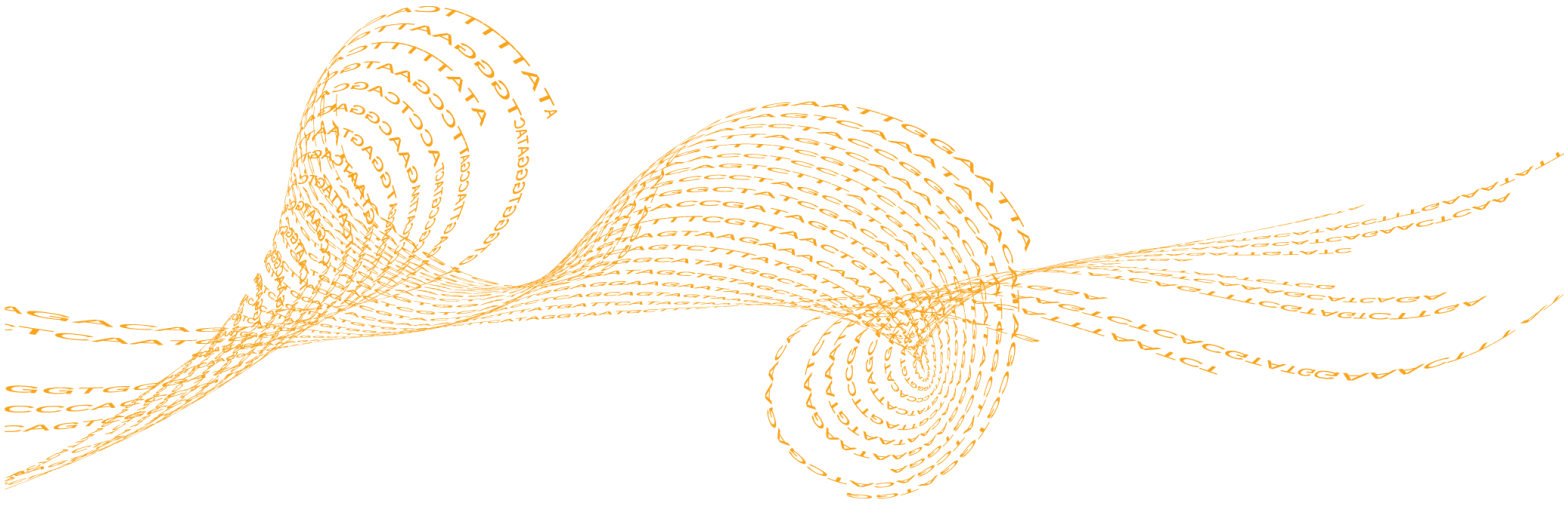
BeadArray Controls Reporter

Software Guide

For Research Use Only. Not for use in diagnostic procedures.



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Document # 1000000004009 v00
October 2015

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Introduction

The BeadArray Controls Reporter software is designed to analyze the sample controls for Illumina BeadArray products. The software outputs an excel file with summary values for each control for each sample in a project.

Software Requirements

The BeadArray Controls Reporter can be installed on any computer running Windows XP, or later.

Running the BeadArray Controls Reporter requires a parent folder that contains the following files:

- IDAT files, include *.sdf files
- A user-supplied sample sheet
- A manifest file (*.bpm)

Make sure that you have read-write access to the parent folder and the specified output folder.

To open the Excel output file, you need Microsoft Excel 2007, or later.

Installation

1. Download the BeadArray Controls Reporter software from the Illumina website.
2. Double-click the MSI installer.
3. Follow the on-screen instructions to accept the terms of agreement and specify a preferred installation folder.
   By default, the software is installed on your local drive in Program Files\Illumina\BeadArray Controls Reporter.
   A desktop icon is created automatically.
4. Click **Install**.
5. After installation is complete, click **Finish**.
6. To open the software, double-click the BeadArray Controls Reporter desktop icon.

Analysis Overview

The BeadArray Controls Reporter software analyzes a set of IDAT files and generates an Excel file that summarizes controls.

When analysis begins, the software processes information in the manifest file and the sample sheet to match each sample name with the appropriate BeadChip and BeadChip location. Then, the software parses IDAT files for the red and green channels for a given sample and generates the control values.

When analysis is complete, the software generates the output file in Excel format, which lists the control values for each sample. The sample names provided in the sample sheet are used in the output file.

Control values below the specified threshold are highlighted in the output file. You can reanalyze highlighted values using visualization tools in the GenomeStudio Methylation module.

The output file is named ParentFolderNameControls.xlsx. The default folder location is Libraries\Documents. If a file of the same name is already present in the folder, another file is written with a sequential number appended to the file name.

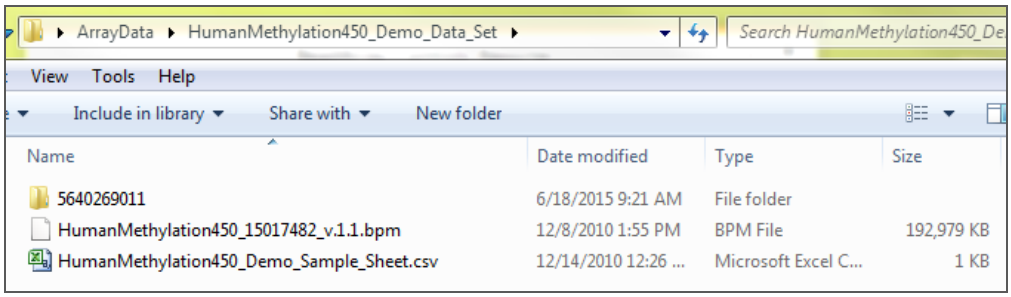
For more information about the controls, see *Control Descriptions* on page 6 and the documentation for the BeadChip kit you are using.

Start Analysis

1 Click **Browse** to select a parent folder for the IDAT file location.

2 Navigate to the parent folder.

Figure 1 Example of Folder Containing IDAT Files for All Samples on a BeadChip



NOTE
You can group the IDAT files into their own folders by BeadChip Sentrix ID. Figure 1 shows a folder containing IDAT files for all samples on a BeadChip. The IDAT files can also be placed in the parent folder without being placed in a subfolder.

3 Click **Start Analysis**.

NOTE
If using the FFPE restore kit, see *Control Descriptions* on page 6.

Modifying the Output Directory

The software writes the output file in the Libraries\Documents folder unless otherwise specified.

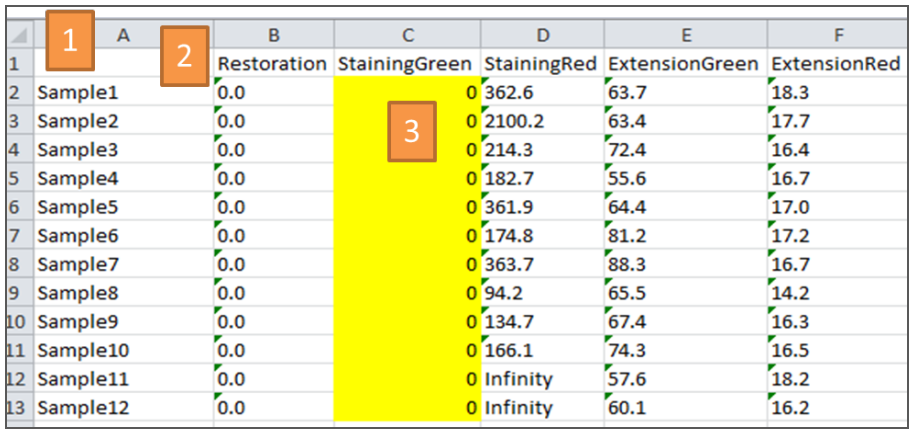
1 Click **Options**.

2 Click **OutputDirectory**, and then browse to the preferred folder.

Output File

The BeadArray Controls Reporter output file summarizes the intensities of the controls across all samples.

Figure 2 Example of Output File



	A	B	C	D	E	F
1		Restoration	StainingGreen	StainingRed	ExtensionGreen	ExtensionRed
2	Sample1	0.0	0	362.6	63.7	18.3
3	Sample2	0.0	0	2100.2	63.4	17.7
4	Sample3	0.0	0	214.3	72.4	16.4
5	Sample4	0.0	0	182.7	55.6	16.7
6	Sample5	0.0	0	361.9	64.4	17.0
7	Sample6	0.0	0	174.8	81.2	17.2
8	Sample7	0.0	0	363.7	88.3	16.7
9	Sample8	0.0	0	94.2	65.5	14.2
10	Sample9	0.0	0	134.7	67.4	16.3
11	Sample10	0.0	0	166.1	74.3	16.5
12	Sample11	0.0	0	Infinity	57.6	18.2
13	Sample12	0.0	0	Infinity	60.1	16.2

The output files have the following features:

1 The column headings list the name of each control.

2 The row headings list the name of each sample.

3 The values in each cell indicate the calculated value for each sample at its corresponding control. If the value is below the set threshold, the cell is highlighted.

Output File Thresholds

If a control is below the default thresholds, the software automatically highlights the control in the Excel output file. A highlighted value can indicate an issue with the sample data, but can also indicate that the default threshold for this control value is not optimal for an individual scanner.

The control values are calculated from the intensity of the bead associated with the individual control probe, and each scanner shows different levels of intensity. The default values are guides that apply to most scanners. Review control values across many samples that performed well in secondary analysis and modifying the thresholds as necessary for each scanner.

The thresholds control values are calculated using the following parameters.

Key Term	Description
Baseline	Extension Green or Red Channel Intensity
Offset Correction	An added value above the baseline to generate a standard window of background. The default is 3000 (intensity) and can be changed for some scanners.
Background	Baseline + Offset Correction

Control Descriptions

Control	Description
Restoration	• The default threshold is 0. • If using the FFPE DNA Restore Kit, the restoration control identifies success of the FFPE restoration chemistry. Change the threshold from 0 to 1; otherwise the failing FFPE Restore controls are not highlighted. If any other samples are highlighted, confirm the results with the GenomeStudio Methylation module for visualization of the controls. • The green channel intensity is higher than Background. Therefore, the metric provided is the Green Channel Intensity/Background.
Staining	• Staining controls are used to examine the efficiency of the staining step in both the red and green channels. These controls are independent of the hybridization and extension step. • The green channel shows a higher signal for biotin staining when compared to biotin background, whereas the red channel shows higher signal for DNP staining when compared to DNP background. • The metric provided for green is the ***(Biotin High value)/ (Biotin Bkg)*** and the metric provided for red is ***(DNP High value)/(DNP Bkg value)*** • The default threshold is 5. This threshold can be increased on some scanners.
Extension	• Extension controls test the extension efficiency of A, T, C, and G nucleotides from a hairpin probe, and are therefore sample independent. • In the green channel, the lowest intensity for C or G is always greater than the highest intensity for A or T. • The metric provided is the ***(lowest of the C or G intensity)/ (highest of A or T extension)*** for a single sample. • The default threshold is 5. This threshold can be increased on some scanners.
Hybridization	• Hybridization controls test the overall performance of the Infinium Assay using synthetic targets instead of amplified DNA. These synthetic targets complement the sequence on the array, allowing the probe to extend on the synthetic target as a template. Synthetic targets are present in the Hybridization Buffer (RA1) at 3 levels, monitoring the response from high-concentration (5 pM), medium concentration (1 pM), and low concentration (0.2 pM) targets. All bead type IDs result in signals with various intensities, corresponding to the concentrations of the initial synthetic targets. • The value for high concentration is always higher than medium and the value for medium concentration is always higher than low. • The metric provided is the value of high/medium and the value of medium/low. • The default thresholds are 1. Do not change the default threshold.

Control	Description
Target Removal	• Target removal controls test the efficiency of the stripping step after the extension reaction. In contrast to allele-specific extension, the control oligos are extended using the probe sequence as a template. This process generates labeled targets. The probe sequences are designed such that extension from the probe does not occur. All target removal controls result in low signal compared to the hybridization controls, indicating that the targets were removed efficiently after extension. Target removal controls are present in the Hybridization Buffer RA1. • The Background for the same sample is close to or larger than either control. • The metric provided is ***Background/Control Intensity***. • The default threshold is 1. Do not change the default threshold; however, the offset correction can be changed.

Bisulfite Conversion I	• These controls assess the efficiency of bisulfite conversion of the genomic DNA. The Infinium Methylation probes query a [C/T] polymorphism created by bisulfite conversion of non-CpG cytosines in the genome.
	• These controls use Infinium I probe design and allele-specific single base extension to monitor efficiency of bisulfite conversion. If the bisulfite conversion reaction was successful, the "C" (Converted) probes matches the converted sequence and get extended. If the sample has unconverted DNA, the "U" (Unconverted) probes get extended. There are no underlying C bases in the primer landing sites, except for the query site itself.
	• The calculation is done in both the green and red channels separately to provide 2 unique sets of values:
	  • Green Channel
	  • ***Lowest value of C1, C2, or C3 / Highest value of U1, 2, or 3***. The default threshold is 1. This value can be increased for some scanners.
	  • ***Background/(U1, U2, or U3)***. The default threshold is 1. Do not change the default threshold; however, the offset correction can be changed.
	  • Red Channel
	  • ***Lowest value of C4,5, or 6 / Highest value of U4, 5, or 6***. The default threshold is 1. This value can be increased for some scanners.
	  • ***Background /(Highest value of U4, U5, or U6)***. The default threshold is 1. Do not change the default threshold; however, the offset correction can be changed.

Control	Description

Bisulfite Conversion II	• These controls assess the efficiency of bisulfite conversion of the genomic DNA. The Infinium Methylation probes query a [C/T] polymorphism created by bisulfite conversion of non-CpG cytosines in the genome.
	• These controls use Infinium II probe design and single base extension to monitor efficiency of bisulfite conversion. If the bisulfite conversion reaction was successful, the "A" base gets incorporated and the probe has intensity in the red channel. If the sample has unconverted DNA, the "G" base gets incorporated across the unconverted cytosine, and the probe has elevated signal in the green channel.
	• The calculation is done using both channels for 1 set of numbers returned.
	• The following metrics are provided:
	  • ***(Lowest of red C 1, 2, 3, or 4) / (Highest of green C 1, 2, 3, or 4)***. The default threshold is 1. This value can be increased for some scanners.
	  • ***Background/(Highest C1, C2, C3, or C4 green)***. The default threshold is 1. Do not change the default threshold; however, the offset correction can be changed.

Specificity I	• Specificity controls are designed to monitor potential nonspecific primer extension for Infinium I and Infinium II assay probes. Specificity controls are designed against nonpolymorphic T sites. • These controls are designed to monitor allele-specific extension for Infinium I probes. The methylation status of a particular cytosine is carried out following bisulfite treatment of DNA by using query probes for unmethylated and methylated state of each CpG locus. In assay oligo design, the A/T match corresponds to the unmethylated status of the interrogated C, and G/C match corresponds to the methylated status of C. G/T mismatch controls check for nonspecific detection of methylation signal over unmethylated background. PM controls correspond to A/T perfect match and give high signal. MM controls correspond to G/T mismatch and give low signal. • The metrics provided are the ratio of the ***lowest PM/highest MM*** in each channel. • The default threshold is 1. Do not change the default threshold.

Control	Description

Specificity II	• Specificity controls are designed to monitor potential nonspecific primer extension for Infinium I and Infinium II assay probes. Specificity controls are designed against nonpolymorphic T sites.
	• These controls are designed to monitor extension specificity for Infinium II probes and check for potential nonspecific detection of methylation signal over unmethylated background. Specificity II probes incorporate the "A" base across the nonpolymorphic T and have intensity in the Red channel. If there was nonspecific incorporation of the "G" base, the probe has elevated signal in the Green channel.
	• The following metrics are provided:
	  • ***(Lowest intensity of S1, S2, or S3 red) / (Highest intensity of S1, S2, or S3 green)***. The default threshold is 1. Do not change the default threshold.
	  • ***Background/(Highest intensity S1, S2, S3, or S4 green)***. The default threshold is 1. Do not change the default threshold; however, the offset correction can be changed.

Nonpolymorphic	• Nonpolymorphic controls test the overall performance of the assay, from amplification to detection, by querying a particular base in a nonpolymorphic region of the genome. They let you compare assay performance across different samples. One nonpolymorphic control has been designed for each of the 4 nucleotides (A, T, C, and G). • In the green channel, the lowest intensity of C or G is always greater than the highest intensity of A or T. • The metric provided is the ***(lowest intensity for C or G) /(highest intensity for A or T)*** for a single sample. • The default threshold is 5. This value can be increased for some scanners.

BeadArray Controls Reporter Calculations

x = Background correction offset. The default value of 3000 can be modified and applies to all background calculations, indicated with (bkg +x).

Control	Calculation	Additional Information
Restoration Green > bkg	(Green/(bkg+x)) > 0*	*If using the FFPE Restore kit, change the default threshold from 0 to 1. • bkg = Extension Green highest A or T intensity

Control	Calculation	Additional Information
Staining Green Biotin High > Biotin Bkg	(High/Biotin Bkg) > 5*	*You can increase this threshold. • Green channel—Lowest C or G intensity is used; highest A or T intensity is used. • Red channel—Lowest A or T intensity is used; highest C or G intensity is used.
Staining Red DNP High > DNP Bkg	(High/DNP Bkg) > 5*	
Extension Green Lowest CG/Highest AT	(C or G/A or T) > 5*	
Extension Red Lowest AT/Highest CG	(C or G/A or T) > 5*	
Hybridization Green High > Medium > Low	(High/Med) > 1	• bkg = Extension Green highest A or T intensity
	(Med/Low) > 1	
Target Removal Green ctrl 1 ≤ bkg	((bkg + x)/ctrl) > 1	
Target Removal Green ctrl 2 ≤ bkg	((bkg + x)/ctrl) > 1	
Bisulfite Conversion I Green C1, 2, 3 > U1, 2, 3	(C/U) > 1*	*You can increase this threshold. • Lowest C intensity is used. Highest U intensity is used. • Green channel—bkg = Extension Green highest AT • Red Channel—bkg = Extension Red highest CG
Bisulfite Conversion I Green U ≤ bkg	((bkg + x)/U) > 1	
Bisulfite Conversion I Red C4, 5, 6 > U4, 5, 6	(C/U) > 1*	
Bisulfite Conversion I Red U ≤ bkg	((bkg + x)/U) > 1	
Bisulfite Conversion II C Red > C Green	(C Red/ C Green) > 1*	
BiSulfite Conversion II C green ≤ bkg	((bkg + x)/C Green) > 1	
Specificity I Green PM > MM	(PM/MM) > 1	• Lowest PM intensity is used • Highest MM intensity is used • bkg = Extension Green highest A or T intensity
Specificity I Red PM > MM	(PM/MM) > 1	
Specificity II S Red > S Green	(S Red/ S Green) > 1	
Specificity II S Green ≤ bkg	((bkg + x)/ S green) > 1	

Control	Calculation	Additional Information
Nonpolymorphic Green Lowest CG/ Highest AT	(A or T/ C or G) > 5*	*You can increase this threshold. • Green channel—Lowest C or G intensity is used; highest A or T intensity is used • Red channel—Lowest A or T intensity is used; highest C or G intensity is used
Nonpolymorphic Red Lowest AT/ Highest CG	(C or G/ A or T) > 5*	

Modifying Threshold Values

1 In the Options menu, click Set **Thresholds**.

2 Modify the threshold values as needed.

3 Click **OK**.
The changed values are saved each time you open the software.

Sample Sheet Specifications

The sample sheet is a *.csv file that identifies the name, chip, and location of each sample in a project. For an example, see the Infinium HD Methylation Sample Sheet.

- Make sure that the format of the sample matches the template, including the complete header section, the data section, and all column names.
- If the sample sheet is not recognized, rename the sample sheet with a simple name, such as Sample_Sheet.csv.
- Make sure that the Sentrix_ID and Sentrix_Position are provided.
- Make sure that the ID and Position match the IDAT files used in analysis.
- The Sample Name is optional, and the remaining columns only appear in GenomeStudio.
- List only samples that the IDAT files in the parent folder. Do not list any other samples.

Figure 3 Example of Sample Sheet

	A	B	C	D	E	F	G	H	I
1	[Header]								
2	Investigat	Scientist		*Column A and B are required					
3	Project Na	DNA Methylation		*column C is optional					
4	Experimer	Test		*Column D-G are optional and only appear in GenomeStudio					
5	Date	8-Dec-10							
6									
7	[Data]								
8	Sentrix_ID	Sentrix_P	Sample_N	Sample_W	Sample_P	Sample_G	Pool_ID		
9	2.12E+11	R01C01	NA17105-M_Rep1			NA17105-M			
10	2.12E+11	R01C02	A431_Rep1			A431			
11	2.12E+11	R01C03	NA17018-F_Rep1			NA17018-F			
12	2.12E+11	R01C04	MCF7_Rep1			MCF7			
13	2.12E+11	R01C05	Raji			Raji			
14	2.12E+11	R01C06	Hemi-methylated			Hemi-methylated			
15	2.12E+11	R01C07	Unmethylated			Unmethylated			
16	2.12E+11	R01C08	Methylated			Methylated			
17	2.12E+11	R01C01	NA17105-M_Rep2			NA17105-M			
18	2.12E+11	R01C02	A431_Rep2			A431			
19	2.12E+11	R01C03	NA17018-F_Rep2			NA17018-F			
20	2.12E+11	R01C04	MCF7_Rep2			MCF7			

FAQs and Troubleshooting

FAQ/Issue	Troubleshooting
The software did not generate an output file or cannot find the output file.	If the software cannot match the files in the sample sheet with the IDAT files, the software cannot generate an Excel file output. Make sure that the following requirements are met: • The parent folder contains IDAT files (including the *.sdf files), the *.bpm manifest file, and a sample sheet *.csv file • The sample sheet contains a line for each sample in the IDAT folder and for no additional samples • Files names only contain letters, numbers, and underscores and do not contain spaces, dashes, or alternate characters.
The output files shows NaN or Infinity.	NaN or Infinity indicates that no number or an impossible number was formed during the threshold calculation. Reanalyze with the GenomeStudio Methylation Module Controls Dashboard.
I cannot view all the threshold parameters.	Some screen resolutions only show a portion of the threshold parameters in the Options—Set Thresholds menu. With your mouse, grab the dotted triangle in the lower-right portion of the Set Thresholds screen to enlarge the viewing window until you see all parameters.
Where do I get a *.bpm manifest?	The *.bpm manifest is specific to the array. For a fixed content array, see the downloads section of the Illumina support page associated with the product. For custom products, the *.bpm manifest is available on the MyIllumina account of the individual who ordered the array.
I do not have Microsoft Excel. Can I still use the software?	Yes. Illumina has not thoroughly tested alternative spreadsheet software, but general freeware such as Google Sheets can open the output file.
Is BeadArray Controls Reporter compatible with my Mac running OSX?	Not currently.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

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**.