks are included in the DecisionTrack pane.</p> <ul style="list-style-type: none"> • Current LOH—The LOH regions for the current experiment. • Bead LOH—A history track for the BeadChip array SNP experiments in the current database.

The following definitions are used to understand the LogR and B-Allele chart calculations.

Concept	Definition
LogR Ratio	<p>The LogR ratio measure in a BeadArray experiment is similar to the Log₂ ratio of an array CGH experiment.</p> $LRR = \log_2 \left(\frac{R}{R_{exp}} \right)$ <p>There are 2 important differences:</p> <ul style="list-style-type: none"> • Instead of a ratio between cohybridized test and reference samples, the ratio is between the test sample and an <i>in silico</i> reference sample produced from the BeadArray cluster file. • The BeadArray uses both red and green channels to measure the signal due to A and B alleles separately. These signals are then combined to produce an R value for each locus. From the cluster file, corresponding R_{exp} values are calculated so that the <i>in silico</i> reference sample has the same genotype at each locus as the test sample.
B-Allele Frequency (BAF)	<p>The value θ is derived from the signals for the A and B alleles. Ideally, the AA, AB, and BB loci give θ values close to 0, 0.5 and 1. In practice, the θ values associated with each genotype vary from locus to locus, making it difficult to compare directly θ values between loci. The BAF value for a locus is obtained by normalizing the θ values to compensate for differences between SNP loci in the θ values associated with each genotype. The BAF values can therefore be compared between loci and are interpreted as the proportion of signal at a locus due to the B allele.</p> <p>The BAF data are helpful for visualizing copy number imbalances as different copy number states each have their own distinctive BAF distribution.</p> <p>The B-Allele horizontal and vertical frequency charts can be configured to include BAF duplication and deletion guidelines. These guidelines show the fixed, theoretical 25% and 50% mosaic frequencies. The following nonlinear equations are used to calculate the upper and lower BAF bands for mosaic duplications and deletions. Here, f is the fraction of abnormal cells (0.25 or 0.5).</p> <ul style="list-style-type: none"> • Duplication: Upper = AB + ABB; Lower = AB + AAB $BAF_{upper} = \frac{(1-f) + 2f}{2(1-f) + 3f}$ $BAF_{lower} = \frac{(1-f) + f}{2(1-f) + 3f}$ <ul style="list-style-type: none"> • Deletion: Upper = AB + B; Lower = AB + A $BAF_{upper} = \frac{(1-f) + f}{2(1-f) + f}$ $BAF_{lower} = \frac{(1-f)}{2(1-f) + f}$

24sure Views

24sure experiments are performed in a single channel format. BlueFuse Multi combines the data from the sample hybridization with male and female reference hybridizations. BlueFuse Multi generates charts and tables that are unique to 24sure.

Compared to other array types, the 24sure report is reduced because large-scale aneuploidy is the main interest. The report does not include the detail of the genes or disease regions that are included in the regions of imbalance.

The 24sure report includes the results of the selected embryo (G for gain, L for loss for whole chromosome imbalances) and a confidence value (between 0.5 to 1.0, where 1.0 is more confident).

The following table describes the shortcuts that are unique to 24sure views.

Shortcut	Description
Single Channel View	Includes the following: <ul style="list-style-type: none"> • Fused Chart—Sample plotted against male references • Fused Chart—Sample plotted against female references • Fused Chart Combined—Sample plotted against a male and a female reference. The sex-matched reference is shown as data points and the sex-mismatched reference is displayed as blue line (male) or pink line (female).
Genome View	Includes the following: <ul style="list-style-type: none"> • Fused Chart (Combined) • Karyotype View • Report
Cycle Report	Appears when the top-level Cycle ID is selected. This report summarizes the results from all the embryos within a cycle. The report can be exported.

VeriSeq PGS Views

Data from VeriSeq PGS is shown using the standard CytoChip shortcuts. There are some charts, tables, and reports that are specific to VeriSeq PGS. The genome view shortcut includes the CNV Chart, Report View, and Karyotype View.

The VeriSeq PGS sample report includes similar content to the 24sure report, but includes the Sequencing QC report, which is described in the following table.

Chart/Table/Report	Description
CNV Chart Vertical CNV Chart	Plots the data using true copy number instead of the log ₂ ratio that is used for array data. For more information about the data points on the CNV charts, see the following definitions table.
Data Table	Lists all bins with their genomic locations (midpoints), the processed number of reads per bin, and the assigned copy number.
CNV Table	Equivalent to the CGH Table and includes region confidence.
Sequencing QC Report	Provides sequencing QC measures for the sequencing run of the selected sample.
VeriSeq Cycle Report	Available when the Cycle ID is selected. This report summarizes the results from all the embryos within a cycle.

The following definition is used to understand the data point calculations on the CNV charts.

Concept	Definition
Sequencing Bin	Regions of the genome are divided into bins. Bins are nonoverlapping regions typically slightly longer than 1 million base pairs. Bin coordinates were chosen so that each bin contains a defined number (N) of unique sequences of a specific length (L). Therefore, repetitive regions of the genome are covered by larger bins. The current specifications for all versions of VeriSeq PGS are set to N=1 million, L=36 bp. Each sequencing bin serves as a data point on the CNV charts.

Set a Custom Shortcut View

Different configurations of views are stored as custom shortcuts. Only administrators can set a shortcut view.

- 1 Select views in the side pane.
- 2 Select and drag the boundaries and title bars of the views to configure the sizes and locations on the screen.
- 3 Select **Tools | Manage Shortcuts**.
- 4 Enter a name for your custom view and select **Add Current**.
- 5 You can also reorder the list or remove any shortcuts.
- 6 Select **OK** to save the new settings to the database.

Experiments and Samples

Add an Experiment

To add an experiment, you can use the **Add Experiment (Express)** toolbar icon, or add a sample and then add an experiment to the existing sample.

Add an Experiment (Express)

Use the Add Experiment (Express) option to create experiments for the following experiment types.

- ▶ 24sure
- ▶ BeadArray
- ▶ VeriSeq PGS

24sure Experiments

- 1 Select the Add Experiment (Express) icon on the toolbar or select **File | Add | Add Experiment (Express)**.
- 2 In the Express Entry Manager, enter the Sample ID, and then select **24sure**.
- 3 In the Sample-Cy3 field, select the TIFF image file. For more information, see *Image Files in 24sure Experiments* on page 15.
- 4 Click through the wizard. When prompted, browse to the relevant GAL file.
- 5 Select **Finish**.

BeadArray Experiments

- 1 Select the Add Experiment (Express) icon on the toolbar or select **File | Add | Add Experiment (Express)**.
- 2 In the Express Entry Manager, enter the Sample ID, and then select **BeadArray**.
- 3 Add an experiment description.
- 4 **[Optional]** Select a BeadArray protocol.
- 5 Select **Next**.
- 6 Browse to the sample GTC file.
- 7 Browse to the SNP manifest file and the SNP cluster file.
- 8 Select the latest annotation database, and then select **Next**.
- 9 Select **Finish**.

VeriSeq PGS Experiments

- 1 Select the Add Experiment (Express) icon on the toolbar or select **File | Add | Add Experiment (Express)**.
- 2 In the Express Entry Manager, enter the Sample ID, and then select **VeriSeq PGS**.
- 3 Browse to the BAM file and sample sheet.
- 4 If necessary, select the Summary QC (Summary.xml) file.
- 5 Select the latest annotation database, and then select **Next**.
- 6 Confirm or enter the Cycle ID, and then select **Next**.
- 7 Confirm or select the cell type and kit lot number, and then select **Next**.
- 8 Select **Finish**.

Add a Sample

You can add a sample with or without an associated experiment.

- 1 Select **Add a Sample** or select **File | Add | Add Sample**.
- 2 Enter the sample ID. If necessary, complete the other fields, select pick lists, or locate the sample information file. For more information on importing sample information, see [Import Sample Information on page 33](#).
- 3 Click through the wizard. If necessary, deselect **Also create an experiment based on the sample**.
- 4 When you have completed the wizard, select **Finish**.

Add an Experiment to an Existing Sample

The following experiment types can be added to existing samples.

- ▶ 24sure
- ▶ BeadArray
- ▶ VeriSeq PGS

24sure Experiments

- 1 Select a sample in the selection group of the side pane.
- 2 Select **Add Experiment** or select **File | Add | Add Experiment**.
- 3 In the Experiment Editor, select **24sure**. All other fields are optional.
- 4 If necessary, complete the other fields, select pick lists, or enter dates.
- 5 In the Sample-Cy3 field, select the TIFF image file. For more information, see [Image Files in 24sure Experiments on page 15](#).
- 6 Click through the wizard. If necessary, complete fields, select pick lists, or locate the NanoDrop file. For more information, see [Import Experiment \(NanoDrop\) Information on page 34](#).
- 7 When prompted, browse to the relevant GAL file.
- 8 Select **Finish**.

BeadArray Experiments

- 1 Select a sample in the selection group of the side pane.
- 2 Select **Add Experiment** or select File | **Add** | **Add Experiment**.
- 3 In the Experiment Editor, select BeadArray. All other fields are optional.
- 4 If necessary, complete the other fields, select pick lists, or enter dates.
- 5 Click through the wizard. If necessary, enter the reagent kit information.
- 6 Browse to the sample GTC file.
- 7 Browse to the SNP manifest file and the SNP cluster file.
- 8 Select the latest annotation database, and then select **Next**.
- 9 Select **Finish**.

VeriSeq PGS Experiments

- 1 Select a sample in the selection group of the side pane.
- 2 Click **Add Experiment** or click File | **Add** | **Add Experiment**.
- 3 In the Experiment Editor, select VeriSeq PGS. All other fields are optional.
- 4 Browse to the BAM file and sample sheet.
- 5 If necessary, select the Summary QC (Summary.xml) file.
- 6 Select the latest annotation database, and then select **Next**.
- 7 Confirm or enter the Cycle ID, and then select **Next**.
- 8 Confirm or select the cell type and kit lot number, and then select **Next**.
- 9 Select **Finish**.

Import Sample Information Files

Import Sample Information

- 1 Create a tab-delimited text file (*.txt) containing the required fields of information. The first row contains column headings. Match the column headings to the fields in the Sample Editor wizard, but remove spaces and use first letter capitalization (eg, Field—Type of Sample; Column Heading—TypeOfSample). Enter dates in YYYY-MM-DD format. SampleID is the only required field. Each following row describes 1 sample.

Figure 2 Example of Sample Information File

SampleID	TypeOfSample	DateReceived	SubjectID	PedigreeNumber	FirstName	LastName	DOB	Sex
Example 1	Blood	2009-04-29	18	31	Jo	Blugs	2010-03-10	Male
Example 2	Blood	2009-08-12	19	18	Donald	Duck	2010-03-10	Male
Example 3	Blood	2007-09-18	20	12	Ted	Bear	2010-03-10	Male
Example 4	Blood	2009-12-12	21	34	Fran	Parker	2010-03-10	Female
Example 5	Blood	2009-08-01	22	44	Gi	Jane	2010-03-10	Female
Example 6	Blood	2004-04-02	23	465	Huey	Lewie	2010-03-10	Male
Example 7	Blood	2005-07-23	24	654	Fred	Kruger	2010-03-10	Male

- 2 To import the entire sample information file, perform the following steps:

- a Select **File | Batch | Import Sample Information**.
- b Select the file created in step 1.

Information is added to existing samples whose Sample ID is in the file, with previous information being overwritten. Entries that match all details of existing samples are ignored. New samples are created for Sample IDs that do not exist in the database.

If any columns contain invalid pick list values, the import will not validate. For more information, see [Configuring Pick Lists on page 37](#).

- 3 To import using the wizard, when you add a sample, select the icon to the right of the Sample ID and locate the sample information file. If the Sample ID in the wizard dialog matches a row in the file, the relevant information is imported.

Import Experiment (NanoDrop) Information

If a NanoDrop is used to assess the fluorescent nucleotide incorporation of the sample and reference during labeling, you can import this information directly into BlueFuse Multi. The NanoDrop file has the extension *.ndj, *.ndv, *.txt or *.tsv.

- 1 When using Nano software, use the same Sample ID (SID) used in BlueFuse Multi. Save the NanoDrop file to a chosen location.
 - ▶ **Sample**—NanoDrop ID is SID_Cy3 or SID_Cy5, depending on type of label used.
 - ▶ **Reference samples**—NanoDrop ID is ref_SID_Cy5 or ref_SID_Cy3, depending on type of label used.
- 2 When you add an experiment, select the icon to the right of Add Information About the Labeling QC in this Experiment.
- 3 Locate the NanoDrop file (saved as a .ndj, .ndv, .txt or .tsv file). BlueFuse Multi validates the NanoDrop file and imports the relevant information.

Import VeriSeq PGS Summary Information

The summary.xml file is created automatically as part of the secondary analysis workflow for VeriSeq PGS sequencing data. It contains sequencing metrics for the entire run and every sample of the run, and for the different physical locations of the flow cell. The summary.xml file is located with the BAM files in the run folder of the MiSeq (<output folder>\Data\Intensities\BaseCalls\Alignment\). During batch import, it can be automatically discovered from this file location. Alternatively, you can provide the location of the file during sample import.

Import 24sure and BeadArray Experiments

Use a batch import file to import and automatically process multiple 24sure or BeadArray samples and experiments.

Create a Batch Import File

Create a batch import file by using the Excel-based laboratory planner for your array type.

Download library planners using the following links.

- ▶ [24sure PGS \(single channel\) planner](#)
- ▶ [24sure+ \(dual channel\) planner](#)
- ▶ [CytoSNP-850K planner](#)

If a planner is not available for your array type, you can create a batch file manually. The batch import file is a tab delimited text file (*.txt) that contains the following information:

- ▶ **Directory**—The folder location of all files to be imported.
- ▶ **Annotation**—Annotation database used for all imported experiments.
- ▶ **[BeadArray only, Optional] Mask**—Name of a mask in the current database. This mask is applied to all imported experiments.
- ▶ **Column Headings**—Required fields are Sample ID, Barcode (full string), and experiment type.
- ▶ **[BeadArray only] Data Directory (DATADIR) Column**—The location for the .gtc file for each sample.
- ▶ Each following row describes 1 sample or experiment.

Figure 3 Example of 24sure Batch Import File

```
DIRECTORY: C:\scan_data\201611
ANNOTATION: BG_Annotation_Ens74_20160127.db
```

CYCLEID	SAMPLEFILE	BARCODE	EXPTYPE	EMBRYOID	SAMPLECH	REFERENCEDB	CELLTYPE	NANODROPPFILE	DESCRIPTION
0667L	066709L	24sure Top		Male/Male	Male/Male	Lot0667L_20161208_Reference_1.db	Genomic DNA		
0667L	066710L	24sure Bottom		Female/Female	Female/Female	Lot0667L_20161208_Reference_1.db	Genomic DNA		
Cycle1	066709L	24sure Single Channel Bottom		Embryo1	Cy3	Lot0667L_20161208_Reference_1.db	Blastomere		

Figure 4 Example of BeadArray Batch Import File with Mask

```
DIRECTORY: \\depts\Development\Masking
ANNOTATION: BG_Annotation_Ens74_20141105 - Masking.db
MASK: An Inclusion Mask
```

SAMPLEID	SAMPLEFILE	BARCODE	SUBARRAY	EXPTYPE	DESCRIPTION	DATADIR
Sample2	5605754140	R03C02	BeadArray			

Import the Batch File

- 1 In BlueFuse Multi, select **File | Batch | Batch Import**.
- 2 Locate the batch import file, and then select **Open**.
- 3 If necessary, select one or both of the following options:
 - ▶ Start processing files automatically after import.
 - ▶ Files are not yet available. Poll directory until files are present.



NOTE

Make sure that the directory path in the batch import file is directed to the location where the image or .gtc files are written.

- 4 Select **Import**.
If there are errors, select **View Log** for more information. Correct any errors, and then select **Import** again.

Import VeriSeq PGS Experiments

Use a sample sheet to import VeriSeq PGS Experiments.

Create a Sample Sheet

Create a sample sheet using BlueFuse Workflow Manager. Use the sample sheet to drive the sequencing run. Do not edit the sample sheets outside of BlueFuse Workflow Manager. For more information, see the *BlueFuse Workflow Manager Quick Ref Guide (part # 15056206)*.

Download the BlueFuse Workflow Manager from the Illumina support site.

Import the Sample Sheet

- 1 In BlueFuse Multi, click **File | Batch | Batch Import**.
- 2 Locate the sample sheet, and then select **Open**.
- 3 Verify the displayed Flow Cell ID and Cartridge Number.
- 4 Select the annotation database.
- 5 If required, select one or both of the following options:
 - ▶ Start processing files automatically after import.
 - ▶ Files are not yet available. Poll directory until files are present.
- 6 Select **Import**.
If there are errors, click **View Log** for more information. Correct any errors, and then select **Import** again.

Processing Single Experiments

- 1 Select the experiment in the selection group of the side pane. The experiment can be previously processed or unprocessed.
- 2 Select **BlueFuse** in the **Algorithm** section of the side pane.

Batch Processing a Group of Experiments

- 1 **[Optional]** Create a filtered list of experiments using the selection group options in the side pane. For more information, see *Filtering Experiments and Samples on page 38*.
- 2 Using the drop-down arrow next to Run Batch Processing, select **Unprocessed** or **Filtered Experiments**.
- 3 Select **Run Batch Processing** or select **File | Batch | Run Batch**.

Editing Existing Samples, Experiments, or Queries

- 1 In the selection group of the side pane, select a sample, experiment, or query.
- 2 Select **Edit the Sample/Experiment/Query** or select **File | Edit**.
- 3 Click through the wizard and make the appropriate additions, selections, or updates.
To change the smoothing parameters and select chromosomes for recentering, deselect **Use default algorithms for this array type**.
- 4 If the change made requires the experiment to be reprocessed, the experiment icon reverts to orange.
To reprocess the experiment, select **BlueFuse** in the Algorithm section of the side pane or select **Run Batch Processing**.

Setting List of Available Microarray Types

The Experiment Editor and Express Entry Manager wizards contain a long drop-down list of microarray types that can be analyzed using BlueFuse Multi. This list can be configured to include only the microarray types that are used in the local setting. Only Supervisors and Administrators can configure the microarray type list.

- 1 Select **Tools | Array Configuration | Array Type Configuration**.
- 2 Select the array types that you want to include. Move array types to either the Allowed or Hidden column. Use the Shift or Ctrl key to select multiple entries.

If an experiment is loaded into the wizard and the microarray type is hidden, the experiment entry cannot proceed.

- 3 Select **OK**.

Configuring Pick Lists

Wizards used to create or edit Samples, Experiments, and Queries contain dropdown fields with configurable entries called pick lists. Only Supervisors and Administrators can configure pick lists.

The pick lists are stored in the database location and initially use default settings. When in Classic mode, read-write access is required to be able to configure pick lists.

- 1 Select **Tools | Global Configuration | Configure Pick Lists**.
- 2 Select a pick list and edit the entries.
 - ▶ If using BlueFuse Multi for the first time, set up the Operator and Consultant pick lists. These lists are not preconfigured and are initially empty.
 - ▶ To add, enter the value into the edit box, and use the Enter key.
 - ▶ To delete, select an entry and use the Delete key.
- 3 Select **OK**.

Interpretation Workflow

The following workflow describes the procedure to interpret processed CytoChip experiments. Most of these functions are accessed using the context menus. For more information on interpreting a 24sure case, see the *Technical Guide on Aneuploidy Calling with 24sure (part # 15056973)*.

- 1 Use the Genome View shortcut to get an overall view of the whole genome and to view the general noise of the data. If necessary, use the Zoom Reset option.
- 2 Check the quality of the data.
 - ▶ Use the Subarray QC shortcut.
 - ▶ Check the QC measures in the report.
 - ▶ Refer to the QC measure guideline documents. For more information, see the Quality Measures technical notes on the 24sure PGS Microarray and BeadArray Microarray support sites.
- 3 Use the CGH table to click through the imbalances or zoom to the regions in the Fused Chart. Make sure that regions are correctly called.
- 4 If necessary, use the Change Type or Create Region context menu options.
- 5 Assess the significance of the called imbalances.
 - ▶ Check for normal variation or disease region content of the imbalances.
 - ▶ Use the DecisionTracks to help with decision making.
 - ▶ If necessary, view external web resources using the context menu options.
 - ▶ Use the Overlapping Regions option to see if the imbalance is identified in other samples in the same database.
 - ▶ Use the Reported Genes option to identify genes present in each imbalance.
 - ▶ If further opinions are required, use the Email Region context menu option to share imbalance details from DecisionTrack with colleagues.
- 6 Check and verify the assessment. If necessary, use Change Assessment option.
- 7 Use the Mark Reported/Not Reported option to decide what regions to include in the report. Alternatively, change the Region View pane.

- 8 Use the Sign Off function to record the changes made. Add the details of the array QC, make comments, and assign an overall experiment status. Sign off to an appropriate level.
- 9 Export the Report. Use the Export the Results to File function or copy and paste a selected portion of the report to an appropriate program. Alternatively, export in formats appropriate for upload to Decipher or Nexus.

Filters

Filtering Experiments and Samples

When large numbers of samples, experiments, and queries have been created, you can filter the list by certain criteria. To filter the list of experiments or samples, select from following options in the side pane.

Option	Description
Unfiltered Experiments	All samples and experiments in the current database. You can further filter selections by category, date range, or text. When switching between filters, the currently selected sample or experiment is remembered. If the sample or experiment appears in the list after the new filter is applied, it is automatically reselected. If it does not appear in the list, then the first item in the list is selected
Queries	All queries. When switching between queries, the currently selected query is remembered and is automatically selected.
Expanded Queries	All queries and the samples and experiments relevant for each query in the selection group box. When switching between expanded queries, the currently selected query is remembered and is automatically selected.

To return to all experiments and samples in the database, select the side pane header and select **Unfiltered Experiments**.

Filtering by Category

After you click Unfiltered Experiments in the side pane, you can apply additional filters in the Category dropdown menu.

Option	Description
Samples	All samples and any linked experiments.
Unprocessed	All unprocessed experiments and any linked samples.
Processed	All processed experiments and any linked samples.
Sign Off 1	All experiments signed off to level 1.
Sign Off 2	All experiments signed off to level 2.
Modifications Without Sign Off	All experiments that have been manually modified, but not signed off.
Reference Hybridization	All reference hybridizations in a database. Relevant for 24sure only.
Experiment Status	Reveals the following subcategories that can be selected during the sign-off procedure — Unknown, Pathogenic, Probably Pathogenic, Probably Benign, Benign.

Filtering by Date Range

You can choose a date range using the following filters. The specified start and end dates are inclusive. The default date range is the last 7 days.

Option	Description
Modified Date	All samples and experiments with last modified dates within the specified range. If either the sample or its linked experiments satisfy the criteria, the sample and experiments are displayed. For example, if the sample was modified in the last week but the experiment was not, the experiment is still shown.
Sample Date	All samples and experiments with the Date Received field within the specified range.
Experiment Date	All experiments and linked samples with the Experiment Date field within the specified range.

Filtering by Text

After you click Unfiltered Experiments in the side pane, you can apply additional filters in the Text dropdown menu.

These filters allow you to choose a search string of text. The search string of text is used either as a substring or a comma-separated list of text strings to search. The substring option searches for that text anywhere within the text field. It does not have to match the first part of the text and wildcards are not required. The comma-separated list search option must be an exact match.

Option	Description
Consultant, SampleID, Subject Surname, Clinical Features	All samples and linked experiments with fields matching the given search string.
Operator, Barcode, Experiment Description	All experiments and linked samples with fields matching the given search string.
Barcode/Flow Cell ID	All experiments and linked samples with fields matching the given search string. Available for VeriSeq PGS experiments only.

Filtering Chart and Table Views

You can filter data points in chart and table views using the Apply Filter to Selection toolbar icon. This feature allows you to track particular data points through the different BlueFuse Multi views. Available filter types are shown in the drop-down adjacent to the toolbar icon.

- Select a region.
 - ▶ Charts and images—Click and drag to select a region.
 - ▶ Tables—Click a row to highlight.
- Select a filter option from the drop-down adjacent to the Apply Filter to Selection toolbar icon.

Option	Description
Selected Clone IDs	All spots with the same clone ID are selected, whether they have passed through the fused normalization processing or not. All included and excluded raw and post-processed spots and shown. This filter can retain selected spots when switching between experiments.
Selection	All spots that have contributed to the fused result. This option does not retain selected spots when switching between experiments.

Option	Description
Clone ID	<p>Opens a Filter by Text dialog box. Enter text that is either a substring or a comma-separated list, and then click OK.</p> <ul style="list-style-type: none"> • Substring—Allows a text search anywhere within the field without the need for wildcards. • Comma-separated list—Returns the specified item or clone name. <p>For example, when viewing a24sure microarray, enter ": RP3-442A17,RP11-441G8" to return only clones with IDs : RP3-442A17 or RP11-441G8.</p>

- 3 Select **Apply Filter to Selection**. When the filter is applied, all other data points in chart views disappear. To remove the filter, select **Apply Filter to Selection** again.

Queries

Queries provide a more sophisticated alternative to using filters to search the current database and display data. Use queries to search for specific information in the database and to show the information in an easily comparable format. You can also use queries to check array performance over time.

Queries created during a session appear in the Selection group box side pane with an orange query icon. Queries that are not saved are lost after you close the current session. A query that is saved to the database appears in the Selection group box with a green query icon.

To save a query to a database, select **Save Permanently** in the Data Management Wizard. To edit an existing query, select the query and select **Edit**, or select **File | Edit**.



NOTE

Queries can include results from different technologies (eg 24sure and VeriSeq PGS). However, some requested data types are not available for all technologies and scales of data are sometimes incompatible.

Creating a Query

- 1 Select **Add a Query**, or select **File | Add | Add Query**.
- 2 In the Data Management Wizard, select the initial query conditions from the drop box.
- 3 Include all relevant information to complete the query for the first condition. For more information, see [Query Conditions on page 41](#).
- 4 Select **Add AND** or **Add OR** to confirm the query. Add up to three conditions to the list. You cannot mix Add AND and Add OR within one query.
- 5 Select **Next**, and then **Finish** to run the query. Queries are shown with a specific set of views. For more information, see [Query Views on page 42](#).

Creating a Region Query

Use a region query to search the current database for other experiments that have an imbalance within the same region.

- 1 From the Fused Chart, CGH Chart, DecisionTracks, or CGH Table view, select a region.
- 2 Right-click within the pane and select **Create Region Query**.
- 3 Select **Gain/Loss/Any Change** to run the query. View the query results as Stacked Region Results or Stacked Results. The query results plot the regions of imbalance that cover at least part of the searched region.

Query Conditions

Query results are returned based on the conditions set in the Data Management Wizard. The dropdown options change depending on the initial query condition selected.

The following conditions are available.

Fields	Available Conditions	Additional Information
Subject	<ul style="list-style-type: none"> • Surname • Sex • Subject ID • Pedigree • Consultant • Clinical Features 	<p>Test that these fields are equal to a text string. The wildcard character "*" can be used anywhere in the string to substitute characters in the search string for any 0 or more unspecified characters, eg "Br*" searches for any strings beginning with "Br."</p>
Experiment	<ul style="list-style-type: none"> • Operator • ISCA ID • QC level • Status • Date • Description 	<ul style="list-style-type: none"> • Test that Operator or Description is equal to a text string. You can use a wildcard character. • ISCA ID opens all experiments exported in ISCA format with that ISCA ID. • QC level options: Not Set, Any Pass, QC Fail, Suboptimal Pass, Pass. • Status options: Unknown, Pathogenic, Probably Pathogenic, Probably Benign, Benign. • Date allows you to search for results that are ≥, =, or ≤ the selected date.
Sample	<ul style="list-style-type: none"> • Sample ID • Sample Type 	<ul style="list-style-type: none"> • Use the special In List case to test that the Sample ID fields match a comma-separated list of values. For example, to return results where sample IDs are "S001" and "S002," enter the string "S001, S002." You can also test that the Sample ID field is equal to a single text string. You can use a wildcard character. • The Sample Type condition includes the following options: Blood, Amnio, CVS, Bone marrow, Buccal Cells.
Microarray	<ul style="list-style-type: none"> • Barcode • Lot • Array Type 	<ul style="list-style-type: none"> • Test that the array type is equal to a string. You can use a wildcard character, eg "*v3.0." • Test that the Barcode In List matches a comma-separated list of values, eg "602002,616084,001085F," or test that it is equal to string. You can use a wildcard character. • Test that the Lot In List matches a comma-separated list of values, eg "602, 684, 001F." • The Barcode and Lot can include the product character code.
Sequencing	<ul style="list-style-type: none"> • Flow Cell ID 	<ul style="list-style-type: none"> • Test that the flow cell ID is equal to a string. You can use wildcard characters. • Test that the flow cell ID In List matches a comma-separated list of values.
Region	<ul style="list-style-type: none"> • Change Type 	<ul style="list-style-type: none"> • Test a specified region against a change type. The following change type values are available: Any Change, Gain, Loss, No Change. Any Change can be either a gain or loss. • Regions can be entered as chromosome number: genomic base position range (eg 2:1670000–1900000, where the latter position must be greater than the first). • This query type is more easily performed using the context menu from a fused chart. For more information, see Creating a Region Query on page 40.

Query Views

The following shortcut views are available.

Shortcut	Description
Individual Results	Opens the Query Results table, which contains all the results returned by the query, along with a fused results chart and DecisionTrack with the data for the selected experiment. Double-click a different row to change the selection in the query results table.
Stacked Region Results	Opens a stacked region view of all the returned experiments as a compressed track view. Regions of gain are shown as blocks of green and regions of loss are shown as blocks of red. Compare different experiments easily. DecisionTracks are also shown with this shortcut.
Stacked Results	Opens a stacked chart view with all the experiment results shown as comparable fused charts. Up to 4 results can be viewed in this view. If there are more than 4 results of the query, you see a message stating that there are too many results to be viewed.
QC Charts	Shows the query results table, along with charts of QC metrics plotted for all the experiments returned from the query. This shortcut can reveal changes in performance over time that can be attributed to other factors. Selecting a data point in these charts highlights the experiment it pertains to in the query results table. The QC metrics include the following: <ul style="list-style-type: none"> • DLR Fused Chart—Measure of noise • Mean Spot Amplitude Chart—Measure of fluorescence signal • SD Robust Chart—Standard Deviation • SBR Chart—Signal to Background ratio For BeadArray, the following charts are available. For more information, see BeadChip Array QC Measures on page 45 . <ul style="list-style-type: none"> • Median LogR Deviation • Median BAF Deviation • Median Call Rate
Experiment Infinium® Control Data	Shows charts that relate to BeadChip and reagent performance, efficiency of hybridization, and the staining process. For more information, see BeadChip Array QC Measures on page 45 .
Sample Infinium Control Data	Shows charts that relate to sample quality and performance. For more information, see BeadChip Array QC Measures on page 45 .

Displaying QC Measures for the Database

- 1 Select **Add a Query**, or select **File | Add | Add Query**.
- 2 Select **All** from the drop-down in the Data Management Wizard.
- 3 Select **Add AND** to confirm the query.
- 4 Select **Next**, and then **Finish** to run the query.
- 5 Select the QC Charts shortcut to view the query results and plotted QC metrics for the database.

Filtering to View Queries

- 1 Choose the selection group drop-down in the side pane to reveal the filter options.
- 2 Select **Queries** or **Expanded Queries**.
 - ▶ **Queries**—Opens all queries.
 - ▶ **Expanded Queries**—Opens all queries, the sample, and the experiments relevant for each query.

Quality Control

Quality control is critical to the workflow of many laboratories. This section includes the following information:

- ▶ Sign-off procedures
- ▶ QC Measures
- ▶ QC Metrics



NOTE

The default QC status is left blank. Make sure to update the status manually after the values are checked.

QC Sign-Off Overview

The sign-off feature allows laboratories to implement a hierarchy of validation for experiment results, depending on the user level. The laboratory size and setup can dictate how many levels of sign-off are required. Administrator, Supervisor, and User levels have different sign-off permissions. For more information, see [Database Permissions on page 3](#).

This feature allows you to record, track, and sign off the comments and manual changes made to experiments. Any previously entered information can be viewed in the sign-off history, along with the associated user and the time of the comment. This feature can confirm experiment inspections and speed up the process of results analysis and senior staff sign-offs.

Experiments are automatically included in the history tracks when processed. After that, manual changes made to an experiment are not submitted to the central database for inclusion in the history tracks until the experiment has been signed off to either level 1 or 2.

The QC status of an experiment is included in the experiment report and cycle report. By default, experiments are not assigned a QC status when processed – it must be selected manually.

See the following table for more information about the sign-off levels.

Sign-Off Level	Description
Level 1	<ul style="list-style-type: none"> • Any user can perform level 1 sign-off. • If experiments that signed off to level 1 are reprocessed, a warning appears. • After sign-off, the chromosome icon next to the experiment is light blue.
Level 2	<ul style="list-style-type: none"> • Only supervisors or administrators can perform level 2 sign-off. • Requires a password. • Requires a QC Status to be selected. • After sign-off, a user can no longer modify the experiment or sign off the experiment at level 1. • After sign-off, only supervisors or administrators can reprocess the data. • After sign-off, a password is required to delete or reprocess experiments. • After sign-off, the chromosome icon next to the experiment is dark blue.

Sign-Off Procedures

Identifying Artifacts on your Array Image

- 1 View your experiment using the Subarray QC shortcut.
- 2 Select the outlying spots on the Raw Chart.
- 3 Inspect the highlighted fluorescent spots on the composite image that contribute to the outliers.

Sign-Off Per Experiment Basis

- 1 Select **Sign-Off** from the Algorithm group box in the side pane.
- 2 Use the drop-down menu to classify the array QC. Choose from the following options:
 - ▶ **Pass**—Allows the experiment to be stored in the history tracks.
 - ▶ **QC Fail**—Removes all calls from the history tracks. Data from the experiment are not included in region searches.
 - ▶ **Suboptimal Pass**—Stores the experiment data in history tracks, but indicates that the quality of the data was slightly noisy.
- 3 **[Optional]** Enter any comments, and then select **Add** or press Enter. Comments are shown in the report with your user name and the date and time of the comment.
- 4 **[Optional]** Enter a molecular karyotype, and then select **Add** or press Enter. Molecular karyotypes are shown in the report with your user name and the date and time of the comment.
- 5 Supervisors or administrators only—Enter an Overall Experiment Status. The default status is Unknown. If known, change to Pathogenic, Probably Pathogenic, Probably Benign, or Benign.
- 6 Perform a save option:
 - ▶ **Save only**—Saves any comments made, but the experiment is not signed off. In the Selection group box in the side pane, a red chromosome icon next to the experiment indicates that modifications have been made, but not signed off.
 - ▶ **Save, Sign-Off, and Update history tracks (Level 1)**—Saves comments and modifications and signs off the experiment to level 1. A light blue chromosome icon indicates a level 1 sign-off.
 - ▶ **Save, Sign-Off, and Update history tracks (Level 2)**—Saves comments and modifications and signs off the experiment to level 2. A dark blue chromosome indicates a level 2 sign-off.
- 7 Select **OK**. When signed off to level 2, the sign-off process is complete and the Sign-Off algorithm button turns green. Only a Supervisor or an Administrator can make any further changes. Changes require a password, although all viewers can view the experiment.

Batch Sign Off for Active Experiments

- 1 **[Optional]** Select experiments for batch sign-off using filters. For more information, see [Filtering Experiments and Samples on page 38](#).
- 2 Select **File | Batch | Batch Sign-Off**.
- 3 Select sign-off level 1 or 2.
- 4 **[Optional for level 1 sign-off]** Use the drop-down menu to set the array QC. This does not affect experiments where an array QC is already assigned.
- 5 Enter your password.
- 6 Select **Sign-Off**.



NOTE

24sure—Experiments set as reference hybridizations cannot have array QC status changed. Regions do not appear in history tracks and are not made available for searching.

QC Measures

24sure QC Measures

The following QC measures are provided in the report. These QC measure guidelines are specific to array type.

QC Measure	Description
SD Autosome/Robust	<p>A measure of the dispersion of the log₂ ratio of all clones on the array. A low value indicates that the log₂ ratios of the autosome clones are tightly clustered around 0, which is the expected value. The measure is calculated on the normal, but unsmoothed data.</p> <ul style="list-style-type: none"> • SD Autosome—Calculated using all autosomal clones on the array. • SD Robust—Calculated using the middle 66% of the data. BlueFuse Multi uses the SD robust value to call regions and determine the gain and loss thresholds.
% Included Clones	<p>The percentage of all clones that were not excluded because of inconsistency between the clone replicates on an array. This measure is used for BAC arrays (24sure). A low percentage indicates a major artifact on the array. For VeriSeq PGS, the percentage of reads after filtering in relation to the total read per sample is reported.</p>
Mean Spot Amplitude Ch1/Ch2	<p>The mean fluorescent signal intensities for the channels.</p> <ul style="list-style-type: none"> • Channel 1—Sample (Cy3). • Channel 2—Reference (Cy5). <p>This metric is variable due to differences between available scanners. The balance between channels can be assessed, but the Cy5 signal usually provides higher intensity than Cy3.</p>
SBR Ch1/Ch2	<p>The overall signal to background ratio for each fluorescent channel. SBR indicates the clarity of spot detection above the background and can indicate the quality of laboratory labeling and washing.</p>
DLR Raw/Fused	<p>The derivative log ratio measures the spread of the difference in log₂ ratios between all pairs of adjacent clones along the genome. For simple profiles, this measure is similar to autosome standard deviation. For complex profiles, many step changes can cause the autosome standard deviation to become inflated. The DLR measure is less sensitive to this problem, and can be a more useful measure. The DLR Fused measure is the DLR calculated on normalized, but unsmoothed data.</p>

BeadChip Array QC Measures

The following QC measures are provided for Illumina BeadChip arrays. The QC Status, SD Autosome/Robust, and DLR measures are also included.



NOTE

Illumina BeadArray technology incorporates system controls to evaluate the quality of specific steps in the laboratory workflow.

QC Measure	Description
Median LogR Deviation	Robustly estimates the noise in the LogR Ratio data. On a high-quality array, the median LogR deviation is < 0.2. Probes from the autosome are arranged in ascending genomic position. Then the probes are divided into nonoverlapping bins, each containing 1000 probes. The standard deviation of the LogR Ratios of the probes in each bin is calculated. The median LogR Dev measure is the median of these bin standard deviations. The raw value is the one reported in BlueFuse Multi.
Normalized LogR Deviation	Listed in the log file during processing.
Median B Allele Frequency Deviation (Median BAF Deviation)	Robustly estimates the noise in the B allele frequency. Probes from the autosome with genotype AB are arranged in ascending genomic position. Then the probes are divided into nonoverlapping bins, each containing 1000 probes. The standard deviation of the B allele frequency of the probes in each bin is calculated. The median B Allele Frequency Dev statistic is the median of these bin standard deviations.
Median Call Rate	Robustly estimates the performance of the genotype calling. On a high-quality array, the median call rate is 0.98–1.00. Probes from the autosome are arranged in ascending genomic position. Then the probes are divided into nonoverlapping bins, each containing 1000 probes. The call rate for each bin is calculated. The median call rate statistic is the median of the bin call rates.
Infinium Genotyping Controls	The Infinium Control Data Charts include the following: Staining Red, Staining Green, Extension Red, Extension Green, Target Removal, Hybridization, Stringency, Nonspecific Binding Red, Nonspecific Binding Green, Nonpolymorphic Red, Nonpolymorphic Green, Restoration Red, and Restoration Green. For more information, see <i>Evaluation of Infinium Genotyping Assay Controls</i> on the Illumina support site and the BlueFuse Multi Color Key.

VeriSeq PGS QC Measures

BlueFuse Multi creates two reports with QC measures for VeriSeq PGS data.

For more detailed data quality information or to analyze any other possible issues, use the Illumina Sequence Analysis Viewer (SAV).

Sequencing QC Report

This report contains flow cell level QC measures. These values are identical for all samples processed on a single flow cell.

QC Measure	Description
Cluster Number	The number of positions on the flow cell with detectable DNA molecules on the surface. The optimal number is between 25–30 million (M) raw clusters. A flow cell that contains clusters outside this range does not provide ideal output for reliable analysis and requires optimization for subsequent sequencing runs.
Cluster Number Passing Filter	A subset of clusters that are considered reliable for downstream processing. The optimal amount is ~85% of total raw clusters.
Yield (kb)	A calculation of clusters passing filter and read length (36 base pairs). The optimal yield is ~900,000 kb.
Aligned Reads (PF) %	The percentage of reads passing filter that aligns to 1 region of the reference human genome. The percentage ranges between 80–85%, or is at least > 70% of the total number of reads. Lower values indicate contamination of your DNA source of suboptimal whole genome amplification.

QC Measure	Description
% Included	The percentage of reads after filtering in relation to the total read per sample. This measure is only reported in Query view.

Report

This report contains sample QC measures regarding the quality of data for a specific sample.

Table 1 Report

QC Measure	Description
Average Quality Score	The base quality (Q) score is a measure of confidence in the base that was called at a given base position. A Q-score 30 estimates that there are 0.1% incorrect bases in the result. For this report, this score is recalculated after filtering. The optimal average quality score is 35, and is at least 30.
Average Alignment Score	The alignment software calculates this score and takes into account the Q-score, read length, and the number of alignments. The optimal average quality score is 35, and is at least 30.
Number of Total Reads	The total number of DNA sequence tags created. For a well-balanced library of 24 samples, there are ~1 million reads reported for each sample (minimum 700,000 reads).
Number of Mapped Reads	The number of mapped reads. The optimal number is 80% of the total number of reads (~800,000). Lower values indicate contamination in your DNA source or suboptimal whole genome amplification.
Number of Reads After Filtering	The number of filtered reads usable for copy number calling. The optimal number is 60% of the total reads (~600,000 reads).
Overall Noise (DLR—Derivative Log Ratio)	The spread of the difference in copy number values between all bins of a chromosome.

QC Metrics

Dual Channel Processing QC Metrics

These metrics are used for 24sure+ and dual channel processed experiments.

QC Metric	Status	Criteria
SD Autosome/Robust	Good	0.05 - 0.11
	Too low	< 0.05
	Too high	> 0.17
% Included in clones	Good	>95%
Mean Spot Amplitude (signal)*	Good	700–3000
	Too low	< 500
	Too high	> 3000

QC Metric	Status	Criteria
SBR	Good	3–12
	Too low	< 3
	Too high	> 15
DLR Raw/Fused	Good	< 0.2
	OK	< 0.22
	Fail	> 0.24

*Dependent on type of scanner.

24sure Reference QC Metrics (Sex-Matched Reference Strategy)

These metrics are basic QC criteria settings to assess 24sure reference subarray results. If reference subarrays fail in a batch, then it is likely that the sample subarrays also fail.

The following examples are from sex-matched references.

QC Metric	Status	Criteria
SD Autosome/Robust	Good	0.05 - 0.11
	Too low	< 0.02
	Too high	> 0.17
% Included Clones	Good	> 95%
Mean Spot Amplitude (signal)*	Good	700–3000
	Too low	< 500
	Too high	> 3000
SBR	Good	3–12
	Too low	< 3
	Too high	> 15
DLR Raw/Fused	Good	< 0.2
	OK	< 0.22
	Fail	> 0.24

*Dependent on type of scanner.

Illumina BeadChip QC Metrics

Median LogR Deviation and Median call rate are the standard QC criteria settings to assess the performance of your CytoSNP-12 or CytoSNP-850K BeadChip. Values for SD Autosome/Robust and DLR Raw/Fused are included in the BeadChip report, but are not considered pass or fail criteria.

QC Metric	Status	Criteria
Median LogR Deviation	Good	< 0.2
Median Call Rate	Good	> 0.98

VeriSeq PGS QC Metrics

The following table contains a summary of VeriSeq PGS QC values.

	Parameter	Optimum Value	Acceptable Value	Source of Values
Amplification Test	Gel electrophoresis	Positive	Positive	Laboratory tests.
	Concentration dsDNA ng/μl (single cells)	25–35	> 10	
Flow Cell-based Parameter	Density (K/mm ²)	1200–1400	1100–1600	Run summary page on BaseSpace® or Summary in the Sequencing Analysis Viewer (SAV).
	% of clusters passing filter (PF)	85	75	
	Total number of reads	30,000,000	25,000,000	
	Total number of reads (PF)	25,000,000	19,000,000	
	% of reads reaching Q30	95	90	
	Maximum phasing/pre-phasing	0.5/0.05	0.65/0.1	
	% of reads identified (PF)	95	85	
Sample-based Parameter	% of reads identified (PF) per sample	5	2.5–8	
	% of reads identified (PF) for empty wells	< 0.025	< 0.05	Downstream analysis from BlueFuse Multi.
	Number of total reads	1,000,000	700,000	
	Number of reads after filtering	500,000	250,000	
	% of total reads after filtering	> 50	> 35	
	Average quality score (Q-score)	> 35	> 30	
	Average alignment score	> 35	> 30	
	Sample noise score (DLR)	0.2	< 0.4	
Chromosome-based Scores	Region Confidence (value in CNV table)	1.0	> 0.7	Downstream copy-number analysis from BlueFuse Multi.

Genome Masking

Genome masking is a feature that allows areas of the genome to be excluded from region calling and visualization. This feature allows you to reduce the probability of incidental findings in areas of the genome that are not required for a particular application.

When using the genome masking feature, note the following items:

- ▶ Only available for Illumina CytoSNP BeadArray experiments.
- ▶ Only supervisors and administrators can create and enable or disable genome masks.
- ▶ To reduce incidental findings further, LOH regions are not automatically called when a mask is used.
- ▶ Masking must be applied before processing an experiment.
- ▶ Masks are stored in the BlueFuse Multi database.
- ▶ Called regions that straddle a mask boundary are fully retained.
- ▶ Mask deletion is *not* supported with this release. Always conduct mask design and verification in a temporary database before using the mask in the production environment.

Procedures

Creating a Mask

- 1 Create a genome mask using the standard BED file format.



NOTE

This tab-delimited text file can be created using any text editor and then saved with the suffix .bed.

- ▶ Use the following header.

Header Value	Example	Description
db = <i>Genome build name</i>	db = GRCh37	Genome build version

- ▶ Use the following columns.

Column	Example	Description
chrom	chr3	Chromosome number
chromStart	1000	The start position of the mask region in the chromosome. The first base in a chromosome is numbered 0. Note: BlueFuse Multi numbers bases in chromosomes from 1.
chromEnd	2000	The end position of the mask region in the chromosome.



NOTE

Make sure that mask regions do not overlap.

Figure 5 Example of Genome Mask File

```
db=GRCh37
chr7 1000000 62500000
chr11 0 134934063
chr14 0 107287663
```

- 2 Import an annotation database that includes the genome masking feature. The standard BlueFuse Multi v4.3, and later, annotation database supports genome masking.
- 3 Select **Tools | Genome Mask**.
- 4 Select **Create New Mask**.

- 5 In the Create Genome Mask window, enter the following information.
 - a **Mask Name**—Enter a mask name that is unique within the database.
 - b **Mask definition BED file**—Browse to the mask definition created in step 1.
 - c **Mask type**—Select **Exclusion Mask** (regions within the BED file are *not* shown in the visualization and reports) or **Inclusion Mask** (only regions within the BED file are shown in the visualization and reports).
 - d **[Optional] CNV region size to unmask threshold**—This parameter determines the minimum size of a region that overrides the masking rules. This parameter is used to make sure that the mask does not hide large regions. For example, to define a minimum region size of 1.5 Mb, enter 1.5.
- 6 Select **Create**.
- 7 Select **OK** to acknowledge creation of the mask.

Applying a Mask to a New Experiment

- 1 Select **Add Experiment**, **Add Experiment (Express)**, or select **File | Add | Add Experiment**.
- 2 Click through the Experiment Editor wizard until you reach the Analysis Parameters section.
- 3 In the Analysis Parameters section, select the mask name from the Mask drop-down list.
- 4 Continue clicking through the wizard, and then select **Finish**.
The mask track is included in the DecisionTrack pane for the experiment. Regions of an exclusion mask are shown in gray. Regions of an inclusion mask are shown in green. For more information, see the Sample Prep Color Key.
- 5 Process the experiment to apply the mask.
The mask name is included in the Algorithm settings section of the report.

Applying a Mask Using a Batch Import File

You can apply a mask to experiments listed in a batch import file.

- 1 Follow the procedure described in [Creating a Mask on page 50](#).
- 2 Create a batch import file that includes the mask name under the annotation database. For more information, see [Create a Batch Import File on page 34](#).

Disable a Mask

A mask can be disabled when it is not needed for future use. Disabled masks are not shown when adding a mask to a new experiment, and cannot be used in batch import.

Disabling masks only affects new experiments; an existing experiment with a mask that has been disabled can continue to use that mask. For information on enabling a mask, see [Enable a Mask on page 51](#).

- 1 Select **Tools | Genome Masks**.
- 2 From the Available Masks list, select the mask to be disabled.
- 3 Select **Disable Selected**.

Enable a Mask

Disabled masks can be enabled if it is determined they are needed for use with new experiments. For information on disabling masks, see [Disable a Mask on page 51](#).

- 1 Select **Tools | Genome Masks**.

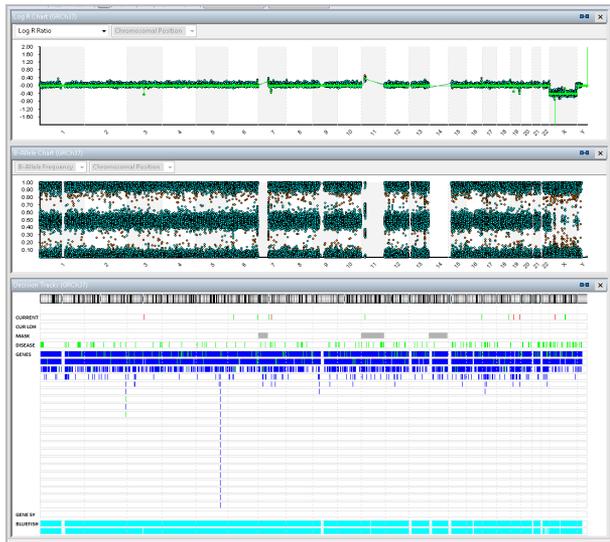
- 2 From the Disabled Masks list, select the mask to be enabled.
- 3 Select **Enable Selected**.

Examples of Masks

Applied Mask

Log R and B-allele data are not plotted in loci that are excluded by a mask to prevent interpretation of these regions. When an experiment is masked, no LOH regions are automatically called. The CGH table does not list any regions that are from the excluded masked regions, unless they are larger than the set mask threshold. LOH regions are excluded from the report. The DecisionTracks show the loci of mask regions.

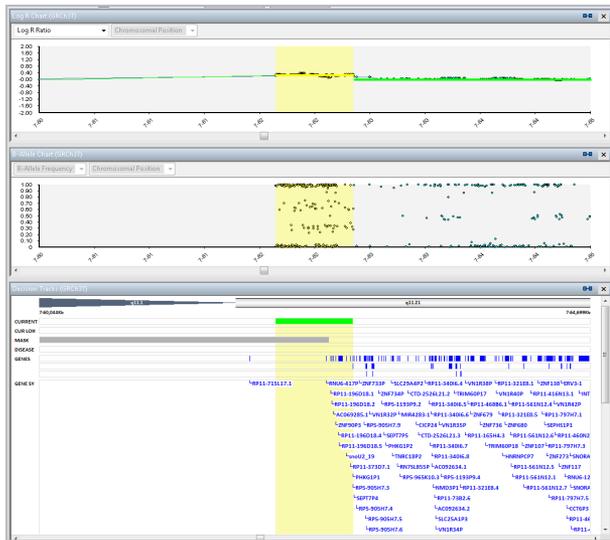
Figure 6 Genome View of an Experiment with an Applied Mask



Regions Straddle Mask Boundary

Regions that straddle a mask boundary are retained. In the following example, the mask overlaps the gain on chromosome 7 and the region is called and displayed.

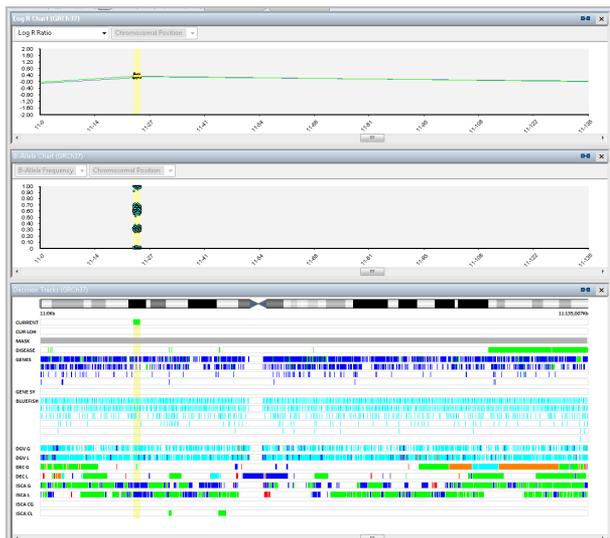
Figure 7 Example with Region Straddling Mask Boundary



Unmask Threshold

You can set an unmask threshold to make sure that masks do not hide large regions. The following example shows a 1.8 Mb region of gain on chromosome 11 that exceeds the 1.5 Mb threshold set for the mask.

Figure 8 Example with Unmask Threshold



Removing a Mask from an Experiment

To remove a mask from the experiment, use the Experiment Editor wizard and reprocess the experiment.

Genotype Analysis

Two genotype analysis views are available in the BlueFuse Multi side pane for BeadArray experiments: Duo Genotype Analysis and Trio Genotype Analysis. For more information, see [Duo Genotype Analysis on page 54](#), [Trio Genotype Analysis on page 55](#), and [Select Experiments for Genotype Analysis on page 56](#).

When using the Duo Genotype Analysis and Trio Genotype Analysis, note the following items:

- ▶ In chart views, the genotype analyses are not remembered as a last selected view.
- ▶ It is recommended that genotype analysis charts are used in combination with the Log R chart and B Allele chart for optimal visualization of the comparative analysis. A custom shortcut can be created that displays the desired set of views.
- ▶ Genome masks applied to any of the experiments involved in an analysis are applied to the analysis. However, the DecisionTrack view only indicates masks applied to the currently selected experiment.
- ▶ It is recommended that you use the Comments box in the Sign-Off feature to record interpretations observed via the genotype analyses.

Duo Genotype Analysis

The Duo Genotype Analysis view compares two genotyping experiments. This view displays the number of shared alleles for each probe that is common to both experiments and informative in each. For example, has a genotype call of either AA, AB, or BB. The experiment currently selected defaults to proband. Each SNP is classified as follows:

- ▶ **Two**—Both experiments are similarly homozygous or heterozygous
- ▶ **One**—One experiment is homozygous and the other is heterozygous
- ▶ **Zero**—The experiments are oppositely homozygous

Proband Genotype	Parental Genotype	Shared Alleles
AA	AA	Two
	AB	One
	BB	Zero
AB	AA	One
	AB	Two
	BB	One
BB	AA	Zero
	AB	One
	BB	Two

The chart title identifies the experiment selected for comparison with the proband listing the sample ID, experiment barcode, experiment location, and the sex.



NOTE

The Y-position of each spot has been randomized within a certain range to provide an impression of density. The Y value of a spot has no precise meaning, only in the general band in which a spot falls. For example Zero, One, or Two.

For information on selecting experiments, see [Select Experiments for Genotype Analysis on page 56](#).

For additional information on Duo Analysis, see the *BlueFuse Multi v4.4: Introducing Duo and Trio Analysis Views* bulletin on the Illumina support site.

Trio Genotype Analysis

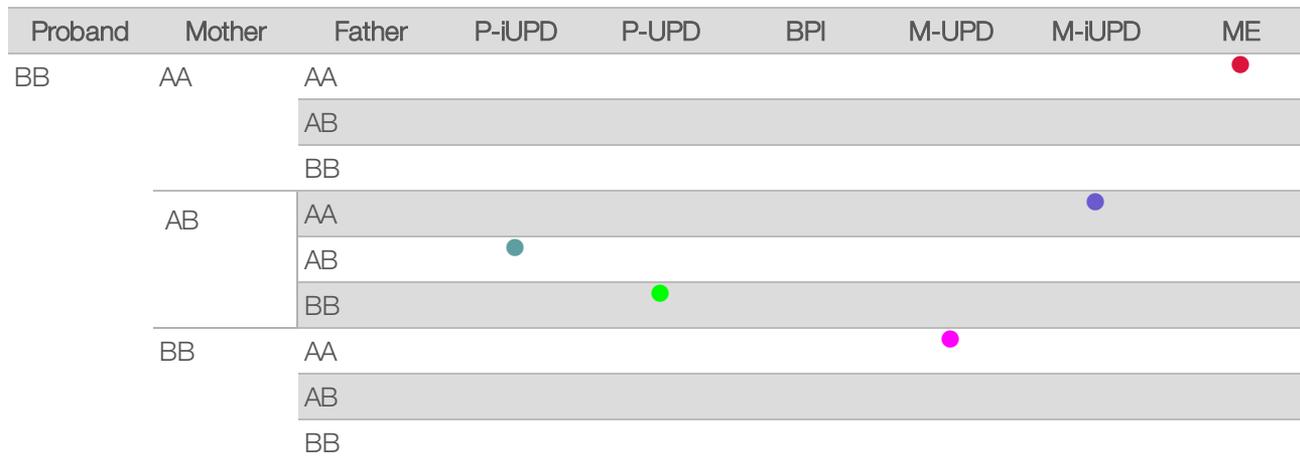
The Trio Genotype Analysis view compares a child against its parents and displays patterns of biparental inheritance, uniparental inheritance, and Mendelian errors. The default experiment is proband. The Trio Analysis dialog lets you select the experiments relating to the mother and father.

Probes are classified into the following categories:

- ▶ ME—Mendelian Error
- ▶ M-iUPD—Maternal Uniparental Isodisomy
- ▶ M-UPD—Maternal Uniparental Disomy*
- ▶ BPI—Biparental Inheritance
- ▶ P-UPD—Paternal Uniparental Disomy*
- ▶ P-iUPD—Paternal Uniparental Isodisomy

*Probes in this category could be either a heterodisomy or an isodisomy but it is not possible to determine which.

Proband	Mother	Father	P-iUPD	P-UPD	BPI	M-UPD	M-iUPD	ME
AA	AA	AA						
		AB						
		BB					●	
	AB	AA						
		AB						
		BB						●
	BB	AA			●			
		AB	●					
		BB						●
AB	AA	AA						●
		AB						
		BB			●			
	AB	AA						
		AB						
		BB						
	BB	AA				●		
		AB						
		BB						●



The chart title identifies the experiments selected for comparison with the proband and the role each was assigned to, mother or father. After assigning the familial roles, the Trio Genotype Analysis feature identifies SNPs that are informative for the analysis, that are the SNPs where the maternal and paternal genotypes are oppositely homozygous. The proband and parental genotypes for the informative SNPs are then compared to assign the classification.



NOTE

Similar to the Duo Genotype Analysis, each spot's Y position has been randomized within a certain range to provide an impression of density.

For information on selecting experiments, see *Select Experiments for Genotype Analysis* on page 56.

For additional information on Trio Analysis, see the *BlueFuse Multi v4.4: Introducing Duo and Trio Analysis Views* bulletin on the Illumina support site.

Select Experiments for Genotype Analysis

There is no restriction on the types of BeadArray experiments that can be directly compared whether of different types or versions.

- 1 Select a BeadArray experiment and then select either **Duo Genotype Analysis** or **Trio Genotype Analysis** from the View side pane.
- 2 In the search text box(es) do one of the following:
 - ▶ Type the Sample ID to list all experiments that contain the search term as a prefix, and select the appropriate experiment.
 - ▶ To refine the experiment list further, follow the Sample ID with the barcode of the desired experiment, separated by a space using the following format:
 <Sample ID> <Experiment Barcode> <Experiment Location>
 Where <Experiment Location> refers to the hybridization area on the array.
 The list is pre-filtered to only include BeadArray experiments that have been processed.
 Once selected, the experiment's details and those of the associated sample are displayed, including an indication if a genome mask has been applied. These details can be reviewed to confirm that the correct experiment has been chosen.
- 3 Click **OK**.

BlueFuse Multi Reports

The end point of the BlueFuse Multi analysis is the report. There are three main types of report—Experiment Report, Sample Report, and Report. For more information about the sections included in the Report, see [Report Contents on page 57](#).

The information contained in the report can be managed so that the most useful and relevant information is provided. For example, 24sure users require less information than users of BeadArray for constitutional applications, where the specific gene content of regions of imbalance is of the utmost importance. BlueFuse Multi can help users decide what information to include in the report using the data included in the DecisionTrack pane. For more information about customizing and exporting your report, see [Customizing the Report Configuration on page 58](#).

Report Contents

The following table shows the report sections that are required for each experiment type.

Section	24sure/ VeriSeq PGS	Cycle Report	BeadArray
Subject Information	√	√	√
Sample Information	√		√
Experimental Details	√		√
Reagent Kit Lot Numbers			√
Algorithm Settings	√		√
QC Measures	√		√
Results	√	√	
Structural Aberration Summary	√		
Sign Off History			√
Karyotype View			√
Tabular View			√
Detailed Region Summary			√
ISCN			√
LOH Region Tabular View			√

*These experiments are no longer supported.

Report Section Descriptions

Section	Description
Sample Information	Fields about the subject or individual—Subject ID, Pedigree, Name, DOB, Sex, Features, Karyotype, Consultant, and Other.
Sample Information	Fields about the sample—Sample Type, Date Received, Extraction Method, Extraction Date, Storage Location, Sample QC, Gel Quality, and Gel Image Location.
Experiment Details	Information relating to the experiment—Microarray Barcode and Lot, Microarray Type, Genome Build, Operator, and Files Used During Analysis.
Reagent Kit Lot Numbers	Relevant to the Infinium protocol reagents for BeadArray.

Section	Description
Algorithm Settings	The BlueFuse Multi algorithm and the specific parameter settings used during analysis.
QC Measures	Metrics that provide evidence of the data quality following analysis of the array, adapted for each experiment type.
Results	A summary of results included in 24sure, VeriSeq PGS, and cycle reports. A 24sure report includes an ideogram representation of the results and a detailed results table with a confidence measure for the called imbalances.
Structural Aberration Summary	For 24sure and Veriseq PGS reports only. This field is populated when a structural aberration on a PGS case is created.
Sign Off History	The time and level of sign offs, and any comments made.
Karyotype View	Shows the karyotype image as displayed in Karyotype Chart (Reported).
Tabular View	Provides summary information for all the copy number imbalances in the experiment. Includes data from the CGH table that are marked as "Reported."
Detailed Region Summary	A separate section for each reported imbalance. This section includes the following: <ul style="list-style-type: none"> • Region statistics, as reported in the Tabular View section. • Details considered when calculating the assessment. • Region comments added to the Region View tab. • HGNC genes, OMIM genes, and OMIM or other diseases present in the region. • Details of DGV studies that overlap the region. • Details of gains and losses reported by the ISCA/dbVar consortium that overlaps the region.
ISCN	The results reported using ISCN nomenclature.
LOH Region Tabular View	Summary information for all the LOH regions in the experiment, including data from the SNP LOH table that are marked as "Reported." For BeadArray, this section provides the amount of autosomal LOH in the sample (Mb and the percentage of LOH).
Detailed LOH Region Summary	A separate section for each reported LOH region. This section includes the following: <ul style="list-style-type: none"> • Region statistics, as reported in the LOH Tabular View section. • Details considered when calculating the assessment. • HGNC genes, OMIM genes, OMIM diseases, and BG diseases present in the region.

Customizing the Report Configuration

Administrators and supervisors can set a custom report configuration.

- 1 Click **Tools | Global Configuration | Custom Report Configuration**.
- 2 Select **Experiment Type**.
- 3 Select the information required in the report. For more information, see [Report Contents on page 57](#).
- 4 Click **Save All** or **Save Current** to save the new settings to the database.

Removing a Region from a Report

If necessary, you can remove a region of imbalance from the report after assessing the relevance of the region.

- 1 Select a region in the CGH/CNV table, the current track of DecisionTrack, or the Detailed Region tab of Region View. You can also remove genes from the Reported Genes tab of Region View.
- 2 Right-click the selection, and then select **Mark Reported/Not Reported**. In the Detailed Region Tab, remove a region using the dropdown menu.
The region is then marked "no" in the reported column and "yes" in the manual change column. Genes are removed from the Reported Genes tab.

After regions have been marked as Not Reported, they are excluded from the report, Karyotype Chart (Reported), CGH Chart (Reported), and CNV Chart (Reported).

Including Maximum Region Contents in a Report

Due to the discrete sampling of the genome by microarrays, the exact size of a detected region is unknown. The minimum region includes the last most extreme probes of the imbalance that are shifted from 0 (on the log₂ scale). The maximum region extends on either side to the adjacent probe that is not included in the imbalance.

When it is enabled, the maximum region function remains on for the subsequent processing of samples. Reprocess past experiments with this option enabled to report the maximum region statistics. This setting affects all users of the database.

- 1 Click **Tools | Global Configuration | Report Configuration**.
- 2 Select **Show Max Region Statistics**, and then click **OK**.
- 3 In the report, the minimum and maximum sizes for each region are provided. Any genes, disease regions, or ISCA/dbVar regions that fall between minimum and maximum regions or overlap the maximum boundary are included in curly brackets.



NOTE

Maximum region contents cannot be provided for experiments processed with a mask. For more information, see [Genome Masking on page 49](#).

Showing ISCN Results in Long and Short Formats

This feature is relevant to cytogenetics reports. By default, new experiments show ISCN results in short form, but you can configure the report to show the long form.

- 1 Click **Tools | Global Configuration | Report Configuration**.
- 2 Select **Show long ISCN result**, and then click **OK**.



NOTE

Long format ISCN results cannot be provided for experiments processed with a mask. For more information, see [Genome Masking on page 49](#).

Exporting a Report

Exporting a Cytogenetics Report

- 1 Select a sample or experiment from the selection group in the side pane.
To export all experiments in a filtered list, select **Filtered Experiments** from the drop-down next to the Export Results to File toolbar icon.
If a sample is selected, BlueFuse Multi creates a report for each experiment associated with the sample.
- 2 Click **Export Results to File** or click **File | Export | Export Results**.
- 3 Select from the following options—**All**, **CGH summary only (full)**, or **CGH summary only (custom)**.
- 4 Select a folder location for the results files. The last used file location is remembered.
- 5 Click **OK**.

Exporting a 24sure or VeriSeq PGS Report

- 1 Select a cycle or embryo from the selection group in the side pane. To export all experiments in a filtered list, selecting **Filtered Experiments** from the dropdown next to the Export Results to File toolbar icon.
- 2 Select **Export Results to File** or select **File | Export | Export Results**.
- 3 Select from the following options:
 - ▶ **Individual embryo reports**—All, CGH summary only (full), CGH summary only (custom), or None. If you select CGH summary only, BlueFuse Multi creates separate reports for each embryo.
 - ▶ **Cycle reports**—Cycle report (full), Cycle report (custom), or None.
 - ▶ **[Optional]** Select the option to include Fused Chart images. Fused charts are only included in the report if a CGH summary report is selected. To configure the Fused Chart, click **Tools | Global Configuration | Export Chart Configuration**.
- 4 Click **OK**.
- 5 Select a folder location for the results files. The last used file location is remembered.
- 6 Click **OK**.

Exporting Results in Specific Formats

Processed data in a BlueFuse Multi database can be exported in formats suitable for direct upload to International Collaboration for Clinical Genomics (ICCG) and Nexus websites.

Data Export for Nexus

Exporting experimental data with this feature provides proper file formatting to easily share results with the public data repository hosted by Nexus software.

- 1 Select either the sample or experiment for export from the selection group in the side pane. Alternatively, select **Filtered Experiments** from the dropdown next to the Export Results to File toolbar icon.
- 2 Click **File | Export | Export Results (Nexus)**.
- 3 Select a folder location.
- 4 Click **OK**.

Karyomapping Module

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Annotation

The Karyomapping module only supports GRCh37 annotation and cannot import a GRCh38 annotation database.

User Interface

BlueFuse Multi uses a standard Windows user interface. The interface comprises of 6 main components:

- ▶ **Menus**—Provides access to all functions in BlueFuse Multi.
- ▶ **Toolbar**—Contains icons for quick access to commonly used functions.
- ▶ **Side pane**—Contains options to select samples and cases, the ability to add samples to the workflow, and shortcuts.
- ▶ **Genome build**—Unavailable for this module.
- ▶ **Views**—The different ways of viewing the karyomapping data.
- ▶ **Module selector**—A dropdown to select CytoChip or karyomapping module.

Icons

Refer to the following table for descriptions of the icons in the karyomapping module. To change the size of the Toolbar icons, click **View | Large Toolbar Icons**.

Toolbar Icons	Toolbar Icon Description	Side Pane Icons	Side Pane Icon Description
	Add Case (Guided)		Case—Unprocessed or only Parents/Parents + Reference processed Sample—Unprocessed
	Add Case		Case/Sample—Processed
	Edit Case		Case/Sample—Manually modified but not signed off
	Clone Case		Case/Sample—Signed off to level 1

Toolbar Icons	Toolbar Icon Description	Side Pane Icons	Side Pane Icon Description
	Delete Case	 	Case/Sample—Signed off to level 2
	Sign Off Case		Workflow step—Action completed
	Run Batch Processing		Workflow step—Action available
	Karyomapping Algorithm Configuration		
	Log in to a Database		

Side pane

The Side pane contains the following groups.

Group	Description
Selection	Opens the list of created cases and their samples, in the order of most recently modified. This list can be filtered so that items that meet specified criteria are displayed. For more information, see Filtering Experiments and Samples on page 38 . After a case is loaded, a case icon represents the case. A chromosome icon represents each sample contained within the case. Samples include embryos and trios (mother, father, and reference). Select a sample to switch to an equivalent CytoChip module view and to visualize the CNV information.
Shortcuts	Lists the common configurations of karyomapping views—Case Report, Case View, and Close All. Each option opens 1 or more views from the View group box, except for the Close All option, which closes all views. The current shortcut is maintained when you change between the selected case in the Selection group box.
View	Lists all possible views that can be opened within the application for the experiment. The current view, including chart zoom levels, is maintained when you change between the selected case in the Selection group box.

Views

This section describes the options for viewing your experiment. The software views are also described in the Karyomapping Walkthrough video guide on the [BlueFuse Multi support pages](#). For more information on using the views to examine a karyomapping case, see the *Technical Guide to Karyomapping Calling (part # 15052496)*.

Case View

The following views are included in this shortcut.

View	Description
Karyotype Chart	Offers an overview of each case and shows all chromosome ideograms in the standard configuration. Each region defined in a case is highlighted with a blue rectangle.
Haploblock Chart	A vertical chromosome view that shows the sample chromosome pairs for all samples in the case. If there are multiple regions in a case, the view is populated with the first region defined in the case. The chart includes a pair of chromosomes for father (blue and red), mother (orange and green), and reference (P1 – blue, M1 – orange) followed by a pair of chromosomes for each embryo. Blue shading indicates the region of interest and flanking region. Double-click a chromosome in the karyotype chart to repopulate the Haploblock Chart with data from all samples for that chromosome.
Detailed Haploblock Chart	Populates when a haploblock on a chromosome in the Haploblock Chart is double-clicked. The Detailed Haploblock Chart shows the pair of chromosomes for a single embryo above the pair of chromosomes for the reference. Key and non-key SNPs are displayed above and below each of the embryo chromosomes respectively and are colored according to their phase—M1 or M2, P1 or P2. Informative SNPs that have not been successfully assigned to genotype (NC) are plotted down the center of the haploblocks.

Case View Context Menu Options

The following menu options are available for Case View.

Option	Description
Zoom Options	Available for the Haploblock Chart and the Detailed Haploblock Chart. Zoom options include: <ul style="list-style-type: none"> • Zoom Out—Doubles the zoom around the center of the zoomed x/y axis. • Zoom to Chromosome—Zooms to the whole of the selected chromosome.
Copy/Print/Save	Available for the Karyotype Chart, Haploblock Chart, and Detailed Haploblock Chart. <ul style="list-style-type: none"> • Copy—Copy the charts to the clipboard to paste into other applications. • Print—Send charts to a printer. • Save—Save charts in *.jpeg or *.bmp formats.
Links to Web Resources	Available for Haploblock Chart. This option provides integration of results with web databases. Click a web link to jump to the selected region in Ensembl and UCSC databases.

Zoom Options

By default, the view is zoomed to the region of interest. You can zoom out to the whole chromosome using the context menu.

- ▶ Right-click and drag to zoom into a region in the x direction only (Detailed Haploblock Chart) or the y direction only (Haploblock Chart).
- ▶ Right-click the chart to reveal the context menu zoom options.
- ▶ Karyotype Chart zoom options:
 - ▶ Double-click any chromosome to zoom to that chromosome in all displayed views.
 - ▶ Double-click the blue rectangle, which indicates the disease region of interest, to zoom to that region in all views.
- ▶ Double-click an embryo chromosome in the region of interest on the **Haploblock Chart** to zoom in to the **Detailed Haploblock Chart** for that embryo.

Viewing SNPs in Haploblock Chart

- 1 Select Tools | Configuration | Karyomapping | Chart Configuration.

- 2 Select **Show SNPs in Haploblock View**, and then select **OK**.
SNPs in the haploblock chart are represented in the following ways:
 - ▶ Filled circles—Key SNPs.
 - ▶ Open circles—Nonkey SNPs.
 - ▶ X—Informative SNPs that have not been successfully assigned to a genotype.

Case Report

The Case Report includes all the information about the case and is broken down into the following sections:

Section	Description
Case Level Warnings	Any warnings relating to the whole case (eg, contamination or sample mishandling).
Case Summary	Includes data entered during the case creation. Includes the following information— Data about the disease, parent details, software build, annotation database, algorithm settings, attached files, and sign-off status. During case sign-off, the user assigns the overall QC status for each array as Pass, Suboptimal Pass, or QC fail.
QC	Includes a table of array QC metrics for each sample, including overall array QC status, call rate, ADO rate, and miscalls. This section also includes a second table of DNA QC measures that you enter during case creation.

For each region in the case, the Case Report includes the following information.

Section	Description
Region Warnings	Any warnings relating to the region (eg, there is a CNV in 1 of the parents within the main or flanking regions).
Region Statistics	Includes the following: <ul style="list-style-type: none"> • Genomic location of the region of interest. • Disorder type. • Available SNPs in main and flanking regions. • Available paternal and maternal informative SNPs in the main and flanking regions.
Region Statistics for Each Embryo	Predicted phase for each embryo. Also includes the supporting and opposing evidence for the predicted phase (key and nonkey maternal and paternal informative SNP statistics).

Case Report Context Menu Options

The following menu options are available for Case Report.

Option	Description
Select All/ Copy/Print/Save	<ul style="list-style-type: none"> • Select All—Select the whole view. Alternatively, select regions to using the mouse. • Copy—Copy the charts to the clipboard to paste into other applications. • Print—Send charts to a printer. • Save—Save charts in *.jpeg or *.bmp formats.

Cases

Adding a Case (Guided)

The Add Case (Guided) icon allows the user to build a case in a wizard in separate steps:

- ▶ Create Case
- ▶ Add Parents

- ▶ Add Reference
- ▶ Add Samples

Create Case

- 1 Select **Add Case (Guided)**.
- 2 Select **Next**.
- 3 Enter the case ID, cycle ID, and disease name, and then select **Next**. All other fields are optional.
- 4 Locate the manifest and cluster files, and then select **Next**.
After creating a case for the first time, the manifest and cluster file options are prefilled with the files that were last used.
- 5 **[Optional]** Deselect **Use Default Flanking Region Size**, and then set the flanking region size.
- 6 Enter the parent details and patient ID fields, and then select **Next**.
- 7 Enter the HGNC gene name for the region of interest. For regions that are not related to a specific gene, enter user-defined coordinates.
For more information, select **HGNC** to search for the name in the HGNC website. Multiple regions can be added to the same case.
- 8 Select the disorder type, status of mother, status of father, reference pedigree, reference gender, and status of the reference, and then select **Add**.
- 9 Select **Next**, and then select **Finish**.



NOTE

To remove an incorrectly added case, right-click the region in the grid, and then select **Delete**.

Add Parents

- 1 Select **Add Parents**.
- 2 Enter the sample ID and locate the *.gtc file for the first parent.
- 3 Select the designation and sample type, and then select **Add**.
- 4 Repeat steps 2 and 3 to add the second parent. Select **Next**.
- 5 To remove an item from the table, right-click the row, and then select **Delete**.
- 6 **[Optional]** Select a sample ID and enter DNA QC information, and then select **Add**. Repeat for the other sample, and then select **Next**.
- 7 **[Optional]** Select the reagent and enter the lot ID. Select **Add**, and then select **Next**.
- 8 **[Optional]** Browse to any files that you want to associate with the case. Add a description, and then select **Attach file**.
- 9 Select **Next**, and then select **Finish**.
- 10 **[Optional]** To process case with only parental samples, select **Process Case** in the workflow box of the side pane.

Add Reference

- 1 Select **Add Reference**.

- 2 To add a reference sample, repeat the steps that you performed to add the parental samples.
- 3 Select **Next**, and then select **Finish**.
- 4 **[Optional]** To process the parental and reference samples before adding the embryo samples, select **Process Case** in the workflow box of the side pane. Examine the reported maternal and paternal informative SNPs in the region.
This step is recommended to provide information on the availability of informative SNPs in the region of interest. For more information, see the *Technical Guide to Karyomapping Calling (part # 15052496)*.

Add Samples

- 1 Select **Add Samples**.
- 2 To add each embryo sample, repeat the steps that you performed to add the parental and reference samples.
- 3 Select **Next**, and then select **Finish**.

Process Case

- 1 Select **Process Case** in the selection or workflow box of the side pane. After the case is processed, the case view is opened. The icons for the case changes from unprocessed (orange) to processed (green).

Adding a Case (Experienced Users)

If you have experience with creating karyomapping cases, you can create a case with parental, reference, and embryo samples in 1 step.

- 1 Select **Add Case** or click **File | New Case**.
- 2 Click through the wizard and add parental, reference, and embryo sample information. For more information, see *Adding a Case (Guided)* on page 64.

Batch Processing a Group of Cases

- 1 From the **Run Batch Processing** drop-down, select **Unprocessed** or **Filtered**.
- 2 Create a filtered list of cases. For more information, see *Filter Cases* on page 67.
- 3 Select **Run Batch Processing** or select **File | Run Batch**.
- 4 **[Optional]** If the filtered option was selected, choose one of the following:
 - ▶ **Unprocessed cases only**
 - ▶ **Unprocessed and Processed (but not signed off)**
- 5 Select **OK**.
- 6 If warning appears regarding reprocessing processed case samples, do one of the following:
 - ▶ Select **Yes** to process all samples.
 - ▶ Select **No** to process only nonprocessed samples.
 Cases with manual changes are not reprocessed.
- 7 The karyomapping cases selected for batch processing are shown in the batch progress dialog. Select **Run** to finish.

Editing an Existing Case

- 1 In the selection group of the side pane, select a case.
- 2 Select **Edit Case**.
- 3 Click through the wizard and make the appropriate modifications.
- 4 If the change made requires the case to be reprocessed, the case icon reverts to orange. To reprocess the experiment, select **Process Case** in the **Workflow** section of the side pane.

Cloning a Case

You can copy a case using the Clone Case function and then make any appropriate modifications.

- 1 In the selection group of the side pane, select a case.
- 2 Select **Clone Case**.
- 3 Enter the new case ID and cycle ID.
- 4 **[Optional]** Enter a description.
- 5 Select whether to include the reference and embryo samples.
- 6 Select **OK**. The cloned case appears in the side pane.
- 7 Select **Edit Case** and modify as needed.

Interpretation Workflow

For more information on interpreting a karyomapping case, see the *Technical Guide for Karyomapping Calling (part # 15052496)*.

Filter Cases

You can filter a list of cases by certain criteria. To filter the list of cases, click **Unfiltered Experiments** in the side pane, and then select **Category**, **Date Range**, or **Text** from the drop-down menu.

The following category filters are available.

Option	Description
Unprocessed	All unprocessed cases.
Processed	All processed cases.
Sign Off 1	All cases signed off to level 1.
Sign Off 2	All cases signed off to level 2.
Modifications Without Sign Off	All cases that have been manually modified, but not signed off.

The following date range filters are available.

Option	Description
Case Creation Date	All cases that were created within the specified range.
Case Last Modified Date	All cases with last modified dates within the specified range.

The following text filters are available.

Option	Description
Referring Centre, Disease Name, Subject Surname, Patient ID, Case ID	All cases with fields matching the given search string.

Quality Control

Signing Off a Case

The Sign Off feature allows you to assign a Pass or Fail status to case. You can select the quality of each sample within the case—Pass, Suboptimal Pass, or Fail. The sign-off history is shown in the case report.

See the following table for more information about the sign-off levels.

Sign Off Level	Description
Level 1	<ul style="list-style-type: none"> Any user can perform level 1 sign-off. If experiments that signed off to level 1 are reprocessed, a warning appears. After sign-off, the chromosome icon next to the experiment is light blue.
Level 2	<ul style="list-style-type: none"> Only supervisors or administrators can perform level 2 sign-off. Requires a password. Requires a QC status to be selected for every sample. After sign-off, a user can no longer modify the experiment or sign off the experiment at level 1. After sign-off, the chromosome icon next to the experiment is dark blue.

Sign-Off Procedure

- 1 Select **Sign-Off Case**.
- 2 Choose the sign-off level—**Level 1** or **Level 2**.
- 3 Choose the case status—**Case Failed** or **Case Passed**.
- 4 **Optional** Select a default QC status.
This status is populated to each sample whose QC has not yet been set.
- 5 Enter any comments, and then select **OK**.
Comments are shown in the report with your user name and the date and time of the comment.
- 6 Set the status of each sample—**Pass**, **Suboptimal Pass**, or **QC Fail**.
- 7 Choose your user name and enter your password.
- 8 Select **Finish**.
Level 2 indicates that the sign-off process is complete.

QC Measures

The following QC measures are used in the karyomapping module.

QC Measure	Description
Call Rate	The fraction of SNPs with a successfully called genotype.
AB Rate	The fraction of called SNPs with an AB genotype.

QC Measure	Description
ADO Rate	The estimated fraction of SNPs affected by allele dropout. This rate is calculated from the proportion of loci where the embryo is homozygous but was expected to be heterozygous, based on the parental genotypes.
Miscall Rate	The estimated fraction of SNPs affected by genotyping errors. This rate is calculated from the proportion of loci where the embryo is heterozygous but was expected to be homozygous, based on the parental genotypes. The measured miscall rate is used to adjust the estimate of the ADO rate.

QC Metrics – SNP Call Rates

The QC section in the karyomapping case report includes metrics about the quality of the karyomapping data. The sample type has an influence on the call rate, AB rate, ADO, and miscall rate. The following table lists the recommended values for good quality genomic DNA, often obtained from parental and reference samples (blood), and for amplified DNA from embryo biopsies (trophectoderm and blastomere biopsies).

Sample type	Call rate	AB rate	ADO [§]	Miscall rate
Blood	95–99%	25–29%	~0	~0%
Blastomere biopsy	75–95%	~15%*	0–80%	< 5%
Trophectoderm biopsy	85–99%	20–30%	0–80%	< 5%

*Can be lower when case has a good quality reference.

§The software is designed to account for ADO. A high ADO rate alone does not stop the calling of a case

Exporting Case Results

The Case Report is used to assess each karyomapping case. The report is divided into sections detailed in [Case Report on page 64](#). You can view the report using the Case Report shortcut and export the information.

- 1 Select a case from the selection group in the side pane.
 - 2 Select **File | Export Results | Export Case Results**.
 - 3 Select from the following options:
 - ▶ **All**—Includes 3 excel (*.xls) files for each sample in the case.
 - ▶ CGH summary—Report of CGH regions in each sample.
 - ▶ Fused—Data for each probe on the array.
 - ▶ Output—Information on the subject, sample, microarray, reagent kit, algorithm setting, BeadArray QC measures, and the sign-off history for each sample.
 - ▶ **CGH summary only (full)**—Includes the CGH summary excel (*.xls) file for each sample in the case.
 - ▶ **None**
- For all these options, you can include the Case Report, LogR Chart, and a *.jpg image of each LogR chart for each sample in the case. An *.xls file containing the case summary information is included in the export.
- 4 Select **OK**.
 - 5 Select a folder location for the results files. The last used file location is remembered.
 - 6 Select **OK**.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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