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VeraCode® ADME Core Panel Assay Guide

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Revision History

Part #	Revision	Date	Description of Change
15007510	C	June 2011	• Revised Titanium Taq DNA Polymerase part and lot numbers
15007510	B	March 2011	• Changed VW2 buffer bottle volume to 52 ml in the 32 sample kit • Revised control DNA suggested vendor • Specified Titanium Taq DNA Polymerase lot number • Incorporated instructions for two plate processing • Replaced incubating microplate shaker manual mode instructions with program mode instructions • Added PSC controls to the plate controls report • Changed “PSC Control” to “process control” • Added troubleshooting workflow diagram • Added Appendix C - Microplate Shaker • Added process control and sample tracking control error troubleshooting • Added Best Practices • Added Index
15007510	A	March 2010	Initial Release

Revision History

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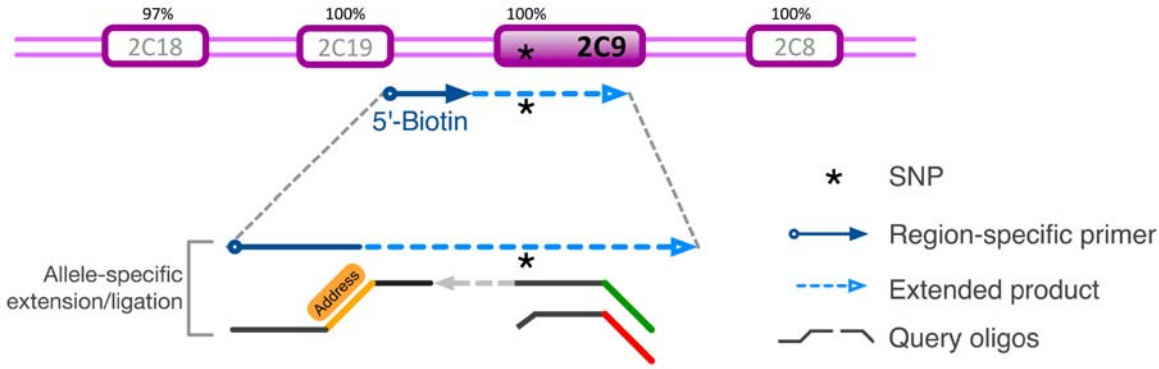
Overview

Chapter 1

Introduction

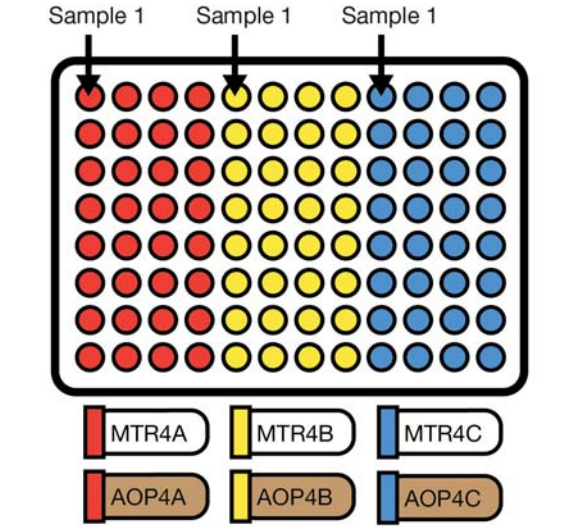
The Illumina® VeraCode®ADME Core Panel on the BeadXpress®System genotypes genetic variations in a human genomic DNA (gDNA) sample. This assay interrogates genes associated with drug absorption, distribution, metabolism and excretion (ADME) using allele-specific extension and ligation followed by PCR with fluorescently labeled primers. For each locus, there is a biotinylated oligonucleotide that copies a specific genomic region. Genotype determination is then accomplished by performing the allele-specific extension and ligation on the copied region.

Figure 1 Approach to Genotyping Variants in Homologous Genomic Regions



The content of the VeraCode ADME Core Panel is assayed in three highly optimized reaction subpools across a 96-well plate enabling 32 samples per bead plate. A targeting mix (MTR) and assay oligo annealing reagent (AOP) are added to each sample. Color-coded tube caps distinguish these reagents for each of the three subpools.

Figure 2 ADME Assay Plate



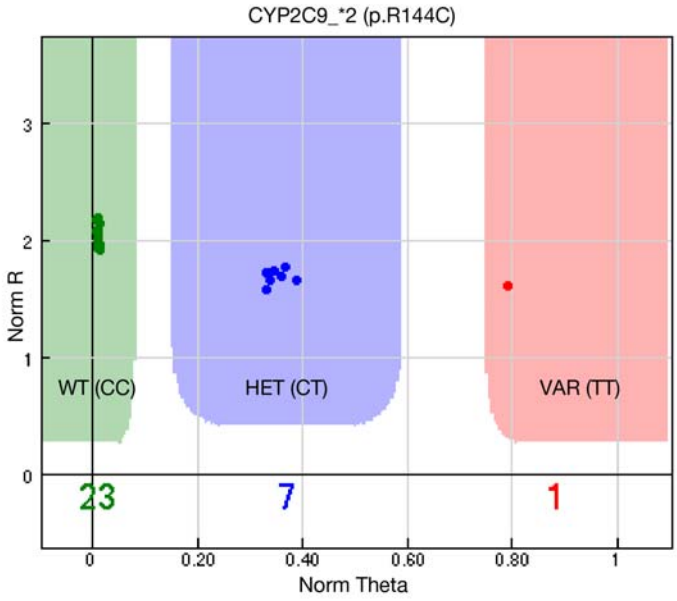
MTR = Subpool specific Targeting Mix
AOP = Subpool specific Assay Oligo Annealing Reagent

The VeraCode bead types used in this assay and the genotyping results are described in the ADME Beadtypes and Translations document available on the Illumina website (http://www.illumina.com/icom).

Genotyping

There are several homologous genes involved with drug ADME included in this panel. For example, by using the specific locus targeting of the VeraCode ADME Core Panel assay (see Figure 1), CYP2C9*2 can be genotyped accurately without interference from its close neighbors CYP2C19, CYP2C8, and CYP2C18.

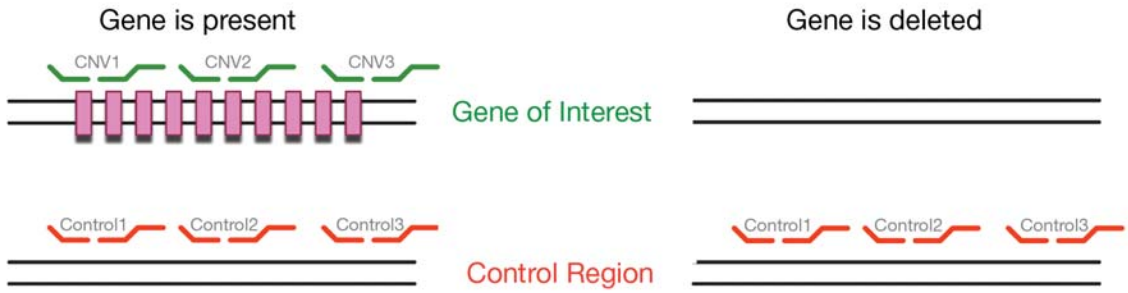
Figure 3 Genotyping Example for A Challenging ADME Core Variant



Copy Number Variation

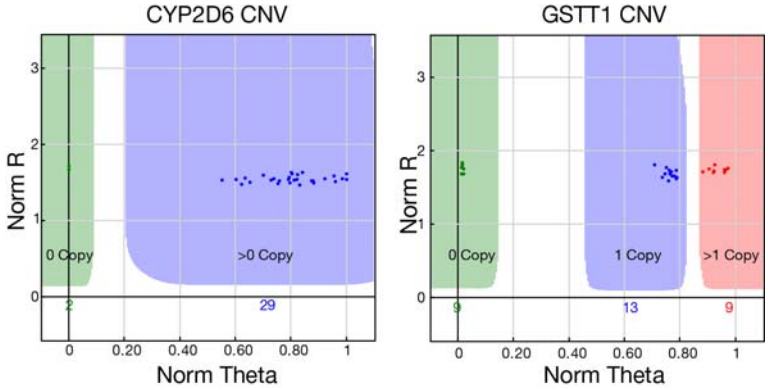
The VeraCode ADME Core Panel contains assay designs which detect copy number variation (CNV) in six genes: CYP2A6, CYP2D6, GSTM1, GSTT1, SULT1A, and UGT2B17. Oligonucleotide probes are designed to target non-polymorphic regions in the gene with CNV and a control region elsewhere in the genome (e.g., CNV1 and Control1).

Figure 4 Multiple Probe Sets for Enhanced Precision of CNV Assays



The signals resulting from the CNV and control probes are detected on the same VeraCode bead type, but in different channels (red and green). As a result, the red to green ratio (theta) represents the copy number of the gene of interest relative to the copy number (two per genome) of the control region. This allows the CNV assay to be internally controlled for performance and visualized on a genoplot. For improved precision, multiple probe sets are used for each gene and the individual results are aggregated for a single graphic output. Examples of CYP2D6 and GSTT1 CNV assay results are shown below.

Figure 5 Examples of CNV Assay Results



Controls

The VeraCode ADME Core Panel includes multiple types of internal controls in each sample, outlined below.

Table 1 VeraCode ADME Core Panel Internal Control Types

Probe ID	Category	Expected Result
STC	Sample Tracking Control	Pass = Internal sample barcode (e.g., A9EBF9F) can be verified for each of the three subpools
PSC	Process Control	Pass = No mix-up of subpool specific reagents is detected. All ADME process steps completed successfully.
SPC	Subpool Position Control	Pass = Samples are run in designated sections A,B, and C of the assay plate
Hyb Control 1	Hybridization Control	Pass = Successful hybridization to VeraCode beads
Hyb Control 2	Hybridization Control	
Mismatch Control 1	Assay Control	Pass = Successful allele-specific extension and ligation
Mismatch Control 2	Assay Control	

Sample Tracking Control (STC)

A panel of high minor allele frequency SNPs, included in each assay pool, provide added sample traceability when translated to a unique barcode for each sample. The control is used to identify sample pipetting errors, cross contamination during sample processing, and DNA quality issues in a run.

Process Control (PSC)

A set of internal negative controls identify proper pairing of targeting oligo pools (MTRs) with their corresponding assay oligo annealing reagent (AOPs) as depicted in Figure 2. The same control bead for each possible MTR/AOP mis-pairing exists in each of the subpools. All results for this control should give background signals as long as all MTRs and AOPs are appropriately matched. If the wrong MTR and AOP are matched this control gives off a high signal alerting VeraScan data analysis and the user that a processing error occurred.

This control can also give elevated signal if there are deviations for critical steps in the protocol. Intensity data shifts seen in the PSC controls are representative of shifts seen in assay genoplots. Therefore, no data is generated for samples that fail this control.

Subpool Position Control (SPC)

This control works alongside the PSC to ensure that correctly paired oligo reagents were pipetted to the correct subpool location on the plate. This control specifically tests for the AOP being analyzed in each subpool. There are three control bead types: one each for pool A, B, and C. Each bead type is in all three subpools. When the assay is run correctly, VeraScan expects to see a high signal for the bead type corresponding to the specific AOP for that subpool (i.e., high signal for AOPA in subpool A). The other two bead types for the other AOPs should have signal close to background (i.e., low signal for AOPB and AOPC in subpool A). This analysis is done by the VeraScan software ADME module and only a Pass is reported in the output data for a successful run.

Hybridization Controls

There are two hybridization control bead types, which report efficient hybridization of fluorescently labeled products to VeraCode beads. The hybridization controls test the hybridization of single-stranded assay products to address sequences specific to VeraCode beads. The address sequence corresponding to the Cy3 labeled probe should result in a green signal and the sequence corresponding to the Cy5 labeled probe should result in a red signal.

Figure 6 Hybridization Controls

Mismatch Controls

Two mismatch controls (one for the green channel and the other for the red channel) are included to verify allele-specific extension and ligation. Similar to the oligonucleotide designs for genotyping the ADME Core variants, each of the two mismatch controls has two upstream oligos targeting a non-polymorphic region in the genome. Only one of the two oligonucleotides has a 3' end base match. The other oligonucleotide has a 3' end base mismatch which, under normal circumstances, cannot be extended and ligated to the downstream oligonucleotide. In addition, the mismatch controls help verify the functionality of assay reagents and the balance of BeadXpress Reader.

Figure 7 Mismatch Control Green

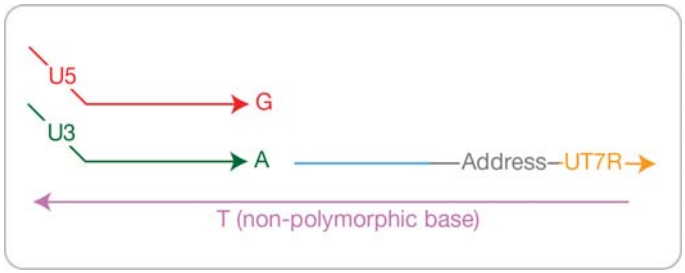
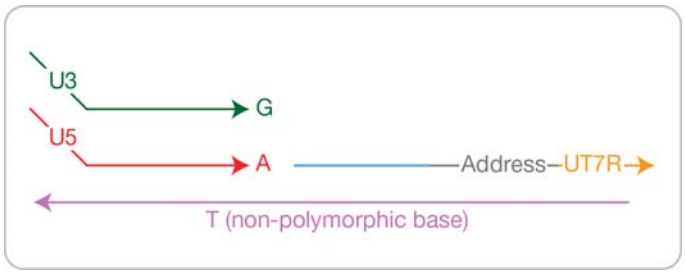


Figure 8 Mismatch Control Red



Control Samples

Good laboratory practices recommend that positive control DNA samples and negative (no-template) control samples are included in every run. See the *Wells Section* on page 21 of *Create Sample Sheet* for information on how to properly mark samples as positive and negative controls in the VeraScan Software.

ADME Core Panel Kit Contents

Check to ensure that you have all of the reagents and materials identified in this section before proceeding with the ADME Core Panel protocol. Depending on the number of samples being prepared, you need one or more of the VeraCode ADME Core Genotyping Kits in which are shipped on dry ice.

Table 2 VeraCode ADME Core Genotyping Kits

Number of Samples	Illumina Catalog #
32	VC-901-0201
160	VC-901-0200

Kit Contents, Box A

As soon as you receive this box, store the components in the freezer (-15° to -25°C) in the pre-PCR lab area, as indicated on the container and in the following table.

Table 3 Kit Contents, Box A

Item	Label Color	Number Supplied 32 Sample Kit	Number Supplied 160 Sample Kit	Volume	Storage Temperature
AB1 reagent	Purple label	1 tube	5 tubes	4.0 ml	-15° to -25°C
UB3 buffer	Orange label	2 tubes	10 tubes	4.8 ml	-15° to -25°C
AE1 reagent	Yellow label	2 tubes	10 tubes	4.8 ml	-15° to -25°C
ELM2 reagent	Navy blue label	1 tube	5 tubes	4.8 ml	-15° to -25°C
MAM1 reagent	Green label	1 tube	5 tubes	4.8 ml	-15° to -25°C
AOP0 reagent	Red label	1 tube	5 tubes	3.6 ml	-15° to -25°C

Kit Contents, Box B

As soon as you receive this box, store the components in the pre-PCR lab area at the storage temperature indicated on the container and in the following table.

Table 4 Kit Contents, Box B

Item	Tube/Cap Color	Number Supplied 32 Sample Kit	Number Supplied 160 Sample Kit	Volume	Storage Temperature
MTR4A reagent	Clear tube/Red cap	1 tube	5 tubes	0.6 ml	-15° to -25°C
MTR4B reagent	Clear tube/Yellow cap	1 tube	5 tubes	0.6 ml	-15° to -25°C
MTR4C reagent	Clear tube/Blue cap	1 tube	5 tubes	0.6 ml	-15° to -25°C
AOP4A reagent	Amber tube/Red cap	1 tube	5 tubes	1.8 ml	-15° to -25°C
AOP4B reagent	Amber tube/Yellow cap	1 tube	5 tubes	1.8 ml	-15° to -25°C
AOP4C reagent	Amber tube/Blue cap	1 tube	5 tubes	1.8 ml	-15° to -25°C
Pool Guide Label	–	2 labels	6 labels	–	Room Temperature

Kit Contents, Box C

As soon as you receive this box, store the components in the post-PCR lab area at the storage temperature indicated on the container and in the following table.

Table 5 Kit Contents, Box C

Item	Label Color	Number x Volume Supplied 32 Sample Kit	Number x Volume Supplied 160 Sample Kit	Storage Temperature
MSS reagent	Lavender label	1 tube x 4.8 ml	5 tubes x 4.8 ml	-15° to -25°C
VW2 buffer	Sea foam green label	1 bottle x 52 ml	1 bottle x 250 ml	Room temperature

Kit Contents, Box D

As soon as you receive this box, store the components in the post-PCR lab area at 2° to 8°C as indicated on the container and in the following table.

Table 6 Kit Contents, Box D

Item	Number Supplied 32 Sample Kit	Number Supplied 160 Sample Kit	Storage Temperature
VeraCode ADME Bead Plate	1 plate	5 plates	2° to 8°C

User-Supplied Materials

Check to ensure that you have all of the following materials in the appropriate lab area (pre- and/or post-PCR) before proceeding with the ADME Core Panel protocol.

NOTE

When processing two plates simultaneously, two magnetic plates are required and the incubating microplate shaker must be able to accommodate two plates.

NOTE

Do not use electronic or repeat pipettes when performing the ADME Core Panel assay.

Table 7 User-Supplied Materials

Item	Suggested Vendor	Pre-PCR	Post-PCR
0.1N NaOH solution	General lab supplier	X	
10% (w/v) Potassium Hydroxide solution	General lab supplier		X
20 µl multichannel pipettes	General lab supplier	X	
20 µl single channel pipettes	General lab supplier	X	
20 µl pipette tips	General lab supplier	X	
96-well skirted PCR microplate	General lab supplier	X	
200 µl multichannel pipettes	General lab supplier	X	X
200 µl single channel pipettes	General lab supplier	X	X
200 µl pipette tips	General lab supplier	X	X
1,000 µl single channel pipettes	General lab supplier	X	
1,000 µl pipette tips	General lab supplier	X	

Table 7 User-Supplied Materials (Continued)

Item	Suggested Vendor	Pre-PCR	Post-PCR
Adhesive microplate sealing film	Beckman Coulter, catalog # 538619 or Applied Biosystems, catalog # 4306311	X	
Adhesive seal applicators (5 per package)	MicroAmp, catalog # 4333183	X	
Benchtop centrifuge for microplates	General lab supplier	X	X
Control DNA [Optional]	Coriell Institute (www.coriell.org)	X	
Heat block for skirted PCR microplates (Note: If processing two plates simultaneously, two heat blocks are required.)	General lab supplier	X	
Incubating microplate shaker (shaking speed 1,400 rpm, temperature range 45° to 68°C, cooling rate (above ambient): 3.5°C per minute) (Note: This shaker can hold up to two skirted plates.)	VWR, catalog # 97027-346	X	
Incubating microplate shaker power cord (Note: Select one and only if applicable)	VWR, catalog #: VWRI444-2853 (Europe) VWRI444-2854 (United Kingdom) VWRI444-2855 (Switzerland)	X	
Kimwipes	General lab supplier	X	X
Magnetic plate (Note: If processing two plates simultaneously, two magnetic plates are required in both the pre-PCR and post-PCR labs.)	Dynal, catalog # MPC-96	X	X
PCR sealing film	BioRad, catalog # MSA-5001	X	
Reagent alcohol	General lab supplier		X

Table 7 User-Supplied Materials (Continued)

Item	Suggested Vendor	Pre-PCR	Post-PCR
Reagent reservoirs	General lab supplier	X	X
Shaking incubator (with shaking speed 850–1,200 rpm, temperature 45°C)	LabNet, catalog # VorTemp 56		X
Tachometer/stroboscope, combination optical [Optional]	Cole-Parmer, catalog # A-87700-06 www.coleparmer.com	X	
Thermocycler compatible with skirted PCR microplates	General lab supplier		X
Titanium Taq DNA Polymerase (5 U/µl)	Clontech, catalog # 639293	X	
Vacuum flask assembly with regulator	QIAGEN, catalog # 19530 or equivalent		X
Vacuum manifold	V&P Scientific, catalog # VP180I		X
VeraCode Test & Calibration Bead Plate	Illumina, catalog # VC-321-1000		X
VR1 buffer (10x Reader Buffer)	Illumina, catalog # VC-400-1001		X

DNA Input Requirements

The VeraCode ADME Core Panel requires extracted genomic DNA from EDTA-anticoagulated whole blood. Commercially available or laboratory validated DNA extraction methods typically yield DNA that is compatible with this test. Extracted DNA purity should range from an A260/A280 ratio of 1.8–2.0. 15 µl of DNA at 50 ng/µl is required for this assay (5 µl of DNA per sample for each of the three assay pools) for a total input DNA quantity of 750 ng per sample.

Optimal assay performance is dependent on using the recommended concentrations and volumes. It is also important to ensure that equal volumes and concentrations of DNA are used in each of the three reaction pools for a given sample.

Safety Precautions

CAUTION

Ensure that the user supplied equipment is properly calibrated. Out of tolerance equipment can negatively impact assay performance.

CAUTION

Please refer to the governmental and facility safety standards applicable to your site.

CAUTION

To minimize aerosols when disposing of the VeraCode Bead Plates, refer to your state waste disposal requirements.

Lab Protocols

Chapter 2

Introduction

This protocol describes how to process the Illumina VeraCode ADME Core Panel on the BeadXpress System. The instructions assume that you have already familiarized yourself with Appendix A, *Standard Operating Procedures* and have set up the lab area appropriately (reference *Control Samples* on page 8 and *ADME Core Panel Kit Contents* on page 9).

CAUTION

It is very important to prevent the contamination of the laboratory with polymerase chain reaction (PCR) product during this assay. To learn about safe lab practices for Illumina assays, see Preventing PCR Product Contamination on page 101. In addition, follow all of the safety procedures described in this document.

NOTE

Calibrate the pre-PCR incubating microplate shaker according to the manufacturer's instruction. The calibration tolerance (allowable deviation from nominal), plus the uniformity tolerance (provided by the manufacturer), plus the error associated with the measurement equipment should not exceed +/- 0.5°C. The shaking speed of the pre-PCR shaker should also be calibrated so that the actual speed of the shaker matches the ADME Core Panel protocol requirement of 1,400 rpm. See Appendix C, *Qualification*.

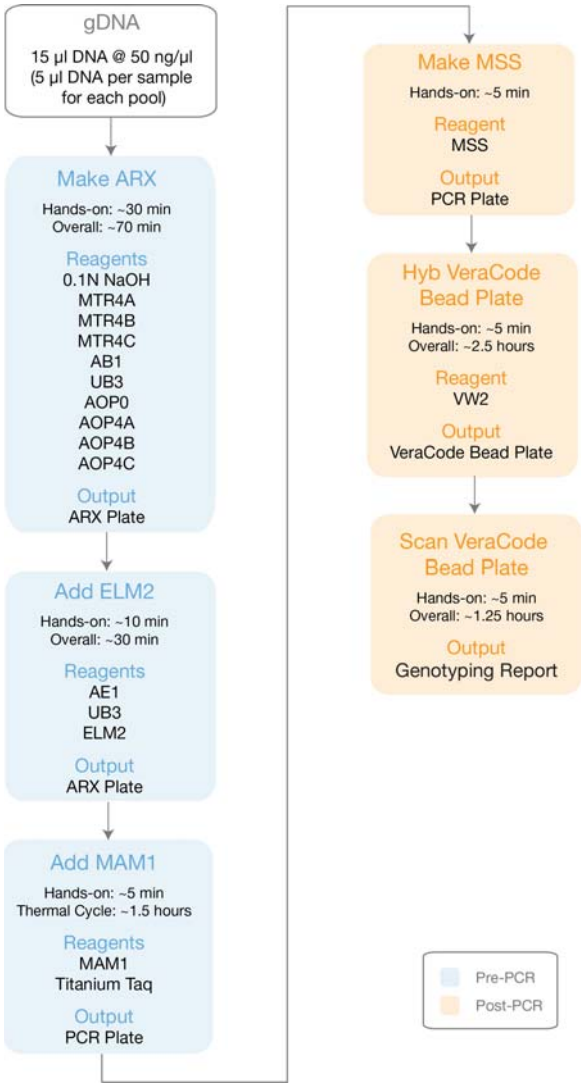
NOTE

Calibrate the post-PCR incubating microplate shaker according to the manufacturer's instruction. The calibration tolerance (allowable deviation from nominal) plus the error associated with the measurement equipment should not exceed +/- 0.5°C. See Appendix C, *Qualify Temperature*.

Workflow

The following graphically represents the VeraCode ADME Core Panel Assay which must be performed in the order shown.

Figure 9 VeraCode ADME Core Panel Workflow



Create Sample Sheet

Before starting the VeraCode ADME Core Panel assay, create a sample sheet. A sample sheet is a comma-separated values (*.csv) file that contains the sample name and related information that describes the location of each sample in the assay reaction plate.

The sample sheet template for the VeraCode ADME Core Panel is located on your BeadXpress Reader at C:\Documents and Settings\All Users\Documents\Illumina\VeraScan\ScanSettings. Fill in your sample sheet according to the guidelines provided in this section.

Figure 10 Example: Sample Sheet

	A	B	C	D	E	F
1	[Header]					
2	Title	CK008H007-VBP				
3	Comments	CK008H007-VBP				
4	KitPN	15011035				
5						
6	[Wells]					
7	Column	Row	Kit_Number	Sample	Comments	Control
8	1	A	ADMECore	NA10842		
9	2	A	ADMECore	NA17120		
10	3	A	ADMECore	NA18978		
11	4	A	ADMECore	NA17246		
12	1	B	ADMECore	NA10843		
13	2	B	ADMECore	NA17127		
14	3	B	ADMECore	NA18971		
15	4	B	ADMECore	NA17280		
16	1	C	ADMECore	NA10844		
17	2	C	ADMECore	NA17129		
18	3	C	ADMECore	NA12006		
19	4	C	ADMECore	NA12003		

ADMECore_samplesheet_all

Sample Sheet Sections

The Sample Sheet is separated into sections that have different uses. These are the Header and the Wells sections. All fields are required except Comments and Control.

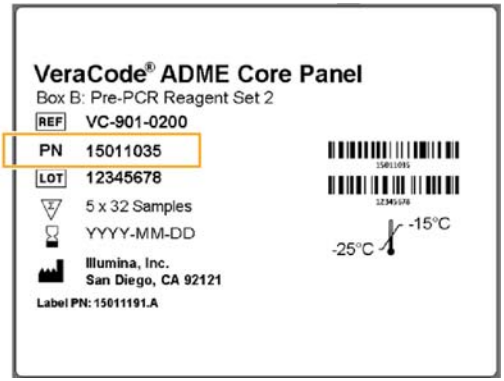
Header section

The Header section contains a title for this run, and room for comments on this run. This information appears in the genotyping report.

Table 8 Header Section

Header Field	Description
Title	The plate ID used to name the data files and folders
Comments	[Optional] Comments for this run (e.g., laboratory name, technician)
KitPN	You must enter the kit part number (PN) printed on Box B of the ADME kit.

Figure 11 Box B Kit Part Number Location



Wells Section

Each well in the plate that contains a sample is represented as a row in the Sample Sheet. In addition to the sample name, other sample information can be included. The VeraScan software identifies samples you have included as positive or negative controls in the genotyping report. The genotyping report also includes any comments you wish to make. This table has the following columns:

Table 9 Wells Section

Column Header	Description
Column	The plate column number (1–4). The sample information for columns 5–12 are automatically completed by VeraScan for the duplicate samples for the 3 assay pools on the plate.
Row	The letter representing the row number (A–H)
Kit_Number	**ADMECore** (this text must not be changed)
Sample	The name of the sample
Control	[Optional] Whether or not the sample is identified as a control. You must enter **Positive** or **Negative**: Positive - sample is a positive control, indicated by **P** following the sample name in the results report Negative - sample is a negative control, indicated by **N** following the sample name in the results report, and are not plotted in graphs and do not contribute to call rate calculations
Comments	[Optional] Comments about the sample

CAUTION

To avoid misidentifying samples, ensure that the sample names entered in the sample sheet correctly correspond to the DNA samples used.

Lab Tracking Form

A Lab Tracking Form (LTF) may be used to track your progress in the protocol and to ensure that all of the protocol steps of the VeraCode ADME Core Panel have been completed. You can fill out the form electronically and save a copy under a new name, or print it and fill it out by hand. Use a new LTF for each assay.

The LTF can be downloaded via http://www.illumina.com/support/documentation.ilmn.

NOTE

The LTF should be used while referencing the protocol in this guide which is more inclusive and detailed. The LTF is intended for use as a tracking tool and not a replacement for the user guide.

Make Assay Reaction (ARX)

In this pre-PCR process, the gDNA and reagents are added to a PCR microplate to create the assay reaction (ARX) plate in which the assay is performed. The gDNA is denatured, copied, captured on paramagnetic beads, and washed. The selected DNA sequences are then annealed to oligonucleotides.

Begin the assay in the pre-PCR laboratory. Check to ensure that you have all of the consumables identified in this section before proceeding with the assay protocol.

NOTE

Processing two plates simultaneously requires appropriate preparation of consumables and equipment. Therefore, it is important to plan ahead before proceeding with the assay protocol to avoid unintentional delays.

Estimated Time

Hands-on: ~30 minutes

Overall: ~70 minutes

Consumables

Item	Label/Tube/ Cap Color	Quantity	Storage	Supplied By
gDNA	–	15 µl (50 ng/µl)	-15° to -25°C	User
0.1N NaOH solution	–	2 ml per plate	Room temperature	User
MTR4A reagent	Clear tube/Red cap	1 tube per plate	-15° to -25°C	Illumina
MTR4B reagent	Clear tube/Yellow cap	1 tube per plate	-15° to -25°C	Illumina
MTR4C reagent	Clear tube/Blue cap	1 tube per plate	-15° to -25°C	Illumina
AB1 reagent	Purple label	1 tube per plate	-15° to -25°C	Illumina
UB3 buffer	Orange label	1 tube per plate	-15° to -25°C	Illumina

Item	Label/Tube/ Cap Color	Quantity	Storage	Supplied By
AOP0 reagent	Red label	1 tube per plate	-15° to -25°C	Illumina
AOP4A reagent	Amber tube/Red cap	1 tube per plate	-15° to -25°C	Illumina
AOP4B reagent	Amber tube/Yellow cap	1 tube per plate	-15° to -25°C	Illumina
AOP4C reagent	Amber tube/Blue cap	1 tube per plate	-15° to -25°C	Illumina
96-well skirted PCR microplate	–	1	Room temperature	User
Adhesive microplate sealing film	–	5 per plate	Room temperature	User
Pool Guide Label	–	1 label per plate	Room temperature	Illumina

NOTE

The 0.1N NaOH solution must not be more than two weeks old.

Preparation

- Thaw all of the kit reagents on the benchtop.
- Vortex each reagent for 5 seconds immediately before using the reagent.
- To avoid switching pipette settings back and forth, use a designated pipette set at 60 µl to remove the supernatant and use an additional pipette for adding reagents.

NOTE

Do not use electronic or repeat pipettes when performing the ADME Core Panel assay.

- Set the pre-PCR centrifuge to 15° to 25°C, if refrigerated.
- Turn on the heat block and let it equilibrate to 95°C.

- Turn on the pre-PCR incubating microplate shaker. The display should show Pr2 step 01. Press **start** to initiate preheating the shaker to 68°C. The shaker will beep when the temperature is stabilized and the display will show step 02 settings.

NOTE

When the incubating microplate shaker is turned off with Pr2 selected, the next time it is turned on it will display the Pr2 Step 1.

- Read *Pipetting and Sealing* on page 90 for recommended reagent pipetting and adhesive seal application techniques for the VeraCode ADME Core Panel Assay.

CAUTION

Program 1 (Pr1) on the incubating microplate shaker is not setup to run ADME incubating conditions. Do not use this program when running the ADME assay.

CAUTION

The VWR incubating/cooling shaker must be qualified and programmed for automated use and to avoid potential ADME Core Panel protocol errors. See Appendix C, *Microplate Shaker* before proceeding with the protocol.

NOTE

During the pre-PCR portion of the assay, all steps on the incubating shaker are performed at 1,400 rpm, which is also the incubating shaker's default setting.

NOTE

If you are only processing 1 plate, place a balance on the empty plate position on the incubating shaker.

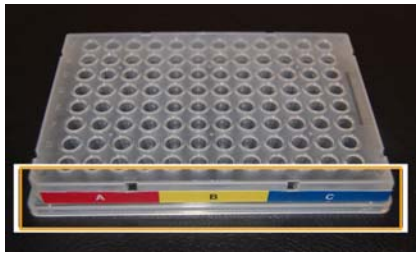
NOTE

Proceed diligently through each step of the protocol. Do not allow the plates to remain at any one step longer than is necessary for standard assay processing. When processing two plates simultaneously, Illumina recommends that you are experienced with the protocol, reagents, and time required for each step.

Steps

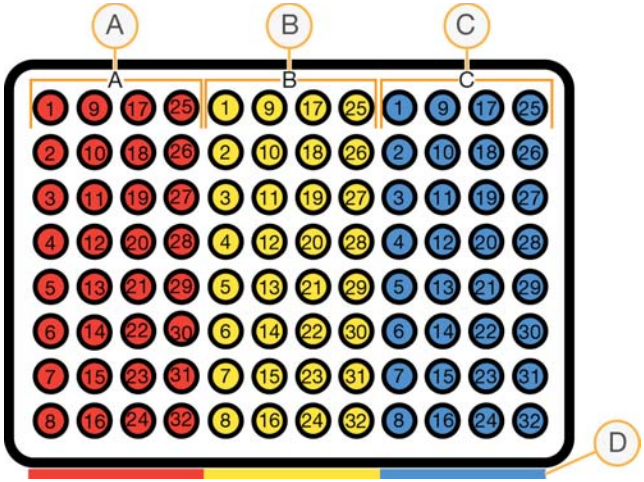
1 Attach the pool guide label to the long side of the PCR microplate.

Figure 12 Pool Guide Label



2 Label the sample sections A, B, and C on the PCR microplate with a smudge resistant pen.

Figure 13 PCR Microplate



A Sample section A
B Sample section B
C Sample section C
D Pool guide label

3 Add DNA to ARX plate.

NOTE
When processing two plates simultaneously, add the gDNA to both plates before proceeding to step 4.

a For each DNA sample to be processed, add 5 µl gDNA (at 50 ng/µl) to the bottom of each of the 3 designated wells of the PCR microplate.

b After dispensing, inspect the tips and bottom of the PCR microplate to make sure the gDNA samples have been properly dispensed into the PCR microplate.

4 Denature DNA.

a Vortex the 0.1N NaOH solution for 5 seconds.

b Add 5µl 0.1N NaOH solution to each well of the PCR microplate. NaOH should be aspirated from the top of the reagent reservoir and the tips should touch the bottom of the well when dispensing.

Gently pipette up and down twice to mix the solutions. Do not pass the first stop on the pipette.

Change the tips after each column.

NOTE

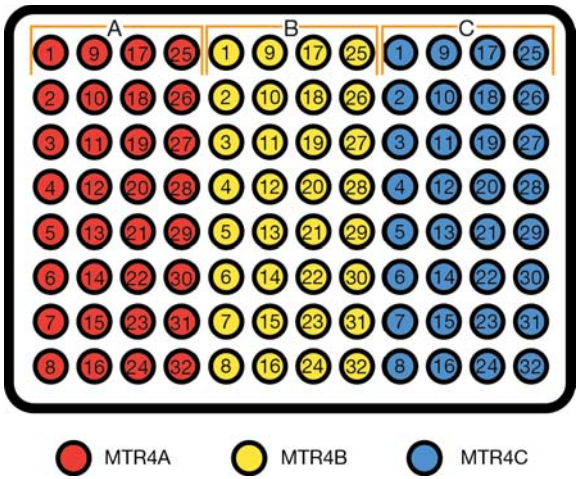
To avoid prolonged exposure of gDNA to DNA when processing two plates simultaneously, add the 0.1N NaOH solution to one plate before adding it to the second plate, then quickly proceed to step 5.

5 Add MTR while referencing the following figure.

NOTE

When processing two plates simultaneously, complete the addition of each MTR reagent to one plate before adding them to the second plate.

Figure 14 MTR Well Distribution



a Vortex each MTR reagent for 5 seconds, then centrifuge each briefly.

b Transfer the contents of each MTR reagent tube to a separate reagent reservoir using a single channel pipette.

c Add 10 µl MTR4A to each well of columns 1–4 of the PCR microplate.

The tips should touch the bottom of the well when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

d Add 10 µl MTR4B to each well of columns 5–8 of the PCR microplate.

The tips should touch the bottom of the well when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

e Add 10 µl MTR4C to each well of columns 9–12 of the PCR microplate.

The tips should touch the bottom of the well when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

6 Spin and Incubate.

a Seal the PCR microplate with a adhesive microplate sealing film.

NOTE

Make sure the sealing film is on securely in order to reduce sample evaporation and cross contamination during incubations. Use an adhesive seal applicator to apply force to the seal and ensure the seal is secured. See *Sealing Microplates* on page 92 for proper seal application techniques.

b Centrifuge the PCR microplate to 1,000 xg for 1 minute.

NOTE

When processing two plates simultaneously, they must be vortexed simultaneously on the incubating shaker throughout the entire protocol.

c Place the PCR microplate on the incubating/cooling shaker and press **start** to begin the incubating/cooling shaker Pr2 program step 02 (1,400 rpm at 68°C for 5 minutes).

7 Heat and Spin.

a Transfer the PCR microplate to the 95°C heat block and incubate for 1 minute.

NOTE

When processing two plates simultaneously, they must incubate on two separate heat blocks simultaneously.

b Transfer the PCR microplate to the bench top and incubate at room temperature for 3 minutes.

c Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 03 (1,400 rpm at 68°C for 5 minutes).

d Centrifuge the PCR microplate to 1,000 xg for 1 minute.

8 Denature Mixture.

NOTE

When processing two plates simultaneously, complete the addition of 0.1N NaOH to one plate before adding it to the second plate.

a Remove the adhesive seal and add 5 µl 0.1N NaOH to each well of the PCR microplate.

NaOH should be aspirated from the top of the reagent reservoir and the tips should touch the bottom of the well when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

b Seal the PCR microplate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

c Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 04 (1,400 rpm at 68°C for 1 minute).

9 Add Paramagnetic Beads.

a Vortex the AB1 reagent for 5 seconds. Visually ensure that the paramagnetic bead pellets are well dispersed in the solution and no crystal structures are present.

NOTE

If crystals are observed, vortex the AB1 solution until no crystal structures are visible.

CAUTION

The paramagnetic particles will settle after some time. Make sure to properly vortex the AB1 solution right before the addition to the reagent reservoir.

b Remove the adhesive seal and add 30 µl AB1 to each well of the PCR microplate.

NOTE

When processing two plates simultaneously, complete the addition of AB1 to one plate before adding it to the second plate.

NOTE

AB1 should not spill out of the wells and on the plate. Accurate pipetting and proper plate sealing will prevent any spillage. An improperly functioning thermal shaker can also produce excess plate agitation leading to spillage.

AB1 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells or just above the liquid when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

CAUTION

Avoid introducing any bubbles into the solution.

CAUTION

The AB1 reagent contains formamide and must be properly disposed of in a hazardous waste container.

c Seal the PCR microplate with a adhesive microplate sealing film. Use an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

d Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 05 (1,400 rpm at 68°C for 5 minutes).

e Centrifuge the PCR plate to 1,000 xg for 1 minute.

10 Collect and Wash Paramagnetic Beads.

NOTE

When processing two plates simultaneously, each step must be performed on both PCR microplates simultaneously using a separate magnetic plate for each PCR microplate.

a Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

b Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

The paramagnetic particles in the plate wells should never dry. Therefore, when processing two plates simultaneously, remove the supernatant from one plate and add UB3 (step 10e) to that same plate before removing the supernatant from and adding UB3 to the second plate.

c Change the tips after each plate if your are processing more than 1 plate. When you remove the supernatant you do not have to change tips after each column of a plate.

d Vortex the UB3 for 5 seconds.

e Add 40 µl UB3 to each well of the PCR microplate. UB3 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing.

f Seal the plate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

g Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 06 (1,400 rpm at 68°C for 1 minute).

h Place the PCR microplate on the magnetic plate for 1 minute.

11 Prepare AOP reagents.

a Vortex the AOP0 reagent for 5 seconds.

b Add 1,200 µl AOP0 to each tube of AOP4A, AOP4B, and AOP4C.

c Vortex each AOP4 reagent for 5 seconds, then centrifuge each briefly.

d Remove the adhesive seal from the PCR microplate.

e Remove all of the supernatant from each well with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

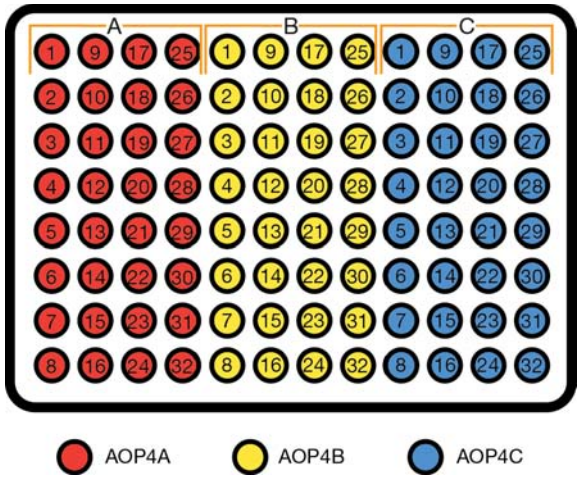
The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

The paramagnetic particles in the plate wells should never dry. Therefore, when processing two plates simultaneously, remove the supernatant from one plate and add the AOP reagents to that same plate before removing the supernatant from and adding the AOP reagents to the second plate. When both plates are ready, proceed to anneal oligos.

12 Add AOP while referencing the following figure.

Figure 15 AOP Well Distribution



a Pour each AOP4 reagent into a separate reagent reservoir.

b Add 40 µl AOP4A to each well of columns 1–4 of the PCR microplate.

AOP4A should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

c Add 40 µl AOP4B to each well of columns 5–8 of the PCR microplate.

AOP4B should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

d Add 40 µl AOP4C to each well of columns 9–12 of the PCR microplate.

AOP4C should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing. Do not pass the first stop on the pipette.

No up/down mixing is necessary.

Change the tips after each column.

e Seal the plate with a adhesive microplate sealing film. Use an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

CAUTION

The AOP reagents contain formamide and must be properly disposed of in a hazardous waste container.

13 Anneal Oligos.

Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 07 (1,400 rpm at 68°C for 15 minutes, with a cooling-rampdown to 45°C).

14 Proceed immediately to *Add Extension and Ligation Mix (ELM2)* on page 37.

Add Extension and Ligation Mix (ELM2)

In this pre-PCR process, AE1 reagent and UB3 buffer are added to the PCR microplate to wash away non-specifically hybridized and excess oligonucleotides. An enzymatic extension and ligation mix (ELM2) is then added to each DNA sample.

Check to ensure that you have all of the consumables identified in this section before proceeding with the assay protocol.

Estimated Time

Hands-on: ~10 minutes

Overall: ~30 minutes

Consumables

Item	Label Color	Quantity	Storage	Supplied By
AE1 reagent	Yellow	2 tubes per plate	-15° to -25°C	Illumina
UB3 buffer	Orange	1 tube per plate	-15° to -25°C	Illumina
ELM2 reagent	Navy blue	1 tube per plate	-15° to -25°C	Illumina
Adhesive microplate sealing film	–	4	Room temperature	User

Preparation

- Vortex each reagent for 5 seconds immediately before using the reagent.

Steps

1 Collect and Wash Paramagnetic Beads.

NOTE

When processing two plates simultaneously, each step must be performed on both PCR microplates simultaneously using a separate magnetic plate for each PCR microplate.

a Centrifuge the PCR microplate to 1,000 xg for 1 minute.

b Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

c Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

When processing two plates simultaneously, remove the supernatant from one plate and add AE1 to that same plate before removing the supernatant from and adding AE1 to the second plate.

d Vortex the AE1 reagent for 5 second or until no crystal structures are visible.

e Add 40 µl AE1 to each well of the PCR microplate. AE1 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing.

f Seal the plate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

CAUTION

The AE1 reagent contains formamide and must be properly disposed of in a hazardous waste container.

g Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 08 (1,400 rpm at 45°C for 1 minute).

h Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

i Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

When processing two plates simultaneously, remove the supernatant and complete the following stringent wash for one plate before doing the same to the second plate.

2 Repeat Stringent Wash.

a Add 40 µl AE1 to each well of the PCR microplate. AE1 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing.

b Seal the plate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

c Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 09 (1,400 rpm at 45°C for 1 minute).

d Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

e Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

When processing two plates simultaneously, place both plates on separate magnetic plates simultaneously, then remove the supernatant and complete the following mild wash for one plate before doing the same to the second plate.

3 Mild Wash.

a Vortex the UB3 reagent for 5 seconds.

b Add 40 µl UB3 to each well of the PCR microplate. UB3 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing.

c Seal the plate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

d Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 10 (1,400 rpm at 45°C for 1 minute).

e Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

NOTE

When processing two plates simultaneously, aspirate the UB3 from and add ELM 2, as follows, to one plate before doing the same to the second plate.

4 Add ELM2.

a Vortex the ELM2 reagent for 5 seconds.

b Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

c Add 40 µl ELM2 to each well of the PCR microplate. ELM2 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing.

d Seal the plate with a adhesive microplate sealing film, using an adhesive seal applicator to ensure the seal is secure. See *Sealing Microplates* on page 92 for proper seal application techniques.

e Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 11 (1,400 rpm at 45°C for 20 minutes).

5 Proceed immediately to *Add Make Amplification Mix (MAM1)* on page 42.

NOTE

When processing two plates simultaneously, aspirate ELM 2 and add MAM1, as follows, to one plate before doing the same to the second plate.

Add Make Amplification Mix (MAM1)

In this pre-PCR process, the MAM1 reagent (containing nucleotides and fluorescent PCR primers) and Titanium Taq DNA Polymerase are added to the PCR microplate. The extended and ligated DNA product from the previous step is amplified by PCR in a thermal cycler.

Check to ensure that you have all of the consumables identified in this section before proceeding with the assay protocol.

Estimated Time

Hands-on: ~5 minutes

Thermal Cycle: ~1.5 hours

Consumables

Item	Label Color	Quantity	Storage	Supplied By
Titanium Taq DNA Polymerase	–	48 µl of 5 U/µl per plate	-15° to -25°C	User
MAM1 reagent	Green	1 tube per plate	-15° to -25°C	Illumina
PCR sealing film	–	1	Room temperature	User

Steps

1 Add Titanium Taq to MAM1.

a Add 48 µl of 5 U/µl Titanium TaqDNA Polymerase to the tube of MAM1.

WARNING

Titanium TaqDNA Polymerase is a critical component. Be sure you have added this enzyme to the MAM1 reagent.

NOTE

MAM1 contains fluorescently-labeled PCR primers. Make sure to limit its exposure to bleach fumes and light to avoid degradation of the Cy5 dye.

b Vortex the tube of MAM1containing Titanium Taq for 5 seconds.

WARNING

Vortexing for a full 5 seconds is essential for uniform distribution.

2 Add MAM1 containing Titanium Taq.

a Centrifuge the PCR microplate to 1,000 xg for 1 minute.

b Place the PCR microplate on the magnetic plate for 1 minute, then remove the adhesive seal.

c Remove the supernatant with a pipette set at 60 µl. Take care to not disturb the paramagnetic bead pellets.

CAUTION

The paramagnetic beads collect to alternate sides of the wells. When aspirating the supernatants, take care to not disturb the paramagnetic bead pellets by pointing the pipette tips away from the pellets.

NOTE

When processing two plates simultaneously, remove the supernatant from and add MAM1, as follows, to one plate before doing the same to the second plate.

d Add 40 µl MAM1 containing Titanium Taq to each well of the PCR microplate. MAM1 should be aspirated from the top of the reagent reservoir and the tips should touch the side of the wells, above the liquid level line, when dispensing. Do not pass the first stop on the pipette.

e Seal the plate with the appropriate sealing film for your thermocycler.

f Place the PCR microplate on the incubating/cooling shaker and press **start** to begin Pr2 program step 12 (1,400 rpm at 45°C for 2 minutes).

g Transfer the PCR microplate to the post-PCR laboratory.

3 Cycle PCR Microplate.

NOTE

Ensure that the thermocycler is set for "calculated" as opposed to "block" temperature measurement.

a Program your thermocycler as follows:

Table 10 MJ/BioRad Thermocyclers

Thermocycler Model	Temperature Mode	Lid Temperature	Vessel Type	Number of Samples	Sample Volume	Additional Information
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated constant at 100°C	Polypropylene plates and tubes	96	40 µl	Set lid to turn off when temp. drops below 30°C; When setting up cycling program, no options are selected (only temperature and time).
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate	96	40 µl	
Bio-Rad MyCycler	Algorithmic	Heated	Plate	96	40 µl	

Table 11 Eppendorf Thermocyclers

Thermocycler Model	Lid Temperature	Control	Vessel Type	Number of Samples	Sample Volume	Additional Information
Eppendorf MasterCyclerPro Gradient	105°C; TSP/ESP Heated Lid (activated); Switch lid off at low temperature (activated)	Simulated Tube; Simulate Mastercycle gradient (activated)	Plate	96	40 µl	When setting up cycling program, no options are selected (only temperature and time). (Ramp = 100% & no gradient)
Eppendorf MasterCyclerPro						
Eppendorf MasterCycler	105°C; WAIT (At the start); AUTO (At the end)	Tube	Plate	96	40 µl	Ramp = 3°/s +0.0°/s when setting up the cycling program steps

	Temperature	Time
	95°C	3 minutes
X 32	95°C	20 seconds
	56°C	10 seconds
	72°C	20 seconds
	72°C	10 minutes
	4°C	5 minutes
	10°C	Hold

CAUTION

Prolonged incubation at 4°C may damage your thermocycler.

b Place the PCR microplate into the thermocycler and run the thermocycler program.

4 Do one of the following:

- Proceed immediately to *Make Single Stranded DNA (MSS)* on page 47.
- Optionally, store the ARX plate in the dark at 2° to 8°C overnight before proceeding to *Make Single Stranded DNA (MSS)*.

Make Single Stranded DNA (MSS)

In this post-PCR process, the MSS reagent is added to generate single-stranded DNA for VeraCode Bead hybridization.

Continue to perform the assay in the post-PCR laboratory. Check to ensure that you have all of the consumables identified in this section before proceeding with the assay protocol.

Estimated Time

Hands-on: ~5 minutes

Consumables

Item	Label Color	Quantity	Storage	Supplied By
VeraCode Bead Plate	–	1	2° to 8°C	Illumina
MSS reagent	Lavender	1 tube per plate	-15° to -25°C	Illumina

Preparation

- Thaw the MSS reagent on the benchtop.
- Vortex each reagent for 5 seconds immediately before using the reagent.
- Remove the PCR microplate from the thermocyler upon completion of cycling.
- If you have stored the PCR microplate overnight, remove the PCR microplate from the refrigerator and allow it to come to room temperature in the dark.
- Set the post-PCR incubating microplate shaker to 47°C.

Steps

1 Collect Paramagnetic Beads.
   a Centrifuge the PCR microplate to 680 xg for 1 minute.
   b Place the PCR microplate on the magnetic plate for 1 minute, then remove the PCR sealing film.

2 Prepare VeraCode Bead Plate
   a Centrifuge the VeraCode Bead Plate to 680 xg for 1 minute.

b Slowly and carefully remove the VeraCode Bead Plate cap mat. Do not discard the cap mat.

c Add 40 µl MSS to each well of the VeraCode Bead Plate.

CAUTION

The MSS reagent contains dimethyl sulfoxide (DMSO) and must be properly disposed of in a hazardous waste container.

CAUTION

Keep pipette tips well above the VeraCode bead pellet to avoid dislodging and losing beads.

3 Transfer PCR Products.

Set the pipette to 45 µl and transfer all contents from each well of the PCR microplate to the corresponding well of the VeraCode Bead Plate.

Change the tips after each column.

NOTE

Take care to not disturb the paramagnetic beads in the PCR plate when transferring the PCR product to the VeraCode Bead Plate.

CAUTION

Keep pipette tips well above the VeraCode bead pellet to avoid dislodging and losing beads.

4 Seal the VeraCode Bead Plate.

Seal the VeraCode Bead Plate with the cap mat that the plate was supplied with from step 2b. Use a Corning seal press to ensure the plate is completely sealed.

5 Proceed immediately to *Hybridize VeraCode Bead Plate* on page 49.

Hybridize VeraCode Bead Plate

In this post-PCR process, a shaking incubator is used to hybridize the PCR products to the VeraCode Bead Plate. Once the PCR products are transferred to the VeraCode Bead Plate, they are ready for hybridization. After hybridization, they are washed with VW2, and the resulting VeraCode Bead Plate is ready for scanning in the BeadXpress Reader.

Check to ensure that you have all of the consumables identified in this section before proceeding with the assay protocol.

Estimated Time

Hands-on: ~5 minutes

Overall: ~2.5 hours

Consumables

Item	Label Color	Quantity	Storage	Supplied By
VW2 buffer	Sea foam green	45 ml per plate	Room temperature	Illumina

Steps

1 Hybridize VeraCode Bead Plate.

a Vortex the VeraCode Bead Plate on the shaking incubator at 47°C at 1,200 rpm. Set the shaking incubator to “Hold” and use a timer to track the incubation time for 2.5 hours.

NOTE

Do not exceed the 2.5 hour incubation time. When processing two plates simultaneously, following hybridization, promptly wash the VeraCode Beads, as follows, in both plates simultaneously.

NOTE

Start the BeadXpress Reader so it can initialize while you hybridize the VeraCode Bead Plate (reference Prepare BeadXpress Reader on page 26)

2 Centrifuge the VeraCode Bead Plate to 680 xg for 1 minute.

3 Pour 45 ml of VW2 into a non-sterile, disposable reservoir.

4 Wash VeraCode Beads.

a Remove the VeraCode Bead Plate seal.

b Add 200 µl VW2 to each well of the VeraCode Bead Plate, then gently swirl the VeraCode Bead Plate on the benchtop 2–3 times.

CAUTION

Keep pipette tips well above the VeraCode bead pellet to avoid dislodging and losing beads.

c Wait 1 minute for the VeraCode beads to settle.

d Remove the supernatant with the aspiration manifold under 40–60 mbar vacuum. Take care to not disturb the VeraCode bead pellets. Make sure the liquid is the same level in all wells.

5 Repeat VeraCode Bead Wash.

a Add 200 µl VW2 to each well of the VeraCode Bead Plate, then gently swirl the VeraCode Bead Plate on the benchtop 2–3 times.

CAUTION

Keep pipette tips well above the VeraCode bead pellet to avoid dislodging and losing beads.

b Wait 1 minute for the VeraCode beads to settle.

c Remove the supernatant with the aspiration manifold under 40–60 mbar vacuum. Take care to not disturb the VeraCode bead pellets. Make sure the liquid is the same level in all wells.

6 Do one of the following:

- Proceed immediately to *Prepare BeadXpress Reader* on page 51.
- Optionally, seal and store the washed VeraCode beads in the dark at room temperature for up to three days before scanning them in the BeadXpress Reader.

Prepare BeadXpress Reader

In this post-PCR process, the BeadXpress Reader is prepared for operation and the BeadXpress Reader and the VeraScan software are initialized in preparation for scanning the VeraCode Bead Plate.

Estimated Time

Hands-on: ~5 minutes

Overall: ~35 minutes

Preparation

- The operator of the BeadXpress Reader should be trained by qualified personnel on the correct operation of the instrument, and be aware of the safety issues involved. Reference the Specifications and Cautions and Warnings sections of the *BeadXpress Reader System Manual*.
- Ensure that the VeraScan data output location has been properly configured. Consult your System Administrator.

Steps

Powering Up the BeadXpress Reader

1 After the BeadXpress Reader has been off for at least two minutes, press the power switch on the back panel of the instrument.

CAUTION

After powering up the instrument, the lasers must stabilize for 15 minutes before the VeraScan software responds to commands. An error message appears in the VeraScan software if you attempt to initialize the scanner before the lasers have stabilized.

Power Up the BeadXpress Reader Computer

1 Press **Power** on the computer.

2 Log in to Windows.

Starting the VeraScan Software

1 Wait until the **Power** and **Ready** lights on the front panel of the BeadXpress Reader are the only lights on.

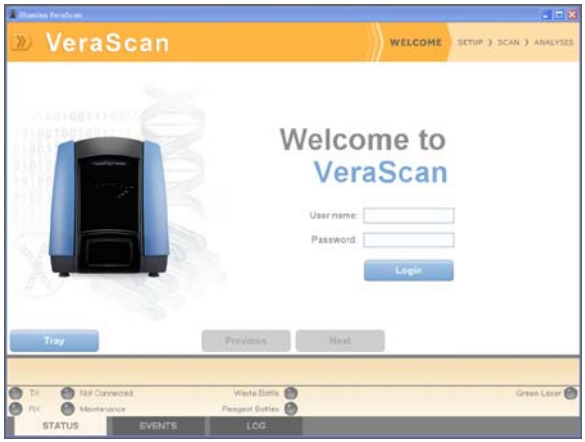
If you do not wait for this condition, an error message may appear when you start the software.

2 Do one of the following:

- From the Windows **Start** menu, select **All Programs** | **Illumina** | **VeraScan**.
- Double-click the VeraScan icon on the desktop.

The VeraScan application opens on the computer desktop.

Figure 16 VeraScan Welcome Screen



NOTE

The VeraScan Software checks whether preventive maintenance has been performed. The Maintenance indicator light on the VeraScan Welcome Screen is green when all maintenance is current. For information about maintaining your BeadXpress Reader reference the section on Maintenance in the *BeadXpress Reader System Manual*.

3 Enter your username and password, then click **Login**.

NOTE

VeraScan user accounts, account options and alerts must be set up by your system administrator. Reference the *BeadXpress Reader System Manual and* Appendix B, *VeraScan Administration and VeraReport*.

Connecting the Reader

- Click the Menu button in the upper-left corner of the screen and select **Reader | Connect**.

  This action connects the BeadXpress Reader to the VeraScan software.
- Click the Menu button in the upper-left corner of the screen and select **Reader | Initialize System**.

NOTE

VeraScan can be configured to automatically connect to and initialize the BeadXpress Reader. Consult your system administrator.

Scan VeraCode Bead Plate

In this post-PCR process, the BeadXpress Reader uses lasers to excite the fluors of the PCR products bound to the VeraCode beads. Light emissions from these fluors are then recorded in a data file. Fluorescence data are analyzed to derive genotyping results using Illumina's VeraScan software.

Estimated Time

Hands-on: ~5 minutes

Overall: ~1.25 hours

Preparation

- If the VeraCode Bead Plate was sealed and stored, remove the seal before scanning.

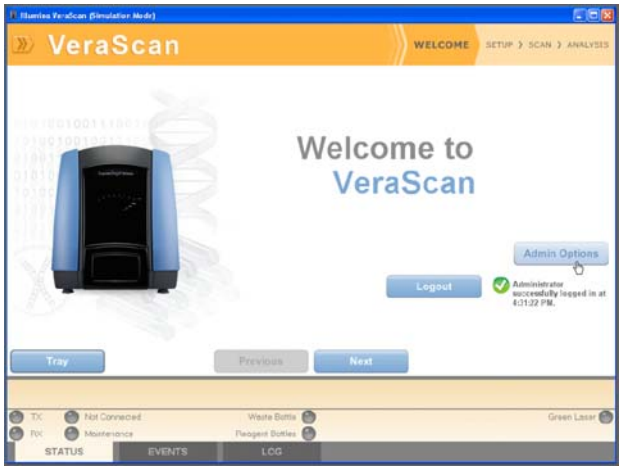
Steps

Set Up the Scan

1 Load the VeraCode Bead Plate.

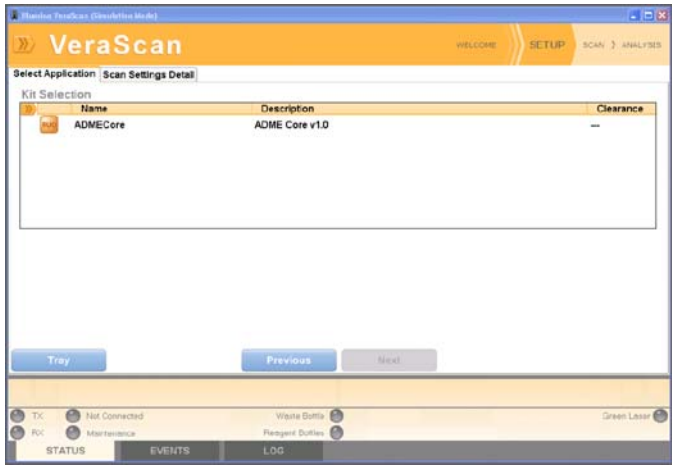
a Click **Next** in the VeraScan window.

Figure 17 Successful VeraScan Login



The VeraScan Setup screen displays the Select Application tab.

Figure 18 VeraScan Setup Screen



b On the Select Application tab, click **Open Tray**. A message with a diagram appears displaying the correct plate orientation.

c Place a VeraCode Bead Plate in the correct orientation so that well A1 is located in the corner indicated by the A1 orientation mark stamped into the BeadXpress Reader plate tray.

Figure 19 A1 Orientation Mark

d Hold the plate by the outer edges of the longer side and place it on the adaptor tray.

e Close the tray to insert the VeraCode Bead Plate into the BeadXpress Reader by clicking **Close Tray** on the Select Application tab.

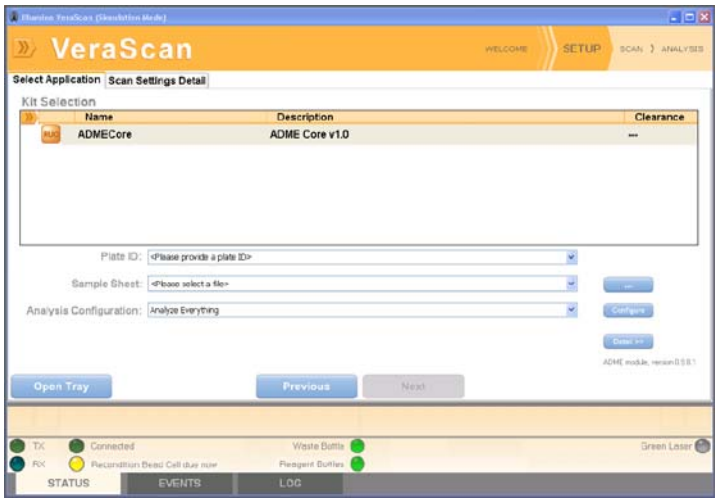
WARNING

Be careful when closing the adaptor tray. Move your hand away from the drawer before closing.

2 Select Scan Properties.

a Select the **ADME Core** kit from the Select Application tab. The scan input data fields are displayed.

Figure 20 VeraScan Select Application Tab



b Enter the **Plate ID** or select from the drop down menu of recently run plates. The plate ID is used to name the data files and the data folders.

NOTE

Do not include the underscore character "_" or comma "," in the Plate ID name.

NOTE
A plate barcode cannot be used as the plate ID more than once.

NOTE
There is no maximum number of characters for the plate ID. However, the plate ID is used in the Windows path where the output files are saved and that entire path must be shorter than 260 characters.

c Select the **Sample Sheet** from the dropdown menu or browse for the file by clicking '**...**' to the right of the text box.

NOTE
The plate ID must match the name of the plate in the sample sheet.

d The default **Analysis Configuration** is set to Analyze Everything or, if available, select from the drop down menu of analysis configurations. (Consult your System Administrator to set up configuration templates.)

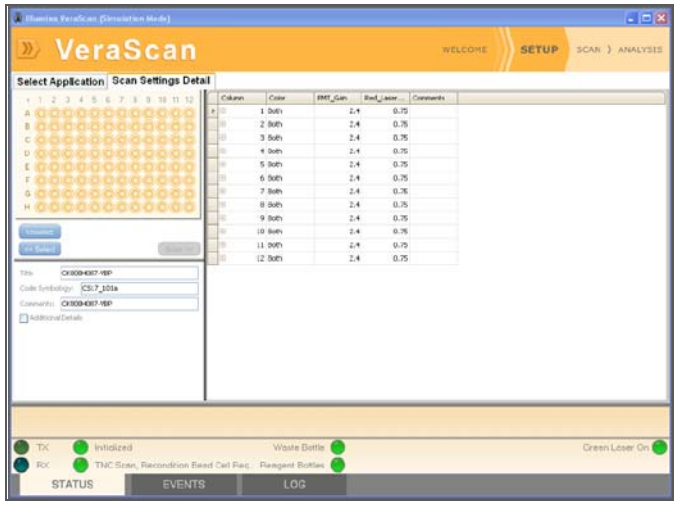
NOTE
The **Configure** button is only available for system administrators.

3 At any time, click **Previous** from the Setup screen to view the Welcome screen.

4 To view scan setting details:

a In the Select Application tab, click **Details** or select the Scan Settings Detail tab. The wells highlighted in orange indicate the well columns to be scanned.

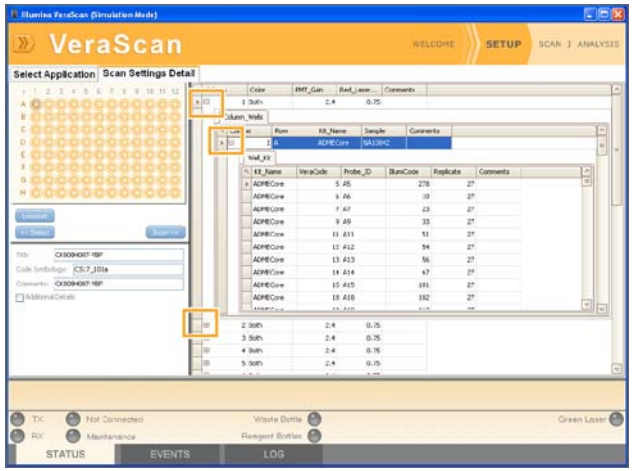
Figure 21 Scan Settings Detail Tab



b Highlight the well column you want to review by clicking it.

c Click the plus (+) to expand cells and view column and well details or click the minus (-) to close the view of cell details.

Figure 22 Expanded Scan Settings Detail Tabs



d Click **Unselect** to clear the view of the data for the selected well.

e Click **Select** to return to the Select Application tab.

f Check **Additional Details** to display format, plate type and plate attributes.

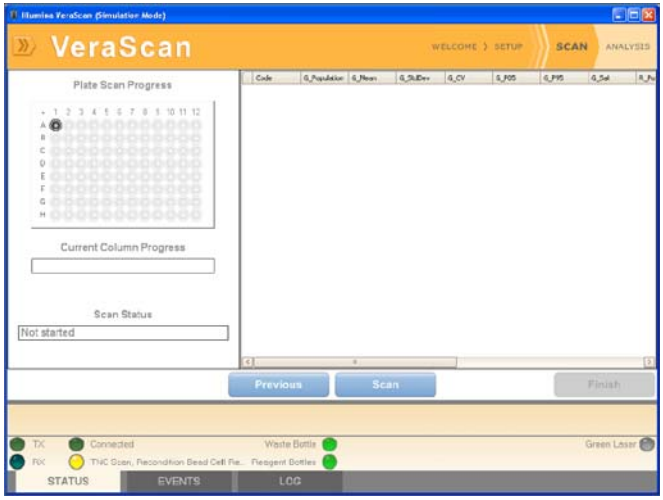
g Click **Scan** to continue to the Scan screen.

5 Click **Next** from the Setup screen to continue to the Scan screen.

Start and Control the Scan

1 Click **Scan** from the Scan screen to begin the scan.

Figure 23 Scan Screen



2 While a scan is in process, you can:

- Monitor scan progress
- Pause the scan
- Abort the scan
- Resume the scan

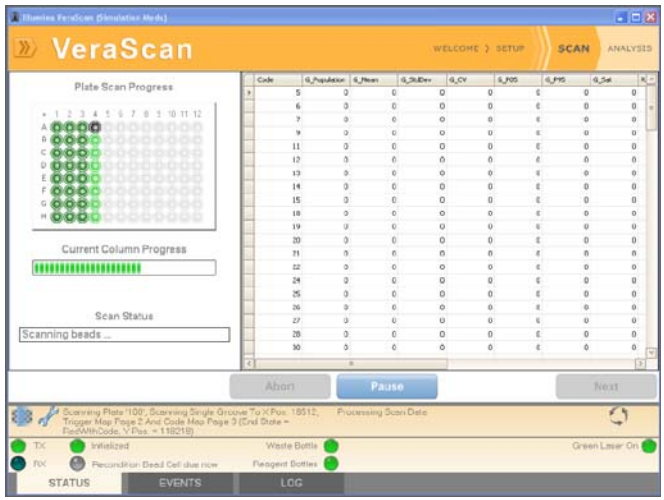
The information bar located along the bottom of the main application window includes tools for monitoring scans. Click Status, Events or Log to view details.

Monitor the Scan Progress

The VeraScan application checks available hard drive space to ensure that sufficient space is available to record the selected sections. If sufficient disk space is not available, an error message is displayed, and the arrays are not scanned. Otherwise, the BeadXpress Reader begins scanning. For each sample, the green intensity data is populated in the table first and then the red intensity data.

The progress through the selected columns, and the status of each section, is displayed in the **Current Column Progress** and **Scan Status** areas. The column in the plate being scanned is indicated by flashing light-green wells in the Plate Scan Progress area. Solid dark-green wells indicate columns that have already been scanned. A status bar representing the progress of the current scan is displayed below the plate.

Figure 24 Scan Screen Progress



NOTE

Serious errors halt the scan process. Errors that may be recoverable, such as network or other hardware errors, cause the scan to pause until you click **OK** in the error message box.

View the Scan Status Information

The Status bar displays current status information as the scan progresses, including status messages and the ID of the current plate being scanned. Reference the *BeadXpress Reader System Manual* for details on status information.

Pause, Abort, or Resume a Scan

If for any reason a scan is aborted, the VeraScan software allows you to recover the data for the scanned columns and continue scanning the remaining columns.

During a scan, the data table is dynamically populated with intensity data. If the data appear faulty (for example, if the intensities are much too low), you may wish to interrupt the scan to consult your supervisor.

- **Pause:** When clicked, the scan remains suspended until you click **Resume Scan** or **Abort**.
- **Abort:** Click to cancel the scan.
- **Resume Scan:** Click to resume the scan after pausing.

Conclude the Scan

To conclude the scanning process do one of the following:

- After a successfully completed scan, click **Next** to proceed to the Analysis screen and *Analyze Scan Data* on page 63.
- If a scan was aborted or otherwise interrupted, click **Finish** to return to the Welcome screen to start over.

Analyze Scan Data

The VeraCode ADME Core Panel assay is processed by VeraScan, the ADME Core module, and the ADME Core Panel kit manifest. VeraScan is the software that runs the BeadXpress reader acquiring and processing green and red fluorescence signals from VeraCode beads. The ADME Core module interprets green and red fluorescence signals from VeraScan. The ADME Core module analyzes data on a per well and per bead type basis using the ADME Core Panel kit manifest which contains genotyping parameters.

Table 12 Bead Type Outcomes

Assay ID	Valid Outcomes	Invalid Outcomes
ADME variant	Genotype call and translated allele call	**Low Signal** **No Call** **Low Beads**
ADME copy number variant	Copy number call	
Control		
• Sample Tracking Control	Sample specific barcode displayed in Controls column	**Failed STC** No results provided
• Process Control		**Failed PSC** No results provided
• Subpool Position Control	Results provided	No results provided Message: Assay pooling control failed. Analysis will be aborted.
• Hybridization Control 1	Pass	**Failed Hyb** Results highlighted in red require review to determine acceptability
• Hybridization Control 2		
• Mismatch Control 1		**Failed Mismatch** Results highlighted in red require review to determine acceptability
• Mismatch Control 2		

Interpret Sample Results

The ADME Core module reports the outcome for each of the bead types on a per well basis.

- A well is considered valid (the genotypes for ADME Core Panel may be reported) if all assay controls (i.e., STC, SPC, and PSC) have valid outcomes.
- A well is considered invalid (the genotypes for ADME Core Panel should ***not*** be reported) if any control bead type has an invalid outcome.

No Template Controls (NTCs) should generate the following outcomes:

Table 13 No Template Control Valid Outcomes

Assay ID	Valid Outcomes
ADME variant	Low Signal or NA
ADME copy number variant	
Control	Pass

NOTE

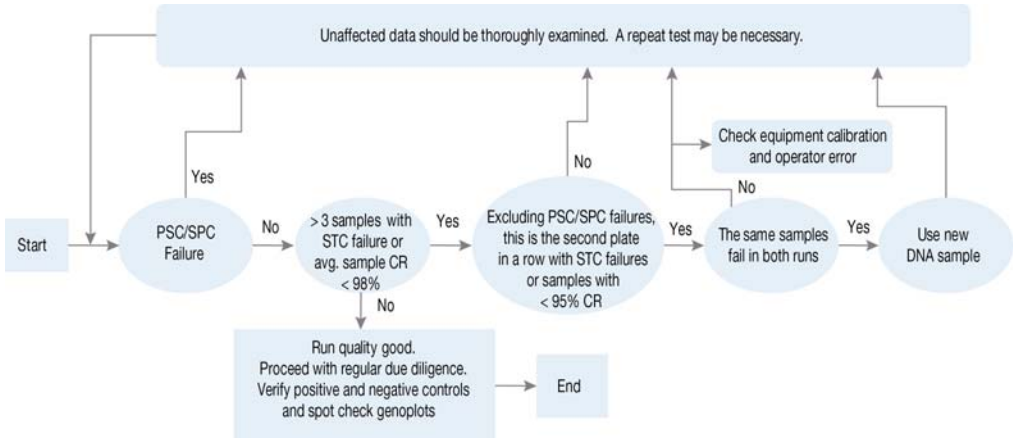
No Template Control is not included in call rate calculations and is not plotted in SNP graphs on the Details tab

No Template Controls may generate some, but usually insubstantial signals in a low number of assays due to the complexity of the content. Ideally, less than 10% of the loci should generate signals above the background in NTCs. If any NTC has more than 24 assays with a genotypable signal, the following possibilities should be considered:

- Cross-well contamination may have occurred during the processing of the plate
- Lab environment may be contaminated with amplicon (See “Best Practices” on page 86.)
- BeadXpress Reader may need routine cleaning

It is important to assess the quality of each run to determine the validity of the outcomes generated from the ADME Core Panel. The following flowchart can be used to help assess the data quality for each plate run. For further assistance, see *Assay Protocol Troubleshooting* on page 122.

Figure 25 Data Assessment Workflow



Display Results

To display the results for analysis:

1 From the Scan screen click **Next**.

The Analysis screen appears and is populated with a Summary Report of the just-completed scan.

NOTE

When specific assay controls fail, the Analysis screen does not appear and the analysis is aborted. See Table 12 on page 63 for invalid outcomes for controls.

Figure 26 Plate Analysis Summary Report

Table 14 Plate Analysis Summary

Column Header	Description
Sample ID	The sample ID from the sample sheet
Well	The well number row and column (e.g. A1)
Call Rate	The sample call rate
Controls	Control result for the sample: Passed samples - displays STC barcode Failed samples - displays failure mode: **Failed PSC**, **Failed STC**, **Failed Mismatch**, or **Failed Hyb**
Gene Result (one column per Gene Name)	All distinct variants detected for the given gene. If present, no Call results are appended to the end of the string. ***1A/*1A** - genes with star nomenclature (no variants were detected) **NMD** - genes without star nomenclature **RAL** - rare allele likely

2 At any time click **Previous** to view the previous screen.

Summary Report

The Summary Report table allows both row and column selection.

1 To view the details of a single sample, select the sample row or a table cell from within the sample row in the Summary Report. The selected row is highlighted in grey and the selected cell is highlighted orange. The report details of the selected sample are displayed in the lower portion of the screen

Figure 27 Plate Summary Report with Sample Selected

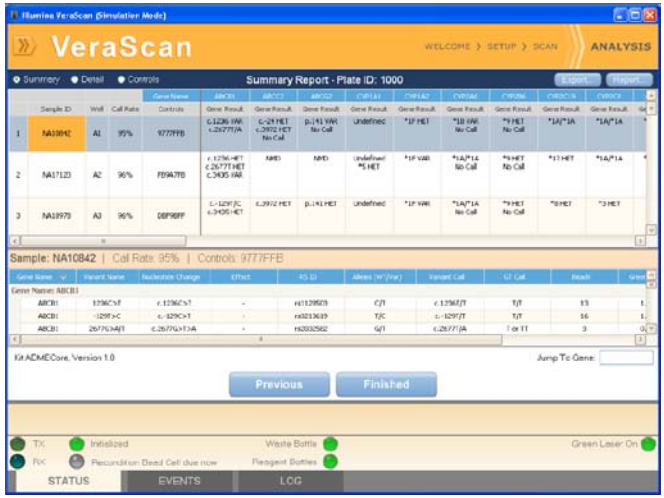


Table 15 Plate Sample Details

Column Header	Description
Gene Name	Gene name
Variant Name	Variant name
Nucleotide Change	Nucleotide change
Effect	Amino acid change or functional change
RS ID	RefSeq ID of locus
Alleles (WT/Var)	Possible alleles

Table 15 Plate Sample Details (Continued)

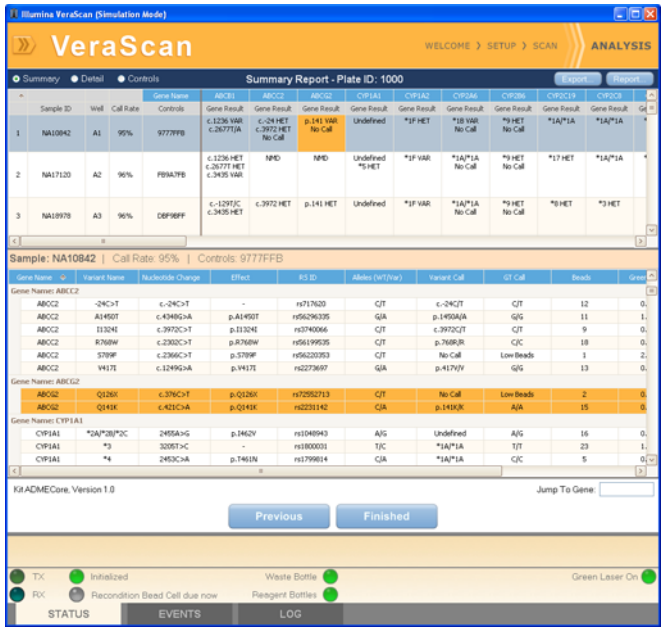
Column Header	Description
Variant Call	Translated variant call result
GT Call	Genotype call result
Beads	Bead population for a locus in the well where the sample is assayed
Green Signal	Raw green signal intensity for negative control samples. Normalized green signal intensity for positive control samples
Red Signal	Raw red signal intensity for negative control samples. Normalized red signal intensity for positive control samples
Theta	Red fluorescence intensity to green fluorescence intensity ratio. The value should be close to 0 or 1 for homozygous genotypes.

2 To jump to the sample details for a specific gene in the selected sample do one of the following:

- Select the cell from the column that displays the gene details
- Enter a gene name in the **Jump to Gene** field

The corresponding rows for the selected gene are highlighted in orange in the sample detail table in the bottom panel.

Figure 28 Plate Summary Report with Gene Selected



- The sample detail table allows row selection but not column selection. When a row is selected, the corresponding cell in the Summary Report and sample detail tables is selected.

Detail Report

1 To view plate details, select **Detail** from the display options in the top portion of the Analysis screen. The Detail Report is displayed on the Analysis screen. The detail table allows both row and column selection.

Figure 29 Plate Detail Report

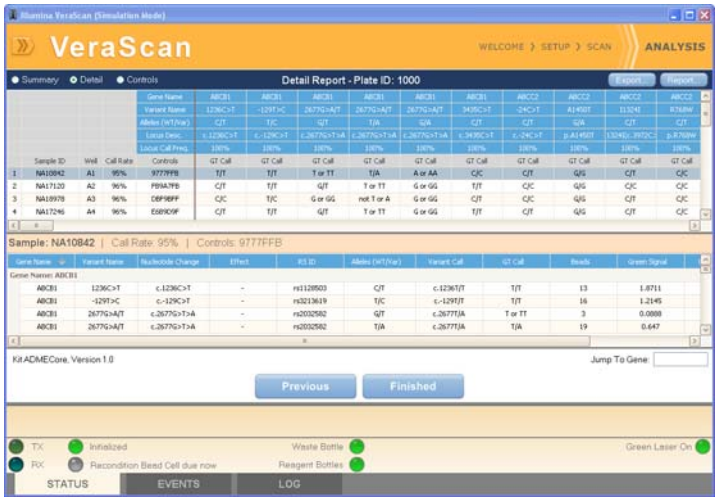


Table 16 Plate Detail

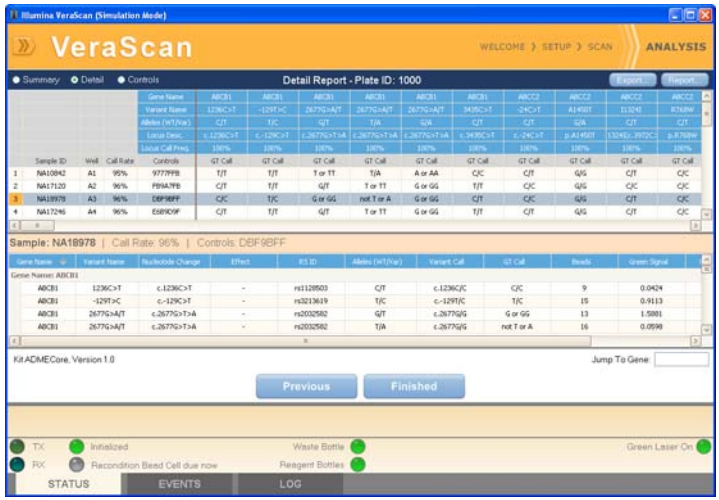
Column Header	Description
Sample ID	The sample ID from the sample sheet
Well	The well number row and column (e.g. A1)
Call Rate	Sample call rate
Controls	Control result for the sample: Passed samples - displays STC barcode Failed samples - displays failure mode: **Failed PSC**, **Failed STC**, **Failed Mismatch**, or **Failed Hyb**
GT Call (one column per Gene Name)	Genotype call result

Table 17 Gene Detail

Row Header	Description
Gene Name	Gene name
Variant Name	Variant name
Alleles (WT/Var)	Possible alleles
Locus Desc.	Nucleotide change or amino acid change, depending upon the locus
Locus Call Freq.	The locus call rate

Select a sample row from the detail table to view the corresponding sample table in the bottom panel. When a cell inside a selected row is selected, the corresponding rows in the sample table is selected.

Figure 30 Plate Detail Report with Sample Selected



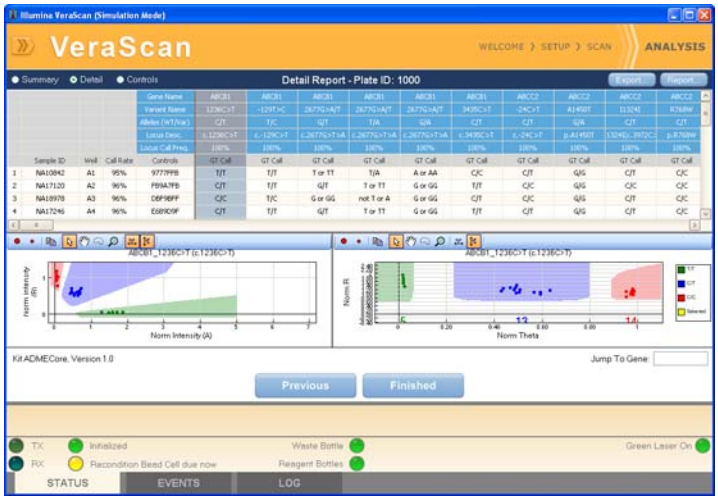
2 To view the details of a genotype, select the genotype column from the Detail Report. The results are displayed in the lower portion of the screen.

- For non CNV loci, two plots are displayed. The left plot shows Cartesian coordinates, while the right plot shows polar coordinates.

  Cartesian plot – displays each data point by its normalized green (x-axis) and red (y-axis) fluorescence intensities.

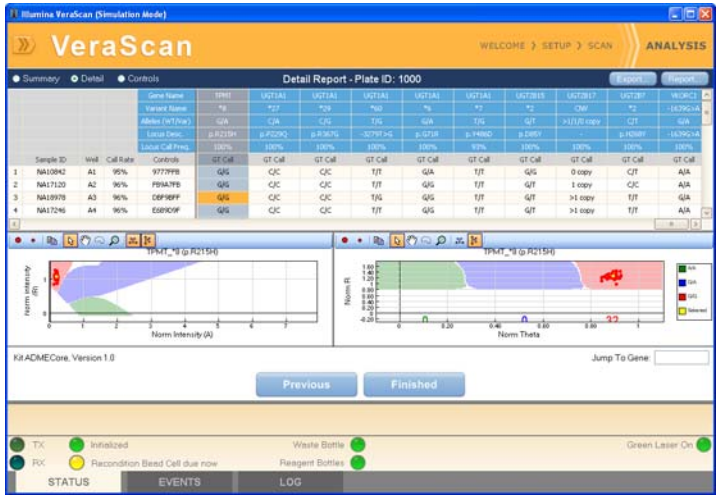
  Polar plot – converts the green and red intensities to radius (the sum of green and red) and theta (red fluorescence intensity to green fluoresceins intensity ratio).
- For CNV aggregate results, only the polar plot is displayed.
- Call zones for heterozygote and homozygote results are colored red, green, and blue. A gray zone represents a call zone for a rare allele, where the call zone has been estimated due to an insufficient number of data points to set empirically because of locus rarity.
- The genotype plot allows point selection. When a point is selected, the corresponding cell in the detail table is selected.

Figure 31 Plate Detail with Gene Selected



3 When a cell inside a selected column is selected, the corresponding point in the plot is highlighted in orange.

Figure 32 Plate Detail with Sample and Gene Selected



The following defines the plot tools:

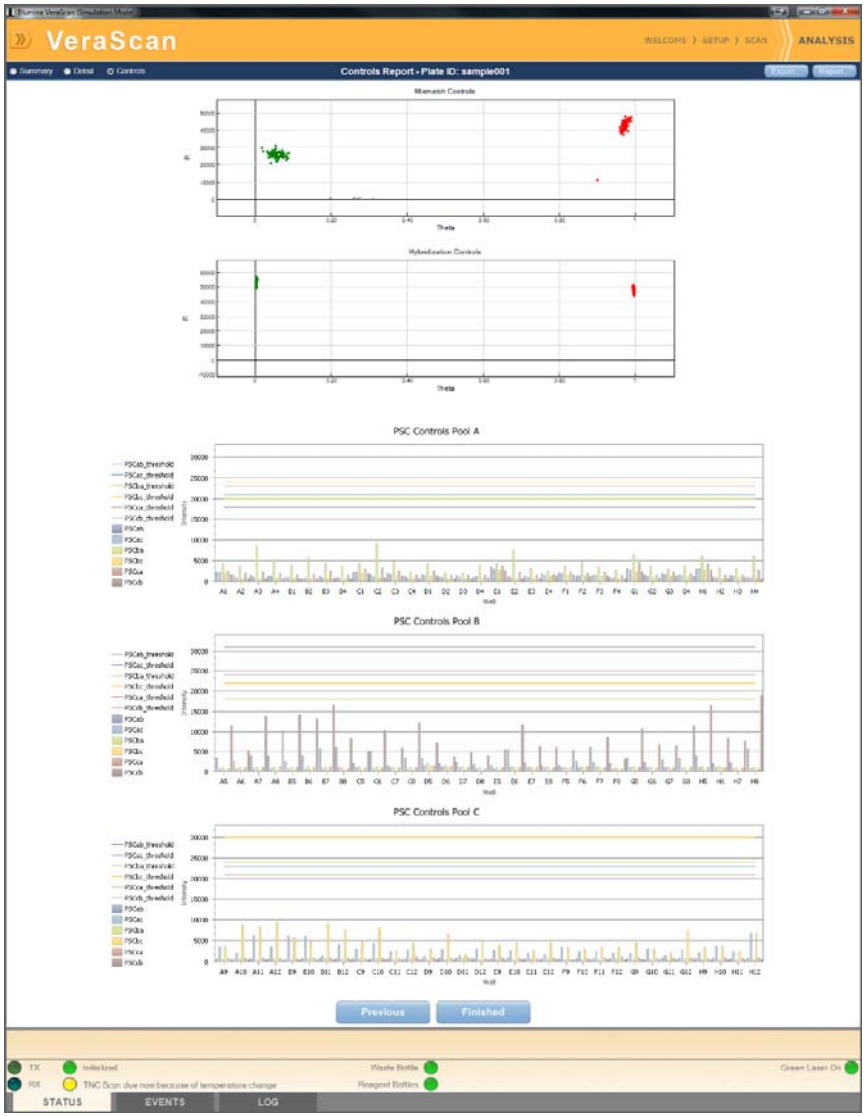
Table 18 Plot tools

Tool	Function	Tool	Function
	Make dots larger		Lasso mode
	Make dots smaller		Zoom mode
	Copy plot to clipboard		Auto-Scale X-Axis
	Default mode		Auto-Scale Y-Axis
	Pan mode		

Controls Report

1 To view plate controls, select **Controls** from the display options in the top portion of the Analysis screen. The Controls Report is displayed on the Analysis screen.

Figure 33 Plate Controls



The following plate control displays are shown:

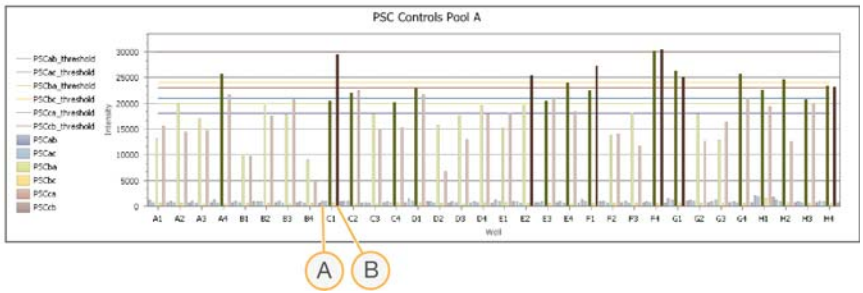
Mismatch Controls and Hybridization Controls - Displays genotype plots

- Green—Homozygous for green allele
- Red—Homozygous for red allele

PSCControls - Displays well intensity and PSC threshold for each pool

- Columns display intensity value of each PSC per well
- Place cursor over column to view numeric value
- Horizontal lines indicated PSC failure threshold
- Column color darkens if PSC intensity exceeds threshold

Figure 34 Plate Detail with Sample and Gene Selected



A Example: Good PSCab control for well C1
B Example: Failed PSCcb control for well C1

2 Manipulate the Controls display data as follows:

- Zoom in or out of the display by scrolling the mouse wheel.
- Pan the display by pressing Shift and moving the mouse.

3 At any time click **Previous** to view the previous screen.

Conclude Analysis

To conclude the plate analysis, perform one of the following:

- Proceed to *Report Genotypes* on page 76.
- Proceed to *Exit Results* on page 83.

Report Genotypes

Reports in .csv Format

Scan results of the individual sample and summary data are automatically saved after each scan in .csv format which can be used in other commercial applications, such as Microsoft Excel. If not modified by the System Administrator, the default file settings are:

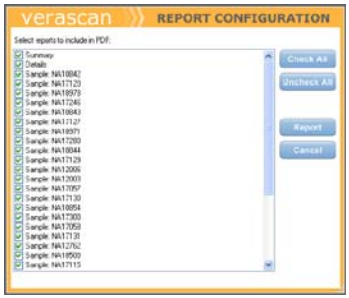
- **Location**—C:\AllUsers\Illumina\VeraScan\Output\Research
- **Filename**—Year-month-plateID

Reports in PDF Format

Scan results can also be saved PDF format. To save and view a PDF report:

1 Click **Report** from the Analysis screen.
The Report Configuration window is displayed

Figure 35 Report Configuration

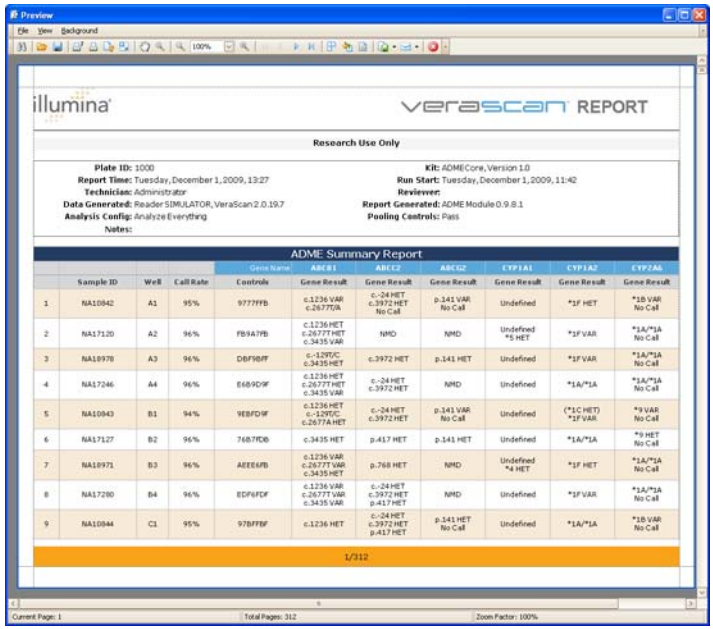


To exclude results from the default Report Configuration setting, uncheck the results to be excluded from the report.

2 Click **Report** from the Report Configuration screen.

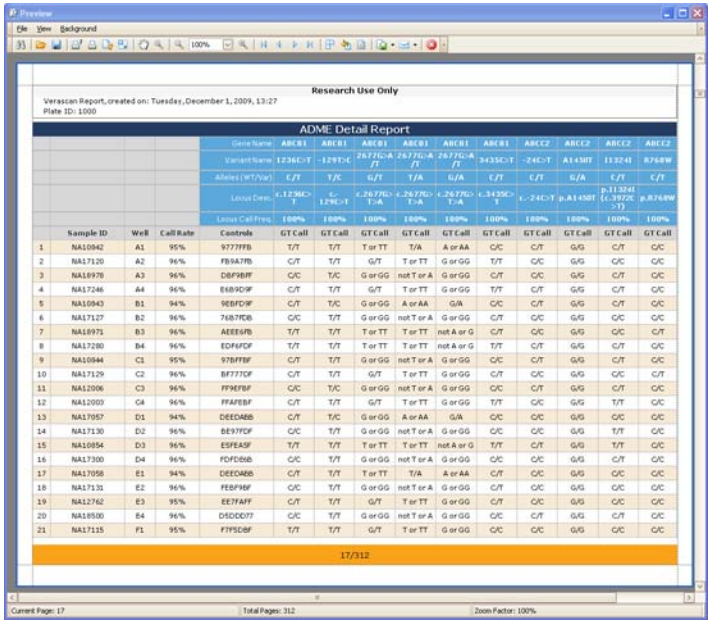
The VeraScan Report window is displayed with the summary of the report results specified in the Setup screen. The contents of the summary are described in Table 14 on page 66.

Figure 36 Report Summary



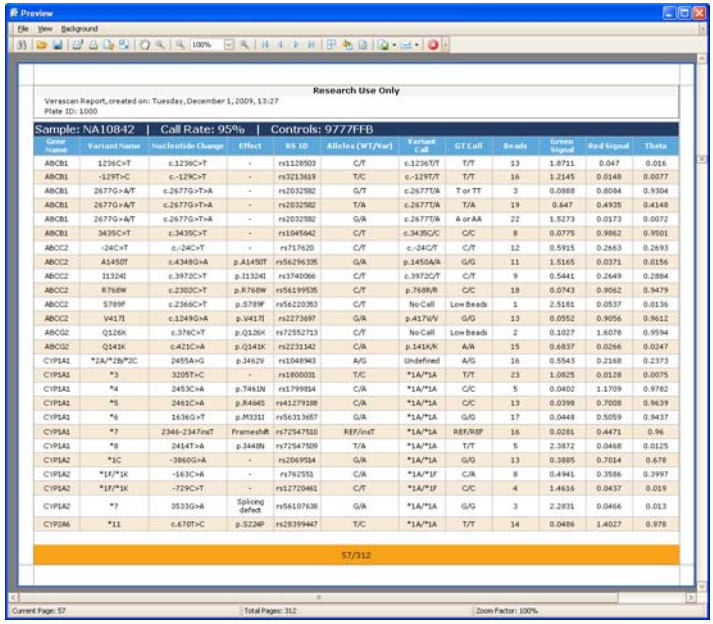
Scroll down to view the detailed report results for each sample. The sample details are described in Table 15 on page 67 and Table 16 on page 70.

Figure 37 Report Detail



Scroll down to view the table of results for each sample. The plate details are described in Table 15 on page 67.

Figure 38 Report on Sample



3 The report can be exported via the VeraScan Report window using one or more of the following options:

- Export the report to a new format by selecting **File | Export Document**.
- Print the report by selecting **File | Print**.
- Send the report by selecting **File | Send via E-mail**.

Export Data

Scan results of the individual sample and summary data are automatically saved after each scan in .csv format as described in *Reports in .csv Format* on page 76. Scan results in .csv format can also be exported to a location other than the default.

1 Click **Export** from the Analysis screen.

2 Name and save the file in the desired location using the file selection window.

The scan results are separated into Plate, Results, and Controls sections, as can be seen in the figure below. The plate data is described in Table 19, the results data is described in Table 20, and the controls data is described in Table 21.

Figure 39 Exported File

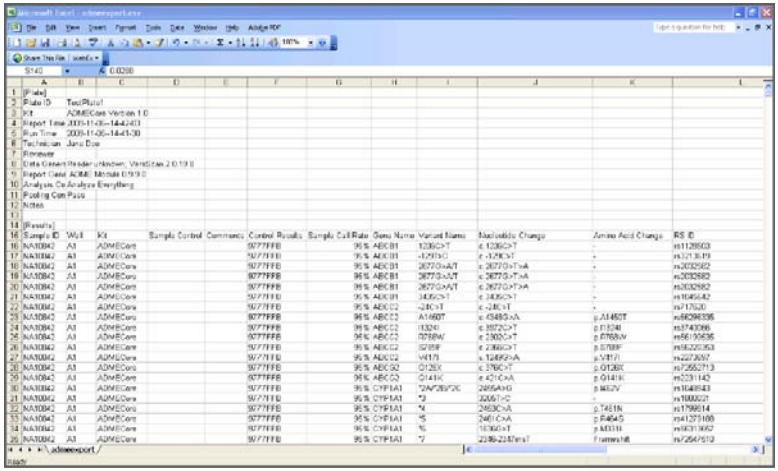


Table 19 Plate Section

Plate Field	Description
Plate ID	The plate ID
Kit	The kit name and version number
Report TIme	The date and time the report was generated
Run TIme	The date and time the report was run
Technician	The name of the user
Reviewer	This field is empty
Data Generated	The name of the BeadXpress Reader and the VeraScan software version
Report Generated	The version of the ADME module
Analysis Configuration	The name of the analysis configuration used

Table 19 Plate Section (Continued)

Plate Field	Description
Pooling Controls	**Pass** indicates the SPC controls passed
Notes	This field is empty

Table 20 Results Section

Column Header	Description
Sample ID	The sample ID from the sample sheet
Well	The well number row and column (e.g. A1)
Kit	The kit name
Sample Control	The control column value from the sample sheet (**Positive**, **Negative**, **Synthetic**, or empty)
Comments	Comments from the sample sheet
Control Results	Control result for the sample: Passed samples - displays STC barcode Failed samples - displays failure mode: **Failed PSC**, **Failed STC**, **Failed Mismatch**, or **Failed Hyb**
Sample Call Rate	Sample call rate
Gene Name	Gene name
Variant Name	Variant name
Nucleotide Change	Nucleotide change
Amino Acid Change	Amino acid change or functional change
RS ID	RefSeq ID of locus
Alleles (WT/Var)	Possible alleles

Table 20 Results Section (Continued)

Column Header	Description
Gene Result	A semi colon separated text string of all distinct variants detected for a given gene. The detected variant value is taken from comments section of the translation file. No Call results are appended to the end of the string. ***1A/*1A** - no variants were detected (genes with star nomenclature) **NMD** - genes without star nomenclature
Variant Call	Variant call result
GT Call	Genotype call result
Beads	Bead population for a locus in the well where the sample is assayed
Green Signal	Raw green signal intensity for negative control samples Normalized green signal intensity for positive control samples
Red Signal	Raw red signal intensity for negative control samples Normalized red signal intensity for positive control samples
Theta	Red fluorescence intensity to green fluorescence intensity ratio
R	Combined intensity
Scale	**Raw** - negative control sample **Normalized** - positive control sample

Table 21 Controls Section

Plate Field	Description
Sample ID	The sample ID from the sample sheet
Well	The well number row and column (e.g., A1)
Control Name	The control name indicating the analysis method and probe ID (e.g., STC (A512)) **PSC** for process control
Result	**Pass** or **Fail** for PSC and Functional and Hyb controls Generic AA, AB, BB result for STC controls

Table 21 Controls Section (Continued)

Plate Field	Description
Beads	Bead population (empty for PSC)
Green Signal	Raw green intensity for Functional and Hyb Normalized green intensity for STC Empty for PSC
Red Signal	Raw red intensity for Functional and Hyb Normalized red intensity for STC Empty for PSC
Theta	Red fluorescence intensity to green fluorescence intensity ratio (empty for PSC)
R	Combined intensity
Scale	**Raw** - Functional and Hyb **Normalized** - STC Empty - PSC

Exit Results

To conclude the scan and analysis session:

1 Click **Finished** on the Analysis screen and the Welcome screen is displayed.

2 Click **Open Tray** to open the BeadXpress Reader plate tray.

3 Take the VeraCode Bead Plate out of the BeadXpress Reader, discard it and perform one of the following:

- Scan a subsequent VeraCode Bead Plate, by inserting the plate into the BeadXpress Reader and repeating the *Scan VeraCode Bead Plate*, *Analyze Scan Data* and *Report Genotypes* procedures.
- Close the empty BeadXpress Reader plate tray by clicking **Close Tray** and log out of the VeraScan software and the BeadXpress Reader computer. If the BeadXpress Reader will no longer be used today, proceed to *Shutting Down the BeadXpress Reader System* on page 84.

Shutting Down the BeadXpress Reader System

To shut down the BeadXpress Reader system:

1 Do one of the following, depending on how long the Reader will be idle:
- If the BeadXpress Reader will be idle for ***less than 24 hours***, proceed to 2. You do not need to do anything with the fluidics system.
- If the BeadXpress Reader will be idle for ***more than 24 hours*** but ***less than two weeks***, click the Menu button in the upper-left corner of the screen and select **Reader | Purge Fluidics**.
- If the BeadXpress Reader will be idle for ***more than two weeks***, click the Menu button in the upper-left corner of the screen and select **Reader | Shut Down Fluidics**.

2 Close the VeraScan software by doing one of the following:
- Click the Menu button in the upper-left corner of the screen and select **Exit**.
- Click the Close button in the VeraScan title bar.

3 Shut down the computer.

4 Turn off the BeadXpress Reader by pressing the power switch on the back panel of the instrument.

NOTE

Leave the power off for at least two minutes before restarting the BeadXpress Reader.

Standard Operating Procedures

Appendix A

Best Practices

While performing the ADME Core Panel assay, you should always adhere to good molecular biology practices.

- When running two plates simultaneously, follow these best practices and alert your field application specialist (FAS):
  - It is important to plan ahead for optimal assay performance and optimal data quality
  - Perform washes and pipetting steps straight through from one plate to the next, to ensure the best timing.
  - Ensure that you have two heat blocks
  - Two magnets are required during pre-PCR. One is sufficient for post-PCR, but two are recommended.
- Do not use electronic or repeat pipettes. A manual pipette provides added control and leads to optimal assay results.
- Have tip boxes readily available.
- Optimal assay results are obtained when samples fall within the concentration and volume guidelines recommended in the protocol. It is important to ensure that equal volumes and concentration of DNA are applied to each reaction pool.
- When performing the denaturation procedures, be sure to stay within the times recommended. Prolonged exposure to NaOH can impact assay performance. To prevent prolonged exposure to NaOH prepare MTR and AOP mixtures in the pipette trough prior to applying NaOH to the reaction plate, to allow you to proceed with the MTR and AOP steps promptly at the conclusion of denaturation.
- When incubating the reaction plate on the heat block of the incubating shaker, promptly remove the reaction plate from the heat source at the conclusion of the step. Prolonged exposure to heat can impact assay performance.
- When performing the hybridization procedures, wash the plate promptly at the end of the 2.5 hour hybridization. A prolonged hybridization can impact assay performance.
- Take advantage of wait time during incubation steps to prepare reagents for the next step in the protocol.

Preventing PCR Product Contamination

The PCR (polymerase chain reaction) process is commonly used in the laboratory to amplify specific DNA sequences. Unless you exercise sufficient caution, PCR products may contaminate reagents, instrumentation, and samples, causing inaccurate and unreliable results.

PCR product contamination can shut down lab processes and significantly delay normal operations. The following sections outline practices that help reduce the risk of PCR product contamination.

Physical Separation of Pre- and Post- PCR Areas

The laboratory space where pre-PCR processes (DNA extraction, quantification, and normalization) are performed should be physically separate from the laboratory space where PCR products are made and processed (post-PCR processes).

Ideally, pre-PCR processes should be performed in a separate, dedicated laboratory space. For example:

- Never use the same sink to wash pre- and post-PCR reservoirs.
- Never share the same water purification system for pre- and post-PCR processes.
- Store all supplies used in the protocols in the pre-PCR area, and transfer to the post-PCR area as needed

Separate full sets of instruments (pipettes, centrifuges, incubating shakers, etc.) should be dedicated to pre- and post-PCR lab processes, and must never be shared between processes.

Daily and Weekly Bleaching

Use the following guidelines for daily and weekly bleaching of the pre- and post-PCR areas. Provide training for personnel responsible for cleaning the lab areas so that they know how to prevent pre- and post-PCR product contamination.

Post-PCR Area

Reducing the amount of product in the post-PCR area reduces the risk of contamination in the pre-PCR area.

CAUTION

You must establish procedures for preventing PCR product contamination before you begin work in the lab.

CAUTION

To prevent sample or reagent degradation, ensure that all bleach vapors that remain after cleaning have fully dissipated before starting any processes.

Identify post-PCR area "hot spots" that pose the highest risk of contamination and clean these items daily with a 10% bleach solution.

Typical hot spots include:

- Bench space used to process amplified DNA
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards
- Centrifuges
- Vortexers
- Thermal cyclers

Once a week, thoroughly bleach the entire post-PCR area, including bench tops and instruments that are not cleaned daily. Mop the floors with a 0.5% sodium hypochlorite (10% bleach) solution as well.

Pre-PCR Area

Establish a daily and weekly bleaching schedule for the pre-PCR area similar to the one in post-PCR. This helps to eliminate product that may have entered the pre-PCR area. Identify high-risk pre-PCR items such as the ones listed below, and clean them with a 0.5% sodium hypochlorite (10% bleach) solution each morning before beginning any pre-PCR processes:

- Bench tops
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards

Once a week, thoroughly clean all laboratory surfaces and instruments, including bench tops and instruments that are not cleaned daily. Mop the floors with a 0.5% sodium hypochlorite (10% bleach) solution as well.

Items Falling to the Floor

The floor is contaminated with PCR product transferred on the shoes of individuals coming from the post-PCR area; therefore, anything that has fallen to the floor should be treated as contaminated. Throw away any disposable items that fall to the floor, such as empty tubes, pipette tips, gloves, lab coat hangers, etc. Individuals handling anything that has fallen to the floor, disposable or not, must throw away their lab gloves and put on a new pair.

Non-disposable items that fall to the floor (such as a pipette, an important sample container, etc.) should be immediately and thoroughly cleaned with a 10% bleach solution to remove PCR product contamination.

NOTE

Be sure to clean any lab surface with which a contaminated item has come into contact.

Pipetting and Sealing

This section outlines recommended reagent pipetting and adhesive seal application techniques for the VeraCode ADME Core Panel Assay. Avoid using electronic or repeat pipettes. Specific pipetting techniques are known to improve the ability to process samples successfully. Some reagents can affect the adhesive microplate sealing film's ability to stay adhered during incubating shaking steps if they are exposed to the top surface of the PCR microplate. Pipetting techniques can also cause bubbles that can impact reagent performance or interfere with the adhesive seal's ability to stay adhered to the microplate. Proper technique to apply adhesive seals is especially important to ensure the seal stays adhered during the incubating shaking steps to avoid cross contamination or evaporation of samples.

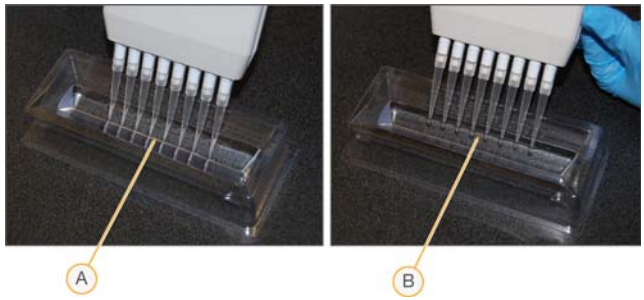
Aspirating from Reservoirs

When aspirating reagents from their respective reservoirs, it is important to not submerge the pipette tips to the bottom of the reagent reservoir. Dipping pipette tips into the bottom of the reservoir wets the pipette tips and can transfer excess reagents onto the top of the PCR microplate during dispensing, interfering with the adhesive seal's ability to adhere to the microplate.

If liquid is noticed on the top surface of the plate during processing, the top of the plate should be blotted dry with a Kimwipe to ensure the best possible adhesion between the top surface of the microplate and the seal. However, the composition of some reagents may interfere with the ability of the microplate seal to adhere to the top surface, even if the top of the plate is blotted dry with a Kimwipe prior to placing the seal.

The best technique is to pull ADME Core Panel reagents by placing the pipette tips just under the top surface of the reagent prior to aspiration. This technique helps draw the correct volume of reagent from the reservoir, with less liquid sticking to the exterior of the pipette tips.

Figure 40 Pipette Tip Placement



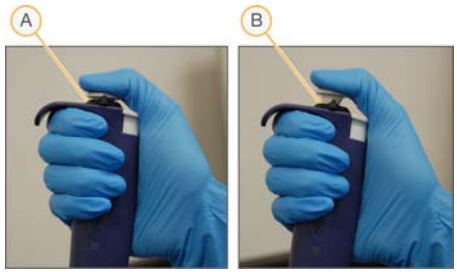
A Incorrect tip placement
B Correct tip placement

Dispensing Reagents

It is important to not introduce bubbles into the assay wells when pipetting the ADME Core Panelreagents. Illumina recommends that the pipette stopper is only moved to the first dispense stop and not to the hard stop. This empties all reagent out of the tips without introducing bubbles in the sample wells. This technique should be employed if excessive foaming is found when pipetting all ADME Core Panelreagents, but is especially important for the following:

- NaOH
- MTR4A, MTR4B, MTR4C
- AB1
- AOP4A, AOP4B, AOP4C
- ELM2
- MAM1

Figure 41 Pipette Stopper



A Incorrect - pipette hard stop
B Correct - pipette first stop

Sealing Microplates

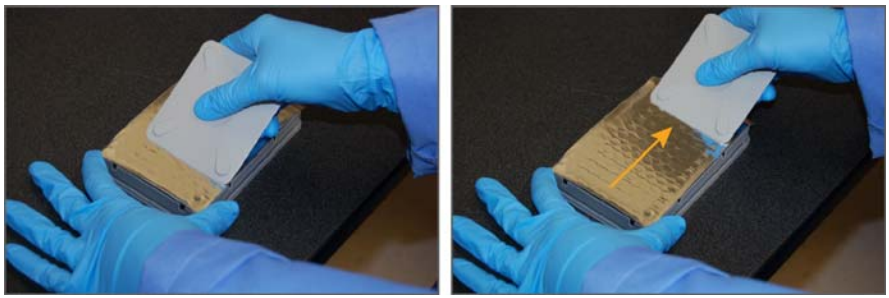
When applying adhesive seals to the PCR microplate, it is important that the right technique be used to ensure a good seal. This allows the wells to stay separated during the incubating shaking steps and avoids sample cross contamination. Illumina recommends that you have an adhesive seal applicator to apply force to the seal (see *User-Supplied Materials* on page 12). Proper PCR microplate sealing procedures are as follows:

1 Peel the backing from the adhesive seal and place the adhesive seal, sticky side down, gently onto the PCR microplate surface.

Figure 42 Adhesive Seal on Microplate Surface

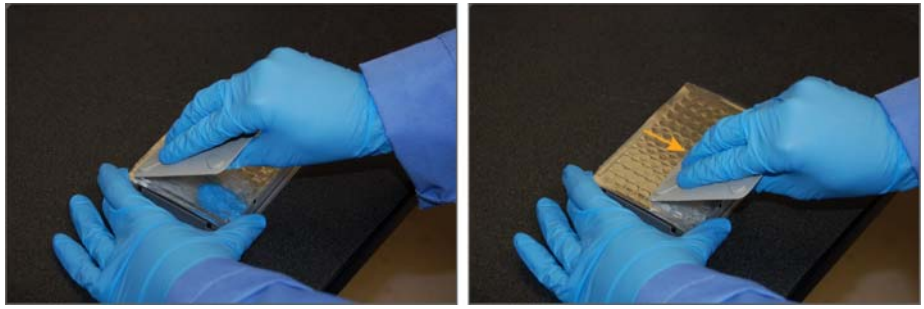
2 Hold the edge of the microplate with one hand and the adhesive seal applicator at an angle with the other hand. Press down with the edge of the adhesive seal applicator and swipe across the top of the PCR microplate, lengthwise, 2–3 times.

Figure 43 Swipe Adhesive Seal Applicator Over Length of Microplate



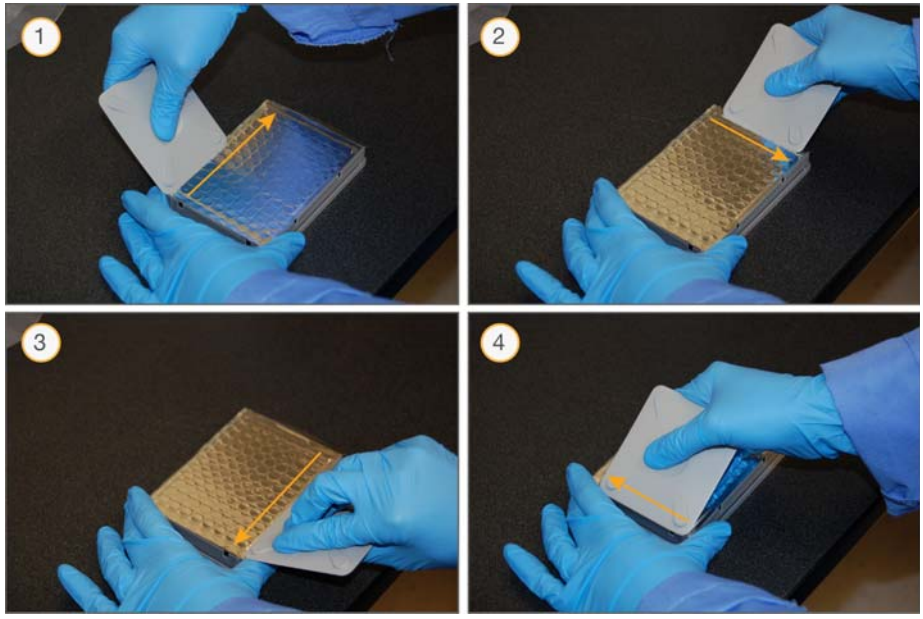
3 Press down with the edge of the adhesive seal applicator and swipe across the top of the PCR microplate, width wise, 2–3 times.

Figure 44 Swipe Adhesive Seal Applicator Over Width of Microplate



4 Press the edge of the adhesive seal applicator along each edge of the microplate to seal the edges in the order shown below.

Figure 45 Run Adhesive Seal Applicator Along Microplate Edge

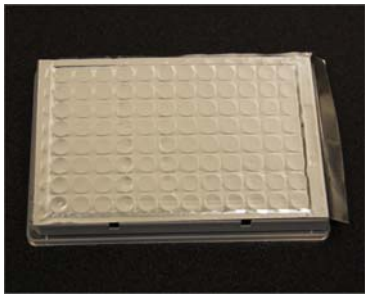


NOTE

Do not run the adhesive seal applicator between each row and column to separate each well. This reduces the seal around the well and can create small pockets where reagents can splash onto the plate surface during vortexing.

5 The microplate is now properly sealed.

Figure 46 Properly Sealed Microplate



VeraScan Administration and VeraReport

Appendix B

Introduction

General VeraScan administration is described in the VeraScan Administration chapter in the *BeadXpress Reader System Manual*. The VeraScan analysis configuration specific to the ADME Core Panel and instructions for regenerating a genotyping report specifically from ADME Core Panel results are described below.

Set Up VeraScan ADME Analysis Configuration

A VeraScan system administrator can set up ADME Core Panel analysis configuration templates. To start the VeraScan Program and access the analysis configuration tool:

1 Wait until the **Power** and **Ready** lights on the front panel of the BeadXpress Reader are the only lights on. See *Power Up the BeadXpress Reader Computer* on page 51.

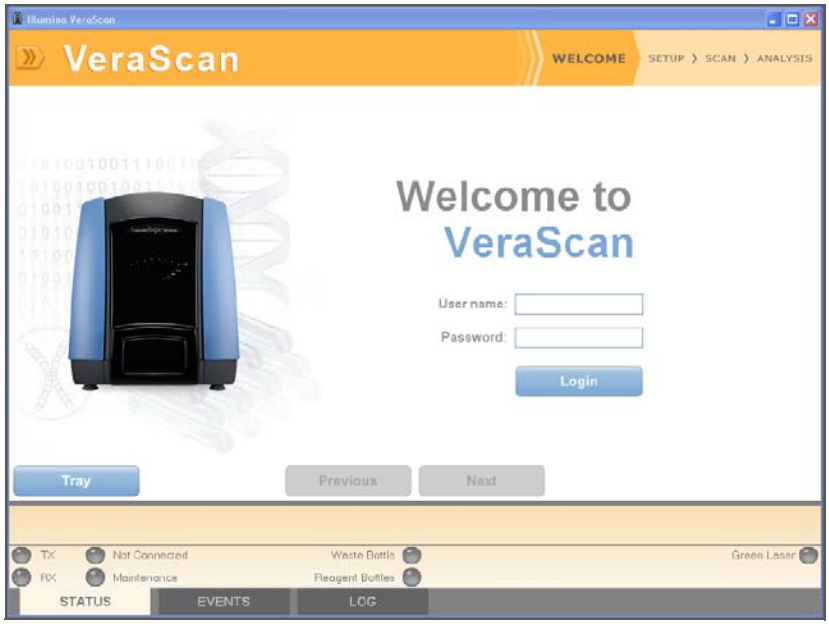
If you do not wait for this condition, an error message may appear when you start the software.

2 Do one of the following:

- From the Windows **Start** menu, select **All Programs** | **Illumina** | **VeraScan**.
- Double-click the VeraScan icon on the desktop.

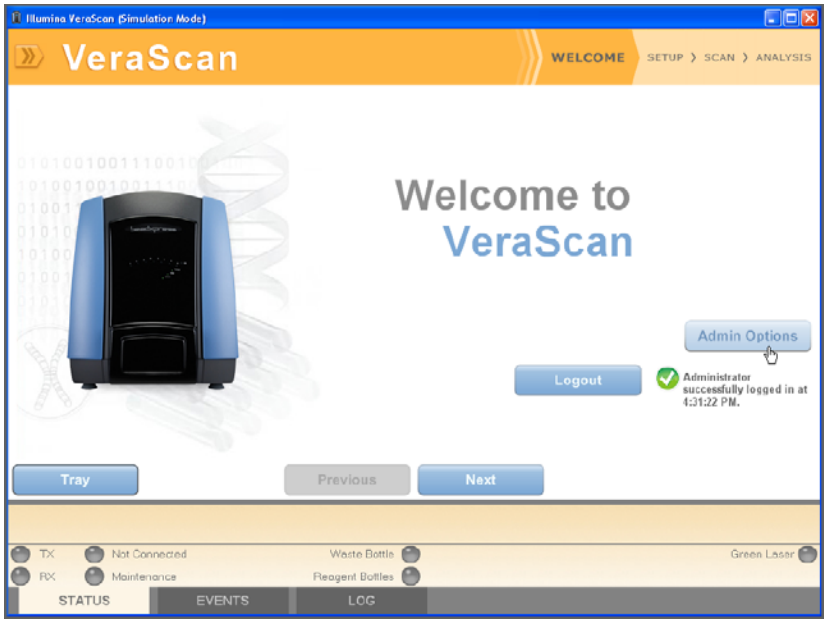
The VeraScan application opens on the computer desktop.

Figure 47 VeraScan Welcome Screen



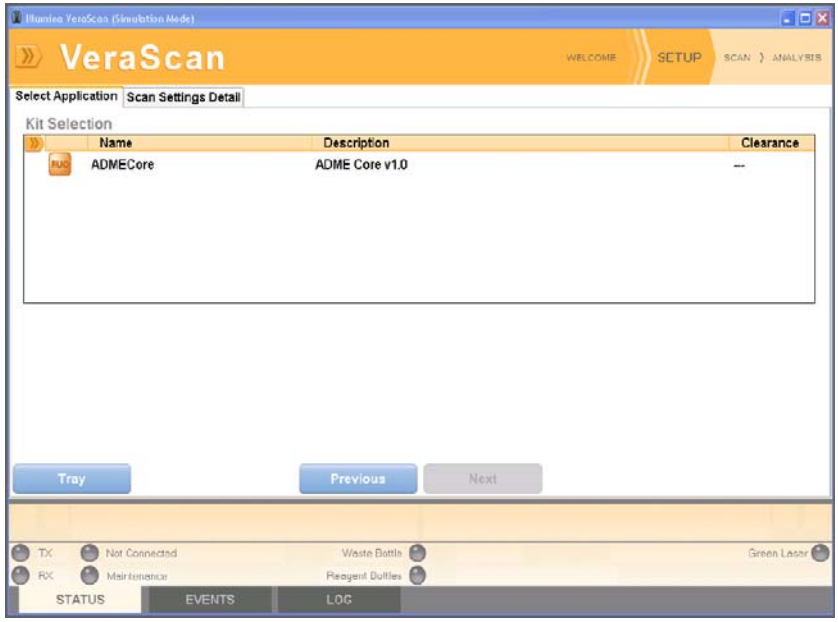
3 Enter your username and password, then click **Login**.

Figure 48 VeraScan Login



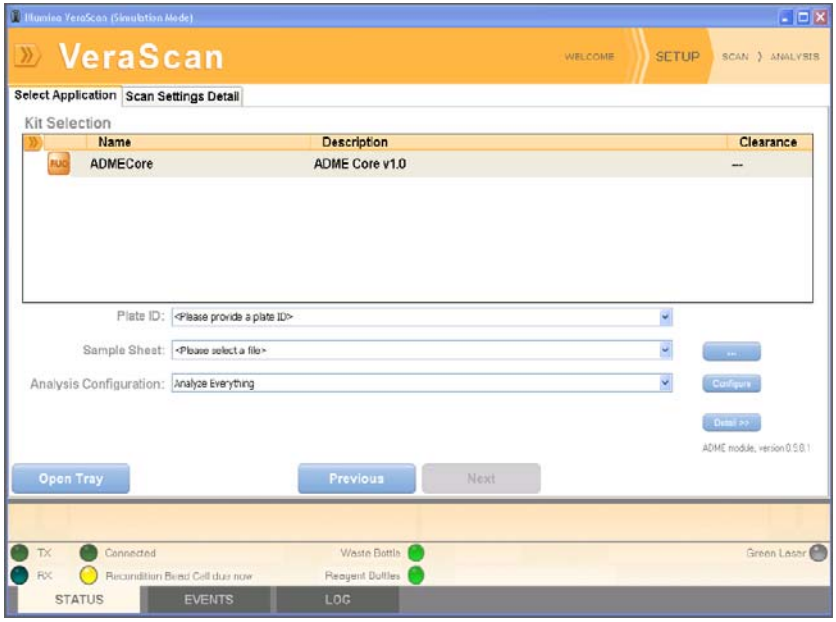
4 Click **Next**.
The VeraScan Setup screen displays the Select Application tab.

Figure 49 VeraScan Setup Screen



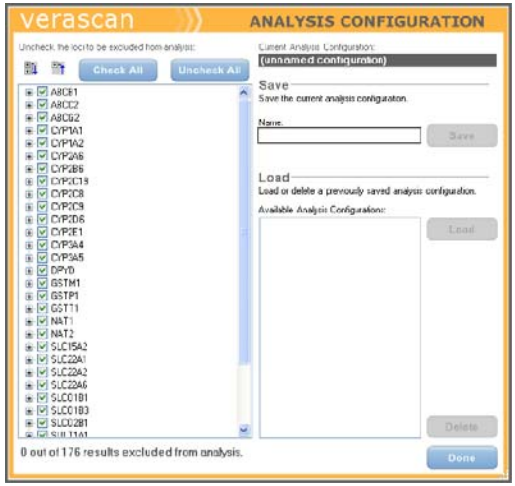
5 Select the **ADME Core** kit from the Select Application tab. The scan input data fields are displayed.

Figure 50 VeraScan Select Application Tab



6 Click **Configure** and the Analysis Configuration window is displayed. Do one or more of the following:

Figure 51 Analysis Configuration



- Uncheck the loci to be excluded from analysis. Click **Check All** to select all loci. Click **Uncheck All** to deselect all loci. Click the plus  icon to view loci details or click the minus  icon to close loci details.
- To save the configuration, enter the configuration **Name** and click **Save**. The configuration name is displayed as the Current Analysis Configuration and is added to the Load list.
- To load a previously saved configuration, select the configuration from the Load list and click **Load**. The configuration is shown in the list of checked and unchecked loci.
- To delete a previously saved configuration, select the configuration from the Load list and click **Delete**. The configuration is removed from the Load list.

7 Click **Done** to exit the Analysis Configuration window.

Regenerate Genotyping Report using VeraReport

VeraReport is a stand-alone application that provides the capability to review data generated by VeraScan and regenerate genotyping reports.The application does not connect to the BeadXpress Reader.

VeraReport supports the same report functionality as described in *Report Genotypes* on page 76, however the only changes that can be made to the report are to select the results to be included or excluded.

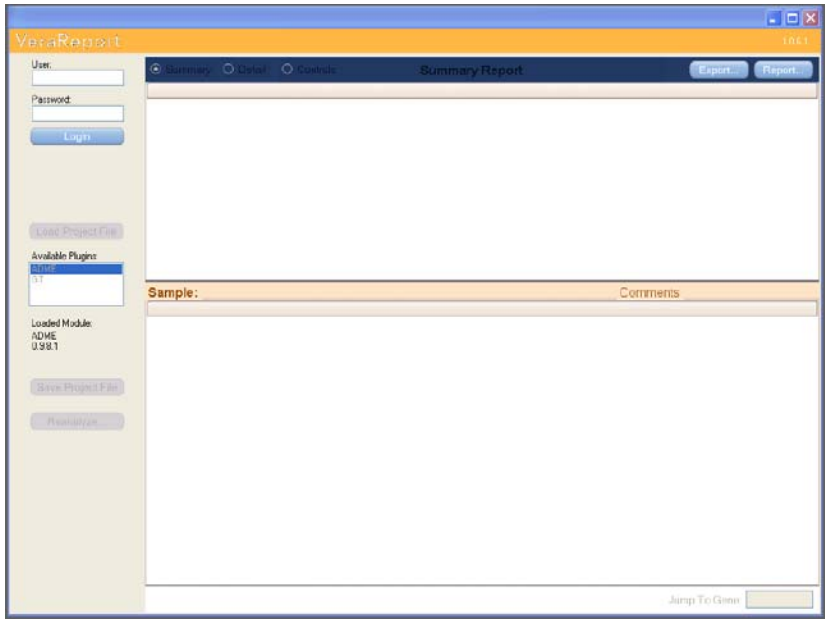
Preparation

In order to be able to review data and regenerate a genotyping report, you must have VeraReport and the corresponding ADME analysis modules installed locally. VeraReport and the ADME analysis modules can be downloaded via the Illumina website (See "Technical Assistance" on page 141.)

Start the VeraReport Program

1 From the Windows **Start** menu, select **All Programs** | **Illumina** | **VeraReport**, or double-click the VeraReport icon  on the desktop.
The VeraReport window opens on the computer desktop.

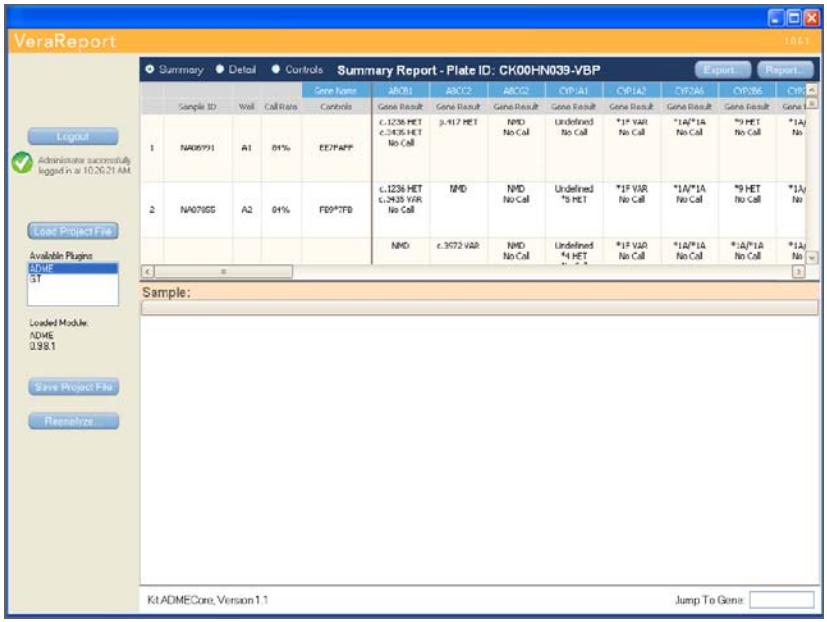
Figure 52 VeraReport Screen



1 Enter your VeraScan username and password and click **Login**.
The Available Plugins installed are listed.

2 Click **Load Project File** to select and view a BeadXpress project file (.bxp).

Figure 53 VeraReport Sample ADME Summary Report



3 To analyze and review the data see *Analyze Scan Data* on page 63.

4 Click **Save Project File** to save the BeadXpress project file (.bxp) with a new name and/or in a different directory from the original analysis.

5 To reanalyze the data, click **Reanalyze** from the VeraReport window. The Reanalyze Existing Data dialog box opens.

Figure 54 Reanalyze Screen

a To reanalyze the data using the default, original **Kit Manifest** and **Sample Sheet** click **Reanalyze**.

b To select different data for analysis:

- Click  to navigate to and select an alternate **Kit Manifest** to use to reanalyze the original scan data.
- Click  to navigate to and select an alternate **Sample Sheet** to use to reanalyze the original scan data.
- Click **Reanalyze**.

View Report

To view the report in PDF format, click **Report...** from the VeraReport screen. Reference *Reports in PDF Format* on page 76 for functional details.

Export Data

To export the report to .csv format for use in other commercial applications, such as Microsoft Excel, click **Export...** from the **VeraReport** screen. Reference *Export Data* on page 105 for functional details.

Exit VeraReport

Exit the VeraReport application by clicking **Logout,** then clicking **Close**  in the upper-right corner of the VeraReport window.

Part # 15007510 Rev. C

Microplate Shaker

Appendix C

Introduction

The incubating microplate shaker must be qualified and then programmed for automated use.

Qualification

The incubating microplate shaker must be qualified prior to programming and use. Follow these procedures to qualify the incubating microplate shaker.

CAUTION

Programming the incubating microplate shaker must be performed exactly as described in the following procedures to ensure optimal assay performance. Incorrect programming can lead to failure of the ADME Core Panel assay. Please contact your Field Applications Scientist or see *Technical Assistance* on page 141 prior to programming your unit.

Equipment

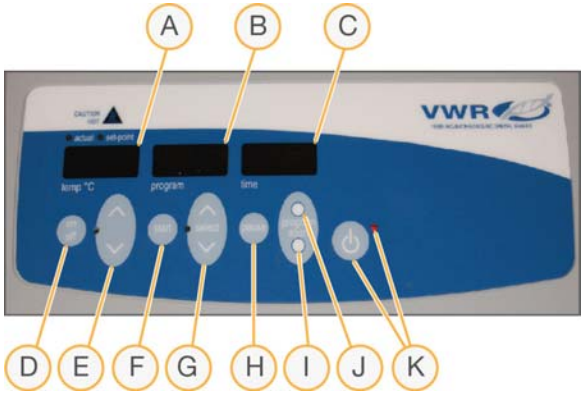
The following user-supplied equipment is required to qualify the incubating microplate shaker:

- Fluke thermometer with thermocouple
- Extech Photo Tachometer Stroboscope

Shaker Controls

Reference the following figure when performing the qualification procedures.

Figure 55 Incubating Shaker Control Panel



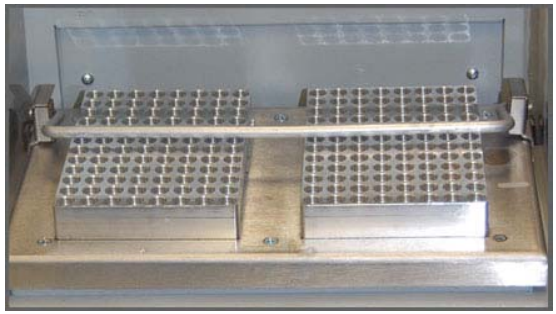
A Temp °C (temperature) display
B Program display
C Time display
D Temperature display on/off button
E Temperature Up/Down buttons
F Speed start button
G Speed select buttons
H Time pause button
I Abort -button
J Program button
K Main on/off and standby indicator light

Power On Shaker

1 Power on the incubating microplate shaker by pushing the main on/off button.

2 Lower the 96-well plate retaining bar onto the incubation blocks.

Figure 56 96-Well Plate Retaining Bar on Incubation Blocks



Qualify Shaking Speed

1 Press the main on/off button to place the unit in standby mode. The standby indicator light turns red and the displays are blank.

2 Press and hold the **select** down arrow and **abort** at the same time. While pressing these two buttons, quickly press and release the main on/off button. Only the **program** display should be visible. If all three display screens are on, press the main on/off button to turn the unit off and repeat step 2.

NOTE

If the **program** displays a program step number instead of rpms, exit to standby mode by pressing **start**, then **abort**.

3 Display the speed setting by pressing the speed **start** button.

4 Set the shaking speed display to 1,400 rpm using the speed **select** up/ down arrows.

5 Allow the system to run for at least 1 minute.

6 Check for any mechanical interference or error codes.

- Any rattling or ticking sounds may indicate a loose screw on the tray, tray attachment, or any accessory.
- If there is any mechanical interference that can not be resolved contact your VWR representative.
- If an error code is displayed, reference the following table to resolve the problem.

Table 22 Incubating Shaker Error Codes

Code	Software Test	Cause	Solution
E01	N/A	Lid open or temperature over 100°C	Switch the unit off and contact your VWR representative.
E02	N/A	Lid open or temperature over 0°C	
E03	Drive system failure	• Loose foot (suction cup) • Mechanical obstruction • Ceased bearing • Drive belt broken	If loose foot, press main on/off button. Adjust foot, then restart. If the error persists, switch the unit off and contact your VWR representative. For all other causes switch the unit off and contact your VWR representative.
E04	Unit overload	• Maximum load exceeded • Loose foot (suction cup)	Press main on/off button. Be sure the load is within the maximum load specification before restarting. If the error persists, switch the unit off and contact your VWR representative.
E06		Program mode was interrupted by loss of power	Unplug unit and reapply power.

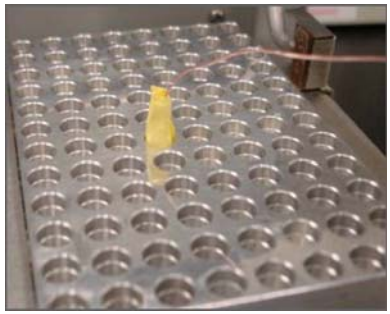
- If there is an error code that can not be resolved contact your VWR representative.

7 When the shaking speed has been qualified press the speed **start** button to turn off the speed display.

Qualify Temperature

1 Place the probe from a calibrated thermometer in one of the center wells of the right incubation block, so that the tip of the probe makes contact with the base of the well.

Figure 57 Temperature Probe



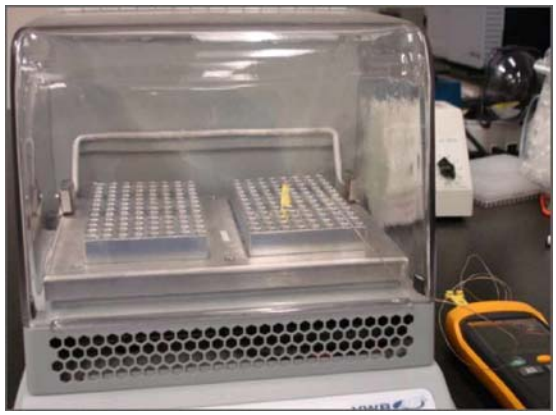
2 Turn on the thermometer if it is not already on.

3 Display the temperature setting by pressing the temperature display **on/off** button.

4 Set the temperature display to 45°C by using the temperature up/down arrows.

5 Close the lid and wait for the set temperature to stabilize.

Figure 58 Incubating Microplate Shaker Lid Closed



6 The readout on the calibrated thermometer should be within +/- 0.5°C of the target temperature. If the target temperature is not within +/- 0.5°C of the actual temperature then the incubating microplate shaker will have to be calibrated.

7 To calibrate the incubating microplate shaker, wait until the temperature has stabilized, then hold down the main on/off button and press temperature up arrow.

8 Press the temperature up/down arrows to specify the calibrated thermometer temperature, the press the main on/off button.

9 Allow the incubating microplate shaker's temperature to stabilize again and verify that the set temperature reaches the target temperature according to the calibrated thermometer +/- 0.5°C. If it does not reach the target temperature, repeat steps 7–9.

10 Repeat steps 1–9 for the left incubation block 45°C target temperature.

11 If after setting the right incubation block to 45°C and the left incubation block is not 45°C +/-0.5°C, and the system can not be calibrated to split the difference between the 2 blocks so that the real temperature measure by the calibrated thermometer is 45°C +/-0.5°C for both left and right incubation blocks, contact your VWR representative.

12 Repeat steps 1–9 for the right incubation block 68°C target temperature.

13 Repeat steps 1–9 for the left incubation block 68°C target temperature.

14 If after setting the right incubation block to 68°C and the left incubation block is not 68°C +/-0.5°C, and the system can not be calibrated to split the difference between the 2 blocks so that the real temperature measure by the calibrated thermometer is 68°C +/-0.5°C for both left and right incubation blocks, contact your VWR representative.

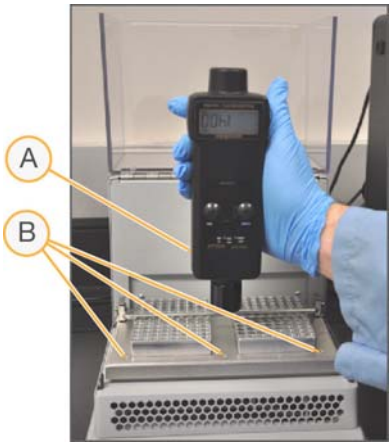
Calibrate Speed

1 Display the current incubating microplate shaker speed by holding down the main on/off button and pressing the speed **start** button once. The speed will flash in the **program** display.

2 Measure the speed of the incubating microplate shaker with a photo tachometer stroboscope.

3 Point the strobe to one of the screws on the incubating microplate shaker. Adjust the course and fine adjustment knobs on the stroboscope until the screw stops rotating and appears stationary.

Figure 59 Photo Tachometer Stroboscope Readout



A Stroboscope
B Screws

4 Press the speed **select** up/down arrows to match the setting of the stroboscope.

5 Press the main on/off button to save the calibration. The standby indicator light turns red and the displays are blank.

Set Cooling Ramp Rate

1 With the system on standby, press and hold the speed **select** down arrow ***and*** the **abort** button and press the main on/off button. The system is now in **std** (standby) mode.

2 Return to standby mode by pressing the main on/off button. This saves **std** mode.

3 Hold the temperature on/off button and press and release the main on/off button.

4 Use the temperature up/down arrows to scroll through the ramp rates on the temperature display until **r2** is displayed.

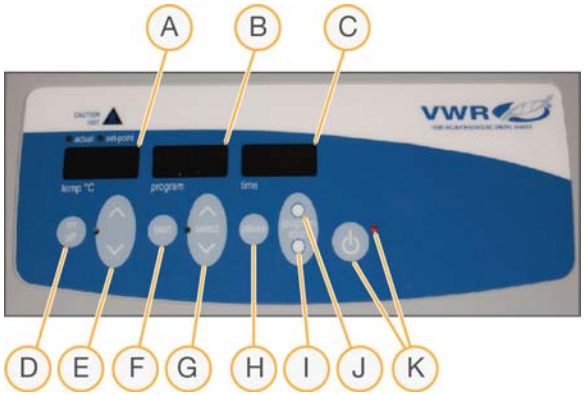
5 Press the main on/off button to save the cooling ramp rate.

6 Once the system has been qualified, power off the system by pressing the main on/off button.

Programming

The incubating microplate shaker must be programmed for automated use and to avoid potential ADME Core Panel protocol errors. Reference the following figure when performing the programming procedures.

Figure 60 Incubating Shaker Control Panel



A Temp °C (temperature) display
B Program display
C Time display
D Temperature display on/off button
E Temperature Up/Down buttons
F Speed start button
G Speed select buttons
H Time pause button
I Abort -button
J Program button
K Main on/off and standby indicator light

1 Press the main on/off button to place the unit in standby mode. The standby indicator light turns red and the displays are blank.

2 Press and hold the **select** down arrow and **abort** at the same time. While pressing these two buttons, quickly press and release the main on/off button. Only the **program** display should be visible. If all three display screens are on, press the main on/off button to turn the unit off and repeat step 2.

NOTE

If the **program** displays a program step number instead of rpms, exit to standby mode by pressing **start**, then **abort**.

3 Press the **select** up arrow until **Pr2** is displayed on the **program** display. This is the memory position where the ADME Core Panel program steps will be stored.

4 Press **program** to edit the Pr2 memory position.

5 The **program** displays **2-01**, to indicate that you are editing step 1 of memory position Pr2. The ADME Core Panel uses 12 steps in memory position Pr2, with the final step being 2-12.

6 Press the temperature up/down arrows to adjust the temperature to 68°C.

7 Press **abort** to adjust the time down to 00:00, then press **start**.

8 The **program** display indicates the incubating microplate shaker speed. Use the **select** up/down arrows to set the speed to 1,400 rpm, then press **start**.

9 The **program** display indicates if the incubating microplate shaker is **On** or **Off**. Press the **select** up/down arrows to set the incubating microplate shaker to **Off**, then press **start**.

10 The **time** display indicates if the timer is **On** or **Off**. Press **program** or **abort** to set the timer to **Off**, then press **start**.

11 The **temp °C** display indicates if the temperature control is **On** or **Off**. Press the temperature up/down arrows to turn temperature control **On**, then press **start**.

12 The **temp °C** display indicates **Phet= On** or **Off**. Press **program** or **abort** to set **Phet= On**, then press **Start**.

13 The **temp °C** display indicates the ramp rate. Press the temperature up/down arrows to adjust the ramp rate to **R2**, then press **start**.

14 The **temp °C** display indicates **Beep= On** or **Off**. Press **program** or **abort** to set Beep= **On**, then press **start**.

15 The **temp °C** display indicates **End= On** or **Off**. Press **program** or **abort** to set End= **On**, then press **start**.

Programming **2-01** is now complete.

16 Press the **select** up arrow to set the **program** display to **2-02**.

17 Repeat steps 6–16 to program each of the 12 ADME Core Panel memory program steps for memory position Pr2. Reference the following table to program each memory program step:

Table 23 Incubating Microplate Shaker Memory Step Settings

Step	Temp (°C)	Timer (Min)	Speed (rpm)	Shaker	Timer	Temp Control	Phet	Ramp Rate	Beep	End
01	68	0:00	1,400	Off	Off	On	On	R2	On	Off
02	68	5:00	1,400	On	On	On	Off	R2	On	Off
03	68	5:00	1,400	On	On	On	Off	R2	On	Off
04	68	1:00	1,400	On	On	On	Off	R2	On	Off
05	68	5:00	1,400	On	On	On	Off	R2	On	Off
06	68	1:00	1,400	On	On	On	Off	R2	On	Off
07	45	15:00	1,400	On	On	On	Off	R2	On	Off
08	45	1:00	1,400	On	On	On	Off	R2	On	Off
09	45	1:00	1,400	On	On	On	Off	R2	On	Off
10	45	1:00	1,400	On	On	On	Off	R2	On	Off
11	45	20:00	1,400	On	On	On	Off	R2	On	Off
12	45	2:00	1,400	On	On	On	Off	R2	On	On

18 When programming steps 1–12 of memory position Pr2 is complete, press the main on/off button. The **program** displays **Pr2** and the **time** displays **USEd**.

19 Place the incubating microplate shaker in standby mode by pressing **start**, then **abort**.

20 Verify programmed steps:

a Repeat steps 1–4 to place the unit in standby mode.

b Press the **select** up/down arrows to scroll through the 12 Pr2 programmed steps and reference Table 23 to verify their settings. Press **start** at each step to verify all settings.

c When verification of all memory program steps for memory position Pr2 is complete, press the **Standby** button. The **program** displays **Pr2** and the **time** displays **USEd**.

d Press **start** to display the first step of Pr2. Press **start** again to initiate the program.

e Test all of the memory position Pr2 programmed steps one time with no samples present to fully verify the programming.

NOTE

The Step 7 cooling ramp-down should take 7–8 minutes. If the ramp down occurs much faster or slower, check your program settings.

21 To edit a memorized program step:

a Repeat steps 1–4.

b Press the **select** arrows to navigate through the program to the step that requires modification.

c Modify the step as needed.

d When the program modification is complete, press the main on/off button. The **program** displays **Pr2** and the **time** displays **USEd**.

22 To run the incubating microplate shaker, press **start** to display **Pr2** in the **program** display.

NOTE

When the incubating microplate shaker is turned off with Pr2 selected, the next time it is turned on it will display the Pr2 Step 1. Press start to initiate the first step of the program.

NOTE

For general operating instructions, reference the *Troemner Incubating Shaker Manual*.

Microplate Shaker

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Troubleshooting

Appendix D

Assay Protocol Troubleshooting

This section provides solutions to issues that may appear when assessing the quality of each run to determine the validity of the outcomes generated from the ADME Core Panel. Issues fall into the following general categories:

- *Sample Processing*
- *VeraCode Bead Hybridization*
- *Data Quality*

Sample Processing

This section addresses causes and resolutions for potential processing issues.

Table 24 Troubleshooting Problems During Sample Processing

Symptom	Probable Cause	Resolution
Evaporation of corner wells after PCR.	Poor plate sealing during pre-PCR processing or in the thermocycler.	Ensure the edges of the ARX plate are well sealed. See "Sealing Microplates" on page 92.
Incomplete resuspension of paramagnetic beads.	Overspeed centrifugation.	Check centrifuge speed setting. The assay is not normally affected by incomplete resuspension.
	Beads left overexposed during wash steps when supernatant removed.	Complete reagent transfers as quickly as possible.
Incomplete capture of paramagnetic beads.	Poor position of ARX plate on magnet or insufficient time.	Position the ARX plate with the wells between the bars of the magnet.

Table 24 Troubleshooting Problems During Sample Processing (Continued)

Symptom	Probable Cause	Resolution
Splashing of samples out of wells after shaking steps.	Poor application of adhesive plate seal.	Use seal applicator to ensure seal is covering all wells and recommended sealing techniques described in *Sealing Microplates* on page 92.
	Shaking incubator is out of calibration and is not shaking correctly.	Perform routine calibration of the shaking incubator.
Not enough volume in the AOP tubes to process samples.	Missing addition of AOP0 reagent to individual AOP4 tubes.	Repeat assay and add AOP0 to individual AOP4 tubes.
Seal does not stay adhered to plate during incubation steps.	Poor application of adhesive plate seal.	Follow recommended seal application technique. See "Sealing Microplates" on page 92.
	Reagents wicking onto plate surface from pipette tips during dispense steps.	Dab top of plate with a Kimwipe before placing seal on plate and follow recommended pipetting techniques. See "Dispensing Reagents" on page 91.

VeraCode Bead Hybridization

This section addresses causes and resolutions for potential issues while hybridizing the VeraCode beads.

Table 25 Troubleshooting Problems During VeraCode Bead Hybridization

Symptom	Probable Cause	Resolution
VeraCode beads are not in the wells.	Insufficient centrifugation.	Centrifuge the VeraCode Bead Plate before unsealing.
	Inadvertent removal of VeraCode beads by touching with pipette tips.	Keep tips above VeraCode beads when pipetting MSS reagent.
	Rapid removal of seal causes bead pellet to flip out of wells.	Slowly and carefully remove cap mat.

Data Quality

This section addresses causes and resolutions for potential data quality issues.

Table 26 Troubleshooting Problems with Data Quality

Symptom	Probable Cause	Resolution
Very low signal intensity for all bead types except hybridization controls.	Titanium Taq DNA polymerase was omitted from MAM1 tube.	Repeat assay with Titanium Taq DNA polymerase.
	Very low DNA input.	Re-extract DNA.
	Incorrect PCR program selected.	Repeat assay and check program.
	Plate failure during PCR.	Repeat assay.
Low red signal for all bead types.	Cy5 degradation due to excess bleach fumes or overexposure to light.	Protect MAM1 reagent from bleach and light.

Table 26 Troubleshooting Problems with Data Quality (Continued)

Symptom	Probable Cause	Resolution
Low signal reported for Sample Tracking Control output - no data outputted for sample.	Cross contamination of at least one of the three wells of sample or incorrect sample location on the plate.	Ensure proper distribution of samples to the ARX plate before starting the assay protocol.
	Poor sealing of plate during vortexing steps lead to sample cross-contamination.	Follow recommended plate sealing techniques. See "Sealing Microplates" on page 92.
Strong signal from the no-template control samples.	Cross-contamination may have occurred.	Take care to avoid PCR amplicon contamination. For example, treat lab work surfaces with 10% bleach and allow them to air-dry.
	Poor sealing of plate during vortexing steps lead to sample cross-contamination.	Follow recommended plate sealing techniques. See "Sealing Microplates" on page 92.
	Pipetting error.	Pipette carefully and according to procedures.
	Splashing during vortexing.	Take care to avoid splashing.
	BeadXpress Reader needs routine cleaning.	Run KOH cleaning. Reference the section on Maintenance in the BeadXpress Reader System Manual.
Poor Fluorescence-BeadXpress Reader producing low-intensity values.	Optical system out of alignment.	Re-initialize the BeadXpress system. If the issue persists, contact Illumina Customer Support.
Poor Fluorescence - low signal result for a majority of loci.	Thermocycler settings were set incorrectly.	Adjust thermocycler settings according to the assay protocol.

Table 26 Troubleshooting Problems with Data Quality (Continued)

Symptom	Probable Cause	Resolution
Low call rate on a small number of loci over multiple runs.	Possible drift in temperature calibration of thermocycler, incubating shakers, or heat block.	Re-check calibration of thermocycler, incubating shakers, or heat block.
Data failed to generate in VeraScan due to SPC failure.	MTR or AOP reagents were added to the wrong column on the plate or location of AOP reagents on plate were out of order (not A, B, then C).	Label ARX plate according to assay protocol and only handle one MTR or AOP subset at a time.
	VeraCode Bead Plate placed into BeadXpress Reader in the wrong orientation.	Place VeraCode Bead Plate into BeadXpress Reader in the correct orientation according to assay protocol.
	BeadXpress Reader was not properly balanced.	Check test and calibration log to verify scanner balance point. Re-run test and calibration beads to ensure BeadXpress Reader is balanced appropriately.
Expected outcome for samples do not match historical data.	Samples inputted into sample sheet incorrectly.	Reanalyze data using VeraReport and a correct sample sheet.
Failed Mismatch control generated during data analysis.	PCR or incubating shaker failure.	Check calibration of thermocycler and incubating shaker.
Mismatch Controls decrease in intensity and fall toward center of plot.	NaOH or MTR are expired or stored improperly.	Make fresh NaOH and repeat assay. Check reagent storage and expiration date.

Table 26 Troubleshooting Problems with Data Quality (Continued)

Symptom	Probable Cause	Resolution
High number of STC failures in a plate.	Poor sealing of plate during incubation steps led to sample cross-contamination.	Follow recommended plate sealing techniques. See "Sealing Microplates" on page 92.
	Incorrect thermocycler program used.	Check thermocycler program used for correct parameters.
	BeadXpress Reader had errors during scanning.	Contact Illumina Customer Support.
PSC error	Pool switching of MTRs and AOPs	
	Cross-contamination of samples during assay run (e.g. AB1 splashing between wells, pipette tips not properly changed between steps, paramagnetic particles accidentally transferred between wells during pipetting/washing.	Follow recommended pipetting techniques. See "Dispensing Reagents" on page 91.
	VeraCode beads were transferred between wells during VW2 wash.	
	VeraCode bead plate hybridization was longer than the specified 2.5 hour incubation time.	

Table 26 Troubleshooting Problems with Data Quality (Continued)

Symptom	Probable Cause	Resolution
High number of PSC errors in a plate	A critical processing step was not performed correctly or might have been missed	Determine the validity of the results obtained for unaffected samples, then reprocess the failed samples and possibly the samples that passed based on further investigation.
	Incubating microplate shaker may be out of calibration or the wrong thermocycler program was used.	Check the instrument programs and calibrations.

BeadXpress Reader System Troubleshooting

This section provides solutions to issues that may appear when using the BeadXpress Reader system and information about how to manage BeadXpress Reader errors.

Issues fall into the following general categories:

- *Data Generation and Storage*
- *Fluidics System*
- *BeadXpress Reader*
- *Test and Calibration Beads*

Data Generation and Storage

This section addresses causes and resolutions for potential data generation and storage issues.

Table 27 Troubleshooting Problems with Data Generation and Storage

Symptom	Cause	Resolution
Cannot find data files/no data files are created.	Network error may have prevented files from being created. NOTE: This only applies to networked BeadXpress Readers.	Use Windows Explorer or another application to verify network accessibility. If network errors exist, ask IT for assistance.

Table 27 Troubleshooting Problems with Data Generation and Storage (Continued)

Symptom	Cause	Resolution
Low bead representation.	The ADME Core Panel Core kit was not selected from the menu.	Repeat assay.
	VeraCode beads were not read properly because green laser is out of factory specification.	Contact Illumina Customer Support.
	Code read laser beam out of alignment.	Initialize the system. If issues persist, contact Illumina Customer Support.
	Plate not seated properly in the plate tray.	Reload plate in plate tray.
	Plate seal covering wells.	Remove plate seal and re–initialize the BeadXpress system twice.
	Air in the fluidics system.	Prime the fluidics system.
	Reagents may be in wrong reagent bottles.	Clean reagent bottles and fill with correct reagents.
	BeadXpress Reader fails to locate the same number of beads in the red laser scan as the green laser scan (data consolidation).	Contact Illumina Customer Support.
	Beads are not being loaded into the BeadXpress Reader in the expected position.	Contact Illumina Customer Support.
	VeraCode beads are not in the wells.	See *VeraCode Bead Hybridization* on page 124

Fluidics System

This section addresses causes and resolutions for potential fluidics system issues.

Table 28 Troubleshooting Problems with Fluidics System

Symptom	Cause	Resolution
Fluidics system won't prime properly.	Reagents may be in wrong reagent bottles.	Clean reagent bottles thoroughly and fill with correct reagents.
	Reagent bottles may be empty.	Refill reagent bottles.
	Read buffer concentration may be too high.	Remix read buffer per Illumina instructions.
	Fluidics tubes may be loose or not connected.	Check fluidics connections to bottles, reagent carrier, and BeadXpress Reader.
	Reagent flow to the BeadXpress Reader may be restricted.	Check to make sure the tubes are not crimped and that nothing is placed on top of the tubes.
Fluid leaking from BeadXpress Reader.	The waste bottle tube may be loose or not connected.	Check waste bottle fluidics connections to bottles, reagent carrier, and BeadXpress Reader.
	Reagent flow to the waste bottle may be restricted.	Check to make sure the waste bottle tube is not crimped and that nothing is placed on top of the tube.
	Internal sensors may be inoperable.	Contact Illumina Customer Support.
	Too much buffer in the well plate at the start of the scan.	Check assay protocols and well plate starting volumes.
Fluidics system obstructed.	Bead mass or foreign matter in fluidics lines or pumps.	Remove the blockage from the system. Reference the section on Maintenance in the BeadXpress Reader System Manual.

BeadXpress Reader

This section addresses causes and resolutions for potential BeadXpress Reader issues.

Table 29 Troubleshooting Problems with the BeadXpress Reader

Symptom	Cause	Resolution
BeadXpress Reader is not connected on VeraScan startup.	VeraScan is configured for manual connection to the BeadXpress Reader.	Select the configuration settings tab in the Administration interface and set the BeadXpress connection to automatic.
Cannot connect to BeadXpress Reader.	Cable connection between BeadXpress Reader and PC may be unplugged.	Inspect the connection between the BeadXpress Reader and the BeadXpress Reader computer to confirm that the cable is securely plugged in.
	The BeadXpress Reader may not be powered up.	Power up the BeadXpress Reader.
System reports mechanical error, will not scan.	BeadXpress Reader detects a possible mechanical error and immediately disables all motors.	If there is no apparent physical problem, either re–initialize the BeadXpress Reader, or cycle the power. To re–initialize the BeadXpress Reader, right-click the BeadXpress logo and select **Reader**	**Initialize System**.

Test and Calibration Beads

This section describes what to do if the BeadXpress Reader does not pass the test and calibration cycles.

Table 30 Troubleshooting Problems with Test and Calibration Beads

Symptom	Cause	Resolution
BeadXpress Reader does not pass Test and Calibration Cycle.	Too few beads in the Test and Calibration column.	Spin down the Test and Calibration plate and rerun the application with a new column.
	Test and Calibration beads exposed to out-of-specification conditions.	Rerun the application with new Test and Calibration beads.
	BeadXpress Reader is out of specification.	Contact Illumina Customer Support.
	Incorrect values inputted for target red and green counts.	Review Test and Calibration log in the BeadXpress Calibration folder and rerun Test and Calibration.

View and Report Errors

The VeraScan application records system events, messages, and errors as they occur in an event log. The event log provides a record of system events that you can view or email to Illumina Customer Solutions for evaluation if an error occurs. If an error occurs, you can view error details in the error message box as well as on the Events tab.

Viewing Error Details as they Occur

If an error occurs while using the BeadXpress Reader system, an error message is displayed.

For information on accessing information about system events (errors and warnings), see the *Viewing Events* section. For information about diagnostic messages, see the *Viewing the Log* on page 135. For information about sending error details to Customer Solutions, see the *Reporting Errors* on page 135.

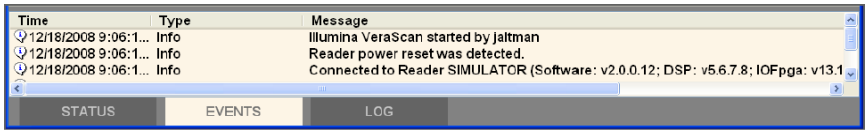
Viewing Events

The Events pane displays errors that have occurred during the current session, including the time the event occurred, the event code, and a description of the event. The Events pane also displays major system actions and warnings.

1 Click **Events** at the bottom of the screen (Figure 61).

The Events pane is displayed.

Figure 61 Events Pane



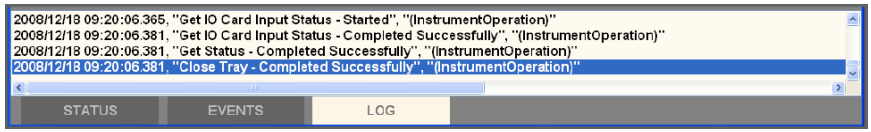
2 Double-click any icon in the Events pane to view more information about that event or warning.

Viewing the Log

As the BeadXpress Reader records images and the software runs, system diagnostic messages are recorded in a system log and saved to a file named VeraScan.log.

1 Click **Log** at the bottom of the screen (Figure 62).
The Log pane is displayed.

Figure 62 Log Pane



2 To pause the Log display, click any line other than the last one. The display stops scrolling until you re-select the last line (press CTRL+END).

Reporting Errors

If an error occurs, call your Illumina Customer Support contact or email a description of the error and attach the BeadXpressLog.txt and ErrorsLog.txt files with a time stamp close to the time of the error (but after the error occurred).

The most recent log files are automatically saved in the Execution Logs folder. All log files use the following naming convention:

BeadXpressLog20091109-232403.txt, where the date (20091109) is year/month/day and the time (232403) is hour/minute/second.

Frequently Asked Questions

For answers to frequently asked questions (FAQs), go to http://www.illumina.com.

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Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 31 Illumina General Contact Information

Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

Table 32 Illumina Customer Support Telephone Numbers

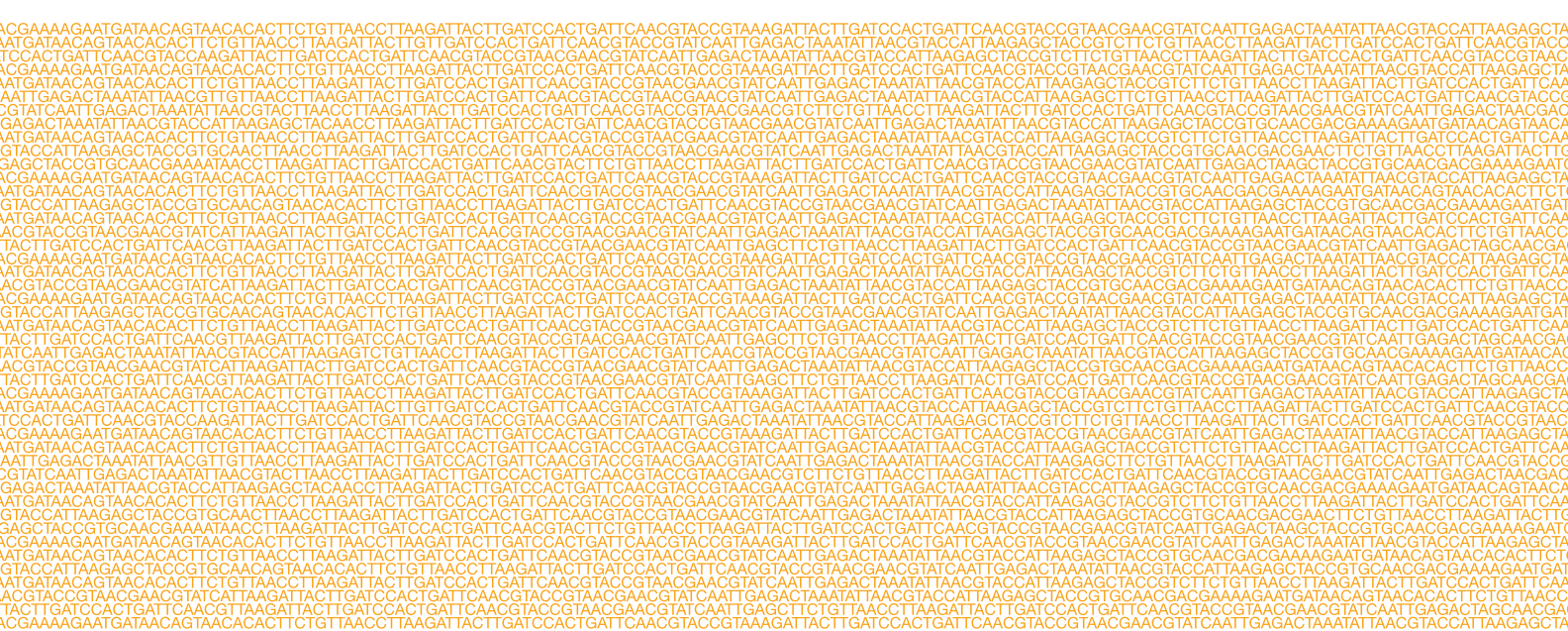
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994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

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at http://www.illumina.com/msds.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to http://www.illumina.com/support/documentation.ilmn. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF. To register for an iCom account, please visit https://icom.illumina.com/Account/Register.



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