



MassARRAY® User's Guide for PLEX™ and hME

SEQUENOM®

MassARRAY®

User's Guide for

iPLEX™ and hME

MassARRAY® Typer 3.4 Software User's Guide for iPLEX™ and hME

Document Number: 11546
Doc 11546, R03 CO 060094
June 30, 2006

Dear Customer,

Thank you for purchasing MassARRAY® from SEQUENOM®. This software was created specifically for use with the MassARRAY system and has been quality control tested to provide the most state-of-the-art algorithms to assist you in your research efforts.

Use this manual as a guide to assist you in mastering the features and tools of this software package. If for any reason you need assistance in using the features of the software or your MassARRAY system, contact your SEQUENOM Customer Support Scientist by phone or email.

Your institution's use of this software is governed by a Terms of Use clause. Please refer to this clause on page 155 of this manual to understand your institution's rights and responsibilities.

At SEQUENOM, we are continually focused on developing the best products to increase the research success of our family of customers.

SEQUENOM, Inc.
San Diego, California

"SEQUENOM's mission is to be the leading provider of genomic systems and knowledge for personalized medicine and the life science industry."

Corporate Headquarters
3595 John Hopkins Court
San Diego, CA 92121
Tel. (858) 202-9000
Fax (858) 202-9001
Sales (877) 4GENOME

US East Coast Office
189 Wells Avenue
Newton, MA 02459
Tel. (617) 244-8777
Fax (617) 868-4975

Asia Pacific Office
300 Herston Road
Herston, QLD 4006 Australia
Tel. (61) 7 3845 3683

European Office
Mendelssohnstrasse 15D, D-22761
Hamburg, Germany
Tel. (+49) 40-899676-0
Fax (+49) 40-899676-10

Copyright 2006. All rights reserved. No part of this publication may be reproduced, distributed, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, or stored in a database or retrieval system, for any reason other than a licensee's internal use without the prior written permission of SEQUENOM.

Printed in the United States of America.

TRADEMARKS

SEQUENOM, MassARRAY, and SpectroCHIP are registered trademarks of SEQUENOM, Inc. MassARRAY 20K, MassARRAY 200K, RealSNP, RealSNP.com, SNPCredits, and iPLEX are trademarks of SEQUENOM, Inc.

Autoflex is a registered trademark of Bruker.

MS DOS, Windows, and Microsoft are registered trademarks of Microsoft Corporation.

UNIX is a registered trademark of UNIX System Laboratories.

This page is intentionally blank.

Contents

Chapter 1 Introduction	MassARRAY System	1
	System Administrator	2
	Procedure Overview	3
Chapter 2 Defining Assays	Introduction	5
	Multiplex and Uniplex	5
	Assay Database Hierarchy	5
	AssayEditor Basics	6
	Opening AssayEditor	6
	To open the AssayEditor window	6
	Exiting AssayEditor	6
	To exit AssayEditor	6
	Navigating AssayEditor	7
	The Navigation Tree	7
	Assay Group tab	7
	To view items on the Assay Group tab	7
	To rename items on the Assay Group tab	7
	The Work Window	8
	Details tab	8
	Edit Assay tab	8
	Edit Group tab	8
	Importing Assays	8
	To import assays	8
	Searching for Assays	10
	To search for assays	10
	Creating and Editing Assays	10
	Creating Assays	10
	To create a new assay	10
	Adding Items to the Expected Peaks Grid	11
	To add probe, sequence, and sequence mass	11
	To add analytes	12
	To add contaminants	12
	Copying and Pasting Items in the Expected Peaks Grid	12
	Deleting Items from the Expected Peaks Grid	12
	Adding Genotype Calls	12
	To specify homozygous calls	13
	To specify heterozygous calls	13
	Viewing Grid Colors	13

To turn off visual aid colors	13
Copying Assays	14
Copying Assays for Editing	14
To edit assays associated with a design	14
Editing Assays	14
To edit assays	15
To edit description text	15
Deleting Assays	15
To delete an assay	15
To view design summary	16
 Editing Assay Groups	16
To edit a group	16
Plexes with Numeric Names	17
To reassign numeric plex names	17
 Managing Assay Projects	18
Adding Assay Projects	18
To add assay projects	18
Emptying and Deleting Assay Projects	18
To empty assay projects	18
To delete assay projects	18
 Managing SNPs	19
Opening SNP Manager	19
To open SNP Manager	19
Selecting SNPs	20
To select a SNP from the navigation tree	20
To select a SNP from the Members list	20
To select a SNP using Locate	20
 SNP Groups	20
Creating SNP Groups	21
To create a SNP group	21
Adding SNPs to SNP Groups	21
To add SNPs to a SNP group	21
Removing SNPs from SNP Groups	22
To remove a SNP from a SNP group	22
 SNPs	22
Creating New SNPs	22
To create a new SNP by modifying an existing SNP	22
To create a new SNP	22
Associating SNPs with Assays	23
To associate a SNP with an assay	23
Exporting SNPs	24
To export SNPs	24
Deleting SNP Groups	24
To remove a SNP	24
To delete a SNP group	24
 Moving, Copying, and Deleting Groups	24

Moving Groups	24
To move a group	24
Copying Groups	24
Deleting Groups	25
To delete assay groups	25
To delete locked definition assay groups	25
To delete SNP groups	25
Exporting Groups	26
To export a group	26
Chapter 3	
Defining Plates	
Introduction	29
Physical Plate	29
Plate Database Hierarchy	29
PlateEditor Basics	29
Opening the PlateEditor	29
To open the PlateEditor	29
Closing the PlateEditor	29
PlateEditor Overview	30
PlateEditor Window	30
Menu Bar and Toolbar	30
Message Bar	30
Status Bar	30
Plate tab	30
Assay tab	31
Sample tab	31
Plate Table	31
Plate Layout	31
Plate Properties	31
Right-Click Menu	31
Selecting Plates	31
To select a plate	32
To select an individual well	32
To select a contiguous group of wells	32
To select a non-contiguous group of wells	32
Creating Plates	33
Creating Plates with the New Plate Dialog Box	33
To create a new plate	33
Creating Plates using Templates	33
Creating Templates	33
Creating Plates Using Templates	35
To create a plate using a template	35
Creating Plates via Assay Groups	36
To create plates via assay groups	36
Creating Samples	37
To create a new sample group	37
Sample Order and Applying Sample Groups	39

To specify the sample direction	39
Sample Order	39
Empty Row	40
Repeated Samples	40
Importing Sample Names and Descriptions from Microsoft® Excel	41
To import sample names and descriptions from Microsoft Excel	41
Importing and Exporting Plate Table Information	42
To copy table data to Excel:	42
To import table data:	42
Finding Which Plates Contain a Sample Group	43
To view a list of plates to which a sample group is assigned	43
Editing Samples	43
Editing a Sample Group	44
To add a sample to a sample group	44
To edit a sample group	44
Deleting a Sample Group	45
To delete a sample group	45
Applying Assays and Samples to Wells	46
Assay and Sample Tree	46
To apply plexes, assays, and samples to wells	46
To apply samples to a region	48
Applying Sample Groups	49
To apply a sample group to a plate	49
Applying Sample Groups Using 4(96) to 1(384) Mapping	49
To apply a sample group using the 4(96) to 1(384) mapping	51
Applying Sample Group Mapping	53
Viewing Applied Assays and Samples	54
Working with Plates	54
Clearing Wells	54
To clear a well	54
Opening Plates	54
To open a plate	54
Copying Plates	54
To copy a plate	54
Deleting Plates	55
To delete a plate	55
Projects	55
To create a new project	55
To edit an existing project	56
Customers	56
To create a new customer	56

To edit an existing customer	56
Extend Primer Adjustment	56
To calculate the extend primer adjustment	57
Changing Layout Options	58
To save a layout	58
To load a layout	58
To restore the default layout	58
Chapter 4	
Acquiring Spectra	
Introduction	59
Overview of Acquiring Spectra	59
Starting the MassARRAY RT Software	60
On a Compact Workstation, to start the MassARRAY RT software	60
Using Chip Linker to Associate Chips with Experiments	60
Opening Chip Linker	60
To open Chip Linker	60
Associating SpectroCHIPs and Experiments	61
To associate SpectroCHIPs and experiments	61
Removing Plates from the Selection Table	62
To remove a plate from the selection table	62
Changing Plate Entries	62
To change plate entries	62
Loading SpectroCHIPs	62
Selecting the Number of Shots and Rastering Options	63
To select the number of shots and rastering options	63
Acquisition Parameters Values	64
Turning on the High Voltage	65
To turn on the high voltage	65
To automatically turn off the high voltage after the last SpectroCHIP	65
Setting SpectroCHIP Geometry Options	66
To set SpectroCHIP geometry options	66
Starting an Automatic Run	67
To start an automatic run	67
Unloading SpectroCHIPs from the Genotype Analyzer	70
Stopping an Automatic Run	70
To stop an automatic run	70
Saving Spectra	71
To save the most recently acquired spectra	71

Chapter 5 Reviewing Processed Data with TyperAnalyzer

Recalling Plate Data	71
To recall plate data	71
Autoteaching	72
Saving Parameters	73
To save parameters	73
Tools Menu	73
Quitting ACQUIRE	73
To quit ACQUIRE	73
Introduction	75
Screen Resolution	75
Genotyping	75
Allelotyping	76
Starting	
TyperAnalyzer	77
To start TyperAnalyzer	77
The TyperAnalyzer Window	77
Cluster Plot Pane	77
Spectrum Pane	77
Histogram Pane	78
Traffic Light Pane	78
Plate Data Pane	78
Well Data Pane	78
Status Bar	78
Selecting an Experiment	78
To select an experiment by customer	79
To select a chip by assay	79
To select a chip by date	80
Color Codes	80
Selecting Which Assays Are Shown	81
Viewing Relationships of a Currently Selected Cell	81
Viewing a Results Table	82
To view all calls	82
Viewing Spectra	83
Viewing the Spectrum from a Well	83
To view a spectrum showing all assays for a well	83
Using the Spectrum Display Cross-Hairs	84
Zooming the spectrum display	85
To zoom the spectrum display	85
To un-zoom the spectrum display	85
Viewing the Calibration Spectrum	85

To view the calibration spectrum	85
Adjusting the Spectrum Display	85
To adjust spectrum display	86
Printing a Spectrum Graph	87
To print a spectrum graph	87
Exporting a Spectrum	87
To copy a spectrum to the Clipboard	87
To save the spectrum to a file	88
To export spectrum graph data points	88
 Plate Data Tab Options	89
Manually Calling a Genotype	89
To manually call a genotype in the results table	89
Selecting and Sorting Data	89
To sort the table by a column in ascending order	89
To change the currently selected data	89
Copying the Grid	89
To copy the grid	89
 Viewing Assay Details for a Well	90
ASSAYINFO	91
CALLINFO	91
PEAKINFO	92
AREAINFO (Genotype+Area and Allelotyping only)	93
FREQUENCYINFO (Allelotyping only)	94
 Viewing Cluster Graphs	94
Yield vs Skew	95
Yield	95
Height	96
Log (Height)	97
Checking Assays for Quality	98
To view a cluster graph	98
Manually Calling a Genotype in the Cluster Plot Pane	99
To manually call a genotype in the Cluster Plot pane	99
To select a group of points	99
Printing a Cluster Plot Graph	101
To print a Cluster Plot graph	101
 Viewing Histogram Graphs	102
To view a histogram graph	102
Printing a Histogram	102
To print a histogram	102
 Recalling Plate Data	103
 Filtering the Results Table	103
To hide or show columns	103
 Importing and Exporting Wells	104
To export wells	104
To import a saved XML file	105

Copying Plate Data to the Clipboard	106
To copy plate data to the clipboard	106
Generating Reports	106
To generate a report	106
Customizing the Window Layout	110
To save the current window layout	110
To reset the window layout to the default layout	110
To load a saved layout	110
To add menu items to the tool bar	110
Customizing the Displays	110
To customize the title or subtitles	110
To customize the plot axis or style	111
Creating Subsets	111
To create a subset	111
Setting Fonts	111
To specify the font	111
Customizing Subsets, Points, and Axis Labels	112
To customize subsets, points, and axis labels	112
Customizing Colors	112
To customize colors	112
Customizing Styles	113
To customize colors	113
Accessing the Export Dialog Box	113
To access the Export dialog box	113
Logging Debug Messages	113
To turn on the Log Debug Message Option	113
Quitting	
TyperAnalyzer	113
To quit TyperAnalyzer	113
Chapter 6	
Reviewing	
Processed Data	
with Genotype	
Analyzer	
Introduction	115
Starting Genotype Analyzer	116
To start Genotype Analyzer	116
Finding and Selecting Data	116
By Project	116
By Test	117
By Date	117
Selecting Data in the Tree	117
Using the Results Table (Genotyping)	117
Color-Coding	117
Genotypes and Peaks	118
To view only the calls	119
To view the calls for all experiments on a plate	119
To view only the best call from the experiments on a plate	120

To view only the no calls	120
To view the no calls for all experiments on a plate	120
To sort the results table	120
To view information about a call	121
To view a history of calls	121
To view calibration and mass shift information	121
To hide calibration and mass shift information:	121
Using the Results Table (Genotype+ Area)	122
Using the Results Table (Allelotyping)	122
Color-Coding	122
Data Columns	122
Viewing Bad Spectrum Rows	124
Viewing Data for all Experiments on a Plate	124
To view data for all experiments on a plate	124
Viewing Detailed Assay Results	124
To view detailed assay results	124
Filtering the Results Table	125
To hide or show columns	126
Printing the Results Table	127
To print an entire table	127
To print from the Print Preview window	127
To print one row and the spectrum	127
Viewing Spectra	128
Splitting the Screen	128
To split the screen	128
Unsplitting the Screen	129
To unsplit the screen	129
Zooming	129
To vertical zoom in	129
To vertical zoom out	129
To horizontally zoom in on an area	129
To revert to the default size	129
Setting an Absolute Y-Axis Maximum	129
To set a y-axis maximum for the spectrum display	129
Viewing the Calibration Spectrum	130
To view the calibration spectrum	130
Viewing All Spectra	130
To view all spectra in succession	130
Generating Reports	131
To generate a report	132
Manually Calling a Genotype	135
To manually call a genotype in the table	135

Viewing a Pie Chart	135
To view a pie chart of selected data	135
Recalling Plate Data	136
Appendix A Reports	
Allelotype Report	137
Allelotype Correction Report	138
Assay Type Count Report	139
Best Call Probability Report	139
Call Probability Report	139
Description Count Report	139
Genotype Area Report	140
Creating a Skew Correction File	140
Skew Correction File	141
Plate Definition Report	141
Plate Result Report	141
Primer Adjustment Report	141
iPLEX	141
Adjusting iPLEX Primer Mixes	141
To adjust iPLEX primer mixes	142
MassEXTEND	143
Adjusting MassEXTEND Primer Mixes	143
To adjust MassEXTEND primer mixes	144
Appendix B Configuring the MassARRAY Software	
Starting the Configuration Software	147
Navigating the Configuration Interface	147
To navigate to the settings you want to modify	147
Configuring Plate Editor	148
Configuring General Settings	148
To configure general settings	148
Configuring the Grid	149
To configure the grid	149
Configuring Plex Settings	149
To configure plex colors	149
To configure plex layout	150
Configuring Sample Colors	150
To set the sample colors	150
Configuring TyperAnalyzer	150
Configuring the Display	150

To configure the display options	151
Configuring the General Options	151
To configure the general options	152
Configuring the Help	152
To configure the help file	152
Configuring the Program Layout	152
To configure the help file	152
Configuring Reports	153
To configure reports	153
Configuring the Traffic Lights	153
To configure traffic lights	153
Quitting the Configuration Software	154
To apply the changes and quit the Configuration Software	154
To quit the Configuration Software without saving the changes	154

Appendix C

Terms and Conditions

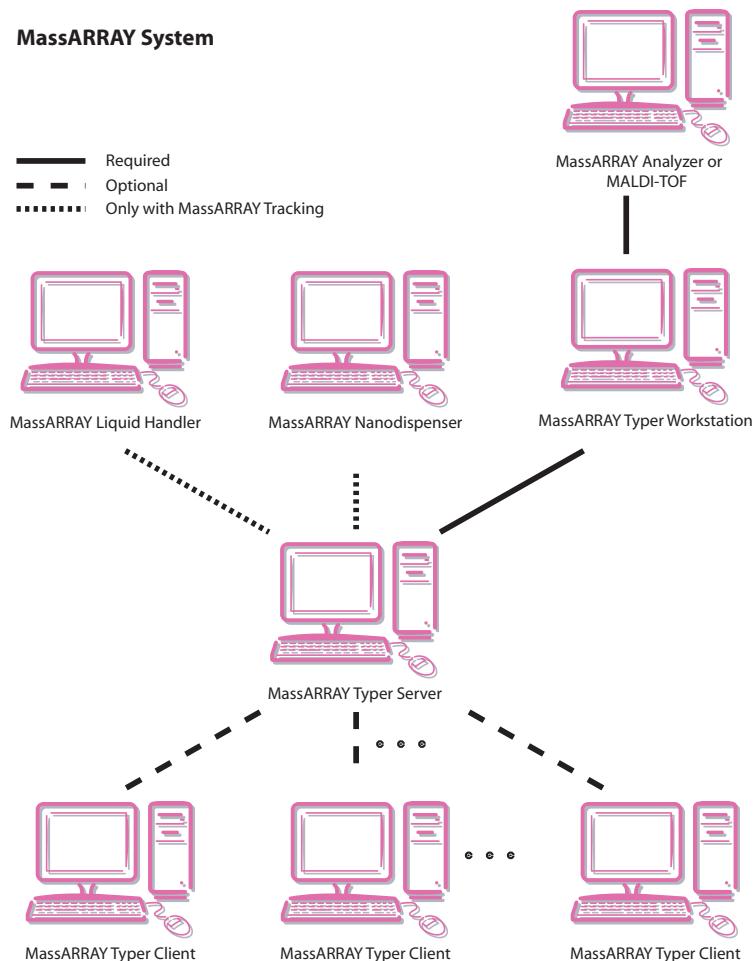
Chapter 1

Introduction

MassARRAY® Typer (Typer) is software for analyzing spectral data acquired from SpectroCHIPs. Typer analyzes each spectrum based on the assay or assays applied to it. An assay establishes where mass peaks are expected in a spectrum and how to interpret the presence of each peak. Based on the peaks present in a spectrum, Typer automatically identifies the genotype in genotyping experiments or estimates the relative frequencies of alleles in allelotyping experiments.

MassARRAY System

The following illustration shows how computers and instruments in the MassARRAY system are networked. An instrument (i.e. liquid handler, nanodispenser, and analyzer or analyzer compact) is represented by the computer that directly controls it. All computers are networked using TCP/IP.



There are three types of the Typer software: Server, Workstation, and Client (referred to as *Typer Server*, *Typer Workstation*, and *Typer Client*, respectively). Each type runs on a separate computer and serves different purposes. The computers themselves are identified as Typer Server, Typer Workstation, or Typer Client depending on which Typer software is installed.

A **Typer Server** is the informational “heart” of the MassARRAY system. It contains the *MassARRAY database* (an Oracle relational database management system). All data generated by the MassARRAY system is stored in this database.

A **Typer Workstation** controls the operation of the analyzer to acquire spectra from SpectroCHIPs. Spectral data is sent from the Typer Workstation to the Typer Server.

A **Typer Client** is used to set up experiments (e.g. create a plate definition specifying the samples in a physical microplate and the assays to be applied to those samples). It is also used to view and analyze spectral data. Any computer that is networked to a Typer Server (via TCP/IP) may be set up to be a Typer Client. For example, the computer at your desk may be set up as a Typer Client. You could then set up experiments and view data on your computer.

System Administrator

At least one person at your facility or company is designated as the *MassARRAY system administrator*. This person is trained and has the computer-access privileges to maintain the MassARRAY system and perform installations and upgrades. For questions about the MassARRAY system, especially issues relating to user IDs/passwords and installing Typer Clients, see your MassARRAY system administrator.

The *MassARRAY Typer System Administration Guide* contains instructions for many of the functions typically carried out by the MassARRAY system administrator.

Procedure Overview

The following table is a very brief outline of the main steps in using the MassARRAY system to process samples.

Table 1: Processing Samples

Step		Computer or Instrument	Notes
1	Define assays	Typer Server, Typer Workstation, or Typer Client	Use the Assay Editor module of Typer. An assay definition specifies the mass peaks you expect to see in spectra and how to interpret those peaks. See “Chapter 2 Defining Assays” on page 5.
2	Create a plate definition	Typer Server, Typer Workstation, or Typer Client	Use the Plate Editor module of Typer. A plate definition consists of a representation of the physical microplate of samples you intend to process plus assays you want applied to each sample. See “Chapter 3 Defining Plates” on page 29.
3	Amplify samples	In-house amplification equipment	Use your established amplification methods to amplify your samples. For PCR guidelines, see <i>MassARRAY Liquid Handler User’s Guide</i> .
4	Process the iPLEX or MassEXTEND reaction	MassARRAY liquid handler	Use the MassARRAY liquid handler to add iPLEX or MassEXTEND reagents to your amplified samples and process the reaction. See <i>MassARRAY Liquid Handler User’s Guide</i> .
5	Transfer iPLEX or MassEXTEND reaction products to a SpectroCHIP	MassARRAY nanodispenser	Use the MassARRAY nanodispenser to transfer your samples—which have been processed through the iPLEX or MassEXTEND reaction—to a SpectroCHIP. See <i>MassARRAY Nanodispenser User’s Guide</i> .
6	Acquire spectra	MassARRAY analyzer (use the MassARRAY Typer Workstation to operate the analyzer) or MassARRAY analyzer compact	Acquire spectra from the SpectroCHIP containing your processed samples. Spectral data is automatically sent to the MassARRAY Typer Server. Use the SpectroACQUIRE module of Typer to operate the analyzer. (The SpectroACQUIRE module is available only on a Typer Workstation.) See “Chapter 4 Acquiring Spectra” on page 59.
7	Analyze data	Typer Server, Typer Workstation, or Typer Client	Use the TyperAnalyzer or Genotype Analyzer modules of Typer. You can view spectra and genotype calls. You can also generate reports on the data. See “Chapter 5 Reviewing Processed Data with TyperAnalyzer” on page 75 or “Chapter 6 Reviewing Processed Data with Genotype Analyzer” on page 115.

Notes:

Chapter 2

Defining Assays

Introduction

Assay Design Software

For more information about Assay Design Software, see the *MassARRAY Assay Design Software User's Guide*.

The AssayEditor module is used to define assays and store them in a SEQUENOM database. In addition to manual editing of assays, AssayEditor allows for the importing and exporting of assay groups along with the associated SNP sequences and design parameters in accordance with the MassARRAY Assay Design Software (Assay Designer) file formats. AssayEditor also allows for the manual creation of subsets of (multiplexed) assays, called reference assay groups.

This chapter covers the following information:

- Basics of AssayEditor (See "Multiplex and Uniplex" on page 5 through "Exiting AssayEditor" on page 6 for information.)
- Working with Assays (See "Searching for Assays" on page 10 through "Editing Assays" on page 14 for information.)
- Working with SNPs (See "Managing SNPs" on page 19 through "Deleting SNP Groups" on page 24 for information.)
- Working with groups (See "Moving, Copying, and Deleting Groups" on page 24 through "Exporting Groups" on page 26 for information.)

Multiplex and Uniplex

An Assay is defined as the procedure that yields a single genotype outcome. Assays can be run together in the same reaction well to allow DNA sequences to be analyzed for multiple genotype determination, but each assay is still defined individually. Assays that are designed to be run together are referred to as *multiplexed assays*.

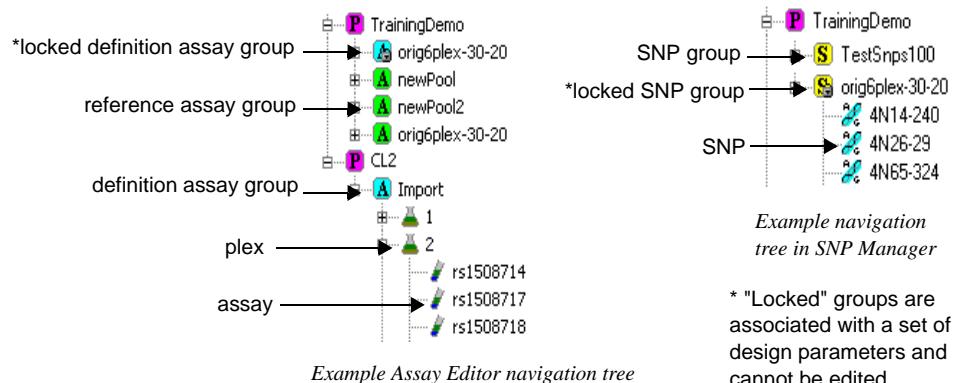
A run of a single assay is referred to as a *uniplex assay*. Generally, multiplexed assays may be separated into smaller multiplexes or uniplexes using AssayEditor or Plate Editor, but uniplex assays may not be multiplexed together without considering potential interactions of the reactants and peak overlaps in the resulting mass spectra.

Assay Groups

In the database, an Assay Group is synonymous with a "panel of assays," and can represent any logical grouping of Assays. For example, an Assay Group can group all the Assays relevant to a particular disease (e.g. Hypertension), or to a particular project, or to a particular gene or amplicon. The goal is to keep the Assay Group definition general enough so it can be used for any of these groupings.

Assay Database Hierarchy

In the MassARRAY Server database hierarchy, each Assay belongs to a Plex, which belongs to an Assay Group. Assay Groups and SNP Groups are stored in Assay Projects, which are the top level of the hierarchy. There are three types of assay group, as shown below.



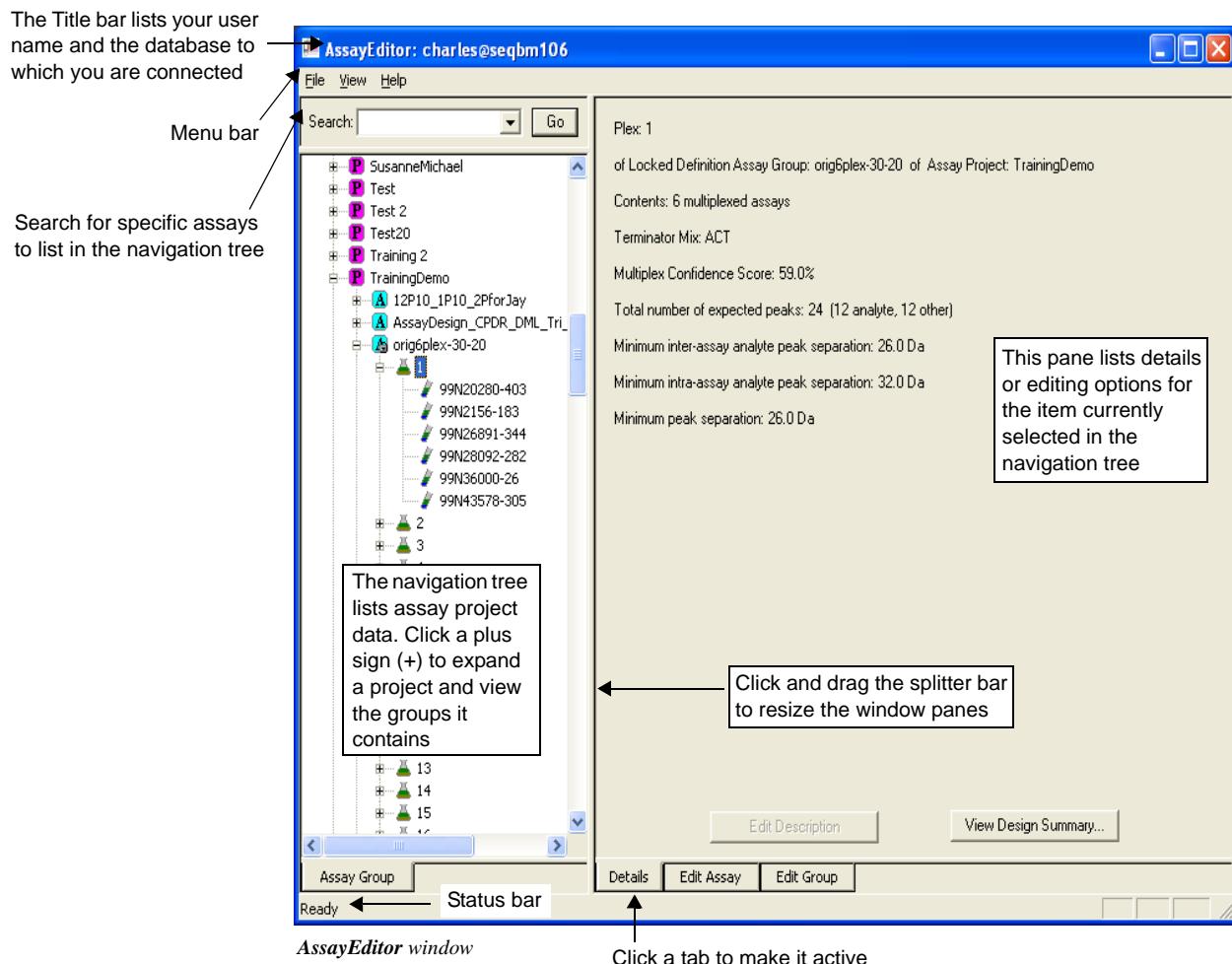
AssayEditor Basics

Opening AssayEditor

► To open the AssayEditor window

1. In the MassARRAY Typer window, click the **AssayEditor** button.
2. If you have not yet connected to the database, the **Connect to Database** dialog box opens. Enter the appropriate information.

Once connected, the **AssayEditor** appears.



Exiting AssayEditor

► To exit AssayEditor

- On the File menu, choose **Exit**.

Navigating AssayEditor

The Navigation Tree

The left pane of the AssayEditor window contains the navigation tree for the **Assay Group** tab. Navigate to assay projects, assay groups, plexes, and assays using the navigation tree.

Note: The general term "assay group" is used in this chapter to refer to any type of assays grouped together in AssayEditor. When necessary, specific terms are used instead; these terms are "definition assay group," "locked definition assay group," and "reference assay group." These specific terms are described below.



Definition Assay Group

A collection of assays not associated with any design parameters. An assay in an assay group may not be edited or deleted from the group if the assay has already been run. New assays may be added to an assay group.



Locked Definition Assay Group

A set of assays associated with a set of design parameters and with a Locked SNP Group. Assays within a Locked Assay Definition Group may not be edited or deleted.



Reference Assay Group

A group of references to assays stored in the database. Assays deleted from Reference Assay Groups are not deleted from the database; they are merely deleted from the group.

Assay Group tab The **Assay Group** tab lists definition assay groups, locked definition assay groups, and reference assay groups. These items are described as follows:

- A *Definition Assay Group* is a set of assay definitions. Assay groups with a blue assay group icon are assays that are not associated with a design or SNP group, although the individual assays may be associated with SNP sequences. Manually created assays may be added to these groups. An assay may only be edited or deleted from a definition assay group if the assay has not yet been associated with an experiment. Editing or deleting assays from a definition assay group will permanently change the assay definition or remove it from the database.
- A *Locked Definition Assay Group* is a set of assays associated with a set of design parameters and with a locked SNP group. A locked definition assay group has a blue padlock assay group icon, which indicates the assays within this group may not be edited. These assay groups are always imported from an assay design file in combination with a design parameter file and design SNP group file.
- A *Reference Assay Group* is a group of references to assays stored in the database. A reference assay group has a green assay group icon. Reference assay groups may be edited, and references to additional assays may be added to them. When you edit an assay in a reference assay group, it alters the contents of that assay globally. Meaning, if the assay is referenced in multiple assay groups, all instances of the assay are affected by the edits. Typically, a reference assay group contains a subset of assays from definition assay groups, or it is used to manually create multiplexes of assays within AssayEditor for the purpose of defining plates in Plate Editor.

► To view items on the Assay Group tab

- Click the plus symbol [+] beside any item on the **Assay Group** tab navigation tree to display its contents.

► To rename items on the Assay Group tab

1. Click a project, assay group, plex, or assay on the Assay Group tab so it becomes highlighted. Then, click it again so a blinking cursor appears at the end of the item's name.
2. Type a new name, and then press the **Enter** key.

A message appears, asking you to confirm the name change.



Example confirmation message box for a renamed plex

3. Click **OK** to confirm the name change or **Cancel** to maintain the original name.

The Work Window

The right pane of the AssayEditor window lists details or task options for whatever item is currently selected in the navigation tree; any work to be performed (editing, creating new items, etc.) is done in this window pane. The right pane provides three tabs, which are described in the following sections.

Details tab The **Details** tab displays information about the currently selected item in the navigation tree. This information may be copied and pasted into another application. The description text displayed for certain items may be edited. (See “To edit description text” on page 15 for details.) For assays and assay groups that have associated design parameters, you may view the associated Design Summary file. (See “To view design summary” on page 16 for details.)

Edit Assay tab The **Edit Assay** tab displays editable information for the assay currently selected in the navigation tree. If the assay is part of a locked definition assay group or has been associated with experimental data, some information may not be available for editing.

Edit Group tab On the **Edit Group** tab, reference assay groups may be viewed, edited, or created. Assays, plexes, and assay groups from the **Assay Group** tab may be dragged into the **Edit Group** tab to become part of the currently selected reference assay group.

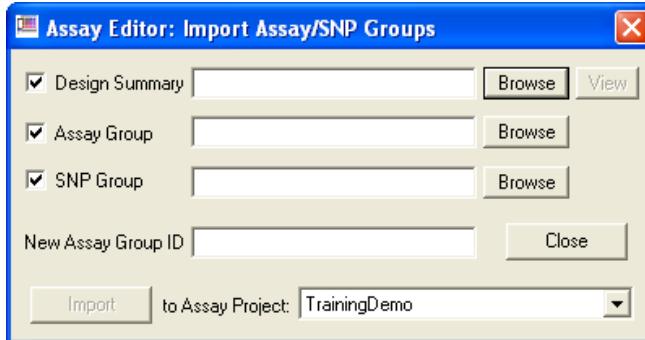
Importing Assays

Assays can be directly imported into the database using AssayEditor, as long as they follow the Assay Design software Assay group file format. (See the *MassARRAY Assay Design Software User’s Guide* for information on assay group files.) Typically, SNP sequences that these assays were designed against and the parameters used in the design would be imported at the same time from a SNP Group file and Design Summary file in the same directory. However, assay groups may be imported without a design summary file, and SNP groups may be imported independently.

► To import assays

1. On the File menu, choose **Import Assay Group**.
Or, on the **Assay Group** tab, right-click a project and choose **Import Assay Group**.

The **Import Assay/SNP Groups** dialog box appears.



Import Assay/SNP Groups dialog box in AssayEditor

2. Click the check box of any item you want to import.
Selecting one of the file types will generally cause all three file types to be specified, if these files were produced as a result of a Assay Designer run.
3. Click **Browse** to locate the Design Summary (.trs file), Assay Group, or SNP Group you want to import.
4. If desired, type or update the name in the **New Assay Group ID** box.
By default, the ID for the imported assay group is the same as the name of the local assay group file.
If the selected group ID conflicts with an existing ID in the database, a message appears prompting you to enter a different ID.
5. On the **Import to Assay Project** drop-down list, select the Assay Project where the imported items will be stored.
6. If desired, click **View** to preview the Design Summary file to ensure you have selected the correct set of assay design files for importing.
7. Click **Import**.
The selected files are imported into the database.

Note: Importing large assay groups into the database may take some time. It is recommended that assay groups contain no more than 10,000 assays. Working with larger assay or SNP groups may hamper performance when opening items in the database navigation trees and could possibly result in an *out-of-memory* problem on smaller systems.

8. Click **Close** to exit the **Import Assay/SNP Groups** dialog box.

Searching for Assays

You can search for any assay name listed on the **Assay Group** tab. You cannot search for an assay project name or an assay group name.

► To search for assays

1. Type an assay name in the **Search** box.

Use a percentage sign (%) as a wildcard to find one or more characters in the assay name. For example, searching on the name **MyAssay%** will return an assay named MyAssay, as well as MyAssays, MyAssays1, MyAssays2, etc.

2. Click **Go** to start the search.

The search results are listed in the navigation tree.

3. Click **Go** again to find the next occurrence of an assay name matching the search query.

Creating and Editing Assays

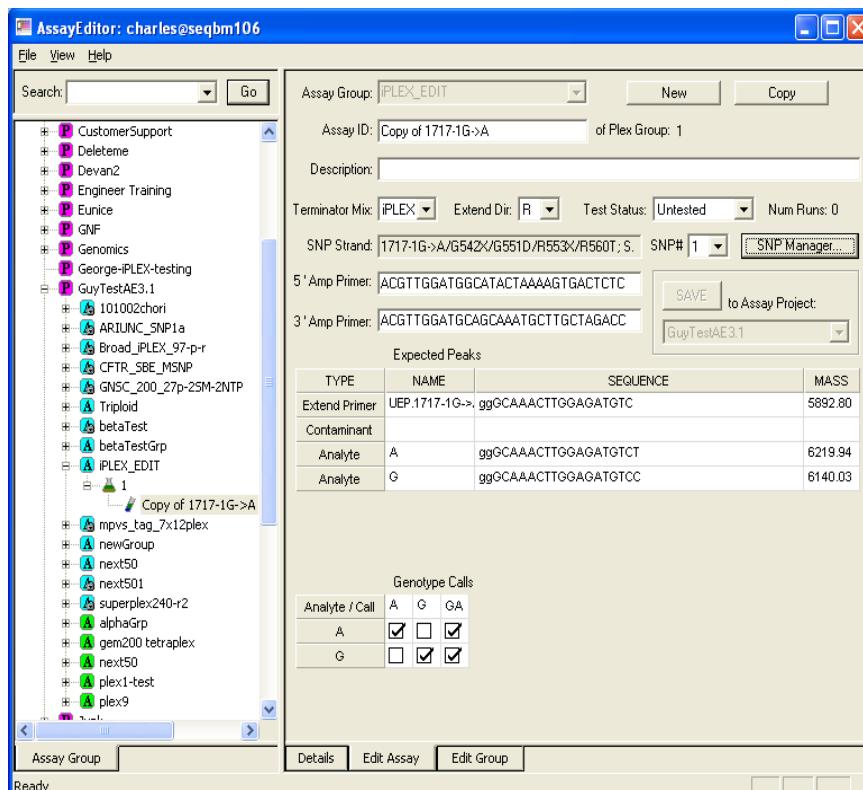
Create and edit assays using the **Edit Assay** tab.

Creating Assays

New assays may be created "from scratch" or by copying an existing assay and modifying its contents.

► To create a new assay

1. In the right pane, click the **Edit Assay** tab.



Edit Assay tab

2. Click **New**.
3. On the **Assay Group** drop-down list, select an assay group into which the new assay will be added.
4. In the **Assay ID** box, type a name for the new assay.
5. Type a description for the assay.
6. Specify the contents of the assay.
(See “Adding Items to the Expected Peaks Grid” on page 11 and “Adding Genotype Calls” on page 12 for instructions.)
7. Click **SAVE** to save the assay.

If the **SAVE** button is unavailable, it means either no changes have been made to the assay or certain required data is missing. Review the **Edit Assay** tab and make the necessary changes.

Adding Items to the Expected Peaks Grid

Review the information in this section to add a probe sequence, analytes, contaminants, sequence, and sequence mass to the **Expected Peaks** grid.

Expected Peaks			
	NAME	SEQUENCE	MASS
Probe	probe	CGCAGCGATAGCAGC	4587.0
Contaminant			
Analyte	A-ANALYTE	CGCAGCGATAGCAGCA	4900.2
Analyte	G-ANALYTE	CGCAGCGATAGCAGCG	4916.2

Analyte peaks on the *Expected Peaks* grid

In the example illustration above, the analyte masses are colored pink. This is a warning that their masses are close together (<= 30 Da for iPLEX or <= 50 Da for MassEXTEND). At least 30 Da from allele peaks and peaks from other assays is recommended for iPLEX (50 Da for MassEXTEND). However, an allele peak from the same assay can be as close as 16 Da in the iPLEX or MassEXTEND chemistry. The software does not distinguish between the two cases and will flag any mass pair with less than 30 Da (50 Da for MassEXTEND) difference between them. If a particular grid cell value is missing or invalid, its background color is red. Or, if the masses between two analytes are closer than 5 Da, the grid cell is colored red.

► To add probe, sequence, and sequence mass

1. On the **Edit Assay** tab, double-click inside the **Expected Peaks** grid, and then type the appropriate values for probe, sequence, and sequence mass.
An assay must have at least a probe sequence defined, with an ID, sequence, and sequence mass.
When you tab away from editing a sequence, its value is tested for syntax and the mass of the sequence is calculated in the **Sequence Mass Calculator** dialog box.

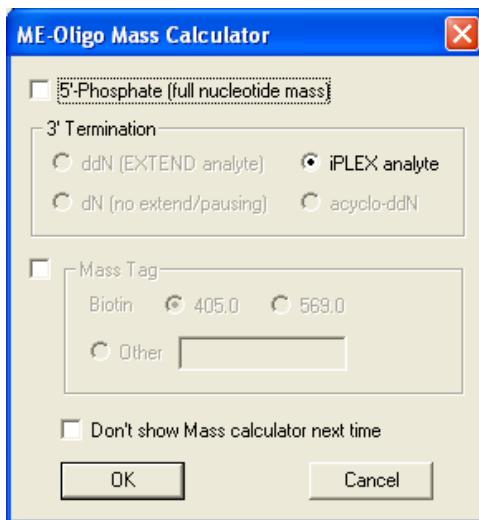
Note

Not all oligo mass calculation options may be available. Only those appropriate for the type of Expected Mass Peak and assay type, as specified by the Terminator Mix, are available for selection. For example, after typing in a sequence for a new Analyte with the Terminator Mix set at iPLEX only the iPLEX analyte 3' termination mass offset option is available, as shown.

Note

To disable Mass Calculator from automatically appearing after typing in oligo sequences check the

Don't show Mass calculator next time option before closing the Mass Calculator dialog box. You can also do this or re-enable the automatic Oligo Mass Calculator using the **Display Mass Calculator** option under the **View** menu.



2. If you do not plan to specify a DNA sequence, you must type the mass value in the **Mass** cell.

► **To add analytes**

1. On the **Edit Assay** tab, double-click inside the **Expected Peaks** grid, and then type the appropriate value for the first analyte.
You should name the analyte with the SNP sequence to which it corresponds.
2. To add additional analytes, right-click the existing analyte and choose **Add New Analyte**.

► **To add contaminants**

1. On the **Edit Assay** tab, double-click inside the **Expected Peaks** grid, and then type the appropriate value for the contaminant.
2. To add another contaminant, right-click the existing contaminant and choose **Insert New Contaminant**.
3. Type the value for the contaminant.

Copying and Pasting Items in the Expected Peaks Grid

1. Right-click an item in the **Expected Peaks** grid, and choose **Copy**.
2. Right-click inside another cell of the grid, and then choose **Paste**.
The selected item is pasted into the cell.

Deleting Items from the Expected Peaks Grid

- Right-click an item in the grid, and choose **Delete**.

Adding Genotype Calls

When an analyte is added to the **Expected Peaks** grid, a row is added to the **Genotype Calls** grid. (See “To add analytes” on page 12 for instructions on adding analytes.) The rows of the **Genotype Calls** grid represent the analytes associated with a particular

genotype call. The columns of this grid are where you specify what combination of observed analyte peaks lead to a particular genotype call. After defining two analytes, you should specify the corresponding homozygous calls for each of those peaks.

► To specify homozygous calls

1. Type the analyte peak combination call name (the genotype).
2. Click the check boxes to associate calls with the analyte peaks.

Now, the **Genotype Calls** grid looks similar to the illustration below:

Genotype Calls		
	A	G
A-ANALYTE	<input checked="" type="checkbox"/>	<input type="checkbox"/>
G-ANALYTE	<input type="checkbox"/>	<input checked="" type="checkbox"/>

Example of Genotype Calls

► To specify heterozygous calls

1. In the **Genotype Calls** grid, right-click an existing genotype and choose **Add New Genotype**.
(If you have not yet added a genotype, see “To specify homozygous calls” for instructions.)
2. Type a name for the heterozygous genotype.
3. Select the checkboxes for the analyte peaks that would result in this call.

Now, the **Genotype Calls** grid appears similar to the illustration below:

Genotype Calls			
	A	G	AG
A-ANALYTE	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
G-ANALYTE	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Example of Genotype Calls

Viewing Grid Colors

As you define genotype calls, some cells, rows, or columns of the grid may be colored red. This indicates errors such as a missing genotype ID or multiple genotypes specified by the same combination of analyte peaks. If you click a genotype call ID, its column is colored green, indicating the item is selected. The analytes involved in the selected genotype call also become colored green in the **Expected Peaks** grid. These colors are intended to be used as a visual aid. If you prefer, you may turn off this visual aid.

► To turn off visual aid colors

- Click the empty cell above the analyte names in the **Genotype Calls** grid.

You can define assays with more than two analytes by continuing the process detailed under “To add analytes” on page 12. As an example, the **Genotype Calls** grid for a tri-allelic SNP would look similar to the illustration below.

Genotype Calls						
	A	G	AG	T	AT	GT
A-ANALYTE	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
G-ANALYTE	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
T-ANALYTE	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Example of Genotype Calls for tri-allelic SNP

Copying Assays

You can copy and paste an assay into another assay group on the **Edit Assay** tab. The steps for copying the contents of an assay are the same as those for editing assays already associated with a design run. See the section below for instructions.

Copying Assays for Editing

You may not change the definition of assays that are associated with designs or that have been run. The only way to edit assays associated with a design or that have been run is to copy these assays to a new assay group and modify them.

► To edit assays associated with a design

1. On the **Edit Assay** tab, click **Copy**.
A copy of the currently selected assay is created. The copy (unlike the original) is available for editing.
2. If you plan to save this copied assay to the same assay group as its original, you must change the **Assay ID** to a new name.
Or, if you plan to save this copied assay to a different assay group than its original, select an assay group from the **Assay Group** drop-down list.
3. Make changes to the assay as needed.
(See “Adding Items to the Expected Peaks Grid” on page 11 and “Adding Genotype Calls” on page 12 for instructions.)
4. Click **SAVE** to save the assay.
If the **SAVE** button is unavailable, it means either no changes have been made to the assay or certain required data is missing. Review the **Edit Assay** tab and make the necessary changes.

Editing Assays

You may edit existing assays (those already in the left pane navigation tree) or new assays on the **Edit Assay** tab.

Note: If an assay has already been associated with a design or if it has already been run, you may not edit it. Instead, you must copy the assay (or assays) to a new assay group, and then edit the assay. See “Copying Assays for Editing” on page 14 for details.

► To edit assays

1. On the **Assay Group** tab, click an assay to select it.
2. In the right pane, click the **Edit Assay** tab, which displays the assay definition for the assay you just selected.
3. Make changes to the assay as needed.

The **Assay Group** box cannot be changed when editing an existing assay. Other fields may not be editable if the currently selected assay belongs to a locked definition assay group or if it has experimental data associated with it.

4. Click **SNP Manager** to associate the assay with a SNP sequence.

The **SNP Manager** dialog box appears. See “Managing SNPs” on page 19 for details on using this dialog box.

Once you associate a SNP with the assay, the SNP ID and SUSID appear in the **SNP Strand** box.

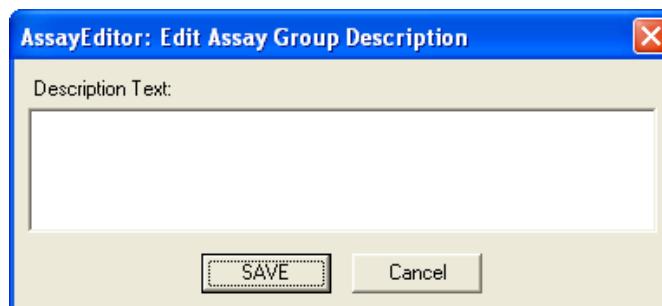
5. If the SNP sequence has multiple SNPs defined, click the **SNP#** drop-down list and select the particular SNP to associate with the assay.
6. Continue specifying the contents of the assay.
(See “Adding Items to the Expected Peaks Grid” on page 11 and “Adding Genotype Calls” on page 12 for instructions.)
7. Click **SAVE** to save the assay.

If the **SAVE** button is unavailable, it means either no changes have been made to the assay or certain required data is missing. Review the Edit Assay tab and make the necessary changes.

► To edit description text

1. On the **Details** tab, click **Edit Description**.

The **Edit Description** dialog box appears.



Edit Description dialog box

2. Type a new description, and then click **SAVE**.

Deleting Assays

► To delete an assay

1. On the **Assay Group** tab, click an assay to select it.
2. Right-click the selected assay, and choose **Delete Assay**.

► **To view design summary**

1. On the **Assay Group** tab, select an assay or assay group by clicking it.
2. In the right pane, click the **Details** tab if it is not already selected.
3. On the **Details** tab, click **View Design Summary**.

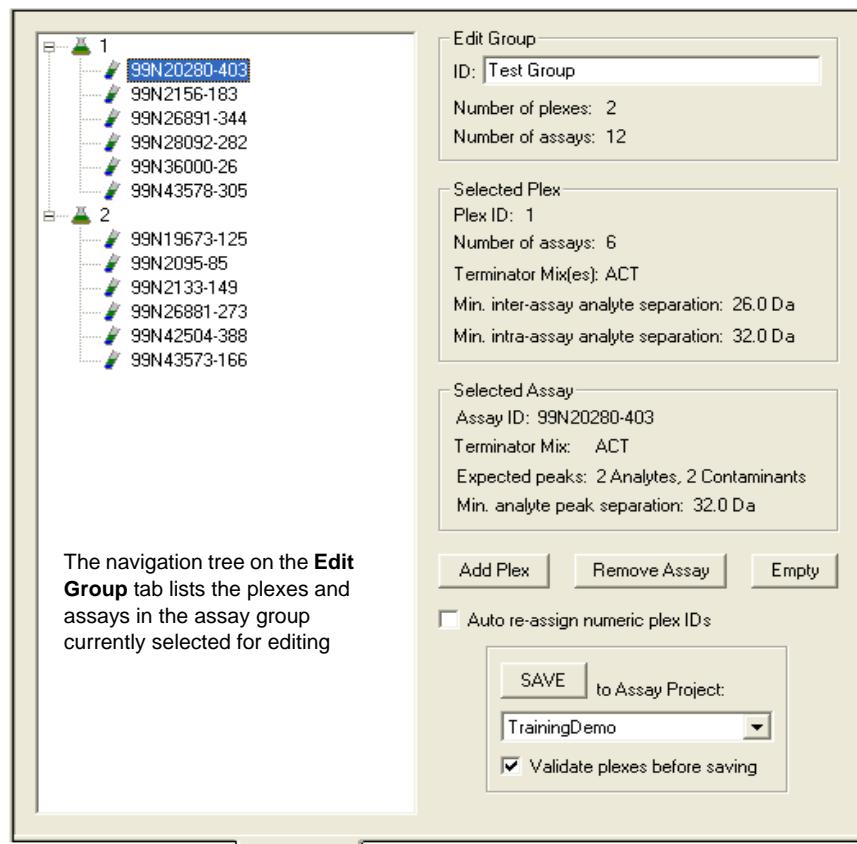
If there is a Design Summary file associated with the currently selected assay or assay group, the file is displayed.

Editing Assay Groups

► **To edit a group**

1. On the **Assay Group** tab, right-click a reference assay group (green icon) and choose **Edit Assay Group**.

The **Edit Group** tab appears (on the right side of the AssayEditor window), displaying the selected assay group.



Tip: Drag and Drop

To drag and drop an item, click it and hold down the mouse button. Then, drag the cursor across the screen and into the desired location. Release the mouse button to drop the selected item into the desired location.

2. To add to the group, drag and drop assays, plexes, or assay groups from the **Assay Group** tab into the **Edit Group** tab navigation tree.
(Assays must be added to an existing plex in the assay group currently selected for editing.)
3. Click **Add Plex** to add a new, empty plex to the assay group.

4. Drag and drop to add assays to the new plex.

As assays are added to a plex, the **Selected Plex** box provides information about the termination mixes and the minimum peak separation between any assay analyte and any other expected assay mass peak in the multiplex. A multiplex is invalid if there is more than one common terminator mix for the assays or the minimum separation is too small relative to resolving the mass peaks for iPLEX or MassEXTEND spectra. In this case, an exclamation point ("!") appears next to the display, indicating the multiplexing of the assays may be invalid.

5. To remove an assay from a plex, select it in the navigation tree, and then click **Remove Assay**.
6. To remove a single plex, select it in the navigation tree, and then click **Remove Plex**.
7. To remove all the plexes from the currently selected assay group, click **Empty**.
8. Type the name of the assay group in the **Edit Assay Group ID** box, or select an assay project from the **to Assay Project** drop-down list.
The assay group will be saved to the selected assay project.
9. To check for multiple terminator mixes and minimum peak separation, check the **Validate Plexes Before Saving** option.
If any plex is found to be invalid, the Save process is canceled.

Note: Selecting this option may cause the save process to take more time. Do not check this option if you want to save time or if you want to save the assay group as it is currently defined.

10. Click **SAVE**.

The assay group is saved to the selected assay project with the given ID. If an assay group with this name already exists in the selected assay project, a message box appears prompting you to either overwrite the existing assay group or not.

Plexes with Numeric Names

When you drag and drop assay groups into the **Edit Group** tab, duplicate numeric plex names are appended with a number to indicate which duplicate it is. For example, a plex named "5" with two duplicates would appear as "5," "5(1)," and "5(2)" in the **Edit Group** tab to distinguish the three different plexes.

You can automatically reassign numeric plex names to remove duplicate names.

► **To reassign numeric plex names**

- On the **Edit Group** tab, select the **Auto re-assign numeric plex IDs** option so a check mark appears in the box.



All duplicate numeric plex names are reassigned. Duplicate naming is removed, and duplicate plex names are assigned new numeric names.

Managing Assay Projects

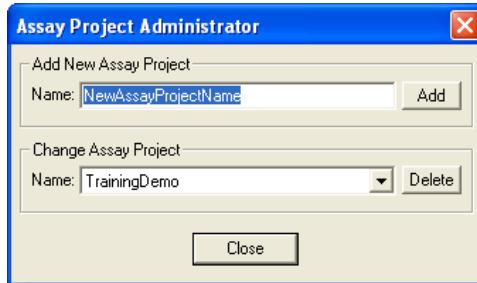
You may add or delete entire assay projects in the database. Before you can delete an assay project, it must first be emptied. (See “Emptying and Deleting Assay Projects” on page 18 for instructions.)

Adding Assay Projects

► To add assay projects

1. On the File menu, choose **Project Administrator**.

The **Assay Project Administrator** dialog box appears.



Assay Project Administrator dialog box

2. Type a name for a new assay project, and then click **Add**.
3. Click **Close** to exit the dialog box.

Emptying and Deleting Assay Projects

An assay project must be emptied of its contents (assays, assay groups, and SNP groups) before it can be deleted from the database. Only assays or assay groups that have not been associated with experimental data may be deleted.

You may move an assay group or SNP group into another assay project, which removes it from its original assay project. See “Moving Groups” on page 24 for information.

► To empty assay projects

1. On the **Assay Group** tab, select an assay group. Then, right-click the selected assay group and choose **Delete Assay Group**.
Or, select an individual assay, and then right-click it and choose **Delete Assay**.
2. In the message box that appears, click **Yes** to confirm the deletion.

► To delete assay projects

1. On the File menu, choose **Project Administrator**.
The **Assay Project Administrator** dialog box appears.
2. Select a name from the **Change Assay Project** drop-down list.
3. Click **Delete** to delete the selected assay project.
4. Click **Close** to exit the dialog box.

Managing SNPs

Use the **SNP Manager** dialog box to work with SNPs in AssayEditor. In the **SNP Manager** dialog box, you can manually create and edit SNP sequences and SNP groups, and you can locate SNPs and associate them with assays.

AssayEditor stores raw SNP sequences to the database to complete the information associated with an assay design. Every SNP stored in the database has a SNP_ID, a DNA sequence, and a SUSID (Sequenom Unique SNP ID). Each SNP must belong to at least one SNP group. Typically, a SNP group is imported into the database with a definition assay group. A new SNP is stored in the database only if it has a unique combination of sequence and SNP_ID. Hence, several SNPs in the database may have the same SNP_ID, e.g. "rs128986," if they have different sequences. Alternatively, two SNPs may have the same sequence but a different SNP_ID. Otherwise, a SNP is identified as already existing in the database.

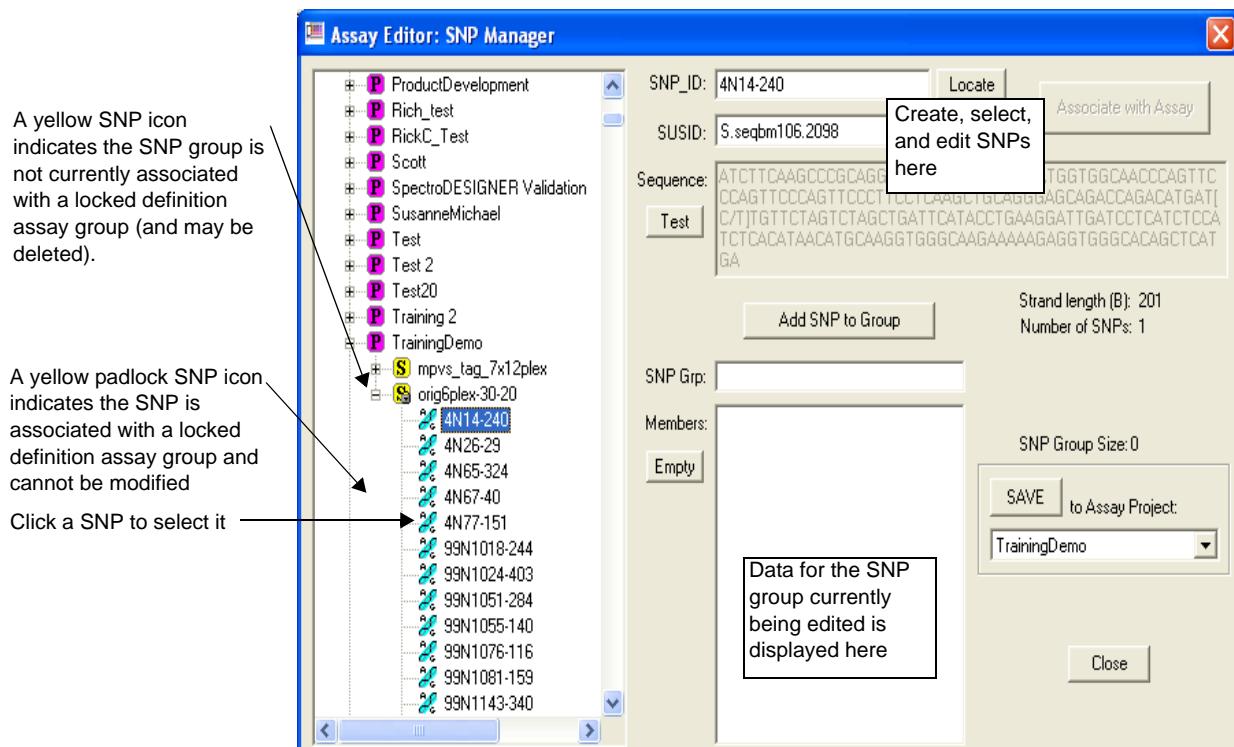
Opening SNP Manager

► To open SNP Manager

- On the View menu, choose **SNP Manager**.

Or, if you are on the **Edit Assay** tab, click **SNP Manager** to open the **SNP Manager** dialog box.

The **SNP Manager** dialog box appears.



SNP Manager dialog box

Selecting SNPs

SNPs may be selected for viewing in any of the following ways:

- By clicking them in the navigation tree
- By clicking them in the **Members** box
- By searching for them with the **Locate** button

Once a SNP is selected, its SUSID and sequence appear. If the SNP is in the SNP group currently selected for editing, then the SNP is also selected in the **Members** box.

► To select a SNP from the navigation tree

- In the **SNP Manager** dialog box, click a SNP_ID in the navigation tree.
The SNP becomes highlighted, indicating it is selected.

► To select a SNP from the Members list

- In the **Members** box, click a SNP_ID.
The SNP becomes highlighted, indicating it is selected.

► To select a SNP using Locate

1. Type a SNP_ID in the **SNP_ID** box.

Note: The **Locate** function requires the exact name of the SNP_ID to locate a SNP. Partial names and the wildcard character (%) may not be used.

2. Click **Locate**.

If the located SNP is a member of the SNP group currently selected for editing, its entry in the **Members** box becomes highlighted.

Otherwise, the SNP is searched for in the database, and the assay project and SNP group containing the SNP are opened with the SNP selected. If the SNP_ID was associated with more than one SNP (having different sequences), then the **SUSID** box contains multiple values from which to select.

If the SNP_ID was not located in any SNP group in the database, then the **SUSID** box contains the value <New>, and the current sequence becomes editable.

3. Click **Locate** again if you want the search to continue from the currently selected SNP.

If the same SNP is a member of more than one SNP group, these occurrences are highlighted in the navigation tree as they are located.

4. If the SNP_ID is associated with multiple DNA sequences, click the **SUSID** drop-down list and select an individual SNP.

SNP Groups

SNPs must belong to at least one SNP group. New SNPs cannot be saved to the database until they are assigned to a SNP group. While you are creating a new SNP or editing an existing SNP, you can create and/or edit a SNP group in the SNP Manager dialog box. (See “Creating New SNPs” on page 22 for instructions on creating SNPs from scratch or by modifying an existing SNP.)

Creating SNP Groups

Create a new SNP group while creating or editing a SNP.

► To create a SNP group

1. Select an existing SNP.

(See “Selecting SNPs” on page 20 for instructions.)

Information for the selected SNP is loaded into the right side of the **SNP Manager** dialog box.

2. In the **SNP Grp** box, type a name for the new SNP group.
3. To add the currently selected SNP to the new SNP group, click **Add SNP to Group**.
4. To add other SNPs to the SNP group, drag and drop SNPs from the **SNP Manager** dialog box navigation tree into the **Members** box.
5. Select an assay project from the **Assay Project** drop-down list.
The new SNP group will be saved to the selected assay project.
6. Once the current SNP group contains all the SNPs you want, click **SAVE** to save it to the database.

Adding SNPs to SNP Groups

While creating or editing a SNP, you can add SNPs to the current SNP group.

► To add SNPs to a SNP group

1. Select an existing SNP.

(See “Selecting SNPs” on page 20 for instructions.)

Or, create a new SNP.

(See “Creating New SNPs” on page 22 for instructions.)

Information for the selected SNP is loaded into the right side of the **SNP Manager** dialog box.

2. To add SNPs to the current SNP group, do either of the following:
 - Click **Add SNP to Group** to add the selected SNP to the SNP group currently being edited.
 - Or, drag and drop SNPs from the **SNP Manager** dialog box navigation tree into the **Members** box.
3. Click **SAVE** to save your changes.

Tip: Drag and Drop

To drag and drop an item, click it and hold down the mouse button. Then, drag the cursor across the screen and into the desired location. Release the mouse button to drop the selected item into the desired location.

Removing SNPs from SNP Groups

► To remove a SNP from a SNP group

1. In the **Members** box, select a SNP_ID.
The **Add SNP to Group** button changes to the **Remove SNP from Group** button.
2. Click **Remove SNP from Group**.
3. To remove all SNPs from the **Members** box, click **Empty**.
4. Click **SAVE** to save your changes.

You may not save an empty SNP group to the database. Add SNPs to the SNP group if it is currently empty. Then, click **SAVE**.

SNPs

Creating New SNPs

New SNPs may be created by updating existing SNPs or "from scratch."

► To create a new SNP by modifying an existing SNP

1. Select an existing SNP.
(See "Selecting SNPs" on page 20 for instructions.)
2. In the **SUSID** drop-down list, select <New>.
3. In the **SNP_ID** box, type a new SNP_ID.
Or, leave the SNP_ID as it appears for the selected SNP. Then, modify the sequence in the **Sequence** box.
4. Click **Test** to test the DNA sequence for sequence, (maximum) strand length, and the number of SNPs to be reported.
If any errors are found, a message box appears. Make any necessary corrections to the sequence.
5. Click **Add SNP to Group** to add the SNP to the SNP group currently being edited.
The new SNP cannot be saved to the database until it belongs to a SNP group.
The SNP_ID is added to the **Members** box, and it is assigned a temporary value in the **SUSID** box. (The SUSID will be updated once the SNP is saved to the database.)

Note: Once a SNP has been added to a SNP group, the SNP may no longer be edited. Instead, you must make a copy of it; the copied SNP may be edited. Also, if any assays were associated with the original SNP, they must be re-associated with another SNP before the original SNP can be deleted.

6. Click **SAVE** to save your changes.

► To create a new SNP

1. Type the ID for the new SNP in the **SNP_ID** box.
2. Click **Locate**.

If the SNP_ID does not yet exist in the database, the **SUSID** box displays <New>, indicating a new SNP is being created.

If a SNP (or SNPs) already exist in the database with this same SNP_ID, it is selected. To create a new SNP with this same SNP_ID, select <New> from the **SUSID** box, and then modify the **Sequence** value.

3. Type or paste the sequence into the **Sequence** box.
4. Click **Test** to test the syntax of the SNP sequence you entered.
If the SNP syntax is valid, then the total SNP sequence length and number of SNPs are reported below the sequence.
If any errors are detected during **Test**, a message box appears.
5. In the **SNP Grp** box, type the name of the SNP group where the SNP will be stored.
A new SNP cannot be saved to the database until it belongs to a SNP group.
6. Click **Add SNP to Group** to add the new SNP to the currently selected SNP group.
A temporary value (indicated by "") appears in the **SUSID** box. This value gets updated once the currently selected SNP group is saved to the database.
7. If you want to add additional SNPs to the SNP group, click and drag them from the navigation tree into the **Members** box. Repeat this step to add other new SNPs to the group or to add other existing SNPs to the group. (See “Adding SNPs to SNP Groups” on page 21 for details.)
8. Select an assay project from the **Assay Project** drop-down list.
The new SNP and its SNP group will be saved to the selected assay project.
9. Once the current SNP group contains all the copied, edited, and/or new SNPs you want, click **SAVE** to save it to the database.

Associating SNPs with Assays

Note: The **Associate with Assay** button is only available if you opened the **SNP Manager** dialog box by clicking **SNP Manager** on the **Edit Assay** tab.

New or existing SNPs may be associated with assays. Before a new SNP may be associated with an assay, it must first be added to a SNP group and saved to the database. (See “To create a new SNP by modifying an existing SNP” on page 22 for instructions.)

► **To associate a SNP with an assay**

1. With an editable assay loaded in the **Edit Assay** tab, click **SNP Manager**.
The **SNP Manager** dialog box appears.
2. Select a SNP.
(See “Selecting SNPs” on page 20 for instructions.)
3. Click **Associate with Assay** to associate the selected SNP with the assay being edited.
The **SNP Manager** dialog box closes. In the main AssayEditor window, the SNP_ID and SUSID for the SNP appear in the **SNP Strand** box.

Exporting SNPs

SNPs and SNP groups may be exported to an Assay Designer file.

► To export SNPs

- In the navigation tree, right-click a SNP group or SNP_ID, and then choose **Export**.

Deleting SNP Groups

SNPs may not be deleted; you may only delete the SNP groups that contain them. (SNPs may be removed from a SNP group, but they are not deleted from the database.)

The SNP group that contains the last reference to a particular SNP may not be deleted. Such a SNP group may only be deleted after its associated assays have been deleted. (See “Editing Assays” on page 14 for instructions.)

► To remove a SNP

- See “Removing SNPs from SNP Groups” on page 22 for instructions.

► To delete a SNP group

1. In the **SNP Manager** dialog box navigation tree, select a SNP group.
2. Right-click the selected SNP group and choose **Delete SNP Group**.

The selected SNP group is deleted.

Moving, Copying, and Deleting Groups

Moving Groups

You can move assay groups from one project to another by simply dragging them within the navigation tree on the **Assay Group** tab. Projects, plexes, and individual assays may not be moved in this way. In the **SNP Manager** dialog box, you may move SNP groups by dragging them to a new location on the navigation tree. You may not move individual SNPs this way.

► To move a group

- On the navigation tree, click and drag a group into its new location.

Copying Groups

In order to limit the number of copies of particular assays in the database, it is not possible to copy definition assay groups or locked definition assay groups using the drag and drop method. However, any assay group may be dropped into the navigation tree of the **Edit Group** tab view to create a reference assay group.

You may copy individual assays to different groups using the **Copy** button on the **Edit Assay** tab, but this is intended for creating new assays based on modifications to existing assays. (See “Copying Assays for Editing” on page 14 for instructions.) SNP groups may be copied by opening them for editing and saving them back to the database with a different ID or into a different project.

Deleting Groups

Review information in this section for instructions on deleting assay groups or SNP groups.

You cannot delete an assay or locked definition assay group if any of the assays in the group have been associated with an experiment plate. An assay group that does not have design settings associated may be deleted as described below.

► **To delete assay groups**

1. On the **Assay Group** tab, right-click an assay group and choose **Delete Assay Group**.

A confirmation dialog box appears.

2. To delete the assay group, click **Yes**.

For instructions on deleting assays, see “Editing Assays” on page 14.

► **To delete locked definition assay groups**

1. On the **Assay Group** tab, right-click a locked definition assay group (blue padlock icon) and choose **Delete Assay Group**.

Individual assays in a locked definition assay group may not be deleted.

2. If a confirmation dialog box appears, click **Yes** to delete the locked definition assay group.

Note: A locked SNP group becomes unlocked once its associated locked assay definition assay group has been deleted.

If the locked definition assay group cannot be deleted, the following message box appears.



3. Click **OK** to close the message box. The selected locked definition assay group is not deleted.

Note: After deleting a locked definition assay group, the associated Design Summary data is also deleted, but an associated SNP group is not deleted. The SNP group may be deleted separately (in the **SNP Manager** dialog box), if it is not associated with any other locked definition assay groups. (See “Deleting SNP Groups” on page 24.)

► **To delete SNP groups**

- For instructions, see “Deleting SNP Groups” on page 24.

Exporting Groups

Assay groups and SNP groups may be exported to local files. Plexes may not be exported directly. To export a plex, add it to a definition assay group or reference assay group, and then export the assay group.

► To export a group

1. In the navigation tree, right-click a group and choose **Export**.

The navigation tree may vary, depending upon the type of group you are selecting. For example, if you are exporting a SNP group, you must be in the **SNP Manager** dialog box navigation tree.

The **Export** dialog box appears.



The options available on the **Export** dialog box may vary depending upon the type of group selected for export and the export options checked

In this example illustration, the resulting export files **test50.xls** and **test50.txt** would be created

Export Assay/SNP Groups dialog box

2. Select export options by clicking the checkboxes.

Up to three export files are created during export, depending upon the type of group selected for export and the export options selected. The **Export Options** are as follows:

- **Design Parameters** writes the design parameters for an assay group to a text file that has a .trs file name extension. Typically, this file is a MassARRAY Assay Design Software (Assay Designer) format file that describes the full set of parameters used to design a set of assays previously imported in to the database via AssayEditor. More generally, this is any text file that was imported with an assay group that serves as a description of how those assays were designed or collected together.
- **Assay Group** writes the set of selected assays out to an Assay Designer assay group format file, which is a tab-delimited text file that has a .xls file name extension. This option is available whenever any assay group or individual assay is selected for exporting.
- **SNP Group** writes out a complete SNP group to an Assay Designer SNP group format file, which is a tab-delimited text file with a .txt file name extension. This option will be available when a SNP group, individual SNP, or locked definition assay group is selected for exporting. For locked definition assay groups, the whole SNP group associated with the locked definition assay group is exported even though this group may contain SNPs that are not associated with individual assays.

- **Assay SNPs** writes a SNP group file for only those assays in the assay group or for the individual assay selected for exporting. This is the only SNP group export option available for assays that were not associated with a design.
 - **Undesigned SNPs** writes a SNP group file for a set of SNPs within a SNP group that is not associated with assays. This option is only available when exporting a locked definition assay group associated with a SNP group. This option is equivalent to exporting the SNPs in the group that failed to be designed by Assay Designer.
3. Type the file name for the exported file in the **File name prefix** box.
By default the **File name prefix** uses the name of the group or individual assay or SNP you are exporting.
-
- Note:** The directory used to write the exported assay files will be the last one selected via the File Open dialog box that appears when the Browse button is clicked. If you Export without first using the Browse button the assay files will be saved in directory the AssayEditor.exe program was installed to.
4. Click **Export**.
The selected assays, SNPs, and design settings are saved and exported.
 5. Click **Close** to exit the **Export** dialog box.

Notes:

Chapter 3

Defining Plates

Introduction

The PlateEditor module is used to define samples and plates and to apply samples and assays to a plate's wells.

This chapter explains how to use the PlateEditor by providing an overview of what you will see followed by sections on how to perform specific tasks, such as: select a plate, create a plate, define samples, apply assays and samples to wells, create an assay group, and edit plates.

Physical Plate

A Plate in the MassARRAY Server database (database) represents the physical microtiter plate that is spotted onto the SpectroCHIP. Since the Plate layout (96 wells or 384 wells) also mirrors the SpectroCHIP layout, in a sense the definition of the Plate also serves as a definition for the SpectroCHIP. Each well contains a single Sample (which can be a pooled sample) and one or more Assays that are run simultaneously on that Sample. Assays which are run simultaneously are called *multiplexed assays*.

Plate Database Hierarchy

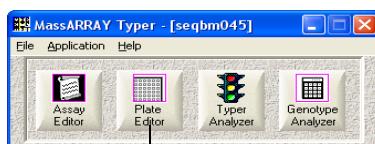
The Plate exists within a hierarchy in the database. As defined in the database hierarchy, a Customer has Projects, and a Project has Plates associated with it. A Plate must exist within a single Project. The Plate hierarchy can be used to associate Plates in any manner that is useful to you; that is, the hierarchies do not need to actually be Customers and Projects, but can reflect some other organization of similar form (e.g. Project and Subproject).

PlateEditor Basics

Opening the PlateEditor

► To open the PlateEditor

1. From the MassARRAY Typer window, click **PlateEditor**.



Click to open the PlateEditor

The **Connect to Database** dialog box opens.

2. Enter the appropriate information, and then click **OK**.
Once connected, the **PlateEditor** opens.
3. Select the plate you want to work on, and then assign assays and samples.
See "Applying Assays and Samples to Wells" on page 46 for instructions.

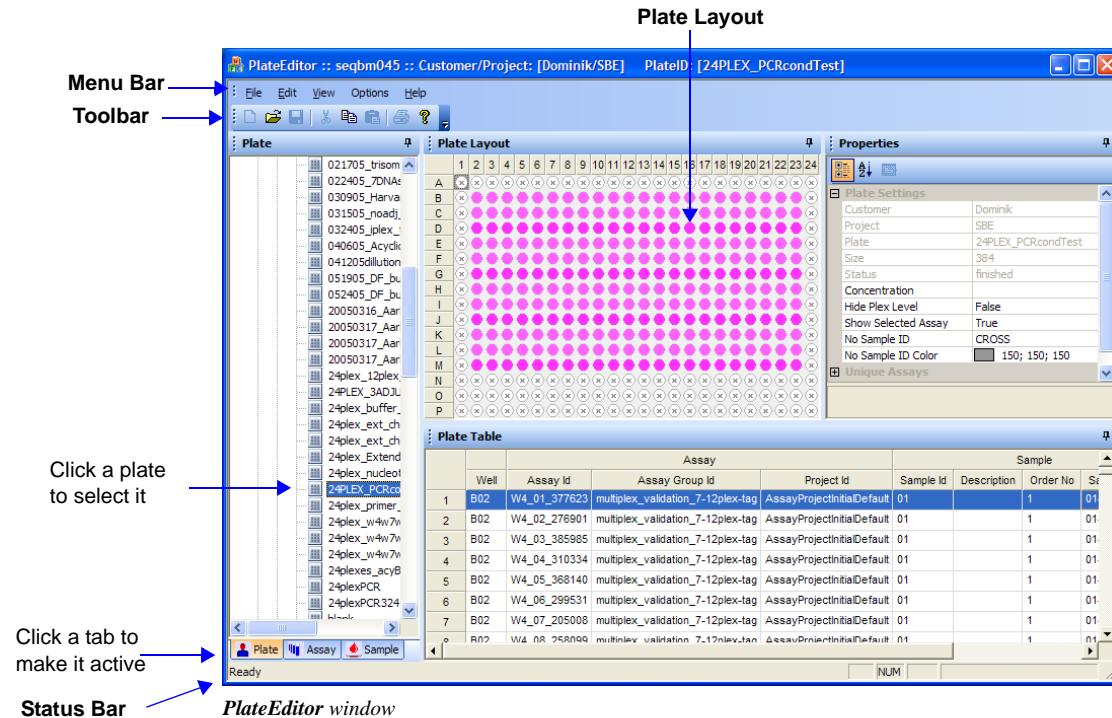
Closing the PlateEditor

- On the File menu, select **Exit**.

PlateEditor Overview

This section provides a general overview of where features are located on the PlateEditor window and instructions for more commonly used tasks. Many PlateEditor attributes can be customized. For more information on customizing PlateEditor, see Appendix B “Configuring the MassARRAY Software” on page 147.

The PlateEditor includes a menu bar, toolbar, tree tabs, and areas displaying the plate table, plate layout, and plate properties. To view all of these, the first step is to select a plate. Plates are defined in the PlateEditor. Once the plate is open, it shows graphically in the window. Each well is identified by the plate layout cell. If there is nothing assigned to a well, the well is marked with an X and has a white background.



PlateEditor Window

The main areas that you work with in the PlateEditor are explained below.

Menu Bar and Toolbar Apply commands from the menu items. The more frequently used commands are also represented in toolbar buttons.

Message Bar This area is located in the lower part of the window. It displays messages regarding the current state of the program.

Status Bar When opening or saving a plate, the status bar indicates the progress. Do not do anything until the task is completed.

Plate tab Click the **Plate** tab to show all the plates in the database, organized by customer and project. Select and highlight a Plate ID and right-click the mouse. A popup menu of options appears.

Assay tab Click the tab to make its information accessible. The **Assay** tab includes all the assays defined using the Assay Editor. Assay projects are organized into assay groups, and within the assay groups are plexes and assays. Highlight the assay that you want to apply to selected wells. Right-click and select **Add** to apply the assay. (See “Assay and Sample Tree” on page 46.) One or more assays can be applied to the same well.

Sample tab Click the tab to make its information accessible. The **Sample** tab shows all defined samples.

Plate Table This area displays the contents of each well shown in the plate layout.

Plate Layout This area displays each well. Highlight one or more wells that you want to select. This can be a single well, a contiguous group of wells, or a non-contiguous group of wells. If a group of wells are selected, a window showing the assays and sample ID displays.

Plate Properties This area displays the selected plate with each well identified by the grid. Highlight one or more wells that you want to select. This can be a single well, a contiguous group of wells, or a non-contiguous group of wells. Once wells are selected, choose an assay or sample and apply it.

Right-Click Menu Right-click the mouse on either the project name, plate name, or a well within the plate layout to open a menu of commands. Point to the command you want. This is a quick method to add a customer, add, move, or delete a plate, and to copy or clear well contents.

Selecting Plates

The first step in using PlateEditor is to select a plate. This can be a blank plate that needs assays and samples applied to its wells, or a plate that already has assays and samples applied to its wells.

After a plate’s data is transferred and used in the Genotype Analyzer, the plate can be opened, copied, or viewed, but not edited.

► To select a plate

1. On the **Plate** tab, select and expand the Customer\Project\Plate tree. Continue down the tree to select the plate you want.

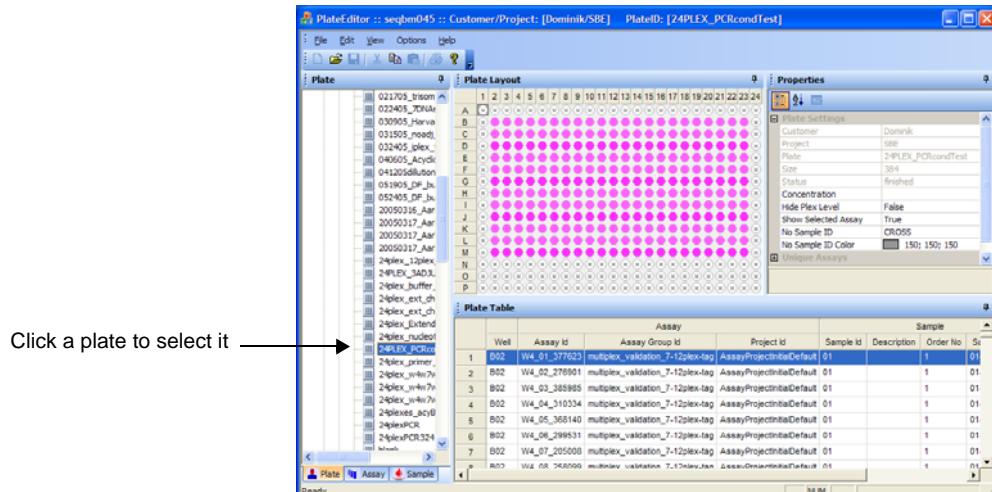


Plate tab with plate selected

► To select an individual well

- Click the individual well.

► To select a contiguous group of wells

1. Click the first well, and hold down the left mouse button.
2. Drag the cursor across and down to cover the wells you want to select.
As you drag, the selected wells turn grey.
3. Release the left mouse button.
The wells remain selected.

► To select a non-contiguous group of wells

1. Select the first well or group as explained in the preceding instructions.
2. Press and hold down the **[Ctrl]** keyboard key.
3. Click additional wells.
You can also click and drag to select more than one additional well.
4. Once all the wells are selected, release the **[Ctrl]** key and left mouse button.

Creating Plates

There are three ways to create plates:

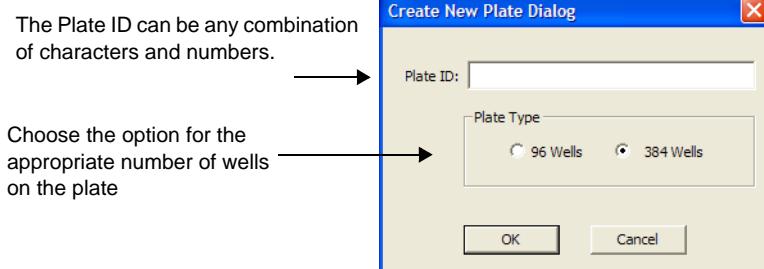
- Using the **New Plate Dialog** box (See “Creating Plates with the New Plate Dialog Box” below for instructions.)
- From a template file (See “Creating Plates using Templates” on page 33 for instructions.)
- By selecting an assay group (See “Applying Sample Groups” on page 49 for instructions.)

Creating Plates with the New Plate Dialog Box

► To create a new plate

1. On the **Plate** tab, select the customer and project in which the new plate will be stored.
2. Right-click a project name, and choose **New Plate** from the menu that appears.
OR, click on the **File** menu and select **New**, followed by **New Plate**.

The **Create a New Plate** dialog box appears.



Create a New Plate dialog box

3. Type the **Plate ID** using any combination of alpha or numeric characters.
4. Click the option for **96 Wells** or **384 Wells**.
5. Click **OK**.

The new blank plate opens.

Creating Plates using Templates

Creating Templates A plate template is a tab-delimited text file that specifies the layout of a plate. The text file may be created in Microsoft Notepad or Microsoft Excel, as long as it is saved as a tab-delimited file. Templates are used to create plates in PlateEditor. The syntax of a plate template file is as follows:

Well Number P Pool Number

Type one well of data per line, and separate each data entry (well number, P, pool number) by pressing the Tab key.

Example plate template files are shown in Figure 1 below through Figure 3 on page 35.

Figure 1: Template file defining quadrants on a 384-well plate

The screenshot shows a Windows Notepad window titled "PT_384Quads.txt - Notepad". The menu bar includes File, Edit, Search, and Help. The main text area contains the following data:

	P	1
A01	P	1
A02	P	1
A03	P	1
A04	P	1
A05	P	1
A06	P	1
A07	P	1
A08	P	1
A09	P	1
A10	P	1
A11	P	1
A12	P	1
B01	P	1
...		

To the right of the data, a descriptive comment is present:

This example defines quadrants on a 384-well plate. Quadrant regions are indicated by the third data column ("1" in this example); only a portion of the first quadrant is shown in this example.

Figure 2: Template file defining column layout on a 96-well plate

The screenshot shows a table template for a 96-well plate. The columns are labeled A, B, and C. The rows are numbered 1 through 15. The data is as follows:

	A	B	C
1	A01	P	1
2	A02	P	2
3	A03	P	3
4	A04	P	4
5	A05	P	5
6	A06	P	6
7	A07	P	7
8	A08	P	8
9	A09	P	9
10	A10	P	10
11	A11	P	11
12	A12	P	12
13	B01	P	1
14	B02	P	2
15	B03	P	3

Below the table, two vertical lines with arrows point downwards, labeled "well numbers" and "pool numbers".

Figure 3: Template file defining one plex per well on a 96-well plate

	A	B	C
1	A01	P	1
2	A02	P	2
3	A03	P	3
4	A04	P	4
5	A05	P	5
6	A06	P	6
7	A07	P	7
8	A08	P	8
9	A09	P	9
10	A10	P	10
11	A11	P	11
12	A12	P	12
13	B01	P	13
14	B02	P	14
15	B03	P	15
16	B04	P	16
17	B05	P	17
18	B06	P	18
19	B07	P	19
20	B08	P	20
21	B09	P	21
22	B10	P	22
23	B11	P	23
24	B12	P	24

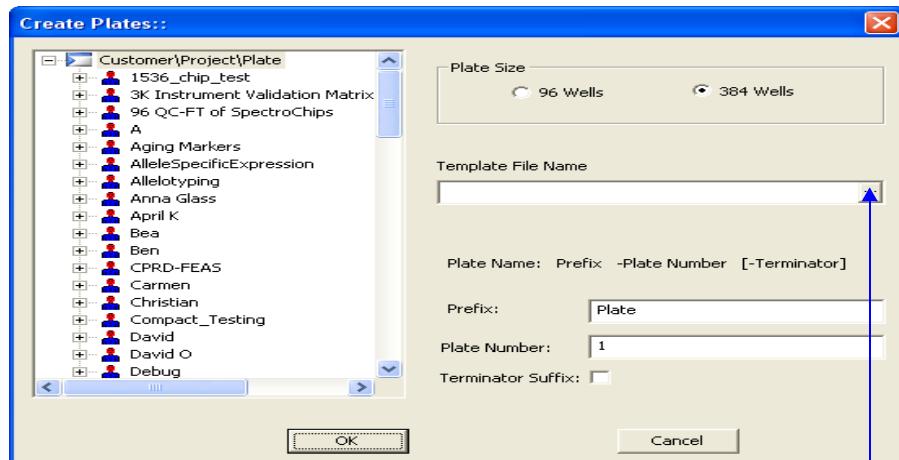
In this example template file, each well of the 96-well plate contains a different plex (indicated by the third data column, which contains pool numbers)

Creating Plates Using Templates Once you have created a template file, you can use it to create a plate.

► **To create a plate using a template**

1. On the **Assays** tab, right-click an assay group and choose **Create Plates** for the collection.

The **Create Plates** dialog box appears.



Create Plates dialog box

Click to browse for a template

Template File

Create a template file in Microsoft Excel or Notepad. The tab-delimited file must be formatted with three tab-delimited columns: well, the letter "P," and the pool number. For details, see "Creating Templates" on page 33.

2. On the Customer\Project\Plate tree, select a project to which the new plate will be added.
3. Click the **Template Browse** button.
A **Browse for File** dialog box appears.
4. Select a properly formatted template file, and then click **OK**.
5. Select a plate size.
6. Under **Plate Name**, edit the plate prefix and plate number as desired.
7. Then, check the **Term Suffix** box if you want to include the terminator at the end of the plate name.
8. Click **OK**.

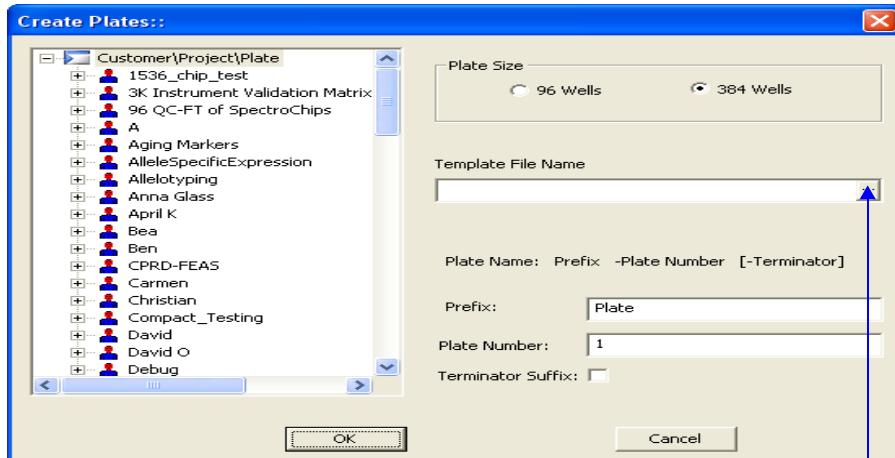
Creating Plates via Assay Groups

You can create plates from the **Assays** tab.

► **To create plates via assay groups**

1. On the **Assays** tab, select an assay group.
2. Right-click the selected assay group and choose **Create Plates** for the collection.

The **Create Plates** dialog box appears.



Create Plates dialog box

Click to browse for a template

3. On the Customer\Project\Plate tree, select a project to which the new plate will be added.
4. If using a template, click the **Template Browse** button and select a properly formatted template file, then click **OK**.
5. Select a plate size.
6. Under **Plate Name**, edit the plate prefix and plate number as desired.
7. Then, check the **Term Suffix** box if you want to include the terminator at the end of the plate name.
8. Click **OK**.

Creating Samples

The samples in Typer relate to your physical samples. For each physical sample, you should create a sample in Typer. In Typer, there are only two items of information associated with a sample: its name and an optional, short description.

A sample cannot be created by itself. It must belong to a sample group.

There are two ways to create a new sample. Either it is created as part of a new sample group or it is added to an existing sample group. For instructions, see the following:

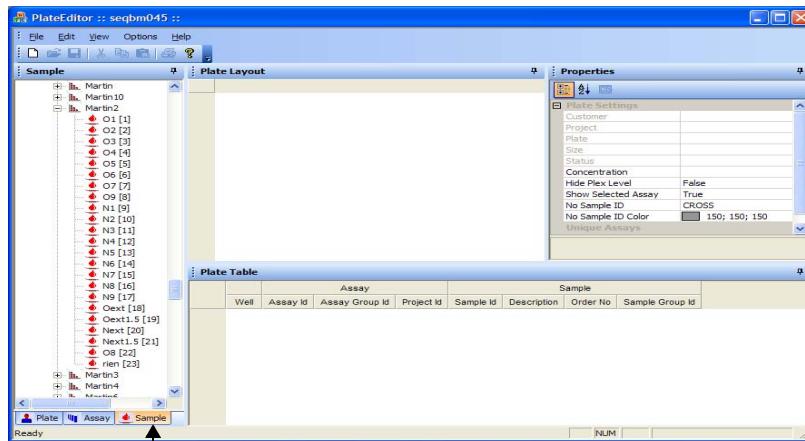
- To create a new sample group, see “To create a new sample group” below.
- To add a sample to an existing sample group, see “Editing a Sample Group” on page 44.

► **To create a new sample group**

1. If it is not currently running, start the PlateEditor.

(For instructions on starting the PlateEditor, see “Opening the PlateEditor” on page 29.

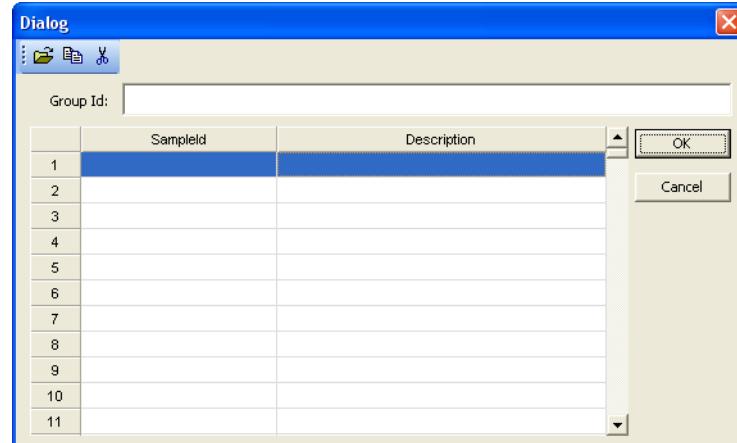
2. In the PlateEditor, click the **Sample** tab.



Click the **Samples** tab

3. Click the plus (+) symbol next to **Sample: Sample Group: Sample**.
Sample Group appears.
4. Right-click **Sample Group**, and choose **Add New Sample Group**.

The dialog box to add a sample appears.



New Sample Group dialog box

5. In the **Group ID** box, type a name for the sample group.
This name appears in the tree-list of the PlateEditor to identify the sample group.
Sample group names can have a maximum of 20 characters and must not contain any single quote marks.
6. For each sample in the sample group, type a name (in the **SampleID** column) and an optional description (in the **Description** column). Use one row for each sample.
When you apply an entire sample group to a plate, the order of the samples determines to which well each sample is applied. The samples are applied in the order in which they appear in the sample group. For more information see "Sample Order and Applying Sample Groups" on page 39.

Note: You can import sample names and descriptions from a Microsoft Excel file. See "Applying Sample Group Mapping" on page 53 or "Importing and Exporting Plate Table Information" on page 42.

7. Click **OK**.

The sample group is saved and you are returned to the **PlateEditor** window. Click the plus symbol (+) next to **sample**. Under **sample**, the name of the new sample group you created appears.

Note: If you want to return to the **PlateEditor** window without saving the sample group, click **Exit**.

Sample Order and Applying Sample Groups

The order in which samples are entered in a sample group determines how they are applied to a plate. For information about applying sample groups to a plate, see “Applying Sample Groups” on page 49. Samples can be applied to a plate either horizontally or vertically.

Note: You can individually apply any sample to any well. This section describes how sample order affects the application of an entire sample group to a plate.

► To specify the sample direction

- In the **Properties** pane, open **Sample Tab Settings** and select **Horizontal** or **Vertical**.

Sample Order When you apply an entire sample group to a plate, the order of the samples in the sample group determines the order in which they are applied to the wells of the plate. See the following illustration.

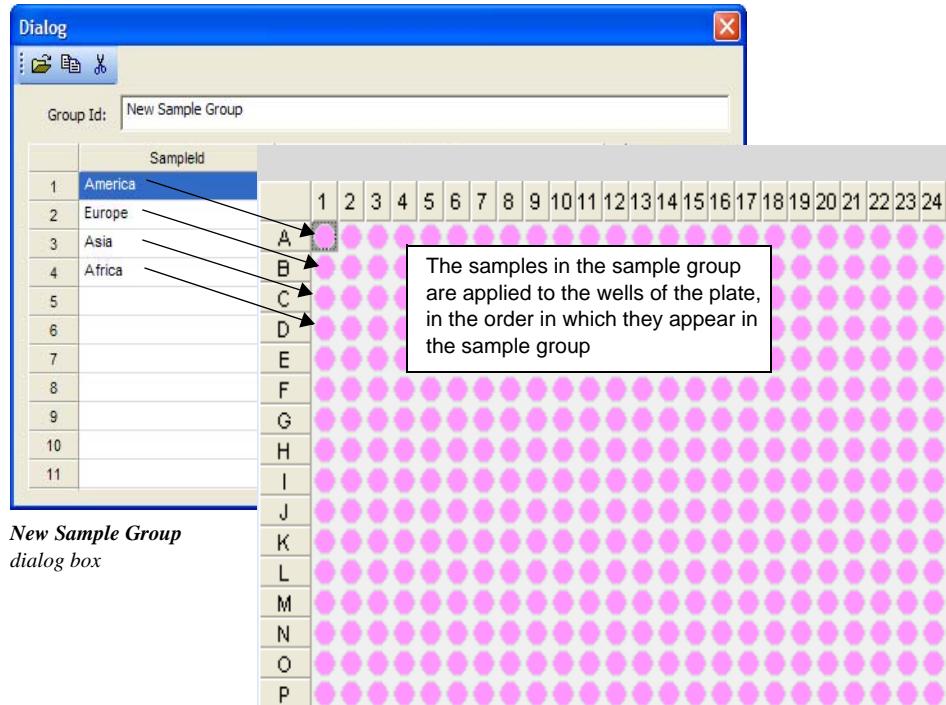
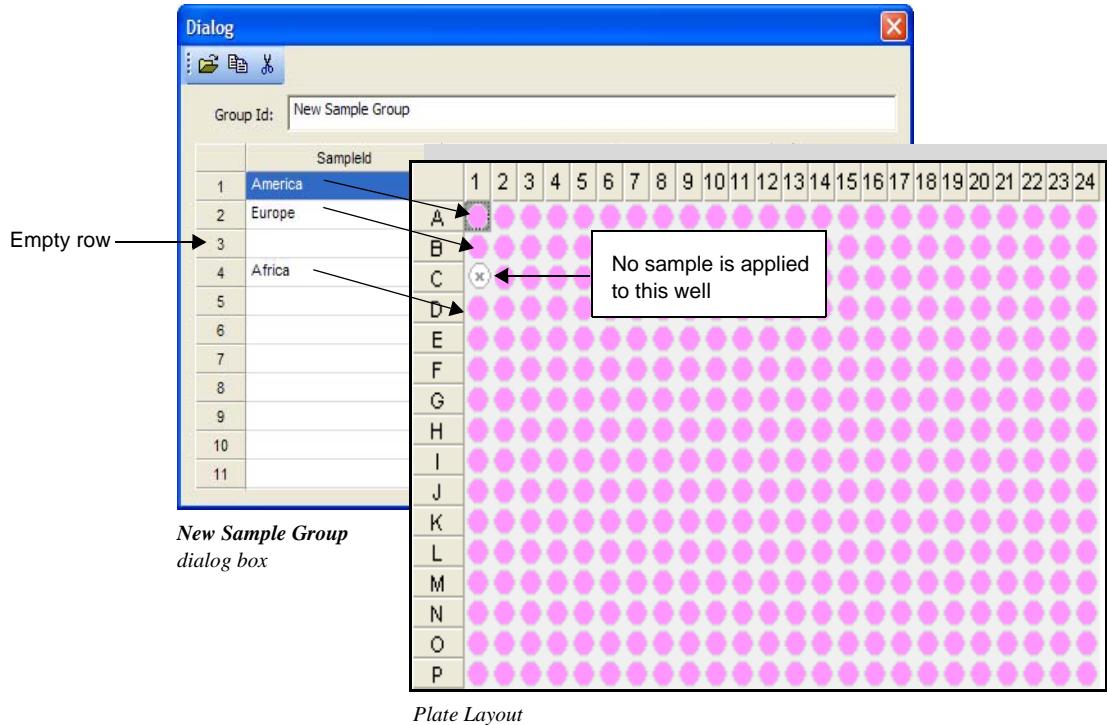


Plate Layout

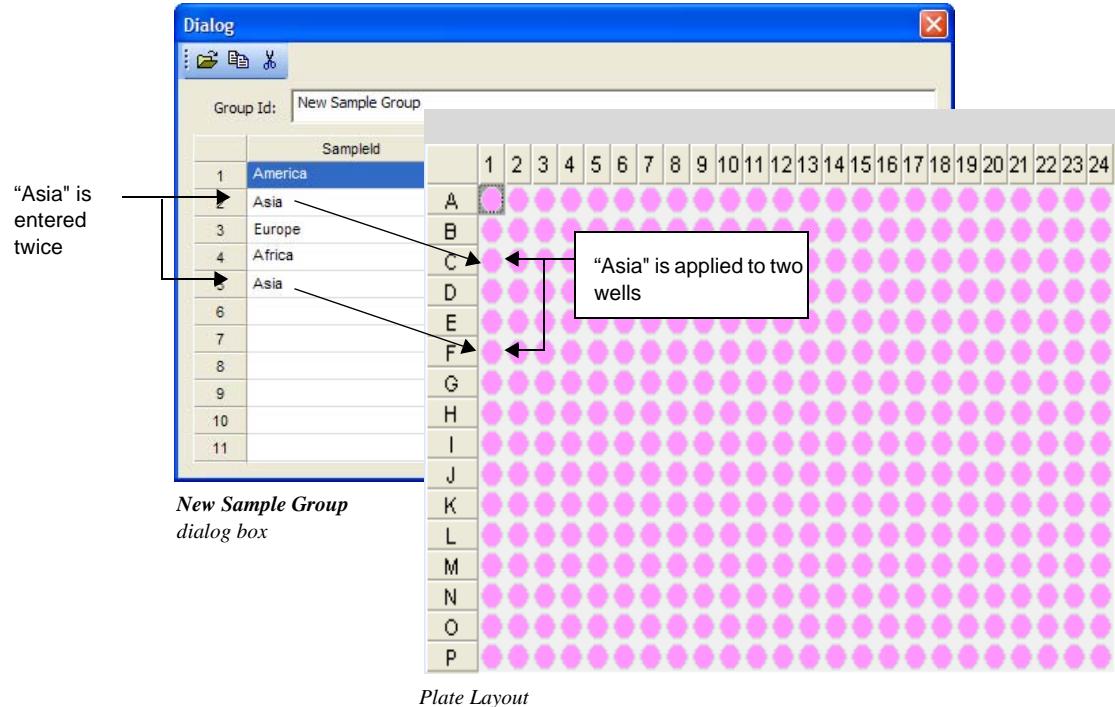
Note: This illustration depicts a sample group applied vertically. You can also apply a sample group horizontally. In that case, the samples would be applied across the rows, rather than down the columns. For information, see “Applying Sample Groups” on page 49.

Empty Row When entering the samples of a sample group, you can leave a row empty. When the sample group is applied to a plate, the well that corresponds to the empty row will not have a sample applied to it. See the following illustration.



Repeated Samples When entering the samples of a sample group, you can enter the same sample name (SampleID) multiple times. When you apply the entire sample group

to a plate, multiple wells on the plate have the same sample applied to it. See the following illustration.



Importing Sample Names and Descriptions from Microsoft® Excel

You can import sample names and descriptions from Microsoft® Excel into the **New Sample Group** dialog box.

This feature is very useful in two situations:

- You want to prepare sample names and descriptions in Microsoft Excel and quickly import them into Typer.
- You already have a Microsoft Excel file containing the sample names and descriptions.

► **To import sample names and descriptions from Microsoft Excel**

Note: It is assumed you are currently at the **New Sample Group** dialog box. If you are not, see the steps under “To create a new sample group” on page 37.

1. Start Microsoft Excel.
2. If you already have a Microsoft Excel file containing the sample names and descriptions, open it.

If you do not have an existing file containing the sample names and descriptions, enter them on a new worksheet. Make sure the file includes SampleID and Description column headings. If not, create the column headings.

3. Save the Excel spreadsheet as a tab-delimited text file.
4. On the **Edit** menu, select **Copy**.

5. In the **New Sample Group** dialog box, click the **Open Folder** button. In the **Open** dialog box, select the text file that contains the sample names and descriptions and click **Open**.

The sample names are imported into the **New Sample Group** dialog box.

Note: For more information about using Microsoft Excel, refer to its documentation or online Help system.

Importing and Exporting Plate Table Information

You have the option of copying the well, assay and plate information from the Plate Table and pasting it into Microsoft Excel in order to manipulate its data. Assuming the spreadsheet retains its original structure and saved in the proper format, the data can be imported back into the Plate Table within PlateEditor. Files may be created from scratch or by copying existing plate data, or a blank plate to establish the structure for the file.

► **To copy table data to Excel:**

1. Select the plate containing the contents to copy to Excel.
2. On the **Edit** menu, select **Copy Table Grid**. This copies all of the well, assay and group data contained in the Plate Table.
3. Start Microsoft Excel, if not already open.
4. Right-click the first cell and select **Paste**.
5. Manipulate the data as desired.
6. When complete, use the **Save As** option to save the file in delimited text format (Tab delimited).

Note: The table data will not import unless saved in text delimited format only.

► **To import table data:**

1. Select the plate to contain the imported data.
2. On the **File** menu, select **Import Into Grid**.
3. At the prompt, select the text delimited file to import, and click **Open**.

Note: A prompt will appear if the format of the file is not supported, or if the file contains data issues. Data will not import in either scenario.

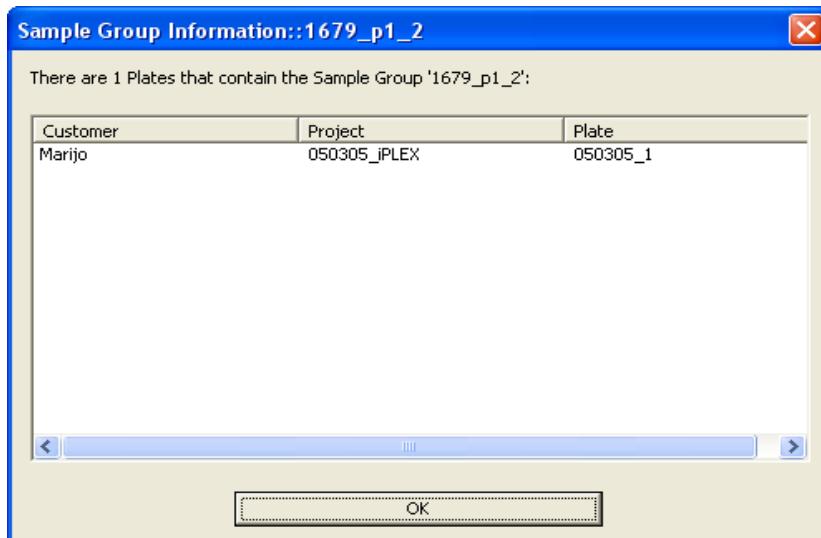
Finding Which Plates Contain a Sample Group

You can select a sample group and view a list of the plates to which it has been assigned. Plates in the list do not have to contain the whole sample group—a plate appears in the list if it contains at least one of the samples from the sample group.

► To view a list of plates to which a sample group is assigned

1. In the PlateEditor, click the **Sample tab**.
2. If necessary, expand the tree-list to find the sample group you want.
Click the plus symbol (+) next to a node to view its contents.
3. Click the sample group to highlight it. Then, right-click the sample group and choose **Sample Group Info**.

The **Sample Group Info** dialog box appears.



Sample Group Info dialog box

The **Sample Group Info** dialog box indicates the number of plates containing samples from the sample group. It also lists the customer, project, and name for each plate containing samples from the sample group.

4. Click **OK** to close the **Sample Group Info** dialog box.

You can add a new sample to an existing sample group. To do so, you must edit the sample group to which you want to add the new sample. See “Editing a Sample Group” below.

Editing Samples

You can change description of a sample. To do so, you must edit the sample group to which the sample belongs. See “Editing a Sample Group” below.

Note: It is not possible to rename a sample.

Editing a Sample Group

You can add a new sample, edit the samples in an existing sample group, or delete a sample group if it is not in use and the user has permission. The editing options available are to change or add a description for a sample.

Note: You can edit a sample group only if none of its samples has been assigned to a plate. If any plate uses samples from the sample group, you must first clear those samples from the plates. To find out which plates use the sample group, see “Importing and Exporting Plate Table Information” on page 42. To clear samples from a plate, see “Clearing Wells” on page 54.

► To add a sample to a sample group

1. In the PlateEditor, click the **Sample** tab.
2. If necessary, expand the tree-list to find the sample group you want.
3. Click the sample group to highlight it. Then, right-click the sample group and choose **Edit Sample Group**.
4. To add a new sample, enter its name and (optional) description in a blank row.

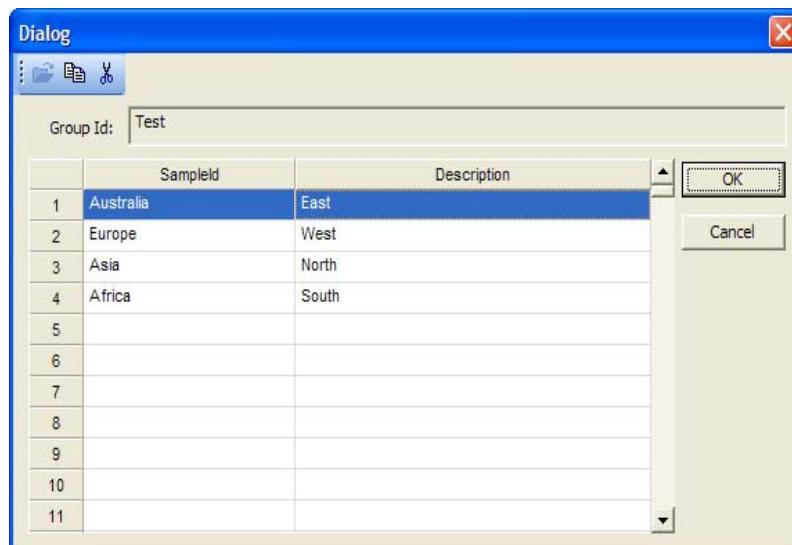
Note: If you do not want to save the changes you made, click **Exit** to close the **Edit Sample Group** dialog box and return to the **PlateEditor** window.

5. When you are done making changes, click **Save**.
6. Click **Exit** to return to the **PlateEditor** window.

► To edit a sample group

1. In the PlateEditor, click the **Sample** tab.
2. If necessary, expand the tree-list to find the sample group you want.
Click the plus symbol (+) next to a node to view its contents.
3. Click the sample group to highlight it. Then, right-click the sample group and choose **Edit Sample Group**.

The **Edit Sample Group** dialog box appears.



Edit Sample Group dialog box

If a message similar to the following appears, click **OK** to dismiss it.



The message can appear for two reasons, either the plate has already been run, or at least one plate uses samples from the sample group you want to edit.

Note: You cannot edit the samples in a sample group if a plate currently uses samples from that group. You must clear those samples from the plates before you can edit the sample group. After closing the message, find the plates that use samples from the sample group and then clear those samples from the plates. See “Importing and Exporting Plate Table Information” on page 42. After clearing the samples from the plates, you can repeat this procedure to edit samples. For information about clearing samples from plates, see “Clearing Wells” on page 54.

4. Make the changes you want to the samples.
To change a name (SampleID) or description, double-click it and make the changes you want.
To add a new sample, enter its name and (optional) description in a blank row.

Note: If you do not want to save the changes you made, click **Exit** to close the **Edit Sample Group** dialog box and return to the **PlateEditor** window.

5. When you are done making changes, click **Save**.
6. Click **Exit** to return to the **PlateEditor** window.

Deleting a Sample Group

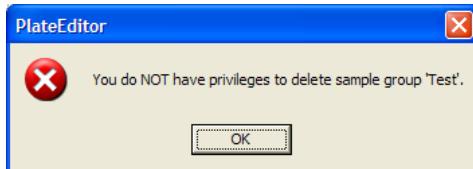
You can delete a sample group only if none of its samples are currently assigned to a plate. If a plate uses any of the sample group's samples, you must clear those samples from the plate before deleting the sample group. To find out which plates use samples from the sample group, see “Importing and Exporting Plate Table Information” on page 42. To clear samples from a plate, see “Clearing Wells” on page 54.

Note: You must be logged in with database privileges in order to delete a sample group. Contact your database administrator for information.

► **To delete a sample group**

1. In the PlateEditor, click the **Sample** tab.
2. If necessary, expand the tree-list to find the sample group you want.
Click the plus symbol (+) next to a node to view its contents.
3. Click the sample group to highlight it. Then, right-click the sample group and choose **Delete Sample Group**.

4. If the following message appears, click **OK** to dismiss it. (If this message does not appear, skip to the next step now.)



This message appears because there are plates that use samples from the sample group you want to delete. To delete the sample group, you must first clear its samples from any plates using them. To find out which plates use samples from the sample group, see “Importing and Exporting Plate Table Information” on page 42. To clear samples from a plate, see “Clearing Wells” on page 54.

After you have cleared its samples from plates, repeat this procedure to delete the sample group.

5. You are asked if you are sure you want to delete the sample group. Click **Yes**.
The sample group is deleted.

Applying Assays and Samples to Wells

With a plate selected, apply plexes (or individual assays) and samples to the wells by highlighting the specific plexes, assays, and samples that you want applied to specific wells. More than one assay can be applied to a single well. After applied, use the **List Items** tab to show what exactly is applied to the plate. Click a sample or assay in the tree and its location is identified graphically in the plate grid by a change in color.

Assay and Sample Tree

When working with the information in the trees on these tabs, click a plus symbol (+) to expand a node. A minus symbol (-) means that the node is open. When a tree is open all the way down to the assay or sample level, there are no plus or minus symbols, just the name of the assay or sample.

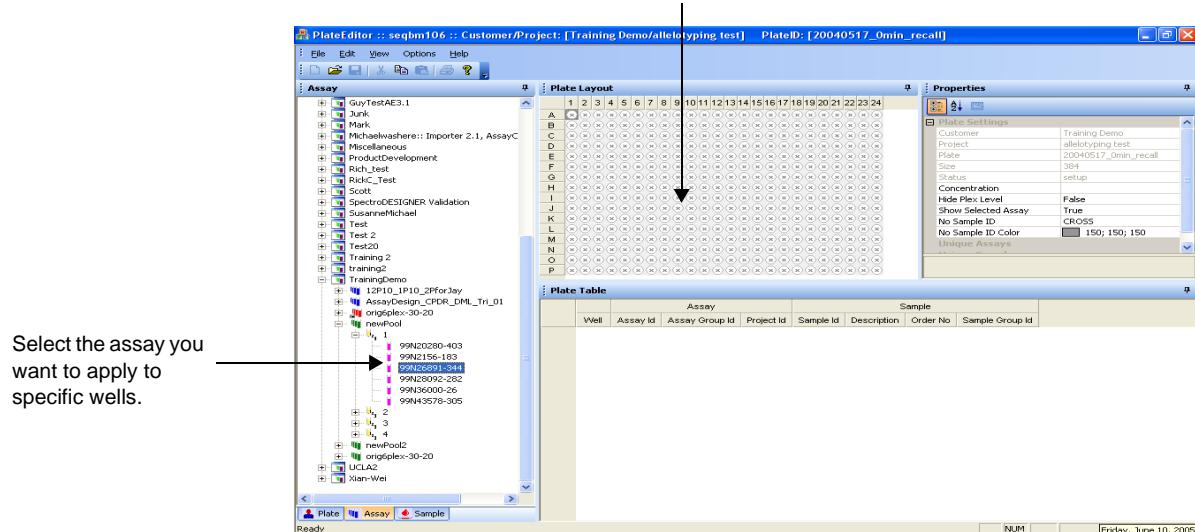
» **To apply plexes, assays, and samples to wells**

1. On the **Plate** tab, select and highlight the plate you want.

The plate opens automatically. Empty wells are marked with an X and filled with a white background.

2. Click the **Assay** tab to make it active.

When the plate is empty, it appears with the grid cells empty to signify there are no assays or samples applied to the wells



Assay Groups tab with an assay selected in the navigation tree

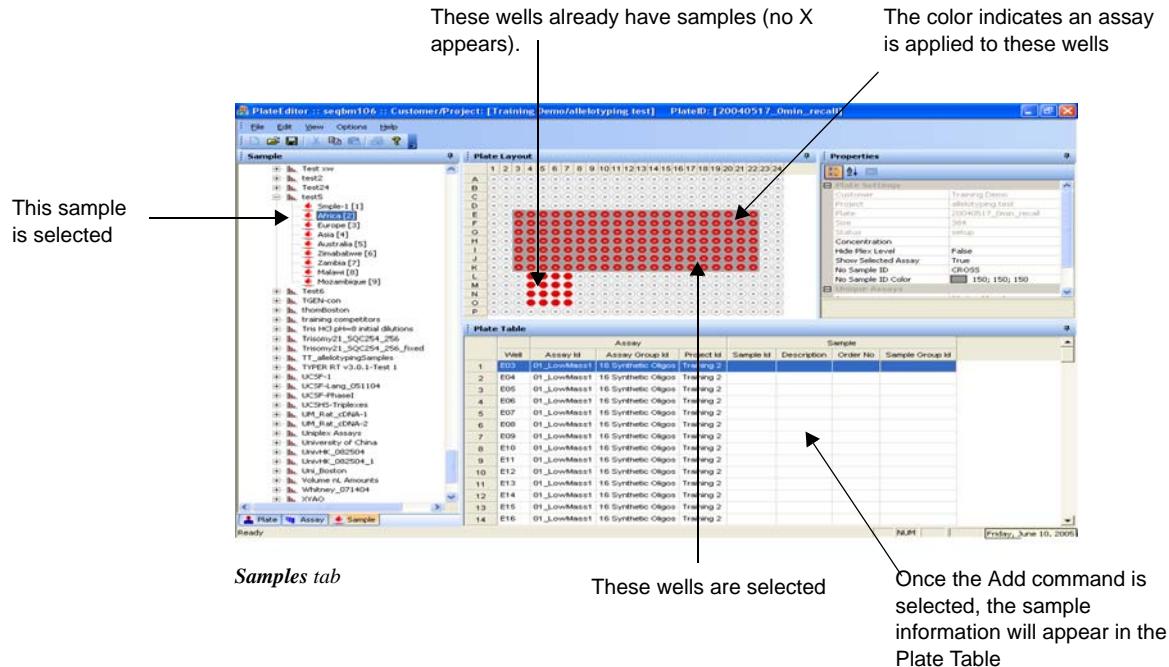
3. Apply selected plexes or individual assays to the appropriate wells. More than one assay can be applied to the same well.

Note: To apply a plex or an assay to the entire plate, select all the wells, and apply the plex or assay.

- Highlight the target well(s).
- In the navigation tree, locate the plex or individual assay that you want to apply to one or more wells, and click it.
- Back in the navigation tree, right-click the plex or individual assay, and choose **Add**.

The selected plex or individual assay is applied to the wells, and the wells change color.

4. Click the **Sample** tab to make it active.
5. Apply selected samples to the appropriate wells.
 - Highlight the target wells.
 - In the tree, locate the sample that you want to apply to one or more wells, and click it.
 - Back in the navigation tree, right-click the plex or individual assay, and choose **Add**.



The sample is applied to the wells. Once applied, the X within the well contained in the Plate Layout disappears, and the name of the sample appears in the Plate Table.

6. From the **File** menu, choose **Save**.
- OR
- Click the **Save** toolbar button.
- Right-click the selected wells and choose **Save Plate**.
- The status bar indicates the progress.

► To apply samples to a region

1. On the **Samples** tab, select the samples you want to apply.
2. In the **Properties** pane, set **Keep in Selected Region** to **True**.
3. On the plate layout, click a well, and then drag the cursor across a region of the plate. The selected region becomes highlighted.
4. Right-click the sample group, and choose **Apply**.

Applying Sample Groups

If you have samples in a group, you can quickly apply the entire group to a plate. There are four options: **192 Bottom Half Horizontal**, **192 Left Half Vertical**, **192 Right Half Vertical**, and **192 Top Half Horizontal**.

If a horizontal sample group option is selected, the samples fill each well in order of left to right in the first selected row, then left to right in the second row, and continues until the plate is filled or the sample group ends. If you prefer to fill the wells vertically, then select a vertical sample group option. This will apply the samples in the group from top to bottom in the first column, then top to bottom in the second column until the plate is filled or the sample group ends.

► **To apply a sample group to a plate**

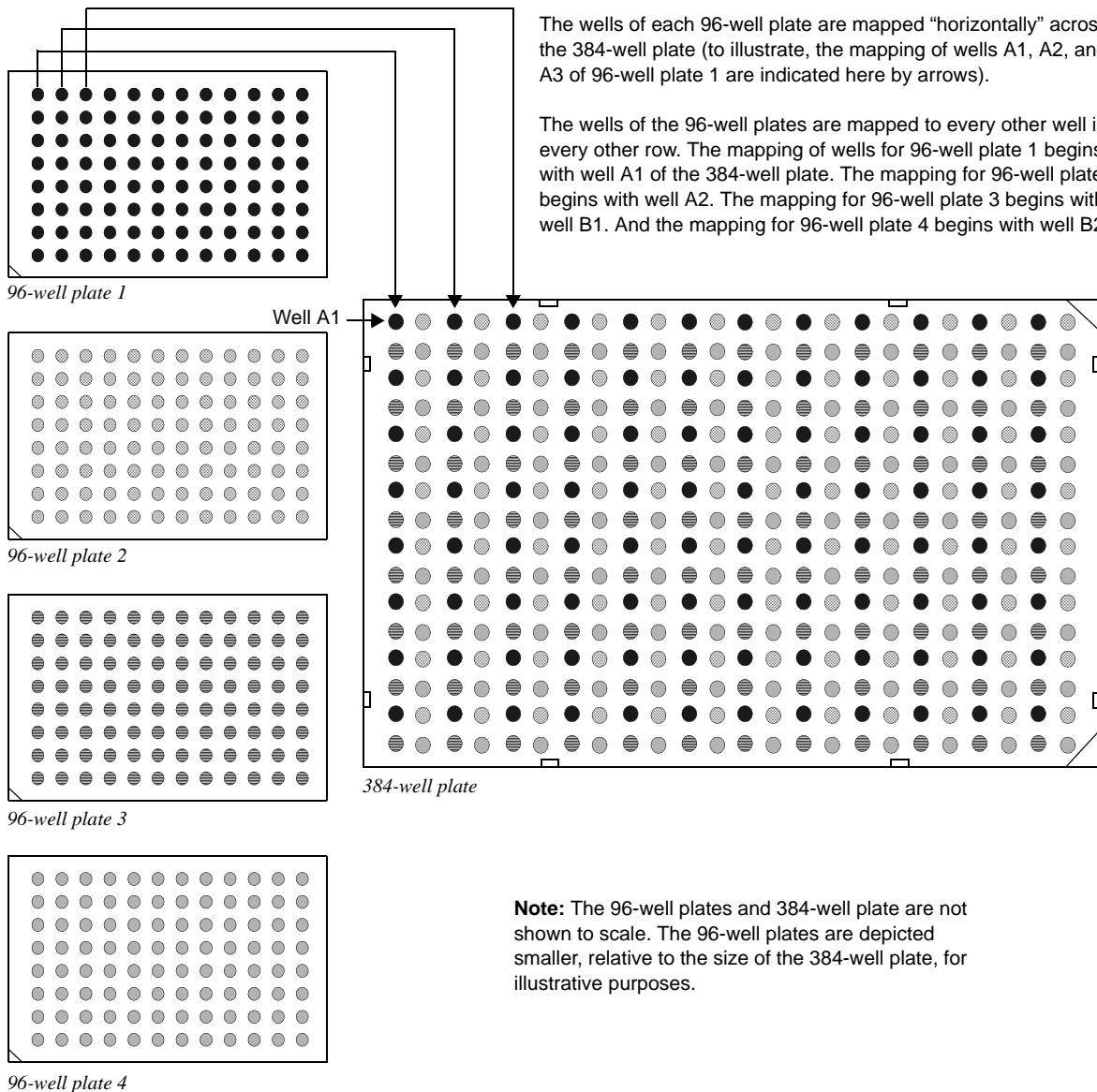
1. Open a plate.
2. Select the **Sample** tab.
3. In the navigation tree, highlight the sample group you want to apply.
4. Right-click the sample group and choose the desired **Apply** option.
The samples apply to each well.
5. From the File menu, choose **Save**.
OR, click the **Save** toolbar button.
OR, right-click a well and choose **Save Plate**.
The status bar indicates the progress.

Applying Sample Groups Using 4(96) to 1(384) Mapping

The 4(96) to 1(384) mapping option applies a sample group to the wells of a plate in the same way that certain automated pipettors transfer material from four 96-well plates to a single 384-well plate. This transfer scheme is illustrated below.

Defining Plates

Applying Sample Groups Using 4(96) to 1(384) Mapping

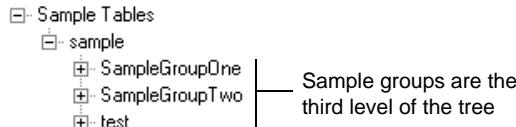


In Typer, you can apply samples to a plate in the manner depicted above by having a separate sample group represent each of the 96-well plates and then applying the sample groups using the 4(96) to 1(384) mapping option. See the following instructions.

► To apply a sample group using the 4(96) to 1(384) mapping

Note: The 4(96) to 1(384) mapping should be used only with sample groups containing 96 samples. Additionally, the samples should be applied to only 384-well plates.

1. In the **Plate** tab, select a plate.
For more information about selecting a plate, see “Selecting Plates” on page 31.
2. Click the **Samples** tab.
3. In the **Samples tables** tree, click the sample group you want to apply.



Sample Samples tables tree

4. In the **Properties** pane, select **Horizontal**.
5. In the **Properties** pane, set **4(96)--> 1(384) Mode** to **True**.

Note: If a check mark already appears next to **4(96)--> 1(384) Map Sample Group**, do not select it. It is already selected—selecting it again would de-select it.

6. Determine from which well you want to start applying the samples.
You should start from well A1, A2, B1, or B2. See the following illustration.

Starting from well A1, samples would be applied as shown here (the numbers are sample names)

	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
B																							
C	13	14	15	16	17	18	19	20															
D																							
E	25	26	27	28	29	30	31	32	33	34	35	36											
F																							
G	37	38	39	40	41	42	43	44	45	46	47	48											
H																							
I	49	50	51	52	53	54	55	56	57	58	59	60											
J																							
K	61	62	63	64	65	66	67	68	69	70	71	72											
L																							
M	73	74	75	76	77	78	79	80	81	82	83	84											
N																							
O	85	86	87	88	89	90	91	92	93	94	95	96											
P																							

Note

Samples are applied in the order in which they appear in the sample group. In the illustration to the right, “1” is the first sample in the sample group, “2” is the second, “3” is the third, and so on.

(Continued on next page)

Defining Plates
Applying Sample Groups Using 4(96) to 1(384) Mapping

(Continued)

Starting from well A2, samples would be applied as shown here (the numbers are sample names)

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B																									
C	13	14	15	16	17	18	19	20	21	22	23	24													
D	25	26	27	28	29	30	31	32	33	34	35	36													
E																									
F																									
G	37	38	39	40	41	42	43	44	45	46	47	48													
H																									
I	49	50	51	52	53	54	55	56	57	58	59	60													
J																									
K	61	62	63	64	65	66	67	68	69	70	71	72													
L																									
M	73	74	75	76	77	78	79	80	81	82	83	84													
N																									
O	85	86	87	88	89	90	91	92	93	94	95	96													
P																									

Starting from well B1, samples would be applied as shown here (the numbers are sample names)

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B	1																								
C																									
D	13	14	15	16	17	18	19	20	21	22	23	24													
E	25	26	27	28	29	30	31	32	33	34	35	36													
F																									
G	37	38	39	40	41	42	43	44	45	46	47	48													
H																									
I	49	50	51	52	53	54	55	56	57	58	59	60													
J																									
K	61	62	63	64	65	66	67	68	69	70	71	72													
L																									
M	73	74	75	76	77	78	79	80	81	82	83	84													
N																									
O	85	86	87	88	89	90	91	92	93	94	95	96													
P																									

Starting from well B2, samples would be applied as shown here (the numbers are sample names)

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B	1																								
C																									
D	13	14	15	16	17	18	19	20	21	22	23	24													
E	25	26	27	28	29	30	31	32	33	34	35	36													
F																									
G	37	38	39	40	41	42	43	44	45	46	47	48													
H																									
I	49	50	51	52	53	54	55	56	57	58	59	60													
J																									
K	61	62	63	64	65	66	67	68	69	70	71	72													
L																									
M	73	74	75	76	77	78	79	80	81	82	83	84													
N																									
O	85	86	87	88	89	90	91	92	93	94	95	96													
P																									

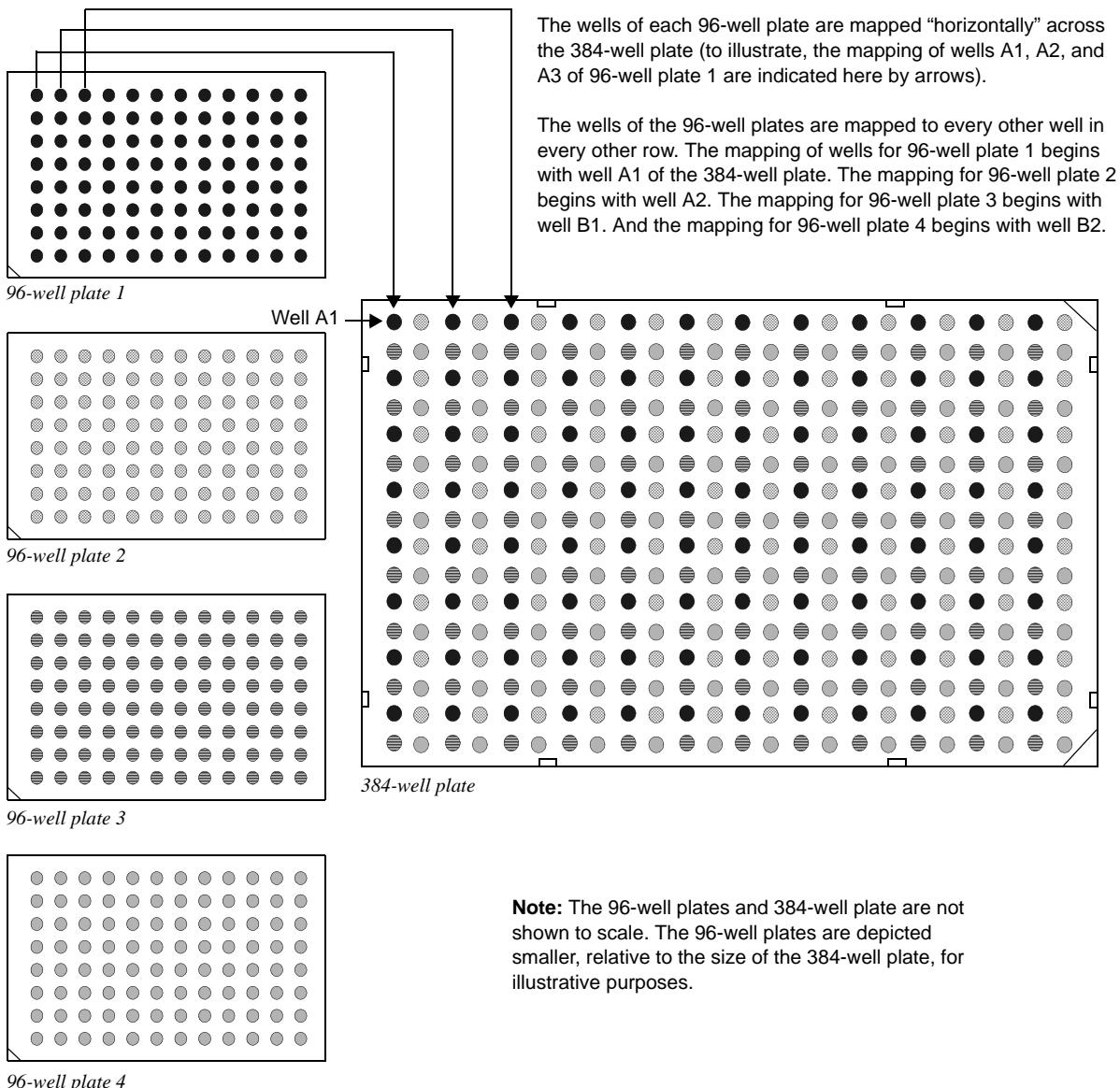
- In the plate diagram, right-click the well from which you want to start applying the samples and choose **Apply**.

The samples are applied to the plate wells.

8. To apply additional sample groups to the current plate, repeat these steps (from step 3).

Applying Sample Group Mapping

The mapping option applies a sample group to the wells of a plate in the same way that certain automated pipettors transfer material from four 96-well plates to a single 384-well plate. This transfer scheme is illustrated below.



In Typer, you can apply samples to a plate in the manner depicted above by having a separate sample group represent each of the 96-well plates and then applying the sample groups using the mapping option. See the following instructions.

Viewing Applied Assays and Samples

Use the **Plate** tab to identify the assays and samples that are applied to the plate. Expand the tree nodes and click a specific plate to view. Once selected, its contents are indicated by Plate Layout and detail appears in the Plate Table. Assays appear different colors, depending on the amount of assays added. A well that is solid in color indicates the presence of a sample.

Working with Plates

Clearing Wells

You can remove the assays or samples that are currently applied to wells. If you want to change what is applied to a well, clear the well before applying the proper assay or sample. If an existing plate can be used to create a new plate, make a copy.

► To clear a well

1. On the navigation tree, select the plate whose wells you want cleared.
2. On the plate grid, highlight the wells you want cleared.
3. Right-click the selected wells, and then choose **Clear**.
4. Select to clear either the assay, the sample or all.

The assays or samples are removed from the selected wells, as indicated by an empty well.

Opening Plates

The **Plate** tab shows all the plates present in the database in a navigation tree organized by Customer and Project.

► To open a plate

- On the **Plate** tab navigation tree, select a plate.
The contents of the plate are displayed in the plate grid.

Copying Plates

If you want to create a copy of an existing plate, use the **Copy to new plate** command. See the following steps.

► To copy a plate

1. On the Customer\Project\Plate tree, select a project to which the new plate will be added.
2. Right-click and select **New Plate**.
3. Type the **Plate ID** using any combination of alpha or numeric characters.
4. Click the option for **96 Wells** or **384 Wells**.
5. Click **OK**.
6. On the Customer\Project\Plate tree, select the plate that contains the contents you want to copy.
7. From the Edit menu, select **Copy**.
OR, press and hold down the [Ctrl] keyboard key and press **C**.
8. Click the new plate to which the copied contents will be added.
9. From the Edit menu, select **Paste**.
OR, press and hold down the [Ctrl] keyboard key and press **P**. The contents of the plate copied populate the newly created plate.
10. Select **Save** on the File menu.

Deleting Plates

Note: If a plate has data in it, you must have database administrator privileges to delete the plate.

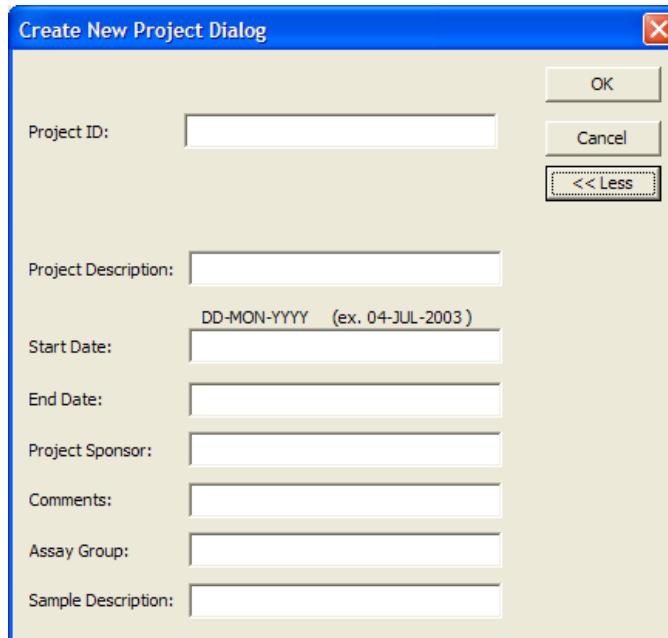
► To delete a plate

1. On the **Plate** tab, select the plate to be deleted on the **Customer\Project\Plate** navigation tree.
2. Right-click the selected plate, and choose **Delete Plate**.
3. In the confirmation dialog box that appears, click **OK** to delete the plate or **Cancel** to discontinue.

Projects

► To create a new project

1. On the **Plate** tab, select an existing customer.
2. Right-click the selected customer, and choose **New Project**.
OR, click on the **File** menu and select **New**, followed by **New Project**.
The **New Project** dialog box appears.



New Project dialog box

3. Type a name in the **Project ID** box.
4. The remaining boxes are optional. Type values in these boxes if desired.
5. Click **OK** to save the project or **Cancel** to discontinue.

Tip: Rename Project

It is possible to change the name of a project without using the **Project** dialog box. In the navigation tree, click the project name in the tree to highlight it, and click again so a cursor appears. Type a different name, and then press the <Enter> key. A confirmation dialog box appears; click **Yes** to change the name or **No** to discontinue.

Tip: Change Customer ID

To change a customer ID, select the customer name in the navigation tree to highlight it, and click again so a cursor appears. Type a different name, and then press the <Enter> key. A confirmation dialog box appears; click **Yes** to change the name or **No** to discontinue.

To edit any other customer information, see the steps under "To edit an existing customer".

► **To edit an existing project**

1. On the **Plate** tab, select a project.
2. Right-click the selected project, and choose **Edit Project**.
The **Project** dialog box appears.
3. Make any changes you want.
4. Click **OK** to save the changes or **Cancel** to discontinue.

Customers

► **To create a new customer**

1. On the **Plate** tab, select the root of the Plate tree labelled Customer\Project\Plate (the root level of the navigation tree) to highlight it.
2. Right-click the root level, and choose **New Customer**.
OR, click on the **File** menu and select **New**, followed by **New Customer**.
The **New Customer** dialog box appears.
3. Fill in the required fields and any optional fields you want.
4. Click **OK** to save the customer or **Cancel** to discontinue.

► **To edit an existing customer**

1. On the **Plate** tab, select an existing Customer to highlight it.
2. Right-click the selected customer, and choose **Edit Customer**.
The **Customer** dialog box appears.
3. Make any changes you want.
4. Click **OK** to save changes or **Cancel** to discontinue.

Extend Primer Adjustment

Due to the inverse relationship between peak intensity and analyte mass, it is recommended that extend primers in iPLEX assays are adjusted by concentration to address this issue. The highest mass primer (~8500 Da) has a peak intensity 25% less than the average of the lower mass primers. Because of this, analyte signal-to-noise ratio estimation throughout the spectrum can pose a significant challenge to the Caller software. In the context of a genotyping reaction, analyte peaks can be missed, leading to genotyping errors. Therefore, it is recommended that extend primers in iPLEX assays are adjusted by concentration and tested prior to use in order to help address these issues.

There are three options for preparing iPLEX primer cocktails provided, labelled options A, B, and C. Option A represents the most simple method and is favored for a simpler reaction process; however, this may result in lower call rates. For maximum call rates, option B or C is preferred. See "Appendix A" of the *iPLEX Application Guide* for more information.

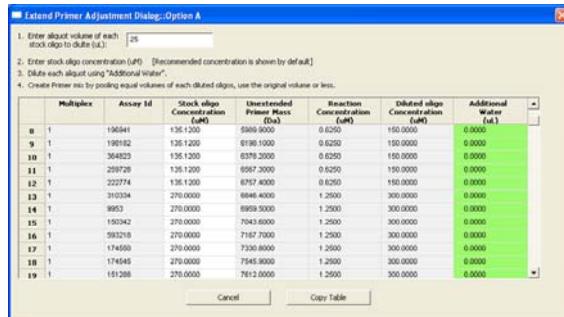
You can copy the contents of the Primer Adjustment grid to send data to Microsoft Excel. The spreadsheets can be printed and used to help set up reactions within the lab environment.

NOTE: For options A and B, you can use the default values to determine the stock oligo ordering concentrations that require dilutions.

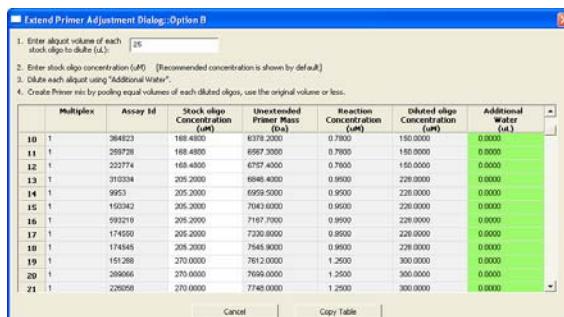
► To calculate the extend primer adjustment

1. On the **Plate** tab, select the desired plate on the **Customer\Project\Plate** navigation tree.
2. On the **Options** menu, select **Primer Adjustment**.
3. Select either **Option A**, **Option B**, or **Option C**.

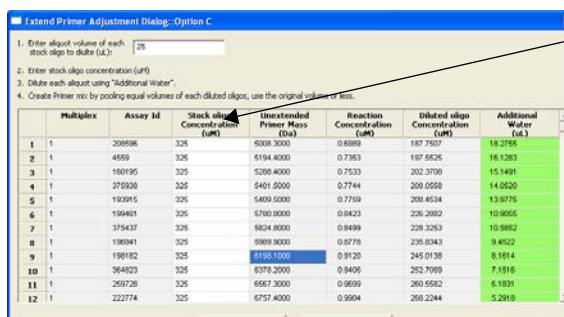
Option A



Option B



Option C



Stock oligo Concentration (uM) must be entered.

Right-click on a cell to open the context menu and use the copy and paste options for entering stock oligo concentration. You can then select groups of cells and paste the concentrations.

Note: The default values for options A and B show the recommended stock oligo concentrations which require no adjustment.

4. If option C is selected, change the aliquot volume to reflect the amount of fluid used to pipette in step 1. This value is not applicable if using options A or B.

5. In the **Stock Oligo Concentration** column, click in the applicable fields and type the appropriate concentration values.
6. To copy the contents to a spreadsheet, click the **Copy Table** button. Open a spreadsheet in Excel and paste the contents. Use the printouts to help set up reactions.
7. Click **Cancel** to close the screen.

Changing Layout Options

You can rearrange the positioning of the Plate Table, Properties, Plate Layout and Plates areas to create one customized layout. Do so by clicking and dragging the title bar of each area to its desired position. Resize the area as necessary by clicking and dragging a corner or edge of the window. It is possible to change back and forth between a customized layout and the original default layout.

» **To save a layout**

1. On the Options menu, select **Layouts**.
2. Click **Save Layout**.
3. Click **Yes** to make the layout the default.

» **To load a layout**

1. On the Options menu, select **Layouts**.
2. Click **Load Layout**.

The screen changes to the layout last saved.

» **To restore the default layout**

1. On the Options menu, select **Layouts**.
2. Click **Reset Layout**.

Acquiring Spectra

Introduction

The ACQUIRE module of Typer controls a MassARRAY analyzer (compact, autoflex, or biflex) to acquire spectra from SpectroCHIPS. As each SpectroCHIP is processed by the analyzer, the spectral data is automatically processed and saved to the MassARRAY database (which resides on a MassARRAY Typer Server).

For specific information on using the MassARRAY analyzer, see the documentation for the specific system that you are using:

- MassARRAY Analyzer Compact: See the *MassARRAY Analyzer Compact User's Guide* (Part Number 11533) for instructions.
- For users of the Bruker Autoflex, see the *MassARRAY Typer User's Guide (Autoflex)* (Part Number 11530).
- For users of the Bruker Biflex, see the *MassARRAY Typer User's Guide (Biflex)* (Part Number 11531).

Note: ACQUIRE is available only on a Typer Workstation. It is not available on a Typer Server or on Typer Clients.

Overview of Acquiring Spectra

This section provides an overview of the main steps involved in acquiring spectra from SpectroCHIPS and saving the spectral data to the MassARRAY database.

The main steps are:

1. Start the MassARRAY RT software.
2. Associate SpectroCHIPS and experiments using Chip Linker (if you have MassARRAY Tracking, this is not necessary).
3. Load SpectroCHIPS.
4. Select the number of shots and raster positions.
5. Turn on the high voltage.
6. Set SpectroCHIP geometry options.
7. Start the automatic run.
8. Unload SpectroCHIPS.

The remaining sections in this chapter cover each of these main steps in detail. Follow the instructions in the sections, in the order in which they appear.

Starting the MassARRAY RT Software

For information on starting the MassARRAY RT software, see the user's guide for the analyzer that you are using as described at the beginning of this chapter.

► On a Compact Workstation, to start the MassARRAY RT software

- On the analyzer computer, double-click the MassARRAY RT icon.



MassARRAY RT icon on the Windows desktop

Using Chip Linker to Associate Chips with Experiments

Use the Chip Linker module to associate SpectroCHIPs with experiments to be processed. This module generates the input files required by MassARRAY Typer RT Workstation for processing the SpectroCHIPs.

Opening Chip Linker

► To open Chip Linker

1. In the **MassARRAY** folder on the Windows desktop, double-click the **Typer 3.3** folder or the **RT-Workstation** folder to open it. (Open either folder. Each one contains a copy of the Chip Linker shortcut icon.)



2. Double-click the **Chip Linker** shortcut icon to open the application.

The **Database Info** dialog box appears.

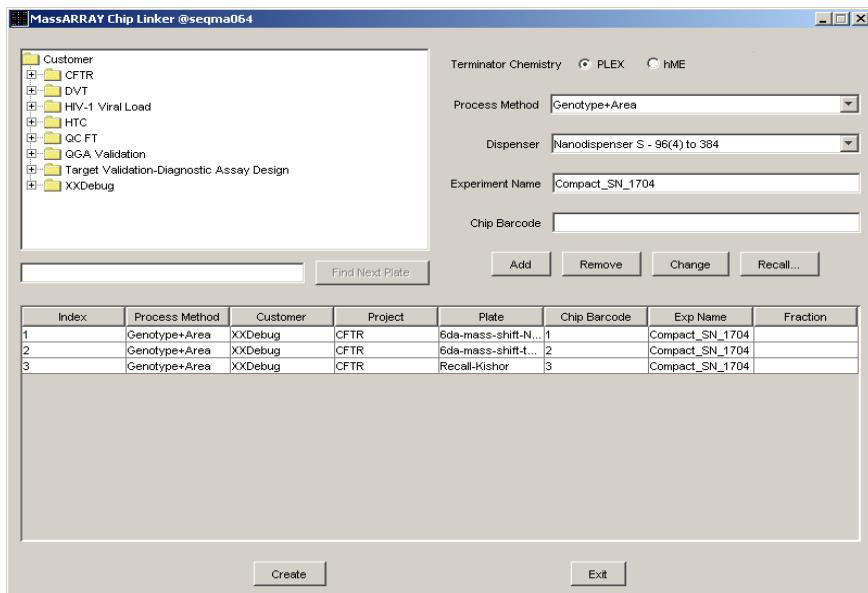
3. Type a user name and password.
4. Select a server from the **DB Host** drop-down list.
5. Click **Enter**.

The **MassARRAY Typer Chip Linker** window appears.

Associating SpectroCHIPs and Experiments

► To associate SpectroCHIPs and experiments

1. In the **MassARRAY Typer Chip Linker** window, select a plate from the navigation tree.



MassARRAY Typer Chip Linker window

2. For the **Terminator Chemistry** option, select **iPLEX** or **hME**.

NOTE: At the end of the run, you can confirm that the correct option was selected by looking at the status bar as described in “Selecting an Experiment” on page 78.

3. Select Genotype or Genotype + Area from the **Process Method** drop-down list.
4. Select the nanodispenser used to dispense to the SpectroCHIPs from the **Dispenser** drop-down list.

“Nanodispenser R” indicates a Robodesign pintool; “Nanodispenser S” indicates a Samsung pintool.

5. Type a name for the group of SpectroCHIPs in the **Experiment Name** box.
6. In the **Chip Barcode** box, type a name for the chip.

After typing the name, copy it to the Windows clipboard. Later, in ACQUIRE, you will paste this name into the **Plate** box.

7. Click **Add** to add the plate to the selection table.

8. Continue to add plates as needed. (Follow Steps 1-6 above.)
9. After you have added all plates to be included in the ACQUIRE run, click **Create** to create the input files used by the MassARRAY Typer RT Workstation.

The selection table is cleared after the input files are created.

Removing Plates from the Selection Table

You can remove plates from those listed in the selection table.

► To remove a plate from the selection table

1. In the **MassARRAY Typer Chip Linker** window, click a plate in the selection table.

A plate is selected when its row in the selection table becomes highlighted.

2. Click **Remove**.

The selected plate is removed from the selection table. This plate will not be included when you create input files for the MassARRAY Typer RT Workstation.

Changing Plate Entries

After a plate has been added to the selection table, you can change the information associated with it.

► To change plate entries

1. In the **MassARRAY Typer Chip Linker** window, click a plate in the selection window.

A plate is selected when its row in the selection table becomes highlighted.

2. Click **Change**.

Details for the selected plate appear in the boxes on the top-right of the **MassARRAY Typer Chip Linker** window.

3. Change the plate information as needed.
4. Click **Add** to add the updated plate to the selection table.

Loading SpectroCHIPs

Place the SpectroCHIPs from which you want to acquire spectra into the SCOUT plate. In ACQUIRE (on the Typer Workstation), name each SpectroCHIP. For detailed instructions, see the user's guide for the specific MassARRAY Analyzer that you are using as described at the beginning of this chapter.

Important: Use only the SCOUT plate supplied with the analyzer. Do not use any other SCOUT plate (for example, from the MassARRAY nanodispenser).

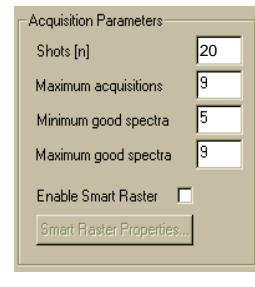
Selecting the Number of Shots and Rastering Options

In ACQUIRE, on the **Auto Run Set Up** tab, under **Acquisition Parameters**, set the number of shots you want, the number of raster positions, and rastering options. See the following for detailed instructions.

To select the number of shots and rastering options

1. On the **Auto Run Set Up** tab, under **Acquisition Parameters**, type the number of shots you want in the **Shots [n]** box.

For the recommended number of shots for your MassARRAY Analyzer, see the appropriate MassARRAY Analyzer user's guide as described at the beginning of this chapter.

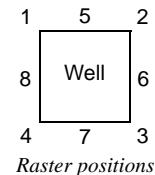


Acquisition Parameters

2. In the **Maximum Acquisitions** box, type the maximum number of raster attempts you want.

Enter a number between 1 and 9. Entering 1 means you do not want the analyzer to raster at all—spectra is acquired from only the center of the well.

By default, the spectra is acquired from the center of the well. In addition, rastering proceeds around eight different positions around the SpectroCHIP well. First around the corners and then around the sides. The illustration to the right shows the raster positions (the numbering indicates the order of rastering). The number you type in the **Maximum Acquisitions** box determines how many of the raster positions you want attempted. For instance, typing 5 means you want the center of the well plus the first four positions attempted.



Raster positions

Note: Not all raster positions you specify may be attempted. You are specifying only the maximum number of raster positions you want to try. The analyzer will move on to the next SpectroCHIP well when either good spectrum is acquired or the maximum number of raster positions has been attempted. For multiplexed assays, this criteria is applied to each assay individually; i.e. the analyzer moves on when good spectrum is acquired or the maximum rasters have been attempted for each assay.

3. Type the minimum and maximum number of good spectra to acquire.

See “Acquisition Parameters Values” on page 64 for a description of these values.

4. Check **Enable Smart Raster** to automatically disable rastering if too many SpectroCHIP wells are failing.

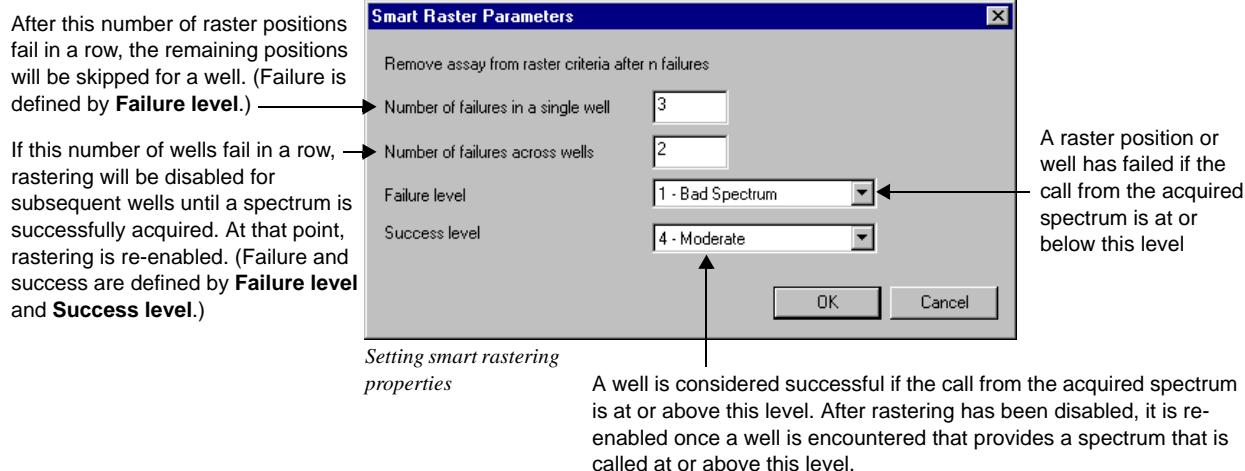
Smart rastering saves time by disabling rastering when a SpectroCHIP is found to have too many wells providing bad spectra. By default (when smart rastering is enabled), after two wells in a row fail, rastering is disabled. Also, in a given well,

Rastering

If the initial acquisition yields an unacceptable spectrum, you may have the analyzer “raster.” When the analyzer rasters, it aims the laser a little off the center of the SpectroCHIP well and acquires another spectrum.

if three raster positions in a row fail, then remaining raster positions on that well are skipped.

Use the **Smart Raster Properties** button to set the criteria that determines when rastering will be disabled. Typically, you should use the default settings of two failed wells and three failed raster positions. However, if you want to change the settings, see the following illustration.



Smart rastering occurs on a per-assay basis. That is, when multiplexing, the smart rastering criteria are applied to each assay individually. It is possible to have rastering disabled for one assay while it is still enabled for other assays in the same well.

Acquisition Parameters Values

The Acquisition Parameters values represent the following:

- **Shots** indicates the number of laser shots attempted during processing.
- **Maximum acquisitions** indicates that the acquisition will stop when the specified number of acquisitions is complete.
- **Minimum good spectra** means collect the specified number of spectra before starting analysis.
- **Maximum good spectra** means to stop acquisition once the specified number of spectra is collected.
- **Enable Smart Raster** applies to genotyping only. It indicates that the system will stop calling assays that fail consecutively.

Table 2 on page 65 provides recommended values for the options under Acquisition Parameters (except **Shots**, which you should determine separately).

Note: The values listed are the suggested defaults to use with Typer 3.3. (If you are using a different version of Typer software, these values do not apply.)

Table 2: Recommended Values for Acquisition Parameters

Option	iPLEX Recommended Value	Genotyping Value	Allelotyping Value
Maximum Acquisitions	9	5 (uniplex) 9 (multiplex*)	9
Minimum Good Spectra	5	1 (uniplex) 5 (multiplex*)	5
Maximum Good Spectra	5	5 (uniplex) 5 (multiplex*)	5
Enable Smart Raster	no	yes (uniplex) no (multiplex)	no

* For optimal results, these values (9, 5, 5) are recommended when you run 8-plexes or higher.

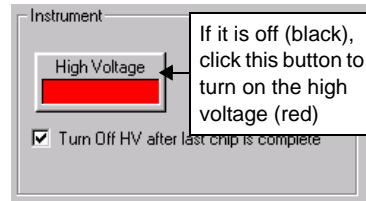
Turning on the High Voltage

Make sure the high voltage is on. If you are leaving the analyzer to run overnight, choose to have the high voltage turned off automatically after the last SpectroCHIP is run. See the following for detailed instructions.

The high voltage should turn on automatically when you click **Start Auto Run**. To turn on high voltage yourself, follow the instructions below.

► To turn on the high voltage

- In the **Auto Run Set Up** tab, under **Instrument**, make sure the **High Voltage** button is red.



Turning on the high voltage

► To automatically turn off the high voltage after the last SpectroCHIP

- Check the **Turn Off HV after last chip is complete** option.

After the last SpectroCHIP has been run, the high voltage will be shut off automatically. This is useful if you will leave the analyzer to run overnight.

Setting SpectroCHIP Geometry Options

SpectroCHIP geometry refers to the format, whether calibrant wells are used, and the order of processing the sample wells. See the following for detailed instructions on setting SpectroCHIP geometry options.

► **To set SpectroCHIP geometry options**

1. On the **Auto Run Set Up** tab (under **Geometry**), **Auto Teach Geometry** should be checked.

When this option is checked, the positioning of each SpectroCHIP in the SCOUT plate is checked. ACQUIRE can correct for small variations in the positioning of each SpectroCHIP.

2. Check the **Use Calibration Wells** option if you want the calibrant wells on the SpectroCHIPS to be used.

If you choose to use calibration wells, make sure calibrant has been dispensed onto at least one of the calibration wells on the SpectroCHIPS. (If you check **Use Calibration Wells** and there is no calibrant on the calibration wells, ACQUIRE will automatically use the last good calibration values.)

Starting an Automatic Run

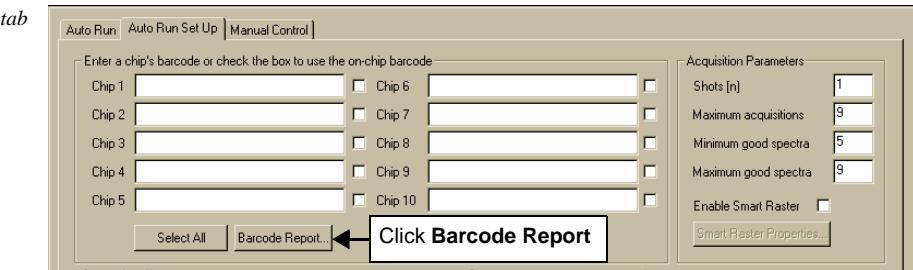
To start an automatic run, click **Start Auto Run** on the **Auto Run** tab. See the following for detailed instructions.

► To start an automatic run

1. In the **Auto Run Set Up** tab, click **Barcode Report**. The **Barcode Report** dialog box appears.

Note: Running a bar code report is optional. However, it is strongly recommended. It allows you to check all chip positions before starting a run. During a run, each chip position is checked only as it is processed. All chip positions are not checked at the beginning of the run. If an error-status chip position is encountered, the run is stopped and any remaining SpectroCHIPs are not processed.

*Auto Run Set Up tab
in ACQUIRE*



*Barcode Report
dialog box*

CHIP ID	STATUS	MAPPING	CHIP TYPE	ANALYSIS
Chip 1 Assay12_Plate5_020517	FOUND	SpectroPoint 384 to 384	384	Genotyping
Chip 2 Assay12_Plate6_020517	FOUND	SpectroPoint 384 to 384	384	Genotyping
Chip 3 Assay12_Plate7_020517	FOUND	SpectroPoint 384 to 384	384	Genotyping
Chip 4 Assay12_Plate8_020518	Error: Reading file D:\V			
Chip 5 Assay12_Plate9_020518	FOUND	SpectroPoint 384 to 384	384	Genotyping
Chip 6				
Chip 7				
Chip 8				
Chip 9				
Chip 10				

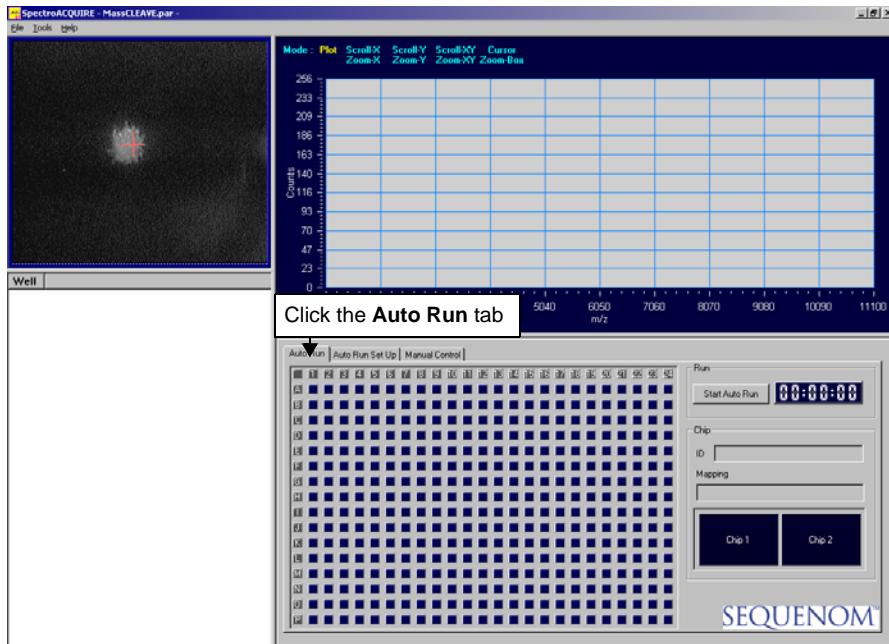
Each chip position should have a “FOUND” status, which means the SpectroCHIP is properly associated with an experiment in the MassARRAY database.

An “Error” status means the SpectroCHIP has not been properly associated with an experiment (in this illustration, Chip 4 has an “Error” status). See “Using Chip Linker to Associate Chips with Experiments” on page 60.

2. Click **CLOSE** and correct any chip positions with an “Error” status.

Caution: You may start a run without correcting chip positions with an “Error” status. However, once an error-status SpectroCHIP is encountered the run will stop. Any remaining SpectroCHIPS will not be run.

3. Click the **Auto Run** tab.



ACQUIRE window with **Auto Run** tab selected

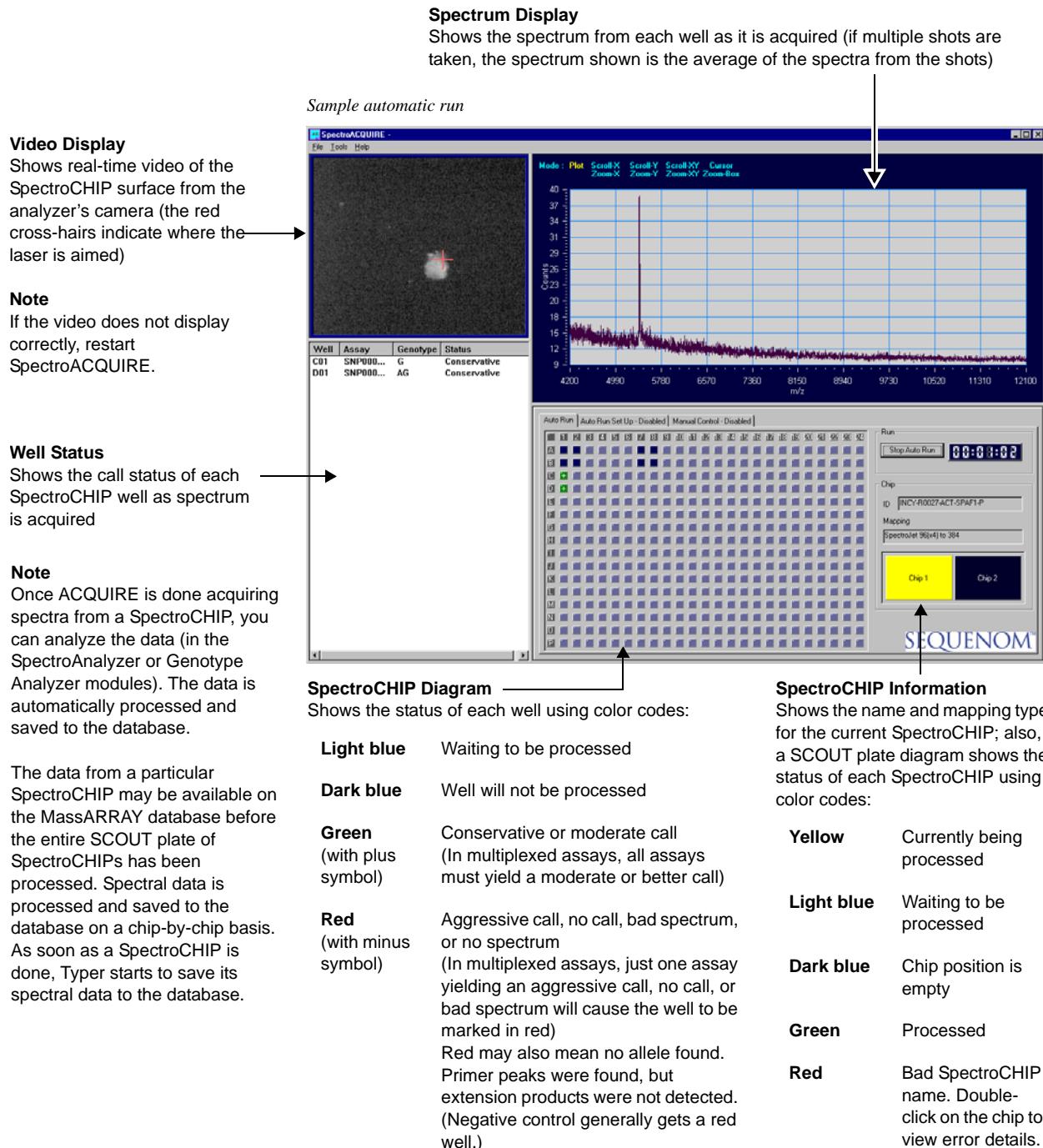
4. Under **Auto Run**, click **Start Auto Run**.

The automatic run begins. Each SpectroCHIP is processed as follows:

- SpectroCHIP name is checked. There must be an experiment associated with the SpectroCHIP name. If there is none, an error message appears and the run stops. (For information about associating SpectroCHIPS with experiments, see “Using Chip Linker to Associate Chips with Experiments” on page 60.)
- Positioning of the SpectroCHIP is checked (for more information, see “Autoteaching” on page 72).
- If calibrant wells are used, calibration spectra are acquired. Using the calibrant is recommended.
- Spectra are acquired from the wells on the SpectroCHIP.

Caution: The SpectroCHIP names are not all checked at the beginning of the run. The name of each SpectroCHIP is checked individually as ACQUIRE begins to process it. If you leave an automatic run to run overnight and ACQUIRE encounters a bad SpectroCHIP name, the run will stop at that SpectroCHIP. Click **Barcode Report** (on the **Auto Run Set Up** tab) to check all SpectroCHIPS before running them.

The following illustration shows a sample ACQUIRE window during an automatic run.



Unloading SpectroCHIPs from the Genotype Analyzer

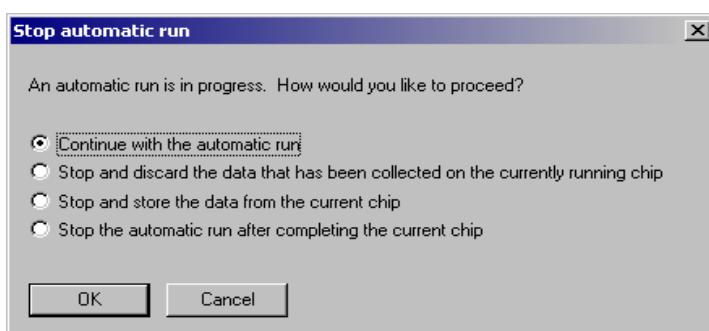
For detailed instructions on unloading SpectroCHIPs, see the user's guide for the specific MassARRAY Analyzer that you are using as described at the beginning of this chapter.

Caution: Do not unload SpectroCHIPs while ACQUIRE is acquiring spectra. Wait until the acquisition run is complete. If you want to stop the run before it is done, see the next section.

Stopping an Automatic Run

To stop an automatic run

1. Under **Run**, click **Stop Auto Run**.
2. If spectra has been acquired from any SpectroCHIP wells, the following dialog box appears. (If spectra has not yet been acquired for the current SpectroCHIP, this dialog box does not appear; the run stops. Do not complete the remaining steps.)



Continue with the automatic run

Resumes the run from where it left off.

Stop and discard the data that has been collected on the currently running chip

Stops the run. Any spectral data acquired for the current SpectroCHIP is discarded. **Important:** If you want to rerun the SpectroCHIP later and save it to the experiment currently associated with it, select this option.

Stop and store the data from the current chip

Stops the run. Any spectral data acquired for the current SpectroCHIP is saved to its associated experiment in the MassARRAY database. Since data is saved to the experiment, the experiment will no longer be available to receive future data. If you want to rerun the SpectroCHIP at a later time you will have to save its spectral data to another experiment.

Stop the automatic run after completing the current chip

Stops the run after completing the current chip.

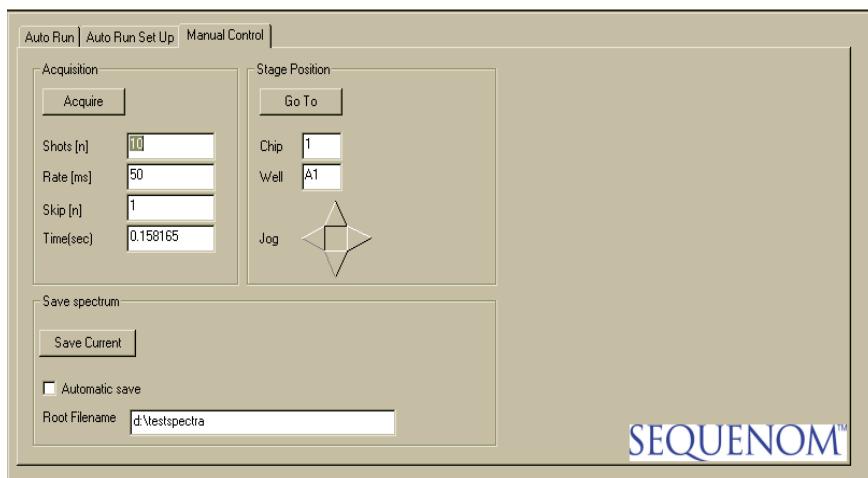
3. Select how you would like to proceed and click **OK**.

Saving Spectra

When manually acquiring spectra, you can save the most recently acquired. The spectra is saved to a file.

► To save the most recently acquired spectra

1. Click the **Manual Control** tab.



Manual Control tab in ACQUIRE

2. Click **Save Current** to save what is currently shown on the screen.
3. Click **Automatic Save** if you want to automatically save the results after each well is processed.
This option is “on” when a check mark appears in the box.
4. Enter the path to save the data in the **Root File Name** box.

Recalling Plate Data

After a SpectroCHIP has been processed, if you find that an assay has been incorrectly applied to a plate or well of a plate, you can recall the plate data and copy it to a new plate with the correct assays applied.

Note: This feature only applies to MassARRAY CALLER used with Typer 3.3. If you are using CALLER with a different version of Typer, you cannot recall plate data. For assistance, contact Sequenom Customer Support toll free at 1 877 4 GENOME.

► To recall plate data

1. On the Windows desktop, double-click the **MassARRAY** folder to open it.
2. Double-click the **Typer 3.3** folder or the **RT-Workstation** folder. (Open either folder. Each one contains a shortcut icon for Chip Linker.)
3. Then, double-click the **Chip Linker** icon to start the application.

The **MassARRAY Typer Chip Linker** window appears.

4. In the **MassARRAY Typer Chip Linker** window, click a plate in the selection window.

A plate is selected when its row in the selection table becomes highlighted.

5. Click **Recall**.

The **Select Recall Chip** dialog box appears.

6. Select the SpectroCHIP to be recalled.
7. Click **Recall**.

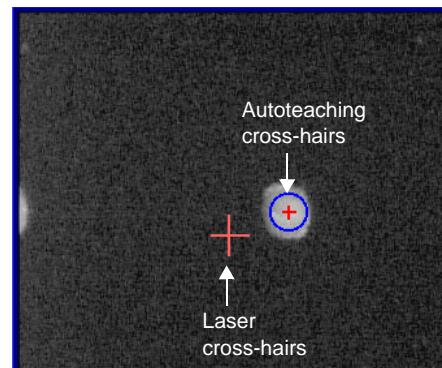
Note: If the **Recall** button is grayed out, MassARRAY Caller is not set to use Typer 3.1. For assistance, contact Sequentom Customer Support toll free at 1 877 4 GENOME.

The recall process may take a few moments to complete. Once the recall is completed, you can return to using Chip Linker.

Autoteaching

When autoteaching is turned on (recommended), the positioning of each SpectroCHIP is checked before spectra are acquired from it. ACQUIRE corrects for small variations in the positioning of SpectroCHIPS.

The positioning of each SpectroCHIP is checked by examining wells A2 to H2, forward and back. The video display, in the upper left of the ACQUIRE window, will show autoteaching cross-hairs in addition to the laser cross-hairs (see right). ACQUIRE finds the location of each well and places the auto teaching cross-hairs on it. The difference between the location of the auto teaching cross-hairs and the laser cross-hairs is found for each of the wells A2 to H2. The differences between the laser and autoteaching cross-hairs for the wells are averaged. This average difference is used as an offset to correct the positioning of the SpectroCHIP. After autoteaching, the autoteaching cross-hairs disappear.



Video display
(upper left of ACQUIRE window)

Note: If ACQUIRE is unable to find one of the autoteaching wells (A2 to H2), it will skip the well. You can tell that a well is not found by watching the video display during autoteaching. The autoteaching cross-hairs will not appear when a well cannot be found.

At least four of the autoteaching wells must be found for autoteaching to work. If less than four are found, no correction is applied to the SpectroCHIP.

Saving Parameters

Unless you save parameters, the auto run settings will revert to original default values if you quit and restart ACQUIRE.

To save parameters

- On the **Tools** menu, select **Save Parameters**.

The current settings are saved. If you quit ACQUIRE and restart it, the current settings will still be in place.

Tools Menu

The **Tools** menu of the ACQUIRE window contains three options: **Configure**, **Image Processing**, and **Save Parameters**.

Unless instructed to do so by SEQUENOM, do not select **Configure** or **Image Processing**. These options access configuration settings for ACQUIRE. These configuration settings should not be changed.

The **Save Parameters** option may be used to save the current auto run settings. See “Saving Parameters” above.

Quitting ACQUIRE

To quit ACQUIRE

- On the **File** menu, select **Exit**.

Notes:

Reviewing Processed Data with TyperAnalyzer

Introduction

This chapter covers using TyperAnalyzer to view results after data is processed from the analyzer by the ACQUIRE module (on the Typer Workstation).

Similar to the Genotype Analyzer module (see “Chapter 6 Reviewing Processed Data with Genotype Analyzer” on page 115), TyperAnalyzer is used to view and analyze data from the analyzer. The TyperAnalyzer and Genotype Analyzer modules complement each other—they provide alternative ways to view the same data.

Note: Some TyperAnalyzer features apply only to genotyping, some only to allelotyping, and others to both types of analysis. Sections in this chapter applicable to only genotyping have “(Genotyping)” after their headings. Sections applicable to only allelotyping have “(Allelotyping)” after their headings. Sections applicable to both have plain headings, with no analysis type indicated.

Screen Resolution

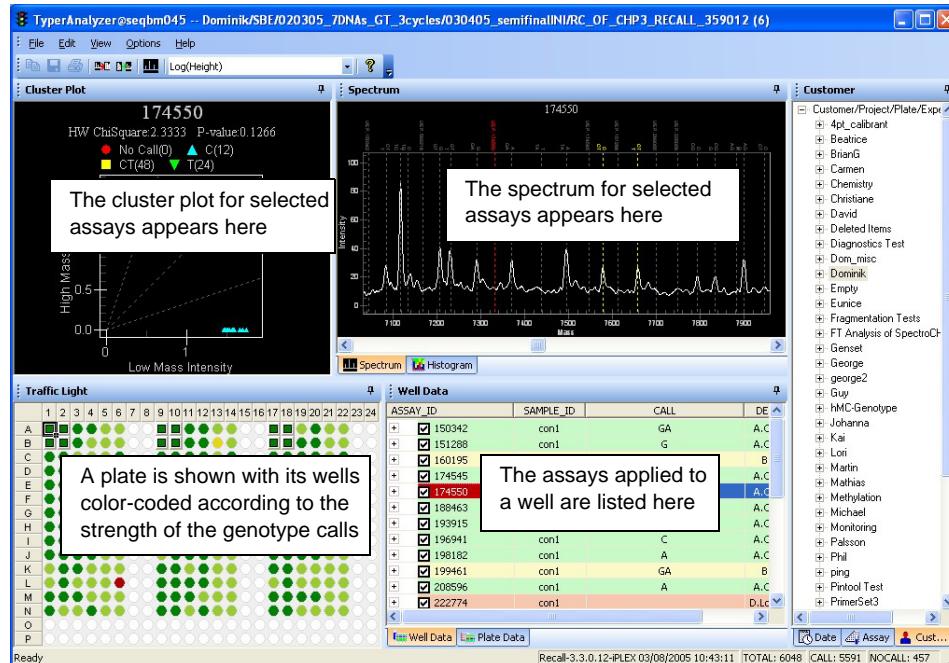
When viewing results data in TyperAnalyzer, it is recommended you set the resolution of your computer screen to 1024 by 768. This screen resolution settings provides the best possible viewing of well data and processing results. (See the online help provided with Microsoft Windows for instructions on setting screen resolution.)

Genotyping

When genotyping, TyperAnalyzer is particularly well suited to viewing and analyzing multiplexed assays. It may also be used to view uniplex assays.

A selected plate is shown with its wells color-coded according the strength of the genotype calls for each well relative to the optimal threshold for a conservative to moderate call. Dark green indicates a call above the optimal threshold, light green indicates a call above the success threshold but below the optimal threshold, yellow indicates a call below the success threshold but above the failure threshold, and red indicates a call below the failure threshold. The colors and thresholds, along with other TyperAnalyzer attributes, can be

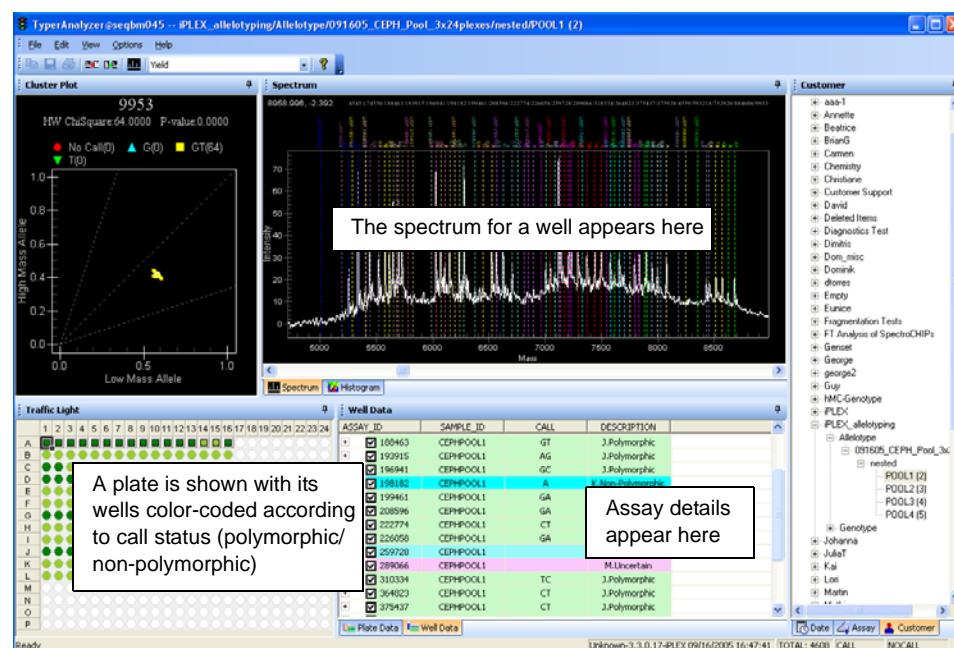
customized using the Configuration software. For more information, see Appendix B “Configuring the MassARRAY Software” on page 147.



Example of TyperAnalyzer window

Allelotyping

When allelotyping, you can quickly determine which wells are polymorphic (i.e. both alleles found) and non-polymorphic (only one allele found).



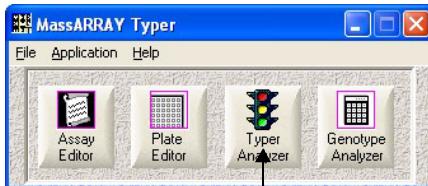
Example of Allelotyping

Starting TyperAnalyzer

Note: To perform allelotyping you must have an allelotyping license. Contact SEQUENOM, Inc. for information.

► To start TyperAnalyzer

1. In the Typer window, click **TyperAnalyzer**.



Click the **TyperAnalyzer** button to start TyperAnalyzer

2. In the **Connect To Database** dialog box, enter your user ID, password, and a data source name, and click **OK**.

The **TyperAnalyzer** window appears.

The TyperAnalyzer Window

The TyperAnalyzer window includes the following panes:

- Cluster Plot
- Spectrum
- Histogram
- Traffic Light
- Plate Data
- Well Data

Data among each of the panes are always synchronized in real time. For example, clicking on a well in the Traffic Light pane changes the display information or currently active selections in the Plate Data and Cluster Plot panes.

Many attributes of the TyperAnalyzer window can be modified from their defaults. For a detailed description of how to customize TyperAnalyzer, see Appendix B “Configuring the MassARRAY Software” on page 147.

Cluster Plot Pane

The Cluster Plot pane shows the data currently selected in either the Plate Data or Well Data tabs of the Plate Data pane. If there is no valid data in the Plate Data pane due to lack of good alleles measured, an empty plot is displayed.

Spectrum Pane

The Spectrum pane shows the raw spectrum for the well currently selected in the Traffic Light pane. The color markers indicate the peak information for the currently selected assay in Well Data. The gray markers indicate the peak information for the rest of assays in the same well.

Histogram Pane

The Histogram pane provides information about the distribution of calls made for each assay on a chip. Use the histogram graph in conjunction with the cluster graph in the Cluster Plot pane and the spectrum graph in the Spectrum tab to analyze assays.

Traffic Light Pane

The Traffic Light pane shows all the wells that have data. You can click on a well or use the arrow keys to change the currently selected well. The shape and color of each well indicates its relationship to the currently selected well.

Plate Data Pane

The Plate Data pane lists the calls for the plate and displays information for each call, including sample ID, well position, and description.

Well Data Pane

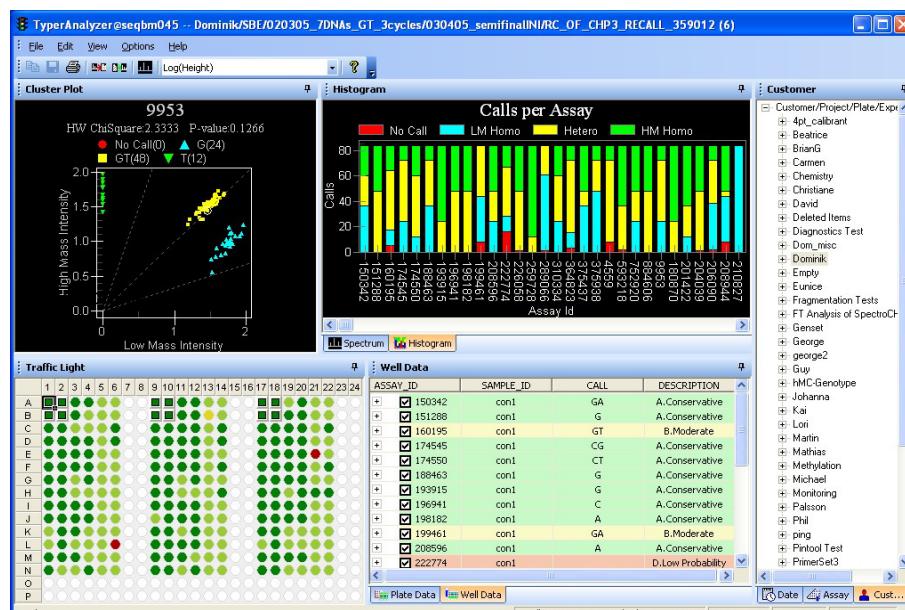
The Well Data pane shows all the assays in the currently selected well. You can click each row or use the Up and Down arrow keys to change the selected assay. For each assay, it also contains detailed information, such as peak information. You can access this detailed information by clicking the + sign next to the assay name.

Status Bar

Displays the caller version, selected chemistry, and date and time of the current plate.

Selecting an Experiment

On the right side of TyperAnalyzer is a *tree control*. Use the tree control to select the experiment under a plate for which you want to view data.



Example of TyperAnalyzer window

Be sure the correct Terminator Chemistry—iPLEX or hME—is displayed in the status bar. If the incorrect chemistry is displayed, the incorrect Terminator Chemistry may have been selected in the Chip Linker module.

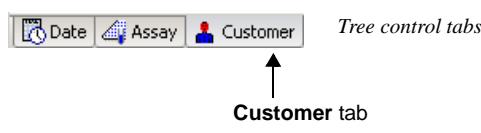
You can view an experiment in the following three ways:

- Customer
- Assay
- Date

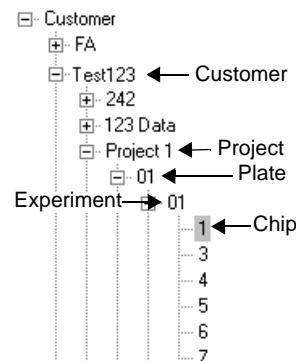
Note: While looking for an experiment, you can use the *, +, and - (asterisk, plus, and minus) keys on the numeric keypad, of the keyboard, to expand and collapse the whole tree or parts of it.

► To select an experiment by customer

- At the bottom of the tree control box, click the **Customer** tab.



The illustration to the right shows the organization of the tree in the **Customer** tab. Plates are organized by customer and project. First expand the customer containing your plate. Next, expand the project containing your plate. Under each plate are experiments and chips. To view spectra, select a chip under one of the experiments.

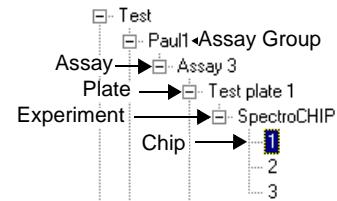


► To select a chip by assay

- At the bottom of the tree control box, click the **Assay** tab.



The illustration to the right shows the organization of the tree in the **Assay Group** tab. Plates are organized by assay group and assay.



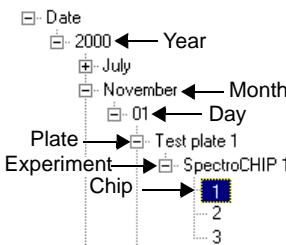
Expand the assay group containing the assay you want. Under each assay are the plates to which the assay has been applied. Under each plate are experiments and chips. To view spectra, select a chip under one of the experiments.

► **To select a chip by date**

- At the bottom of the tree control box, click the **Date** tab.



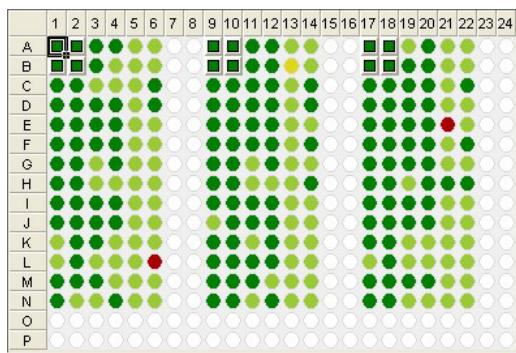
The illustration to the right shows the organization of the tree in the **Date** tab. The tree is organized by year, month, then day. Under a day are the plates run on that day. Under each plate are experiments and chips. To view spectra, select a chip under one of the experiments.



Sample date tree control in
TyperAnalyzer

Color Codes

Once you select a genotyping experiment (see “Selecting an Experiment” on page 78), its plate diagram appears with wells color-coded according to the strength of genotype calls.



Example of a plate diagram for a genotyping plate

Each well is color-coded:

Dark Green Above the optimal threshold for a conservative to moderate call (default: 85-1--%)

Light Green Above the success threshold but below the optimal threshold for a conservative to moderate call (default: 50-84%)

Yellow Below the success threshold but above the failure threshold for a conservative to moderate call (default: 15-49%)

Red Below the failure threshold for a conservative to moderate call (default: 0-14%)

White No data (i.e., spectrum) stored and/or no assays applied to the well

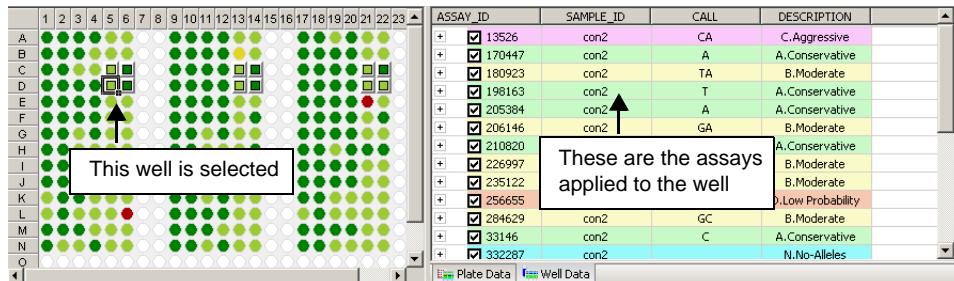
Wells are color-coded according to the weakest call of all selected assays that were applied to it. Default colors and values are assigned as described above. To change the these defaults, see “Configuring TyperAnalyzer” on page 150.

Selecting Which Assays Are Shown

When you select a well in the plate diagram, the assays applied to the well appear in a table to the right (called the “assay table”).

Note

The coloring in the assay table is not related to the color-coding of the plate diagram. The coloring of the assay table follows the coloring convention of the results table in the Genotype Analyzer module (see Table 3, “Genotype Analyzer Color Codes for Genotyping,” on page 118).



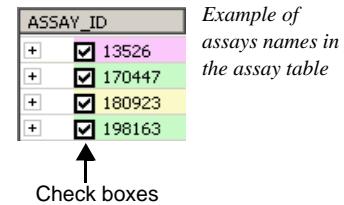
Example of a plate diagram and assay table

The check boxes next to the assay names control how wells are color-coded in the plate diagram. The wells are color-coded according to all of the selected assays; selected assays are checked. If you clear the check box for one of the assays, then the wells are color-coded according to only those assays that remain checked.

De-select assays (by clearing the check box) to “filter”

them out of the plate diagram. For instance, you may have a failed assay that results in weak calls for all wells. Filter out the failed assay by de-selecting it. The wells will be color-coded according to the remaining, selected assays.

Also, use the check boxes to view the strength of the calls for individual assays by de-selecting all but the assay in question. The wells will be color-coded according to only the selected assay.



Example of assays names in the assay table

Viewing Relationships of a Currently Selected Cell

You can click on a well or use the arrow keys to change the currently selected well. The shape of each well indicates its relationship to the currently selected well.

The shape of each well in the Traffic Light pane indicates the following:

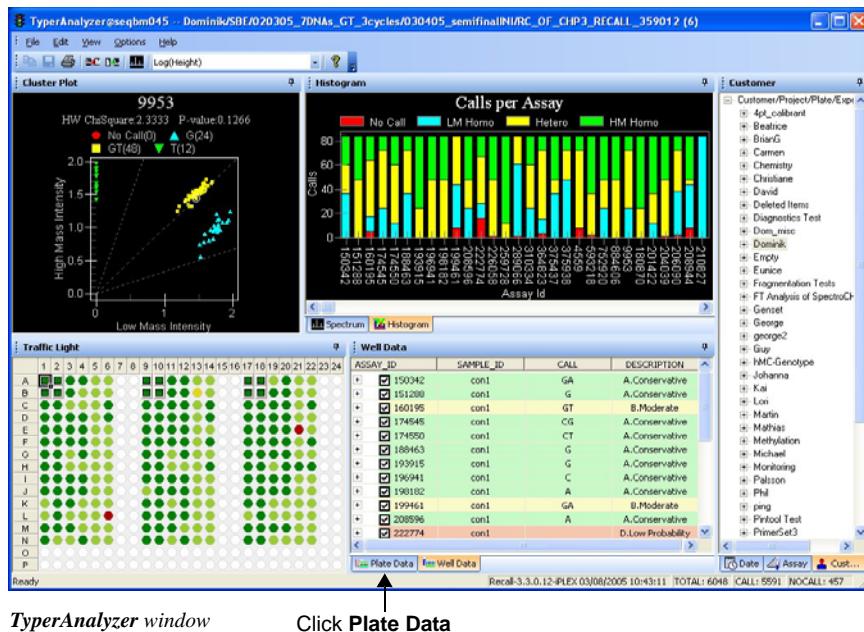
- Squares indicate wells that contain the same sample and assay as the currently selected well
- Circles indicate wells that contain different samples and assays than the currently selected well

Viewing a Results Table

Rather than view a color-coded plate diagram, you can view a table of all the calls for the plate (similar to the results table in the Genotype Analyzer module).

► To view all calls

1. Click the **Plate Data** tab.



TyperAnalyzer window

Click **Plate Data**

The **Plate Data** tab shows a results table of genotype calls for the plate wells.

The figure shows a detailed view of the 'Plate Data' table. The table has columns: SAMPLE_ID, CALL, ASSAY_ID, WELL_POSITION, DESCRIPTION, CALIBRATION, MAX_SHIFT, and RASTERS. The data includes rows for samples like 150342, 151288, 160195, 174545, 174550, 188463, 193915, 196941, and 198182, with calls such as GA, G, GT, CG, CT, A, C, and D. An example row is shown with a blue background. A vertical arrow points to the table area. To the right of the table, a text box reads 'Example of a results table'.

All calls are listed. Note that both calls and no calls are listed.

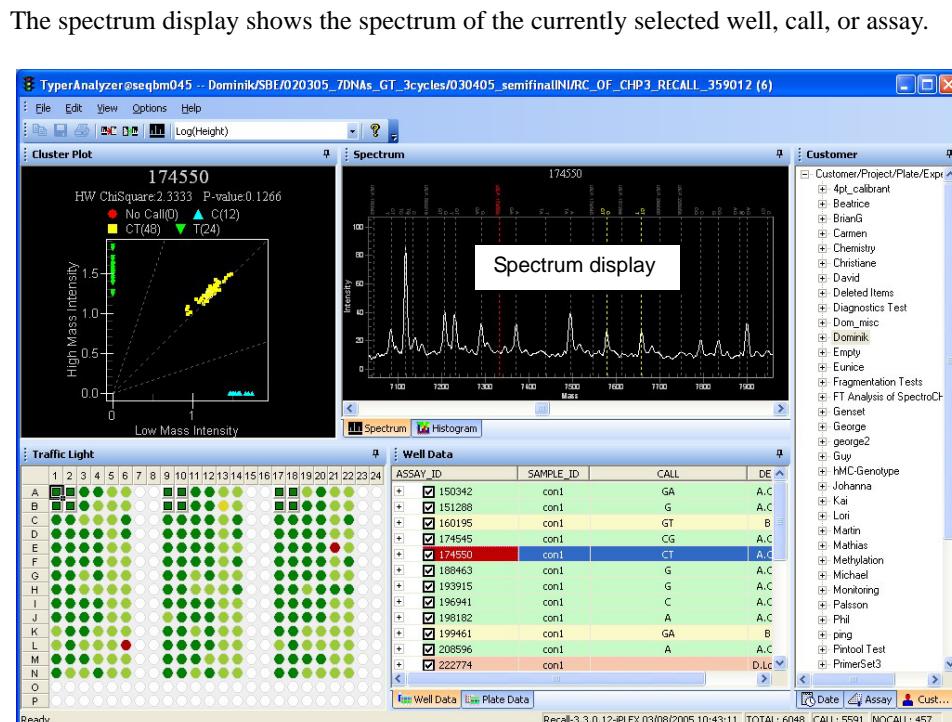
2. To view the spectrum for a call, click the call (i.e., row).

The spectrum appears in the spectrum display (upper right area of the **TyperAnalyzer** window).

You can manually call a genotype, i.e., you may choose the genotype yourself. Right-click the row and choose the genotype call you want to make. (See “Plate Data Tab Options” on page 89 for information.)

Note: When you manually call a genotype, the call will be noted as a “user call” even if you change it back to the original call.

Viewing Spectra



Sample TyperAnalyzer window

Viewing the Spectrum from a Well

When you select a plate well, the spectrum display shows the spectrum acquired from the well. The spectrum is annotated with the expected location of allele peaks and the unextended-primer peak. In some cases, contaminant peaks may be indicated (depending on whether you defined them in Assay Editor when creating the assay; see “Defining Assays” on page 5).

If the well is multiplexed (i.e. multiple assays are applied to it), then annotations for all assays are shown.

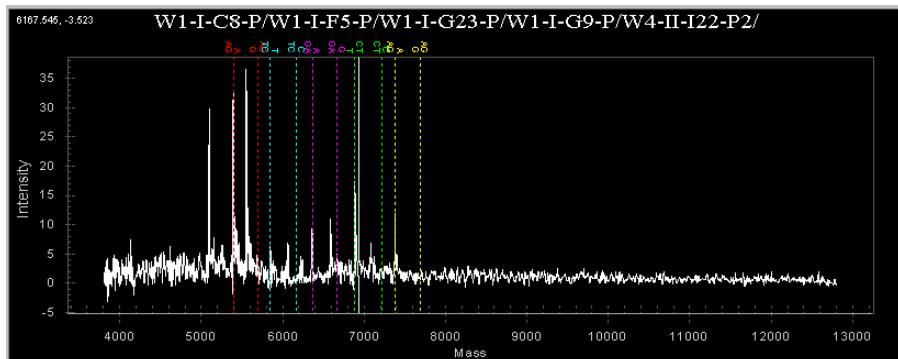
► To view a spectrum showing all assays for a well

- In the plate diagram click a well.

The spectrum appears. All assays applied to the well are shown. Each pair of alleles for each assay are shown in the same color. Each assay is shown as a different color.

Note

When viewing a spectrum showing multiple assays, the color assigned to each assay does not indicate anything. Different colors are assigned to each assay simply to differentiate it from the other assays.

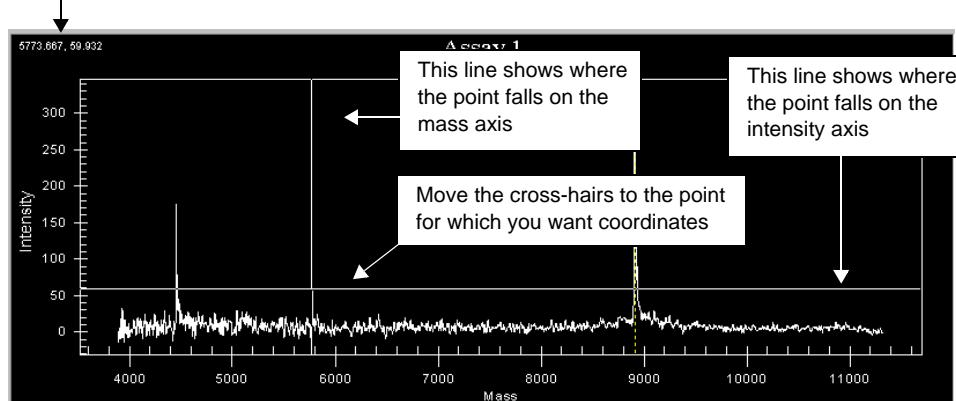


Sample spectrum display showing all assays for a well

Using the Spectrum Display Cross-Hairs

When you point the mouse cursor over the spectrum display it turns into cross-hairs (a cross) with lines extending across and down the display. Use the cross-hairs to find the coordinates of a point in the graph. See the following illustration.

Coordinates of the current location of the cross-hairs are listed here (*mass, intensity*)



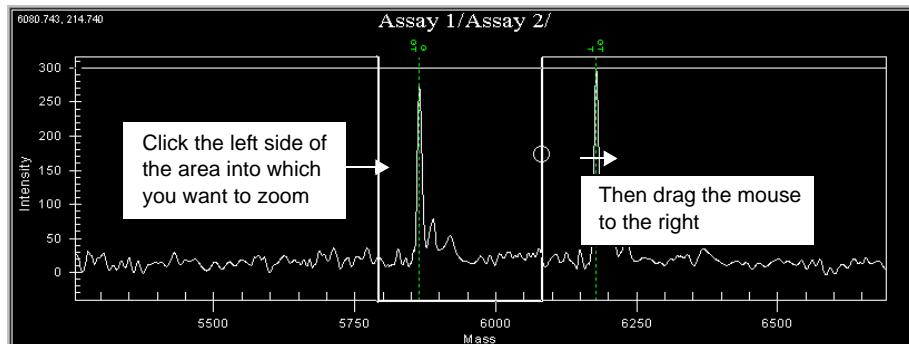
Using the cross-hairs to find point coordinates

Zooming the spectrum display

You can zoom in on an area of the spectrum display.

► To zoom the spectrum display

1. In the spectrum display, click the left side of the area into which you want to zoom and drag the mouse to the right.



Zooming the spectrum display

2. When the zooming box encloses the area you want, release the mouse button.

The zoomed view remains for any subsequent spectra you view. To turn off the zoomed view, follow the instructions under “To un-zoom the spectrum display” on page 85.

► To un-zoom the spectrum display

- Right-click the spectrum and select **Undo Zoom**.

You are returned to the top zoom level (that is, where no zooming is applied).

Viewing the Calibration Spectrum

You can view the calibration spectrum for the chip in the Spectrum pane if it is available.

► To view the calibration spectrum

- On the View menu, select **Display Calibration Spectrum** or click the Display Calibration Spectrum button in the tool bar.

The current well spectrum will be replaced by the chip calibration spectrum. The calibration spectrum display is temporary. Any changes that cause the well spectrum to be changed will replace the calibration spectrum with the corresponding well spectrum in the Spectrum pane.

Adjusting the Spectrum Display

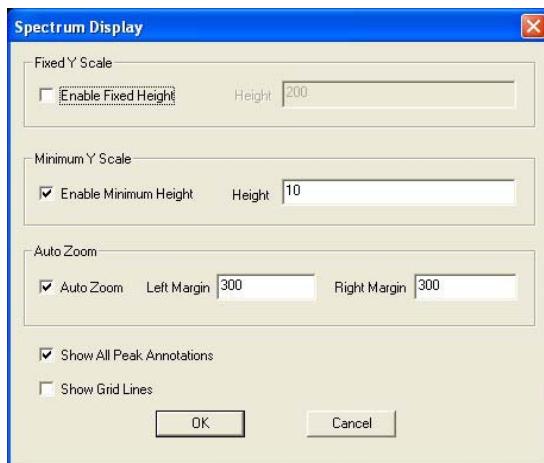
The spectra are not shown to the same scale along the y-axis (intensity). In one spectrum, the y-axis may go up to 500. In another, it may only go up to 350. Each spectrum is displayed with the y-axis scaled to best show the spectrum.

You can choose to view all spectra scaled to the same maximum y-axis value, allowing you to better judge the relative intensities of peaks in different spectra.

► **To adjust spectrum display**

1. On the **View** menu, select **Spectrum Display**.

The **Spectrum Display** dialog box appears.



Spectrum Display dialog box

2. To specify a fixed height for the Y axis, click the **Enable Fixed Height** option so a checkmark appears. Then, in the **Height** box, specify a Y axis height.

All spectra graphs will now use this height for the Y axis.
3. To specify a minimum height for the Y axis, click the **Enable Minimum Height** option so a checkmark appears. Then, in the **Height** box, specify a Y axis height.
4. To automatically zoom on all spectrum graphs, click the **Auto Zoom** option so a checkmark appears.

This option may already be selected. If so, clicking it again removes the checkmark and turns off the option.

5. Specify a mass margin in the **Left Margin** and **Right Margin** boxes.

The zoomed view will have the selected mass margin around it.

6. To show all peak annotations, click the **Show All Peak Annotations** option so a checkmark appears.
7. To show grid lines, click the **Show Grid Lines** option so a checkmark appears.
8. Click **OK** to save your changes.

Your settings are applied to all spectra displayed, unless you return to the **Spectrum Display** dialog box again to make changes.

Printing a Spectrum Graph

► To print a spectrum graph

1. Click on the **Spectrum** tab to make it active.
2. From the **File** menu, select **Print Graph**.
3. In the **Printing** dialog box, choose a printing style—**Color**, **Monochrome**, or **Mono Plus Symbol**.
4. Click **Setup** to set up the printer.
5. Click **OK** to print the graph.

Exporting a Spectrum

You can paste the spectrum to the Windows Clipboard for copying into another program such as Microsoft Word, Excel, or PowerPoint. You can also save the spectrum to a file.

► To copy a spectrum to the Clipboard

1. Right-click the spectrum and select **Export Dialog**.

The **Exporting <assay name>** dialog box appears (where *<assay name>* is the name of the assay).

2. Under **Export**, select the file format in which you want to copy the spectrum to the Clipboard.

Metafile is recommended for copying to the Clipboard.

Note: Selecting **Text / Data Only** copies text data values defining the spectrum graph. For more information, see “To export spectrum graph data points” on page 88.

3. Under **Export Destination**, select **Clipboard**.

4. (Optional) If you need the spectrum in a specific size, specify the size under **Object Size**. Otherwise, leave Object Size at No Specific Size.

Note: Changing the size may “distort” the image if you do not change the width and height proportionally.

5. Click **Export**.

An image of the spectrum is now available on the Windows Clipboard for pasting into any program that will accept pasting of a Windows metafile.

► To save the spectrum to a file

You can save an image of the spectrum as either a Windows metafile (.wmf) or a bitmap (.bmp) file.

1. Right-click the spectrum and select **Export Dialog**.

The **Exporting <assay name>** dialog box appears (where *<assay name>* is the name of the assay).

2. Under **Export**, select a file format.

Note: Selecting **Text / Data Only** saves text data values defining the spectrum graph. For more information, see “To export spectrum graph data points” below.

3. Under **Export Destination**, select **File**.

4. Click **Browse**.

A **Save As** dialog box appears.

5. Choose a folder and enter a name for the file.

.Wmf (Windows metafile) or .bmp (bitmap) extensions are automatically added to the file name you type.

6. Click **Save**.

You are returned to the **Exporting <assay name>** dialog box.

7. Click **Export**.

► To export spectrum graph data points

You can copy the data points defining the spectrum graph as text to the Windows Clipboard or you can save them to a file. If you copy the data points to the Clipboard, you can then paste them from the Clipboard to a spreadsheet or graphing program. If you save the data points to a file, you can import them into another program.

1. Right-click the spectrum and select **Export Dialog**.

The **Exporting <assay name>** dialog box appears (where *<assay name>* is the name of the assay).

2. Under **Export**, select **Text / Data Only**.

3. Under **Export Destination**, select **File or Clipboard**.

4. If you selected **File** as the **Export Destination**, click **Browse**.

A **Save As** dialog box appears. Choose a folder and enter a name for the file. Then click **Save**.

5. Click **Export**.

The **Export <assay name>** dialog box appears.

6. Select the export options you want.
7. Click **Export**.

Plate Data Tab Options

Manually Calling a Genotype

You can override an assay call (or no call) by manually calling a genotype. You can manually call a genotype in the results table (on the **Plate Data** tab).

► **To manually call a genotype in the results table**

1. Click the **Plate Data** tab to view the results table.
2. In the results table, right-click on the row of the assay call you want to override and select the call you want to make.

The screenshot shows a Microsoft Excel spreadsheet with a grid of data. A context menu is open over the fifth row of the grid. The grid has columns labeled '29', 'con1', 'CT', 'L47450', and 'A0'. The fifth row contains the values '33', 'con1', 'A', 'No Call', and 'A0'. The context menu is titled 'Right-click menu for manually calling genotypes' and includes options: 'C', 'CT', 'T', and 'No Call'. The 'No Call' option is highlighted with a green background.

29	con1	CT	L47450	A0
30	con1	G	C	A0
31	con1	G	CT	A0
32	con1	C	T	A0
33	con1	A	No Call	A0

Right-click menu for manually calling genotypes

Calls are made locally and not saved to the database unless you save them.

3. To save the change to the database, click the Save icon or select **Save Changes** from the **File** menu.

Selecting and Sorting Data

► **To sort the table by a column in ascending order**

- Click on the column header.

► **To change the currently selected data**

- Click on a row or use the Up and Down arrow keys

Copying the Grid

You can copy the Plate Data grid to Microsoft Excel.

► **To copy the grid**

1. On the **Plate Data** tab, click inside the grid. (Click any row.)
2. On the **Edit** menu, select **Copy Plate Data Grid**.
3. Open Microsoft Excel.
4. Paste the grid data into Excel.

Press the CTRL and V keys simultaneously to paste the data.

Viewing Assay Details for a Well

The assay table contains information about the assays applied to a well. Under each assay are sections of detailed information about the assay. The sections are initially collapsed (i.e. hidden).

First, in the plate diagram, click the well about which you want information. The assay table lists the assays applied to the well. Click the plus (+) next to an assay to view its details.

When you click the plus (+) next to an assay, details about the assay appear below it

ASSAY_ID	SAMPLE_ID	CALL	DESCRIPTION
+ <input checked="" type="checkbox"/> 500277	DNA Control 19	A	A.Conservative
+ <input checked="" type="checkbox"/> 546106	DNA Control 19	C	A.Conservative
+ <input checked="" type="checkbox"/> 662714	DNA Control 19	TC	A.Conservative
+ <input checked="" type="checkbox"/> 90389	DNA Control 19	G	A.Conservative

Example of an assay table

The following illustration shows an example of the assay details that appear.

ASSAY_ID	SAMPLE_ID	CALL	DESCRIPTION
+ <input checked="" type="checkbox"/> C11-48170	1	GA	J.Polymorphic
-	ASSAYINFO	NAME	SEQUENCE
-	Analyte	A	CAGAACACTTAGCACCCCACTG
-	Analyte	G	CAGAACACTTAGCACCCCAACC
-	Analyte	GA	CAGAACACTTAGCACCCCAACC
-	Analyte	GA	CAGAACACTTAGCACCCCACTG
-	Probe	C11-48170-P	CAGAACACTTAGCACCCCAAC
-	CALLINFO	PROBABILITY	SND
-	Calibrated	1.000000	1.144805
-	PEAKINFO	HEIGHT	SNR
-	5999.900000	5.926464	2.970775
-	6273.100000	115.235081	59.692560
-	6304.100000	4.233089	2.200810
-	6617.300000	105.226049	56.737394
-	AREAINFO	AREA	AREA_VARIANT
-	5999.900000	66.594350	45.111770
-	6273.100000	1108.321027	17.961267
-	6304.100000	80.410700	43.494948
-	6617.300000	998.044321	17.028181
-	FREQUENCYINFO	FREQUENCY	FREQUENCY_UNCERTAINTY
-	6273.100000	0.526177	0.011341
-	6617.300000	0.473823	0.011341

Genotyping Genotyping + Area Allelotyping

ASSAYINFO

CALLINFO

PEAKINFO

AREAINFO

FREQUENCYINFO

Example of assay details

Depending on the type of plate (i.e. genotyping, genotype+area, or allelotyping) only some or all of the information, illustrated here, is shown. For instance, in a genotyping plate, ASSAYINFO, CALLINFO, and PEAKINFO information is shown, but AREAINFO and FREQUENCYINFO are not present.

ASSAYINFO

This part lists information about analytes, MassEXTEND or iPLEX primer (Probe), and contaminants expected in the spectrum.

*Example of
ASSAYINFO
part of assay
details*

<input type="checkbox"/> ASSAYINFO	NAME	SEQUENCE	MASS
<input type="checkbox"/> Analyte	A	CAGAACACTTAGCACCCACTG	6617.300000
<input type="checkbox"/> Analyte	G	CAGAACACTTAGCACCCACC	6273.100000
<input type="checkbox"/> Analyte	GA	CAGAACACTTAGCACCCACC	6273.100000
<input type="checkbox"/> Analyte	GA	CAGAACACTTAGCACCCACTG	6617.300000
<input type="checkbox"/> Probe	C11-48170-P	CAGAACACTTAGCACCCAC	5999.900000
<input checked="" type="checkbox"/> CALLINFO	PROBABILITY	SND	OFFSET

<input type="checkbox"/> Type of expected peak	<input type="checkbox"/> Names, specified when the assay was created in Assay Editor	<input type="checkbox"/> Sequences ("NULL" means no sequence specified, only a mass value was specified)	<input type="checkbox"/> Expected mass values (Da), specified when the assay was created in Assay Editor
------------------------------------------------	--------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------

CALLINFO

This part lists calibration information.

*Example of
CALLINFO
part of assay
details*

<input type="checkbox"/> ANALYSIS	PROBABILITY	SND	OFFSET
<input type="checkbox"/> Calibrated	1.000000	1.144805	-0.177160
<input checked="" type="checkbox"/> DEVIATION	HEIGHT	SND	PROBABILITY

<input type="checkbox"/> "Calibrated" means calibrant was present on the SpectroCHIP and a calibration spectrum was successfully acquired	<input type="checkbox"/> Level of confidence in the assay call expressed as a probability (1.0 means 100%)	<input type="checkbox"/> Standard error of noise	<input type="checkbox"/> Calibration offset applied to spectra
-------------------------------------------------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------------------------------------	--------------------------------------------------	----------------------------------------------------------------

PEAKINFO

This part lists information about the peaks in the spectrum.

Example of
PEAKINFO
part of assay
details

CHARGE	HEIGHT	SNR	PROBABILITY
5999.900000	5.926464	2.970775	0.286040
6273.100000	115.235081	59.692560	1.000000
6304.100000	4.233089	2.200810	0.209770
6617.300000	105.226049	56.737394	1.000000

↑ ↑ ↑ ↑
 Expected mass of a peak Intensity of a peak (i.e. height along the y-axis) Signal-to-noise ratio (ratio of peak height to local noise) Level of confidence that a peak is the actual expected peak, expressed as a probability (1.0 means 100%)

Note: For a bad spectrum, no PEAKINFO information is available

Peaks are identified by mass. To find which analyte, contaminant, or unextended primer a peak represents, match up the mass values. For example, this peak is for the A allele.

ASSAY_ID	SAMPLE_ID	CALL	DESCRIPTION
+ <input checked="" type="checkbox"/> C11-48170	1	GA	J.Polymorphic
- <input type="checkbox"/> ASSAYINFO	NAME	SEQUENCE	MAGS
<input type="checkbox"/> Analyte	A	CAGAACACTTAGCACCCACTG	6617.300000
<input type="checkbox"/> Analyte	G	CAGAACACTTAGCACCCACC	6273.100000
<input type="checkbox"/> Analyte	GA	CAGAACACTTAGCACCCACTG	6617.300000
<input type="checkbox"/> Probe	C11-48170-P	CAGAACACTTAGCACCCAC	5999.900000
- <input type="checkbox"/> CALLINFO	PROBABILITY	SND	OFFSET
<input type="checkbox"/> Calibrated	1.000000	1.144805	-0.177160
- <input type="checkbox"/> PEAKINFO	HEIGHT	SNR	PROBABILITY
<input type="checkbox"/> 5999.900000	5.926464	2.970775	0.286040
<input type="checkbox"/> 6273.100000	115.235081	59.692560	1.000000
<input type="checkbox"/> 6304.100000	4.233089	2.200810	0.209770
<input type="checkbox"/> 6617.300000	105.226049	56.737394	1.000000
- <input type="checkbox"/> AREAINFO	AREA	AREA_VARIANT	RESOLUTION
<input type="checkbox"/> 5999.900000	66.594350	45.111770	268.733247
<input type="checkbox"/> 6273.100000	1108.321027	17.961267	282.752209
<input type="checkbox"/> 6304.100000	80.410700	43.494948	275.282209
<input type="checkbox"/> 6617.300000	998.04321	17.028181	268.523247
- <input type="checkbox"/> FREQUENCYINFO	FREQUENCY	FREQUENCY_UNCERTAINTY	
<input type="checkbox"/> 6273.100000	0.526177	0.011341	
<input type="checkbox"/> 6617.300000	0.473823	0.011341	

AREAINFO (Genotype+Area and Allelotyping only)

This part lists information about the peaks in the spectrum.

*Example of
AREAINFO
part of assay
details*

AREAINFO	AREA	AREA_VARIANT	RESOLUTION
5999.900000	66.594350	45.111770	268.733247
6273.100000	1108.321027	17.961267	282.752209
6304.100000	80.410700	43.494948	275.282209
6617.300000	998.044321	17.028181	268.523247

↑ ↑ ↑ ↑
 Expected Area under Area may be Peak resolution
 mass of a the curve of +/- this amount

Peaks are identified by mass. To find which analyte, contaminant, or unextended primer a peak represents, match up the mass values. For example, this peak is for the G allele.

ASSAY_ID	SAMPLE_ID	CALL	DESCRIPTION
C11-48170	1	GA	J.Polymorphic
	ASSAYINFO	NAME	SEQUENCE
	Analyte	A	CAGAACACTTAGCACCCACC
	Analyte	G	CAGAACACTTAGCACCCACC
	Analyte	GA	CAGAACACTTAGCACCCACC
	Analyte	GA	CAGAACACTTAGCACCCACTG
	Probe	C11-48170-P	CAGAACACTTAGCACCCAC
	CALLINFO	PROBABILITY	SND
		1.000000	1.144805
			-0.177160
	PEAKINFO	HEIGHT	SNR
		5999.900000	2.970775
		6273.100000	59.692560
		6304.100000	2.200810
		6617.300000	0.209770
		6617.300000	56.737394
	AREAINFO	AREA	RESOLUTION
		5999.900000	268.733247
		6273.100000	282.752209
		6304.100000	275.282209
		6617.300000	268.523247
	FREQUENCYINFO	FREQUENCY	FREQUENCY_UNCERTAINTY
		6273.100000	0.011341
		6617.300000	0.011341

FREQUENCYINFO (Allelotyping only)

This part lists allele frequency information.

Example of
FREQUENCYINFO
part of assay details

	FREQUENCYINFO	FREQUENCY	FREQUENCY UNCERTAINTY
	6273.100000	0.526177	0.011341
	6617.300000	0.473823	0.011341

↑
Expected mass of a peak
↑
Average Relative frequency estimate (1.0 means 100%)
↑
Standard error of all frequency estimates

Note: When acquiring spectra, an analyzer attempts to acquire five spectra for each sample. Relative frequency estimates are the average frequency found in the five spectra. The standard error of the frequency estimates is the standard error of the frequencies from all five spectra.

ASSAY_ID	SAMPLE_ID	CALL	DESCRIPTION
+ C11-48170	1	GA	J.Polymorphic
- ASSAYINFO	NAME	SEQUENCE	MASS
Analyte A	CACAACTTACCCAC	6273.100000	
Analyte G	CAGAACACTTAGCACCCACC	6273.100000	
Analyte GA	CAGAACACTTAGCACCCACC	6273.100000	
Analyte GA	CAGAACACTTAGCACCCACTG	6617.300000	
Probe C11-48170-P	CAGAACACTTAGCACCCAC	5999.900000	
- CALLINFO	PROBABILITY	SNR	OFFSET
Calibrated	1.000000	1.144805	-0.177160
- PEAKINFO	HEIGHT	SMR	PROBABILITY
5999.900000	5.926464	2.970775	0.286040
6273.100000	115.235081	59.692560	1.000000
6304.100000	4.233089	2.200810	0.209770
6617.300000	105.226049	56.737394	1.000000
- AREAINFO	AREA	AREA VARIANT	RESOLUTION
5999.900000	66.594350	45.111770	268.733247
6273.100000	1108.321027	17.961267	282.752209
6304.100000	80.410700	43.494948	275.282209
6617.300000	996.044321	17.028181	268.523247
- FREQUENCYINFO	FREQUENCY	FREQUENCY UNCERTAINTY	
6273.100000	0.526177	0.011341	
6617.300000	0.473823	0.011341	

Peaks are identified by mass. To find which allele a peak represents, match up the mass values. For example, this peak is for the G allele.

Viewing Cluster Graphs

Cluster graphs provide a visual description of certain measurements used in the genotype calls for an assay on a SpectroCHIP. Cluster graphs help you to determine if an assay is reliable or not. If there are chemistry problems with an assay, they are likely to be revealed in the cluster graphs.

There are four types of graphs available:

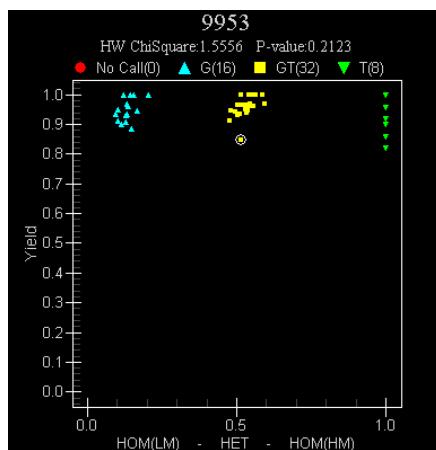
- **Yield vs Skew** plots the combined yield of both low- and high-mass analytes.
- **Yield** plots each assay result by probe of extension rates from 0.0 to 1.0 (0% to 100%).
- **Height** plots each assay result by probe of extension rates using the reported height of allele peak signals
- **Log (Height)** plots each assay result by low mass allele and high mass allele heights on a logarithmic scale.

Each sample of a particular assay produces a point of data on a cluster plot. The color and shape of the point reflects the genotype call for a particular sample. The top of the cluster graph lists Hardy-Weinberg Chi Square and P-values. A P-value of 0.01 corresponds to an approximate Chi Square value of 6.5. (Values listed as "?????" indicate that no calculation

could be made.) When the P-value falls below 0.01, it means the data does not score well on the Hardy-Weinberg Equilibrium (HWE). Use the Log (Height) and Yield cluster graphs to perform quality checks of your assays.

Yield vs Skew

In a Yield vs Skew cluster graph the yield is the combined yield of both low- and high-mass analytes. The skew shows the relative yield of each analyte. Assays that worked well will usually have high yields (closer to 1). Assays that produced homozygous calls for the low- or high-mass allele will cluster around the skew 0 or 1 axes respectively, whereas those that produced heterozygous calls will cluster around the skew 0.5 (50%) axis. Assays that lead to No Calls will usually result in points that have low yield values or that fall somewhere between the homozygous and heterozygous skew axes.



Example of a Yield vs Skew graph for a reliable assay

Yield

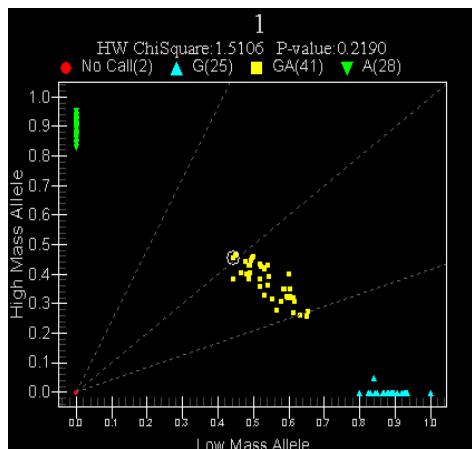
The Yield cluster graph plots the relative yields for the two alleles of an assay directly against each other. In this graph, the total yield and relative yields of the alleles lie along the diagonals of the plot. Points that fall close to one or the other axis are homozygous. Points that are close to zero on both axes are assays where there was little probe primer extension.

Use the Yield graph to understand the reasons for No Call genotype call results (shown as red data points on the cluster graph). Low yield indicates a less efficient extension reaction. The low yield cutoff is approximately 0.25; data points below this cutoff should

Chemistry Issues

Certain assays—those you determine to be poor performers or to have inefficient extension reactions—may need to be re-designed. For information on chemistry and cluster graphs, see the *MassARRAY Typer Reference Manual*.

have genotype calls of “No Call,” which may be the result of low yield and/or other performance factors.



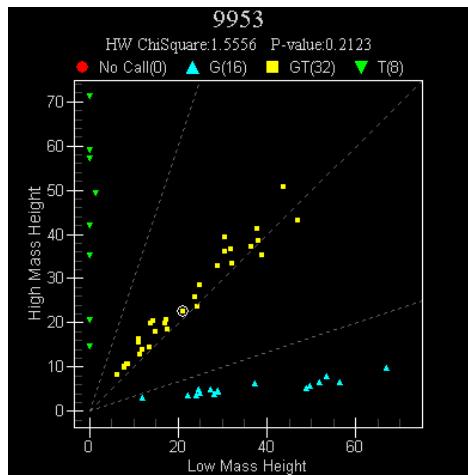
Example of a Yield graph for a reliable assay

Generally, a high quality assay has a Yield graph similar to the example shown here. The homozygous calls are clustered at the outer edge of each axis, and the heterozygous calls are clustered near the middle. You can imagine a diagonal line connecting each of the cluster groups. The Chi Square value and P-value indicate the assay performs well under HWE.

The Log (Height) and Yield graphs provide different types of information. In general, you may want to look at an assay’s Log (Height) graph first and then at its Yield graph. If the Log (Height) graph shows weak clustering or the Yield graph shows low yield, then you may need to examine the spectra to determine if the assay is unstable. Or, if after looking at both Log (Height) and Yield graphs you determine it to be an assay that performs well, you may choose to replace the genotype call for the assay with a manual user call. (See “Manually Calling a Genotype” on page 89 for information.) Or, if after looking at both graphs you determine it to be a good assay, you may choose to keep the genotype call as it was automatically assigned by Typer.

Height

The Height cluster graph is similar to the Yield cluster graph but uses the reported height of allele peak signals directly rather than a measurement relative to the unextended probe peak primer signal. The height axis is not normalized to a maximum of 1.0. The distribution of the data points reflect the variation in peak intensities of the individual mass spectrum recorded.

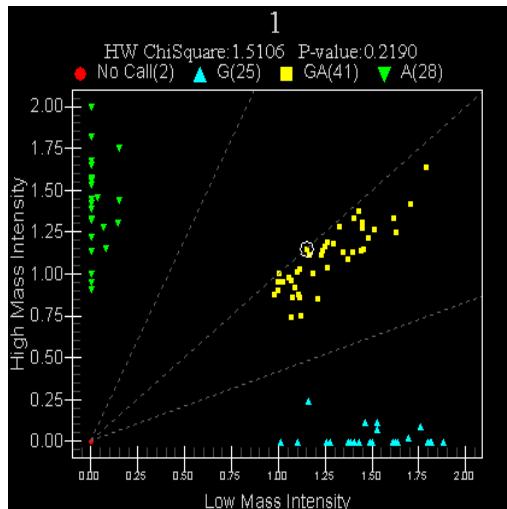


Example of a Height graph for a reliable assay

Log (Height)

The Log (Height) cluster graph is similar to the Height cluster graph except $\log_{10}(\text{height}) - 1$ is plotted instead of the reported allele peak heights. This graph may be more useful for visualizing assay skew clustering where there is a large variation in spectrum peak intensities between wells.

The Log (Height) graph plots each assay result by low mass allele and high mass allele heights on a logarithmic scale. An ideal assay has a Log (Height) graph with data points that fall mostly along each axis and along the diagonal (see figure below). Results from a less reliable assay would show data points scattered across the entire graph region. The quality of the clusters reflects the quality of the assay performance. Good clusters are defined as those where samples assigned common genotypes fall in the same local area on the graph. Outliers may require closer examination of their spectra. On a graph with red data points ("No Call" genotype calls), you may also want to view the Yield cluster graph to determine if the red data points are the result of low yield.



Example of a Log (Height) graph for a reliable assay

Generally, a high quality assay has a Log (Height) graph similar to the example shown here. The homozygous calls are clustered along each axis, and the heterozygous calls are clustered along the diagonal running through the center of the graph. The Chi Square value and P-value indicate the assay performs well under Hardy-Weinberg Equilibrium (HWE).

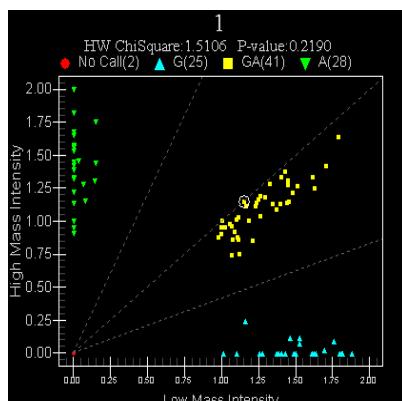
Checking Assays for Quality

Use the Typer cluster graphs and histogram graph to perform quality checks of assays on a SpectroCHIP. The cluster graphs and histogram graph may be used separately or at the same time, and you may also find it helpful to use them in conjunction with the spectrum graph. The section below provides instructions for viewing a cluster graph. See “Printing a Cluster Plot Graph” on page 101 for instructions on viewing a histogram graph.

► To view a cluster graph

1. In the navigation tree, select a chip. Then, click a well to select it.

The cluster graph for the selected well appears in the **Cluster Plot** pane.



Cluster Plot pane

2. On the tool bar drop-down list, select one of the following:
 - **Log (Height)** plots each assay result by low mass allele and high mass allele heights on a logarithmic scale.
 - **Yield** plots each assay result by height of extension products.

The graph is updated according to your selection.

3. To perform a quality check of an assay, do the following:
 - View the Log (Height) graph to determine how well the assay fits HWE and to see how well the genotype calls are clustered.
 - View the Yield graph to see how the extension reaction performed or to determine if the No Call genotype calls (red data points) were made as the result of a lack of extension product.
4. To zoom in on a particular cluster or data point, click and drag the cursor around an area on the graph.

The graph view is updated to display only the selected area.

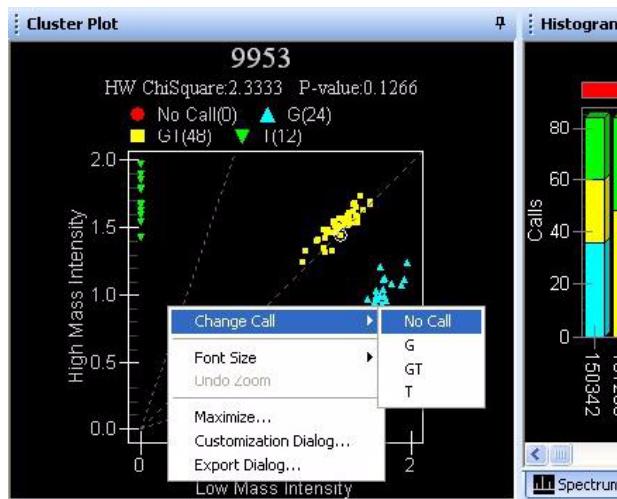
Manually Calling a Genotype in the Cluster Plot Pane

You can override an assay call (or no call) by manually calling a genotype in the Cluster Plot pane.

Caution: When you manually call a genotype, the call will be noted as a “user call” even if you change it back to the original call.

► To manually call a genotype in the Cluster Plot pane

1. To enter Change Call mode, right-click in the **Cluster Plot** pane, and select **Change Call** and the call you want to make from the submenu.



Right-click menu for manually calling genotypes

2. Apply the call to points on the graph as follows:
 - To apply the selected call to a single point on the graph, click on the point.
 - To select multiple points by drawing a lasso around them, see “To select a group of points” on page 99.

Note: To select multiple points by dragging the mouse, the Allow Multiple Call Changes option must be set in Configuration. See “Configuring TyperAnalyzer” on page 150.

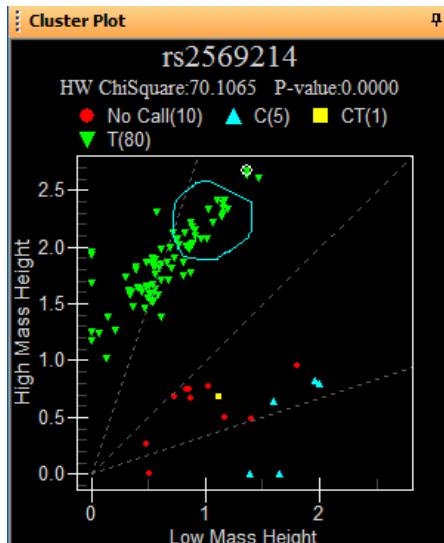
Calls are made locally and not saved to the database unless you save them.

3. To exit Change Call mode, right-click in the **Cluster Plot** pane, and select **Change Call** and reselect the call from the submenu to remove the check box.
4. To save the change to the database, click the Save icon or select **Save Changes** from the **File** menu.

► To select a group of points

1. Place the pointer where you want to begin selecting points, press and hold down the Shift key, and press and hold the left mouse button.

2. Drag the mouse to enclose the desired points.
3. Release the mouse button and the Shift key when done.

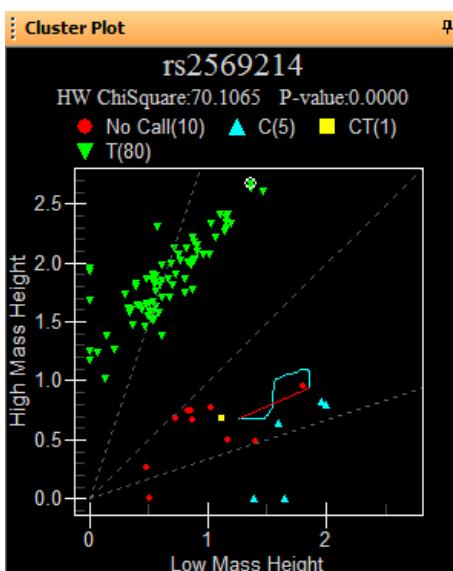


Lassoing points

The region is always closed by a line from the last point to the first point.

Use a simple polygon when lassoing. Line crossings that result in a simple polygon are allowed; however, if a line crossing is not allowed, the crossing line will be displayed in red rather than cyan and the status bar indicates the invalid state: "Invalid Lasso: can't change calls until lasso closing line is not red."

If you terminate the drawing while the crossing line is red, the option for changing the call is disabled. In this case, you must either clear the lasso by clicking elsewhere or finish the drawing by holding down the Shift key and the left mouse button and continuing to draw the line until the line no longer crosses.



Lassoing points

4. To change the calls for the data points in the enclosed region, right-click on the cluster plot and choose the desired call from the Change Call menu.

Printing a Cluster Plot Graph

► *To print a Cluster Plot graph*

1. Click on the **Cluster Plot** pane to make it the active pane.
2. From the **File** menu, select **Print Graph**.
3. In the **Printing** dialog box, choose a printing style—**Color**, **Monochrome**, or **Mono Plus Symbol**.
4. Click **Setup** to set up the printer.
5. Click **OK** to print the graph.

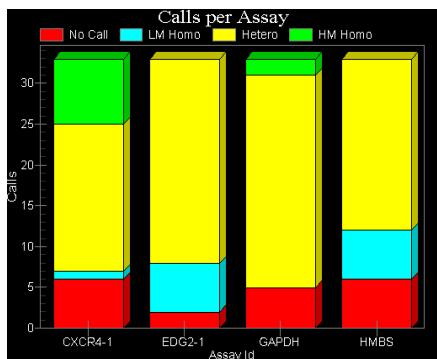
Viewing Histogram Graphs

Histogram graphs provide information about the distribution of calls made for each assay on a chip. Use the histogram graph in conjunction with the cluster graph and the spectrum graph to analyze assays.

► To view a histogram graph

1. In the navigation tree, select a chip. Then click a well to select it.
2. Click the **Histogram** tab.

The histogram for the selected well appears.



Histogram pane

3. Click a color bar on the graph.

The spectrum graph in the Spectrum pane is updated to show peaks for the selected assay.

The Cluster Plot pane displays a graph of the assay you selected on the histogram. The data point representing the selected assay is circled in white on the cluster graph.

4. If there are more than 24 assays in the well, drag the scroll bar along the bottom of the pane to view subsequent assays.

If there are fewer than 24 assays, the scroll bar will not be visible.

Printing a Histogram

► To print a histogram

1. Click on the **Histogram** tab to make it active.
2. From the **File** menu, select **Print Graph**.
3. In the **Printing** dialog box, choose a printing style—**Color**, **Monochrome**, or **Mono Plus Symbol**.
4. Click **Setup** to set up the printer.
5. Click **OK** to print the graph.

Recalling Plate Data

If you find that an assay has been incorrectly applied to a plate or well of a plate, you can recall the plate data and copy it to a new plate with the correct assays applied. Use ChipLinker to recall plate data. You can recall plate data on the RT-Workstation computer or on the server. See “Recalling Plate Data” on page 71.

Caution: Data recall should be used on only genotyping and genotype+area data.
Do **not** recall allelotyping data.

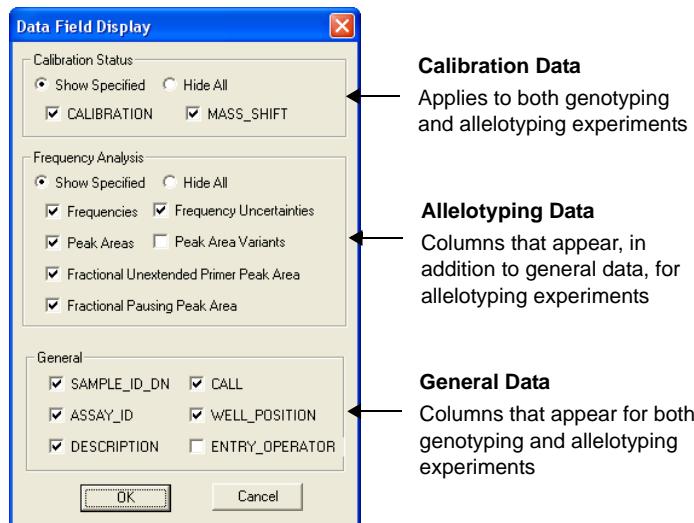
Filtering the Results Table

You can control which columns are shown in the Plate Data tab by hiding individual columns or groups of columns. When you hide a column, it is simply hidden from view. You can easily switch back to showing the column.

► To hide or show columns

1. On the View menu, select **Display Fields Dialog**.

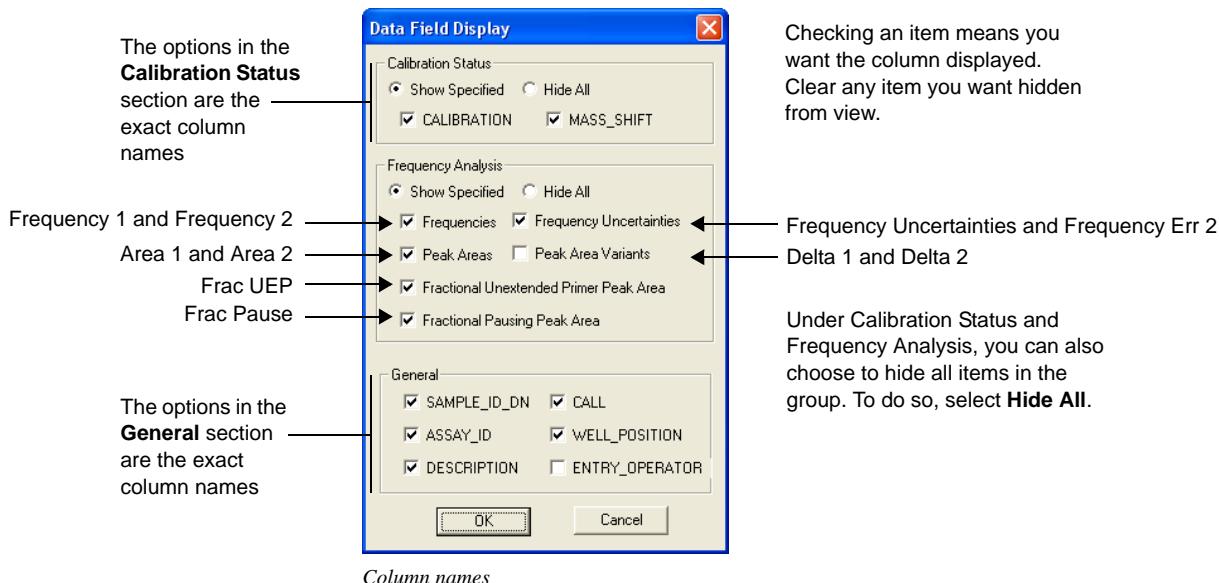
The **Data Field Display** dialog box appears.



Selecting which columns to display

2. Select which columns to display.

Note: The options under **Frequency Analysis** are not necessarily the column names; instead they are the types of data in the columns. Use the following illustration to match an option to a column.



Checking an item means you want the column displayed.
Clear any item you want hidden from view.

Frequency Uncertainties and Frequency Err 2
Delta 1 and Delta 2

Under Calibration Status and Frequency Analysis, you can also choose to hide all items in the group. To do so, select **Hide All**.

Column names

3. Click **OK**.

Importing and Exporting Wells

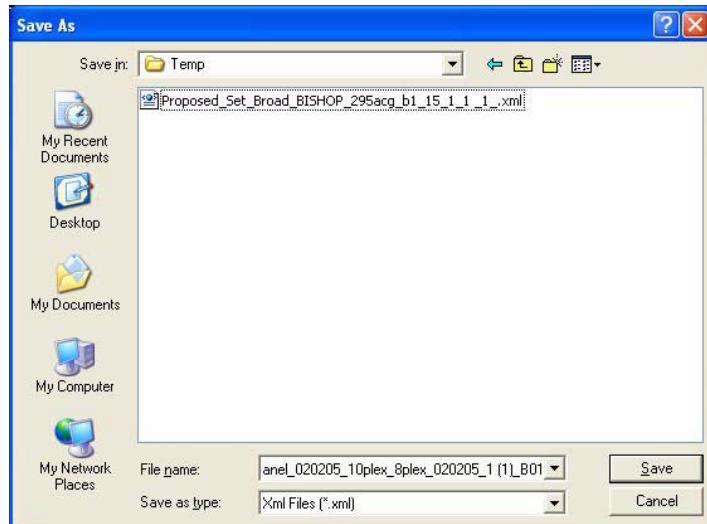
You can export some or all of the wells in the current chip to an XML file. A saved XML file can be imported back into TyperAnalyzer.

► To export wells

1. From the **File** menu, select **Export**.
2. In the **Export Wells** dialog box, enter the wells to be exported separated by commas (",") or check the **Export All Wells** check box, and click **Next**.

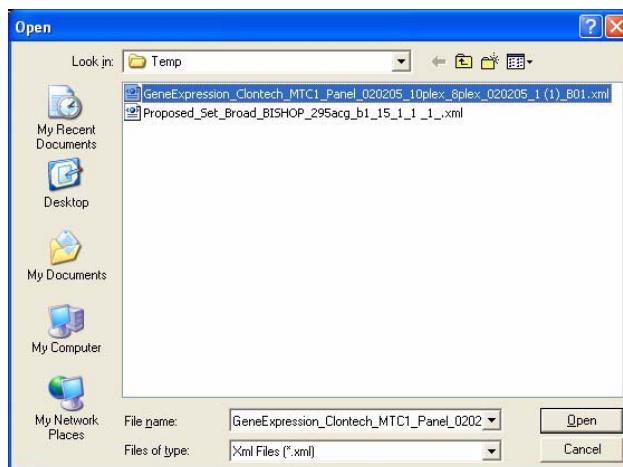


3. In the **Save As** dialog box, enter or select an XML file and click **Save**.



► **To import a saved XML file**

1. From the **File** menu, select **Import**.
2. In the **Open** dialog box, select the XML file to import and click **Open**.



Note: Imported chips are for display only. Although most of the functionality in works properly, the result cannot be saved to a database. Also, the import function is available even if TyperAnalyzer starts without a database connection.

Copying Plate Data to the Clipboard

You can copy plate data to the clipboard, so it can be pasted into other programs such as Excel.

► To copy plate data to the clipboard

1. Click on the **Plate Data** tab to make it active.
2. From the **Edit** menu, select **Copy Plate Data Grid**.

Generating Reports

You may create a report of plate results as a tab-delimited file. When you create a report, the file is automatically displayed in Microsoft® Excel™. The following reports are available:

- Allelotype*
- Allelotype Correction*
- Assay Type Count
- Best Call Probability
- Call Probability
- Description Count
- Genotype Area*
- Plate Definition
- Plate Result
- Primer Adjustment

** To generate these reports, you must have an allelotyping license. For more information, contact SEQUENOM.*

Tab-Delimited File

A file containing plain, text items separated by a tab character. Each line of data is separated by a carriage-return character.

Note: The reporting functions are identical in the TyperAnalyzer and Genotype Analyzer modules.

Note: Do not run Gene Expression and Genotype Cluster reports for iPLEX and hME. Gene Expression and Genotype Cluster reports are not applicable to iPLEX and hME and do not produce meaningful results.

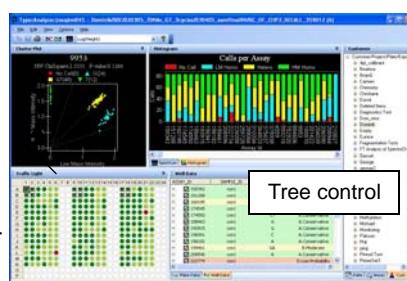
Experiment

In a genotyping or genotype+area plate, an experiment represents a SpectroCHIP and chip represents an analyzer run on the SpectroCHIP. Multiple chips represent multiple analyzer runs on the **same** SpectroCHIP.

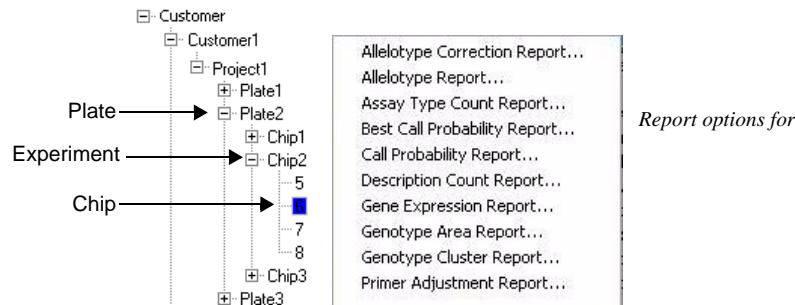
For a description of each report, see Appendix A “Reports” on page 137.

► To generate a report

1. In the tree control, select the chip on which you want a report. Then right-click the chip and select <Name>**Report** where <name> is the name of the report listed.

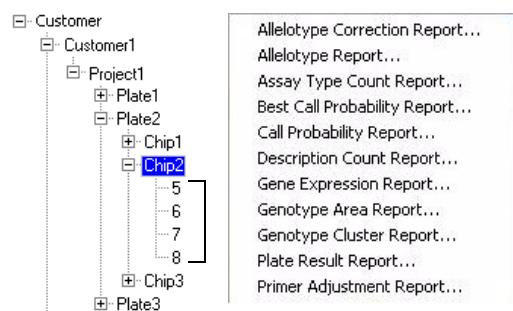


TyperAnalyzer window



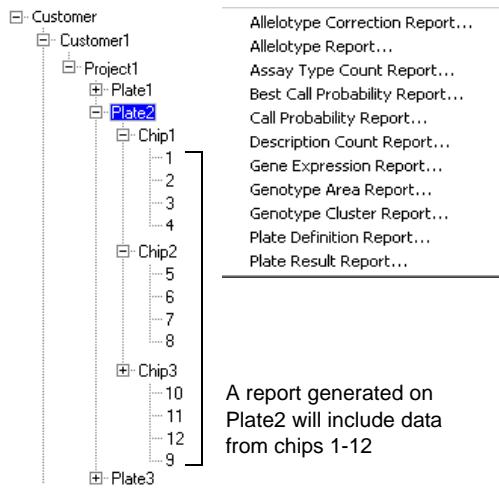
You can generate a report on multiple chips under the same experiment or plate. The report will include data for all of the chips under the experiment or plate.

For example, a report generated on experiment 2 will include data from chips 5, 6, 7, and 8



Allelotyping is typically done using four SpectroCHIPs. The SpectroCHIPs are “copies” of each other—the point of having four copies is to obtain more spectral data points on which to perform statistical analyses. The data from each SpectroCHIP is stored in a chip under the same experiment. So, an experiment should have four chips under it, one for each SpectroCHIP.

In most cases, when generating an Allelotype or Allelotype Correction report, you should generate the report on the experiment containing the four chips. Allele frequency estimates will be based on statistical analyses done on the data from all four chips. You may generate an Allelotype or Allelotype Correction report on an individual chip, but the frequency estimates will be based only on the data from one SpectroCHIP.



Caution

Do not select anything above the plate-level. This would select chips under different plates. Generating a report on chips under different plates does not create valid report data.

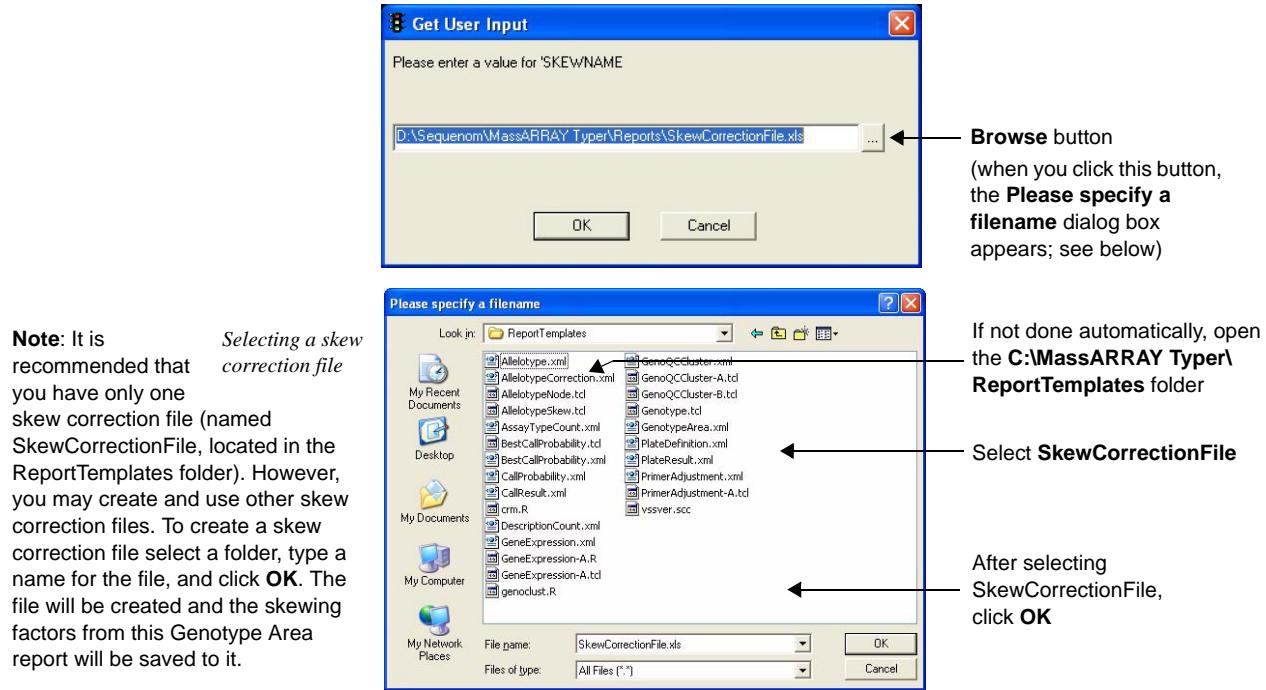
Report options for plates

Generating a Genotype report on multiple chips is equivalent to generating a Genotype report individually for each chip and appending them into a single file. The data for each chip is kept separate from that for the other chips.

Note: To generate an Allelotype Correction report, there must be a skew correction file containing heterozygous skewing factors for the assays applied to your plate. Skew factors are saved to a skew correction file when you generate a Genotype Area report on genotype+area experiments. For more information, see "Skew Correction File" on page 141.

2. In the **Get User Input** dialog box, click the **Browse** button and select a skew correction file. This is the file to which heterozygous skewing factors will be appended.

Note: This dialog box appears for only Genotype Area and Allelotype Correction reports. If you are not generating either kind of report, skip to the next step.

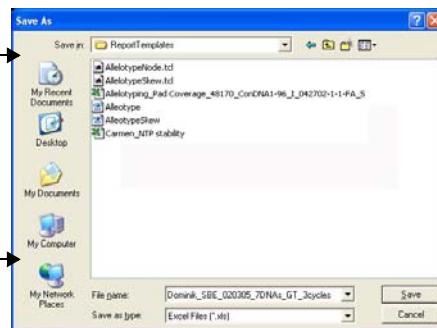


Note: If this is the first time you are running a Genotype Area report, SkewCorrectionFile does not exist. Select the **ReportTemplates** folder and type “SkewCorrectionFile” in the **File name** box. When you click **OK**, a skew correction file named SkewCorrectionFile will be created. From this point on, you should use this skew correction file.

3. In the **Save As** dialog box, select a different folder, type a file name, and click **Save**.

Initially, this dialog box shows the **ReportTemplates** folder. You should **not** save your reports to this folder. Select or create a different folder for your reports.

A default name is supplied for your report based on the experiment, chip, or plate you selected (see right). Edit or replace the name if you wish.



Selecting a report type

4. When you click **Save**, the report is generated. The report is displayed in Excel.

For descriptions of report contents, see Appendix A “Reports” on page 137.

Default Name

A default report file name is in the form: <customer>_<project>_<plate>_<experiment>_<chip>

If you selected a plate, the default name only goes to <plate> (i.e. <customer>_<project>_<plate>). Similarly, if you selected a chip, the name goes to <chip>.

Customizing the Window Layout

You can customize the window layout by resizing, moving, and combining panes. Once you have set the layout as you want it, you can save the layout so it will load each time the program starts. You can also load a saved layout, reset the layout to the original default layout, and add menu items to the tool bar for easy selection of frequently used items.

► **To save the current window layout**

- From the **Options** menu, select **Layout > Save Layout**.

► **To reset the window layout to the default layout**

- From the **Options** menu, select **Layout > Reset Layout**.

► **To load a saved layout**

- From the **Options** menu, select **Layout > Load Layout**.

This removes temporary layout changes. Any unsaved changes to the currently displayed layout will be lost when the program quits unless Automatically Save Layout is set to True in the Configuration program.

► **To add menu items to the tool bar**

- Click the Down arrow on the right edge of the tool bar and select **Add or Remove Buttons > Customize**.

- In the **Customize** dialog box, find the menu item and drag it to the tool bar.

For example, to add the Selection Type command to the tool bar, select **Commands > View > Selection Type** and drag it to the tool bar.

- To remove an item from the tool bar, drag it back to the **Customize** dialog box.

Customizing the Displays

You can customize the following aspects of the Spectrum, Cluster Plot, and Histogram displays:

- Title and subtitles
- Plot axis and style
- Fonts and colors

► **To customize the title or subtitles**

- Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
- In the **Customization** dialog box, click on the **General** tab.
- To change the title, enter a new title in the **Main Title** text box.
- To modify the subtitle, enter a new title in the **Sub Title** text box.
- Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

► **To customize the plot axis or style**

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Cluster Plot** tab.
3. To modify the axis, select the **Axis--Intensity** or **Axis 2** through **Axis 6**.
4. To modify the plot style, select from the following options—Area, Bar, Line, Points, Points+BestFitCurve, Points+BestFitLine, Points+Line, Points+Spline, or Spline.
5. To specify a companion plot style, select from the following options:
Line, Points, Points+BestFitCurve, Points+BestFitLine, Points+Line, Points+Spline, Spline
6. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Creating Subsets

You can specify subsets to include in the graph.

► **To create a subset**

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Subsets** tab.
3. Select a subset from the **Subsets to Graph** list.
4. Use the scrollbar to scroll through 0 through n . Current subset number is displayed in text box.
5. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Setting Fonts

You can set the font and style for the main title or subtitles in the Spectrum or Plot Cluster displays.

► **To specify the font**

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Font** tab.
3. To set the font for the main title, click on the **Main Title** drop-down box and choose the font from the list.
4. To specify a font style for the main title, select any of the following options—bold, italic, or underline.

5. To set the font for the subtitles, click on the **Sub-Title** drop-down box and choose the font from the list.
6. To specify a font style for the subtitles, select any of the following options—bold, italic, or underline.
7. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Customizing Subsets, Points, and Axis Labels

You can define how subsets, points, and axis labels are displayed in the Spectrum or Plot Cluster displays.

► **To customize subsets, points, and axis labels**

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Subsets** tab.
3. Select a subset from the Subsets to Graph list.

A sample appears at bottom of dialog box.
4. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Customizing Colors

You can customize colors in the Spectrum or Plot Cluster displays. Graph Attributes: Desk Foreground, Desk Background, Shadow Color, Graph Foreground, Graph Foreground, Table Foreground, Table Background.

► **To customize colors**

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Color** tab.
3. Select an attribute below, then click on a color:

Graph Attributes: Desk Foreground, Desk Background, Shadow Color, Graph Foreground, Graph Foreground, Table Foreground, Table Background
4. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Customizing Styles

You can assign styles to the subsets created on the Subsets tab, include the color, point type, and line type.

► To customize colors

1. Right-click on either the **Spectrum**, **Cluster Plot**, or **Histogram** pane.
2. In the **Customization** dialog box, click on the **Style** tab.
3. To change the color, click on a color.
4. From the **Point Type** drop-down box, choose a point type.
5. From the **Line Type** drop-down box, choose a line type.
6. Click **OK** to save your changes and close the dialog box or **Apply** to apply your changes and continue customizing.

Accessing the Export Dialog Box

From the Customization dialog box, you can at any time access the Export dialog to export your spectrum or plot cluster diagram to another graphic format.

► To access the Export dialog box

- In the **Customization** dialog box, click **Export**.

Logging Debug Messages

Turning on the Log Debug Message option writes debugging messages to the TyperAnalyzer.log file, located in the same directory as the TyperAnalyzer executable. Restarting the program automatically turns off the Log Debug Message until it is turned on again. Besides writing debug messages, the TyperAnalyzer.log file contains warning and error messages when the program encounters unexpected situations. It is useful for troubleshooting if some operations do not perform as expected.

► To turn on the Log Debug Message Option

- From the **Options** menu, select **Log Debug Message**.

Quitting TyperAnalyzer

► To quit TyperAnalyzer

- On the **File** menu, select **Exit**.

Notes:

Chapter 6

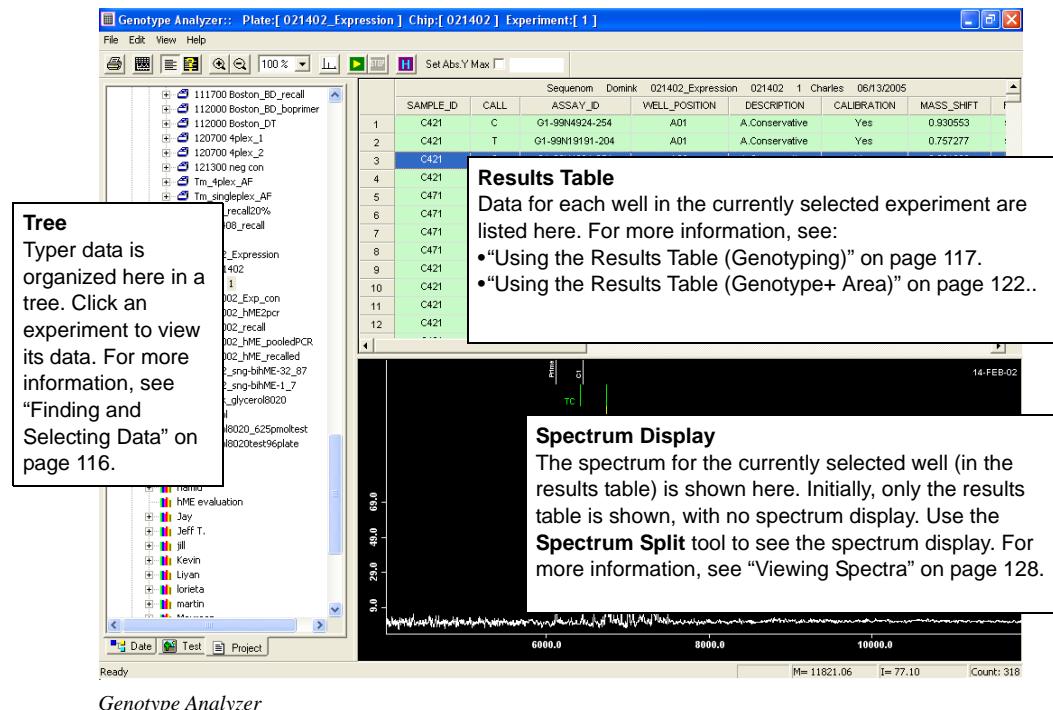
Reviewing Processed Data with Genotype Analyzer

Introduction

This chapter explains how to view the results after receiving data from a MassARRAY analyzer or MassARRAY analyzer compact. The Genotype Analyzer module displays results in both a table format and in a graph view that shows where the genotypes fall in the spectrum.

For genotyping data, you can view the successful *Calls* and the *No Calls*. You can make a judgement on the No Calls and perform a *User Call* if necessary.

For allelotyping data, you can view whether a sample is polymorphic or not (i.e. both alleles or just one) and the relative frequencies of each allele. You can also view additional statistical details about the spectra for each sample.

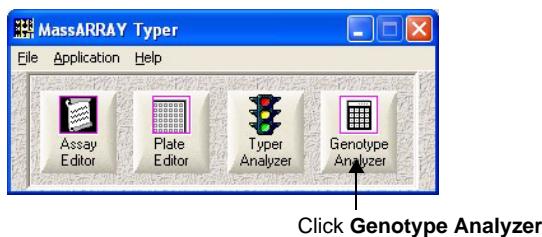


Note: There are slight differences in the way some Genotype Analyzer features work depending on whether you are viewing genotyping or allelotyping data. Sections in this chapter applicable to only genotyping have "(Genotyping)" after their headings. Sections applicable to only allelotyping have "(Allelotyping)" after their headings. Sections applicable to both have plain headings, with no analysis type indicated.

Starting Genotype Analyzer

► To start Genotype Analyzer

1. From the Typer window, select the **Genotype Analyzer** button.

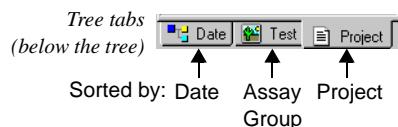


2. If you have not yet connected to the MassARRAY database, the **Connect to Database** dialog box opens. Enter the appropriate information.
3. Once connected, Genotype Analyzer opens.

Finding and Selecting Data

Typer data in Genotype Analyzer is organized into a tree. The tree has multiple levels; each successive level is indented to the right. Each level of the tree represents a different type of item.

There are three different ways to view the Typer data in the tree: sorted by project, assay group (called “test” in Genotype Analyzer), or date. Use the tree tabs to switch between the views.

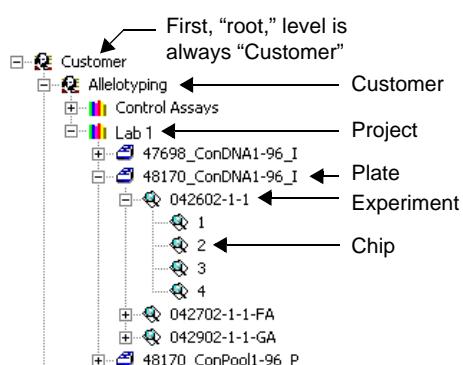


The data is the same; the three views are simply different ways to organize it. For example, use the **Date** tab to find data grouped by the date on which it was created.

By Project

The tree is organized into the following levels:

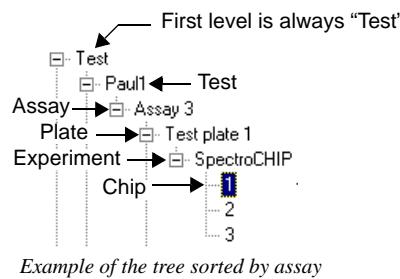
- Customer
- Project
- Plate
- Experiment
- Chip



By Test

The tree is organized into the following levels:

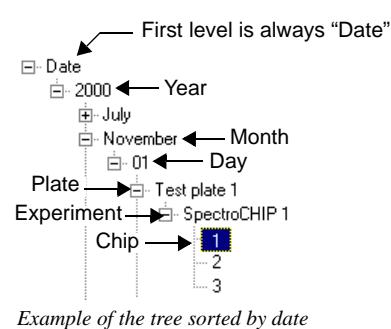
- Test (a collection of assays)
- Assay
- Plate
- Experiment
- Chip



By Date

The tree is organized into the following levels:

- Year
- Month
- Day
- Plate
- Experiment
- Chip



Selecting Data in the Tree

Regardless of whether you sort the tree by project, assay, or date, the last three levels are always the same: plate, experiment, chip.

To view data you must select a chip, the lowest level of the tree. Chips contain the data from a SpectroCHIP. Chips under a genotyping plate contain data from different MassARRAY analyzer runs on the **same** SpectroCHIP. Chips under an allelotyping plate contain data from MassARRAY analyzer runs on **different** SpectroCHIPS. Selecting a chip in the tree will display the data from the chip.

You can also choose to view data from groups of chips. If you select an experiment and choose to view its calls, then data from all chips under the experiment will be displayed. If you select a plate and choose to view its calls, then data from all chips from all experiments under the plate will be displayed.

Using the Results Table (Genotyping)

Color-Coding

Genotype Analyzer uses a three-parameter model to calculate the significance of each putative genotype. Based on the relative significance, a *final* genotype will be called. Different parameter settings can give rise to different results. Thus, based on internal testing and training of the model, three different sets of parameters have been developed for the model.

- **Conservative:** The most conservative set makes no error on the training and test data, but has the most uncalled genotypes.
- **Aggressive:** The most aggressive set makes the most errors (still less than 1 percent), but makes the most number of calls.
- **Moderate:** The moderate set is a compromise between the two extremes.

The non-calls are further categorized:

- **Low Probability:** Implies that the spectrum in question contains peaks that fail any criteria of significance even for the aggressive parameter set.
- **Conflict:** Implies that there is more than one *read* or spectrum corresponding to one well, and these reads give rise to different and conflicting genotypes.
- **Bad Spectrum:** The spectrum for the well doesn't exist above noise level.
- **Bad Assay:** Result of analyst or operator input errors in defining the assays. The most common errors are mass values of analytes or contaminants that are out of range of the spectrum, or contaminants and analytes having the same mass.
- **User Call:** The analyst or operator selects a genotype in the table and performs a manual call. See "To manually call a genotype in the table" on page 135.

The results appear in a table with color coded rows and a column titled *Description*. The following table matches the color to the description. Colors can be edited by your system administrator.

Table 3: Genotype Analyzer Color Codes for Genotyping

Filtering Columns

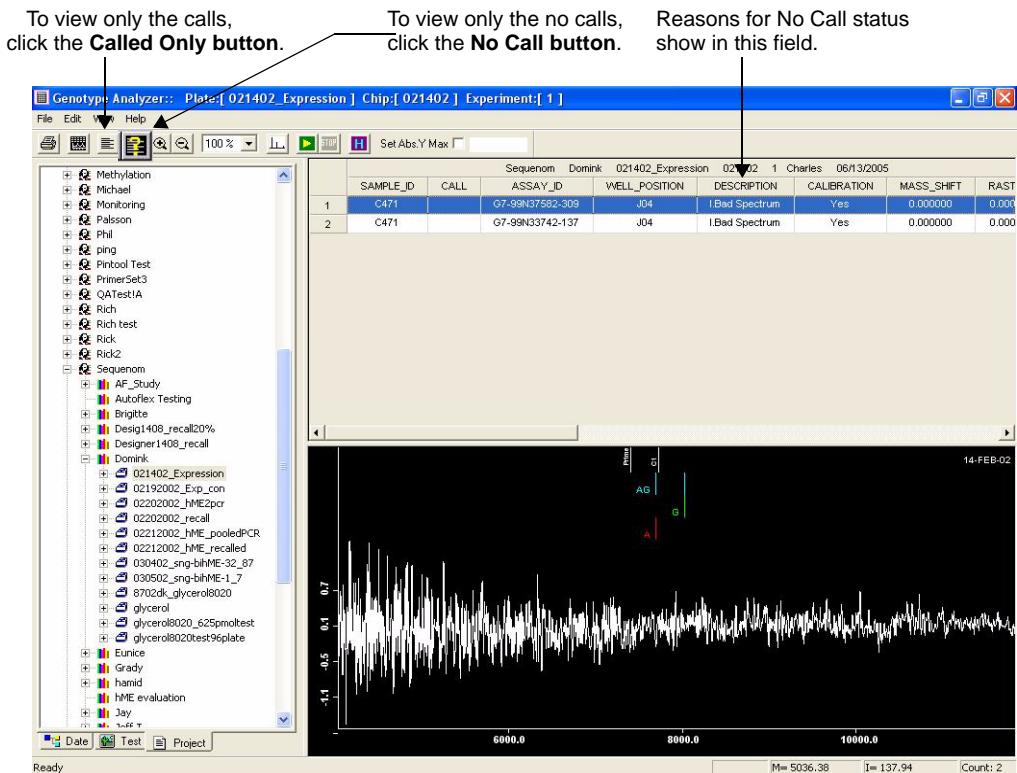
You can choose to view or hide individual columns in a results table. See "Filtering the Results Table" on page 125.

Description	Default Color in Table
Conservative	Green
Moderate	Yellow
Aggressive	Pink
Low Probability	Rose
Conflict	Red
Bad Spectrum	White
Bad Assay	Red
No Alleles	Blue
User Call	Cyan

Genotypes and Peaks

Different genotypes result in a different peak pattern in the spectrum. The configuration of present and absent peaks is used to determine the genotype (*Calls*). In a good assay, most calls can be made automatically. Any genotype determinations that cannot be made (*No Calls*) require manual intervention. (See "To manually call a genotype in the table" on page 135.) The goal of Typer is to have close to 100% accuracy on automatic calls made with conservative criteria. It is required that you manually call the non-call spectra. Furthermore, it is advisable to examine the automatic calls based on the Aggressive criterion. The main function of Genotype Analyzer is to provide the interface to allow making these manual calls.

The following illustration shows the No Calls. Notice the table includes reasons for the No Call status.



Use the **Called Only** and **No Call** toolbar buttons to view the data that you want to work with.

► To view only the calls

- On the toolbar, click the **Called Only** button.

► To view the calls for all experiments on a plate

If you have multiple experiments (that is, MassARRAY analyzer or analyzer compact reads) on a plate, you can view all the calls for all experiments on the plate. To do so, complete the following steps.

- In the left pane (which lists customers, projects, plates, and experiments), select the plate you want. Then, right-click the selected plate and choose **Show All Calls for <plate name>** (where *plate name* is the name of the plate you right-clicked).

The results table lists all calls from all experiments on the plate. Since, all calls from all experiments are listed, you may see multiple calls for the same well position.

You can sort the results table on any column by clicking the column's header at the top of the table. The rows of the table are sorted on the column you select in ascending order.

► **To view only the best call from the experiments on a plate**

You can have multiple experiments (that is, MassARRAY analyzer or analyzer compact reads) on a plate. As a result, there may be multiple calls for the same well position—one from each experiment. For a specific well position, you can choose to view only the call that has the highest score for all the experiments. To do so, complete the following steps.

1. In the left pane (which lists customers, projects, plates, and experiments), select a plate. Then, right-click the selected plate and choose **Show Collated Calls for <plate name>** (where *plate name* is the name of the plate you right-clicked).

The results table lists a single call for each well position. For a given well position, the call that is listed is the one with the highest score amongst all the experiments on the plate.

Note: You may see several calls for the same well position, highlighted in red. This happens if the calls from the experiments do not correspond (that is, if there are different genotype calls for the same well position from the experiments). In this case, all calls from all experiments are listed for the well position in question.

You can sort the results table on any column by clicking the column's header at the top of the table. The rows of the table are sorted, on the column you select, in ascending order.

► **To view only the no calls**

- On the toolbar, click the **No Calls** button. 

► **To view the no calls for all experiments on a plate**

If you have multiple experiments (that is, MassARRAY analyzer or analyzer compact reads) on a plate, you can view all the no calls for all experiments on the plate. To do so, complete the following steps.

1. In the left pane (which lists customers, projects, plates, and experiments), select a plate. Then, right-click the selected plate and choose **Show All NOCalls for <plate name>** (where *plate name* is the name of the plate you right-clicked).

The results table lists all no calls from all experiments on the plate. Since, all no calls from all experiments are listed, you may see multiple no calls for the same well position.

You can sort the results table on any column by clicking the column's header at the top of the table. The rows of the table are sorted, on the column you select, in ascending order.

► **To sort the results table**

- You can sort the results table by any of its columns. Clicking a column header at the top of the results table sorts the calls in ascending order by the column.

For example, clicking the **Well_Position** column sorts the calls by their well positions, in alphanumeric, ascending order. Clicking the same column header a second time sorts the calls in descending order by the column. Clicking the column header again returns to sorting in ascending order, and so on.

► **To view information about a call**

1. On the View menu, select **Call Info Dialog**.

The **Call Information Dialog** dialog box appears.

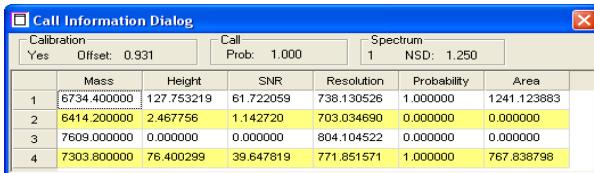


Call Information Dialog dialog box

If necessary, move the **Call Information Dialog** dialog box to view the calls beneath it.

2. In the results table, click the call about which you want information.

The **Call Information Dialog** dialog box shows information about the call you click. See the following illustration.



Example of Call Information Dialog dialog box with call information

You can leave the **Call Information Dialog** dialog box open and click another call to view information about it.

3. When you are done, close the **Call Information Dialog** dialog box by clicking in the upper right corner.

► **To view a history of calls**

- On the toolbar, click (**Show History** tool).
A history of calls appears, indicating who made each call.

► **To view calibration and mass shift information**

You can choose to either show or hide calibration and mass shift columns in the results table.

- On the View menu, select **Show Calibration**.

Note: If a check mark appears next to **Show Calibration**, calibration and mass shift columns are already currently shown. Selecting **Show Calibration** will hide these columns.

► **To hide calibration and mass shift information:**

- On the View menu, select **Show Calibration**.

Note: If there is no check mark next to **Show Calibration**, calibration and mass shift columns are already currently hidden. Selecting **Show Calibration** will show these columns.

Using the Results Table (Genotype+ Area)

For a genotype+area experiment, the color-coding of the rows is done on the same basis as for genotyping experiments, by the strength of calls (see “Color-Coding” on page 117).

The columns in the table, however, are the same as for an allelotyping experiment. It includes data about peak areas and allele frequencies. For instructions on using the results table, see “Using the Results Table (Allelotyping)” below; skip the first section on color-coding.

Using the Results Table (Allelotyping)

Color-Coding

The columns and color-coding in the results table for an allelotyping experiment are different from that for a genotyping experiment. Instead of color-coding by strength of genotype calls, the rows are color-coded according to whether each well is polymorphic (both alleles found) or non-polymorphic (only one allele found). The following table describes the color-coding scheme.

Table 4: Genotype Analyzer Color Codes for Allelotyping

Color	Description
Green	Polymorphic (both alleles present)
Cyan	Non-Polymorphic (one allele present)
Pink	Uncertain (the average frequency of one allele is less than or equal to 3 times the standard error of the frequencies before averaging.)
Yellow	Low-Frequency (one of the alleles has a frequency of 0.06, i.e. 6%, or less and the standard error is less than 2%)
White	Bad Spectrum (a usable spectrum was not acquired from the well; the best spectrum acquired contains only noise)

Note: Low-Probability and Bad Spectrum rows are hidden from view. To view these rows, see “Viewing Bad Spectrum Rows” on page 124.

Data Columns

The columns of data include information about the peak areas and allele frequencies. The following table describes the data columns.

Table 5: Results Table Contents for Allelotyping

Column	Description
SAMPLE_ID	Sample name
CALL	Alleles present in the pool
ASSAY_ID	Assay name
WELL_POSITION	Well number

Filtering Columns

You can choose to view or hide individual columns in a results table. See “Filtering the Results Table” on page 125.

Table 5: Results Table Contents for Allelotyping (Continued)

Column	Description
DESCRIPTION	<ul style="list-style-type: none"> • Polymorphic: both alleles found • Non-Polymorphic: one allele found • Low Frequency: one of the alleles has a frequency of 0.06, i.e. 6%, or less and the standard error is less than 2% • Uncertain: the average frequency of one allele is less than or equal to 3 times the standard error of the frequencies before averaging. • Bad Spectrum: a usable spectrum was not acquired from the well; the best spectrum acquired contains only noise
ENTRY_OPERATOR	<ul style="list-style-type: none"> • Automatic: The call (see CALL above) was made automatically by Typer • User Call: The call (see CALL above) was manually selected by a user
CALIBRATION	<ul style="list-style-type: none"> • Yes: calibration spectrum was acquired and applied • No: calibration was not applied
MASS_SHIFT	Calibration offset applied to the spectrum
RASTERS	Number of raster positions from which spectra were acquired. A MassARRAY analyzer attempts to acquire spectra from five positions (raster positions) on a SpectroCHIP well, the center plus the four corners. "5" means spectra were acquired from all five positions. Anything less means spectra could be successfully acquired from only the indicated number of positions. Frequency estimates for an allele are generated by averaging the frequencies found from the successful raster positions.
Area 1	Average area under the curve for the lower-mass allele. This is an average of areas found at all successful raster positions.
Delta 1	The average difference in area for the values used to calculate Area 1.
Area 2	Average area under the curve for the higher-mass allele. This is an average of areas found at all successful raster positions.
Delta 2	The average difference in area for the values used to calculate Area 2.
Frequency 1	Average frequency of the lower-mass allele. This is an average of the frequencies found at all successful raster positions.
Frequency Err 1	Standard error of the frequencies found for the lower-mass allele.
Frequency 2	Average frequency of the higher-mass allele. This is an average of the frequencies found at all successful raster positions.
Frequency Err 2	Standard error of the frequencies found for the higher-mass allele.

Table 5: Results Table Contents for Allelotyping (Continued)

Column	Description
Frac UEP	Ratio of peak areas of the unextended-primer to unextended-primer+allele1+allele2
Frac Pause	Ratio of peak areas of the pausing peak to pausing peak+allele1+allele2

Viewing Bad Spectrum Rows

Bad Spectrum rows are usually hidden from view. To view them, click the **No Calls** tool.



To view the Polymorphic/Non-Polymorphic/Uncertain rows, click the **Called Only** tool. Switch back-and-forth between the two views by using the **Called Only** and **No Calls** tools.

Viewing Data for all Experiments on a Plate

In a single results table, you can view the data for all chips on a plate. When allelotyping, a chip represents an individual SpectroCHIP. Viewing all chips for a plate shows you the data from all SpectroCHIPS run for the plate.

When viewing all chips for a plate, you can sort the results table by well position. The same well positions on all chips will be grouped together. Since the same well positions on all chips (i.e. SpectroCHIPS) contains the same sample, you can compare the individual results from each SpectroCHIP.

► To view data for all experiments on a plate

- In the left pane (which lists customers, projects, plates, experiments, and chips), click a plate to select it. Then, right-click it again and choose **Show All Calls for <plate name>** (where *plate name* is the name of the plate you right-clicked).

The results table lists the data from all chips on the plate. You can sort the results table on any column by clicking the column's header at the top of the table. The rows of the table are sorted, on the column you select, in ascending order.

To sort data by well position, click the WELL_POSITION column header.

Note: Bad Spectrum rows are hidden from view. To view these rows click the **No Calls** tool. For more information, see "Viewing Bad Spectrum Rows" above.

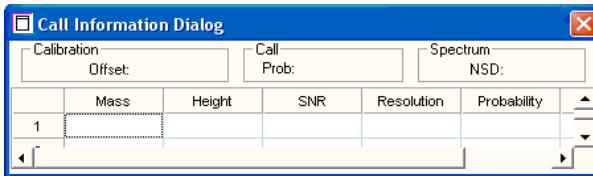
Viewing Detailed Assay Results

For any well, you can view detailed assay results, such as peak heights, peak areas, and signal-to-noise ratios.

► To view detailed assay results

1. On the View menu, select **Call Info Dialog**.

The **Call Information Dialog** dialog box appears.

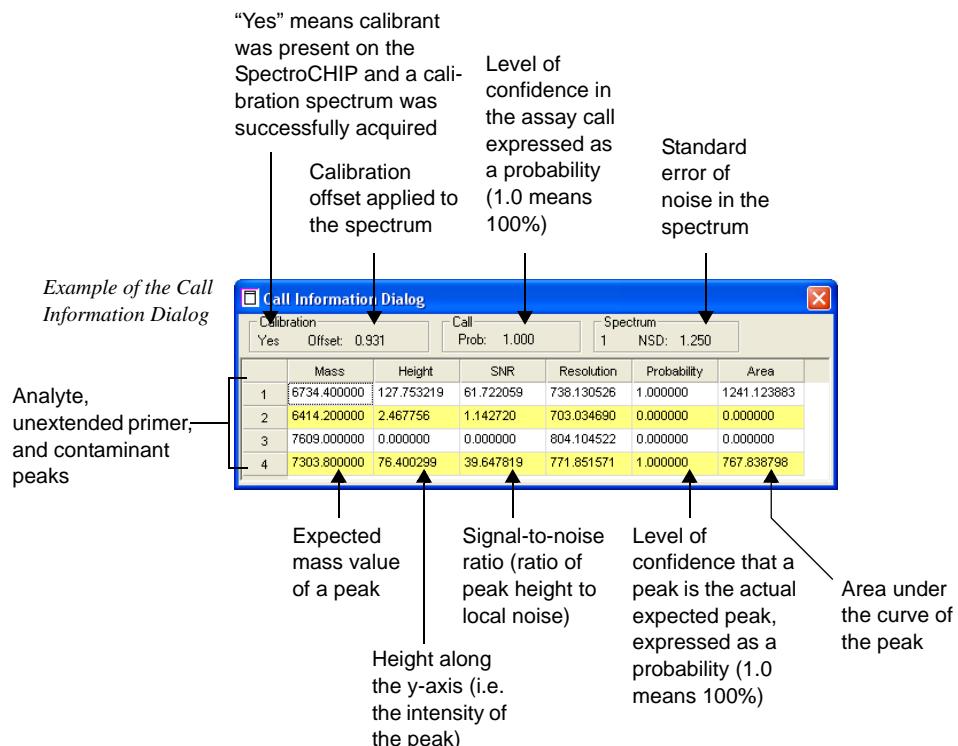


Call Information Dialog dialog box

If necessary, drag the **Call Information Dialog** dialog box to view the results table beneath it.

2. In the results table, click the row (i.e. well) about which you want information.

The **Call Information Dialog** dialog box shows information about the well you click. See the following illustration.



You can leave the **Call Information Dialog** dialog box open and click another well to view information about it.

3. When you are done, close the **Call Information Dialog** dialog box by clicking in the upper right corner.

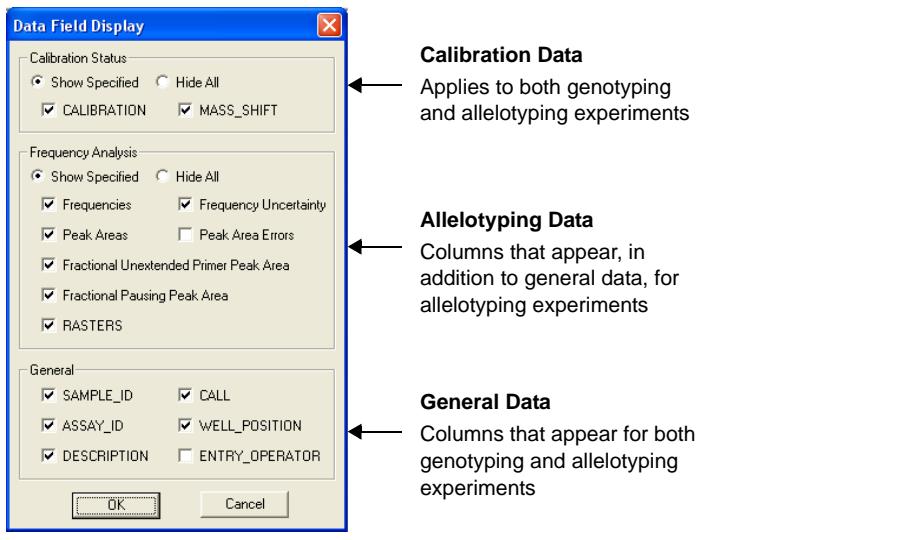
Filtering the Results Table

You can control which columns are shown in a results table by hiding individual columns or groups of columns. When you hide a column, it is simply hidden from view. You can easily switch back to showing the column.

► To hide or show columns

1. On the View menu, select **Display Fields Dialog**.

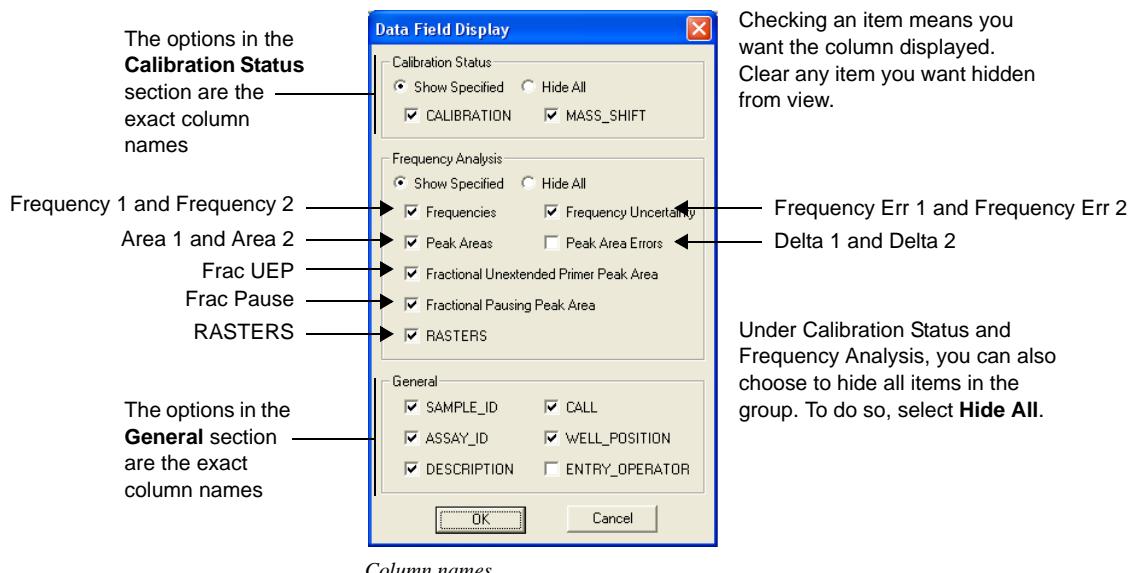
The **Data Field Display** dialog box appears.



Selecting which columns to display

2. Select which columns to display.

Note: The options under **Frequency Analysis** are not necessarily the column names; instead they are the types of data in the columns. Use the following illustration to match an option to a column.



3. Click **OK**.

Printing the Results Table

The information in the table can be printed.

► To print an entire table

- From the File menu, choose **Print Table**.

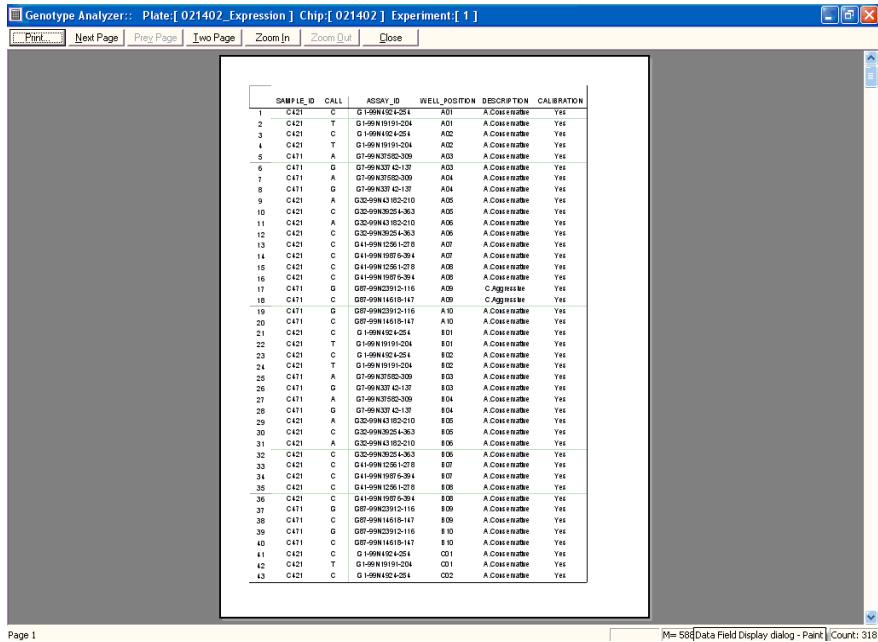
The **Print** dialog box opens.

- Select the appropriate print options.
- Click **OK**.

► To print from the Print Preview window

- Select the plate whose information you want to print.
- From the File menu, choose **Print Table Preview**.

A preview window appears.



- Click the buttons at the top to **Zoom In** or **Zoom Out**, view the previous or next page, and close.

- Click the **Print** button located at the top of the window.

► To print one row and the spectrum

- On the File menu, select **Print Spectrum**.
- Select the print options you want.
- Click **OK**.

Viewing Spectra

Once you have a results table open, you can view the spectrum for each row (i.e. well). Split the screen to view spectra.

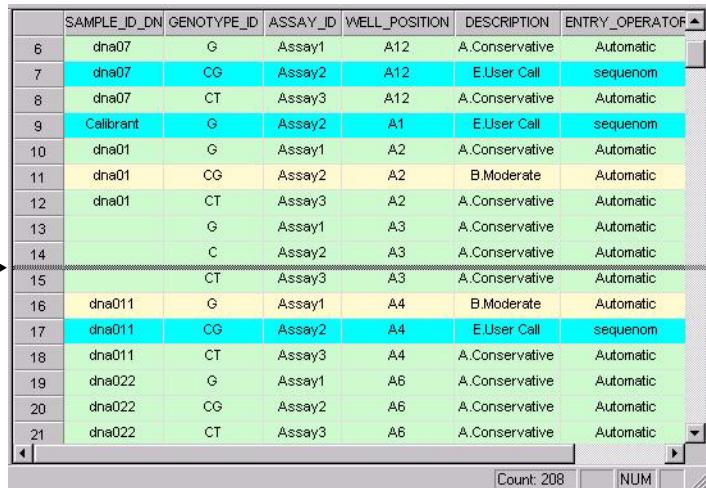
Splitting the Screen

► To split the screen

1. From the Edit menu, choose **Spectrum Split**, or click the **Spectrum Split** toolbar button. 

A horizontal line appears across the view, and the mouse pointer turns into a double line.

Drag this line to the point where you want to split the view and click the mouse button. The result table will be above the line; the spectrum display will appear below the line.



SAMPLE_ID_DN	GENOTYPE_ID	ASSAY_ID	WELL_POSITION	DESCRIPTION	ENTRY_OPERATOR
6 dna07	G	Assay1	A12	A.Conservative	Automatic
7 dna07	CG	Assay2	A12	E.User Call	sequenom
8 dna07	CT	Assay3	A12	A.Conservative	Automatic
9 Calibrant	G	Assay2	A1	E.User Call	sequenom
10 dna01	G	Assay1	A2	A.Conservative	Automatic
11 dna01	CG	Assay2	A2	B.Moderate	Automatic
12 dna01	CT	Assay3	A2	A.Conservative	Automatic
13	G	Assay1	A3	A.Conservative	Automatic
14	C	Assay2	A3	A.Conservative	Automatic
15	CT	Assay3	A3	A.Conservative	Automatic
16 dna011	G	Assay1	A4	B.Moderate	Automatic
17 dna011	CG	Assay2	A4	E.User Call	sequenom
18 dna011	CT	Assay3	A4	A.Conservative	Automatic
19 dna022	G	Assay1	A6	A.Conservative	Automatic
20 dna022	CG	Assay2	A6	A.Conservative	Automatic
21 dna022	CT	Assay3	A6	A.Conservative	Automatic

Splitting the screen

2. To view the spectrum for a well, click it in the results table.

The spectrum appears in the spectrum display. The selected genotype is identified by its underlined yellow text.

Note: Spectra acquired using the ACQUIRE module may appear “less intense” than older spectra acquired by the XACQ software on the MassARRAY analyzer (and processed by the Data Process module in earlier versions of Typer). That is, the intensity peaks in spectra acquired by ACQUIRE may generally not be as high as those in spectra acquired by the XACQ software.

When taking multiple shots of a SpectroCHIP well using ACQUIRE, the intensities in the spectrum of each shot are accumulated and then divided by the total number of shots. The resulting spectrum that is saved to the MassARRAY database is an average of the spectra from the multiple shots.

When taking multiple shots using the XACQ software on the MassARRAY analyzer, the spectra are accumulated also, but not divided by the total number of shots. Thus, in general, the intensities in spectra acquired by the XACQ software are greater than in spectra acquired by ACQUIRE.

Unsplitting the Screen

You can hide the spectrum display by “unsplitting” the screen.

► To unsplit the screen

1. Move the mouse pointer over the top edge of the spectra display.

The pointer will turn to a double line with arrows.

2. Click and drag the border to the bottom of the window.

The spectrum display is hidden from view; only the results table is shown.

Zooming

If you want to see more details in a spectrum, use the mouse to quickly zoom in or revert to the default size. Use the Zoom toolbar buttons to horizontally zoom the spectra view. This is handy when matching peaks to alleles.

► To vertical zoom in

- Click the **Zoom In** toolbar button. 

► To vertical zoom out

- Click the **Zoom Out** toolbar button. 

► To horizontally zoom in on an area

1. Use the mouse to click and drag over the area in the spectrum that you want to zoom in on.
2. Repeat for more zooming.

► To revert to the default size

- Right-click the spectrum.

The spectrum reverts to its original size no matter how much you zoomed in.

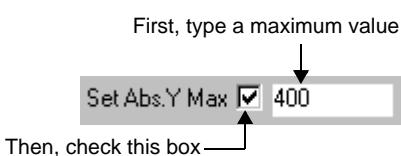
Setting an Absolute Y-Axis Maximum

The spectra are not shown to the same scale along the y-axis (intensity). In one spectrum, the y-axis may go up to 500. In another, it may only go up to 350. Each spectrum is displayed with the y-axis scaled to best show the spectrum.

You can choose to view all spectra scaled to the same maximum y-axis value, allowing you to better judge the relative intensities of peaks in different spectra.

► To set a y-axis maximum for the spectrum display

- On the toolbar, at the top of the **Genotype Analyzer** window, type a maximum value and click the check box for **Set Abs. Y Max**.



Note: Be sure to type the maximum value first and then check the **Set Abs. Y Max** box.

Viewing the Calibration Spectrum

► To view the calibration spectrum

1. If you have not done so already, split the view to see spectra.

Use the  (Split View tool) to split the view. For more information about splitting the view, see “Splitting the Screen” on page 128.

2. On the toolbar, click  (Show Calibration Spectrum tool).

The calibration spectrum appears.

Viewing All Spectra

You can view all spectra in succession (similar to a slide show).

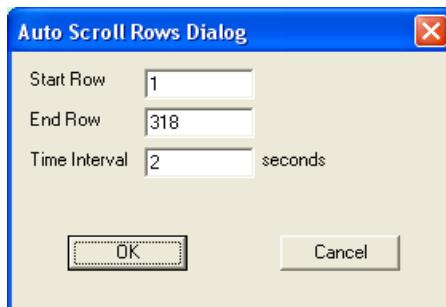
► To view all spectra in succession

1. If you have not done so already, split the view to see spectra.

Use the  (Split View tool) to split the view. For more information about splitting the view, see “Splitting the Screen” on page 128.

2. On the toolbar, click  (Auto Play tool).

The Auto Scroll Rows Dialog appears. See the following illustration.



Auto Scroll Rows Dialog

3. In the **Start Row** box, type the row from which you want to start viewing the spectra.
4. In the **End Row** box, type the last row for which you want to view spectra.
5. In the **Time Interval** box, type the amount of time (in seconds) you want Typer to wait before displaying the next spectra.

It is recommended that you choose a time interval between 2 to 30 seconds.

6. Click **OK**.

The spectra are displayed.

Note: Depending on the number of calls, it may actually take longer than the time interval you set for Typer to display each successive spectra. If there are a large number of calls, there may be a longer time interval between the spectra.

7. If you want to stop the display of the spectra before the last one is shown, click  (**Stop Auto Scroll** tool).

The display of the spectra may not immediately stop. There is a slight “lag time” between your click of the **Stop Auto Scroll** tool and the actual stopping of the spectra display. A few more spectra may be displayed before it stops.

Generating Reports

You may create a report of plate results as a tab-delimited file. When you create a report, the file is automatically displayed in Microsoft® Excel™. The following reports are available:

Tab-Delimited File

A file containing plain, text items separated by a tab character. Each line of data is separated by a carriage-return character.

- Allelotype*
- Allelotype Correction*
- Assay Type Count
- Best Call Probability
- Call Probability
- Description Count
- Genotype Area
- Plate Definition
- Plate Result
- Primer Adjustment

** To generate these reports, you must have an allelotyping license. For more information, contact SEQUENOM.*

Note: The reporting functions are identical in TyperAnalyzer (Traffic Lights) and Genotype Analyzer.

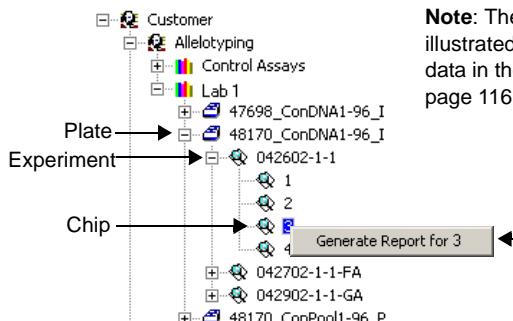
For a description of each report, see Appendix A “Reports” on page 137.

► To generate a report

1. On the **Project** tab, select the experiment on which you want a report. Then right-click the experiment again and select **Generate Report**.

Experiment

In a genotyping or genotype+area plate, an experiment represents a SpectroCHIP and a chip represents a MassARRAY analyzer run on the SpectroCHIP. Multiple chips represent multiple runs on the **same** SpectroCHIP.



Note: The organization of a tree sorted by project is illustrated here. For more information about selecting data in the tree, see "Finding and Selecting Data" on page 116.

After selecting a chip, right-click it and select **Generate Report for n** (where *n* is the chip number you selected).

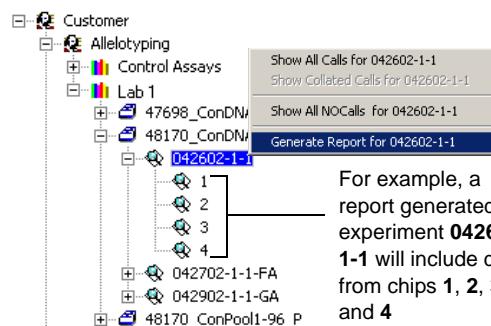
Example of a tree

Note: The names used here are for illustration; you should name your plates and chips according to a consistent convention that allows you to easily identify their contents

Caution

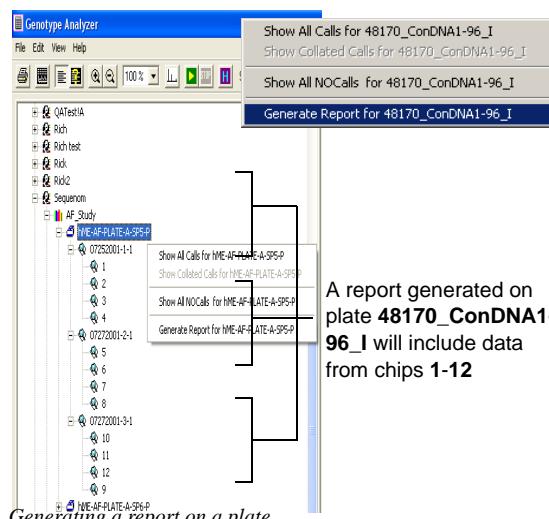
Do not select anything above the plate-level. This would select experiments under different plates. Generating a report on experiments under different plates does not create valid report data.

You can generate a report on multiple chips under the same experiment or plate. The report will include data for all of the chips under the experiment or plate.



For example, a report generated on experiment **042602-1-1** will include data from chips **1, 2, 3, and 4**

Generating a report on an experiment

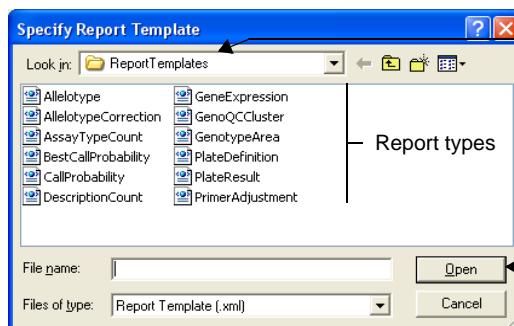


A report generated on plate **48170_ConDNA1-96_I** will include data from chips **1-12**

In most cases, when generating an Allelotype or Allelotype Correction report you should generate the report on the experiment which may contain multiple chips. Allele frequency estimates will be based on statistical analyses done on the data from all identical chips. You may generate an Allelotype or Allelotype Correction report on an individual chip, and the frequency estimates will be based on the data from one SpectroCHIP.

Generating a Genotype Area report on multiple chips is equivalent to generating a Genotype Area report individually for each chip and appending them into a single file. The data for each chip is kept separate from the data for the other chips.

When you select **Generate Area Report**, a **Select Report Template** dialog box appears.



Selecting a report type

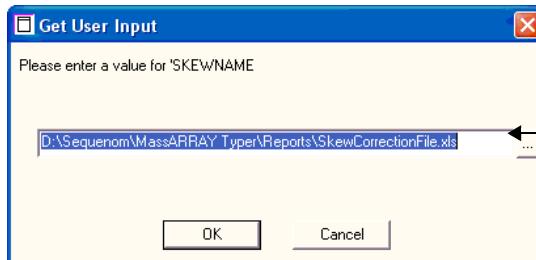
2. Select a template, and then click **Open**.

If you selected a Genotype Area report or Allelotype Correction report, a **Get User Input** dialog box appears. (Proceed to the next step.)

For all other report types, a **Save As** dialog box appears. (Skip to Step 4.)

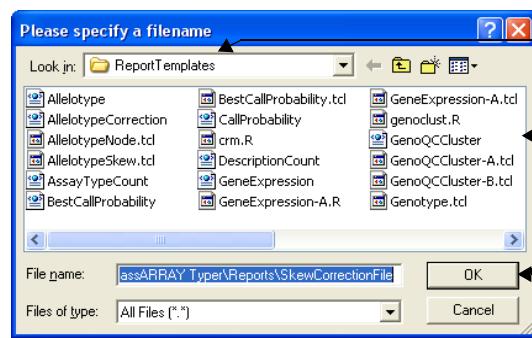
Note: To generate an Allelotype Correction report, there must be a skew correction file containing heterozygous skewing factors for the assays applied to your plate. Skew factors are saved to a skew correction file when you generate a Genotype Area report on genotype+area experiments. For more information, see "Skew Correction File" on page 141.

3. In the **Get User Input** dialog box, click the **Browse** button and select a skew correction file. This is the file to which heterozygous skewing factors will be appended.



Note: It is recommended that you have only one skew correction file (named SkewCorrectionFile, located in the ReportTemplates folder). However, you may create and use other skew correction files. To create a skew correction file select a folder, type a name for the file, and click **OK**. The file will be created and the skewing factors from this Genotype Area report will be saved to it.

Selecting a skew correction file



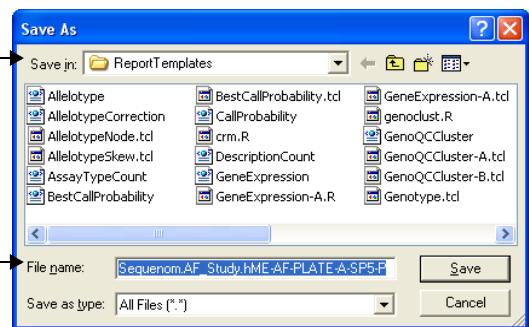
Select **SkewCorrectionFile**
After selecting SkewCorrectionFile, click **OK**

Note: If this is the first time you are running a Genotype report, SkewCorrectionFile does not exist. Select the **ReportTemplates** folder and type "SkewCorrectionFile" in the **File name** box. When you click **OK**, a skew correction file named SkewCorrectionFile will be created. From this point on, you should use this skew correction file.

4. In the **Save As** dialog box, select a different folder, type a file name, and click **Save**.

Initially, this dialog box shows the **ReportTemplates** folder. You should **not** save your reports to this folder. Select or create a different folder for your reports.

A default name is supplied for your report based on the experiment, chip, or plate you selected (see right). Edit or replace the name if you wish.



Default Name

A default report file name is in the form: <customer>_<project>_<plate>_<experiment>_<chip>. If you selected a plate, the default name only goes to <plate> (i.e. <customer>_<project>_<plate>). Similarly, if you selected a chip, the name goes to <chip>.

5. When you click **Save**, the report is generated. The report is displayed in Excel.

For descriptions of report contents, see Appendix A "Reports" on page 137.

Manually Calling a Genotype

You can override the automatic call by manually calling the genotype.

► To manually call a genotype in the table

1. Right-click the genotype row.

A menu opens with the available genotypes (see illustration).

2. Select the proper name for the genotype that you want to call.

The genotype name is replaced in the table and underlined in the spectrum. Manual calls are labeled *User Call*.

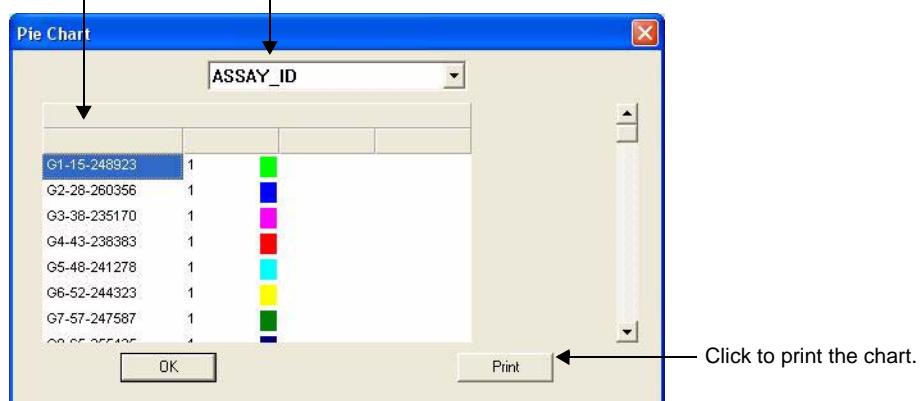
	SAMPLE_ID	CALL	ASSAY_ID	Sequenom AF_Study
1	SP5	CT	G1-15-243923	
2	SP5	G	G2-28-260356	
3	SP5	GA	G3-38-235170	
4	SP5	GA	G4-43-238383	
5	SP5	CT	G5-48-241278	
6	SP5	GA	G6-52-244323	
7	SP5	G	G7-57-247587	
8	SP5	T	G8-65-255425	
9	SP5	GA	G9-76-263848	
10	SP5	GA	G10-83-238314	
11	SP5	A	G11-91-241104	

Viewing a Pie Chart

View the table's data graphically using the pie chart feature.

This is the number of times the genotype was called.

From the drop down list, select the data you want to view in the chart.



► To view a pie chart of selected data

1. Select the proper plate.
2. From the View menu, choose **Pie Chart**.

The **Pie** window opens, initially showing a blank screen.

3. From the drop-down list, select the type of data you want to view.
The data appears in the dialog box.
4. If you want to print the results, click the **Print** button.
5. When finished viewing, click **OK**.

Recalling Plate Data

If you find that an assay has been incorrectly applied to a plate or well of a plate, you can recall the plate data and copy it to a new plate with the correct assays applied. Use ChipLinker to recall plate data. You may recall plate data on the RT-Workstation computer or on the server. See “Recalling Plate Data” on page 103.

Caution: Data recall should be used on only genotyping and genotype+area data. Do not recall allelotyping data.

Appendix A

Reports

Allelotype Report

The reports available in TyperAnalyzer and Genotype Analyzer are identical. Reports of plate data are generated as a tab-delimited output file (.xls) that can be viewed in Microsoft Excel. This appendix provides a description of the output files for each report.

For instructions on how to run a report in TyperAnalyzer, see “To generate a report” on page 106. For instructions on how to run a report in Genotype Analyzer, see “To generate a report” on page 132.

Note: Do not run Gene Expression and Genotype Cluster reports for iPLEX and hME. Gene Expression and Genotype Cluster reports are not applicable to iPLEX and hME and do not produce meaningful results.

You must have an allelotyping license to create an Allelotype report.

The Allelotype report contains allelotyping data, such as estimated frequencies of each allele. The following table describes the contents of an Allelotype report.

Table 6: Allelotype Report Contents

Column	Description
PLATE	Plate Name
WELL	Well Number
SAMPLE	Sample Name Sample name specified in Plate Editor when you created the plate
ASSAY	Assay Name Assay applied to the well
DATA_POINTS	Data Points Number of spectra acquired from the well. Typically, if the report is generated for a four-SpectroCHIP set, there should be 20 spectra (5 from each SpectroCHIP). Each provides a “data point” for frequency estimates. If the report is generated for a single SpectroCHIP, there should be 5 data points. Any value less than 20 (for four SpectroCHIPS) or 5 (for a single SpectroCHIP) means the full number of spectra were not successfully acquired, frequency estimates are still calculated. The estimates are based on the available data points.
NUM_CHIPS	Number of Chips The number of chips from which data was acquired for the well. Typically, allelotyping is done with four SpectroCHIPS. Each SpectroCHIP contains the same sample on corresponding wells. If you generated the report for a single SpectroCHIP, the number of chips should be 1.
AVE_FREQ1	Average Frequency of Allele 1 Weighted average relative frequency found from the data points for the lower-mass allele. 1.0 means 100%.

Table 6: Allelotype Report Contents

Column	Description
AVE_FREQ2	Average Frequency of Allele 2 Weighted average relative frequency found from the data points for the higher-mass allele. 1.0 means 100%.
FREQ_ERROR	Frequency Error The weighted uncertainty of the frequencies for both the lower- and higher-mass alleles.
ASSAY_STATUS	Assay Status <ul style="list-style-type: none"> • Polymorphic: Both alleles found • Non-Polymorphic: One allele found • Uncertain: The average frequency of one allele is less than or equal to 3 times the standard error of the frequencies before averaging • Bad Spectrum: A usable spectrum was not acquired from the well; the best spectrum acquired contains only noise • No Spectrum: No spectrum acquired or no assay applied to the well
PRIMER_FREQ	Primer Frequency Ratio of peak areas: $\frac{\text{Unextended-Primer Area}}{\text{Unextended-Primer Area} + \text{Allele 1 Area} + \text{Allele 2 Area}}$
PAUSE_FREQ	Pause Frequency Ratio of peak areas: $\frac{\text{Pausing Peak Area}}{\text{Pausing Peak Area} + \text{Allele 1 Area} + \text{Allele 2 Area}}$

Allelotype Correction Report

Genotype Area report
See “Assay Type Count Report” on page 139.

Skew Correction File
See “Skew Correction File” on page 141.

You must have an allelotyping license to create an Allelotype Correction report.

The Allelotype Correction report contains the same information as the Allelotype report plus corrections for heterozygous skewing.

In heterozygous samples, some skewing has been observed in spectra acquired by analyzers (skewing can also be caused by multiple reasons). When performing allelotyping, you can adjust for such skewing. Any assay you will use for allelotyping pooled samples should first be used to genotype individual DNA samples. When genotyping the individual DNA, choose **genotype+area** as the analysis type in an analyzer mass spectrometer. The relative frequencies of the alleles in heterozygous samples will be calculated for each spectrum. Then, in TyperAnalyzer or Genotype Analyzer, generate a Genotype Area report on the data. When the Genotype Area report is generated, the relative frequencies of the alleles in heterozygous samples will be averaged and saved to a skew correction file. These average heterozygous frequencies, from individual DNA, will be used as skewing factors to adjust the frequencies found in pooled DNA whenever the assay is used for allelotyping.

Once you have saved skewing factors to a skew correction file, you may view allelotyping results that are adjusted for heterozygous skewing by generating an Allelotype Correction report. The report will show allele frequencies adjusted with the skewing factors from the skew correction file.

Caution: Before generating an Allelotype Correction report, a skew correction file must contain skewing data which are applied to the allelotyping data. If your skew correction file does not have skewing data for the assays applied to the plate, you must add skewing data for those assays. Skewing data for an assay is added to a skew correction file by generating a Genotype Area report on a genotype+area plate on which the assay has been applied (see “Assay Type Count Report” on page 139).

The following table describes the additional columns of data included in an Allelotype Correction report. An Allelotype Correction report contains the same information as an Allelotype report (see Table 6 on page 137), plus these columns.

Table 7: Additional Columns in an Allelotype Correction Report

Column	Description
SKEW_LOWMASS	Skew of Lower-Mass Allele Frequency of the lower-mass allele, found by performing genotype+area analysis on individual DNA sample; this value is used to adjust the frequency of the lower-mass allele in a pooled sample; this value is taken from the skew correction file selected when the report was generated
SKEW_HIGHMASS	Skew of Higher-Mass Allele Frequency of the higher-mass allele, found by performing genotype+area analysis on individual DNA sample; this value is used to adjust the frequency of the higher-mass allele in a pooled sample; this value is taken from the skew correction file selected when the report was generated
CORR_FREQ1	Corrected Average Frequency of Allele 1 Average relative frequency found for the lower-mass allele, adjusted for heterozygous skewing (using the SKEW_LOWMASS value)
CORR_FREQ2	Corrected Average Frequency of Allele 2 Average relative frequency found for the higher-mass allele, adjusted for heterozygous skewing (using the SKEW_HIGHMASS value)

Assay Type Count Report

An Assay Type Count report lists the number of assays for the selected data.

Best Call Probability Report

The Best Call Probability report looks at all the data results and provides the best score result for the same sample and assay from among several results. The Score column in the output file indicates how good the call was, on a scale from 0 to 1.

Call Probability Report

The Call Probability report provides the same information as the Plate Result report, but with additional scoring information. (See “Plate Result Report” on page 141 for information.) The Score column in the Call Probability output file provides a record of how good the call was, based on a scale of 0 to 1.

Description Count Report

The Description Count report provides a count of each call status (Conservative, Moderate, etc.) for the selected data.

Genotype Area Report

You must have an allelotyping license to create a Genotype Area report.

This report contains genotyping data for the selected experiment or set of experiments. The following table describes the contents of a Genotype Area report.

Table 8: Genotype Area report Contents

Column	Description
CUSTOMER	Customer name
PROJECT	Project name
PLATE	Plate name
EXPERIMENT	Experiment name
CHIP	Chip name
WELL	Well number
SAMPLE	Sample name, as specified in Plate Editor when you created the plate
ASSAY GROUP	Assay Group name, as specified in Assay Editor when you created the assay group
ASSAY	Assay ID, as specified in Assay Editor when you created the assay
GENOTYPE	Genotype call

In addition to the contents described in the table above, the Genotype Area report contains summary information at the bottom. See the following illustration.

	A	G	GA	
TOTAL:	64	1	27	
FREQ:	0.8424	0.1576		
AVE HETERO RATIO:	0.5346	0.4654		
EVEN:	Yes			
STABLE:	Yes			
HETERO:	29.35%			

Example of the summary information appearing at the bottom of a Genotype Area report

Note: For genotyping data (with no area information) AVE HETERO RATIO, EVEN, and STABLE will be "0." These values are based on peak-area data, which is not available on plain genotyping data.

- TOTAL: Number of samples called for each allele and heterozygotes
- FREQ: Relative frequency of each allele in all samples
- AVE HETERO RATIO: In heterozygous samples, the average ratio of the alleles
- EVEN: "Yes" means the average heterozygous ratio is not more than 70/30 (i.e. 30 < either allele's frequency < 70)
- STABLE: "Yes" means the standard error of the heterozygous ratios is less than 0.11
- HETERO: Percentage of samples that are heterozygous

Genotype+Area Experiment

A genotyping experiment in Typer whose data includes peak-area data. You choose to capture such data when setting up the analyzer mass spectrometry run for a SpectroCHIP. For more information, see "Using Chip Linker to Associate Chips with Experiments" on page 60.

Creating a Skew Correction File

When you generate a Genotype Area report on a genotype+area experiment, heterozygous skewing data is automatically saved to a *skew correction file* (see below). The skewing data is used in an Allelotype Correction report to adjust the frequency estimates for alleles.

Skew Correction File

A skew correction file contains heterozygous skewing data for assays. It serves as a “repository” of skewing data for various assays; any new data is appended (added) to the existing data. For each assay, the relative frequencies of the lower- and higher-mass alleles are stored. When an Allelotype Correction report is generated, skewing data (from a skew correction file) for the applied assays are used to correct allele frequency estimates. It is recommended that you create and maintain only one skew correction file. Whenever, you generate new skewing data (by generating a Genotype Area report on genotype+area data), you should save it to the same file. This way, you are assured you are applying the most up-to-date skewing correction for an assay.

Note: The reporting functions are identical in the TyperAnalyzer and Genotype Analyzer modules. The same skew correction file should be used regardless of whether you are generating reports in the TyperAnalyzer or Genotype Analyzer module.

Plate Definition Report

The Plate Definition report is useful when you want to see what assays and samples are applied to the wells. This report lists the contents of each well.

Plate Result Report

The Plate Result report lists the call for each well.

Primer Adjustment Report

iPLEX

To get optimal results, it is recommended that you select one of the three options—A, B, or C—in Plate Editor that account for inverse relationship between analyte mass and signal-to-noise ratio. Still non-predictable variations in peak heights can occur. These variations may stem from inconsistent oligonucleotide quality and poor desorption/ionization behavior in MALDI. The Primer Adjustment report allows you to account for these variations and refine your adjustment from the spectra from the actual oligo mixture. This combination offers the best chance of success and is recommended if your plan to use the same primer mixture over a large number of samples. For more information, see Appendix A of the *iPLEX Application Guide*.

After performing the procedures described in the next section, run the Primary Adjustment report. This indicates which primers require further adjustment. It creates an Excel file (.xls) that indicates how to adjust the volume of the primer mixture for each assay in a multiplex.

For all the assays in a well, the one with the highest signal-to-noise ratio (SNR) receives a score of 1. Scores for the other assays in the multiplex are calculated relative to this score. For any assays in the well that fall below 45% (0.45), the primer volume needs to be adjusted. The **FRAC_ADD** column in the Excel file lists the amount of oligonucleotide to add to the mixture.

Adjusting iPLEX Primer Mixes

For best multiplexing results, the concentrations of iPLEX primers should be adjusted to even out peak heights (intensities) in the mass spectrum. **This adjustment must be done prior to preparing the iPLEX reaction cocktail and processing the iPLEX reaction.**

Note: Adjusting iPLEX primer mixes requires the use of a SpectroCHIP® bioarray. Adjusting iPLEX primer mixes is critical to successful multiplexing. An assay with a very low primer peak will systematically fail when applied to samples as part of a multiplex.

► **To adjust iPLEX primer mixes**

1. For each multiplex, prepare a mixture of the required iPLEX primers (referred to as a *primer mix*) according to method A, B, or C as described in Appendix A of the *iPLEX Application Guide*.
2. Pipette 1 µL of the primer mix into a well of a microplate and add 24 µL nanopure water to obtain a 360 nM dilution of the primer mix (referred to as a *primer mix sample*).
3. Repeat steps 1 and 2 for each multiplex, to generate a microplate containing primer mix samples for all of the multiplexes.
4. Add 3 mg Clean Resin to each well of the microtiter plate (MTP) using the dimple plate.

Note: Do **not** add any water. The existing dilutions of the primer mix samples are appropriate.

5. Dispense the primer mix samples to a SpectroCHIP using standard dispensing conditions for iPLEX reaction products.

It is recommended you dispense to two pads per primer mix.

For instructions on operating the Nanodispenser, see the *MassARRAY Nanodispenser User's Guide*. and the *iPLEX Application Guide*.

Note: If the entire SpectroCHIP is not used, you may keep it for future use in adjusting iPLEX primer mixes. Use only those pads on the SpectroCHIP that have not been used before; you cannot reuse previously spotted pads. Store SpectroCHIPS—in their original packaging—in a desiccator. SpectroCHIPS may be stored for **one week** maximum.

6. Acquire spectra from the SpectroCHIP.

For instructions on acquiring spectra, see “Chapter 4 Acquiring Spectra” on page 59.

Use the assay definitions (in Typer) for the actual multiplexes. Each well on the SpectroCHIP will yield no-calls because there is no analyte, only unextended iPLEX primers. A peak should appear at the expected mass for each iPLEX primer in the mix.

Note: At this point, you should “quality-check” the iPLEX primers and the primer mixes by reviewing the spectra. There should be a peak at the expected mass of each primer. A missing peak generally indicates poor primer quality or a primer missing from the mix. An unexpected peak generally indicates poor primer quality or the addition of an unnecessary primer to the mix.

7. Now run the Primer Adjustment report to determine if the primer mix should be adjusted.

If all peaks are at least 45% the height of the highest peak, they are acceptable. If any peak is less than 45% the height of the highest peak, add more of that primer. The **FRAC_ADD** column in the Primer Adjustment report indicates the amount to add (as a fraction of the given primer’s original volume).

Note: Adjust the original **primer mix**, not the primer mix sample in the microplate.

MassEXTEND

To get optimal results, run the Primer Adjustment report **before** preparing and processing the hME reaction. This report indicates which primers require volume adjustment. The peaks in the mass spectrum for a multiplexed reaction may not have comparable heights. Variations in peak height may stem from 1) inconsistent oligonucleotide quality, 2) inconsistent oligonucleotide concentration, and 3) different desorption/ionization behavior in MALDI.

The Primer Adjustment report creates an Excel file (.xls) that indicates how to adjust the volume of the primer mixture for each assay in a multiplex. (For information on multiplexing, see the application note *Multiplexing the homogeneous MassEXTEND Assay* available at www.sequenom.com.)

For all the assays in a well, the one with the highest signal-to-noise ratio (SNR) receives a score of 1. Scores for the other assays in the multiplex are calculated relative to this score. For any assays in the well that fall below 45% (0.45), the primer volume needs to be adjusted. The **FRAC_ADD** column in the Excel file lists the amount of oligonucleotide to add to the mixture.

Adjusting MassEXTEND Primer Mixes

For best multiplexing results, the concentrations of hME primers should be adjusted to even out peak heights (intensities) in the mass spectrum. **This adjustment must be done prior to preparing the hME reaction cocktail and processing the hME reaction.**

Note: Adjusting MassEXTEND primer mixes requires the use of a SpectroCHIP® bioarray. Adjusting MassEXTEND primer mixes is critical to successful multiplexing. An assay with a very low primer peak will systematically fail when applied to samples as part of a multiplex.

► **To adjust MassEXTEND primer mixes**

1. For each multiplex, prepare a mixture of the required MassEXTEND primers (referred to as a *primer mix*). The final concentration of each primer in the primer mix must be 9 µM.

Consider how much primer mix you will need. Each single hME reaction (i.e. a single well in a 384-well microplate) requires 1 µL primer mix.

Note: When ordering MassEXTEND primers from your oligonucleotide supplier it may be useful to consider at what plex-level you will use the primers and ask for the primers to be supplied at a certain concentration. For example, ordering primers for a 12-plex at 108 µM makes preparing primer mixes much easier. You can simply mix equal volumes of each 108 µM primer. Each primer will have a concentration of 9 µM in the final primer mix. Similarly, for a 10-plex, order MassEXTEND primers at 90 µM.

2. Pipette 1 µL of the primer mix into a well of a microplate and add 24 µL nanopure water to obtain a 360 nM dilution of the primer mix (referred to as a *primer mix sample*).
3. Repeat steps 1 and 2 for each multiplex, to generate a microplate containing primer mix samples for all of the multiplexes.
4. Add 3 mg Clean Resin to each well of the microtiter plate (MTP) using the dimple plate.

Note: Do **not** add any water. The existing dilutions of the primer mix samples are appropriate.

5. Dispense the primer mix samples to a SpectroCHIP using standard dispensing conditions for hME reaction products.

It is recommended you dispense to two pads per primer mix.

For instructions on operating the Nanodispenser, see the “Dispensing MassEXTEND Reaction Products onto SpectroCHIPS” chapter in *MassARRAY Nanodispenser User’s Guide*.

Note: If the entire SpectroCHIP is not used, you may keep it for future use in adjusting MassEXTEND primer mixes. Use only those pads on the SpectroCHIP that have not been used before; you cannot reuse previously spotted pads. Store SpectroCHIPS—in their original packaging—in a desiccator. SpectroCHIPS may be stored for **one week** maximum.

6. Acquire spectra from the SpectroCHIP.

For instructions on acquiring spectra, see “Chapter 4 Acquiring Spectra” on page 59.

Use the assay definitions (in Typer) for the actual multiplexes. Each well on the SpectroCHIP will yield no-calls because there is no analyte, only unextended MassEXTEND primers. A peak should appear at the expected mass for each MassEXTEND primer in the mix.

Note: At this point, you should “quality-check” the MassEXTEND primers and the primer mixes by reviewing the spectra. There should be a peak at the expected mass of each primer. A missing peak generally indicates poor primer quality or a primer missing from the mix. An unexpected peak generally indicates poor primer quality or the addition of an unnecessary primer to the mix.

7. Now run the Primer Adjustment report to determine if the primer mix should be adjusted.

If all peaks are at least 45% the height of the highest peak, they are acceptable. If any peak is less than 45% the height of the highest peak, add more of that primer. The **FRAC_ADD** column in the Primer Adjustment report indicates the amount to add (as a fraction of the given primer’s original volume).

Note: Adjust the original **primer mix**, not the primer mix sample in the microplate.

Notes:

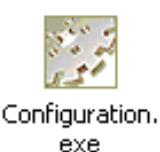
Configuring the MassARRAY Software

Starting the Configuration Software

The Configuration program is a companion program that allows you to customize Plate Editor and TyperAnalyzer components of the MassARRAY software.

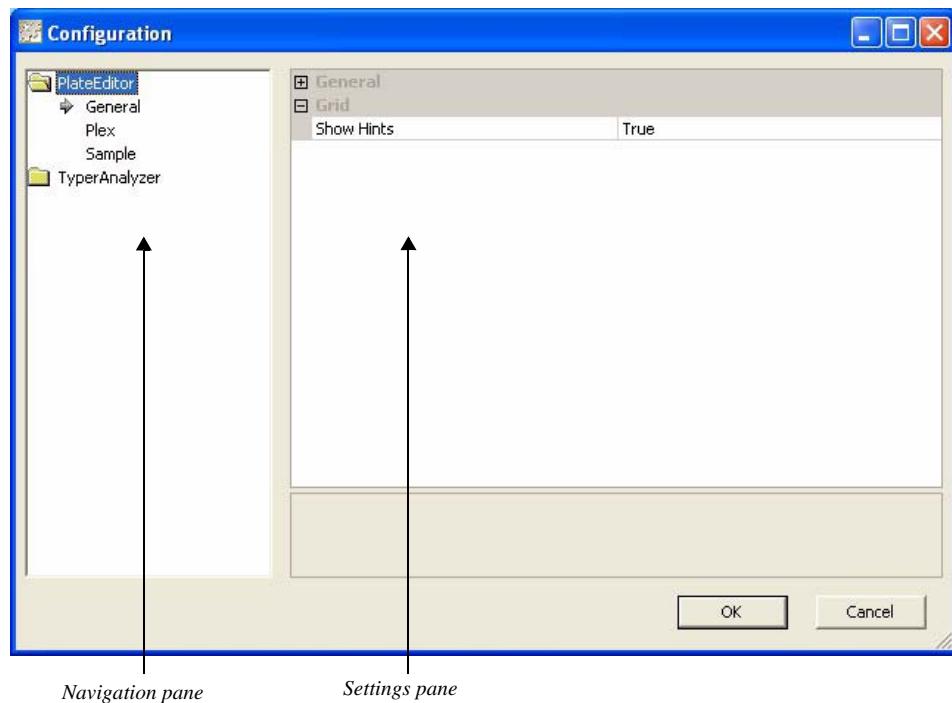
Navigating the Configuration Interface

The Configuration program resides in the same directory as the TyperAnalyzer program. To start the Configuration program, double-click the Configuration icon as shown below:



Configuration.
exe

The Configuration software provides a navigation pane on the left to locate the type of settings you want to configure, and a settings pane on the right for viewing and setting the parameters.



► **To navigate to the settings you want to modify**

1. In the left pane click a folder—PlateEditor and TyperAnalyzer—to open and view the configuration categories.
2. In the left pane, click on the configuration category you want to display the settings subcategories in the right pane. A green arrow in front of the category name in the left pane means the category is currently selected.

3. In the right pane, click on a subcategory to view the settings. Click on the plus symbol [+] of any item to display its contents. Click on the minus symbol [-] of any item to hide its contents.

Configuring Plate Editor

You can configure general Plater Editor properties—such as look and feel, grid height, allow NULL concentration values, and set schema owner. The following general settings can be configured:

- General properties, including look and feel, grid height, allow NULL concentration values, and set schema owner
- Plex properties, including plex colors, plex layout, and sample colors
- Color of the samples displayed

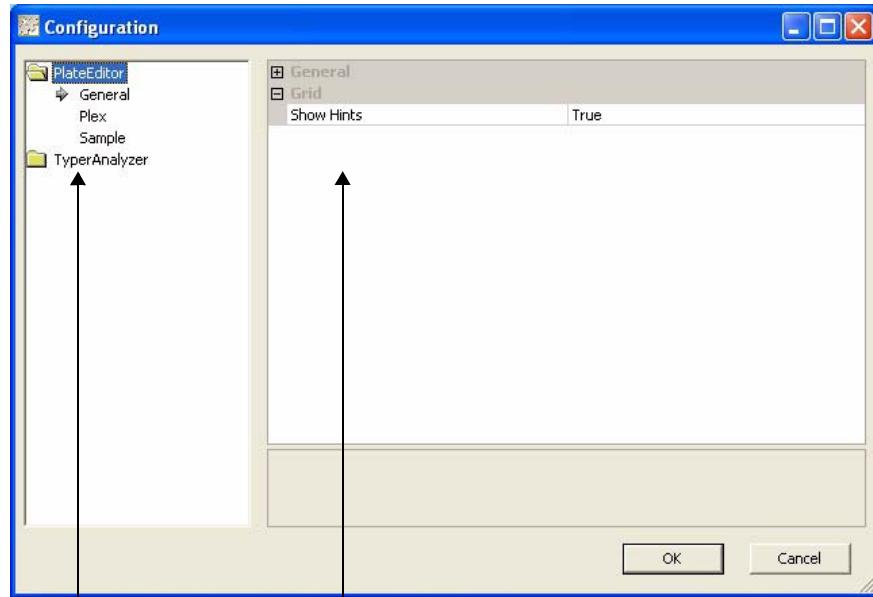
Configuring General Settings

General settings apply to the Plate Editor as a whole. General settings include:

- Look and feel
- Property grid help height
- NULL concentration value
- Schema owner

► To configure general settings

1. In the left pane, click **PlateEditor**, then click **General**.



2. In the right pane, click on **General** to open the General options.

3. Configure the settings as follows:

Look and Feel	Set the look and feel of the program. Click and select one of the following themes from the drop-down list:
	<ul style="list-style-type: none"> • Office 2003 • OfficeXP • NativeWinXP
Property Grid Help Height	Set the height of the property help window. Click on the cell and enter the height.
NULL Concentration Value	Allow a NULL concentration value. Click on the cell and choose True or False .
Schema Owner	Set the owner of the schema. Click on the cell and enter the owner name.

Configuring the Grid

The Grid setting allows you to show hints when a user holds the mouse pointer over a button or icon on the Plate Editor user interface.

► To configure the grid

1. In the left pane, click **PlateEditor**, then click **General**.
2. In the right pane, click on **Grid** to open the Grid options.
3. Configure the settings as follows:

Show Hints	Indicates whether hints are displayed. Click in the cell and select True or False .
------------	---------------------------------------------------------------------------------------------------

Configuring Plex Settings

Using the Plex settings you can configure the plex colors and layout.

► To configure plex colors

1. In the left pane, click **PlateEditor**, then click **Plex**.
2. In the right pane, click on **Colors** to open the Color options.
3. Click on the plex—1-Plex through 12-Plex—whose colors you want to define.
4. Click the ... button.
5. In the **Color** dialog box, define the color and click **OK**.

► To configure plex layout

1. In the left pane, click **PlateEditor**, then click **Plex**.
2. In the right pane, click on **Layout** to open the Layout options.
3. Configure the layout as follows:

Automatically Save Layout	Automatically save the current layout as the default when the program closes. Click in the cell and choose True or False .
Use Docking Stickers	Display docking stickers for easy window docking. Click in the cell and choose True or False .

Configuring Sample Colors

Using the Samples settings you can configure the color of the interactive plot active node.

► To set the sample colors

1. In the left pane, click **PlateEditor**, then click **Sample**.
2. In the right pane, click on **Colors** to open the Color options.
3. Click the ... button.
4. In the **Color** dialog box, define the color and click **OK**.

Configuring TyperAnalyzer

The following general settings can be configured for TyperAnalyzer:

- Display—Set the Cluster Plot and Well Data display settings
- General—Set the look and feel of the TyperAnalyzer program.
- Help—Specify a new help file.
- Layout—Set layout behavior.
- Report—Set reporting options.
- Traffic Light—Configure Traffic Light pane attributes.

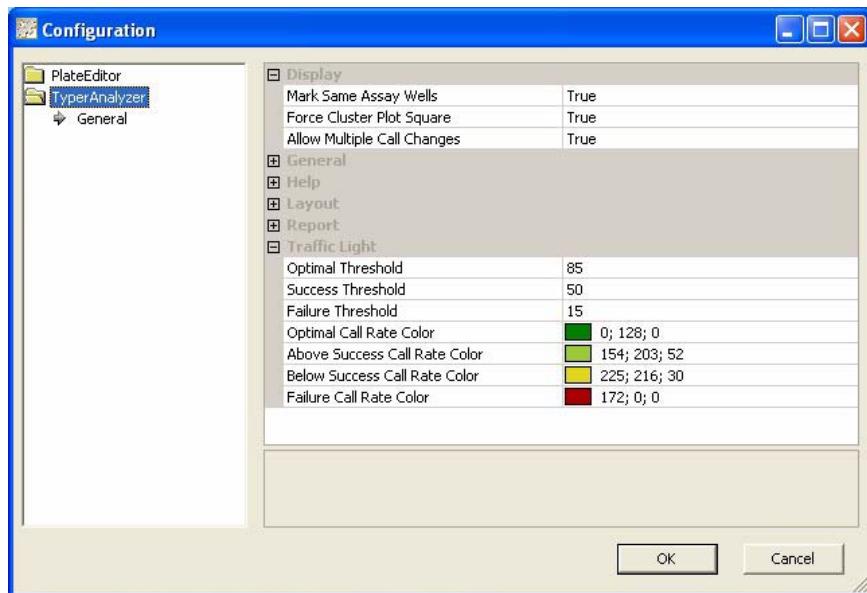
Configuring the Display

You can configure display options:

- Mark same assay wells
- Force cluster plot square

To configure the display options

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **Display** to open the Display options.



3. Set any of the following:

Mark Same Assay Wells	Mark the same assay wells in the same color as the currently selected well. Click in the cell and select True or False .
Force Cluster Plot Square	Make cluster plots square regardless of the window shape. Click in the cell and select True or False .
Allow Multiple Call Changes	Allow a user to select an area in the Cluster Plot and change all of the calls within it. Zoom will be turned off when the target call is set. The default is False. To turn on this feature, click in the cell and select True .

Configuring the General Options

You can configure display options:

- Mark same assay words
- Force cluster plot square

» To configure the general options

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **General** to open the General options:

Look and Feel Allows you to set the look and feel of the program. You can choose from the following themes:

- Office 2003
- OfficeXP
- NativeWinXP

Configuring the Help

You can set which file is displayed when the help button in the TyperAnalyzer software is clicked.

» To configure the help file

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **Help**.
3. Click on the **User Manual** cell, click on the button, and locate and select the file that contains TyperAnalyzer user guide.

Configuring the Program Layout

You can configure which file is displayed when the help button in the TyperAnalyzer software is clicked.

» To configure the help file

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **Layout**.
3. Configure any of the following:

Automatically Save Layout Specify whether to automatically save the current layout as the default when the program exits. Click on the cell and choose **True** or **False**.

Use Docking Stickers Display docking stickers to be used for easy window docking. Click on the cell and choose **True** or **False**.

Cluster Plot Type Combo Width Define the width of the Cluster Plot Type combo box width on tool bar. Click on the cell and enter a number.

Cluster Plot Split Line Color Specify the color of the cluster plot split line. Click ... to open the **Color** box and define a color.

Histogram Display Limit	Set the Histogram assay display limit at startup. Click on the cell and enter a number.
Graph Label Font Size Control	Set the font size for the graph labels. Click in the cell and enter a value between 0.5 and 1.5

Configuring Reports

You can configure TyperAnalyzer keep processing files for debugging in a report.

► **To configure reports**

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **Layout**, then click **Report**.
3. Click in the **Debug** cell and enter **True** or **False**.

Configuring the Traffic Lights

You can configure the threshold, success, and failure values used by the Traffic Lights in the TyperAnalyzer. You can define, in percent, where the following calls are split:

- Success conservative/moderate compared to aggressive/low probability/bad spectra calls
- Conservative/moderate calls compared to aggressive/low probability/bad spectra calls
- Failure conservative/moderate calls compared to aggressive/low probability/bad spectra calls

In addition, you can define the colors of the following:

- Above optimal threshold conservative/moderate calls
- Above success threshold and below optimal threshold conservative/moderate calls
- Below success threshold and above failure threshold conservative/moderate calls
- Below failure threshold conservative/moderate calls

► **To configure traffic lights**

1. In the left pane, click **TyperAnalyzer**.
2. In the right pane, click on **Layout**.
3. Configure any of the following:

Optimal Threshold	Set the value, in percent, that splits optimal and success conservative/moderate compared to aggressive/low probability/bad spectra calls. Click in the cell and enter the value.
Success Threshold	Set the value, in percent, that splits success conservative/moderate calls compared to aggressive/low probability/bad spectra calls. Click in the cell and enter the value.

Failure Threshold	Set the value, in percent, that splits failure conservative/moderate calls compared to aggressive/low probability/bad spectra calls. Click in the cell and enter the value.
Optimal Call Rate Color	Set the display color for above optimal threshold conservative/moderate calls. Click in the cell, click on the ... button, and define the color in the Color dialog box.
Above Success Call Rate Color	Set the display color for above success threshold and below optimal threshold conservative/moderate calls. Click in the cell, click on the ... button, and define the color in the Color dialog box.
Below Success Call Rate Color	Set the display color for below success threshold and above failure threshold conservative/moderate calls. Click in the cell, click on the ... button, and define the color in the Color dialog box.
Failure Call Rate Color	Set the display color for below failure threshold conservative/moderate calls. Click in the cell, click on the ... button, and define the color in the Color dialog box.

Quitting the Configuration Software

Changes you make are applied the next time Plate Editor or TyperAnalyzer are started.

» **To apply the changes and quit the Configuration Software**

- Click **OK**.

» **To quit the Configuration Software without saving the changes**

- Click **Cancel**.

Appendix C

Terms and Conditions

LIMITED LIABILITY

SEQUENOM shall not be liable, to any extent whatsoever, for any damages resulting from or arising out of the use or performance of this system and related documentation or the procedures specified in this manual, regardless of foreseeability or the form of action, whether in contract, tort (including negligence), breach of warranty, strict liability or otherwise, and including but not limited to, damages resulting from loss of data, loss of anticipated profits, or any special, indirect, incidental or consequential damages. In no event shall SEQUENOM's liability to the user exceed the amount paid by the user to SEQUENOM hereunder. The **USER** assumes full responsibility for the results obtained from the use of this system and related documentation and for application of such results. This system is for research use only and is not to be used for diagnostic purposes.

THE **USER** IS HEREBY PUT ON NOTICE THAT SEQUENOM'S MASSARRAY PRODUCTS AND iPLEX™ AND MassEXTEND™ METHODS AND PROCESSES HAVE NOT BEEN SUBJECT TO REGULATORY REVIEW OR APPROVED BY THE FEDERAL FOOD AND DRUG ADMINISTRATION OR ANY OTHER UNITED STATES GOVERNMENTAL AGENCY OR ENTITY, AND HAVE NOT BEEN APPROVED FOR CLIA COMPLIANCE, OR OTHERWISE APPROVED UNDER ANY STATUTE, RULE, LAW, OR REGULATION, FOR ANY PURPOSE, RESEARCH, COMMERCIAL, DIAGNOSTIC, OR OTHERWISE.

LIMITED WARRANTY

Limited Warranty Relating to MassARRAY Products. SEQUENOM warrants that the MassARRAY System will be free from defects in materials and workmanship and will conform to SEQUENOM's current specifications, and perform accordingly, from the time of installation and for a period of one (1) year thereafter, so long as the MassARRAY System remains unchanged and in the original condition supplied by SEQUENOM. SEQUENOM warrants that the MassARRAY Kits will be free from defects in materials and workmanship and will conform to SEQUENOM's specifications and perform accordingly up to the expiration date specified on the MassARRAY Kit packaging, so long as the MassARRAY Kits are stored according to specifications, and remain unchanged and in the original condition supplied by SEQUENOM. The foregoing warranty does not include periodic maintenance or calibration recommended for some MassARRAY Products. This warranty does not apply to defects resulting from improper or inadequate maintenance or calibration by the **USER**; defects resulting from hardware, software, interfacing, or supplies provided by parties other than SEQUENOM; defects resulting from unauthorized modification, maintenance, or repair, or improper use or operation outside of SEQUENOM's specifications for the MassARRAY Products or by personnel not authorized by SEQUENOM, and; defects resulting from abuse, negligence, accident, loss or damage in transit. In addition, this warranty does not apply to damage due to (1) environmental conditions at the site of installation; (2) operator failure to perform standard operating

procedures and routine maintenance as prescribed in the operator manuals; (3) moving (by other than SEQUENOM authorized personnel) the MassARRAY System from its installed location; (4) exposure of the MassARRAY Products to Bio-Safety Level 3 or 4 (as defined by the United States Occupational Health and Safety Administration) agents; or (5) exposure to radioactivity.

SEQUENOM's sole obligation and liability for any breach of the limited warranty set forth herein shall be at SEQUENOM's sole discretion and option: (1) to replace the MassARRAY Products, in whole or in part, provided that the **USER** notifies SEQUENOM of the defects, SEQUENOM directs the **USER** to return the defective MassARRAY Products to SEQUENOM and the **USER** returns the MassARRAY Products as directed, at SEQUENOM's expense; or (2) to repair (and recalibrate as necessitated by repair) the MassARRAY Products in whole or in part. MassARRAY Products may not be returned to SEQUENOM under any circumstances without SEQUENOM's prior authorization. SEQUENOM shall not be liable, to any extent whatsoever, for any damages resulting from or arising out of the use or performance of the MassARRAY Products provided regardless of foreseeability or the form of the cause of action, whether in contract, breach of warranty, tort (including negligence, strict liability, or otherwise), and including but not limited to damages resulting from loss of data, loss of anticipated profits or revenue, or any special, direct, indirect, incidental or consequential damages.

The limited warranty sets forth SEQUENOM's sole and exclusive responsibility with respect to any alleged breach of this limited warranty. Except as provided herein, the MassARRAY Products are provided without warranty of any kind or nature.

SEQUENOM does not warrant, guarantee, or make any representations regarding the use or the results of the use, of the MassARRAY Products in terms of correctness, accuracy, reliability, or otherwise. The **USER** assumes the entire risk as to the results and performance of the MassARRAY Products. The foregoing warranty is exclusive and is made in lieu of and to the exclusion of any other warranties, whether oral or written, express or implied, direct, indirect, by estoppel or otherwise, or created by the Uniform Commercial Code or the usage in the industry or the course of dealings of the parties, as to any matter whatsoever, including but not limited to those concerning merchantability or fitness for a particular purpose.

LICENSING

Sequenom's patented DNA analysis by mass spectrometry methods (including the iPLEX™ and MassEXTEND™ method) are protected under U.S. patent rights including but not limited to 6,500,621; 6,300,076; 6,258,538; and 5,869,242 and their foreign equivalent patent rights. With the purchase of Sequenom SpectroCHIP® array chips (whether purchased as a stand-alone product or in a kit product with reagents), Customer is granted a limited, one time use only per each chip element, right to practice iPLEX™ and MassEXTEND™ methods. Such license right granted is limited to one-time use for the number of elements provided per SpectroCHIP® array chip purchased. For example, Sequenom's SpectroCHIP® 384 array chip is provided with 384 elements. Sequenom's SpectroCHIP® 96 array chip is provided with 96 elements. Such license right is granted for customer's internal research and development purposes only, and not for commercial use. Customer may not practice Sequenom's patented methods for providing services to third parties in exchange for fees or other consideration, without written permission from Sequenom. Re-manufacture or re-use of Sequenom's SpectroCHIP® array chips and elements, with Sequenom's patented

DNA analysis methods, is prohibited. Other than as expressly set forth above, no license rights are granted or implied.

SOFTWARE LICENSING

For purposes of this section, “Software” means computer software or programs supplied by Sequenom to the Customer under this Agreement including but not limited to such software that is embedded in or forms an integral part of Sequenom’s hardware products in addition to separately provided software for application specific or other purposes. For purposes of this section, Software does not include Oracle Corporation software products provided by Sequenom as such Oracle products are separately governed by the attached Oracle End User License Terms. (a) Sequenom grants Customer a non-exclusive and non-transferable license to use the Software for processing data for business purposes only. Customer shall not permit another party to use the Software and Customer shall effect and maintain adequate security measures to safeguard the Software from access or use by unauthorized persons. Customer shall not transfer, rent, lease, sublicense, loan, copy, modify, adapt, merge, translate, reverse engineer, decompile, or disassemble the Software or create derivative works based on the whole or any part of the Software. (b) The Software license shall not be deemed to extend to any of Sequenom’s intellectual property rights, including rights in source code. No copies may be made of the Software without the prior written consent of Sequenom, except that Customer may make a single back up or archival copy. The Software license shall apply to any copy as it applies to the Software. (c) With specific reference to Sequenom’s iPLEX™ application software (the “iPLEX™ Software”) that is part of Sequenom’s Typer 3.3 software product, Customer is provided free of charge, a royalty-free, non-exclusive and non-transferable license to use the iPLEX™ Software subject to the terms and conditions in subsections (a) and (b) above and elsewhere in this section, solely upon the condition that such iPLEX™ Software is used exclusively in conjunction with the specially designated reagent kit product (catalog item no. 10130) purchased from Sequenom. License rights to use the iPLEX™ Software are not granted by or with the iPLEX™ Software by itself, separate and apart from use in conjunction with such specially designated reagent kit product purchased from Sequenom. All Software licenses shall terminate automatically and immediately if Customer fails to abide by any of the terms in this section or under this Agreement.

CONFLICTS

In the event that a conflict arises between the language of this Proposal and any work order, purchase order, billing statement, or invoice related to the purchase, license, or other transfer of any SEQUENOM products or technology or services to be provided, the language of this Proposal shall govern and control, and the conflicting terms, provisions, and conditions of any such other documents shall be deemed non-existent, and shall not obligate or be binding upon Customer or SEQUENOM.

